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23rd Congress of the European Hematology Association

Stockholm, Sweden | June 14 - 17, 2018

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23rd Congress of the European Hematology Association

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ABSTRACT BOOK

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MCL-1, a BCL-2 family member, is an APOPTOSIS INHIBITOR in MCL-1–dependent AML[1-3]

MCL-1 dependence may drive progression of AML[1,2]

Acute myeloid leukemia (AML) is associated with high mortality and is challenging to treat, with an overall 5-year survival rate of 27%.[4,5] Standard therapies often fail to achieve the goal of inducing complete remission in 25%–50% of patients, and relapse is common even in patients who have an initial response to treatment.[1,5] Disease progression and treatment resistance in a subset* of AML have been associated with a key anti-apoptotic protein, myeloid cell leukemia 1 (MCL-1).[1,2] This is referred to as MCL-1 dependence.[6] Understanding the role of MCL-1 can inform therapeutic targeting strategies in AML.[7]

MCL-1 may have multiple roles in sustaining AML blasts[1,3]

MCL-1 is a member of the apoptosis-regulating BCL-2 family of proteins.[8] In normal function, MCL-1 is essential for early embryonic development and for the survival of multiple cell lineages, including lymphocytes and hematopoietic stem cells.[3]

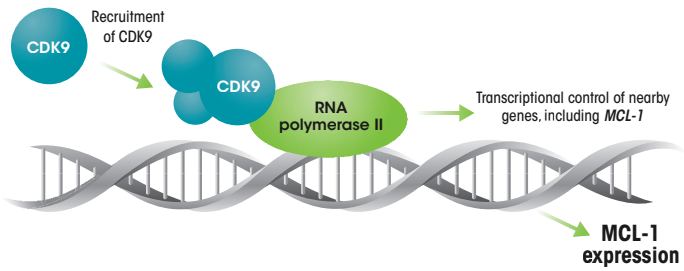
However, in MCL-1–dependent AML, MCL-1 has been shown to sustain the survival of AML cells, which may lead to relapse.[1] MCL-1 dependence is also associated with resistance to agents that typically have activity against leukemic blasts.[8]

A key function of MCL-1 is to inhibit apoptosis[1]

In addition, independently of its anti-apoptotic activity, MCL-1 has a role in mitochondrial function that may promote cancer cell survival and proliferation.[3] The anti-apoptotic and mitochondrial functions of MCL-1 may synergize to promote tumor progression by inhibiting apoptosis and supporting proliferation.[3]

Downregulation of MCL-1 to enable apoptosis of leukemic blasts may be a rational therapeutic strategy in MCL-1–dependent AML[7]

The activity of cyclin-dependent kinase 9 (CDK9) is essential for the transcription of *MCL-1* mRNA in leukemic blasts.[9,10]



Because of the short half-life of MCL-1 (2-4 hours), the effects of targeting upstream pathways are expected to reduce MCL-1 levels rapidly.[11] CDK9 inhibition has been shown to block *MCL-1* transcription, resulting in the rapid downregulation of MCL-1 protein, thus triggering apoptosis.[8,12,13]

*The prevalence of MCL-1–dependent AML is under investigation.

MCL-1 Dependence in AML

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Word of Welcome

On behalf of the EHA Board and the Scientific Program Committee we are pleased to present to you this year's Abstract Program.

The Scientific Program Committee has compiled an exciting program of oral and poster presentations from close to 2600 submitted abstracts representing all fields of hematology. The six best abstracts will be presented during the Presidential Symposium on Friday afternoon. This will be a session not to miss.

The Oral Sessions are spread over three days (Friday to Sunday) providing you with ample opportunity to attend several of them and get updated on a broad range of topics and research areas. Several poster presenters will have the opportunity to pitch their abstract during an Oral Session. These Poster Pitches are an exciting opportunity to promote basic science and research, and to invite delegates to the Poster Sessions.

The Poster Session setup has changed significantly because the Scientific Program Committee has doubled the abstracts accepted for presentation. The posters will be on display for only one day, either during the Friday or Saturday Poster Session. All accepted posters can also be viewed on the e-poster screens from Friday, June 15, 09:30 to Sunday, June 17, 13:00. The Poster Session gives maximum exposure to the scientific work and allows direct interaction between poster authors and congress delegates. Don't miss this opportunity of interesting discussions and networking!

Nurturing the next generation is an important task of EHA and this year we are providing over 150 travel grants to Junior Members who received an abstract presentation in the program. In addition, during the Opening Ceremony, we are awarding the best submitted abstracts of trainees in four categories of career stages. These awardees and the travel grant winners can be found on the next page.

The late-breaking abstract program is intended for abstracts with novel data not available at the time of the regular abstract submission deadline. Only a few abstracts, with the most exciting results are selected for a presentation in the Late-Breaking Oral Session on Sunday morning, and a special slot is reserved during Plenary Session I on Saturday for the best late-breaking abstract.

All the abstracts are also available on the EHA Learning Center, for which you have complimentary access after the congress: learningcenter.ehaweb.org.

On behalf of the EHA Board, the committees and all the people involved in this year's EHA Congress and the abstract program, we trust you will enjoy reading this book, the abstracts and the research presented.

Martina Muckenthaler

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Late-Breaking Oral Session

The best abstracts selected from the late-breaking abstract submission are presented during this oral session on Sunday, June 17 from 11:15 - 12:45 in Room A1, and a special slot is reserved during Plenary Session I on Saturday for the best late-breaking abstract.

A complete session overview is available via the mobile app or the online program at ehaweb.org

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SIMULTANEOUS SESSIONS I

Follicular and marginal zone lymphomas – Clinical

S100

INTERIM UPDATE FROM A PHASE 2 MULTICENTER STUDY OF TAZEMETOSTAT, AN EZH2 INHIBITOR, IN PATIENTS WITH RELAPSED OR REFRACTORY (R/R) FOLLICULAR LYMPHOMA (FL)

F. Morschhauser[1], H. Tilly[2], A. Chaidos[3], T. Phillips[4], V. Ribrag[5], P. Campbell[6], C. Fruchart[7], W. Jurczak[8], P. McKay[9], S. Opat[10], J. Radford[11], A. McDonald[12], H. Howell[12], K. Newberry[12], M. Woodruff[12], A. Clawson[12], J. Larus[12], S. Blakemore[12], H. Miao[12], G. Salles[13,*]

[1]Centre Hospitalier Universitaire, Lille, [2]Centre de Lutte Contre le Cancer Henri Becquerel, Rouen, France, [3]Centre for Haematology, Department of Medicine, Imperial College London, Imperial College Healthcare NHS Trust, Hammersmith Hospital, London, United Kingdom, [4]Division of Hematology and Oncology, University of Michigan, Ann Arbor, United States, [5]Gustave Roussy, Villejuif, France, [6]Barwon Health, Geelong, Australia, [7]Centre François Baclesse, Caen, France, [8]UJCM, Krakow, Poland, [9]Beatson West of Scotland Cancer Centre, Glasgow, United Kingdom, [10]Monash University, Clayton, Australia, [11]The University of Manchester, Manchester, United Kingdom, [12]Epizyme, Cambridge, United States, [13]Lyon-Sud Hospital Centre, Pierre-Bénite, France

Background: R/R FL remains an area of unmet medical need and treatments with novel mechanisms of action are urgently needed. The histone methyltransferase EZH2 is an important regulator of the germinal center (GC) reaction that is involved in prevention of terminal differentiation of GC B-cells. EZH2 activating mutations are not uncommon in FL and are postulated to be oncogenic drivers. Tazemetostat, a potent, selective, oral EZH2 inhibitor has shown antitumor activity in a phase 1 study that included NHL patients with mutated (mt) or wild-type (wt) EZH2 tumors, providing rationale for further investigation.
Aims: This open-label, multicenter phase 2 study is enrolling patients with either mt or wt EZH2 R/R diffuse large B-cell lymphoma or FL (Grade 1-3b); Results of an interim analysis of FL patients are presented here.
Methods: Key inclusion criteria include: age ≥18 years old, ≥2 prior treatment regimens, measurable disease, and adequate organ function. Tazmetostat 800 mg is administered orally, twice daily (BID). Response is assessed every 8 weeks using 2007 IWG-NHL assessment criteria. Tumor tissue is analyzed for EZH2 hot spot activating mutations (Y646X, A682G, A692V) using a **cobas**® EZH2 Mutation Test (Roche Molecular Systems, investigational use only). The primary endpoint is overall response rate (ORR). Secondary endpoints include progression-free survival (PFS) and safety/tolerability.
Results: As of January 16, 2018, interim phase 2 safety and efficacy data were summarized from 76 FL patients (median 3 prior therapies; one patient who had not yet been evaluated at week 8 was excluded from the efficacy analysis). In patients with an activating EZH2 mutation (n=22), the ORR (complete response [CR] + partial response [PR]) was 82%, with best overall response of CR 5% (n=1), PR 77% (n=17), stable disease (SD) 18% (n=4), and no patients with progressive disease (PD). Median PFS was >48 weeks and median duration of response (DOR) was >32 weeks. Fifty-six percent of patients (10/18) have maintained their response and remain on study. In the EZH2 wt group (n=54), ORR was 35% (n=19), with best overall response of CR 6% (n=3), PR 30% (n=16), SD 30% (n=16), PD 30% (n=16); data not available for 3 patients. Median PFS was >30 weeks and median DOR >56 weeks. Fifty-eight percent of patients (11/19) have maintained their response and remain on study. Safety analysis showed that treatment-emergent adverse events (TEAE) leading to study drug discontinuation occurred in 12% of patients. Grade ≥3 treatment-related AEs were reported in 12% of patients. The most common (≥10%) treatment-related TEAEs (all grades) were: nausea (18%), anemia (13%), fatigue (13%), diarrhea (12%), and asthenia (10%).
Summary and Conclusions: Tazemetostat appears to be generally well tolerated at a dose of 800 mg BID with observed meaningful clinical activity and durability of response in FL patients who have had multiple prior therapies, as seen in this interim update. Clinical activity is pronounced in patients with EZH2 activating mutations; 100% had CR, PR, or SD as best response. For wt, 65% had CR, PR, or SD as best response. Late onset responses have been previously reported on tazemetostat, therefore clinical outcome in patients on continuing treatment may evolve from SD to PR and from PR to CR. These encouraging phase 2 data demonstrate that EZH2 inhibition may be an important and effective therapeutic target.

S101

PATIENTS WITH HIGH RISK FEATURES ACCORDING TO PRIMA PI HAVE SIGNIFICANTLY HIGHER RISK TO DIE EVEN IF THEY ARE LATE PROGRESSORS

M. Trneny[1,*], A. Janikova[2], D. Belada[3], H. Mocikova[4], V. Prochazka[5], K. Kopeckova[6], J. Duras[7], J. Pirnos[8], L. Barsova[9], J. Salkova[10], K. Benesova[10], J. Mayer[11], P. Blahovcova[12]

[1]1st Dept of Medicine, 1st Faculty of Medicine, Charles University & General University Hospital Prague, 1st Faculty of Medicine, Charles University & General University Hospital, Prague, [2]Dept. of Internal Medicine, Hematology and Oncology, University Hospital, Brno, [3]4th Department of Internal Medicine - Hematology, University Hospital, Hradec Kralove, [4]Dept. of Clin. Hematology, University Hospital Kralovske Vinohrady, Prague, [5]Department of Haemato-Oncology, University Hospital, Olomouc, [6]Dept of Oncology, University Hospital Motol, Prague, [7]Department of Haematooncology, University Hospital, Ostrava, [8]Dept of Oncology, Hospital C. Budejovice, Ceske Budejovice, [9]Dept of Oncology, Hospital Liberec, Liberec, [10]1st Dept of Medicine, 1st Faculty of Medicine, Charles University & General University Hospital, Prague, [11]Dept. of Internal Medicine, Hematology and Oncology, Faculty Hospital, Brno, [12]Czech Lymphoma Study Group, Charles University & General University Hospital, Prague, Czech Republic

Background: Follicular lymphoma (FL) patients (pts) have high long-term survival probability in majority of cases, however some subgroups especially early progressors and pts with transformation poor outcome. Prognostic indexes could partially help and recently presented PRIMA PI (Bachy ASH2017) is the first one based on population of immunochemotherapy treated pts (PRIMA study).
Aims: Validation of PRIMA PI in large cohort of real world treated pts and analysis of impact of different PRIMA PI risk groups on progression within 24 months since start of therapy (POD24).
Methods: This analysis was part of NiHiL project (GovTrial No: NCT03199066). NiHiL project is based on prospective collection and analysis of diagnostic, epidemiologic and therapeutic data of NHL patients. All patients signed the informed consent approved by EC. The eligibility criteria were: all consecutively pts with confirmed FL dg, with confirmation of essential data (clinical characteristic, therapy, follow-up) treated with immunochemotherapy. Progression free survival (PFS) and Overall survival (OS) were counted since the therapy initiation. Pearson's Chi-square Test was used for group comparison, log rank test for survival analysis.
Results: Altogether 1179 pts diagnosed 1999-2015 and treated with

immunochemotherapy in 1st line 2000-2015 fulfilled eligibility criteria. The majority (893) had grade 1 or 2, followed by gr 3A (188) or without given grade (98). Median age was 59y (26-87), BM was involved in 50.4% cases, LDH was elevated in 42.1% cases and beta2microglobulin >3mg/l in 31.8% of pts. Low risk group (LRG) according to FLIPI, FLIPI2 and PRIMA PI resp. represent 20.5%, 35.5% and 39.0% pts resp., intermediate risk group (IRG) 31.3%, 25.6% and 29.2% pts resp., high risk group (HRG) 48.1%, 38.9% and 31.8% pts resp. All patients were treated with rituximab and majority of pts (76.3%) were treated with CHOP, CVP was used in 13.3% pts, bendamustine 2.8%, fludarabine 2.6% and the rest with other regimens according to centres policy (PACEBO, sequential protocol and others). ORR (Cheson 1999) was reached in 92.4% of pts, out of these 66.7% received rituximab maintenance. With the median follow-up 6.1y there was 7y PFS and OS probability 58.7% and 82.3%. According to FLIPI, FLIPI2 and PRIMA PI resp. there was 7y PFS probability in LRG 76.1%, 71.3% and 72.6% resp., in IRG 63.7%, 61.1% and 57.7% resp., in HRG 46.9%, 45.6% and 42.4% resp. (Figure 1A). OS probability at 7y according to FLIPI, FLIPI2 and PRIMA PI resp. was in LRG 90.8%, 90.8% and 88.2% resp., in IRG 87.0%, 82.7% and 89.2% resp. and in HRG 75.2%, 73.1% and 68.0% resp. OS probability at 7y was 51.9% for those with POD24 *vs* 91.9% (median 7.1 *vs* nr) for those without POD24 (HR 7.1, p <0.0001). We tested impact of HR *vs* LG and IG (pooled into one group) PRIMA PI characteristic on OS separately in those pts with and without POD24. Among POD24 pts There was trend (p<0.11) for shorter OS for HRG pts *vs* others with median 6.2y *vs* 9.1y (HR 1.5). More importantly among pts without POD24 there was significantly higher risk to die for HRG patients *vs* others, HR 2.6 (p<0.0001) with 7y OS 83.4% *vs* 93.0% (Figure 1B).

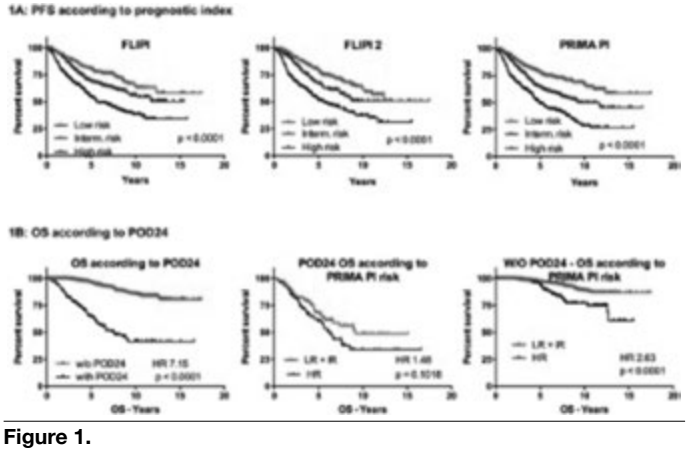

Figure 1.

Summary and Conclusions: PRIMA PI represents feasible prognostic index which works in real world practice. Worse outcome of HRG pts is not given only by higher proportion of POD24 pts, but HRG pts compared to the others have greater risk to die (2.5x) even if they are late progressors.
Supported by grant AZV 16-31092A and Progres Q28 UK.

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TWO YEARS RITUXIMAB MAINTENANCE *VS* OBSERVATION AFTER FIRST LINE TREATMENT WITH BENDAMUSTINE PLUS RITUXIMAB IN PATIENTS WITH MARGINAL ZONE LYMPHOMA (MZL): RESULTS OF THE STIL NHL7-2008 MAINTAIN TRIAL

M. Rummel[1,*], M. Koenigsmann,[2], K.U. Chow[3], W. Knauf[4], C. Lerchenmueller[5], C. Losem[6], M. Goerner[7], B. Hertenstein[8], T. Decker[9], A. Ganser[10], T. Gaska[11], M. Heike[12], E. Lange[13], R. Weide[14], W. Willenbacher[15], A. Burchardt[1], F. Kauff[1], J. Barth[1], A. Hinke[16], R. Greil[17]

[1]Hematology and Oncology, University Hospital Giessen, Giessen, [2]Onkologisches Ambulanzzentrum, Hannover, [3]AKS Frankfurt, [4]Centre for Haematology & Oncology Bethanien, Frankfurt, [5]Joint Practice for Hematology and Oncology, Greven, [6]Joint Practice for Hematology and Oncology, Neuss, [7]Bielefeld Hospital, Bielefeld, [8]Hematology and Oncology, Bremen Hospital, Bremen, [9]Ravensburg Oncology, Ravensburg, [10]Hematology, Hemostasis, Oncology, and Stem Cell Transplantation, Hannover Medical School, Hannover, [11]Bruderkrankenhaus St. Josef, Paderborn, [12]Dortmund Hospital, Dortmund, [13]Evangeisches Krankenhaus Hamm, Hamm, [14]Practice for Hematology and Oncology, Koblenz, Germany, [15]University Hospital of Innsbruck, Innsbruck, Austria, [16]CCRC, Duesseldorf, Germany, [17]Internal Medicine III, Salzburg, Austria

Background: Rituximab (R) maintenance is part of a standard treatment for follicular lymphoma. In MZL, however, it is not yet common practice.
Aims: In this study we compared the effect of 2 years of R maintenance *vs* observation after first-line treatment with B-R in patients with previously untreated MZL.
Methods: Patients had stage II (bulky disease >7 cm), III, or IV disease. Nodal and splenic MZL were included but not MALT lymphomas. Primary endpoint was progression free survival (PFS). Secondary endpoints included response rates, overall survival (OS), and toxicity. For induction patients were treated with up to 6 cycles of B-R plus 2 additional R cycles. Only patients responding to B-R were then randomized to either R maintenance (q 2 months for 2 years) or observation.
Results: Median time of follow-up after registration was 76 months at the time of this analysis (February 2018). 119 patients with a median age of 65 years were evaluable for response. 108 (91%) responded to B-R induction, with 23 patients (19%) achieving a complete remission. Of 104 randomized patients, 53 (51%) were randomized to R maintenance and 51 (49%) to observation. Median age of randomized patients was 64 years, patient characteristics and toxicity were similar for both groups. PFS was superior for 2 years of R maintenance, with the median not yet reached *vs* 92.2 months for observation (hazard ratio (HR) 0.35, 95% CI 0.17 – 0.76, p=0.008). The OS rate at 6 years was 92% for R maintenance *vs* 86% for observation. The difference in OS was not statistically significant (HR 0.52, 95% CI 0.20 – 1.39).
Summary and Conclusions: Our results demonstrate a statistically significant PFS improvement of a 2-year R-maintenance *vs* observation after B-R induction in patients with MZL.

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TP53 MUTATIONS, BUT NOT NOTCH2 OR KLF2 MUTATIONS, IDENTIFY PATIENTS WITH POOR PROGNOSIS IN SPLENIC MARGINAL ZONE LYMPHOMA – INTERIM ANALYSIS OF THE IELSG46 STUDY

F. Guidetti[1,*], A. Bruscaggin[1], A. Condoluci[12], V. Spina[1], L. Terzi di Bergamo[1], G. Forestieri[1], F. Bertoni[12], L. Cascione[1], M. Mollejo[3], V. Meignin[4], A. Traverse-Glehen[5], J.T. Geyer[6], A. Broccoli[7], A. Tucci[8], S. Bea[9], R. Boldorini[10], G. Tapia[11], A. Maiorana[12], M. Gomes da Silva[13], J.F. Garcia[14], F. Hitz[15], A. López[9], E. Santambrogio[16], G. Bastidas[9], M. Merli[17], F. Piazza[18], S. Pizzolitto[19], S. Dietrich[20], L. Devizzi[21], L. Marco[22], M. Ponzoni[23], A. Rambaldi[24], P. Corradini[21], C. Tarella[25], P. Zinzani[7], U. Vitolo[16], F. Zaja[19], F. Passamonti[17], A. Tzankov[26], S. Cogliatti[15], C. Montalban[14], R. Marasca[12], C. Visco[27], M. Joao Baptista[28], V. Canzonieri[29], E. Matutes[9], F. Facchetti[8], M. Paulli[30], E. Campo[9], D. Oscier[31], E. Sabattini[7], S.A. Pileri[25], G. Inghirami[6], G. Gianluca[10], G. Salles[5], C. Thieblemont[4], M. A. Piris[32], C. Franco[2], E. Zucca[2], L. Arcaini[30], D. Rossi[12]

[1]Institute of Oncology Research, [2]Oncology Institute of Southern Switzerland, Bellinzona, Switzerland, [3]Hospital Virgen de la Salud, Toledo, Spain, [4]Saint Louis Hospital, Paris, [5]Centre Hospitalier Lyon Sud, Lyon, France, [6]Weill Cornell Medical College, New York, United States, [7]Policlinico Sant'Orsola, Bologna, [8]Spedali Civili, Brescia, Italy, [9]Hospital Clínic i Provincial de Barcelona, Barcelona, Spain, [10]University of Eastern Piedmont, Novara, Italy, [11]Universitat Autònoma de Barcelona, Barcelona, Spain, [12]Università di Modena e Reggio Emilia, Modena, Italy, [13]Portuguese Institute of Oncology, Lisbon, Portugal, [14]MD Anderson Cancer Center, Madrid, Spain, [15]Kantonsspital St. Gallen, St.Gallen, Switzerland, [16]AOU Città della Salute e della Scienza di Torino, Turin, [17]Ospedale di Circolo e Fondazione Macchi, Varese, [18]Ospedale Universitario di Padova, Padova, [19]Policlinico Universitario, Udine, Italy, [20]National Center for Tumor Diseases, Heidelberg, Germany, [21]Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Milan, [22]Azienda Ospedaliera SS Antonio e Biagio, Alessandria, [23]Ospedale San Raffaele, Milan, [24]Ospedale Bolognini, Bergamo, [25]Istituto Europeo di Oncologia, Milan, Italy, [26]Universitätsspital Basel, Basel, Switzerland, [27]Ospedale San Bortolo, Vicenza, Italy, [28]Josep Carreras Leukaemia Research Institute, Barcelona, Spain, [29]Centro di Riferimento Oncologico, Aviano, [30]Fondazione IRCCS Policlinico San Matteo, Pavia, Italy, [31]Royal Bournemouth Hospital, Bournemouth, United Kingdom, [32]Instituto de Investigación Marqués De Valdecilla, Santander, Spain

Background: Although the majority of SMZL displays an indolent course, the disease is still incurable. Moreover, a significant proportion of patients (~25-30%) experience poor outcome and survive <5 years. Molecular aspects of SMZL have been claimed as promising biomarkers and their incorporation into prognostic models for SMZL might improve risk stratification of patients.
Aims: The main objective of the study is to test the impact of molecular alterations on overall survival (OS) prognostication in newly diagnosed SMZL.
Methods: IELSG46 is a multicentre, international, retrospective, observa-

tional study in which already existing and coded health-related personal and biological material is further used. The study included adults, who received a diagnosis of SMZL on spleen histology, and for whom tumor material collected before initiation of medical therapy and clinical annotations were available. Mutation analysis was performed by CAPP-seq targeted deep next generation sequencing of tumor genomic DNA was performed on the NexSeq500 sequencer (Illumina). A stringent bioinformatic pipeline was applied to suppress the background noise allowing to call variants with a sensitivity of $5x10^{-2}$ in FFPE derived DNA. Deletion of 17p and of 7q were identified by using the sequencing reads-based GATK4-CNV algorithm. The adjusted association between exposure variables and OS was estimated by Cox regression. Cox regression included exposure variables showing an univariate association with OS with a Bonferroni corrected significant level <0.1.

Results: This interim analysis included 162 of the 316 cases enrolled in the study. The sample size allowed to identify 30% differences in OS for genetic lesions represented in at least 5% of cases. Median follow-up was 10 years. At 10 years, 74.6% of cases were alive, consistent with the general indolent behavior of this lymphoma. Genes recurrently affected by non-synonymous somatic mutations in >10% of SMZL included KLF2 (25.9%), NOTCH2 (22.2%), KMT2D (15.4%), TNFAIP3 (13.5%), ATM (11.1%) (Figure 1A). Deletion 7q was documented in 31.5% of cases. OS was not affected by either KLF2 mutations (Figure 1B), NOTCH2 mutations (Figure 1C), or the co-occurrence on NOTCH2 and KLF2 mutations. The only recurrent (>5%) lesion associated with inferior OS was TP53 mutation, which occurred in 7.4% cases (Figure 1D). Among TP53 mutated cases, the median OS was 6 years (10-year OS: 46%), while it was not reached among TP53 wild type cases (10-year OS: 76.9%, p=.01).

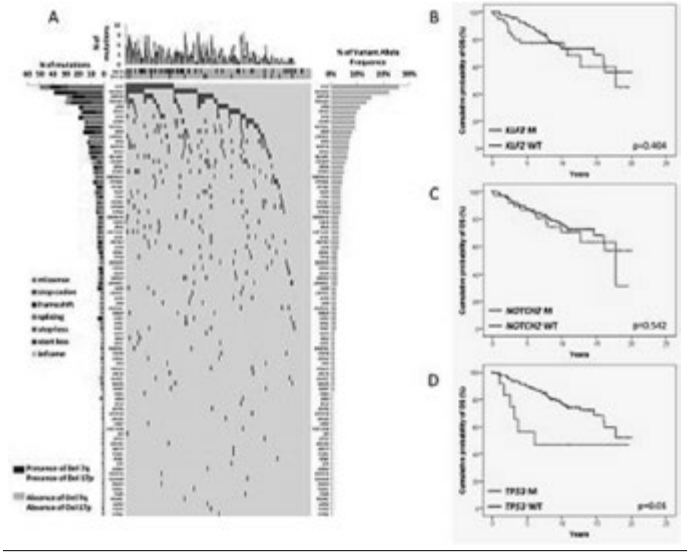

Figure 1.

Summary and Conclusions: The large sample size and the inclusion of SMZL confirmed on spleen pathology allowed to precisely estimate the prevalence of KLF2 and NOTCH2 mutations in this lymphoma, which were previously reported in the range of 10-40%. NOTCH2 and KLF2 mutations do not affect disease course, consistent with the hypothesis that they are early and founding molecular events in SMZL. As in other mature B-cell tumor, also SMZL outcome is affected by TP53 mutations.

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CC-122, A NOVEL CEREBLON-MODULATING AGENT, IN COMBINATION WITH OBINUTUZUMAB (GA101) IN PATIENTS WITH RELAPSED AND REFRACTORY (R/R) B-CELL NON-HODGKIN LYMPHOMA (NHL)

J.-M. Michot[1,*], R. Bouabdallah[2], J.K. Doorduijn[3], U. Vitolo[4], M.J. Kersten[5], A. Chiappella[4], P.L. Zinzani[6], G. Salles[7], R. Sarmiento[8], S. Mosulen[8], C. Mendez[8], M. Petrarca[9], M. Pourdehnad[9], L. Li[10], Z. Nikolova[8], V. Ribrag[1]

[1]Institut Gustave Roussy, Villejuif, [2]Institut Paoli-Calmettes, Marseille, France, [3]Erasmus MC Cancer Institute (on behalf of the HOVON/Lunenburg Lymphoma Phase I/II Consortium [HOVON/LLPC]), Rotterdam, Netherlands, [4]Azienda Ospedaliera Universitaria Città della Salute e della Scienza di Torino, Torino, Italy, [5]Academic Medical Center (on behalf of the HOVON/Lunenburg Lymphoma Phase I/II Consortium [HOVON/LLPC]), Amsterdam, Netherlands, [6]Institute of Hematology "Seràgnoli," University of Bologna, Bologna, Italy, [7]Centre Hospitalier Lyon-Sud, Pierre-Bénite, France, [8]Celgene Institute for Translational Research Europe, Seville, Spain, [9]Celgene Corporation, San Francisco, [10]Celgene Corporation, Berkeley Heights, United States

Background: There is an unmet need for more effective therapeutic options for patients (pts) with non-Hodgkin lymphoma (NHL), including pts with relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL) and indolent lymphomas (Crump *et al., Blood* 2017). Pts with follicular lymphoma (FL) experiencing early relapse (ER) within 2 years of initial diagnosis and those double refractory (DR) to both rituximab and chemotherapy, have limited treatment options and particularly poor outcomes (Casulo *et al., JCO* 2015). CC-122 is a novel oral agent that modulates cereblon, resulting in the selective ubiquitination and degradation of the hematopoietic transcription factors, Aiolos and Ikaros (Hagner *et al., Blood* 2015; Ribrag *et al., Blood* 2014). Preliminary results from the CC-122-NHL-001 trial, the first study of CC-122 in combination with obinutuzumab, showed promising response rates in pts with R/R B-cell NHL (Michot *et al., Blood* 2017).

Aims: To report updated safety and efficacy from the CC-122-NHL-001 phase Ib study of CC-122 plus obinutuzumab in pts with R/R B-cell NHL (EUDRACT 2014-003333-26; NCT02417285).

Methods: Eligible pts had histologically/cytologically confirmed $CD20^+$B-cell R/R NHL after ≥1 prior regimen for FL/marginal zone lymphoma (MZL) or after ≥2 regimens and/or ASCT for DLBCL. Pts received dose escalated CC-122 active ingredient in capsule formulation (AIC) 1, 2, 3, or 4 mg and formulated capsule (F6) 3 or 4 mg for 5 out of 7 days (5/7d) per week in 28-day cycles with a fixed dose of obinutuzumab IV 1000 mg on days 2, 8, 15 of cycle 1 and day 1 of cycles 2-8. Primary endpoints included safety, NTD, and MTD. Response was assessed by Cheson 2007 criteria.

Results: As of January 10, 2018, 49 R/R B-cell NHL pts were enrolled, including 29 pts with FL (59%), 19 (39%) with DLBCL, and 1 with MZL. Of the 29 FL pts, 16 were characterized as ER or DR and considered high-risk pts. For all pts, the median age was 60 y (range, 26–83), 32 (65%) were male, and 38 (78%) had stage III/IV disease. The median number of prior anticancer therapies was 3 (range, 1–11), and 19 pts (39%) had 1 prior ASCT. As of data cutoff, 16 pts (33%) were ongoing. The median number of cycles of CC-122 was 6 (range, 1-29). Although 16 pts (33%) had ≥1 dose reduction (31% due to AEs), these pts remained on treatment and experienced clinical benefit. Forty-one pts (84%) had dose interruptions (71% due to AEs). Two pts had a DLT, consisting of grade 4 neutropenia (n=1, CC-122 AIC 3 mg) and grade 5 tumor flare (n=1, CC-122 F6 4 mg). The most common any-grade AEs included neutropenia (65%), thrombocytopenia (39%), and diarrhea (29%). Grade 3/4 TEAEs occurred in 41 (84%) pts; the most common (≥15%) were neutropenia (55%) and thrombocytopenia (22%). 47% of pts had SAEs. The ORR was 65% with a CR rate of 29%; median PFS was 13.8 mo (95% CI, 3.7-21.2) and the median DOR was 10.2 mo (95% CI, 8.4-NR) (Table 1). Among pts with FL, the ORR was 77% with a CR rate of 40%. A subgroup analysis of efficacy was performed to further examine the activity of the combination in standard-risk (no ER/DR) and high-risk (ER or DR) pts with FL, demonstrating similar outcomes in the FL subgroups (Table 1).

Table 1. Efficacy with CC-122 in combination with obinutuzumab in evaluable patients by subgroup.

	n	ORR	CR	Median PFS mo (95% CI)	6-mo PFS rate %, (95% CI)	Median DOR mo, (95% CI)
All patients	49	65%	29%	13.8 (3.7-21.2)	60 (43-73)	10.2 (8.4-NR)
DLBCL	19	47%	11%	4.7 (1.8-13.8)	40 (16-63)	10.2 (1.8-10.2)
FL + MZL	30	77%	40%	16.6 (5.4-NR)	72 (50-86)	19.4 (8.4-NR)
High-risk FL (ER or DR)	16	75%	38%	21.2 (3.7-NR)	64 (34-84)	19.4 (1.9-NR)
Standard-risk FL*	13	77%	46%	16.6 (5.4-16.6)	80 (39-95)	8.4 (3.6-NR)

Data cutoff was January 10, 2018. *Defined as neither early relapse (ER) nor double-refractory (DR).

Summary and Conclusions: The chemotherapy-free combination of CC-122 and obinutuzumab was well-tolerated and had favorable clinical activity and durable remissions in R/R B-cell NHL. Notably, subgroup analysis demonstrated that high-risk and standard-risk FL pts have comparable efficacy in response to CC-122 plus obinutuzumab. An expansion (Part B) study is ongoing for pts with R/R FL.

Advances in front-line treatment of newly diagnosed multiple myeloma

S105

A TRIPLET BORTEZOMIB- AND IMMUNOMODULATOR-BASED THERAPY BEFORE AND AFTER DOUBLE ASCT IMPROVES OVERALL SURVIVAL OF NEWLY DIAGNOSED MM PATIENTS: FINAL ANALYSIS OF PHASE 3 GIMEMA-MMY-3006 STUDY

P. Tacchetti[1,*], F. Patriarca[2], M.T. Petrucci[3], M. Galli[4], L. Pantani[1], L. Dozza[1], F. Di Raimondo[5], M. Boccadoro[6], M. Offidani[7], V. Montefusco[8], N. Pescosta[9], A. Ledda[10], T. Caravita[3], N. Cascavilla[11], B. Gamberi[12], P.M. Stefani[13], C. Terragna[1], G. Marzocchi[1], F. Ferrara[14], V. De Stefano[3], E. Zamagni[1], M. Cavo[1]

[1]Bologna University School of Medicine, Seràgnoli Institute of Hematology, Bologna, [2]GIMEMA Italian Myeloma Network, Udine, [3]GIMEMA Italian Myeloma Network, Roma, [4]GIMEMA Italian Myeloma Network, Bergamo, [5]GIMEMA Italian Myeloma Network, Catania, [6]GIMEMA Italian Myeloma Network, Torino, [7]GIMEMA Italian Myeloma Network, Ancona, [8]GIMEMA Italian Myeloma Network, Milano, [9]GIMEMA Italian Myeloma Network, Bolzano, [10]GIMEMA Italian Myeloma Network, Cagliari, [11]GIMEMA Italian Myeloma Network, Foggia, [12]GIMEMA Italian Myeloma Network, Reggio Emilia, [13]GIMEMA Italian Myeloma Network, Treviso, [14]GIMEMA Italian Myeloma Network, Napoli, Italy

Background: The phase 3 GIMEMA-MMY-3006 study comparing bortezomib-thalidomide-dexamethasone (VTD) *versus* thalidomide-dexamethasone (TD) as induction therapy before, and consolidation after, double autologous stem-cell transplantation (ASCT) for newly diagnosed multiple myeloma (MM) provided demonstration of prolonged PFS, but not OS, for patients randomized to the VTD arm (Cavo M *et al.*, Lancet 2010; Blood 2012). Based on superior rates of high quality response and PFS, a bortezomib-based triplet is currently considered as the standard induction therapy for ASCT-eligible MM patients. However, no data from prospective phase 3 trials have so far shown an OS benefit from incorporation of bortezomib and an immunomodulatory into ASCT.
Aims: The current analysis was aimed at evaluating long term results of the GIMEMA-MMY-3006 study.
Methods: Overall, 474 patients were included in the trial, and of these 236 were randomized to VTD and 238 to the TD arm. Median follow-up for surviving patients was 92.8 months (IQR: 59.6-123.0). Analyses were performed on an intention-to-treat basis.
Results: Median PFS was 56.5 months for patients randomly assigned to the VTD arm, and 41.3 months for those in the TD group (HR=0.66, p<0.001). PFS benefit with VTD was seen for patients with ISS stage II-III (HR=0.68, p=0.007) and ISS stage I (HR=0.60, p=0.005), as well as for those with t(4;14) and/or del(17p) positivity (HR=0.45, p<0.001) and negativity (HR=0.66, p=0.003). Median OS was not yet reached in the VTD arm and was 118.6 months in the TD arm (HR=0.71, p=0.024), representing a 29% reduction in the risk of death with incorporation of VTD into double ASCT (Figure 1).

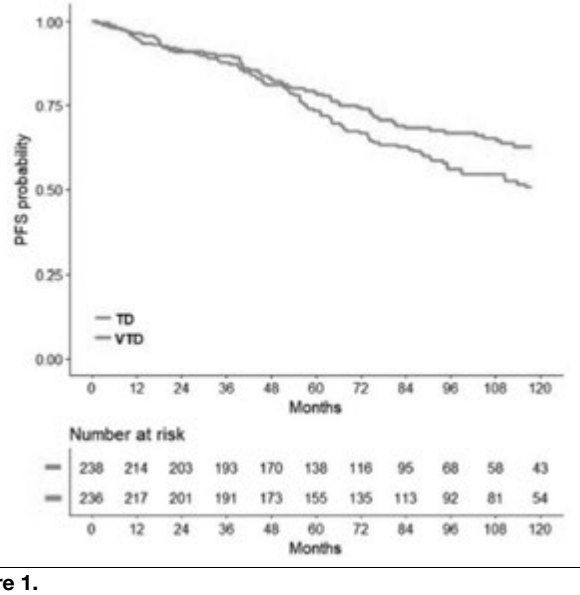

Figure 1.

Estimated rates of OS at 93 months were 67.6% and 58.5%, respectively. Superior OS benefit with VTD over TD was retained across prespecified subgroups of patients with both high-risk and low-risk disease, including those with ISS stages III (HR=0.52, p=0.056), ISS stage I (HR=0.56, p=0.033) and cytogenetics by FISH. In particular, VTD significantly prolonged the OS of patients with both t(4;14) and/or del(17p) positivity (HR=0.57, p=0.031) and negativity (HR=0.66, p=0.034). On multivariate Cox regression analysis, randomization to VTD was an independent factor predicting for prolonged PFS (HR=0.62, p<0.001) and OS (HR=0.61, p=0.001). Additional disease-related variables with a favorable impact on PFS and OS were absence of t(4;14) and/or del(17p) (HR=0.50, p<0.001; HR=0.49, p<0.001), and β2-microglobulin <3.5 mg/L (HR=0.60, p<0.001; HR=0.51, p<0.001). Further analyses of PFS2, time to second anti-MM therapy, and second primary malignancies will be presented at the meeting.
Summary and Conclusions: With an extended median follow-up of 7.6 years, a persistent PFS benefit with incorporation of VTD into ASCT was confirmed. Moreover, a longer OS from primary randomization to VTD *versus* TD was demonstrated in the overall population, as well as in subgroups of patients with high risk and low risk MM.

S106

AUTOLOGOUS STEM CELL TRANSPLANTATION IS SAFE AND EFFECTIVE FOR OLDER MYELOMA PATIENTS: RESULTS FROM THE MYELOMA XI TRIAL

C. Pawlyn[12,*], F. Davies[3], A. Striha[4], D. Cairns[4], J. Jones[1], B. Kishore[5], M. Garg[6], C. Williams[7], K. Karunanithi[8], J. Lindsay[9], M. Jenner[10], G. Cook[11], N. Russell[7], M. Drayson[12], M. Kaiser[12], R. Owen[13], W. Gregory[4], G. Morgan[3], G. Jackson[14]

[1]The Institute of Cancer Research, [2]The Royal Marsden Hospital, London, United Kingdom, [3]Myeloma Institute, University of Arkansas for Medical Sciences, Little Rock, United States, [4]Clinical Trials Research Unit, Leeds Institute of Clinical Trials Research, Leeds, [5]Heart of England Foundation Trust, Birmingham, [6]Leicester Royal Infirmary, Leicester, [7]Centre for Clinical Haematology, Nottingham University Hospital, Nottingham, [8]University Hospital of North Midlands, Stoke-on-Trent, [9]Kent and Canterbury NHS Trust, Canterbury, [10]University Hospital Southampton NHS Foundation Trust, Southampton, [11]Leeds Institute of Cancer and Pathology, Leeds, [12]Institute of Immunology and Immunotherapy, Birmingham, [13]St James's University Hospital, Leeds, [14]Department of Haematology, Newcastle University, Newcastle, United Kingdom

Background: The introduction of high-dose therapy with autologous stem cell transplant (ASCT) for myeloma patients in the early 2000s led to a significant improvement in outcomes. Despite the further advances made with the introduction of novel agents, ASCT remains the standard of care for all eligible patients. Initial ASCT studies recruited only patients aged less than 65 years, but registry data suggests a recent increase in transplants for patients aged 65-75. There are few studies that directly compare ASCT to no ASCT in this age group and the upper age limit for ASCT in routine clinical practice varies around the world.
Aims: We sought to evaluate the outcomes of older patients receiving ASCT within the UK NCRI Myeloma XI trial.
Methods: The Myeloma XI trial, recruited patients to two pathways; one for younger/fitter patients considered transplant eligible (TE) and one for transplant ineligible patients (TNE). The choice of pathway was determined on an individual basis, based on performance status, clinician judgment and participant preference. Patients were randomized to receive either thalidomide or lenalidomide combined with cyclophosphamide and dexamethasone (CTD *vs* CRD, dose attenuated for TNE) for a minimum of 4 (TE) or 6 (TNE) cycles and to maximum response. Some patients with a suboptimal response received further induction therapy with a proteasome inhibitor based triplet (CVD). TE patients were planned to receive 200mg/m^2 melphalan prior to ASCT. There was a maintenance randomization to lenalidomide, lenalidomide/vorinostat or observation. This post-hoc analysis compared outcomes for TE patients of different ages and those of age-matched TNE patients.
Results: 2042 patients were recruited to the TE pathway of the study with median age 61 years(y), range 28-75. 27% (546/2042) of TE patients were aged 65-69y and 5% (101/2042) aged 70-75y. Older patients were less likely to ultimately undergo ASCT than younger patients, despite having entered the TE pathway, (<65y 67%, 65-69y 55%, ≥70y 44%). The reasons given for not undergoing ASCT were more often related to patient fitness in those over 65y. Median CD34+ cell collection also decreased with age from 4.6x10^6 <65y to 3.8 x10^6 65-69y to 3.1x10^6≥70y. For those patients who underwent ASCT having entered the TE pathway (<65y n=931, 65-69y n=299 and ≥70y n=44) median PFS shortened with increasing age but overall survival was not significantly different (Median PFS: <65y

46.5months, 65-69y 38.7m, ≥70y 30.1m and OS: <65y 69m, 65-69y 63.8m, ≥70y not reached). There was no difference in 100-day post ASCT mortality between age groups.
1852 patients were concurrently recruited to the TNE pathway with median age 74y, range 54-92. The TE and TNE patients' ages overlapped as shown in Figure 1. We defined a group of best-age-matched patients between the TE and TNE pathways that comprised 389 TE patients who received ASCT (TE-ASCT), 310 TE patients who did not (TE-noASCT) and 382 TNE patients. Patients undergoing transplantation had significantly improved outcomes compared to those that did not, irrespective of whether ASCT had been initially planned (Median PFS: TE-ASCT 42.9m, TE-noASCT 15.9m, TNE 17.2m and Median OS: TE-ASCT 63.8m, TE-noASCT 46,1m, TNE 45.8m). These differences were independent of the use of ongoing maintenance therapy.

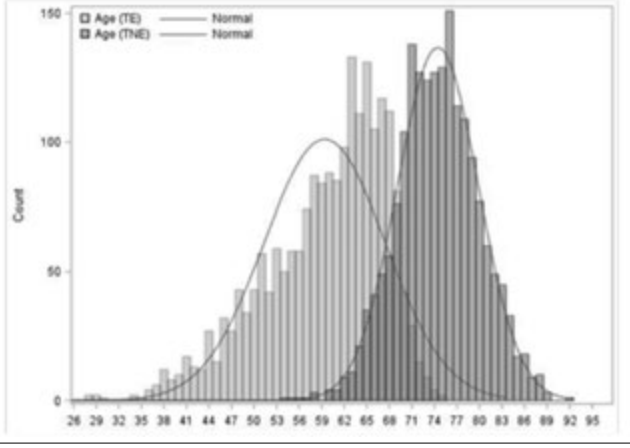

Figure 1. Histogram of age distribution in the TE and TNE pathways of the Myeloma XI trial.

Summary and Conclusions: Our data suggests that ASCT improves outcomes for newly diagnosed myeloma patients and supports its use as standard of care for all considered able to tolerate the procedure, without strict age limits.

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DARATUMUMAB PLUS BORTEZOMIB-MELPHALAN-PREDNISONE (VMP) IN ELDERLY (≥75 YEARS OF AGE) PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA INELIGIBLE FOR TRANSPLANTATION (ALCYONE)

J. San-Miguel[1,*], S. Iida[2], J. Blade[3], M.-V. Mateos[4], J.-J. Lee[5], M. Garg[6], V. Hungria[7], M. Beksac[8], I. Spicka[9], S. Knop[10], L. Pour[11], P. Campbell[12], A. Jakubowiak[13], M.K. Bauer[14], J. Wang[15], S. Wroblewski[16], R. Kobos[15], M. Qi[16], M. Cavo[17]

[1]Clínica Universidad de Navarra-CIMA, IDISNA, CIBERONC, Pamplona, Spain, [2]Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan, [3]Servei d'Hematologia, Hospital Clínic de Barcelona, Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), University of Barcelona, Barcelona, [4]University Hospital of Salamanca/IBSAL, Salamanca, Spain, [5]Department of Hematology-Oncology, Chonnam National University Hwasun Hospital, Hwasun, Korea, Republic Of, [6]Leicester Royal Infirmary – Haematology, Leicester, United Kingdom, [7]Irmandade Da Santa Casa De Misericordia De São Paulo, São Paulo, Brazil, [8]Ankara University School of Medicine Department of Hematology, Ankara, Turkey, [9]Clinical Department of Haematology, 1st Medical Department, Charles University in Prague, Prague, Czech Republic, [10]Würzburg University Medical Center, Würzburg, Germany, [11]University Hospital Brno, Brno, Czech Republic, [12]Andrew Love Cancer Centre, Geelong, Australia, [13]University of Chicago Medical Center, Chicago, United States, [14]Genmab A/S, Copenhagen, Denmark, [15]Janssen Research & Development, Raritan, NJ, [16]Janssen Research & Development, Spring House, PA, United States, [17]"Seràgnoli" Institute of Hematology, Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy

Background: Daratumumab (D) plus VMP (D-VMP) prolonged progression-free survival compared with VMP and was well-tolerated in the phase 3 ALCYONE study (NCT02195479).
Aims: We examined the efficacy and safety profiles of D-VMP *vs* VMP in elderly (≥75 years of age) and non-elderly (<75 years of age) newly diagnosed multiple myeloma patients in ALCYONE.
Methods: Patients were 65 years of age or older or otherwise ineligible for high-dose chemotherapy with autologous stem cell transplantation. Patients received up to nine 6-week VMP cycles (V: 1.3 mg/m^2 subcutaneously Days 1, 4, 8, 11, 22, 25, 29, 32 [Cycle 1] and Days 1, 8, 22, 29 [Cycles 2-9]; M: 9 mg/m^2 orally and P: 60 mg/m^2 orally Days 1-4 [Cycles 1-9]) with or without D (16 mg/kg intravenously QW for Cycle 1, Q3W for Cycles 2-9, and Q4W for Cycles 10+ [post VMP-treatment phase] until progression). Minimal residual disease was assessed by clonoSEQ® assay (Adaptive Biotechnologies).
Results: 706 (350 D-VMP; 356 VMP) patients were randomized, including 211 patients ≥75 years of age (104 D-VMP; 107 VMP) and 495 patients <75 years of age (246 D-VMP; 249 VMP). For D-VMP *vs* VMP, the median duration of study treatment was 14.5 months *vs* 12.0 months for patients ≥75 years of age and 15.0 months *vs* 12.0 months for patients <75 years of age, respectively. The cumulative dose of bortezomib was 43.1 mg/m^2 and 34.1 mg/m^2 with D-VMP and VMP, respectively, for patients ≥75 years of age, and 48.6 mg/m^2 and 46.2 mg/m^2 with D-VMP and VMP, respectively, for patients <75 years of age.After median follow-up of 16.5 months, progression-free survival was prolonged with D-VMP *vs* VMP both in patients ≥75 years of age (median not reached [NR] *vs* 20.4 months; hazard ratio [HR] 0.53; 95% confidence interval [CI] 0.32-0.85) and patients <75 years of age (median NR *vs* 17.9 months; HR 0.49; 95% CI 0.36-0.68). Overall response rate (ORR) and ≥complete response (CR) rates were consistently higher for D-VMP *vs* VMP in patients ≥75 years of age (ORR: 88% *vs* 70%; ≥CR: 41% *vs* 24%) and <75 years of age (ORR: 92% *vs* 76%; ≥CR: 43% *vs* 25%). Minimal residual disease-negative rates (10^{-5}threshold) also increased with D-VMP *vs* VMP in patients ≥75 years of age (24% *vs* 8%) and <75 years of age (22% *vs* 6%).Rates of most common grade 3/4 (≥10%) treatment-emergent adverse events, peripheral sensory neuropathy, and infections are presented in the Table 1. D-associated infusion-related reactions were 36% (9% grade 3/4) in patients ≥75 years of age and 24% (3% grade 3/4) in patients <75 years of age.

Table 1.

	Total population		≥75 years of age		<75 years of age	
Grade 3/4, %	D-VMP	VMP	D-VMP	VMP	D-VMP	VMP
Most common TEAEs						
Neutropenia	40	39	52	42	35	38
Thrombocytopenia	34	38	51	43	28	35
Anemia	16	20	24	23	13	19
Leukopenia	8	9	13	9	6	9
Lymphopenia	8	6	10	10	7	4
Pneumonia	11	4	18	9	9	2
Peripheral sensory neuropathy	1	4	0	6	2	3
Infections	23	15	28	20	21	13

Summary and Conclusions: The efficacy of D-VMP *vs* VMP in patients ≥75 years of age was consistent with the overall study population. Furthermore, D in combination with VMP demonstrated acceptable tolerability regardless of age.

S108

CONSOLIDATION FOLLOWED BY MAINTENANCE *VS* MAINTENANCE ALONE IN NEWLY DIAGNOSED, TRANSPLANT ELIGIBLE MULTIPLE MYELOMA: A RANDOMIZED PHASE 3 STUDY OF THE EUROPEAN MYELOMA NETWORK (EMN02/HO95 MM TRIAL)

P. Sonneveld[1,*], M. Beksac[2], B. vanderHolt[1], M. Dimopoulos[3], A. Carella[4], H. Ludwig[5], C. Driessen[6], R. Wester[1], R. Hajek[7], P. Cornelisse[1], R. Troia[8], L. Dozza[9], F. Gay[10], A. Cafro[11], L. De Rosa[12], G. Fiontoni[13], G. Fiontoni[13], U. Mellqvist[14], H. Johnsen[15], S. Zweegman[16], K. Wu[17], J. Parreira[18], F. Schjesvold[19], J. D'Rozario[20], A. Palumbo[21], M. Boccadoro[21], M. Cavo[22]

[1]Hematology, Erasmus Medical Center, Rotterdam, Netherlands, [2]Hematology, Ankara University School of medicine, Ankara, Turkey, [3]Hematology, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece, [4]Hematology, IRCCS AOU San Martino-IST, Genoa, Italy, [5]medicine, Wilhelminen Cancer Research Institute, Vienna, Austria, [6]Oncology and Hematology, Kantonsspital St.Gallen, St Gallen, Switzerland, [7]Oncology and Hematology, Faculty Hospital Brno, Ostrava, Czech Republic, [8]Hematology, University of Turin, Azienda Ospedaliera Citta` della Salute e della Scienza di Torino, Torino, [9]Hematology, Seràgnoli" Institute of Hematology, Bologna University School of Medicine, Bologna, [10]Hematology, University of Torino, A.O.U. S. Giovanni Battista, Torino, [11]Hematology, ; Niguarda Ca' Granda Hospital, Milan, [12]Hematology, San Camillo, Rome, [13]hematology, Ospedale San Sprito, Pescara, Italy, [14]hematology, South Elvsborg Hospital, Boras, Sweden, [15]hematology, Clinical Cancer Research Center, Aalborg University Hospital, Aalborg, Denmark, [16]Hematology, VU University Medical Center, Amsterdam, Netherlands, [17]Hematology, ZNA Stuivenberg, Antwerpen, Belgium, [18]Hematology, Institute Oncology Hematology, Lisboa, Portugal, [19]Hematology, Center for Immune Regulation, Uni-

versity of Oslo and Oslo University Hospital, Oslo, Norway, [20]Hematology, Canberra Hospital, Woden, Australia, [21]hematology, University of Torino, Torino, [22]Hematology, "Seràgnoli" Institute of Hematology, Bologna University School of Medicine, Bologna, Italy

Background: The role of up-front consolidation therapy for newly diagnosed, transplant-eligible MM (NDMM) patients has not been prospectively addressed in the novel agent era.
Aims: To investigate the efficacy of consolidation therapy in NDMM patients following intensification therapy with VMP or HDM.
Methods: The EMN02/HOVON-95 trial was designed to compare [randomization (R) 1] intensification therapy with 4 cycles of bortezomib-melphalan-prednisone (VMP) *vs* high-dose melphalan (HDM) and autologous stem cell transplantation (ASCT), either single or double, after induction with bortezomib-cyclophosphamide-dexamethasone (VCD) (M. Cavo *et al.* ASCO 2016, abstract #8000; ASH 2017, abstract #397). A second randomization to consolidation therapy with bortezomib-lenalidomide-dexamethasone (VRD) *vs* no consolidation (R2) was performed after intensification, to be followed by lenalidomide maintenance until progression or toxicity in both arms. Primary study end points were progression-free survival (PFS) from R1 and PFS from R2. The second planned interim analysis for R2 was performed in February 2018 when at least 66% (= 343) of the required events for PFS had been observed.
Results: From February 2011 to April 2014, 1510 pts aged ≤65 years with symptomatic MM were enrolled, of whom 1499 were eligible. Of these, 1211 were randomized (stratification by ISS stage) to VMP (505 pts) or HDM (1 or 2 ASCT) (706 pts). For R2 892 eligible patients were randomized to no consolidation (arm A; 437 pts) or VRD consolidation (arm B; 455 pts). Median follow up from R2 was 42 months (mo) (IQR 32-49, maximum 71). Investigator assessed response status at time of R2 was ≥CR (20%), ≥VGPR (67%), ≥PR (92%). At the time of analysis, 366 events for PFS after R2 had been reported. 5-year PFS from R2 was 44% in all patients (median 55 mo), 41% in arm A (median 45 mo) and 48% in arm B (median 59 mo). PFS from R2 with adjustment for R1 was prolonged in pts randomized to VRD consolidation (HR=0.77; 95% CI=0.63-0.95; P=0.014), which is consistent with results of the first interim analysis (P. Sonneveld *et al.* ASH 2016, abstract #242). The PFS benefit from VRD was retained across most predefined subgroups, including revised ISS stage I (Hr=0.77, 95% CI 0.47-1.27) and III (HR=0.76, 95% C 0.40-1.45), low-risk cytogenetics (HR=0.79, 95% CI 0.60-1.05), in patients randomized to either VMP (HR=0.67, 95% CI 0.48-0.94) or HDM (HR=0.84, 95% CI 0.65-1.09), but not in patients with high-risk cytogenetics (del(17p) and/or t(4;14) and/or t(14;16) (HR=1.06, 95% CI 0.70-1.61). At 5 years, OS from R2 was 72% in arm A and 77% in arm B, respectively. Toxicity from VRD was limited with 5% CTCAE grade 4, mainly neutropenia (2%) and thrombocytopenia (2%). The actuarial probability of SPM at 4 years from R2 was 5% *vs* 6% in both arms.
Summary and Conclusions: This second interim analysis confirms the initial promising results of consolidation treatment with VRD followed by lenalidomide maintenance until progression or toxicity as compared to maintenance alone for younger NDMM patients, but further study follow-up is needed. This trial was supported by the Dutch Cancer Society (grant 2010-4798) and by unrestricted grants from Celgene and Janssen.

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UPDATED EFFICACY AND MRD DATA ACCORDING TO RISK-STATUS IN NEWLY DIAGNOSED MYELOMA PATIENTS TREATED WITH CARFILZOMIB PLUS LENALIDOMIDE OR CYCLOPHOSPHAMIDE: RESULTS FROM THE FORTE TRIAL

F. Gay[1,*], D. Rota Scalabrini[2], A. Belotti[2], S. Oliva[1], A. Rambaldi[2], E. Zamagni[2], M. Offidani[2], P. Omedé[1], C. Cerrato[1], R. Troia[1], A. Gozzetti[2], G. Pietrantuono[2], V. Pavone[2], L. De Rosa[2], S. Palmieri[2], F. Narni[2], A. Pascarella[2], R. Zambello[2], V. Montefusco[2], A. Palumbo[3], P. Musto[2], M. Cavo[2], M. Boccadoro[1]

[1]Myeloma Unit, Division of Hematology, University of Torino, A.O.U. Città della Salute e della Scienza di Torino, Torino, [2]Italian Multiple Myeloma Network, GIMEMA, [3]Myeloma Unit, Division of Hematology, University of Torino, A.O.U. Città della Salute e della Scienza di Torino - Currently Takeda Pharmaceuticals Co., Torino, Italy

Background: Proteasome inhibitor-based induction is the standard of care in newly diagnosed multiple myeloma (NDMM) patients eligible for melphalan 200 mg/m^{2}followed by autologous stem cell transplant (MEL200-ASCT), but the treatment of patients with high-risk disease still represents an unmet medical need. The second-generation proteasome inhibitor Carfilzomib proved to be effective, either in combination with Lenalidomide-Dexamethasone (KRd) or with Cyclophosphamide-Dexamethasone (KCd).
Aims: The primary endpoint of this analysis was to evaluate the response rate of KRd *vs* KCd induction. Secondary endpoints were: 1) rate of minimal residual disease (MRD) negativity with KRd *vs* KCd; 2) efficacy of KRd *vs* KCd in different subgroups of patients according to baseline prognostic features: International Staging System (ISS), chromosomal abnormalities, and Revised-ISS (R-ISS).
Methods: NDMM patients ≤65 years were randomized (1:1:1; stratification ISS and age) to ARM A: 4 28-day induction cycles with KCd (carfilzomib 20/36 mg/m^2 IV days 1,2,8,9,15,16; cyclophosphamide 300 mg/m^2 days 1,8,15; dexamethasone 20 mg days 1,2,8,9,15,16) followed by MEL200-ASCT and consolidation with 4 KCd cycles; ARM B: 4 28-day cycles with KRd (carfilzomib 20/36 mg/m^2 IV days 1,2,8,9,15,16; lenalidomide 25 mg days 1-21; dexamethasone 20 mg days 1,2,8,9,15,16) followed by MEL200-ASCT and 4 KRd cycles; ARM C: 12 KRd cycles. Primary endpoint was very good partial response (VGPR) rate with KRd *vs* KCd induction. MRD evaluation - 8 color second generation flow cytometry, sensitivity 10^{-5} - was performed. For this analysis, the 2 KRd groups were pooled (2:1), since the treatment was the same until that point. Enrollment was completed in March, 2017. Data cut-off was November 30, 2017.
Results: 474 patients were randomized (KRd, n=315; KCd, n=159) and analyzed. Patients characteristics were well balanced with 49% KRd *vs* 49% KCd patients presenting at baseline with ISS Stage II/III, 31% *vs* 35% with high-risk chromosomal abnormalities [del17 and/or t(4;14) and/or t(14;16) detected by FISH], and 68% *vs* 74% with R-ISS Stage II/III disease. Rates of stringent complete response (sCR)/complete response (CR) (14% *vs* 4%; P=0.0004), ≥near CR (nCR) (33% *vs* 21%; P=0.0106) and ≥VGPR (75% *vs* 60%; P=0.0017) were significantly higher with KRd *vs* KCd. Partial response rate was 97% with KRd and 91% with KCd and only 1% of patients in each group was primary refractory. The advantage of KRd *vs* KCd was consistent in all the analyzed subgroups (Figure 1, Panel A). MRD evaluation was available in a subset of patients (144 KRd patients and 56 KCd patients). Rate of MRD negativity on evaluable patients was 56% with KRd *vs* 29% with KCd (P=0.008). MRD negativity in high-risk patients treated with KRd was comparable to the overall population (Figure 1, Panel B). Treatment was well tolerated, as previously presented (Gay F. ASCO 2017).

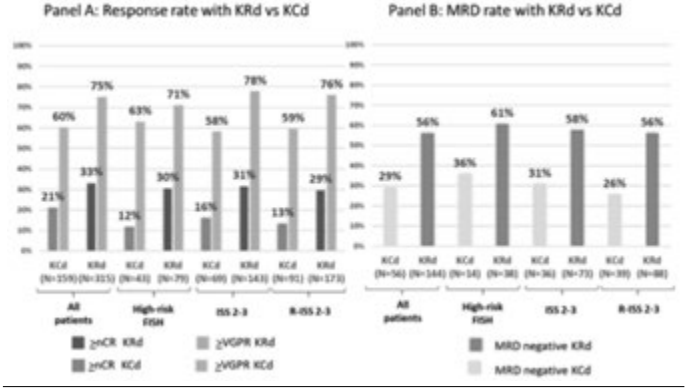

Figure 1.

Summary and Conclusions: K-based induction in transplant-eligible patients is well tolerated and induces deep responses. KRd induction significantly improved sCR/CR, ≥nCR and ≥VGPR *vs* KCd. Rate of MRD negativity on evaluable patients was also higher with KRd. The regimen was similarly effective in high-risk patients, currently representing an unmet medical need.

Hodgkin lymphoma: Clinical studies

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FINAL ANALYSIS OF THE AHL2011 RANDOMIZED PHASE III LYSA STUDY COMPARING AN EARLY PET DRIVEN TREATMENT DE-ESCALATION TO A NOT PET-MONITORED STRATEGY IN PATIENTS WITH ADVANCED STAGES HODGKIN LYMPHOMA

This abstract is embargoed until Friday, June 15, 08:30 local time.

O. Casasnovas[1,*], P. Brice[2], R. Bouabdallah[3], G. Salles[4], A. Stamatoulas[5], J. Dupuis[6], O. Reman[7], T. Gastinne[8], B. Joly[9], K. Bouabdallah[10], E. Nicolas-Virelizier[11], P. Feugier[12], F. Morschhauser[13], R. Delarue[14], A. Berriolo-Riedinger[15], V. Edeline[16], A. Traverse-Glehen[17], M. André[18], N. Mounier[19], M. Meignan[20]

[1]Hematology, CHU Le Bocage and INSERM 1231, Dijon, [2]Hematology, APHP, Hopital Saint Louis, Paris, [3]Hematology, Institut P. Calmette, Marseille, [4]Hematology, Hospices Civils de Lyon, Centre Hospitalier Lyon-Sud, et Université Claude Bernard Lyon-1, Pierre Bénite, [5]Hematology, Centre H. Becquerel, Rouen, [6]Hematology, Hopital H. Mondor, Creteil, [7]Hematology, Institut d'hématologie de basse normandie, Caen, [8]Hematology, University Hospital of Nantes, Nantes, [9]Hematology, Hospital Sud Francilien, Corbeil-Essonnes, [10]Hematology, University Hospital of Bordeaux, Bordeaux, [11]Hematology, Centre L. Bérard, Lyon, [12]Hematology, University Hospital of Nancy, Vandoeuvre les Nancy, [13]Hematology, CHU Lille, Unité GRITA, Université de Lille 2, Lille, [14]Hematology, Hopital Necker, Paris, [15]Nuclear Medicine, Centre G.F. Leclerc, Dijon, [16]Nuclear Medicine, Hopital R. Huguenin, Institut Curie, St-Cloud, [17]Pathology, Hospices Civils de Lyon, Centre Hospitalier Lyon-Sud, et Université Claude Bernard Lyon-1, Pierre Bénite, France, [18]Hematology, CHU UCL Namur, Université catholique de Louvain, Yvoir, Belgium, [19]Hematology, University Hospital of Nice, Nice, [20]Nuclear Medicine, LYSA IM, University Hospital H. Mondor, Creteil, France

Background: Escalated BEACOPP (BEAesc) improves PFS but not OS in pts with advanced Hodgkin Lymphoma (HL) compared to ABVD and is associated to a higher risk of hematological toxicity, secondary leukemia and infertility.
Aims: We hypothesized that PET performed after 2 cycles of upfront BEAesc (PET2) could identify early responding patients who might benefit from a strategy of dose intensity de-escalation, without impairing the disease control.
Methods: The AHL 2011 trial (NCT01358747) was designed to evaluate in 16 to 60 y old HL pts with stage III, IV or high risk IIB, a treatment strategy driven by PET after 2 BEAesc cycles, delivering 4 cycles of ABVD for PET2 negative pts and 4 cycles of BEAesc for PET2 positive pts. This PET driven strategy (arm B) was randomly compared to a standard treatment not adapted by PET delivering 6 cycles of BEAesc (arm A). The allocation of treatment in the experimental arm was based on the central review of PET2 interpreted according to Deauville criteria within 48 hours. PFS was the primary endpoint of the study with a hypothesis of non-inferiority of the PET driven arm compared to the standard arm.
Results: From May 2011 to May 2014, 823 pts were registered including 413 and 410 pts in the arms A and B respectively. Pts characteristics were well balanced in both arms: median age was 30 y, 63% were male, 74% had nodular sclerosis HL, 11% stage IIB, 28% stage III, 60% stage IV, and 58% an IPS ≥3.PET2 positivity rate was similar in arms A (12%) and B (13%). Based on PET2 results, 346 (84%) pts received 4 cycles of ABVD and 51 (12%) 4 additional cycles of BEAesc in the experimental arm.The treatment toxicity was significantly higher in pts receiving 6 cycles of BEAesc compared to those who received 2 cycles of BEAesc + 4 cycles of ABVD with more frequent grade ≥3 AE (anemia (11% *vs* 2%), leukopenia (85% *vs* 74%), thrombocytopenia (44% *vs* 15%), and sepsis (7% *vs* 3%). 204 serious adverse events (SAE) related to treatment occurred in 119 (26%) patients treated with 6 cycles of BEAesc (leading to death in 6 cases), compared to 102 SAE (leading to death in 2 cases) in 62 (17%) patients treated with 2 x BEAesc + 4 x ABVD (p<0.003). In these latter patients most of SAE (66%) occurred during the 2 first cycles of chemotherapy. With a median follow up of 50 months, the estimated 4y-PFS was similar in the standard (87.4%) and the PET driven arms (87.1%; p=0.68). PET2 positivity was related to a significantly lower 4y-PFS compared to PET2 negative patients in the whole population (70.7% *vs* 90.4%; p<0.0001) and in both randomization arms (75.1% *vs* 94% and 70.8% *vs* 91.6% in the standard and PET driven arms, respectively; p<0.0001 for both). OS was similar in both arms.
Summary and Conclusions: PET performed after 2 cycles of BEAesc can be safely used to guide subsequent treatment and supports the response-adapted strategy delivering 4 cycles of ABVD for pts with negative PET2 reducing the treatment-related immediate toxicity without impairing the disease control. PET positivity after 2 cycles of BEAesc is related to a higher risk of disease progression, encouraging to develop new treatment options in patients with PET2 positive advanced stage HL.

S111

PRETREATMENT VITAMIN D DEFICIENCY IS ASSOCIATED WITH LOWER PROGRESSION-FREE AND OVERALL SURVIVAL IN PROSPECTIVELY TREATED HODGKIN LYMPHOMA

S. Borchmann[123,*], H. Görgen[1], S. Sasse[1], S. Kreissl[1], P.J. Bröckelmann[1], B. von Tresckow[1], M. Fuchs[1], V. Diehl[1], A. Engert[1]

[1]Department I for Internal Medicine and German Hodgkin Study Group (GHSG), [2]Center for Molecular Medicine Cologne (CMMC), [3]Else Kröner Forschungskolleg Cologne, University of Cologne, Köln, Germany

Background: Vitamin D (25-OHD, VD) deficiency has been associated with a poor prognosis in a wide range of cancers. For Hodgkin lymphoma (HL), no data on pretreatment VD levels and their correlation with patient characteristics and outcome exists. Biologically, VD deficiency might render the characteristic T-cell rich microenvironment of HL more tumor-supportive due to a diminished anti-tumor T-cell response (von Essen, 2010).
Aims: To test the hypothesis that pretreatment VD deficiency reduces progression-free survival (PFS) and overall survival (OS) in HL.
Methods: Between 1998 and 2003, the GHSG prospectively recruited 2653 patients of all stages in the GHSG trials HD7, HD8 and HD9. The enriched analysis cohort included all patients with available pretreatment serum sample and documented progression or relapse (N=118), as well as two relapse-free patients each matched by trial and treatment arm (N= 236). VD was measured with a commercially available ELISA. Serum VD levels were categorized according to the Institute of Medicine, Food and Nutrition board (IOM) guidelines defining <30nmol/l as deficient, 30 to 50 nmol/l as insufficient and ≥50nmol/l as sufficient. VD levels and their correlation with other baseline characteristics and outcomes were analyzed descriptively and by linear or logistic regression where applicable. PFS and OS were analyzed with Kaplan-Meier methods and Cox regression; analyses were weighted to attain event rates of the total study cohort. All statistical tests were stratified by trial and treatment arm to account for the matched structure of the analysis cohort.
Results: VD levels could be quantified in 351/354 patients in the analysis cohort. VD levels did not correlate with age, sex, clinical stage, a large mediastinal mass, extranodal involvement, presence of 3 or more nodal areas, elevated ESR, B-symptoms, Karnofsky index, IPS, trial or treatment arm and thus were independent of tumor mass, the patients' clinical condition and the received treatment. VD levels were highly dependent on the season of diagnosis with median levels of 27.7, 42.9, 30.8 and 22.0 nmol/l for spring, summer, autumn and winter, respectively. Thus, according to the IOM cutoffs, 55%, 34%, 48% and 55% of patients diagnosed in spring, summer, autumn and winter, respectively, were deficient (p<0.0001 for summer *vs* other). Patients with progression or relapse had a lower median VD level than relapse-free patients (21.4 *vs* 35.5 nmol/l) and were more often deficient (68% *vs* 41%, p<0.0001). Similar trends were observed across all treatment arms. With a median observation time of 156 months, a lower 5-year PFS rate in deficient patients compared to non-deficient patients was observed (74.4% [95%>CI: 66.9-81.8] *vs* 84.6% [95%>CI: 78.9-90.3]) (Figure 1). In a multivariate Cox regression adjusted for season of diagnosis, age and sex, VD deficiency was associated with higher risk for a PFS event (HR: 2.13 [95%>CI: 1.84-2.48], p<0.0001). This difference also translated into a clinically significant OS difference with VD deficiency associated with a higher risk for death in a similarly adjusted Cox regression (HR: 1.82 [1.53-2.15], p<0.0001). More deaths in the VD deficient group were HL-related than in the non-deficient group.

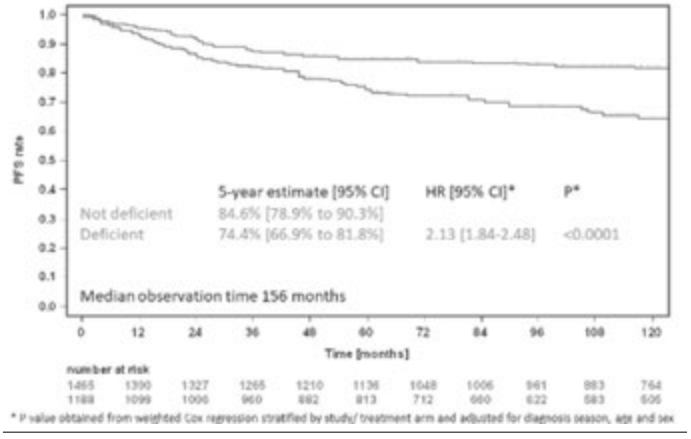

Figure 1.

Summary and Conclusions: Pretreatment VD is a modifiable, independent baseline risk factor for poorer outcome of HL in the present analysis. Supplementation with VD is cheap, generally safe and could potentially improve outcomes in HL patients. Interventional, ideally randomized studies should evaluate VD supplementation as an add-on treatment in HL.

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BRENTUXIMAB VEDOTIN PLUS CHEMOTHERAPY IN HIGH RISK ADVANCED-STAGE CLASSICAL HODGKIN LYMPHOMA (CHL) PATIENTS: RESULTS OF PRE-SPECIFIED SUB-GROUP ANALYSES FROM THE ECHELON-1 STUDY

M. Hutchings[1,*], J. Radford[2], A. Gallamini[3], A. Illes[4], A. Sureda[5], J.M. Connors[6], A. Sykorova[7], H. Shibayama[8], J. S. Abramson[9], N. Chua[10], J.W. Friedberg[11], J. Koren[12], A. LaCasce[13], L. Molina[14], N. Josephson[15], E. Song[15], H. Jolin[16], C. Lee[16], A. Gautam[16], S. Ansell[17]

[1]Department of Haematology, Rigshospitalet, Copenhagen, Denmark, [2]University of Manchester and the Christie NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, United Kingdom, [3]Research, Innovation and statistics department, A Lacassagne Cancer Centre, Nice, France, [4]Department of Haematology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary, [5]Clinical Hematology Department, Institut Català d'Oncologia-Hospitalet, Barcelona, Spain, [6]Centre for Lymphoid Cancer, British Columbia Cancer Agency, Vancouver, BC, Canada, [7]Fourth Department of Internal Medicine – Haematology, Charles University Hospital and Faculty of Medicine, Hradec Kralove, Czech Republic, [8]Department of Hematology and Oncology, Osaka University Graduate School of Medicine, Osaka, Japan, [9]Center for Lymphoma, Massachusetts General Hospital Cancer Center, Boston, MA, United States, [10]Department of Oncology, Division of Medical Oncology, Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada, [11]Wilmot Cancer Institute, University of Rochester, Rochester, NY, United States, [12]Internal Clinic of Hematology, University Hospital; Vinohrady, Third Faculty of Medicine, Charles University, Prague, Czech Republic, [13]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, United States, [14]Service d'hématologie clinique, CHU de Grenoble, Grenoble, France, [15]Seattle Genetics, Inc., Bothell, WA, [16]Millennium Pharmaceuticals Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, MA, [17]Division of Hematology, Department of Internal Medicine, Mayo Clinic, Rochester, MN, United States

Background: Primary results of the randomized, phase 3, ECHELON-1 study demonstrated a significant improvement in modified progression-free survival (mPFS), per independent review facility (IRF), in patients with stage III and IV cHL treated with frontline A+AVD *vs* doxorubicin, bleomycin, vinblastine, dacarbazine (ABVD). At 2 years, the mPFS rates were 82.1% (95% CI, 78.8 to 85.0) *vs* 77.2% (95% CI, 73.7 to 80.4), respectively, a 4.9% point difference (Hazard Ratio [HR] 0.77; 95% CI: 0.60-0.98; p=0.0351). We report data from pre-specified high risk subgroups of the ECHELON-1 data.
Aims: This prespecified subanalysis assessed the efficacy and safety of A+AVD *vs* ABVD in patients with cHL and high risk features including: ≥1 extranodal site of involvement, stage IV disease, or an International Prognostic Factor Project (IPFP) score of 4-7.
Methods: Patients were randomized 1:1 to receive up to six 28-day cycles of A+AVD (brentuximab vedotin 1.2 mg/kg, doxorubicin 25 mg/m^2, vinblastine 6 mg/m^2, dacarbazine 375 mg/m^2) or ABVD (AVD regimen + bleomycin 10 units/m^2) intravenously on Days 1 and 15 of each cycle. Patients were analyzed by Stage at diagnosis (III *vs* IV), IPFP score (0-1 *vs* 2-3 *vs* 4-7), and number of extranodal disease sites (0 *vs* 1 *vs* ≥1). The sub-group analyses were performed on the primary endpoint of mPFS (defined as time to progression, death, or evidence of non-complete response after completion of frontline therapy followed by subsequent anticancer therapy).
Results: In total, 664 and 670 patients were randomized to A+AVD and ABVD, respectively. High risk features at baseline were well balanced between treatment group with 64% and 63% having stage IV disease, 25% and 27% having an International Prognostic Score (IPS) of 4-7 in the A+AVD and ABVD arms respectively, 62% in each arm had ≥1 extranodal sites at baseline. Most prespecified subgroups demonstrated a consistent trend toward benefit with A+AVD. A+AVD showed the most improved mPFS compared with ABVD in the following sub-groups (Table 1): stage IV disease (2-year mPFS 82.0% *vs* 75.3% [HR=0.71, 95% CI: 0.53–0.96; p=0.023]), >1 extranodal sites (2-year mPFS 80.2% *vs* 71.1% [HR=0.67, 95% CI: 0.44-1.00; p=0.049], ≥1 extranodal sites (2-year mPFS 82.4% *vs* 74.9% [HR=0.70, 95% CI: 0.52-0.94; p=0.02]). Patients with an IPS of 4-7 also had a favorable improvement in mPFS with A+AVD (77.0% *vs* 69.2% [HR=0.70, 95% CI: 0.46-1.07; p=0.097]). Efficacy and safety analyses for combinations of high risk sub-groups will be presented in full.
Summary and Conclusions: Compared with standard ABVD, A+AVD in frontline trends favorably for mPFS outcomes for patients with high risk cHL (Stage IV disease, or ≥1 extranodal disease sites, or IPS of 4–7) and the mPFS benefit for high risk patients is more than the ITT population. These results suggest that patients with high risk cHL might have a greater treatment benefit with A+AVD compared with ABVD.

Table 1. mPFS by patient sub-groups.

Baseline characteristics		A+AVD vs ABVD Hazard Ratio (95% CI)	P-value
Ann Arbor stage	Stage III	0.92 (0.60–1.42)	p=0.712
	Stage IV	0.71 (0.53–0.96)	p=0.023
IPS	0–1	0.84 (0.47–1.49)	p=0.548
	2–3	0.79 (0.55–1.12)	p=0.183
	4–7	0.70 (0.46–1.07)	p=0.097
Extranodal sites	0	1.04 (0.67–1.62)	p=0.856
	1	0.75 (0.48–1.16)	p=0.191
	>1	0.67 (0.44–1.00)	p=0.049
	≥1*	0.70 (0.52-0.94)	p=0.018

*ad hoc analysis. IPS, International Prognostic Score.

S113

BRENTUXIMAB VEDOTIN + ESHAP (BRESHAP) FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANT: HIGH COMPLETE REMISSION RATE AND LONG-TERM TIME TO TREATMENT FAILURE IN REFRACTORY/RELAPSED HODGKIN LYMPHOMA

R. Garcia-Sanz[1,*], A. Sureda[2], F. De la Cruz[3], A.P. González[4], A. Rodríguez[5], B. Sánchez-González[6], E. Domingo[2], M. Moreno[7], J. López[8], J.L. Piñana[9], G. Rodríguez-Macías[10], M. Canales[11], A. Gutierrez[12], M.J. Rodríguez Salazar[13], M.D. Caballero[1], C. Martínez[14]

[1]Hematology, University Hospital of Salamanca, Salamanca, [2]Hematology, Hospital Duran i Reynals, Barcelona, [3]Hematology, Hospital Virgen del Rocío, Sevilla, [4]Hematology, Hospital Central de Asturias, Oviedo, [5]Hematology, Hospital 12 de Octubre, Madrid, [6]Hematology, Hospital del Mar, Barcelona, [7]Hematology, Hospital Germans Trias i Pujol, Badalona, [8]Hematology, Hopital Ramón y Cajal, [9]Hematology, Hospital Clínico de Valencia, [10]Hematology, Hospital Gregorio Marañón, [11]Hematology, Hospital Universitario la Paz, Madrid, [12]Hematology, Hopsital Son Espases de Mallorca, Palma de Mallorca, [13]Hematology, Hospital Universitario de Canarias, Santa Cruz de Tenerife, [14]Hematology, Hospital Clínic i Provincial, Barcelona, Spain

Background: Refractory or Relapsed Hodgkin Lymphoma(RRHL) are treated with Salvage therapy and Autologous Peripheral Blood Stem Cell Transplantation (APBSCT). The addition of brentuximab vedotin (BV) is feasible and increases the CR rate.
Aims: To evaluate long-term results of a phase II trial with the combination of BV and ESHAP chemotherapy [BRESHAP] as 2nd line therapy for RRHL prior to APBSCT (ClinicalTrials.gov #NCT02243436).
Methods: Primary efficacy endpoint was complete responses (CR) pre-APBSCT after **BR**entuximab vedotin, **E**toposide, **S**olumedrol, **H**igh dose **A**raC and cis**P**latin.
Results: Patients with relapsed or refractory classical HL (cHL) after one prior line of therapy were eligible. 66 patients were included in the trial. There were 35 females and 31 males, with a median age of 36 years (18-66). At inclusion, 40 patients were primary refractory, 16 early relapses (CR ≤1 year) and 10 as late relapses (CR >1 year). In this update, all patients have completed all the protocol. During the follow-up, there have been 39 Severe Adverse Events (SAEs) reported in 22 patients (hospitalizations and AEs around transplant were not considered SAEs). Most frequent were fever (n=25, 35% neutropenic), hypomagnesemia and gastrointestinal alterations (n=3). There were 3 SAEs, terminating in death: pneumonia, abdominal sepsis and pulmonary embolism. Apart from APBSCT, there grade 3-4 hematologic toxicity presented in 28 cases: neutropenia (n=21), thrombocytopenia (n=14), and anemia (n=7). Grade 3-4 extra-hematologic adverse events present in ≥5% of cases were non-neutropenic fever (n=13) and hypomagnesemia (n=3). All patients except two underwent stem cell mobilization after the 1st (n=15), 2nd (n=36) or 3rd (n=13) cycle using subcutaneous G-CSF 5 mcg/Kg/12 h. for 5 days. All 64 patients collected >2·10e6/Kg peripheral blood CD34+ cells in all cases (median 5.75, range 2.12-33.4). The number of harvesting procedures was one in 48 patients, two in 13, three in 2 and four in 1. The transplant was done after BRESHAP in 61 patients: all engrafted with a median of 11 & 12 days for neutrophil and platelet recovery, respectively. Four other patients were transplanted after receiving

other therapies. No major events were registered during transplant period, except for the patient who died at day +110 due to pneumonia. Overall pre-transplant response was 93%, including a 71% and 22% complete and partial remission rates, respectively. Six patients were considered as non-responders: one who died before any evaluation, two who stable disease and three progressions. Status 3-months after the transplant was CR in 48 patients, PR in 6, SD in 2 and PD in 5. Three doses of BV were given in 50 patients; the remaining 16 patients did not receive the projected consolidation due to toxicity (cytopenias, n=6; neuropathy, n=1), progression (n=6), patient refusal (n=2) and death (n=1). At a mean follow-up of 27 months, 13 patients have progressed and 3 have died without progression, providing 3-year time to treatment failure and progression free survival of 75% and 71%, respectively. Six patients died: 3 due to progression, and the 3 already mentioned. Projected overall survival is 91% at one year.

Summary and Conclusions: BRESHAP is a highly effective regimen for remission induction prior to transplant, which maintains it in patients with refractory or relapsed Hodgkin lymphoma.

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CHECKMATE 205 COHORT D: A PHASE 2 TRIAL OF NIVOLUMAB FOR NEWLY DIAGNOSED ADVANCED-STAGE CLASSICAL HODGKIN LYMPHOMA

R. Ramchandren[1,*], E. Domingo Domenech[2], A. Rueda[3], P. Armand[4], M. Trněný[5], T. Feldman[6], S. Ansell[7], M. Provencio[8], U. Jäger[9], J.B. Cohen[10], K.J. Savage[11], W. Willenbacher[12], M. Sacchi[13], A. Sumbul[13], M. Fanale[14]

[1]Barbara Ann Karmanos Cancer Institute, Detroit, United States, [2]Institut Català d'Oncologia (ICO), Barcelona, [3]Costa del Sol Hospital, Marbella, Spain, [4]Dana-Farber Cancer Institute, Boston, United States, [5]Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic, [6]Hackensack University Medical Center, Hackensack, [7]Mayo Clinic, Rochester, United States, [8]Hospital Universitario Puerta de Hierro, Madrid, Spain, [9]Medical University of Vienna, Vienna, Austria, [10]Winship Cancer Institute, Emory University, Atlanta, United States, [11]British Columbia Cancer Agency Center for Lymphoid Cancer, Vancouver, Canada, [12]Innsbruck University Hospital & OncoTyrol - Center for Personalized Cancer Medicine, Innsbruck, Austria, [13]Bristol-Myers Squibb, Princeton, [14]University of Texas MD Anderson Cancer Center, Houston, United States

Background: Front-line multi-agent chemotherapy cures the majority of patients (pts) with classical Hodgkin lymphoma (cHL). However, pts with advanced-stage (AS) cHL have suboptimal outcomes and the high toxicity of intensive regimens limits their use in elderly/frail pts. Programmed death-1 (PD-1) ligands are commonly overexpressed in cHL and may contribute to an immunosuppressive tumor environment (Roemer MG *et al. J Clin Oncol* 2016;34:2690–7). Nivolumab, an immune checkpoint inhibitor targeting PD-1, enhances T-cell activation and has shown frequent and durable responses in pts with relapsed/refractory cHL after failure of autologous hematopoietic cell transplantation (Armand P *et al.* J Clin Oncol 2018;in press). Addition of nivolumab to chemotherapy may therefore be a promising treatment (Tx) strategy for newly diagnosed (ND) AS cHL.

Aims: To assess the safety and efficacy of nivolumab plus doxorubicin, vinblastine and dacarbazine (N-AVD) in pts with ND AS cHL.

Methods: In CheckMate 205 Cohort D (NCT02181738), pts ≥18 y of age with ND AS (stage IIB with unfavorable risk factors, III, or IV) cHL received 4 doses of nivolumab monotherapy (monoTx; 240 mg IV every 2 wk) followed by N-AVD combotherapy (comboTx; nivolumab 240 mg IV) for 6 cycles (12 doses), after informed consent. Primary endpoint was the proportion of pts with grade (G) 3+ Tx-related adverse events (TRAEs) ≤30 d after last dose. Secondary endpoints included complete remission (CR) rate (International Working Group 2007 criteria); exploratory endpoints included discontinuation rate, objective response rate (ORR, per Independent Radiology Review Committee [IRC]) at end of monoTx, after 2 combocycles, and at end of Tx (EOT), and modified progression-free survival (mPFS; time to progression, death, or first subsequent systemic therapy in pts without CR at EOT).

Results: At database lock (Oct 12, 2017), 51 pts had been treated; median follow-up (FU) was 11 mo. At baseline (BL), median age was 37 y and 31% of pts had bulky disease; at diagnosis, 57% had stage IV disease and 80% had B symptoms. MonoTx was completed by 49/51 (96%) pts and comboTx by 45/50 (90%); 1/5 pts who did not complete comboTx was lost to follow-up. 30 pts (59%) experienced a G3–4 TRAE, including neutropenia/decreased neutrophil count in 25 (49%) and febrile neutropenia (FN) in 5 (10%). 30 pts (59%) received growth factors (GFs), all during comboTx; 90% of GF use was secondary prophylaxis. 8 pts (16%) experienced Tx-related infections (excluding FN). No pneumonitis was reported and median change from BL in hemoglobin-corrected diffusing capacity for carbon monoxide was –3% predicted. The most common G3–4 immune-mediated AE was hepatitis (2 pts; 4%). 4 pts (8%) discontinued due to an AE, 1 G3–4. No G5 TRAEs occurred ≤30 d from last dose; 1 pt (age 68 y) died 38 d after last dose due to study drug toxicity. At EOT, ORR in the ITT population was 84% (67% CR) per IRC and 84% (80% CR) per investigator; 7/12 response-evaluable pts without CR per IRC were deemed in CR per investigator. Nearly all response-evaluable pts showed >50% reduction in tumor burden (Figure 1). Median (range) time to response was 2 (2–5) mo; with a minimum FU of 9 mo, 9-mo mPFS was 94% (95% CI 82, 98).

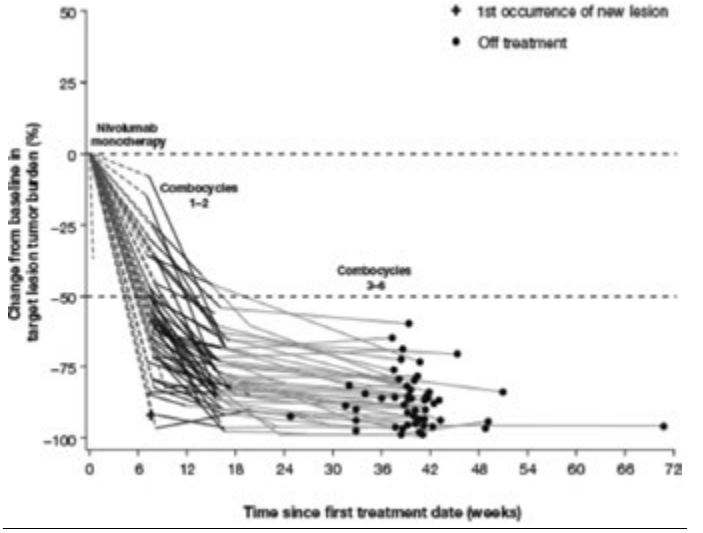

Figure 1. Tumor burden reduction in patients response-evaluable in at least 1 on-study timepoint.

Summary and Conclusions: Nivolumab followed by N-AVD was well tolerated, with a safety profile consistent with previous reports, including a favorable pulmonary toxicity profile. N-AVD had a high ORR, with 67% CR at EOT per IRC. Nivolumab followed by N-AVD warrants further study for ND AS cHL.

Miscellaneous treatments in AML

S115

VERY LONG-TERM RESULTS OF ALL-TRANS RETINOIC ACID AND ARSENIC TRIOXIDE THERAPY IN NON-HIGH RISK ACUTE PROMYELOCYTIC LEUKEMIA: LATEST UPDATE OF THE ITALIAN-GERMAN APL0406 RANDOMIZED TRIAL

L. Cicconi[1,*], U. Platzbecker[2], G. Avvisati[3], F. Paoloni[4], C. Thiede[2], M. Vignetti[4], P. Fazi[4], F. Ferrara[5], M. Divona[6], F. Albano[7], F. Efficace[4], M. Sborgia[8], E. Di Bona[9], M. Breccia[10], E. Borlenghi[11], R. Cairoli[12], A. Rambaldi[13], L. Melillo[14], G. La Nasa[15], W. Fiedler[16], P. Brossart[17], B. Hertenstein[18], H.R. Salih[19], O. Annibali[3], M. Wattad[20], M. Lubbert[21], C.H. Brandts[22], M. Haenel[23], C. Rollig[2], N. Schmitz[24], H. Link[25], C. Frairia[26], C. Fozza[27], A.M. D'Arco[28], N. Di Renzo[29], A. Cortellezzi[30], F. Fabbiano[31], K. Dohner[32], A. Ganser[33], H. Dohner[32], S. Amadori[4], F. Mandelli[4], G. Ehninger[2], R.F. Schlenk[34], F. Lo-Coco[1]

[1]Biomedicine and Prevention, University Tor Vergata, Rome, Italy, [2]Universitatsklinikum Carl Gustav Carus der Technischen Universitat Dresden, Dresden, Germany, [3]University Campus Biomedico, [4]Gruppo Italiano Malattie Ematologiche dell'Adulto Central Office, Rome, [5]Cardarelli Hospital, Naples, [6]Policlinico Tor Vergata, Rome, [7]University of Bari, Bari, [8]U.O. di Ematologia Clinica, Pescara, [9]San Bortolo Hospital, Vicenza, [10]University La Sapienza, Rome, [11]U.O. di Ematologia, Spedali Civili,, Brescia, [12] Ospedale Niguarda, Ca' Granda, Milan, [13]Azienda Opsedaliera Papa Giovanni XXIII, Bergamo, [14]Casa Sollievo della Sofferenza Hospital, IRCCS, San Giovanni Rotondo, [15]Centro Trapianti Midollo Osseo, Ospedale R. Binaghi, Cagliari, Italy, [16]University Hospital Hamburg-Eppendorf, Hamburg, Germany, [17]Innere Medizin mit deSchwerpunkten Onkologie, Haematollogie un Rheumatologie, Bonn, Italy, [18]Klinikum Bremen Mitte, Bremen, [19]University Hospital Tubingen, Tubingen, [20]Kliniken Essen-Sued, Essen, [21]University Medical Center, Freiburg, [22]Goethe University, Frankfurt, [23] Klinikum Chemnitz gGmbH, Chemnitz, [24]Asklepios Klinik St Georg Hamburg, Hamburg, [25]Klinik fur Innere Medizin I, Westpfalz-Klinikum, Keiserslauten, Germany, [26]Hematology-Citta della Salute e della Scienza, Torino, [27]University of Sassari, Sassari, Sassari, [28]U.O. Medicina Interna e Onco-Ematologica P.O. "Umberto I, Nocera Inferiore, [29]Ospedale Vito Fazzi, Lecce, [30]Foundation Istituto di Ricovero e Cura a Carattere Scientifico Ca' Granda Ospedale Maggiore Policlinico and University of Milan, Milan, [31]Ospedali Riuniti Villa Sofia-Cervello, Palermo, Italy, [32]University Hospital Ulm, Ulm, [33]Hannover Medical School, Hannover, [34] Department of Medicine, Hematology, Oncology and Rheumatology, Heidelberg University, Heidelberg, Germany

Background: All-*trans* retinoic acid (ATRA) and arsenic trioxide (ATO) combination therapy has been recently established as the new standard of care for non-high risk acute promyelocytic leukemia (APL). The Italian-German randomized trial APL0406 has first shown that this chemotherapy-free approach is at least not inferior to the ATRA and chemotherapy-based regimen. In addition to significantly improving EFS, OS and CIR rates, ATRA-ATO resulted in considerably reduced hematologic toxicity as compared to ATRA-chemotherapy, while ATO-specific side effects were frequent but manageable.
Aims: We hereby provide a very long-term outcome update (median f.up 66.4 months) on the 276 patients enrolled in APL0406 trial.
Methods: The APL0406 study was a prospective, randomized, multicenter, phase III non-inferiority trial designed by the Italian cooperative group GIMEMA and joined by German groups AMLSG and SAL. Enrolment started in Oct 2007 and was completed in Jan 2013. The present analysis has been performed in February 2018 and data were analyzed following an intent-to-treat principle. The primary objective of the study was EFS. The study enrolled patients aged 18-70 years with newly diagnosed, genetically proven low-intermediate risk APL. Survival distributions were estimated using the Kaplan-Meier method, while cumulative incidence of relapse (CIR) was calculated using the proper nonparametric method. Differences in terms of OS, EFS, and disease-free survival (DFS) were evaluated using the log-rank test. The Gray test was applied to compare cumulative incidence curves. All tests were two-sided.
Results: With a median follow-up of 66.4 months (range: 0.9-116.7), the EFS rate at 72 months for the 263 evaluable patients in the intention-to-treat analysis was 96.6% (95%CI: 93.4-99.9) in the ATRA-ATO group and 77.4% (95%CI: 70.2-85.4) in the ATRA-chemotherapy group (p<0.0001). The total number of events was 28 in the ATRA–chemotherapy (17 relapses, 4 induction deaths, 2 molecular resistant cases and 5 deaths in CR) as compared to the 23 published in the previous report (Platzbecker *et al*., JCO 2016). By contrast, no further events were recorded in the ATRA-ATO group in the present update in addition to the previously reported ones (2 relapses and 2 deaths in CR). Two cases of therapy-related myeloid neoplasms occured in the ATRA-chemotherapy group, while no cases of secondary malignancies were reported in the ATRA-ATO cohort. DFS rate in the ATRA-ATO group was 96.6% (95%CI: 93.4-99.9) and 79.8% (95%CI: 72.7-87.6) in the ATRA-chemotherapy group (p<0.0001) and CIR was 1.7 (95% CI: 0.0-4.0) and 15.5% (95% CI: 9.0-22.0) in the ATRA-ATO and in the ATRA-chemotherapy groups, respectively (p=0.00015). Finally, the OS rate at 72 months was 98.3% (95% CI: 96.0-100.0) and 89.8% (95% CI, 84.3-95.7) in the respective groups (P=0.0040). Survival outcomes are reported in Figure 1.

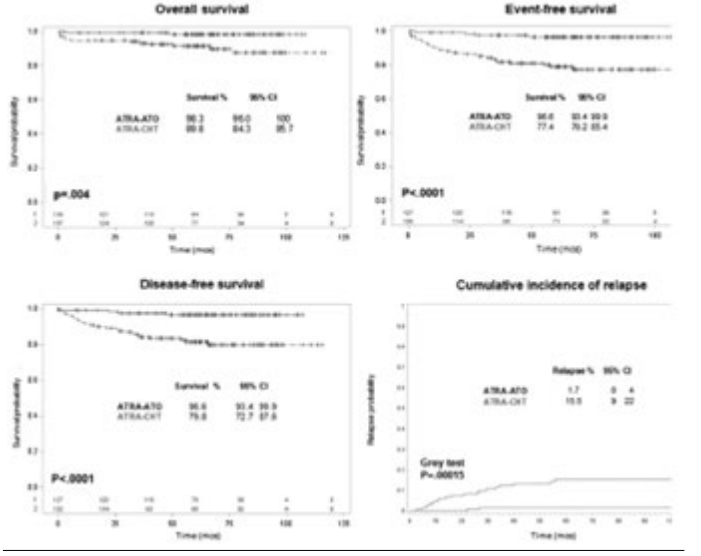

Figure 1.

Summary and Conclusions: This updated analysis of the APL0406 study establishes also in the very long-term the advantage of ATRA-ATO over ATRA-CHT with respect to both efficacy and safety.

S116

RESULTS OF PIVOTAL PHASE 2 TRIAL OF SL-401 IN PATIENTS WITH BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM

N. Pemmaraju[1,*], K. Sweet[2], A. Lane[3], A. Stein[4], S. Vasu[5], W. Blum[5], D. Rizzieri[6], E. Wang[7], M. Duvic[1], S. Spence[8], S. Shemesh[8], J. Chen[8], C. Brooks[8], I. Bergstein[8], P. McDonald[8], T. Goswami[8], J.M. Sloan[9], J. Lancet[2], H. Kantarjian[1], M. Konopleva[1]

[1]The University of Texas MD Anderson Cancer Center, Houston, [2]H. Lee Moffitt Cancer Center, Tampa, [3]Dana-Farber Cancer Institute, Boston, [4]City of Hope National Medical Center, Duarte, [5]The Ohio State University, Columbus, [6]Duke University Medical Center, Durham, [7]Roswell Park Cancer Institute, Buffalo, [8]Stemline Therapeutics, New York, [9]Boston University School of Medicine, Boston, United States

Background: SL-401 is a novel targeted therapy directed to the interleukin-3 receptor-α (CD123), a target expressed on blastic plasmacytoid dendritic cell neoplasm (BPDCN) and other malignancies. BPDCN is a highly aggressive hematologic malignancy with a historical overall survival (OS) of ~8-14 mos from diagnosis. BPDCN has no approved therapies or standard of care, highlighting the need for novel therapeutic approaches. SL-401 was granted Breakthrough Therapy Designation for treatment of BPDCN. Detailed results from the pivotal SL-401 Phase 2 trial in BPDCN will be presented.
Aims: Determine safety and efficacy of SL-401 in patients with BPDCN. Stage 3 was prospectively designed to support potential US registration.
Methods: This pivotal Phase 2 trial of SL-401 is a multicenter, open label, non-randomized, single-arm trial. In Stage 1 (lead-in), first line (1L) and relapsed/refractory (r/r) patients with BPDCN received SL-401 as a daily IV infusion at 7, 9, or 12 mcg/kg/day on days 1-5 of a 21-day cycle. BPDCN patients enrolled in subsequent Stages received SL-401 at the dose determined in Stage 1 (12 mcg/kg). Stage 2 (expansion) enrolled 1L and r/r patients, and Stage 3 (pivotal, confirmatory) enrolled only 1L patients. Enrollment in Stages 1, 2, and 3 has completed.
Results: 45 patients with BPDCN (Stages 1 and 2, n=32; Stage 3, n=13) were enrolled at 7 sites. Median age 70 yrs (range, 22-84 yrs); 82% male. In Stage 1, 12 mcg/kg was the highest tested dose for BPDCN. Median follow-up for all 1L patients treated at 12 mcg/kg (n=29) was 8.7 mos (range

0.2-29.1). The most common treatment-related adverse events (TRAEs) at 12 mcg/kg in Stages 1, 2, and 3 (n=42) were transaminitis (52%), hypoalbuminemia (50%), and thrombocytopenia (38%). TRAEs at 12 mcg/kg across BPDCN and other indications (AML, MPN, and MM) (n=114) were hypoalbuminemia (49%), transaminitis (48%), and thrombocytopenia (29%). Capillary leak syndrome (all grades) has been generally manageable and reversible, and occurred at 12 mcg/kg in 19% (8/42) of patients with BPDCN (Stages 1, 2, and 3) and 20% (23/114) of patients across all indications; 0.9% (1/114) and 2% (3/153) of cases resulted in death across all indications at 12 mcg/kg (n=114) and all doses (n=153), respectively. The Stage 3 pivotal cohort met its primary endpoint with a 54% (7/13) rate of CR+CRc (95% CI: 25.1, 80.8). Across Stages 1, 2 and 3, in 1L patients dosed at 12 mcg/kg (n=29), ORR was 90% (26/29; 95% CI: 72.6, 97.8) with a 72% (21/29; 95% CI: 52.8, 87.3) rate of CR+CRc+CRi (CR=complete response; CRc=clinical CR: absence of gross disease with minimal residual skin abnormality; CRi=CR with incomplete hematologic recovery). 45% (13/29) of patients were bridged to stem cell transplant (SCT) (10 allo+3 auto). Median OS not reached in 1L patients in Stages 1-2 or Stage 3. In r/r patients, ORR was 69% (9/13) with a 38% (5/13) rate of CR+CRc+CRi.

Summary and Conclusions: This pivotal trial of SL-401 in BPDCN met its primary endpoint. In 1L patients dosed at 12 mcg/kg across all 3 stages, ORR was 90% with a 72% rate of CR+CRc +CRi, with 45% of patients bridged to SCT. Across all 3 Stages, SL-401's side effect profile remained manageable and consistent over increasing patient exposure and experience. Based on these positive data, a BLA submission is in preparation. SL-401 is being evaluated in other trials (as single agent and in combination) including CMML, MF, high risk MDS, AML, and MM.

S117

A RANDOMISED EVALUATION OF LOW-DOSE ARA-C PLUS TOSEDOSTAT *VERSUS* LOW DOSE ARA-C IN OLDER PATIENTS WITH ACUTE MYELOID LEUKAEMIA: RESULTS OF THE LI-1 TRIAL

M. Dennis[1,*], R. Hills[2], I. Thomas[3], M. Kallenbach[4], C. Hemmaway[5], P. Greaves[6], M. Copland[7], A. Burnett[8], N. Russell[9]

[1]Department of Haematology, Christie Hospital NHS Trust, Manchester, [2]Centre for Trials Research, Cardiff University, Cardiff, [3]Centre for Trials Research, CARDIFF UNIVERSITY, Cardiff, United Kingdom, [4]Department of Haematology, Aalborg University Hospital, Aalborg, Denmark, [5]Department of Haematology, Auckland City Hospital, Auckland, New Zealand, [6]Department of Haematology, Queen's Hospital, Romford, [7]Institute of Cancer Sciences, University of Glasgow, Glasgow, [8]Blackwaterfoot, Isle of Arran, [9]Department of Haematology, Nottingham University Hospitals, Nottingham, United Kingdom*

Background: Among patients over the age of 60, a considerable number of patients with Acute Myeloid Leukaemia (AML) are not considered for conventional induction chemotherapy, so survival is poor, with only approximately 10% of patients surviving beyond 2 years when treated with standard of care (demethylation agents or low dose ara-C (LDAC)). In the pivotal trials demethylation agents improve median survival, but not overall survival. Therefore there remains a significant unmet need in this patient group. Tosedostat is a selective, oral aminopeptidase inhibitor. Since Phase I/II trials of tosedostat as monotherapy showed acceptable toxicity and potential activity in relapsed AML it was included, combined with LDAC, as an option in the LI-1 "pick-a-winner" trial.

Aims: To assess the efficacy of LDAC+tosedostat *versus* LDAC alone in patients aged 60+ unsuitable for intensive therapy in a "pick-a-winner" design. This design allows several treatments to be assessed simultaneously compared with LDAC in a randomised fashion, with the aim of doubling 2-year survival from 11% to 22% (HR 0.70). There are two interim assessments: after 50 patients per arm are recruited, remission rates must improve by ≥2.5%; the second interim analysis occurs after 170 deaths are seen, when the hazard ratio must be <0.85.

Methods: Tosedostat was given orally at 120mg once a day for up to 6 months. LDAC was given at 20mg bd subcutaneously on days 1-10 of each course, with courses of LDAC occurring at 4-6 wk intervals. To enter the randomisation patients needed to fulfil specific cardiac entry criteria. Toxicities were recorded using NCI-CTC version 3. At the second interim analysis after 183 events tosedostat failed to pass the second assessment, and the arm was therefore closed. Results here are based upon a median follow-up of 18.9 months.

Results: Between 6/2014 and 2/2017, 245 patients, median age 76 years (range 60-88) entered the randomisation. Overall 60% were male; 66% had *de Novo* AML, 28% secondary AML, and 6% high risk MDS; 1% had favourable, 73% intermediate and 26% adverse cytogenetics. By validated Wheatley index, 2% were good risk, 36% standard risk and 63% poor risk. A median of 2 courses was delivered in either arm (mean 2.9 LDAC+tosedostat *vs* 2.3 LDAC).

Overall, complete remission was achieved in 18% of patients (LDAC+tosedostat 22%, LDAC 14%, OR 0.59 (0.31-1.13) p=0.11). Thirty-day mortality was not significantly increased (17% *vs* 13%, HR 1.44 (0.75-2.78) p=0.3); but overall survival showed no difference (2-year OS 16% *vs* 12%, HR 0.99 (0.74-1.32) p=0.9) (Figure 1). Causes of death were: resistant/recurrent disease 39 *vs* 61; infection 20 *vs* 10; haemorrhage 8 *vs* 0; cardiac 5 *vs* 3; multiple 13 *vs* 8; other/unknown 5 *vs* 11. Relapse-free survival did not significantly differ (HR 0.93 (0.41-2.16) p=0.9) with median OS 28.4m *vs* 24.0m in responders (p=0.6); non-remitters had median OS 2.8m *vs* 3.3m (HR 1.20 (0.88-1.63) p=0.2). Stratified analyses failed to identify any subgroup of patients benefitting from tosedostat. Although rates of grade 3+ toxicity were low, tosedostat was associated with diarrhoea, increased cardiac toxicity, and increade use of platelets (mean 5.0 *vs* 3.5 p=0.006).

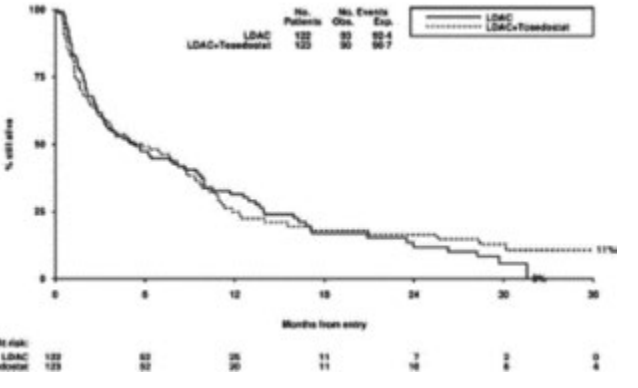

Figure 1. AML LI–1: Overall survival.

Summary and Conclusions: Despite promising early data and acceptable tolerability, we did not find evidence that the addition of tosedostat to LDAC produced a survival benefit in this group of patients, with the anticipated hazard ratio of 0.70 being outside the 95% confidence intervals at second interim analysis. Acknowledgements: We are grateful to CTI Biopharma for providing drug and support for this Investigator Initiated Study.

S118

RESULTS OF A PHASE 1B/2 STUDY OF ENTOSPLETINIB (GS-9973) MONOTHERAPY AND IN COMBINATION WITH INDUCTION CHEMOTHERAPY IN NEWLY DIAGNOSED PATIENTS WITH ACUTE MYELOID LEUKEMIA

A.R. Walker[1,*], J.C. Byrd[1], B. Bhatnagar[1], A. Mims[1], T. Lin[2], H.E. Croswell[3], D. Zhang[4], A.V. Rao[4], M.D. Minden[5], W. Blum[6]

[1]The Ohio State University, Columbus, [2]Kansas City Medical Center, Kansas City, [3]Bon Secours St. Francis Health System, Greenville, [4]Gilead Sciences, Inc., Foster City, United States, [5]Princess Margaret Cancer Centre, Toronto, Canada, [6]Winship Cancer Institute of Emory University, Atlanta, United States

Background: Spleen tyrosine kinase (SYK) is a non-receptor tyrosine kinase primarily expressed in hematopoietic cells. Constitutive activation of SYK in acute myeloid leukemia (AML) has been reported; targeted inhibition of SYK-induced differentiation *in vitro* demonstrated anti-leukemia activity in AML mouse models. SYK promotes leukemogenesis by directly phosphorylating the *FLT3* receptor, and inducing *MEIS1* in conjunction with *HOXA9* to form a regulatory loop in *KMT2A* (mixed lineage leukemia [MLL]) rearranged leukemia. Entospletinib (ENTO) is an orally bioavailable, selective inhibitor of SYK with activity in myeloid and B-lymphoid malignancies.

Aims: Evaluate the efficacy of ENTO in untreated AML patients (pts) as monotherapy followed by ENTO plus standard 7+3 induction chemotherapy (cytarabine 100 mg/m^2/d, days 1-7 plus daunorubicin 60 mg/m^2/d, days 1-3).

Methods: In this phase 1b/2 study (NCT02343939), pts aged 18 to 70 years with previously untreated AML, preserved organ function, and ECOG ≤2 were eligible to receive ENTO 400 mg BID for 14 days as monotherapy lead-in (days -14 to 0) followed by combination with standard 7+3 induction chemotherapy (ENTO+7+3) for a maximum of two induction cycles. Response assessments were made per the modified International Working Group criteria.

Results: Fifty-three pts (n=12 in Phase 1b and n=41 Phase 2) with *de novo*

and secondary AML were enrolled with a median age of 60 (range, 18-78) years. 58% pts were male and per the 2010 European LeukemiaNet guidelines, there were 7 (13%), 16 (30%), 12 (27%) and 18 (34%) pts in the favorable, intermediate I, intermediate II, and adverse risk groups, respectively. ENTO monotherapy and ENTO+7+3 were well tolerated. Most of the adverse events (AEs) that occurred in the ENTO+7+3 phase were consistent with those expected from 7+3 induction chemotherapy. Seven (13%) pts had a grade ≥3 rash and 8 (15%) pts had grade ≥3 transaminitis or hyperbilirubinemia. Overall, 16 (30%) pts needed ENTO dose interruptions or reduction due to AEs, and 8 (15%) pts discontinued study drug due to AEs. Forty-one (77%) pts had grade ≥3 febrile neutropenia. There were no deaths with 30-day induction mortality of 0%. With the 14-day lead-in one pt with t(11;19) translocation achieved a CR with ENTO monotherapy alone, prior to the combination phase. Of note, 15 (28%) pts did not get the full 14-day lead-in due to pt or physician preference, and 12 (23%) pts required concomitant hydroxyurea due to rising white blood cell counts. The CR rate with ENTO+7+3 was 70% (Table 1). Fourteen (26%) pts had secondary AML and the CR rate in this group was 64%. In addition, higher than historical control CR rates were noted in pts with *NPM1* mutation (n=15, CR 87%), *FLT3-ITD* (n=6, CR 83%) and *KMT2A* gene rearrangements (n=10, CR 90%). After a median follow-up (f/u) of 8 months (m), the median event-free survival, relapse-free survival and overall survival were 7m, 7m, and not reached, respectively. Fifteen (28%) pts received allogeneic stem cell transplantation in the post-remission setting.

Table 1. CR rates by risk-groups in newly-diagnosed AML patients treated with ENTO+7+3.

Risk-Group	Phase 1 CR n=12 (%)	Phase 2 CR n=41 (%)	Combined CR n=53 (%)
Favorable-risk	3/3 (100%)	3/4 (75%)	86%
Intermediate-I	0/0 (0%)	13/16 (81%)	81%
Intermediate-II	4/4 (100%)	5/8 (63%)	75%
Adverse-risk	3/5 (60%)	6/13 (46%)	50%
Secondary AML	3/3 (100%)	6/11 (55%)	64%
De novo AML	7/9 (78%)	21/30 (70%)	72%
KMT2A+ (MLL)	3/3 (100%)	6/7 (86%)	90%
NPM1+	3/3 (100%)	10/12 (83%)	87%
FLT3-ITD+	1/1 (100%)	4/5(80%)	83%
TOTAL	**10/12 (83%)**	**27/41 (66%)**	**37/53 (70%)**

Note: Some patients have overlapping mutations P
Phase 1b was the safety, dose finding phase, Phase 2 was for efficacy

Summary and Conclusions: In untreated AML patients, ENTO+7+3 is safe and demonstrates high remission rates in specific molecular subgroups. In our study, pts with secondary AML, *NPM1*, *FLT3-ITD* mutations, and *KMT2A* gene rearrangements were noted to have higher CR rates than historical controls. Durability of response with longer f/u is pending and correlative biomarker studies evaluating *HOXA9/MEIS1* in pts with *NPM1*, *FLT3-ITD* mutated and *KMT2A* rearranged leukemia treated with ENTO are being presented separately.

S119

RELAPSED AML IN CHILDREN: IMPROVED OUTCOME

M. Rasche[12,*], J. Skalska-Sadowska[3], R. Masetti[4], A. Kolenova[5], F. Verwer[6], W. Balwierz[7], U. Creutzig[8], J.-P. Bourquin[9], M. Dworzak[10], H. Hasle[11], L. Sramkova[12], N. Niktoreh[1], K. Kalwak[13], J. Stary[12], J. Wachowiak[14], A. Uyttebroeck[15], C.M. Zwaan[16], B. De Moerloose[17], F. Locatelli[18], G.J. Kaspers[19], D. Reinhardt[12]

[1]Ped. Hematology/Oncology, University Children's Hospital, [2]AML-BFM Study Group, Essen, Germany, [3]Ped . Hematology/Oncology and HSCT, University of Medical Sciences, Poznan, Poland, [4]Pediatrics, Sant'Orsola Hospital, Bologna, Italy, [5]Pediatric Hematology and Oncology, COMENIUS UNIVERSITY, Bratislava, Slovakia, [6]Dutch Childhood Oncology Group (DCOG), Den Hague, Netherlands, [7]Pediatric Hematology and Oncology, University Children's Hospital, Cracow, Poland, [8]AML-BFM Study Group, Essen, Germany, [9]Ped. Hematology/Oncology, UniversityChildren's Hospital, Zürich, Switzerland, [10]Ped. Hematology/Oncology, St. Anna Children's Hospital, Vienna, Austria, [11]Ped. Hematology/Oncology, UniversityChildren's Hospital, Aarhus, Denmark, [12]Ped. Hematology/Oncology, University Hospital Motol and Charles University, Prague, Czech Republic, [13]Ped. Hematology/Oncology and HSCT, University Children's Hospital, Wroslaw, [14]Ped. Hematology/Oncology and HSCT, University of Medical Sciences, Poznan, Poland, [15]Ped. Hematology/Oncology, University Leuven, Leuven, Belgium, [16]Pediatric Oncology/Hematology, Erasmus MC, Rotterdam, Netherlands, [17]Pediatric Hematology-Oncology and Stem Cell Transplantation, University Ghent, Ghent, Belgium, [18]Ped. Hematology/ Oncology, Bambino Gesù Children's Hospital, Rome, Italy, [19]Ped. Hematology/Oncology, Prinses Máxima Centrum, Utrecht, Netherlands

Background: The international AML relapse trial 2001/01 improved significantly the prognosis of children and adolescents with AML (Kaspers *et al.,* JCO 2013). Since the end of recruitment in 2009, most relapsed AML have been enrolled to national or international registries, mainly continuing the established protocol.

Aims: This study analyses the outcome of children with relapsed and refractory AML between 2009 and 2016 (2009 Registry), including patients from Germany, Austria, Czech Republic, Switzerland, Poland, Italy Slovakia, Belgium and the Netherlands.

Methods: In total, 435 patients were enrolled, 395 with relapse and 43 with refractory disease. Children with acute promyelocytic leukemia and myeloid leukemia of Down Syndrome were excluded. For comparison, the updated AML-BFM and Dutch cohort out of the International AMLRelapse Trial 2001/01 was used (n=391; relapse n=351; refractory n=51). The recommended reinduction therapy was liposomal daunorubicin / fludarabine / cytarabine (L-DNR-FLA) followed by FLA course. Allogeneic stem cell transplantation (HSCT) was recommended in all patients.

Results: Most patients (89%) have been treated with the recommended reinduction therapy, however, due to the lack of L-DNR in some countries, idarubicin was used instead of L-DNR. In particular subgroups (mainly children with an early relapse), clofarabine (5%) or gemtuzumab ozogamicin (4%) containing regimens have been applied. The event-free (2nd EFS, 3 years) and overall survival (OS, 3 years) following relapse/ primary refractory AML were 42±2% and 51±3%, respectively. These data show a remarkable improvement in comparison to both 2nd EFS and OS of children enrolled in the International AML-Relapse Trial 2001/01 (2nd EFS 33±3%; OS 38±3%; p<0.005). Duration of first remission and genetic lesions remain significant prognostic factors: Remission <1 year *vs* >1 year: 2nd EFS: 32±3% *vs* 57±4%; OS 36±3% *vs* 60±4%; p<0.00001; Core binding leukemia *vs* others: 2nd EFS: 57±4% *vs* 31±3%; OS 61±4% *vs* 37±3%; p<0.0001. This improved outcome could be partially explained by an amelioration of the results obtained with alloSCT in 2nd CR or refractory disease (2nd CR: 2001/01 EFS 41±6% *vs* 2009 52±6%, p=0.07; refractory AML 19±7% *vs* 33±6%; p<0.003). In addition, 3rd line therapy including 2nd SCT (12%) was applied more often with a curative intention. The approaches to reinduce 3rd remission were heterogenous. They include both conventional chemotherapy schemes such as clofarabine/liposomal daunorubicin/ cytarabine as well as targeted treatment with gemtuzumab ozogamicin (GO) or other experimental approaches. Overall, the patients (n=53) who underwent 2nd SCT showed an EFS of 42± 7% (2001/01: 18±6%, p<0.001). Due to low number in subgroups and selection bias, no significant differences were found in terms of conditioning regimen or type of donor employed (BuCyMel, treosulfan/fludarabine; MSD, MUD, MMD, haplo).

Summary and Conclusions: Children with relapsed AML have a reasonable option to be cured with intensive 2nd and 3rd line therapy. Further research and trials are warranted to identify those children who will experience relapse earlier, for either apply more effective therapy or to for treating patients already when a molecular relapse will be detected. Prospective, randomized trials are urgently needed to identify the best conditioning regimen to be employed.

AML biology and translational research I: Epigenetics

S120

PROGNOSTIC AND BIOLOGIC SIGNIFICANCE OF CIRCULAR RNA PROFILING IN YOUNGER ADULT PATIENTS WITH CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA (CN-AML)

D. Papaioannou[1,*], S. Volinia[2], D. Nicolet[13], M. Swierniak[4], A. Petri[5], K. Mrózek[1], A.J. Carroll[6], J. Kohlschmidt[13], B.L. Powell[7], G.L. Uy[8], J.E. Kolitz[9], E.S. Wang[10], S. Kauppinen[5], R.M. Stone[11], R. Garzon[1], C.D. Bloomfield[1]

[1]Comprehensive Cancer Center, The Ohio State University, Columbus, United States, [2]Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Ferrara, Italy, [3]Alliance Statistics and Data Center, The Ohio State University, Columbus, United States, [4]Centre of new Technologies, University of Warsaw, Warsaw, Poland, [5]Center for RNA Medicine, Department of Clinical Medicine, Aalborg University, Copenhagen, Denmark, [6]Department of Genetics, University of Alabama at Birmingham, Birmingham, [7]The Comprehensive Cancer Center of Wake Forest University, Winston-Salem, [8]Siteman Cancer Center , Washington University School of Medicine, St. Louis, [9]Monter Cancer Center, Hofstra Northwell School of Medicine, Lake Success, [10]Roswell Park Comprehensive Cancer Center, Buffalo, [11]Dana-Farber Cancer Institute, Harvard University, Boston, United States

Background: Circular RNAs (circRNAs) constitute a novel class of non-coding RNAs characterized by an out-of-order arrangement of exons called "back-splicing". While individual circRNAs have been linked to cancer pathogenesis, the prognostic value and biologic implications of circRNA expression in CN-AML pts have not been studied.
Aims: The aims of our study were to determine whether circRNA expression associates with clinical outcome of CN-AML pts, and to gain biological insights into circRNA function in CN-AML.
Methods: We conducted whole transcriptome profiling (RNAseq) in a training (n=254) and a validation (n=111) set of younger adults (aged <60 years) with *de novo* CN-AML. We applied a novel algorithm (MScircRNA) to quantify circRNA expression in both datasets. All pts were treated on frontline Cancer and Leukemia Group B (CALGB)/Alliance trials.
Results: We first performed RNAseq in RNAseR (a linear RNA-degrading exonuclease) v mock-treated samples of 7 AML pts and 3 AML cell lines (EOL-1, K562, OCI-AML3). We found 85% of the MScircRNA algorithm-predicted circRNAs to represent true circularized transcripts, as shown by enrichment upon RNAseR treatment. We next studied 180 circRNAs in the training set, and identified 4 (*circCFLAR*, *circFCHO2*, *circKHLH8*, *circSMC1A*) that associated with disease-free (DFS), overall (OS), and event-free (EFS) survival in both the training and validation sets. *circKHLH8* was most strongly associated with clinical outcome. Using median expression values, we dichotomized the pts into high and low *circKHLH8* expressers. In the validation set, high *circKHLH8* expressers had longer 5-year DFS (50% *vs* 21%, *P*<.001), OS (53% *vs* 29%, *P*<.001) and EFS (45% v 18%, *P*<.001) than low *circKHLH8* expressers. Regarding pretreatment clinical and molecular features, pts with high *circKHLH8* expression had higher platelet counts (*P*=.05) and lower % of blasts in blood (*P*=.002) and bone marrow (*P*=.05). High *circKHLH8* expressers also had more often *FLT3*-TKD (*P*=.05) and less often *FLT3*-ITD (*P*<.001) or high expression of miR-155 (*P*<.001) and *ERG* (*P*<.001) than low *circKHLH8* expressers. In multivariable analyses, high *circKHLH8* expression independently associated with longer DFS (*P*=.02; HR: 0.53), OS (*P*=.03; HR: 0.54) and EFS (*P*=.02; HR: 0.54) after adjusting for other co-variates. To ensure that our findings on *circKHLH8* were not merely reflecting the prognostic significance of the linear *KHLH8*, we confirmed that the correlation between the transcripts was weak (Pearson's r: 0.29) and that linear *KHLH8* expression was not prognostic for clinical outcome in either of the 2 datasets. To study the biologic significance of circRNA expression, we performed knock-down (KD)-based screening of candidate circRNAs associated with outcome or ELN prognostic gene mutations in KG1a and OCI-AML3 cells. We used back-splice site-targeting locked nucleic acid-modified oligonucleotides, which depleted circRNAs without affecting the corresponding linear transcripts. Of the circRNAs tested, *circFBXW7* KD increased the proliferative capacity of KG1a and OCI-AML3 cells, as measured by WST1 reagent degradation (*P*=.001 and *P*<.001, respectively) and bromodeoxyuridine-based cell cycle analysis (*P*=.02 and *P*=.01, respectively). Colony forming unit assays with blasts of 3 AML pts revealed a consistent increase in the number of colonies formed upon *circFBXW7* KD (*P*=.03, *P*=.04 and *P*=.02, respectively). Altogether, our data suggest that *circFBXW7* has a tumor suppressor function in CN-AML.
Summary and Conclusions: We conclude that circRNA expression has prognostic and biologic significance in CN-AML.

S121

PHARMACOLOGIC DISPLACEMENT OF LSD1 FROM GFI1 ACTIVATES PRIMED ENHANCERS TO INDUCE DIFFERENTIATION IN ACUTE MYELOID LEUKEMIA

A. Maiques-Diaz[1,*], G.J. Spencer[1], J.T. Lynch[1], F.M.R. Amaral[1], S. Sahoo[2], T C. Somervaille[1]

[1]Leukaemia Biology Laboratory, [2]Computational Biology Group, Cancer Research UK Manchester Institute, University of Manchester, Manchester, United Kingdom

Background: Treatment of patients with MLL-translocated acute myeloid leukemia (AML) with a tranylcypromine-derivative inhibitor of Lysine Specific Demethylase 1 (LSD1/KDM1A) induces differentiation of blast cells in blood and bone marrow (Somervaille *et al*., Blood 2016). The assumption has been that LSD1 contributes to gene repression by removing monomethyl and dimethyl histone marks from lysine 4 of histone H3 (H3K4me1/me2) and that this is the key activity targeted for potential therapeutic effect. LSD1 also interacts with multiple transcription factors raising the possibility that other mechanisms may be significant.
Aims: We aimed to better characterize and understand LSD1 inhibitors mechanisms of action.
Methods: RNAseq, ATACseq and ChIPseq in human THP1 AML cell line treated with a tranylcypromine-derivative inhibitor (OG86, Oryzon Genomics).
Results: Contrary to what has been assumed, human THP1 AML cells treated with the LSD1 inhibitor OG86 induced rapid transcriptional changes without significant H3K4me1/2 changes in the upregulated LSD1-bound promoters (214/766, 28%) compared with those lacking an LSD1 binding peak (552/766, 72%). Regarding putative enhancer regions, only a modest H3K4me1 reduction in LSD1-bound *versus* LSD1-unbound enhancers was observed, but a highly significant increase in H3K9ac and H3K27ac, consistently with increase activation of LSD1-bound enhancers. Intriguingly, the transcriptional consequences of LSD1 inhibition mimicked the transcriptional changes of SNAG-domain transcription repressor GFI1 knockdown in THP1 AML cells, and we found that GFI1:LSD1/RCOR1 interaction was disrupted by OG86 treatment in THP1 and MV4,11 AML cells using several unrelated inhibitors.
Interestingly all the strongest GFI1 ChIPseq peaks (based on MACS2 pileup value) exhibited coincident LSD1 and RCOR1 binding (*i.e.* 98.6% and 88.4% respectively), and a significant GFI1 consensus-binding motif was found on LSD1 and RCOR1 ChIPseq bound regions surrounding the peak centers (MEME-ChIP, p=10-686 and p=10-55 respectively). We observed a loss of LSD1, RCOR1 and GFI1 ChIPseq signal in OG86-treated THP1 AML cells, with the greatest proportional reduction found at the 1,867 sites co-occupied by the three proteins together. The selective loss of LSD1/RCOR1 from GFI1 sites upon inhibition was further supported by the absence of any GFI1 consensus motif found on the residual LSD1 and RCOR1 binding peak sequences in OG86-treated cells. The loss of chromatin binding of the three proteins was also observed using subcellular fractionation analysis. Moreover, LSD1 inhibition in THP1 AML cells promoted differentiation (*i.e.* increased CD86 expression and reduced clonogenic potential), but cells expressing a doxycycline-regulated GFI1-ZNF DNA binding domain-LSD1 fusion protein completely blocked this differentiation effect, indicating that the myeloid differentiation induced by LSD1 inhibition results from the physical separation of LSD1 and GFI1. Finally, we did not observe any changes on chromatin accessibility (using ATACseq) surrounding sites co-occupied by GFI1, LSD1 and RCOR1 following LSD1 inhibition, but a significant increase of ChIPseq signal for H3K9ac and H3K27ac.
Summary and Conclusions: Our data illustrate a paradigm for epigenetic therapy whereby, through disruption of the protein:protein interaction between a transcription repressor and an epigenetic regulator, repression is released and dynamic enhancer acetylation and gene expression ensue.

S122

ALTERED COHESIN ACTIVITY AT DIFFERENTIATION-SPECIFIC ACTIVE CIS-REGULATORY ELEMENTS PREDICTS THE FATE OF COHESIN-PERTURBED HAEMATOPOIESIS

D. Sasca[1,2,3,4,*], G. Giotopoulos[1,2,3], H. Yun[1,2,3], T. Evan[1,2,3], B.J. Huntly[1,2,3]

[1]Wellcome Trust-MRC Cambridge Stem Cell Institute, [2]Department of Haematology, University of Cambridge, [3]Cambridge Institute for Medical Research, Cambridge,

United Kingdom, [4]Department of Internal Medicine III (Hematology, Oncology, Pneumology), University Medical Center Mainz, Mainz, Germany

Background: Myeloid malignancies are a heterogeneous continuum of neoplasia that share many common characteristics, such as altered transcriptional regulation caused by recurrent mutations of signalling, transcription and epigenetic factors. With a frequency of 15% across all myeloid malignancies, several of these mutations occur in members of the cohesin complex (*STAG2, RAD21, SMC1a* or *SMC3*, less frequent *STAG1).* The mutations are mutually exclusive, are predicted to cause loss of function alleles and a decrease of complex activity.
Aims: To determine a mechanism for the establishment of cohesin-deficient myeloid neoplasia.
Methods: Utilizing a model of haematopoietic stem and progenitor differentiation (HPC-7), we performed integrative analysis of cohesin binding, gene expression, chromatin state and 3D-DNA topology at genome scale, assessing different cellular states - haematopoietic progenitors, erythroid and myeloid cells - followed by matched analysis after inducible shRNA-mediated knockdown (KD) of multiple cohesin members. We finally validated the observed patterns *in vitro* and *in vivo* using a murine model of *Flt3-ITD/Npm1c* AML after inducible KD of Stag2 or Smc1a.
Results: We demonstrated alterations of cohesin member expression upon commitment down the erythroid but not myeloid lineage. This variable expression of cohesin translated into differing dosages at chromatin during differentiation; cohesin binding increased in the erythroid lineage at active *cis*-regulatory elements (enhancers/promoters), while the global cohesin dosage remained low in the myeloid lineage. Binding dynamics correlated to the H3K27 acetylation (H3K27ac), but not to gene expression, Ctcf binding or promoter interaction frequency. Suprisingly, KD of cohesin members only minimally affected binding of the residual cohesin complex to active promoters/enhancers in immature cells, resulting in discrete changes of H3K27ac and gene expression. However, during differentiation of Stag2-perturbed haematopoiesis, significant biases of cohesin binding to active *cis*-regulatory elements were seen, with impaired dosage of cohesin demonstrated at many promoters. In erythroid progenitors, these promoters consistently specified for key erythroid genes, such as Klf1 or Gata1, which we had shown to require increased cohesin binding during erythropoiesis. The lack of cohesin complex binding led to impairment of H3K27ac, promoter interactions, gene expression and, functionally, a block of differentiation. Conversely, in the myeloid lineage, cohesin redistribution following Stag2-KD led to a relative increase at promoters of genes that are crucial for proliferation, including *Myc*, *Id1* and *Foxo1*, inducing myeloid expansion.
We confirmed our findings in a murine model of *Flt3-ITD/Npm1c* AML where limiting concentrations of Stag2, by shRNA-KD, caused genome-wide cohesin and H3K27ac redistribution. This generated significant alterations of transcription, a more primitive and aggressive phenotype and, in limiting dilution transplants, a marked increase in leukemia stem cell frequency (1/2812 for WT-AML to 1/25 for shS2-AML).
Summary and Conclusions: Cohesin member perturbation redistributes the binding of the remaining cohesin proteins to active promoters/enhancers, altering H3K27ac, 3D-DNA interactions and transcription. This leads to distinct and opposite patterns in the erythroid and myeloid lineages and explain the loss of differentiation, increased self-renewal and proliferation phenotypes, thereby providing a mechanistic model for (pre)leukaemic development of cohesin-impaired haematopoiesis.

S123

STAG2 REGULATES HEMATOPOIETIC DIFFERENTIATION AND SELF-RENEWAL

Y. Ochi[12,*], A. Kon[1], M.M. Nakagawa[1], K. Kataoka[1], K. Yoshida[1], H. Koseki[3], M. Nakayama[4], A. Yoda[1], H. Makishima[1], A. Takaori-Kondo[2], S. Ogawa[15]

[1]Department of Pathology and Tumor Biology, Graduate School of Medicine, [2]Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto, [3]Laboratory for Developmental Genetics, RIKEN Center for Integrative Medical Sciences, Yokohama, [4]Chromosome Engineering Team, Department of Technology Development, Kazusa DNA Research Institute, Kisarazu, Japan, [5]Center for Hematology and Regenerative Medicine, Department of Medicine, Karolinska Institutet, Stockholm, Sweden

Background: Cohesin is a multimeric protein complex, which was originally implicated in sister chromatid cohesion and more recently, in long-range regulation of gene expression by stabilizing 3-dememsional structure of the genome. Recently, multiple components of the cohesin complex are identified as recurrent targets of somatic mutations in various myeloid malignancies, of which *STAG2* is most frequently mutated and inactivated; however, the leukemogenic mechanism of mutated-*STAG2* remain largely unknown.
Aims: The aim of the study is to investigate the functional role of *STAG2* in leukemogenesis as well as normal hematopoiesis.
Methods: We generated *Stag2* conditional knockout (cKO) mice having an Mx1-cre allele, in which *Stag2* deletion was induced by polyIC injection.
Results: *Stag2* cKO mice showed a mild leukocytopenia. Flow cytometry of bone marrow cells revealed an increase in the frequency of hematopoietic stem and progenitor cells (HSPCs) defined as $Lin^-Sca\text{-}1^+Kit^+$(LSK) cells in *Stag2* cKO mice. In the cell cycle kinetics analysis, *Stag2* cKO HSPCs were characterized by an increase in the proportion of cells in S/G2/M phases. Within the myeloid progenitor (MP) compartment, we observed increased common myeloid progenitors (CMPs) and granulocyte-macrophage progenitors (GMPs), and decreased megakaryocyte/erythroid (MEPs) and common lymphoid progenitors (CLPs). Moreover, $CD11b^+Gr\text{-}1^+$ myeloid cells were increased in the bone marrow of *Stag2* cKO mice. These results suggest that *Stag2* deficiency causes skewing toward myeloid lineage. In repeated methylcellulose cultures, *Stag2* deficient BM cells showed an enhanced serial replating capacity, suggesting an increased self-renewal potential of *Stag2* deficient hematopoietic stem cells (HSCs). In competitive repopulation assays, *Stag2* cKO-derived cells showed reduced chimerism in the peripheral blood compared to wild-type mice-derived cells. In the bone marrow, by contrast, the chimerism of *Stag2* cKO cells was not significantly changed, but tended to increase in the LSK, CMP and GMP fractions and decrease in the MEP and CLP fractions. These results suggest that *Stag2* deficiency could enhance the self-renewal capacity of HSCs, while differentially impacting on their contribution to different hematopoietic cell fractions due most likely to the deregulated differentiation of HSCs. We next investigated differential expression profiles between *Stag2* WT and cKO cells using RNA sequencing of HSPC fractions. We observed significant up-regulation of key hematopoietic regulators, including *Gata1*, *Gata2* and *Runx1*, which are implicated in the differentiation of HSCs. Of particular interest in this regard, assays for transposase accessible chromatin with sequencing (ATAC-seq) revealed a significant enrichment of the binding sites for Gata1, Gata2, and Runx1 in the open chromatin regions in *Stag2*-deleted HSPCs. We also observed up-regulated expression of genes related to myeloid programs, such as *Mpo* and *Cebpa*, while expression of several genes involved in the lymphoid development such as *Cd9* and *Irf4* were down regulated. These results indicate that *Stag2*-deficient HSCs are primed to differentiate toward myeloid lineage, which is in agreement with *in vivo* hematopoietic phenotypes.
Summary and Conclusions: Our results demonstrate that *Stag2* loss leads to the impaired hematopoietic differentiation and enhances the self-renewal potential of HSCs, likely through the modulation of gene expression and chromatin accessibility, which may contribute to leukemogenesis in *STAG2*-mutated cells.

Stem cell transplantation – Clinical I

S124

BUSULFAN + MELPHALAN WAS ASSOCIATED WITH A LONGER PFS COMPARED TO MELPHALAN ALONE IN HIGH-RISK MULTIPLE MYELOMA IN A RANDOMIZED PHASE 3 CLINICAL TRIAL

M. Qazilbash[1,*], Q. Bashir[1], P. Thall[2], D. Milton[2], N. Shah[3], K. Patel[4], B. Andersson[1], Y. Nieto[1], P. Kebriaei[1], B. Valdez[1], J. Kawedia[5], S. Parmar[4], R. Delgado[1], S. Ahmed[1], C. Hosing[1], U. Popat[1], E. Shpall[1], D. Weber[4], S. Thomas[4], E. Manasanch[4], H. Lee[4], R. Orlowski[4], R. Champlin[1]

[1]Stem Cell Transplantation, [2]Biostatistics, MD Anderson Cancer Center, Houston, [3]Medicine, University of California San Francisco, San Francisco, [4]Lymphoma/Myeloma, [5]Pharmacy Pharmacology Research, MD Anderson Cancer Center, Houston, United States

Background: High-dose melphalan (Mel) 200 mg/m2 is considered the standard of care preparative regimen for autologous hematopoietic stem cell transplantation (auto-HCT) for multiple myeloma. Two recent retrospective analyses suggested that a combination of busulfan (Bu) and Mel (Bu-Mel) may be associated with a longer progression-free survival (PFS) compared to Mel alone. In this randomized phase III trial we compared the safety and efficacy of Bu-Mel *vs* Mel.
Aims: The primary objective was to compare PFS between the two arms.
Methods: Patients were randomized to either Bu-Mel or Mel using the Pocock-Simon method within 12 months of the start of induction therapy. The trial was designed to detect a 14-month increase in median PFS in the Bu-Mel arm with two-sided tests having nominal overall type I error .029 and power .80, with up to 3 tests using O'Brien-Fleming decision boundaries.
Results: Two-hundred and four patients (Bu-Mel: 104, Mel: 100) were enrolled from October 2011 to March 2017. There was no significant difference between the two arms in age, gender, race, cytogenetic risk status, ISS stage, serum LDH, induction regimens, response to induction, or maintenance therapy. Sixty-two patients, 31 each in Bu-Mel (30%) and Mel (31%) arms, respectively, had high-risk (HR) chromosomal abnormalities as defined by IMWG criteria. At day 90 after auto-HCT, 11 (35%) HR MM patients had achieved complete remission (CR) in each arm (p=1.00). At last evaluation, 16 (52%) and 14 (45%) HR MM patients had achieved a CR (p=0.80) in Bu-Mel and Mel, respectively. Minimal residual disease (MRD) analysis by multiparametric flow cytometry (MFC) was performed at day 100 in 26 and 22 patients with HR MM in Bu-Mel and Mel, respectively. Fifteen (58%) and 13 (59%) achieved MRD negative status in Bu-Mel and Mel arms, respectively (p=1.00). -Meier estimates of median PFS for HR MM was not reached in the Bu-Mel arm, and 25.0 months in the Mel only arm (p=0.021) (Figure 1).

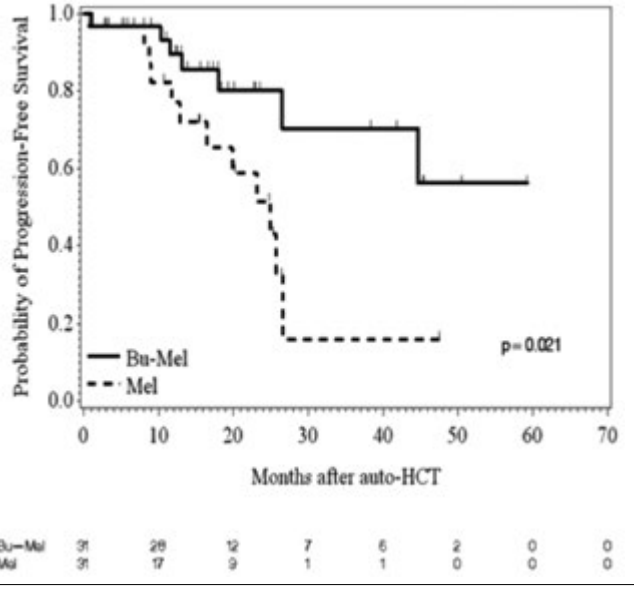

Figure 1.

This significant difference in PFS was maintained after adjusting for the type of maintenance therapy received (p=0.035). There was no difference in OS between HR MM patients in the Bu-Mel or Mel only arms (p=0.37). In a fitted Bayesian regression model that included age, race, ISS stage, and response to induction therapy, the posterior probability that patients receiving Bu-Mel had a smaller risk of progression and/or death compared with patients receiving Mel alone is 0.989, which is highly significant.
Summary and Conclusions: In this phase III trial, Bu-Mel regimen was safe, and associated with a significantly longer PFS than Mel alone. This significant difference in PFS was also observed for patients with high-risk disease. Potential explanations for a longer PFS without a significant difference in response rates include a deeper MRD negativity or selective targeting of clonogenic myeloma progenitor cells by Bu-Mel.

S125

THE ROLE OF MINIMAL RESIDUAL DISEASE AT THE TIME OF TRANSPLANTATION IN PATIENTS WITH AML IN CR2 AFTER ALLOGENEIC STEM CELL TRANSPLANTATION. A STUDY OF THE ACUTE LEUKEMIA WORKING PARTY OF THE EBMT

A. Shimoni[1,*], M. Labopin[2], S. Robinson[3], D. Beelen[4], G. Socié[5], A. Unal[6], A. Ganser[7], A. Vitek[8], H. Sengeloev[9], I. Yakoub-Agha[10], E. Tholouli[11], M. Mohty[12], A. Nagler[1]

[1]CHAIM SHEBA MEDICAL CENTER, Tel-Hashomer, Israel, [2]ALWP office, Paris, France, [3]Bristol Royal Hospital for Children, Bristol, United Kingdom, [4]Essen University Hospital, Essen, Germany, [5]Hopital St. Louis, Paris, France, [6]Erciyes Medical School, Kayseri, Turkey, [7]Hannover Medical School, Hannover, Germany, [8]Institute of Hematology and Blood Transfusion, Prague, Czech Republic, [9]National University Hospita, Copenhagen, Denmark, [10]Hôpital HURIEZ UAM, Lille, France, [11]Manchester Royal Infirmary, Manchester, United Kingdom, [12]Saint Antoine Hospital, Paris, France

Background: The current definition of complete remission (CR) in patients with acute myeloid leukemia (AML) is based on recovery of normal blood counts after treatment and morphological assessment of the marrow. However, patients in CR can still harbor significant number of leukemic cells that may result in relapse. Minimal residual disease (MRD) assessment can establish, by different methods, the presence of $1:10^3$- $1:10^6$ leukemia cells. The level of MRD as assessed at particular time-points during AML treatment, and in particular prior to stem-cell transplantation (SCT), is an independent and important predictor of outcome. However, most of the existing data are available for CR1 and there is only limited data on the role of MRD in patients achieving CR2 following leukemic relapse.
Aims: To assess the impact of MRD status on transplantation outcomes in patients in CR2 at the time SCT.
Methods: We retrospectively analyzed SCT outcomes in a group of 1042 patients with *de-novo* AML in CR2 given SCT between 2006 and 2016 from HLA- matched siblings (n=719) or 10/10 matched unrelated donors (n=293), who had available MRD status data at the time of SCT and were registered in the data base of the ALWP of the EBMT. MRD methodology and allocation to MRD negative or MRD positive groups were determined by the individual participating centers and utilized molecular and/or immunophenotyping criteria.
Results: The median age was 49 years (range, 18-73), 558 males and 484 females. The conditioning regimen was myeloablative (n=610) or reduced-intensity (n=432) and 566 patients (54%) had *in-vivo* T-cell depletion. In all, 749 patients (72%) were MRD negative and 293 (28%) had positive MRD at the time of SCT. The only difference in patient characteristics between the MRD (-) and MRD (+) groups was a longer time from diagnosis to SCT in the MRD (-) group, 18 *vs* 16 months, respectively (P<0.001). In particular, there was no difference in the cytogenetic or molecular characteristics between the groups. The 2-year relapse rate was 24% (95%CI, 21-28) and 40% (95%CI, 34-46) in the MRD (-) and MRD (+) groups, respectively (P<0.001). The predicting factors for relapse in multivariate analysis were MRD (-) status (HR 0.57, P<0.001), good cytogenetics (HR 0.62, P=0.001), longer time from diagnosis to SCT (HR=0.97, P<0.001) and *in-vivo* T-cell depletion (HR 1.35, P=0.03). The 2-year LFS was 57% (53-61) and 46% (40-52%), respectively (P=0.001). The predicting factors for LFS were MRD (-) status (HR 0.76, P=0.01), good cytogenetics (HR 0.79, P=0.04) and longer time from diagnosis to SCT (HR=0.99, P<0.001). Age, gender, donor or conditioning type did not predict relapse or LFS rates.
Summary and Conclusions: Achieving a negative MRD status after second-line treatment for relapsed AML is associated with lower relapse rates and improved LFS after SCT. These observations are similar to the effect of MRD status in the frontline setting. MRD status pre SCT for AML relapsing patients achieving CR2 should dictate therapeutic strategies in this patient population.

S126

THE IMPACT OF RECIPIENT AGE ON ALLOGENEIC STEM CELL TRANSPLANTATION OUTCOME –A COMPARISON BETWEEN SIBLINGS *VERSUS* UNRELATED *VERSUS* ALTERNATIVE DONORS: AN ANALYSIS ON BEHALF OF THE ALWP OF THE EBMT

R. Shouval[1,*], J. Fein[1], M. Labopin[2], N. Kröger[3], R. F. Duarte[4], P. Bader[5], C. Bonini[6], J. Kuball[7], G. Basak[8], C. Dufour[9], A. Lankester[10], S. Montoto[11], J. A. Snowden[12], J. Styczynski[13], M. Mohty[14], A. Nagler[1]

[1]Sheba Medical Center, Tel-HaShomer, Ramat-Gan, Israel, [2]Acute Leukemia Working Party, EBMT, Paris, France, [3]University Hospital Eppendorf, Hamburg, Germany, [4]Hospital Universitario Puerta de Hierro, Madrid, Spain, [5]University Hospital Frankfurt, Frankfurt, Germany, [6]Fondazione San Raffaele Del Monte Tabor, Milan, Italy, [7]UMC Utrecht, Utrecht, Netherlands, [8]University of Warsaw, Warsaw, Poland, [9]G. Gaslini Chidlren's Hospital, Genova, Italy, [10]Leiden University Medical Centre, Leiden, Netherlands, [11]Barts Cancer Institute, London, [12]Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, United Kingdom, [13]Collegium Medicum, Nicolaus Copernicus University, Bydgoszcz, Poland, [14]Hopital Saint-Antoine, Université Pierre & Marie Curie, Paris, France

Background: Contemporary practice in allogeneic stem cell transplantation (SCT) has increased the likelihood of finding donors for nearly all transplantation candidates. Furthermore, older patients (pts) are being transplanted.
Aims: We sought to explore the relationship between increasing age, donor selection, and risk of non-relapse mortality (NRM).
Methods: A retrospective study including 55,941 adult pts treated for hematologic malignancies who underwent first SCT from sibling (MSD), matched (MUD) or mismatched (MMUD) unrelated, cord blood (CB) or haploidentical (Haplo) donors between 2010 and 2015 in European Society for Blood and Marrow Transplantation (EBMT) centers. We compared outcomes across four consecutive age groups (18-39 [I], 40-49 [II], 50-59 [III], ≥60 [IV]). The primary outcome was NRM. Within each age group, using a Cox regression model adjusted for key variables, we studied the risk associated with different donor types. MSDs were the reference category.
Results: Younger patients were more likely to receive MSD grafts (39% [group I], 39% [II], 38% [III], and 26% [IV]) and myeloablative conditioning (76% [I], 61% [II], 40% [III], and 23 [IV]. Unrelated donors were increasingly used with increasing age (49% [I], 53% [II], 56% [III], 66% [IV]). Haplo and CB transplant were more prevalent in ages 18-39 (9% and 4%, respectively), compared to all other age groups. The probability of 3-year NRM increased with age, regardless of donor type. (Table 1).

Table 1.

		18-39	40-49	50-59	60-83
3-year Non-Relapse Mortality (95% CI)	MSD	15.8 (14.7-17.0)	19.7 (18.4-21.1)	24.3 (23.1-25.6)	28.5 (26.9-30.2)
	MUD	21.9 (20.5-23.4)	24.4 (22.8-26.1)	29.4 (28.0-30.8)	34.5 (33.1-36.0)
	Haplo	25.8 (23.2-28.6)	32.0 (28.0-36.5)	36.0 (32.4-40.0)	38.9 (35.2-42.9)
	MMUD	30.8 (28.1-33.7)	34.5 (31.4-38.0)	39.8 (37.1-42.8)	44.0 (41.1-47.2)
	Cord	30.4 (26.6-34.6)	35.8 (30.7-41.7)	39.2 (34.3-44.8)	43.9 (38.6-50.0)
3-year Overall Survival (95% CI)	MSD	59.8 (58.1-61.4)	58.4 (56.7-60.2)	52.4 (50.9-54.0)	45.3 (43.5-47.3)
	MUD	59.4 (57.6-61.2)	55.6 (53.6-57.6)	49.5 (47.9-51.1)	44.5 (43.0-46.0)
	Haplo	49.8 (46.7-53.1)	42.6 (38.1-47.6)	41.5 (37.6-45.8)	38.5 (34.6-42.7)
	MMUD	47.5 (44.5-50.8)	44.2 (40.7-47.9)	41.1 (38.3-44.2)	33.1 (30.2-36.2)
	Cord	25.5 (21.9-29.6)	32.3 (27.2-38.2)	26.7 (22.3-31.9)	22.9 (18.5-28.5)

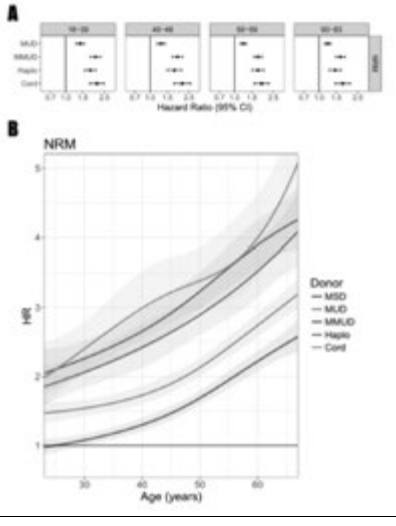

Figure 1.

Across all age groups, NRM was lowest with MSD followed by MUD and Haplo, while the highest incidence was in transplants from MMUD and CB. 3-year overall survival (OS) was also higher with MSDs and MUDs in all age groups. Notably, among older pts (group IV) receiving grafts from alternative donors, Haplo was associated with better OS (38.5%) compared to MMUD (33.1%) and CB (22.9%). MUD transplants had increased risk for NRM compared to MSDs regardless of age group (HR ranging from 1.2 to 1.4, Figure 1A). Alternative donors had an overlapping risk for NRM ranging from 1.5-2.1 (reference MSD). Using cubic splines, we further validated these findings by modeling the risk of donors across age as a continuous factor (Figure 1B).
Summary and Conclusions: MSDs remains the safest option in all age groups. In older pts with no conventional donor available, Haplo may be preferable because of reduced NRM in comparison to MMUD and CB.

S127

TRENDS IN THE USE OF HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THE MANAGEMENT OF RELAPSED/ REFRACTORY HODGKIN LYMPHOMA. A RETROSPECTIVE ANALYSIS OF THE LYMPHOMA WORKING PARTY OF THE EBMT

A. Sureda[1,*], S. Genadieva Stavrik[2], A. Boumendil[3], H. Finel[3], I. Khvedelidze[3], S. Dietrich[4], P. Dreger[4], O. Hermine[5], C. Kyriakou[6], S. Robinson[7], N. Schmitz[8], H. Schouten[9], A. Tanase[10], S. Montoto[11]

[1]Department of Hematology, Institut Catala d'Oncologia-Hospital Duran I Reynals, Barcelona, Spain, [2]Department of Hematology, Medical faculty, Skopje, Macedonia, The Former Yugoslav Republic Of, [3]EBMT, Lymphoma Working party, Paris, France, [4]Department of Internal Medicine V, University of Heidelberg, Heidelberg, Germany, [5]Department of Clinical Haematology, AP-HP, Necker Hospital, Paris, France, [6]University College London Hospital, London, [7]Bone Marrow Transplant Unit, University Hospital Bristol, Bristol, United Kingdom, [8]Asklepious Klinik St Georg, Hamburg, Germany, [9]University Hospital, Maastricht, Netherlands, [10]Department of Stem Cell Transplantation, Fundeni Clinical Institute, Bucharest, Romania, [11]Department of Haemato-Oncology, St Bartholomew's Hospital, Barts Health NHS Trust, London, United Kingdom

Background: Both autologous (auto-HSCT) and allogeneic stem cell transplantation (allo-HSCT) represent well-accepted treatment strategies for patients with relapsed / refractory (RR) Hodgkin lymphoma (HL). Nevertheless, both transplant modalities have evolved over time and the recent advent of new drugs might have modified the indications and timing of HSCT.
Aims: We have therefore analysed the transplant activity for patients with RR HL reported to the EBMT registry over the last three decades.
Methods: Patients were included if they had RR (primary refractory or relapsed disease) HL, were above 18 years of age and had undergone an auto-HSCT as 1st HSCT or an allo-HSCT either as a 1st HSCT or after a prior auto-HSCT between January 1990 to December 2014. Med A level data were retrieved from the EBMT database.
Results: 13639 patients [11435 auto-HSCT and 2204 allo-HSCT (555 1st allo-HSCT and 1649 allo-HSCT after an auto-HSCT)] were registered in the EBMT database during the study period. The number of auto-HSCT steadily increased from 129 in 1990 up to a maximum of 811 in 2010; the number of allo-HSCT also increased from 6 in 1990 up to a peak of 243 in 2014. With regards to autologous recipients, over time there was a significant increase in age at HSCT [31 yrs (1990-1994) *vs* 35 yrs (2010-2014), p<0.0001], time between diagnosis and HSCT was shorter [31 (1990-1994) *vs* 23 mo (2010-2014), p<0.0001], peripheral blood (PB) has become the universally used stem cell source [30% (1990-1994] *vs* 98% (2010-2014), p<0.0001] and total body irradiation has almost been abandoned [4% (1990-1994) *vs* 1.7% (2010-2014), p<0.0001]. 36-mo overall survival (OS) has significantly improved over time [63% (1990-1994) *vs* 79% (2010-2014), p<0.0001] as well as non-relapse mortality (NRM) [12% (1990-12994) *vs* 6% (2010-2014), p<0.0001]. Interestingly, allo-HSCT has been less used as the 1st HSCT [88% (1990-1994) *vs* 23% (2010-1014)] whereas the number of allo-HSCT after a first auto-HSCT has steadily increased [12% (1990-1994) *vs* 77% (2010-2014), p<0.0001]. Time between diagnosis and HSCT has decreased over time also in allogeneic recipients [36 mo (1990-1994) *vs* 34 mo (2010-2014), p<0.04]]. Performance status >80% at HSCT has improved [62% (1990-1994) *vs* 94% (2010-2014), p<0.0001], PB has become the universal source of stem cells [6% (1990-1994) *vs* 84% (2010-2014), p<0.0001], and there has been a more frequent use of reduced intensity conditioning protocols [0% (1990-1994) *vs* 70% (2010-2014), p<0.0001] as well as of matched unrelated donors and haploidentical donors [0% (1990-1994) *vs* 48% (2010-2014) and 0% (1990-1994) *vs* 17% (2010-2014), respectively, p<0.0001]. 36-month OS estimates have also significantly improved [21% (1990-1994) *vs* 61% (2010-2014), p<0.001)] as well

as those for progression free survival [15% (1990-1994) *vs* 43% (2010-2014), p<0.001] and NRM [58% (1990-1994) *vs* 22% (2010-2014), p<0.001].
Summary and Conclusions: Transplantation activity, the clinical pattern of patients undergoing this treatment and the characteristics of the procedure have significantly changed over the study period and results in terms of OS and NRM for both auto-HSCT and allo-HSCT are much better. Improvement of supportive measures as well as in the experience of the transplant centers and a better selection of patients could account for these changes. The impact of the introduction of new treatment modalities (anti-CD30 monoclonal antibodies, check point inhibitors) in the number of HSCT is difficult to ascertain at this point.

S128

REDUCED-INTENSITY ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION FOR DIFFUSE LARGE B-CELL LYMPHOMA: COMPARABLE OUTCOMES OF HAPLO-IDENTICAL *VS* FULLY MATCHED RELATED DONORS. A CIBMTR AND EBMT ANALYSIS

P. Dreger[1,*], A. Sureda[1], K. Ahn[2], H. Finel[1], J. Tischer[1], L. Castagna[1], D. Blaise[1], A. Dominietto[1], C. Schmid[1], F. Ciceri[1], A. Boumendil[1], C. Litovich[2], S. Smith[2], T. Fenske[2], S. Dietrich[1], S. Robinson[1], S. Montoto[1], M. Hamadani[2]

[1]Lymphoma Working Party, EBMT, Paris, France, [2]CIBMTR, Milwaukee, United States

Background: Allogeneic hematopoietic cell transplantation (alloHCT) from haplo-identical donors using immunosuppression with post-transplant cyclophosphamide (ptCY) is increasingly performed in patients with relapsed/refractory diffuse large B-cell lymphoma (DLBCL). Disease-specific outcome data for ptCY-based haplo-transplantation (haploHCT) have not been reported for DLBCL to date.
Aims: To compare the outcomes of haploHCT with that of fully matched sibling transplants (msdHCT) as standard reference donor source in patients with DLBCL.
Methods: Eligible for this retrospective registry study were patients aged 18 or older who had undergone a reduced-intensity conditioning (RIC) haploHCT or msdHCT for DLBCL between January 2008 and June 2015 and were registered with the CIBMTR or the EBMT. Primary endpoint was overall survival (OS); secondary endpoints included engraftment, acute and chronic GVHD, non-relapse mortality (NRM), relapse/progression incidence (REL) and progression-free survival (PFS).
Results: Altogether 657 patients were eligible (haploHCT 132; msdHCT 525). HaploHCT and msdHCT recipients were comparable for gender, comorbidity score, time from diagnosis, and disease status at alloHCT. However, haploHCT patients were significantly older (58 *vs* 55 years), more likely to have a Karnofsky score of 90-100 (73% *vs* 62%), had less often received a prior autoHCT (42% *vs* 55%), and had been allotransplanted more recently. Moreover, haploHCT and msdHCT recipients differed fundamentally in terms of use of TBI-based conditioning (86% *vs* 21%) and graft source bone marrow (76% *vs* 2%).
On univariate and multivariate comparisons, OS, PFS, REL, and NRM were not significantly different between haploHCT and msdHCT with 3-year estimates of 46% *vs* 50%, 38% *vs* 37%, 41% v 47%, and 22% v 17%, respectively. Engraftment was significantly delayed after haploHCT *vs* msdHCT (neutrophils >0.5/nl at d +28 90% *vs* 97%, p=0.01; platelets >20/nl at d +28 61% *vs* 92%, p<0.001). Whilst the cumulative incidence of d +180 grade 3-4 acute GVHD was comparable (haploHCT *vs* msdHCT 7% *vs* 11%, p=0.07), there was a significantly lower 1-year incidence of chronic GVHD after haploHCT *vs* msdHCT (15% *vs* 41%; p<0.001; relative risk (RR) 0.31 (95%CI 0.27-0.47) after multivariate adjustment for confounders). Factors significantly impairing OS, PFS, REL (but not NRM) on multivariate analysis were lower Karnofsky performance status and advanced disease status at alloHCT.
Summary and Conclusions: This data suggests that in DLBCL survival, REL and NRM after RIC haploHCT is comparable to that after RIC msdHCT despite a lower risk of chronic GVHD. Although delayed engraftment has to be taken into account, RIC haploHCT might be a reasonable substitute for RIC msdHCT in the absence of a matched related donor.

Myeloproliferative neoplasms – Clinical

S129

COMPARATIVE PERFORMANCE OF PROGNOSTIC SYSTEMS IN PATIENTS WITH MYELOFIBROSIS SECONDARY TO PV AND ET AFTER ALLOGENEIC STEM-CELL TRANSPLANTATION

N. Gagelmann[1,*], D.-J. Eikema[2], L. Koster[2], C. Wolschke[1], R. Arnold[3], L. Kanz[4], G. McQuaker[5], I. Yakoub-Agha[6], T. Lamy[7], G. Socié[8], J.H. Bourhis[9], M. Mohty[10], J.J. Cornelissen[11], P. Chevallier[12], P. Bernasconi[13], M. Stelljes[14], P.-S. Rohrlich[15], R. Fanin[16], J. Finke[17], J. Maertens[18], D. Blaise[19], M. Itälä-Remes[20], H. Labussière-Wallet[21], M. Robin[8], Y. Chalandon[22], N. Kröger[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [2]EBMT data office, Leiden, Netherlands, [3]Charité, Berlin, [4]University Hospital Tübingen, Tübingen, Germany, [5]Gartnaval General Hospital, Glasgow, United Kingdom, [6]CHU de Lille, Lille, [7]Centre Hospitalier Universitaire de Rennes, Rennes, [8]Hopital St. Louis, Paris, [9]Gustave Roussy, institut de cancérologie, Villejuif, [10]Hospital Saint Antoine, Paris, France, [11]Erasmus MC Cancer Institute, Rotterdam, Netherlands, [12]CHU Nantes, Nantes, France, [13]Fondazione IRCCS Policlinico San Matteo, Pavia, Italy, [14]University Münster, Münster, Germany, [15]CHU Nice, Nice, France, [16]Azienda Ospedaliero Universitaria di Udine, Udine, Italy, [17]University Hospital Freiburg, Freiburg, Germany, [18]University Hospital Gasthuisberg, Leuven, Belgium, [19]Institut Paoli Calmettes, Marseille, France, [20]HUCH Comprehensive Cancer Center, Helsinki, Finland, [21]Centre Hospitalier Lyon Sud, Lyon, France, [22]Hôpitaux Universitaires De Genève, Geneva, Switzerland

Background: A recent model using clinical-molecular features of patients with secondary myelofibrosis (sMF) was developed by MYSEC (MYelofibrosis SECondary to PV and ET project) showing better prediction of outcome compared with the International Prognostic Scoring System (IPSS); and although the dynamic IPSS (DIPSS) is validated for primary myelofibrosis but also currently used for risk stratification in sMF patients receiving transplant, evaluations of the actual performance of these scores are lacking.
Aims: We aimed to validate and compare the MYSEC and DIPSS in sMF patients undergoing allogeneic stem-cell transplantation.
Methods: We identified 159 sMF patients who received stem-cell transplant from related (n=59) or unrelated donors (n=100) between 2007 and 2015 with available data on blood levels at transplant and presence of mutations at diagnosis. The MYSEC model was calculated as follows: one point was assigned to presence of constitutional symptoms and platelets <150 x10^9/L. Two points were assigned to hemoglobin <11 g/dl, circulating blasts ≥3% and a CALR-unmutated genotype, whereas 0.15 points were assigned for each year of age. Risk groups (number of patients) according to MYSEC and DIPSS were: low (n=27 and n=16), intermediate-1 (n=70 and n=59), intermediate-2 (n=40 and n=70), and high (n=22 and n=14). Scores were validated using Kaplan-Meier estimates while C-statistics were applied to evaluate their discriminatory power.
Results: Median follow-up was 41 months (range, 29 to 52) while the median time between diagnosis and transplant was 128 months (range, 2 to 526). The median age of sMF patients was 52 years (range, 32 to 75). Overall survival at three years was 55.9% (95% confidence interval [CI], 47.5 to 64.3). By stratifying sMF according to evolution from either polycythemia vera (post-PV) or essential thrombocythemia (post-ET), no difference was found in survival at three years being 57.7% (95% CI, 45.5 to 70.0) for post-PV and 54.6% (95% CI, 43.0 to 66.2; p=0.92) for post-ET. Overall survival rates at three years according to each risk group of the DIPSS were as follows: 79.5% (95% CI, 58.5 to 100) for the low-risk, 56.3% (95% CI, 42.6 to 70.0) for the intermediate-1-risk, 53.9% (95% CI, 41.2 to 66.6) for the intermediate-2-risk, and 40.8% (95% CI, 14.1 to 67.5) for the high-risk group. Overall, DIPSS was not predictive of outcome (p=0.30). Regarding MYSEC, probabilities of survival at three years was 69.3% (95% CI, 51.5 to 87.1) for the low-risk, 64.5% (95% CI, 52.5 to 76.5) for the intermediate-1-risk, 46.8 (95% CI, 29.7 to 63.9) for the intermediate-2-risk, and 21.7% (95% CI, 0 to 45.4) for the high-risk group (Figure 1). The MYSEC model was predictive of survival overall (p=0.03). When used to assign patients to the four discrete risk categories, the test retained moderate predictability for MYSEC (C-index=0.585). Prognostic ability was improved in comparison with DIPSS (C-index=0.546) leading to a significant re-classification of patients (p <0.001). Due to a considerable difference in age distribution between our transplant cohort and the originally published MYSEC cohort, the score was adjusted by age as continuous variable which even increased the performance of the score showing C-statistics of 0.627.
Summary and Conclusions: In comparison to DIPSS, the clinical-molecular system by MYSEC provides a significant re-classification of patients leading

to improved prognostic capability in sMF after transplantation. Furthermore, transplant-specific age-adjustment of the MYSEC resulted in an even better predictive power.

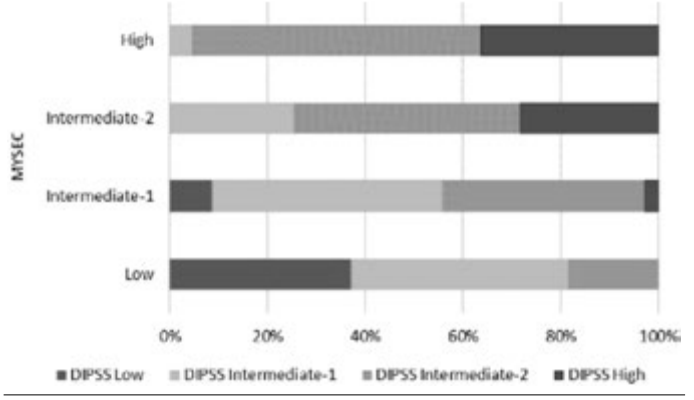

Figure 1.

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FREQUENT IMMUNOGLOBULIN REARRANGEMENT, JAK1/2 INHIBITION AND AGGRESSIVE B-CELL LYMPHOMA DEVELOPMENT IN PATIENTS WITH MYELOFIBROSIS

V. Sexl[1,*], E. Porpaczy[2], S. Tripolt[1], A. Hoelbl-Kovacic[1], B. Gisslinger[3], Z. Bago-Horvath[1,4], E. Casanova-Hevia[5,6], T. Decker[7], S. Fajmann[1], D. Fux[1], S. Gueltekin[2], G. Heller[8], H. Herkner[9], T. Kolbe[10,11], E.M. Putz[1], C. Kornauth[4], M. Mueller[12], M. Prchal-Murphy[1], A.-I. Schiefer[4], C. Schneckenleithner[1], C. Skrabs[2], W.R. Sperr[2,13], B. Strobl[12], P. Valent[2,13], R. Kralovics[14,15], L. Muellauer[4], I. Simonitsch-Klupp[4], E. Clappier[16,17], E. Raffoux[16,17], J.-J. Kiladjian[16,17], M.-T. Krauth[2], P. B. Staber[2], G. Greiner[15], G. Hoermann[13,15,18], U. Jaeger[2,13], H. Gisslinger[2]

[1]Pharmacology and Toxicology, University of Veterinary Medicine Vienna, [2]Internal Medicine, Medical University Vienna, [3]Internal Medicine, Medical University of Vienna, [4]Pathology, Medical University Vienna, [5]Ludwig Boltzmann Institute Cancer Research, [6]Physiology, Medical University Vienna, [7]Max F. Perutz Laboratories, University of Vienna, [8]Oncology, [9]Emergency Medicine, Medical University Vienna, [10]Biomodels Austria, University of Veterinary Medicine Vienna, Vienna, [11]IFA Tulln, University of Natural Resources and Life Sciences, Tulln, [12]Animal Breeding and Genetics, University of Veterinary Medicine Vienna, [13]Ludwig Boltzmann Cluster Oncology, Medical University Vienna, [14]Molecular Medicine, Cemm Research Center, [15]Laboratory Medicine, Medical University Vienna, Vienna, Austria, [16]Centre d'Investigations Cliniques, Hopital Saint-Louis, [17]Université Paris, Paris, France, [18]MLL Munich Leukemia Laboratory, Munich, Germany

Background: Inhibition of Janus-kinase 1/2 (JAK1/2) is a mainstay to treat myeloproliferative neoplasms (MPN). Besides driving MPN, the JAK-STAT pathway is involved in the development of malignant lymphoma. Recent reports point towards a slightly increased risk of lymphoid neoplasms in MPN patients with JAK2 V617F mutations. Moreover, sporadic cases of diffuse large B-cell lymphomas (DLBCL) have been reported in patients with MPN under ruxolitinib treatment. The frequency and potential causes of lymphomas under JAK2 inhibition remain unclear.
Aims: We aimed at identifying the global frequency and the potential causes of aggressive B-cell lymphomas under JAK2 inhibition as well as at early diagnosing patients at risk.
Methods: 626 MPN patients (557 with conventional, 59 with JAK1/2 inhibitor treatment) from Vienna and 929 (872 *vs* 57) from Paris were included in the study. Immunoglobulin rearrangement (IgR) and MPN associated mutations were tested by PCR. IGHV–D–J sequence was assessed by Sanger sequencing according to ERIC recommendations.11 For the detection of BCL2/IGH gene rearrangements, an Identiclone BCL2/JH Translocation Assay Gel Detection Kit was applied according to the manufacturer's instructions. Targeted next generation sequencing was provided by Foundation OneTM Heme (Roche Austria GmbH, Vienna).
Results: 16.7% of JAK 1/2 inhibitor (N=54) and 15.9% of 44 age- and sex-matched conventionally treated patients were positively tested for IgR in the bone marrow of patients with myelofibrosis (MF) (Figure 1). In the Viennese cohort, 4 out of 69 (5.8%) developed aggressive lymphomas upon JAK1/2 inhibitor treatment while only 2 lymphomas evolved in 557 patients (0.36%) without inhibitor (Odds ratio (OR) 16, 95% confidence interval (95%CI) 3 to 87; p=0.0017). These results have been validated in an independent cohort from Paris (5.51 *vs* 0.23%, OR 15, 95%CI 2 - 92, p=0.0205). The median time from start of JAK1/2 inhibitor-treatment to lymphoma diagnosis was 25 months (range 13-35 months). Subgroup analysis of 216 patients with primary myelofibrosis (31 with and 185 without JAK1/2 inhibitor therapy) revealed 3 DLBCL (9.68%) under ruxolitinib and only one (0.54%) in the controls (OR 19, 95%CI 2 - 196, p=0.01). Results remained unchanged after adjustment for age (OR 21, 95%CI 2-218) or sex (OR 25, 95%CI 2-266). 3 of 4 JAK 1/2 inhibitor associated lymphomas were IgR-positive as long as 6 years before overt lymphoma and preceded JAK1/2 inhibition. From one patient no material was available. Sequencing verified clonal identity in 2 patients. The effects of JAK1/2 inhibition were mirrored in *Stat1*$^{-/-}$ mice: 16/24 mice developed a spontaneous myeloid hyperplasia with the concomitant presence of aberrant B-cells. Transplantations of bone marrow from diseased mice unmasked the outgrowth of a malignant B-cell clone evolving into aggressive B-cell leukemia-lymphoma.

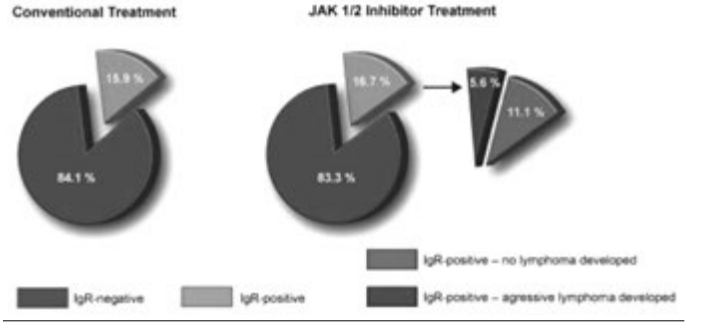


Figure 1.

Summary and Conclusions: Our results indicate that clonal B-cells are detectable in 15-17% of MF patients. Aggressive lymphomas during JAK1/2 inhibitor treatment occur with increased frequency, have uniform clinicpathological features and arise from a pre-existent B-cell clone. Early detection of IgR, at the time point of MPN diagnosis, offers the opportunity to determine patients at risk.

S131

INTERIM ANALYSIS OF THE DALIAH TRIAL - A RANDOMIZED CONTROLLED PHASE III CLINICAL TRIAL COMPARING RECOMBINANT INTERFERON ALPHA-2 *VS* HYDROXYUREA IN MPN PATIENTS

T. Alma Knudsen[1,*], D. Lund Hansen[2], L. Frans Ocias[2], O. Weis Bjerrum[3], M. Brabrand[2], D. el Fassi[45], M. Frederiksen[6], L. Kjær[1], T. Kielsgaard Kristensen[7], T. Kruse[8], H.T. Mourits-Andersen[9], P. Møller[1], U. Malthe Overgaard[3], M. Tang Severinsen[10], V. Henriette Skov[1], J. Stentoft[11], J. Starklint[12], K. de Stricker[13], M. Thomassen[8], T. Stauffer Larsen[2], H. Carl Hasselbalch[1]

[1]Department of Haematology, Zealand University Hospital, Roskilde, [2]Department of Haematology, Odense University Hospital, Odense, [3]Department of Haematology, Copenhagen University Hospital, Rigshospitalet, [4]Department of Haematology, Herlev University Hospital, [5]Institute for Inflammation Research, Copenhagen University Hospital, Rigshospitalet, Copenhagen, [6]Department of Haematology, Hospital of Southern Jutland, Haderslev, [7]Department of Pathology, [8]Department of Clinical Genetics, Odense University Hospital, Odense, [9]Department of Haematology, Hospital of South West Jutland, Esbjerg, [10]Department of Haematology, Aalborg University Hospital, Aalborg, [11]Department of Haematology, Aarhus University Hospital, Aarhus, [12]Department of Haematology, Hospital of West Jutland, Holstebro, [13]Department of Pathology, Odense University Hospital, Copenhagen, Denmark

Background: Hydroxyurea (HU) is considered first line cytoreductive therapy in most parts of the world for patients with Myeloproliferative Neoplasms (MPNs). The drug effectively lowers the risk of thrombosis but there is a concern with regard to its possible leukemogenic potential. Recombinant Interferon Alpha-2a (r-IFNα), which is used off label, is non-leukemogenic and has been demonstrated to induce high rates of both clinical, haematological and molecular responses in MPN patients.
Aims: To investigate efficacy and toxicity of low-dose r-IFNα compared to HU in patients with MPN in a randomized controlled clinical phase III trial.
Methods: Patients with newly diagnosed or previously untreated (cytoreductive agents) MPN according to WHO criteria were enrolled in the DALIAH trial (NCT01387763). Written informed consent was obtained from all patients. Patients >60 years were randomized (I:I:I) to either r-IFNα-2a or r-IFNα-2b at a starting dose of 45 or 35 µg/week, respectively, or to HU at doses of 500 to 2000 mg/day. Patients ≤60 were randomized (I:I) to either r-IFNα-2a or r-IFNα-2b. The protocol allowed addition of HU in patients randomized to r-

IFNα with major thrombosis or platelets >1500 10^9/L. A planned interim analysis was performed after 18 months of therapy. The molecular and hematological response rates were assessed by the European Leukemia Net (ELN) 2009 and the EUMNET 2005 criteria. JAK2 V617F was analysed by qPCR.

Results: A total of 205 patients were enrolled between 2012 and 2015 (Table 1). The interim analysis was performed after a median of 17.7 months (range 17.1 – 18.2). Overall response rates (ORR) were 75% (24/32) for HU and 49% (73/149) for r-IFNα among patients with essential thrombocythemia (ET), polycythemia vera (PV) and prefibrotic myelofibrosis (pre-MF). Partial hematologic response (PHR) and complete hematologic response (CHR) were observed in 6 (19%) and 18 (56%) patients treated with HU and in 20 (13%) and 53 (36%) patients treated with r-IFNα. CHR was significantly higher in patients treated with HU (Fisher's exact test, p=0.04). In primary myelofibrosis ORR was 100% (6/6) for patients on HU and 33% (6/18) for r-IFNα. For patients still on study medication at 18 months the ORR were 88% (30/34) for HU and 81% (79/98) for r-IFNα. The median treatment doses were 811 mg/day, (range 576 – 977) for HU and 53 µg/week (range 44 – 78) and 44 µg/week (range 33 – 46), for r-IFNα-2a or r-IFNα-2b, respectively. Ninety-six patients were available for molecular response analysis at 18 months. A complete molecular response was obtained in one patient treated with r-IFNα. Partial molecular response (PMR) was observed in 23% (7/31) and 16% (19/120) patients treated with HU and r-IFNα, respectively. The observed difference in PMR was not significant (Fisher's exact test, p=0.42). Discontinuation of study medication for any reason after 18 months was 4 (11%) for HU and 69 (41%) for r-IFNα. Toxicity dependent drop-out was 5% for HU and 27% for r-IFNα. Grade 3-4 adverse events occurred in 7 (18%) HU patients and in 58 (35%) r-IFNα patients.

Table 1. Baseline characteristics.

	HU n = 38	r-IFNα n = 167
Age, median years	68 (64-71)	58 (46-67)
Sex		
Men	24 (63%)	89 (53%)
Women	14 (36%)	78 (47%)
MPN subtype		
ET	9 (24%)	63 (48%)
PV	22 (58%)	68 (41%)
Pre-MF	1 (3%)	16 (10%)
PMF	6 (16%)	18 (11%)
History of major thrombotic event	5 (13%)	34 (20%)
Patients positive for JAK2 V617F	31 (82%)	120 (72%)
Median JAK2 V617F allele burden	34 (17 - 47)	34 (19 - 51)
Abnormal karyotype	2/18 (11%)	4/95 (4%)
Biochemistry		
Haemoglobin (mmol/L)	9.6 (8.1 - 12.2)	9.3 (8.4 - 10.7)
EVF (vol%)	46 (42-59)	45 (42-52)
WBC (× 10^9/L)	10.1 (8.4 - 12.2)	9.5 (7.8 - 11.9)
Platelets (× 10^9/L)	636 (540 - 895)	670 (468 - 903)
LDH (U/L)	249 (219 - 323)	238 (189 - 314)
Median spleen size below costal margin (cm)	0.0 (0.0 - 0.0)	0 (0.0 - 0.0)
Spleen size (mm)	130 (110-140)	125 (106-145)
Disease-related symptoms		
Microcirculatory disturbances	9/37 (24%)	44/166 (27%)
Hypermetabolic symptoms	10 (26%)	49 (29%)
Phlebotomy	0 (0-2)	0 (0-2)
Low risk	0	74 (45%)

Data are median (IQR) and n (%)

Summary and Conclusions: This planned analysis of efficacy and toxicity at 18 months shows a significant difference in the CHR rate (p=0.04) for patients treated with HU compared to low-dose r-IFNα. However, ORR was almost similar for patients still on study medication. Toxicity dependent discontinuation from r-IFNα was higher than expected (27%) even in this low-dose setting. Due to the study design patients treated with r-IFNα were younger than those treated with HU.

S132

COMPARISON OF LONG-TERM EFFICACY AND SAFETY OF ROPEGINTERFERON ALFA-2B *VS* HU IN POLYCYTHEMIA VERA PATIENTS AGED BELOW OR ABOVE 60 YEARS: TWO-YEAR ANALYSIS FROM THE PROUD/CONTINUATION PHASE III TRIALS

H. Gisslinger[1,*], C. Klade[2], P. Georgiev[3], D. Krochmalczyk[4], L. Gercheva-Kyuchukova[5], M. Egyed[6], V. Rossiev[7], P. Dulicek[8], A. Illes[9], H. Pylypenko[10], L. Sivcheva[11], J. Mayer[12], V. Yablokova[13], B. Grohmann-Izay[2], G. Maurer[2], H. Hasselbalch[14], R. Kralovics[15], J.-J. Kiladjian[16]

[1]Department of Internal Medicine I, Clinical Division of Hematology and Hemostaseology, Medical University Vienna, [2]AOP Orphan Pharmaceuticals AG, Vienna, Austria, [3]University Multiprofile Hospital for Active Treatment "Sveti Georgi", Clinic of Hematology, Plovdiv, Bulgaria, [4]Teaching Unit of the Hematology Department, University Hospital in Krakow, Krakow, Poland, [5]Multiprofile Hospital for Active Treatment "Sveta Marina", Varna, Bulgaria, [6]Department of Internal Medicine II, Kaposi Mor Teaching Hospital, Kaposvar, Hungary, [7]V.D. Seredavin Samara Regional Clinical Hospital, Samara, Russian Federation, [8]Department of Clinical Hematology, University Hospital Hradec Kralove, Hradec Kralove, Czech Republic, [9]University of Debrecen, Medical and Health Science Center, Debrecen, Hungary, [10] Department of Hematology, Cherkasy Regional Oncology Center, Regional Treatment and Diagnostics Hematology Center, Cherkasy, Ukraine, [11]First Department of Internal Medicine, Multiprofile Hospital for Active Treatment- Hristo Botev, Vratsa, Bulgaria, [12]University Hospital Brno, Clinic of Internal Medicine - Hematology and Oncology, Brno, Czech Republic, [13]Department of Hematology, Yaroslavl Regional Clinical Hospital, Yaroslavl, Russian Federation, [14]Department of Hematology, Roskilde Hospital, University of Copenhagen, Copenhagen, Denmark, [15]CeMM, Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria, [16]Saint-Louis Hospital, Paris, France

Background: Ropeginterferon alfa-2b (Ropeg) is a novel mono-pegylated IFNα, allowing convenient self-administration every 2 to 4 weeks. It is currently being developed for treatment of MPNs in particular PV. Hydroxyurea (HU) is the only licensed first-line therapy in high-risk patients with PV of all ages. Off-label IFNα as first-line therapy is primarily used in patients of younger age, partly because of the misconception that the risk-benefit ratio is not so favorable in elderly patients.

Aims: To analyse the difference in efficacy and safety of Ropeg and HU in two age cohorts (<60 years and ≥60 years).

Methods: 254 PV patients (WHO2008 criteria) had been randomized to receive Ropeg or HU in the PROUD Study. After 12 months of treatment, 89.6% of Ropeg treated patients and 68.5% of HU treated patients continued treatment in the CONTINUATION Study. Efficacy assessment consisted of complete hematological response (CHR) rate according ELN criteria, and the rate of CHR including symptom improvement (disease-related signs including clinically significant splenomegaly and PV-related symptoms). Secondary endpoints included *JAK2*V617F allelic burden assessed as rate of molecular response (modified ELN criteria). Efficacy and safety analysis was done for patients <60 years (Ropeg: n=49; HU: n=39) and ≥60 years (Ropeg: n=46; HU: n=37).

Results: After 24 months of treatment, Ropeg induced higher CHR rates compared to HU, irrespective of age: 77.6% *vs* 55.9% (<60 years); 63.0% *vs* 42.4% (≥60 years). Higher response rates were also shown for Ropeg *vs* HU for CHR including symptom improvement, similar for both age cohorts: 55.1% *vs* 37.1% (<60 years); 43.5% *vs* 36.1% (≥60 years). CHR rate maintenance (response maintained from first occurrence to 24 months assessment) was also higher for Ropeg and age-independent for both study treatments (Ropeg <60 years: 49.0%, ≥60 years: 37.0%; HU <60 years: 17.9%, ≥60 years: 18.9%). A similar observation for response maintenance was shown for CHR rate including symptom improvement (Ropeg <60 years: 32.7%, ≥60 years: 28.3%; HU <60 years: 15.4%, ≥60 years: 18.9%). After 24 months of treatment, partial molecular response rates were higher for Ropeg compared to HU, irrespective of age: 78.1% *vs* 33.3% (<60 years) and 59.5% *vs* 25.0% (≥60 years). Regarding safety, Ropeg and HU treated patients showed comparable numbers of both, adverse events (89.8% *vs* 92.3% <60 years, 93.5% *vs* 91.9% ≥60 years) and serious adverse events (6.1% *vs* 10.3% <60 years, 21.7% *vs* 24.3% ≥60 years) irrespective of age. The number of adverse drug reactions (ADRs) was comparable below 60 years (77.6% *vs* 74.4%) but interestingly in the cohort ≥60 years, a trend towards a lower number of ADRs was evident for Ropeg (63.0%) *vs* HU (89.2%). No serious ADRs were reported for Ropeg, but there were 4 serious ADRs (*Acute Leukemia*, *Anemia*, *Leukopenia*, *Granulocytopenia*) reported for HU (all patients aged ≥60 years).

Summary and Conclusions: A high CHR, symptom improvement and molecular response (*JAK2*V617F) achieved by long-term treatment with Ropeg was shown, with an advantage over HU independent of age. The safety analysis in patients ≥60 years also showed a positive trend regarding less ADRs and less serious ADRs for Ropeg *vs* HU. These data indicate that Ropeg provides a valuable, efficacious and safe new treatment option for PV patients of all ages including elderly.

S133

RESURRECTING RESPONSE TO RUXOLITINIB: A PHASE I STUDY TESTING THE COMBINATION OF RUXOLITINIB AND THE PI3K DELTA INHIBITOR UMBRALISIB IN RUXOLITINIB-EXPERIENCED MYELOFIBROSIS

T. Moyo[12,*], J. Palmer[3], Y. Huang[24], O. Oluwole[12], S. Mohan[12], R. Caza[2], G. Ayers[24], L. Berry[24], C. Dudley[2], L. Dugger[2], H. Miskin[5], A. Cavers[5], P. Sportelli[5], B. McMahon[6], S. Strickland[12], R. Mesa[7], L. Michaelis[8], M. Savona[12]

[1]Department of Medicine, [2]Vanderbilt-Ingram Cancer Center, Vanderbilt University

Medical Center, Nashville, TN, [3]Mayo Clinic, Scottsdale, AZ, [4]Center for Quantitative Sciences, Vanderbilt University Medical Center, Nashville, TN, [5]TG Therapeutics, New York, NY, [6]University of Colorado, Denver, CO, [7]University of Texas Health Science Center, San Antonio, TX, [8]Medical College of Wisconsin, Madison, WI, United States

Background: JAK inhibition with ruxolitinib (rux) reduces spleen size and myelofibrosis (MF)-related symptoms and improves survival, but nearly half of responders relapse within 5 years. Treatment options for MF after failure of rux are scant. Inhibition of PI3Kδ and AKT signaling reduces proliferation and clonogenic potential of JAK2-mutated cell lines, and synergizes with rux in patient samples. Umbralisib, a novel next-generation PI3K inhibitor highly selective for the delta isoform was well-tolerated in clinical trials in lymphoid malignancies. We tested the combination of umbralisib and rux in previously rux-treated MF.
Aims: 1. To assess safety of the combination of umbralisib + ruxolitinib 2. To evaluate preliminary efficacy of the combination in MF
Methods: This study has three stages. In Escalation Stage (ES) 1, 12 MF subjects, on a stable ruxolitinib dose for ≥8 weeks with a suboptimal or lost response at the highest tolerated dose were treated with once daily doses of umbralisib in a 3+3 design. In ES2, 4 additional MF patients were treated with the umbralisib dose from ES1 and escalating rux doses to complete the safety analysis. In the Expansion Stage, 7 additional MF patients continued their stable dose of rux, and umbralisib was added at the ES1 recommended dose. Adverse events (AEs) were graded by NCI-CTCAE v4.03. Efficacy was assessed by IWG-MRT consensus response criteria. Symptoms were assessed by the MPN symptom assessment form total symptom score (MPN-SAF TSS).
Results: All 23 subjects enrolled as of the data cutoff on December 31, 2017 were included in safety analyses. Median number of cycles was 5 (range 1-29). Median age at study entry was 67 years; 61% were male; 91% had ECOG PS<2. Dose-limiting toxicities (DLTs) of asymptomatic pancreatic enzyme elevation were experienced by one subject each treated with 10mg or 15mg rux + 800mg umbralisib in ES1. No DLTs were seen in subjects at lower umbralisib doses. Thus, all subsequently enrolled subjects were treated with 600mg umbralisib. The most common Gr3/4 AE was anemia reported by 3 patients (Table 1). Two patients each experienced Gr 3 diarrhea, neutropenia, and elevations in serum amylase and lipase. There was one case of colitis in a patient with chronic intermittent diarrhea. Three subjects were removed from study due to AEs, 1 to pursue HSCT; 10 remained on study. Of 23 response-evaluable rux-experienced MF subjects, 2 achieved CR after 5 and 15 cycles. An additional 11 met IWG-MRT criteria for clinical improvement based on anemia, spleen and/or symptoms responses. Mean improvement in hemoglobin was 1.2g/dL (range 0-3.7g/dL). Mean reduction in TSS was 33% (Figure 1).

Table 1. Most common (>5%) all-cause AEs and AEs of special interest.

	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)
Anemia	1 (4%)	6 (11%)	3 (13%)	-
Neutrophil decreased	-	-	2 (9%)	-
Platelet count decreased	3 (13%)	2 (9%)	-	-
AST increased	6 (26%)	-	-	-
ALT increased	3 (13%)	-	-	-
Amylase increased	1 (4%)	-	2 (9%)	-
Lipase increased	1 (4%)	-	2 (9%)	-
Diarrhea	-	-	2 (9%)	-
Colitis	-	-	1 (4%)	-
Dyspnea	-	-	1 (4%)	-
Upper respiratory infection	-	2 (9%)	-	-
Pneumonia	1 (4%)	2 (9%)	1 (4%)	-
Sepsis	-	-	-	1 (4%)

Summary and Conclusions: Enhancing suppression of pathologic JAK-STAT signaling with PI3Kδ inhibition represents a viable treatment strategy for MF patients with inadequate or lost response to ruxolitinib. The addition of umbralisib to rux was well-tolerated. Pancreatic enzyme elevation was not seen with doses of umbralisib <800mg. Significant LFT abnormalities were absent and colitis uncommon, distinguishing umbralisib from other PI3Kδ inhibitors. Hematologic AEs were largely unrelated to drug and occurred in the context of extensive marrow fibrosis and disease progression. An IWG-MRT response (CR, PR or CI) was observed in 56.5% of rux-experienced MF subjects. Two patients who had lost response after >1y of ruxolitinib monotherapy achieved CR on study, demonstrating that umbralisib can augment and resurrect a response in subjects with suboptimal or lost response to ruxolitinib. The addition of umbralisib to rux was well-tolerated. Pancreatic enzyme elevation was not seen with doses of umbralisib <800mg. Significant LFT abnormalities were absent and colitis uncommon, distinguishing umbralisib from other PI3Kδ inhibitors. Hematologic AEs were largely unrelated to drug and occurred in the context of extensive marrow fibrosis and disease progression. An IWG-MRT response (CR, PR or CI) was observed in 56.5% of rux-experienced MF subjects. Two patients who had lost response after >1y of ruxolitinib monotherapy achieved CR on study, demonstrating that umbralisib can augment and resurrect a response in subjects with suboptimal or lost response to ruxolitinib alone.

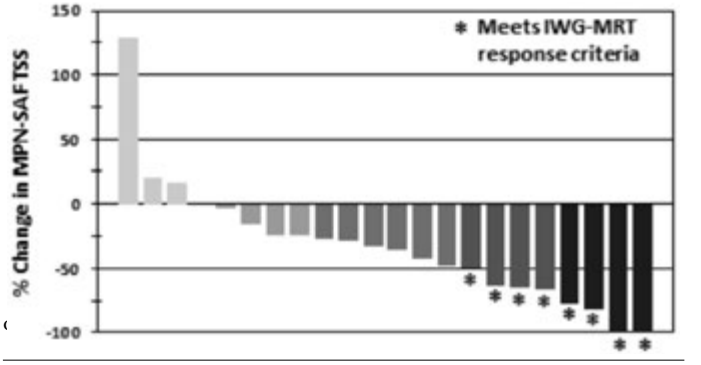


Figure 1. Symptom Assessment.

Gene therapy, cellular immunotherapy and vaccination – Biology & Translational Research

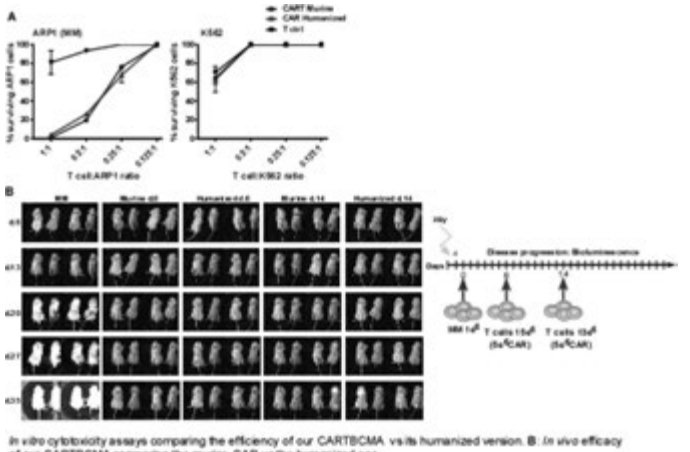
S134

PRE-CLINICAL RESULTS OF A HUMANIZED CART-BCMA FOR MULTIPLE MYELOMA PATIENTS

L. Pérez-Amill[1,*], G. Suñe [1], M. Castella Castella[1], A. Najjar[2], M. Juan[3], C. Fernández-de Larrea[1], A. Urbano-Ispizua[14], B. Martín-Antonio[14]

[1]Hematology, IDIBAPS, Hospital Clínic, Barcelona, Spain, [2]Stem Cell Transplantation & Cellular Therapy, University of Texas, MD Anderson Cancer Center, Houston, United States, [3]Immunology, IDIBAPS, Hospital Clínic, [4]Josep Carreras Leukaemia Research Institute, Barcelona, Spain

Background: B Cell Maturation Antigen (BCMA) has appeared as a promising antigen to be used in CAR immunotherapy against Multiple Myeloma (MM) patients due to specific BCMA expression in plasma cells and its absence in most tissues. Recent clinical results infusing CART BCMA cells in patients with relapsed/refractory MM with a median of 7 prior lines of therapy (*i.e.*: NCT02658929) have shown 94% of objective responses, including 56% of complete remissions at 10 months. Deepening of response over time was detected. One of the main problems limiting the success of CART immunotherapy is the early disappearance of CART cells, occurring in approximately 30% of patients receiving CART19, which might be attenuated by the use of a humanized CART. Moreover, the high inflammatory response, termed cytokine release syndrome (CRS), could be detrimental to the patient and needs to be carefully monitored.
Aims: We decided to create a humanized CART-BCMA for the treatment of MM patients, and investigate possible ways of decreasing inflammation associated to CART expansion without impacting in the anti-MM CART activity.
Methods: We designed a 2nd generation murine CAR against BCMA with 4-1BB as co-stimulatory domain, and after confirming its functionality, we humanized the scFv of BCMA. *In vitro* studies were performed with MM cell lines (RPMI-8226, ARP1, U266) and K562 as a negative control cell line. *In vivo* studies were performed in NSG mice receiving $1{\cdot}10^6$ of MM (ARP1) cells i.v. injected at day 1. Then, mice received $5{\cdot}10^6$ i.v. injected of either CAR-T or Ctrl non-transduced T cells, both murine and humanized. CART cells were infused at day 6 and day 14 of MM injection to have an early and advanced MM model.
Results: We successfully humanized the scFv of BCMA maintaining the cytotoxicity activity obtained by the murine CAR-BCMA. Moreover, both CARs were specific against MM (Figure 1A), as K562 cells were not eliminated by CART cells. *In vivo* results showed that both murine and humanized CART-BCMA injected at day 6 were equally efficient in abrogating MM growth at an early-stage of the MM disease. When CART cells were infused at day 14, both murine and humanized CART-BCMA significantly reduced the advanced-stage of the MM disease but could not avoid the total progression of the disease (Figure 1B). Moreover, a-MSH, a molecule with ant-inflammatory properties which does not decrease CD8 cytotoxic activity was evaluated as anti-inflammatory agent and its potential effect in CART activity. *In vitro* functional assays showed that a-MSH partially increased the cytotoxic activity of the CART-BCMA and decreased the IL6 and TNFa *in vitro* production.



In vitro cytotoxicity assays comparing the efficiency of our CARTBCMA vs its humanized version. B: *In vivo* efficacy of our CARTBCMA comparing the murine CAR vs the humanized one.

Figure 1.

Summary and Conclusions: We generated a humanized CART-BCMA that is as efficient as the murine one, but might favor a longer persistence of CART cells in patients. In the next months, this CAR will be translated into the clinic for MM patients. Furthermore, a-MSH potentially could be used as an adjuvant to ameliorate CRS without impacting negatively in the CART activity.

S135

CLINICAL-GRADE PRODUCTION, PRECLINICAL EFFICACY AND SAFETY OF ALLOGENEIC CD19CAR CYTOKINE-INDUCED KILLER CELLS TRANSFECTED WITH SLEEPING BEAUTY TRANSPOSON FOR ACUTE LYMPHOBLASTIC LEUKEMIA TREATMENT

C.F. Magnani[1,*], C. Mezzanotte[1], C. Cappuzzello[1], F. Benedicenti[2], D. Belotti[3], B. Cabiati[3], M. Bardini[1], G. Fazio[1], G. Cazzaniga[1], L. Cooper[4], E. Montini[2], G. Dastoli[1], G. Gaipa[1], E. Biagi[1], A. Biondi[1]

[1]Tettamanti Research Center, Department of Pediatrics, University of Milano-Bicocca, San Gerardo Hospital/Fondazione MBBM, Monza, [2]San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Milan, [3]Laboratorio di Terapia Cellulare e Genica Stefano Verri, ASST-Monza, San Gerardo Hospital, Monza, Italy, [4]University of Texas, MD Anderson Cancer Center, Houston, United States

Background: Infusion of patient-derived CD19-specific chimeric antigen receptor (CAR) T cells engineered by viral vectors achieved complete remission and durable response in relapsed and refractory (r/r) B-lineage neoplasms. Here, we expanded on those finding by providing a preclinical evaluation of allogeneic non-viral cytokine-induced killer (CIK) cells transfected with Sleeping Beauty (SB) CD19CAR (CARCIK-CD19).
Aims: Since both non-clinical pharmacology studies in patient-derived xenograft (PDX) and toxicology of SB-transfected T cells are lacking, here we have performed a pre-clinical evaluation of donor-derived CARCIK-CD19, including validation of the GMP-compliant protocol, efficacy and biodistribution studies.
Methods: PBMC non-viral modification and CIK cell differentiation were performed according to the method enclosed in the filed patent EP20140192371 with electro-transfer of the SB GMP-grade DNA plasmids using 4D-Nucleofector (Lonza). The manufacturing process was performed in an academic Cell Factory, authorized by Agenzia Italiana del Farmaco (AIFA). Integration site (IS) analysis was performed by Sonication Linker Mediated (SLiM)-PCR and Illumina MiSeq sequencing. Human leukemia xenografts were generated in NOD.Cg-Prkdcscid Il2rgtm1Wj1/SzJ (NSG) mice. The good laboratory practice (GLP) toxicity study was conducted at GLP SR-TIGET (Milan) in absence of tumor.
Results: In a pre-GMP large-scale 18-day manufacturing process, we achieved stable CD19CAR expression (62.425%±6.399, n=8), efficient T-cell expansion (23.36±3.00-fold) and features of *in vitro* T-cell potency. Three lots was GMP manufactured by seeding 46.0x10^6, 56.8x10^6 and 140.4x10^6 PBMCs respectively. After 21-22 days of culture, we harvested 15.998x10^9, 1.436x10^9, and 3.897x10^9 total nucleated cells, with a mean viability of 96.96%. The median expression of CD3+CD19CAR+ cells was 46.90%, median vector copy number (VCN) was 2.0 VCN/cells and average killing was 75.29 (E:T ratio 5:1). Cell products appear to be highly polyclonal and no signs of genotoxicity by transposon insertions could be observed by IS analysis. Frozen/thawed CARCIK-CD19 remained fully functional both *in vitro* and in a established PDX of MLL–ENL rearranged acute lymphoblastic leukemia (ALL). CARCIK-CD19 showed a dose-dependent antitumor response and prolonged persistence in a PDX, bearing the feature of a Philadelphia-like ALL with PAX5/AUTS2 translocation, and in a survival model of lymphoma, achieving complete eradication of disseminated tumors. CARCIK-CD19 induced long-term tumor regression along with a statistically significant survival prolongation compared to untreated mice (n=11, p=0.0495, Log-rank Test). Interestingly, CARCIK-CD19 cells persisted in PB and organs up to 3 months. Finally, the infusion of CARCIK-CD19 proved to be safe and well tolerated in a bio-distribution and toxicity model. In the engrafted animals, transfected cells persisted, mainly with a constant VCN value, in the hematopoietic and post-injection perfused organs untill the end of the study (60 days) and consisted of CD8+, CD56+ and CAR+ T cells.
Summary and Conclusions: Overall, our findings provide important implications for non-viral technology and the proof-of-concept that donor-derived CARCIK-CD19 are indeed effective against relapsed ALL. A clinical trial investigating allogeneic CARCIK-CD19 in r/r pediatric and adult ALL post HSCT is currently ongoing (NCT03389035).

S136

P53 ISOFORM D133P53A: A NEW TRANSCRIPTIONAL ENHANCER OF T-CELL EFFECTOR FUNCTION

K. Legscha[1,*], E. Antunes[1], E. Amann[12], B. Guezguez[12], M. Theobald[123], H. Echchannaoui[12]

[1]Hematology, Oncology, and Pneumology, University Medical Center, Mainz, [2]German Consortium for Translational Cancer Research (DKTK), Frankfurt/Mainz, [3]Research Center for Immunotherapy, University Medical Center, Mainz, Germany

Background: Adoptive transfer of genetically modified T lymphocytes with tumor antigen-specific receptor has proven efficacy in cancer immunotherapy. However, in many patients the overall benefit is still limited due to various tumor escape mechanisms. Cell damage and metabolic/hypoxic stress in the tumor microenvironment (TME) can lead to a dysfunctional anti-tumor T cell response called T cell senescence. The tumor suppressor TP53 is a master molecule in the regulation of cell cycle and senescence. Few studies have demonstrated the critical role of p53 isoforms in the regulation of cellular senescence mainly in tumor cells. However, their role in tumor infiltrating lymphocytes (TILs) remains largely unexplored.
Aims: Strategies to prevent T cell senescence in the TME could improve T cell function leading to a more effective anti-tumor response. To better understand the role of Δ133p53α isoform in regulating the cell cycle and senescence we studied the cellular and metabolic phenotype as well as the effector function of the Δ133p53α-modified tumor-antigen (TA) specific human T cells.
Methods: T cells form healthy donors were retrovirally co-transduced with a TA-specific T cell receptor (TCR) together with the Δ133p53α isoform or an empty control vector. Modified T cells were characterized for the expression of key activating/inhibitory molecules, homing markers and their proliferation capacity by flow cytometry. Additionally, we determined the metabolic phenotype of the cells with an Agilent Seahorse XFp Analyzer. The effector functions *i.e.* cytokine secretion and antigen-specific killing capacity were assessed by Luminex immunoassay and long-term tumor colony-forming assay, respectively.
Results: Our analyses of human T cells simultaneously engineered with Δ133p53α-isoform and a TA-specific TCR revealed reduced cell surface expression of T-cell inhibitory molecules (*i.e.* PD-1 or TIGIT), senescence markers (CD57, CD160) and increased expression of the homing receptor CD62L upon TA stimulation. Interestingly, first comparative analyses between Δ133p53α-modified and control T-cells revealed changes in the cell's metabolic program similar to quiescent/naïve T cells. Δ133p53α-T cells exhibited lower ATP production, oxygen consumption as well as lower glucose utilization. Upon antigen-specific stimulation, however, they increased their metabolic activity up to the levels of control cells or even slightly above. Importantly, while control T cells exhibited cellular senescence after several rounds in culture, Δ133p53α-expressing T cells remained highly proliferative, showed superior cytokine secretion and enhanced tumor-specific killing capacity.
Summary and Conclusions: Genetic modulation of p53 isoforms in human T lymphocytes represents a novel approach to circumvent tumor-mediated T-cell replicative senescence and to preserve long-term effector function of tumor-reactive T cells. We believe that this new approach could improve current adoptive T cell-based therapies.

S137

LEUKEMIA CELL-DERIVED MICROVESICLES INDUCE T CELL EXHAUSTION VIA MIRNA DELIVERY

J. Cui[1,*], Q. Li[2], L. Jiang[1], Y. You[1], Z. Zhong[1], X. Zhu[3], P. Zou[1]

[1]Institute of Hematology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, [2]Department of Hematology,, Wuhan No.1 Hospital, Wuhan, [3]Department of Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Background: Acute leukemia is a heterogeneous malignancy characterized by the clonal expansion of hematopoietic blasts in the peripheral blood, bone marrow, and/or other tissues. A majority of acute leukemia patients have poor prognosis with traditional therapies. T cell therapy, especially with chimeric antigen receptor T (CAR-T) cells, is promising for the treatment of leukemia and other cancers. However, in the context of persistent antigen exposure in chronic viral infections and cancer, both native and adoptive T cells can become exhausted/dysfunctional. Cancer cells can generate large membrane-enclosed structures, known as microvesicles (MVs). Our previous work has demonstrated that BCR-ABL1$^+$ MVs can induce malignant transformation of mononuclear cells from bone marrow.
Aims: The aim of this study was to investigate whether MVs can play a role in leukemia associated T cell exhaustion.
Methods: T cells isolated from healthy donor peripheral were incubated with leukemia-derived MVs. The immune checkpoints inhibitors and function of T cells were examined to explicit the effect of MVs on T cell *in vitro*. In addition, leukemia-derived MVs were injected into BALB/c mice to detect the effect of MVs on T cells *in vivo*. The transcriptomes RNA-seq of T cells on day 0, 3 and 7 after incubation with MVs was conducted to excavate the mechanism of T cell exhaustion. Furthermore, bone marrow of leukemia patients were collection to isolated CD3$^+$ T cells through microbeads, then the isolated T cells were cultured *in vitro* to examined the change of immune checkpoint inhibitors on day 0 and 7.
Results: Following incubation with MVs from various sources, all T cell subtypes exhibited the exhaustion phonotype and impaired cytokine secretion *in vitro*. Mice models also showed the connection between immune checkpoint inhibitors and MV injection. Sequencing and bioinformatics analyses indicated that a number of transcription factors and microRNAs (miRNAs) were attributable to the dysregulation of pathways and exhaustion in T cells. Further work revealed that functional miR-92a-3p, miR-21-5p, miR-16-5p, miR-126 and miR-182-5p in MVs could be delivered into T cells to induce the exhaustion phenotype. SerpinB2, IL-1β and CXCL5, which are mediators of the NF- B pathway, were identified as the targets of the miRNAs mentioned above.Interestingly, the immune checkpoint inhibitors of T cells isolated from leukemia patients reduced after culture *in vitro*, which show that the exhaustion of T cells might be reversed once T cells were separated from tumor environment (Figure 1).

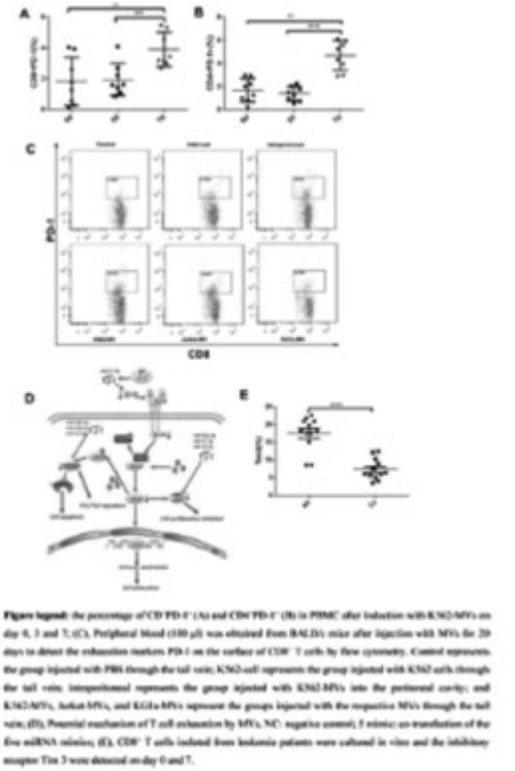


Figure 1.

Summary and Conclusions: We demonstrated that leukemia-derived MVs could initiate T cell exhaustion via the progressive temporal delivery of multiple exogenous miRNAs into T cells and the subsequent interaction of these miRNAs with their targets. Therefore, MVs can be expected not only to become new indicators of the T cell status in patients but also to be used as novel targets for personalized patient treatment.

S138

BB2121 ANTI-BCMA CAR T CELL THERAPY IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA: UPDATED RESULTS FROM A MULTICENTER PHASE I STUDY

This abstract is embargoed until Friday, June 15, 08:30 local time.

N. Raje[1,*], J. Berdeja[2], Y. Lin[3], N. Munshi[4], D. Siegel[5], M. Liedtke[6], S. Jagannath[7], D. Madduri[7], J. Rosenblatt[8], M. Maus[1], A. Turka[9], L.P. Lam[9], R.A. Morgan[9], M.T. Quigley[9], M. Massaro[9], K. Hege[10], F. Petrocca[9], J.M. Kochenderfer[11]

[1]Massachusetts General Hospital Cancer Center, Boston, [2]Sarah Cannon Research Institute and Tennessee Oncology, Nashville, [3]Mayo Clinic, Rochester, [4]Dana-Farber Cancer Institute, Boston, [5]Hackensack University Medical Center, Hackensack, [6]Stanford University Medical Center, Palo Alto, [7]Mount Sinai Medical Center, New York, [8]Beth Israel Deaconess Medical Center, Boston, [9]Bluebird Bio Inc., Cambridge, [10]Celgene Corporation, San Francisco, [11]Experimental Transplantation and Immunology Branch, National Cancer Institute/Institutes of Health, Bethesda, United States

Background: bb2121 is a second-generation chimeric antigen receptor (CAR) T cell therapy targeting B-cell maturation antigen (BCMA) to redirect T cells to recognize and kill malignant myeloma cells. Initial data from the dose-escalation phase of CRB-401, a first-in-human study of bb2121 in relapsed/refractory multiple myeloma (RRMM), have shown promising efficacy and safety.
Aims: Here, we report updated safety and efficacy results on 43 subjects enrolled in this ongoing study.
Methods: CRB-401 (NCT02658929) is a two-part, phase I study of bb2121 in patients with RRMM. Patients in the dose-escalation had received ≥3 prior lines of therapy including a proteasome inhibitor and an immunomodulatory agent, or were double refractory, and had ≥50% BCMA expression on plasma cells. In the dose-expansion phase, patients had to have received daratumumab and been refractory to their last line of therapy; no BCMA expression was required. Following lymphodepletion with fludarabine (30 mg/m^2)/cyclophosphamide (300 mg/m^2) given daily for 3 days, patients received 1 infusion of bb2121.
Results: As of 02 Oct 2017, 21 patients had received bb2121 in the 4 dose-escalation cohorts (median follow-up, 35 weeks); no dose-limiting toxicities and no grade ≥3 neurotoxicities were observed. Cytokine release syndrome (CRS), primarily grade 1-2, was reported in 15 of 21 (71%) patients; 2 patients had grade ≥3 CRS that resolved in 24 hours. There were 2 deaths on study; both patients had achieved complete response (CR) and had not progressed. Overall response rate in the 18 evaluable patients in dose-escalation cohorts ≥150 × 10^6 CAR T cells was 94%; 10 of 18 (56%) patients had CR or unconfirmed CR, and 9 of 10 evaluable patients were minimal residual disease (MRD)-negative. With a median follow-up of 40 weeks in the ≥150 × 10^{6}dose-escalation cohorts, median response duration and progression-free survival (PFS) had not been reached; PFS rates at 6 and 9 months were 81% and 71%, respectively. Doses of 150 to 300 × 10^6 CAR T cells were selected for the expansion phase. Results from an additional 5 months of follow-up and initial data from ~20 patients from the expansion cohort will be presented.
Summary and Conclusions: bb2121 shows promising efficacy at dose levels ≥150 × 10^6 CAR T cells with deep and durable ongoing responses and manageable CRS and neurotoxicities. These data support the potential of bb2121 anti-BCMA CAR T cell therapy as a new treatment paradigm for RRMM.

Platelet and bleeding disorders I

S139

COMPARISON OF STANDARD- AND LOW-DOSE RITUXIMAB IN PRIMARY IMMUNE THROMBOCYTOPENIA (ITP): DATA FROM THE UK ITP REGISTRY

C. Gracie[1,*], A. Zaidi[2], U. Doobaree[1], L. Taylor[2], D. Provan[2], A. Newland[2], V. McDonald[2]

[1]The ITP Registry, Queen Mary University, [2]Clinical Haematology, Bartshealth NHS Trust, London, United Kingdom

Background: Rituximab is reported to give response rates, partial or complete, (platelets >30 x10^9/L and >2 baseline) of up to 60% in patients with ITP. The efficacy of lower doses of rituximab (<375mg/m2) have been published successfully but there have been no large-scale comparative randomised trials.
Aims: To compare the efficacy of low and high dose rituximab in patients with primary ITP.
Methods: We performed a retrospective review of the efficacy of two main dosing regimens in ITP patients (100mg weekly x 4 weeks (low dose) *vs* 375mg/m2 weekly x 4 weeks (standard dose)) using data from the UK Adult ITP registry, a large national registry of primary ITP. Choice of dosing was based on physician preference and funding.
Results: The UK Adult ITP registry database was reviewed for all patients who received rituximab. There were 301 patients who had sufficient data input for analysis. Of these 301 patients, 179 had received low dose rituximab dose and 122 had received standard dose. There was no significant difference in the demographics between the two groups: (female 56% and 46% respectively; median age of 56.6years and 56.4 years respectively). 10% patients in the low dose group and 17% in the high dose group underwent splenectomy prior to receiving rituximab and the average number of courses of treatments given before rituximab was 6 in both groups (including corticosteroids and intravenous immunoglobulin). The median time from diagnosis to rituximab therapy in the low dose group was 13.7 months and in the standard dose group was 14.4 months. There was no difference in the median patient platelet count before treatment between the groups (28.1 and 26.4 x10^9/L respectively for low dose and standard doss regimes). The median platelet count was not significantly different at 2, 4 and 6 months after rituximab therapy between the low and standard doses (46, 72, 74 x10^9/L and 43, 65, 81 x10^9/L respectively). There was, however, some ongoing increase in platelet count between 2 and 4 months for both dosing schedules showing ongoing delayed response. At 2 months after therapy, complete remission (defined as platelets >100 x 10^9/L) was achieved in 21.1% and 32% patients in the low dose and standard dose regimes respectively. This increased to 37.7% and 43.1% at 6 months. Partial remission at 2 months was achieved in 36 and 27.1% patients for 100mg and 375mg/m2 doses. By 6 months this was 34% and 27% respectively. Bleeding episodes (all bleeds) before and after therapy were reported in 63.7% and 44.7% of patients in the 100mg dose group and 63.9% and 39.3% patients in the 375mg/m2 group. The median number of bleeding events per patient prior to treatment was 2 in both groups (range 0-4). After rituximab the median was 1 in both groups (range 0-2). For those that did bleed, the median time was 4.0 months for 100mg (range 1.6 – 18.7) and 2.8 months for 375mg/m2 (range 1.1 – 12.3). There was no statistically significant difference between these two. For those that required another line of treatment, the median time was 4 months for 100mg and 3 months for the 375mg/m2 dose.
Summary and Conclusions: In conclusion, in this large cohort, 100mg rituximab appears to be as effective as 375mg/m2 in improving platelet count and achieving PR. These data show that both doses are equally as effective in reducing bleeds with no difference in the median time to next treatment. This would suggest, that for patients with primary ITP, 100mg rituximab weekly x 4 weeks may be a more cost-effective option than the standard dosing regimen (375mg/m^2 weekly x 4).

S140

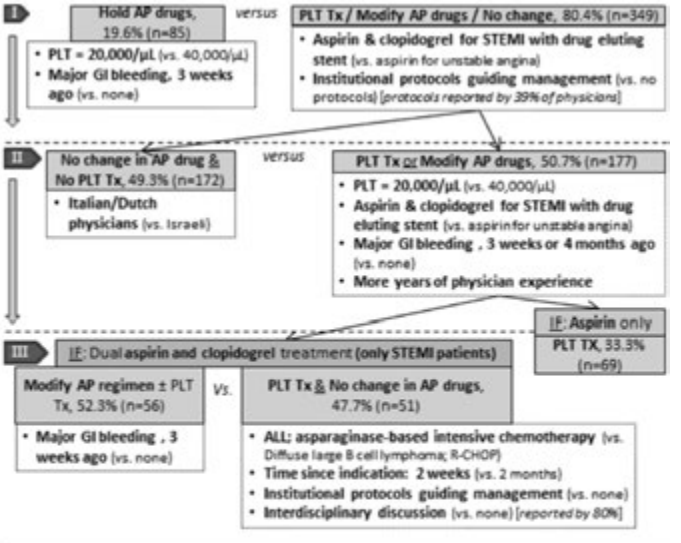
FACTORS INFLUENCING MANAGEMENT OF ANTIPLATELET DRUGS IN THROMBOCYTOPENIC PATIENTS WITH HEMATOLOGICAL MALIGNANCY

A. Leader[1,2,3,*], V. ten Cate[4], A. ten Cate-Hoek[1,5], G. Spectre[3,6], E. Beckers[7], P. Raanani[2,3], H. Schouten[7], A. Falanga[8], H. ten Cate[1,5]

[1]Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, Netherlands, [2]Institute of Hematology, Davidoff Cancer Center, Rabin Medical

Center, Petah Tikva, [3]Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel, [4]Epidemiology Department, Maastricht University, [5]Thrombosis Expert Center, Maastricht University Medical Center (MUMC+), Maastricht, Netherlands, [6]Institute of Hematology, Davidoff Cancer Center, Rabin Medical Center, Petah Tikva, Israel, Petah Tikva, Israel, [7]Department of Hematology, Maastricht University Medical Center (MUMC+), Maastricht, Netherlands, [8]Immunohematology and Transfusion Medicine Department, Hospital Papa Giovanni XXIII, Bergamo, Italy

Background: The use of antiplatelet (AP) drugs in thrombocytopenic (TCP) cancer patients is not uncommon. Balancing the increased risks of arterial thrombosis and bleeding is complex and informed only by retrospective studies on aspirin in acute coronary syndrome. Choice experiments can provide insight into management, in the absence of patient data.
Aims: (1) Identify patient and physician characteristics associated with AP drug management in TCP patients with hematological malignancies; (2) Evaluate whether physician assessments of bleeding risk (bl_risk) and thrombotic risk (thr_risk) correlate with AP drug management.
Methods: We designed a clinical vignette-based choice experiment. *First*, 11 Israeli/Dutch hematologists were interviewed, identifying 5 relevant attributes (*i.e.* malignancy & treatment type, platelet level, AP drug indication, time-since-indication, gastrointestinal bleeding) with 2-3 levels each. The case constants were: 50 year old male with normal renal function and coagulation tests. *Second*, an algorithm was used to create a fractional factorial design using these variables, generating 18 unique case vignettes each comprised of all 5 attributes, but different combinations of levels. The survey was piloted in Italy and distributed in Israel, Italy and the Netherlands (N=886). Each physician received 3 vignettes and was asked to assess bl_risk & thr_risk (scale: 1-10), and AP drug management (*i.e.* hold; no change; transfuse platelets; modify). *Third*, for analysis, management was split into 3 consecutive steps (I, II, III), each with a choice between 2 options (Figure 1). At each step, multivariate mixed-effects binomial logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI) of using one management option (over the other), for each physician/patient variable in comparison to a reference level within that attribute group. This was also done using thr_risk/bl_risk as independent variables.
Results: 145 hematologists or thrombosis/transfusion specialists, mainly from Italy (48), Israel (46) and the Netherlands (44), responded to 434 cases. Figure 1 schematically depicts variables with statistically significant higher odds of choosing one management strategy over the other, compared to the reference variable. For instance, cases with platelet counts of 20,000/μL were 3.45 times more likely to have AP drugs held over not holding, than cases with platelet counts of 40,000/μL [OR for not holding, 0.29 (95% CI, 0.12-0.70)]. Stepwise: (I) continuing AP drugs (*vs* holding) was associated with increasing thr_risk (p<0.0001) and decreasing bl_risk (p<0.0001); (II) modifying AP drugs or transfusing platelets (*vs* neither) correlated with rising bl_risk (p<0.0001); (III) transfusing platelets without modifying AP drugs (*vs* modifying) was associated with increasing thr_risk (p<0.05) and decreasing bl_risk (p<0.005). There was virtually no variance between physicians and countries in how risk assessment affected management (data not shown).



shows the AP management process and associated variables in these thrombocytopenic cases. The 3 steps of management (I→II→III) are depicted in gray boxes, together with the % of cases (n) in which each strategy was selected. The white box shows variables with statistically significant higher odds of choosing the adjacent management strategy over the competing one, compared to the reference variable in parentheses. Odds ratios not shown (multivariate analysis). *ALL*, acute lymphoblastic leukemia; *AP*, antiplatelet; *GI*, gastrointestinal; *PLT*, platelets; *STEMI*, ST elevation myocardial infarction; *Tx*, transfusion

Figure 1.

Summary and Conclusions: Physician-assessed bleeding and thrombotic risk consistently and logically influence all levels of management, with surprisingly little variance between physicians. The decision process is complex and affected by physician characteristics as well as a large number of patient variables. Platelet transfusions were frequently chosen to support continuing AP drugs, although no evidence supports this practice. The factors identified in this analysis should be considered when planning prospective management studies in this population.

S141

LONG-TERM SAFETY AND EFFICACY OF RITUXIMAB IN 248 ADULT IMMUNE THROMBOCYTOPENIA PATIENTS: RESULTS WITH MORE THAN 5 YEARS OF FOLLOW-UP FROM A FRENCH PROSPECTIVE REGISTRY ITP-RITUX

S. Deshayes[1,2,*], M. Khellaf[2], A. Zarour[3], R. Layese[3], O. Fain[4], L. Terriou[5], J.-F. Viallard[6], S. Cheze[7], J. Graveleau[8], B. Slama[9], S. Audia[10], M. Ebbo[11], M. Cliquennois[12], G. Le Guenno[13], G. Salles[14], F. Canoui-Poitrine[3], M. Michel[2], B. Godeau[2]

[1]Internal Medicine, CHU Côte de Nacre, Caen, [2]Internal Medicine, [3]Clinical investigation unit, CHU Henri Mondor, Créteil, [4]Internal Medicine, CHU Saint-Antoine, Paris, [5]Internal Medicine and Hematology, CHRU Lille, Lille, [6]Internal Medicine and Infectious diseases, Hôpital Haut-Lévèque, Pessac, [7]Hematology, CHU Côte de Nacre, Caen, [8]Internal Medicine, C HU Hôtel Dieu, Nantes, [9]Hematology, CH Avignon, Avignon, [10]Internal Medicine, CHU Dijon, Dijon, [11]Internal Medicine, Hôpital de la Timone, Marseille, [12]Hematology, Groupe Hospitalier de l'Institut Catholique de Lille, Lille, [13]Internal Medicine, Centre Hospitalier Universitaire Estaing, Clermont-Ferrand, [14]Hematology, Centre Hospitalier Lyon Sud, Lyon, France

Background: The long-term safety and efficacy of rituximab (RTX) in adult's immune thrombocytopenia (ITP) are not well known and current data are only based on retrospective studies.
Aims: To assess long-term (5 to 7 years of follow-up) safety and efficacy of RTX in ITP throughout the prospective registry set up in France in 2010 in which 248 adult patients were included (ClinicalTrials.Gov: NCT1101295).
Methods: All consecutive patients ≥18 years of age who received RTX for a diagnosis of primary ITP based on international criteria were prospectively included between 2010 and 2012. Patients with secondary ITP or who had received a previous course of RTX were excluded. A prospective and periodic assessment of the safety and the efficacy of RTX were recorded through an electronic case report form. In accordance to international guidelines, complete response (CR) was defined by a platelet count >100x10^9/L and response (R) by a platelet count between 30-100x10^9/L with at least a 2-fold increase from baseline. One-year follow-up data have already been published (Khellaf *et al.*, Blood 2014).
Results: Among the 248 patients included in the registry (64% of females, mean age at ITP diagnosis: 51±20 years), 102 (41%) patients had persistent ITP and 146 (59%) chronic ITP at time of first RTX administration, and 10% were splenectomized. The median follow-up duration after the first RTX infusion was 69 [IQR, 55-79] months, with a follow-up ≥60 months for 177 (71%) patients. In terms of efficacy, at last follow-up visit, 77 (31%) patients had a lasting response (70 CR; 7 R). Among the 177 patients with a follow-up ≥60 months, 50 (28%) had a lasting response (46 CR; 4 R). According to National Cancer Institute Common Terminology Criteria, 34 (14%) grade 3 or 4 infections were observed, but only 10 (4%) of them occurred within the 12 months following the last RTX infusion, including 1 pneumocystis pneumonia and 1 aspergillosis sinusitis. No case of progressive multifocal encephalopathy was observed. Malignancies were observed in 24 (10%) patients and occurred at a median age of 71 [62-79] years of age after a median of 48 [39-62] months from RTX (incidence rate of 1.4 [IC95%, 1.1-2.5] for 100 patient-years, similar to the one observed in the French general population). No over-representation of a type of malignancy was found. Moreover, there were 47 adverse effects (AE) related to RTX infusion; 22 (9%) patients developed or exacerbated another autoimmune disease; 21 (8%) patients had cardiovascular complications; and 16 (6%) experienced at least 1 venous thromboembolic event. Overall, 31 (12%) patients died (median age: 80 [IQR, 71-84] years) after a median time of 30 [IQR, 14-54] months after the first RTX infusion, corresponding to a mortality rate of 2.4 [IC95%, 1.7-3.4] for 100 patient-years. Deaths were mainly related to infections (n=6), malignancies (n=5) or bleeding (n=4). Among these AEs, only 24 AE ≥grade 3 were possibly related to RTX: 10 (4%) infections, 10 (4%) AE related to RTX infusion, 4 (2%) deaths (3 from infectious origin, 1 from unexplained cause). Gammaglobulin levels were not systematically monitored. Among the 142 (57%) patients from whom the data was available, 6 (2%) patients developed a hypogammaglobulinemia <5 g/L during follow-up.

Summary and Conclusions: This large nationwide prospective registry shows that a durable response is achieved in almost one third (31%) of adults with persistent or chronic ITP treated by RTX, and that no unexpected long-term complications occur beyond 12 months of follow-up.

S142

ABNORMAL BLEEDING ASSOCIATED WITH COAGULATION ABNORMALITIES IN SYSTEMIC AMYLOIDOSIS

K. Sun[1,*], X. Nan[1], S. Kasparian[1], C. Gentille[1], L. Rice[2], I. Ibrahim[2], S.R. Pingali[2], S. Iyer[2]

[1]Internal Medicine, [2]Hematology, Houston Methodist Hospital, Houston, United States

Background: Abnormal bleeding is a common complication of systemic Amyloidosis. Proposed mechanisms include Amyloid angiopathy and coagulation abnormalities among which Factor X deficiency is the most common. Prolongation of prothrombin time (PT) and partial thromboplastin time (PTT) were also reported.
Aims: We performed a retrospective review of different coagulation factors of systemic Amyloidosis patients from the Amyloid Database at the Houston Methodist Hospital between 2006 and 2015 to investigate the mechanisms of abnormal bleeding.
Methods: The patient data was queried from METEOR (Methodist Environment for Translational Enhancement and Outcomes Research), a clinical data warehouse that contains records dating back to January 1, 2006. We queried for the diagnosis of systemic Amyloidosis, clinically significant bleeding events, coagulation factors and other laboratory results along with patient demographics. The diagnosis of systemic Amyloidosis was further confirmed by reviewing tissue biopsy reports. The degree of bleeding was categorized into 4 grades according to World Health Organization standardized grading scale. Grade 2 or higher was defined as clinically significant bleeding. Grade 3 or 4 was considered as severe bleeding. Laboratory data including Factor X activity, PT, PTT, international normalized ratio (INR), platelets and fibrinogen were reviewed. Average laboratory test values in each clinical visit were used in univariate logistic regression test to investigate association of coagulation abnormalities with clinical significant bleeding and severe bleeding events.
Results: 230 patients and 2178 encounters were identified after excluding patients on anticoagulation. The type of the Amyloidosis was confirmed in 168 patients, 148 of which are primary Amyloidosis with the rest listed as unknown. 26 patients had at least one clinically significant bleeding event. 140 patients had PT and INR measured; 138 patients had PTT measured; 162 patients had platelet measured; 48 patients had fibrinogen measured and 34 had Factor X level measured. Elevated PT and INR were identified in 86/140 (61%) patients, elevated PTT in 61/138 (44%) patients, thrombocytopenia in 79/162 (48%) patients, abnormal Factor X (<70%) in 19/34 (56%) patients and decreased fibrinogen level (<200) in 5/48 (11%) patients. In univariate logistic regression analysis, prolongation of PT, INR, PTT and thrombocytopenia were independent variables associated with clinically significant bleeding (p=0.0011, p=0.0007, p=0.0044 and p= 0.0002 respectively). The same variables are also independently associated with severe bleeding events (p=0.0006, p=0.0004, p=0.0013 and p<0.0001 respectively). Factor X and fibrinogen level were not significantly associated with clinically significant bleeding or severe bleeding events (Table 1).

Table 1. The percentage of bleeding event with normal and abnormal laboratory studies.

Lab studies		%Bleeding	%Severe bleeding
PT	Normal PT	9.3%	1.9%
	Abnormal PT	23.3%	18.6%
	P value	0.0011	0.0006
PTT	Normal PTT	13.0%	6.5%
	Abnormal PTT	21.3%	18.0%
	P value	0.0044	0.0013
Platelet	Normal platelet	13.3%	8.4%
	Abnormal platelet	19.0%	13.9%
	P value	0.0002	< 0.0001
Factor X	>70%	33.3%	26.7%
	<70%	47.4%	36.8%
	P value	0.411	0.530
Fibrinogen	>200	30.2%	26.8%
	<200	20.0%	20.0%
	P value	0.9850	0.8067

Summary and Conclusions: Prolonged PT, INR and PTT are common in systemic Amyloidosis and correlate with increased bleeding events. Factor X deficiency is common but contrary to observations from previous studies, no correlation of it with abnormal bleeding was seen in our review. This could be explained by our low number of Factor X measurements. Studies with systemic measurements of PT, PTT, platelet and factor X activity are needed to further investigate the bleeding mechanisms in systemic Amyloidosis.

S143

A NOVEL BETA1-TUBULIN-BASED MEGAKARYOCYTE REPORTER SYSTEM IDENTIFIES COMPOUNDS THAT PROMOTE MEGAKARYOCYTE MATURATION AND PLATELET PRODUCTION

S.J. Chen[1,*], H. Seo[1], A. Hotta[1], Y. Nishi[1], A. Ohta[1], H. Hayashi[2], N. Koseki[2], N. Sugimoto[1], K. Eto[1]

[1]Center for iPS Cell Research and Application, Kyoto University, Kyoto, [2]New Drug Research Division, Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan

Background: Transfusion of donor-derived platelets is commonly employed to treat patients with thrombocytopenia, while current supply system confronts challenges including limited donors, short shelf life of platelet products, the risk of bacterial and viral contamination and transfusion refractoriness. These limitations have motivated the development of alternative donor-independent platelet sources to meet the growing need of clinical transfusion. Human induced pluripotent stem cells (iPSCs) have been proposed as a potential source of megakaryocytes (MKs) and platelets to meet the demands of clinical transfusion.
Aims: Our group previously established immortalized megakaryocyte cell lines (imMKCLs) derived from iPSCs as cryopreservable master cells that can be robustly expanded and then produce platelets upon maturation (Nakamura *et al.,* Cell Stem Cell, 2014). However, the low platelet yield and requirement of mouse somatic feeder cells are the major hurdles that must be overcome before clinical realization. This study aimed to efficiently identify small molecules that could facilitate *in vitro* platelet generation from imMKCLs under the feeder-free culture condition.
Methods: We herein established a genetically modified imMKCL that express the β1-tubulin-Venus reporter to identify optimal feeder-free culture condition by monitoring *in vitro* MK maturation in a high throughput manner. The Venus transgene was inserted downstream of the *TUBB1* locus in imMKCLs using CRISPR/Cas9 technology, and the expression was visualized by Venus fluorescence intensity. The imMKCL reporter line enabled visible real-time studies under a confocal quantitative image system. This reporter line was then employed for a high throughput screening (HTS) to identify compounds that significantly improved the efficiency of platelet production.
Results: The HTS of five thousand compounds successfully identified several compounds from diverse categories (*i.e.* a WNT inhibitor and a FLT3 inhibitor) that facilitate *in vitro* MK maturation and platelet release from imMKCLs under feeder-free conditions. The *in vitro* thrombopoietic effects of candidate compounds was also confirmed in two common MK lineages, one was cord blood derived CD34+ cells and another was iPSC directly derived MKs. In addition, candidate compounds improved *in vivo* recovery of thrombopoiesis in two mouse thrombocytopenia models, which were induced by irradiation or anti-GPIbα antibody administration. Given that WNT and FLT3 inhibitors are used to treat leukemia and cancers, that often manifest thrombocytopenia due to the diseases themself or as a result of chemotherapy. This finding may also facilitate clinical settings of cancer therapy. Interestingly, according to a luciferase aryl hydrocarbon receptor (AhR) reporter gene assay, most of these compounds, including a WNT signaling inhibitor, facilitated thrombopoiesis by antagonizing AhR signaling, in accordance with the crucial role of AhR inhibition in MK maturation reported by some groups. Others promoted thrombopoiesis independently of AhR signaling.
Summary and Conclusions: In summary, we have established a comprehensive, real-time monitoring system for MK maturation based on β1-tubulin expression, which thereby allows to identify novel small molecule inducers of MK maturation and platelet production through HTS as well as to perform more in depth studies of thrombopoiesis.

Anemia and quality of life

S144

A ROLE OF PIEZO1 IN ERYTHROID DIFFERENTIATION OF NORMAL AND HEREDITARY XEROCYTOSIS DERIVED HEMATOPOIETIC PROGENITORS

A. Caulier[1,*], N. Jankovsky[1], H. Ouled-Haddou[1], Y. Demont[1], D. Bastit[2], C. Rose[3], H. Guizouarn[4], A. Lahary[5], L. Merlusca[6], C. Guitton[7], D. Lebon[1], M. Thuillier[1], C. Badens[8], J.-P. Marolleau[1], V. Picard[9], L. Garçon[1]

[1]EA HEMATIM, Université Picardie Jules Verne, Amiens, [2]EFS Normandie, Bois-Guillaume, [3]Hématologie, Hôpital Saint-Vincent, Lille, [4]UMR 7277, Institut de Biologie Valrose, Nice, [5]Laboratoire d'hématologie, CHU Rouen, Rouen, [6]Hematologie clinique, CHU Amiens, Amiens, [7]Hématologie pédiatrique, Hôpital Kremlin-Bicêtre, Paris, [8]Département de Génétique Médicale, Hôpital de La Timone, Marseille, [9]Laboratoire d'hématologie, Hôpital Kremlin-Bicêtre, Paris, France

Background: Activation of the mechanotransductor PIEZO1 plays a central role in the red cell dehydration observed in hereditary xerocytosis (HX). Data recently published in epithelial and endothelial cells suggested that PIEZO1 was involved in the balance between cell proliferation and differentiation. Preliminary expression study confirmed that PIEZO1 was expressed in immature erythroblasts. However, its role during erythropoiesis and the consequences of its gain-of-function mutations in HX have not been evaluated so far.
Aims: We studied the role of PIEZO1 activation in erythroid differentiation of normal and hereditary xerocytosis derived hematopoietic progenitors.
Methods: *In vitro* human erythropoiesis was conducted during 21 days using normal peripheral CD34+ cells after PIEZO1 activation by Yoda1 (Y1, n=4) and CD34+ (n=6) or total mononuclear cells (MNC, n=9) from 10 HX patients displaying 9 different mutations. Cell proliferation, apoptosis, and differentiation were assessed using cell count, flow cytometry (FC) and cytology at day 10. The erythroleukemic cell line UT7/EPO was used in parallel for Piezo1 knock-down experiments using shRNA lentiviruses, for calcium signaling and for transduction pathways studies.
Results: PIEZO1 activation by Y1 1µM in normal CD34+ cells induced a slow-down in erythroid differentiation as shown by a decrease in mature CD71+/Glycophorin A (GPA)+expressing cells at day 10 of culture (20.9%±10.8 *vs* 77.6%±8.5, $p<10^{-3}$), with no effect on cell proliferation and a slight increase in the apoptosis rate (Annexin V: 14.7±2.3% *vs* 5.1±0.8, p=0.049). A drastic decrease in GPA expression was also observed in UT7/EPO cells after Y1 exposure (reduction by 88%±0.7; $p<10^{-3}$). This decrease was transcriptional, as shown by RQ-PCR, and was abolished after shRNA-mediated PIEZO1 knock down or after PIEZO1-inhibition using Gadolinium. Functional studies using flow imaging revealed that Y1 induced a calcium influx inside UT7/EPO cells. EGTA reverted the Y1-induced GPA repression, showing that Ca^{2+} signaling mediated the phenotype. Of note, a secondary Ca^{2+} dependant activation of Gardos channel was not involved, since Senicapoc did not correct the phenotype. Investigating the PIEZO1 downstream signaling pathways, we observed that phospho-ERK blockade by either chemical inhibitor UO126 or retroviral dominant-negative MEK transduction prevented Y1-induced GPA repression (76%±3 *versus* 9%±1.1; $p<10^{-3}$), showing that a functional ERK was necessary to the PIEZO1-mediated effects in human erythropoiesis. *In vitro* erythroid differentiation of progenitors from HX patients showed heterogeneous results. A similar phenotype than described after Y1 exposure was clearly observed for 5 mutations. Among them, 1 patient carrying the *PIEZO1* mutation G1792A was tested in triplicate: the mature CD71+/GPA+population at day 10 was significantly decreased in comparison with controls (13.8%±12.6 *versus* 62.2%±20.7; p<0.01). This delay was confirmed by cytology after MGG staining, showing a significant increase in the proportion of immature precursors (pro+basophilic erythroblasts) at day 10 (99%±1 *versus* 58.2%±19.3; p<0.01).
Summary and Conclusions: We described here for the first time a role of PIEZO1 during human erythroid differentiation. PIEZO1 activation repressed erythroid terminal maturation through Calcium entry and secondary phosphorylation of the ERK pathway. A similar phenotype was observed in the majority of PIEZO1-mutated HX patients but not all, underlying the high heterogeneity in the phenotype and the pathophysiology of this disease.

S145

FOSTAMATINIB, A SPLEEN TYROSINE KINASE INHIBITOR, FOR THE TREATMENT OF WARM ANTIBODY AUTOIMMUNE HEMOLYTIC ANEMIA: PRELIMINARY RESULTS OF THE SOAR PHASE 2, MULTICENTER, OPEN-LABEL STUDY

D.J. Kuter[1,*], D. Arnold[2], A. Lichtin[3], R. Agajanian[4], M. Choi[5], K.A. Rogers[6], M.A. Boxer[7], C. Broome[8], J.J. Field[9], I. Murakhovskaya[10], A.M. Lowe[11], B. Cadieux[12], H. Zayed[12], S. Tong[12], A.-M. Duliege[12]

[1]Massachusetts General Hospital, Boston, Massachusetts, United States, [2]Michael G. DeGroote School of Medicine, Department of Medicine, McMaster, Hamilton, Ontario, Canada, [3]Hematologic Malignancies and Blood Disorders, Taussig Cancer Center, Cleveland Clinic, Cleveland, Ohio, [4]The Oncology Institute of Hope and Innovation, Downey, California, [5]University of California, San Diego, San Diego, California, [6]Division of Hematology, The Ohio State University, Columbus, Ohio, [7]Arizona Oncology, Tucson, Arizona, [8]Division of Hematology, Georgetown University, Washington, D.C., [9]Blood Center of Wisconsin, Medical College of Wisconsin, Milwaukee, Wisconsin, [10]Montefiore Medical Center, Bronx, New York, [11]Rigel Pharmaceuticals, Inc., West Lake Hills, Texas, [12]Rigel Pharmaceuticals, Inc., South San Francisco, California, United States

Background: Spleen tyrosine kinase (Syk), a signaling component of Fc receptors, is associated with macrophage destruction of red blood cells (RBCs) in warm antibody autoimmune hemolytic anemia (wAIHA). The Syk inhibitor fostamatinib diminishes antibody-induced anemia in mice (Podolanczuk *et al.*, Blood 2009;113(14):3154-60).
Aims: This Phase 2 study (NCT02612558) assessed the efficacy and safety of fostamatinib in wAIHA.
Methods: Eligible adult patients had primary or secondary wAIHA with more than one prior failed treatment, hemoglobin (Hgb) <10 g/dL, IgG-positive direct antiglobulin test (DAT), haptoglobin <10 mg/dL, and lactate dehydrogenase (LDH) >ULN. Patients received fostamatinib 150 mg BID for up to 24 weeks. In this Simon two-stage design, if ≥4 patients in Stage 1 achieved the primary efficacy endpoint (Hgb >10 g/dL with an increase of ≥2 g/dL from baseline by Week 24 without rescue therapy or RBC transfusion), suggesting a true response rate of greater than 20% (at alpha=0.10), then Stage 2 would begin enrollment. Data as of February 23, 2018, from Stage 1, are presented.
Results: Of 19 patients with post-baseline assessments, 1 (5%) had a history of lymphoproliferative disease, 5 (26%) had prior splenectomy, 12 (63%) had prior steroids, 1 (5%) had prior ESAs, and none had prior rituximab. The median baseline Hgb was 9.1 g/dL (range: 6.8-9.9). The primary endpoint was met: 9 of 17 (53%) patients had a response, including 1 late responder. Response was generally rapid and sustained; 5 of 9 responses were achieved within 4 weeks. The median duration of the first response was 16.6 weeks (range: 0.1 to >30 weeks). Trends for decreasing LDH and reticulocytes and increasing haptoglobin were observed. The most common adverse events were diarrhea and dizziness. Serious adverse events were reported in 3 patients. None were related to fostamatinib. One patient recovered and continued on treatment. Two patients had serious adverse events resulting in fatalities: one with skin necrosis and infection (immunosuppressed due to steroids) and one elderly patient with pneumonia (immunosuppressed due to steroids and prior CLL).
Summary and Conclusions: Fostamatinib markedly improved Hgb levels in some patients with wAIHA. Side effects were manageable and consistent with those previously reported with fostamatinib in other conditions. Based on these results, Stage 2 of this study is currently enrolling patients. Long-term follow-up will provide additional efficacy and safety data for fostamatinib in patients with wAIHA.

S146

LONG TERM SURVIVAL OF PATIENTS WITH EVANS SYNDROME: A POPULATION-BASED COHORT STUDY

D.L. Hansen[1,*], S. Möller[2], N. Mannering[1], K. Andersen[3], D. Gaist[4], H. Frederiksen[1]

[1]Department of Haematology, Odense University Hospital, [2]OPEN, Odense University Hospital and University of Southern Denmark, [3]Department of Psychiatry, Region of Southern Denmark, [4]Department of Neurology, Odense University Hospital, Odense, Denmark

Background: Evans syndrome (ES) is defined by either simultaneous or sequential diagnoses of both immune thrombocytopenia (ITP) and autoimmune haemolytic anaemia (AIHA). The syndrome is rare, and data on frequency and prognosis are lacking. Earlier reports have focused mainly on complications and relapse (*e.g.*: Michel, Blood. 2009, Costallat, Joint Bone Spine. 2012). Here we report novel epidemiological data on primary and secondary ES from a nationwide register.
Aims: To provide population-based estimates of incidence, prevalence and survival of adults with ES.
Methods: To estimate these parameters we followed a nationwide cohort of patients with at least one diagnosis of ITP or haemolysis according to the Danish National Patient Register, January 1977 to December 2015. We

excluded patients less than thirteen years of age, due to the usually self-limiting nature of childhood ITP. A comparison cohort comprised individuals from the general population matched to the patient cohort (50:1) on age, and gender. The patient cohort was classified as either primary or secondary ES based on the following diagnoses (recorded at any time before or up to one year after date of ES diagnosis): hereditary and non-familial hypogammaglobulinemia, systemic lupus erythematosus (SLE), HIV/AIDS, hepatitis C, liver cirrhosis, autoimmune lymphoproliferative syndrome type I and haematological malignancies including myelofibrosis.

Results: The patient cohort included 25,238 patients with a haemolysis diagnosis or ITP. From this cohort we identified 209 patients with ES aged 13-97 years. Simultaneous diagnoses were present in 32.5%, and ITP was the first symptom in 44.5% of the patients. Mean time between the two diagnoses was 3 years [95%CI: 2.2-3.7]. The patients with ES made up 2% [95%CI: 1.8-2.3] of all ITP cases, and 8% [95%CI: 7-9] of the AIHA patients. 54.1% [95%CI: 46.0-62.0] of the primary ES patients, and 46.0% [95%CI: 31.8-60.7] of the secondary cases were women. Age at diagnosis was 57.3 years [95%CI: 54.1-60.6] in the primary ES group and 62.6 [95%CI: 57.6-67.6] years in the secondary ES group. 23.9% [95%CI: 18.3-30.3] were secondary ES, with haematological malignancy as the predominant cause (68.0%). The five-year cumulated incidence of ES (primary or secondary) in number of new cases per 1 million inhabitants, was 6.1 [95%CI: 3.8-9.4] in 1981-1985; 4.5 [95%CI: 2.6-7.3] in 1996-2000; and 12.6 [95%CI: 9.3-16.7] in 2011-2015. The prevalence in number of cases per 1 million inhabitants was in 1985 5.1 [95%CI: 3.2-7.7]; increasing to 8.7 [95%CI: 6.2-11.9] in 2000; and 18.2 [95%CI: 14.6-22.4] in 2015. Age at death was 64.6 years [95%CI: 61.7-67.4] in patients with primary ES and 65.9 years [95%CI: 61.3-70.5] in those with secondary ES. Median survival was 7.9 years [95%CI: 5.5-11.7] in primary ES, and 2.2 years [95%CI: 1.1-10.4] in secondary ES, compared to 21.6 years [95%CI: 20.9-22.4] in the comparison cohort from the general population (Figure 1). Median survival was stable during the period.

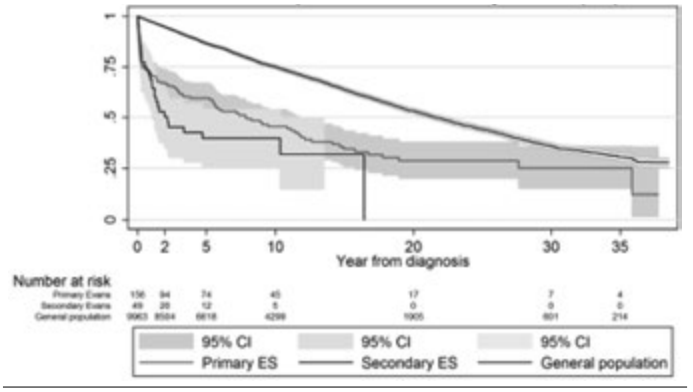


Figure 1. Survival for patients with Evans syndrome (ES).

Summary and Conclusions: ES is associated with a significant increase in mortality, especially among secondary cases. Incidence and prevalence are increasing, but survival does not seem to improve.

S147

IMPROVEMENTS IN CARDIAC BIOMARKERS ARE ASSOCIATED WITH BETTER HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH LIGHT CHAIN AMYLOIDOSIS

K. L. McCausland[1], S. Guthrie[2], T. Quock[3,*], M. Yokota[1], M. Bayliss[1], M.K. White[1], V. Sanchorawala[4]

[1]Optum, Johnston, [2]Biopharma Strategic Consulting, LLC, San Francisco, [3]Prothena Biosciences Inc, South San Francisco, [4]Boston University School of Medicine, Boston, United States

Background: Light-chain (AL) amyloidosis is a rare, progressive and typically fatal disease in which misfolded light chains are deposited in tissues and organs, which may lead to organ failure, disability, and death. The cardiac biomarker B-type natriuretic peptide (BNP) or N-terminal pro B-type natriuretic peptide (NT-proBNP) is used to monitor disease progression and response to treatment among patients with cardiac involvement from AL amyloidosis. The relationships between these cardiac biomarkers and health-related quality of life (HRQoL) are poorly understood in this condition.

Aims: To examine whether HRQoL in patients with cardiac AL amyloidosis differs significantly by changes in BNP or NT-proBNP.

Methods: We obtained cardiac biomarker and HRQoL data from AL amyloidosis patients from two data sources: a community-based sample (n=108) and a clinical sample of patients seen at the Amyloidosis Center at Boston University School of Medicine at Boston Medical Center (n=95). In the community-based sample, HRQoL scores (based on the SF-36® Health Survey [SF-36] and the Kansas City Cardiomyopathy Short Form [KCCQ-12]) were examined by patients with and without a self-reported decline in NT-proBNP ≥30%. Separate generalized linear models, adjusted for complete hematologic response, were used to compare HRQoL scores by NT-proBNP response to treatment. HRQoL scores were reported relative to existing benchmarks, including: 1) mean SF-36 scores from both a general population sample and patients with congestive heart failure (CHF); 2) mean KCCQ scores according to New York Heart Association functional classes. Analysis of variance was used to compare SF-36 scores to age- and gender-adjusted benchmarks. In the clinical sample, both pre- and post-treatment SF-36 scores and BNP lab values were obtained from medical record reviews. Generalized linear models, controlling for baseline SF-36 scores, were used to test for significant differences in SF-36 change scores among patients with and without a decline in BNP ≥30%.

Results: In the community-based sample, patients with an NT-proBNP response had significantly better HRQoL than those without for all SF-36 and KCCQ scales and summary scores (P <0.05 for all; Figure 1). Patients without an NT-proBNP response had SF-36 scores that were comparable to CHF benchmarks. Analyses in the clinical sample further supported longitudinal associations between changes in cardiac biomarkers and changes in SF-36 scores.

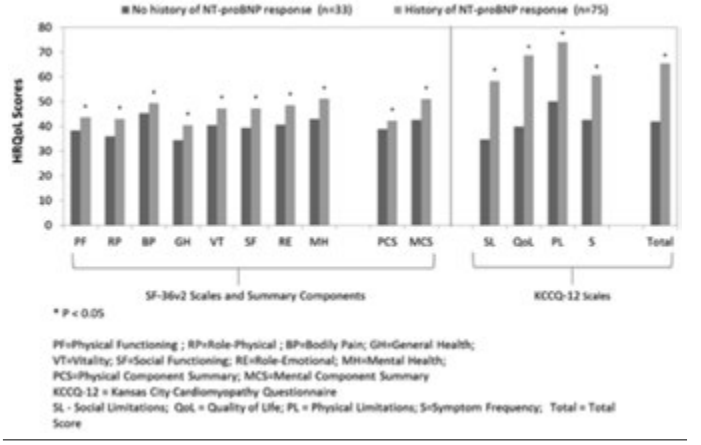


Figure 1. Health-related quality of life (HRQoL) by history of NT-proBNP response in a community-based sample of AL amyloidosis patients with cardiac involvement.

Summary and Conclusions: This study provided consistent preliminary evidence of a relationship between cardiac biomarkers and HRQoL using multiple data sources and different analytic approaches. These findings should be replicated in larger, longitudinal samples. A clearer understanding of the relationship between these frequently-used biomarkers and HRQoL can aid those in the scientific community who want to continue using these biomarkers to develop prognoses, evaluate treatment efficacy, and develop relevant and meaningful endpoints for clinical trials in patients with AL amyloidosis.

S148

FATIGUE AT BASELINE IS ASSOCIATED WITH GERIATRIC IMPAIRMENTS AND PREDICTS SHORTER SURVIVAL IN OLDER PATIENTS WITH A HEMATOLOGICAL MALIGNANCY

F. Hofer[1], K. Koinig[1], L. Nagl[1], R. Stauder[1,*]

[1]Department of Internal Medicine V (Haematology and Oncology) , Innsbruck Medical University, Innsbruck, Austria

Background: Hematological malignancies represent typical diseases at advanced age. The relevance of the patient-reported outcome (PRO) fatigue in decision making and as an endpoint in clinical studies in older persons has been propagated recently. However, prospective data on the distribution and clinical impact on fatigue in elderly patients with a hematological malignancy are rare.

Aims: We set out to determine the prevalence and the association of self-reported fatigue with clinical outcome and geriatric impairments in older individuals newly diagnosed with blood cancer.

Methods: The EORTC QLQ-C30 and a multidimensional geriatric assessment (MGA) were performed in parallel in 149 consecutive patients aged >67 years (median 77.8 years) newly diagnosed with a hematologic malignancy at Innsbruck University Hospital between January 2009 and April 2016.
Results: Self-reported fatigue as defined by EORTC QLQ-C30 was the most prevalent symptom (84 %). Other symptoms namely insomnia, dyspnea, loss of appetite, pain and constipation were reported by about a half to a quarter of patients. Moreover, functional impairments, namely in physical, role and emotional functioning, were observed in a relevant proportion of patients. Increased fatigue was significantly associated with reduced self-reported role, physical functioning as well as global health status as defined by EORTC QLQ-C30. Remarkably, pronounced fatigue was associated with impaired performance status and objective functional capacities in MGA, with altered depression scoring, G8-screening, and elevation of serum markers of inflammation ($p < 0.001$). Patients with minor fatigue had a median overall survival of 26.4 months, whereas those with marked fatigue had a median OS of 7.0 months ($p < 0.001$). The association of fatigue with shortened OS was supported in multivariate analyses (hazard ratio 1.74, CI 1.09 – 2.76; $p=0.021$).
Summary and Conclusions: Fatigue reveals a high prevalence and represents an adverse prognostic factor in elderly patients with a hematological malignancy. The strong impact of fatigue on clinical performance and overall survival emphasizes the relevance of patient reported outcomes for individualized treatment algorithms. Patients will particularly benefit from early identification of fatigue, allowing for timely interventions. The correlation of fatigue, reduced performance capacities, nutritional status and inflammation might suggest an underlying common pathway.

PRESIDENTIAL SYMPOSIUM

Best abstracts

S149

FIRST-IN-HUMAN CLL1-CD33 COMPOUND CAR T CELLS AS A TWO-PRONGED APPROACH FOR THE TREATMENT OF REFRACTORY ACUTE MYELOID LEUKEMIA

This abstract is embargoed until Friday, June 15, 08:30 local time.

F. Liu[1,*], K. Pinz[2], Y. Ma[3], M. Wada[2], K. Chen[2], G. Ma[2], Y. Su[1], S. Zhang[1], G. He[1], Y. Ma[2]

[1]Department of Hematology, Chengdu Military General Hospital, Chengdu, China, [2]Research and Development, iCell Gene Therapeutics, Stony Brook, United States, [3]Manufacturing Division, iCAR Bio Therapeutics Ltd, Zhongshan, China

Background: Anti-CD19 CAR T cells have shown impressive efficacy in B-ALL (acute lymphoblastic leukemia) and lymphoma, and have obtained FDA approval. However, treatment of relapsed/refractory acute myeloid leukemia (AML) remains a substantial clinical challenge. AML bears heterogeneous cells that can offset killing by single CAR-based therapies, resulting in disease relapse. Leukemic stem cells (LSCs) associated with CLL1 expression comprise a rare population that also plays an important role in disease progression and relapse. CD33 is a myeloid marker found on bulk AML disease cells in the majority of AML patients. Here, we report on the robust anti-tumor activity of compound CAR (cCAR) T cells possessing discrete scFv domains targeting two different AML antigens, CLL1 and CD33, simultaneously.
Aims: To develop a first-in-human treatment of refractory acute myeloid leukemia using Compound CAR T Cells simultaneously targeting two AML antigens, in order to avoid antigen escape, and to be safe and well tolerated.
Methods: We have generated a cCAR bearing two complete CAR constructs connected by a self-cleavable peptide linker, P2A. The anti-leukemic activities of CLL1-CD33 cCAR T cells were evaluated *in vitro* with killing assays using multiple AML cell lines, primary human AML samples as well as REH cells expressing either CLL1 or CD33. We tested cCAR in multiple mouse models in which mice were injected with REH expressing CLL1 or CD33 or U937 cell line. We also tested an alemtuzumab safety switch that allows for rapid cCAR therapy termination *in vivo*.
Results: We report on the robust anti-tumor activity of a compound CAR (cCAR) T-cell possessing discrete scFv domains targeting two different AML antigens, CLL1 and CD33, simultaneously. We showed that the CLL1-CD33 cCAR was able to ablate both the CLL1- or CD33-expressing REH cells independently both in co-culture assays and in mouse models. Mice treated with CLL1-CD33 cCAR showed significantly improved survival as compared to control-treated mice. We also showed that CLL1-CD33 cCAR promoted sustained *in vivo* anti-leukemic activity against the AML U937 cell line, as well as superior murine survival in both models. As a safety-switch to protect against the high potency of our cCAR, we developed a strategy to the rapid cCAR therapy termination in mouse models. Our findings indicate that targeting both CLL1 and CD33 on AML cells may be an effective strategy for eliminating both AML bulk disease and LSCs, potentially preventing relapse due to antigen escape or LSC persistence.
In the context of a first-in-human phase 1 clinical trial, informed consent from patients was obtained and CLL1-CD33 cCAR T cells expanded in AML patients. CLL1-CD33 cCAR T therapy was safe and well tolerated, and achieved complete response (CR).
Summary and Conclusions: Our study supports the development of CLL1-CD33 cCAR as a promising immunotherapy for AML. In the first-in-human clinical trial of CLL1-CD33 cCAR T cell therapy, we demonstrated the feasibility and safety of targeting both CLL1 and CD33 to achieve complete response (CR). Our findings suggest further exploration of CLL1-CD33 cCAR T therapy as a stand-alone therapy or "bridge to transplant" for patients with aggressive, relapsing/refractory AML leukemia.

S150

RIBOSOMAL LESIONS PROMOTE ONCOGENIC MUTAGENESIS

S. Sulima[1,*], K. Kampen [1], S. Vereecke[1], J. Verbeeck[1], J. Dinman[2], K. De Keersmaecker[1]

[1]Oncology, KU Leuven, Leuven, Belgium, [2]Cell Biology and Molecular Genetics, University of Maryland, College Park, United States

Background: Ribosomopathies are congenital disorders with mutations in ribosomal proteins (RPs) or assembly factors, characterized by cellular hypo-proliferation which particularly affects blood lineages. Intriguingly, ribosomopathies with hematopoietic insufficiency carry an increased risk to develop cancer, such as AML, later in life. The transition from hypo- to hyper-proliferation fits within an unexplained paradox known as Dameshek's Riddle (Dameshek, *Blood* 1967). Somatically acquired RP mutations have recently also been described, primarily in blood cancers such as T-ALL and CLL. Of these, the R98S mutation in ribosomal protein L10 (RPL10 R98S) is the most recurrent missense mutation, found in 8% of pediatric T-ALL. We have previously shown that this mutation interferes with ribosome function and cell proliferation. Moreover, RPL10 interacts with the SBDS protein during ribosome assembly, and *SBDS* mutations cause similar cellular defects in the ribosomopathy Shwachman-Diamond Syndrome (SDS).
Aims: To investigate how an early ribosomal mutation, exemplified by RPL10 R98S which negatively affects cell proliferation, can ultimately have an oncogenic impact.
Methods: Analysis of isogenic models expressing RPL10 R98S or WT including Ba/F3, lineage-negative cells from transgenic Rpl10-R98S and control mice, and pro-T cell cultures. NOTCH1-hyperactivation was attained by DL4 stimulation or expression of active NOTCH1 forms (NOTCH1-ICN or NOTCH1-L1601P-ØPEST found in T-ALL patients).
Results: RPL10 R98S induced a proliferation deficiency in lymphoid mouse cells, which was rescued with time by acquisition of additional mutations. Specifically, exome sequencing revealed that RPL10 R98S promoted a ~5-fold higher acquisition of additional mutations compared to WT. Analysis of two sets of previously generated T-ALL exomes (De Keersmaecker, *Nat. Genet.* 2013; Liu, *Nat. Genet.* 2017) revealed a ~2-fold higher load of mutations and oncogenic drivers in patients with RPL10 R98S or other RP mutations compared to patients with WT ribosomes. Further, RP-mutant patients from both cohorts displayed a significant enrichment for NOTCH1 pathway activating lesions. NOTCH1 expression rescued the proliferation defect of RPL10 R98S cell lines, which was reversible with NOTCH1 inhibition. In particular, RPL10 R98S specifically increased oxidative stress levels, which in turn promoted DNA damage, and both of these phenotypes were eliminated by NOTCH1 expression. Analysis of CLL patient exomes (Landau, *Nature* 2015) also demonstrated a higher mutational burden in cases with RP lesions (*i.e.* RPS15), and an enrichment in TP53 aberrations in RP mutant CLL cases. A recent study moreover described the acquisition of TP53 mutations in SDS patients as early events in the transformation to AML (Xia, *Blood* 2018). The role of TP53 mutations in RP mutant CLL and SDS is unclear, but it may also alleviate oxidative stress as TP53 activation upon cellular burden (*i.e.* ribosome assembly defects) is known to promote oxidative stress (Figure 1).

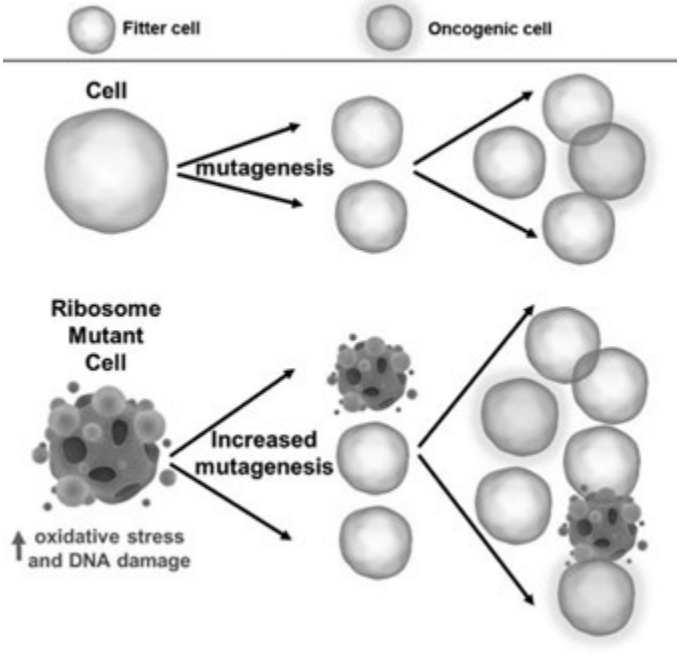


Figure 1.

Summary and Conclusions: We propose that RP lesions in ribosomopathies and cancer cause ribosome dysfunction-driven oxidative stress and consequently DNA damage, hypo-proliferation and hematopoietic insufficiency.

This drives surviving blood cells to acquire rescuing mutations which, potentiated by genomic instability, leads to a larger mutagenic pool. RP mutations may thus act as intrinsic cellular stressors that make transformation more accessible by opening the oncogenic window in disease-specific pathways. This in turn opens the window for novel prognosis and therapy potential for RP mutant patients.

S151

OVERALL SURVIVAL BENEFIT OF OBINUTUZUMAB OVER RITUXIMAB WHEN COMBINED WITH CHLORAMBUCIL IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA AND COMORBIDITIES: FINAL SURVIVAL ANALYSIS OF THE CLL11 STUDY

This abstract is embargoed until Friday, June 15, 08:30 local time.

V. Goede[1,2,*], K. Fischer[1], M.J. Dyer[3], L. Müller[4], L. Smolej[5], M.C. Di Bernardo[6], A. Knapp[7], T. Nielsen[7], M. Hallek[18]

[1]German CLL Study Group, Department I of Internal Medicine, Center of Integrated Oncology, University Hospital, [2]Oncogeriatric Unit, Department of Geriatric Medicine, St Marien Hospital, Cologne, Germany, [3]The Ernest and Helen Scott Haematological Research Institute, University of Leicester, Leicester, United Kingdom, [4]Oncology Practice, Leer, Germany, [5]Charles University Faculty of Medicine, University Hospital, Hradec Králové, Czech Republic, [6]On assignment to F. Hoffmann-La Roche Ltd, [7]F. Hoffmann-La Roche Ltd, Basel, Switzerland, [8]Cologne Cluster of Excellence in Cellular Stress Responses in Aging-Associated Diseases, University of Cologne, Cologne, Germany

Background: Obinutuzumab (GA101; G), a glycoengineered type II anti-CD20 monoclonal antibody, has been developed as an effective treatment for chronic lymphocytic leukemia (CLL). The phase III CLL11 study evaluated the efficacy and safety of G plus chlorambucil (Clb; G-Clb) and rituximab (R) plus Clb (R-Clb) *vs* Clb alone (Stage 1), as well as G-Clb *vs* R-Clb (Stage 2), in patients with previously untreated CLL and comorbidities. Previous pre-planned analyses have established superiority of G-Clb over Clb alone and R-Clb.
Aims: We report the final analysis of the CLL11 study (data cut-off, 10 October 2017), with approximately 2 years of additional follow-up compared with previous analyses.
Methods: Patients were randomised 1:2:2 to receive six 28-day cycles (C) of Clb, R-Clb or G-Clb. Clb (0.5 mg/kg) was administered orally on Day (D) 1 and D15 of C1–6. R was administered intravenously (IV) at a dose of 375 mg/m^2 on D1 of C1 and 500 mg/m^{2}on D1 of C2–6. G (1000 mg) was administered IV on D1 (dose split over two days; 100 mg D1, 900 mg D2), D8 and D15 of C1, and D1 of C2–6. Eligible patients had previously untreated CD20+ CLL, a total Cumulative Illness Rating Scale (CIRS) score of >6 and/or a creatinine clearance (CrCl) of <70 mL/min. The primary endpoint was investigator-assessed progression-free survival (PFS). Secondary endpoints included overall survival (OS), time to new treatment (TTNT) and safety. Comparison of PFS was performed by log rank test. Treatment effects were expressed as hazard ratios (HR) using a stratified Cox regression model.
Results: A total of 781 patients were enrolled and received treatment (median: age, 73 years; CIRS score, 8; CrCl, 62 mL/min). No new safety signals were identified at this update. After a median observation time of 62.5 months, treatment with G-Clb (n=238) was associated with improved outcomes compared with Clb alone (n=118); median: PFS, 31.1 *vs* 11.1 months (HR 0.21, 95% CI 0.16–0.28, p<0.0001); OS, not reached *vs* 66.7 months (HR 0.68, 95% CI 0.49–0.94, p=0.0196); and TTNT, 55.7 *vs* 15.1 months (HR 0.25, 95% CI 0.19–0.35, p<0.0001). After a median observation time of 59.4 months, G-Clb (n=333) also demonstrated a clinically meaningful improvement in outcomes compared with R-Clb (n=330); median: PFS, 28.9 *vs* 15.7 months (HR 0.49, 95% CI 0.41–0.58, p<0.0001) and TTNT, 56.4 *vs* 34.9 months (HR 0.58, 95% CI 0.46–0.73, p<0.0001) (Figure 1A and 1B). Notably, G-Clb also provided a clinically meaningful improvement in OS compared with R-Clb; median OS, not reached *vs* 73.1 months (HR 0.76, 95% CI 0.60–0.97, p=0.0245) (Figure 1C). Two- and five-year survival rates were 91% *vs* 84% and 66% *vs* 57% for G-Clb *vs* R-Clb, respectively. Overall, fewer patients died in the G-Clb arm (37%) than in the R-Clb arm (45%). During the survival follow-up period, the most common cause of death was disease progression (G-Clb, 10%; R-Clb, 15%).
Summary and Conclusions: This final survival analysis from the CLL11 study confirms that G-Clb provides clinically meaningful benefits in CLL patients with comorbidities, including prolongation of PFS and OS, when compared with R-Clb and Clb alone, and an absolute treatment-free duration of approximately four and a half years, while maintaining an acceptable and manageable safety profile. These findings support the use of G-Clb as first-line treatment for CLL patients with comorbidities, and suggest G as the preferred anti-CD20 antibody in future combination regimens for CLL.

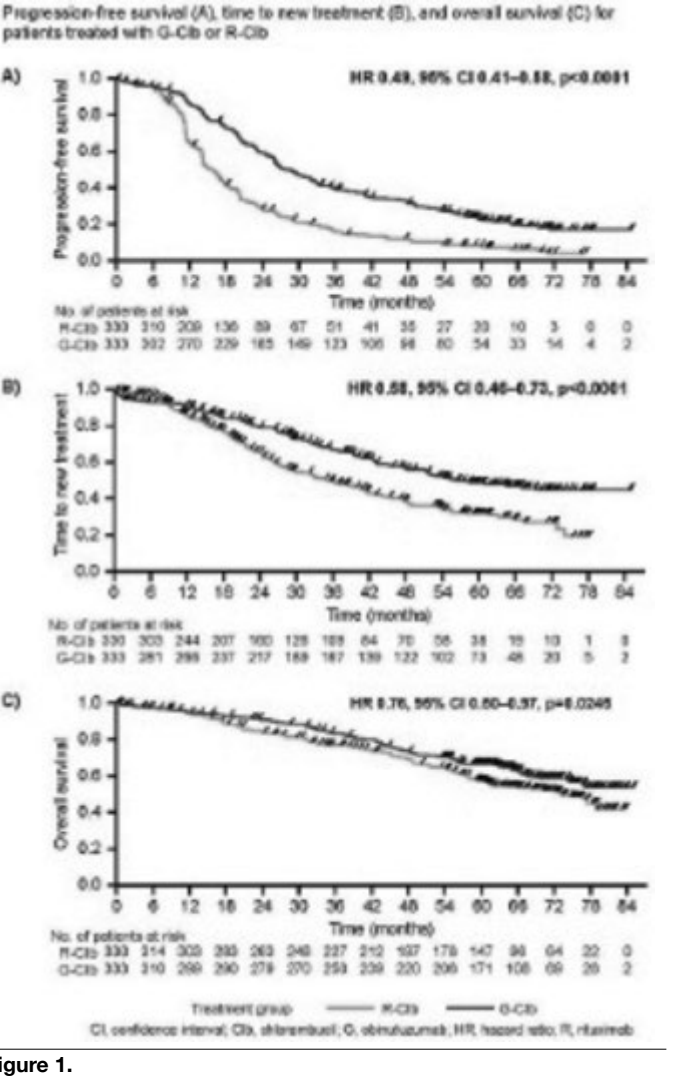


Figure 1.

S152

CHRONIC RED BLOOD CELL TRANSFUSIONS IMPAIR THE INNATE IMMUNE RESPONSE TO INFECTIOUS CUES BY SHAPING MACROPHAGES TOWARDS AN ANTI-INFLAMMATORY FUNCTIONAL PHENOTYPE

This abstract is embargoed until Friday, June 15, 08:30 local time.

F. Vinchi[1,*], R. Sparla[2], H. Weidner[3], M. Rauner[3], U. Platzbecker[3], M. Muckenthaler[2]

[1]Iron Research Program, New York Blood Center, New York, United States, [2]Iron Homeostasis Group, University Hospital Heidelberg, Heidelberg, [3]University Hospital Dresden, Dresden, Germany

Background: Chronic iron overload is common in thalassemia (thal), sickle cell disease (SCD) and myelodysplastic syndromes (MDS), due to red blood cell transfusions and increased intestinal iron absorption to support ineffective erythropoiesis. Chronically transfused patients show increased susceptibility to infections. Iron overload may increase the risk of infections by supporting bacterial growth and altering the response of the adaptive immunity. Likewise, transfusional iron overload may impact on the innate immune response, in particular on macrophage function.
Aims: We aimed to study the impact of transfusions on the phenotypic plasticity of monocytes and macrophages and their responses to infectious cues.
Methods: The functionality of hepatic and splenic macrophages as well as of recruited monocytes was studied in transfused and non-transfused wild-type and myelodysplastic mice, subjected or not to LPS challenge.
Results: Repeated transfusions of fresh RBCs in mice cause macrophage iron overload and cell death as well as recruitment of monocytes to the liver and spleen. After transfusion, macrophages acquire an M2-like anti-inflammatory phenotype, hallmarked by elevated expression of M2 markers

(CD206, Arg-1, Ym1) and reduced expression of M1 markers (MHCII, CD86). Consistently, pro-inflammatory cytokines (IL-6, CCL2, INFg, IL1b) are reduced compared to steady-state levels and anti-inflammatory cytokines (IL-10, IL-4) increased. These results indicate that transfusion-induced erythrophagocytosis likely suppresses the inflammatory response. These findings contrast previous observations, which support a pro-inflammatory role of free heme and iron on macrophage activation. This indicates that not only iron overload but also the route of iron acquisition (*e.g.* RBC phagocytosis *vs* free iron uptake) may shape the macrophage phenotype. After LPS challenge (15hours), macrophages from both transfused and non-transfused mice show an upregulation of M1 markers and pro-inflammatory cytokines. Nevertheless, M1 marker expression is drastically reduced and M2 markers increased in cells isolated from transfused compared to non-transfused mice. This is further reflected by reduced levels of circulating inflammatory cytokines. Our *in vivo* experiments support the concept that transfusions shape macrophages towards an M2-like anti-inflammatory phenotype after LPS stimulation, highlighting a novel adverse anti-inflammatory effect of transfusions upon infections. These findings do not only apply to wild-type mice but also to a mouse model of transfusion-dependent myelodysplastic syndrome.

Summary and Conclusions: Our data provide evidence that transfusions show a negative impact on the innate immune system and blunt the inflammatory response of macrophages to infectious stimuli. Our observations indicate that transfusions dampen macrophage immune effector functions by inducing their switching towards an anti-inflammatory phenotype, which is unlikely to efficiently counteract infections. The weak pro-inflammatory response of the macrophage might contribute to the increased propensity of transfused patients to develop infections, having potential implications for conditions associated with chronic transfusions, as in MDS, thalassemia and SCD. Finally these results suggest that transfusion practice might increase the risk of infections not solely by promoting the growth of microorganisms through increasing iron availability, but also by impairing the innate immune system, through the alteration of macrophage plasticity. The underlying mechanisms are currently under investigation.

S153

MPN CALR MUTANTS PROMOTE CELL-SURFACE LOCALIZATION OF TPOR WHICH IS OBLIGATORY FOR ONCOGENESIS: NOVEL THERAPEUTIC AVENUES AND RESCUE OF CONGENITAL THROMBOCYTOPENIA TPOR MUTANTS

C. Pecquet[1,*], I. Chachoua[1], A. Roy[1], T. Balligand[1], G. Vertenoeil[1], J.-P. Defour[1], C. Marty[2], I. Plo[2], H. Nivarthi[3], E. Xu[4], E. Hug[4], R. Kralovics[3], W. Vainchenker[2], S. N. Constantinescu[1]

[1]Signal Transduction Unit, Ludwig Institut for Cancer Research Ltd, Brussels, Belgium, [2]INSERM U 1170, Institut Gustave Roussy, Villejuif, France, [3]Genetics of Hematological Disorders, CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, [4]Myelopro Diagnostics and Research GmbH, Vienna, Austria

Background: Mutant calreticulins are major drivers of myeloproliferative neoplasms (MPNs) by activating TpoR/MPL and JAK2 signaling. However how exactly this interaction occurs, and in which cell compartment this abnormal TpoR activation will lead to an oncogenic signaling remains unknown.

Aims: Our objectives were: 1) to determine the precise sequences of CALR mutant and TpoR required for interaction, traffic and activation, 2) whether disease-inducing TpoR signaling occurs from intracellular compartments or cell-surface, 3) whether CALR mutants can also interact and activate defective TpoR mutants, such as TpoR mutants in congenital amegakaryocytic thrombocytopenia (CAMT) and 4) whether CALR mutants are secreted.

Methods: Engineered CALR and TpoR mutants were analyzed by a combination of biochemical approaches (thermal shift assay in cells and on protein), functional assay (cell growth assay, luciferase assay, flow cytometry, primary megakaryocytic clonogenic assay) and cell imaging (confocal microscopy).

Results: 1) We map the required sequences for interaction between mutant CALR and TpoR extracellular domain. A minimum of 10 proximal aminoacids of the new CALR tail and sequences of the N-terminal globular domain, but not the P-domain are required for TpoR activation. We also isolated a complex between extracellular domain of TpoR and CALR del52 produced in insect cells and mapped the required N-glycosylation profile required for complex formation. 2) We identify a specific region of 8 aminoacids in D1 TpoR that can confer the ability to activate JAK2-STAT5 to another receptor (EpoR) after binding of mutant CALR. Mutation to alanines of this TpoR region (TpoR 8A) abolishes response to CALR mutant. 3) As a direct consequence of mutant CALR-TpoR interaction, the thermal stability of TpoR was found to significantly increase in the presence of CALR del52. Such an increase was not observed with EpoR. However, engineered EpoR that can induce CALR del52 dependent JAK2-STAT5 activation also showed increased thermal stability with CALR del52. Concurrently, neither TpoR mutated at key asparagine residues involved in N-glycosylation nor TpoR with Alanine mutations could show enhanced thermal stability with CALRdel52. 4) We provide genetic evidence that the cell-surface TpoR-CALR mutant complexes are obligatory for hematopoietic cell transformation. Mutant CALRs and TpoR must pass via the Golgi apparatus to transform, and they co-localize in the Golgi compartments. The cell-surface complexes of active tyrosine phosphorylated forms of TpoR-JAK2 induced by mutant CALRs are internalized and can be detected in early endosomes, suggesting prolonged signaling in that compartment. 5) We show that both CALR del52 and ins5, but not wild type CALR are able to rescue traffic and cell-surface localization and signaling by the CAMT TpoR R102P mutant. Mutant CALRs promote folding and stability of TpoR R102P. This effect can also be seen with TpoR P106L and other defective engineered TpoRs. 6) Mutant CALRs are secreted to levels of 0.1-0.2 g/ml in patients.

Summary and Conclusions: We show that mutant CALRs act as rogue chaperones, leading to folding and cell-surface localization of TpoR, which is obligatory for hematopoietic cell transformation. Mutant CALRs can rescue defective TpoR in CAMT to cell-surface localization and activation. Moreover our data indicate that cell-surface mutant CALR is crucial for oncogenicity. We discuss avenues of targeting mutant CALRs on the cell-surface and potential effects of secreted mutant CALR.

S154

RELEVANCE: PHASE III EFFICACY AND SAFETY STUDY OF LENALIDOMIDE PLUS RITUXIMAB (R2) *VERSUS* RITUXIMAB PLUS CHEMOTHERAPY, FOLLOWED BY RITUXIMAB, IN PREVIOUSLY UNTREATED FOLLICULAR LYMPHOMA

This abstract is embargoed until Friday, June 15, 08:30 local time.

F. Morschhauser[1,*], N.H. Fowler[2], P. Feugier[3], R. Bouabdallah[4], H. Tilly[5], M.L. Palomba[6], C. Fruchart[7], E.N. Libby[8], R.-O. Casasnovas[9], I. Flinn[10], C. Haioun[11], H. Maisonneuve[12], L. Ysebaert[13], N. Bartlett[14], K. Bouabdallah[15], P. Brice[16], V. Ribrag[17], N. Daguindau[18], S. Le Gouill[19], G.M. Pica[20], A. Martin Garcia-Sancho[21], A. López Guillermo[22], J.-F. Larouche[23], K. Ando[24], M. Gomes da Silva[25], M. Andre[26], P. Zachée[27], L.H. Sehn[28], K. Tobinai[29], G. Cartron[30], D. Liu[31], J. Wang[31], L. Xerri[32], G. A. Salles[33]

[1]Department d' Hematologie, Centre Hospitalier Universitaire Régional de Lille, Unité GRITA, Lille, France, [2]Department of Lymphoma and Myeloma, The University of Texas MD Anderson Cancer Center, Houston, United States, [3]Centre Hospitalier Universitaire Régional de Nancy, Service d'Hématologie, Vandoeuvre les Nancy, [4]Department d' Hematologie, Institut Paoli Calmettes, Marseille, [5]Department d' Hematologie, Centre Henri Becquerel, Rouen, France, [6]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, United States, [7]Institut d'Hématologie de Basse Normandie, Caen, France, [8]Department of Medicine, University of Washington, Seattle, United States, [9]CHU Le Bocage Service d'Hématologie Clinique, Dijon, France, [10]Sarah Cannon Research Institute, Nashville, United States, [11]Hôpital Henri Mondor Unité Hémopathies Lymphoïdes, Creteil, [12]CHD Vendée Service d'Onco-Hématologie, [13]IUCT Oncopole Service d'Hématologie, Cedex, France, [14]Washington University School of Medicine, Siteman Cancer Center Hematology Labs, St. Louis, United States, [15]Hôpital Haut Lévêque - Centre François Magendie Service d'Hématologie Clinique et Thérapie Cellulaire, [16]Hôpital Saint Louis Service d'Onco-Hématologie, [17]Gustave Roussy Cancer, [18]Centre Hospitalier Annecy Genevois Service d'Hématologie, Cedex, [19]CHU de Nantes - Hôtel Dieu Service d'Hématologie Clinique, Nantes, [20]CH Métropole Savoie Service Hématologie, Chambery, France, [21]Department of Hematology, Hospital Universitario de Salamanca and IBSAL, CIBERONC, Salamanca, [22]Department of Hematology, Hospital Clinic de Barcelona, Barcelona, Spain, [23]CHU de Québec, Hôpital de l'Enfant-Jésus, Québec, Canada, [24]Department of Hematology and Oncology, Tokai University Hospital, Kanagawa, Japan, [25]Departamento de Hematologia, Instituto Português de Oncologia Lisboa Francisco Gentil (IPOLFG), Lisboa, Portugal, [26]Hematology Department, CHU UCL Namur, Yvoir, [27]Department of Hematology, ZNA Stuivenberg, Antwer, Belgium, [28]British Columbia Cancer Centre for Lymphoid Cancer, University of British Columbia , Vancouver, Canada, [29]Department of Hematology, National Cancer Center Hospital, Tokyo, Japan, [30]Department of Hematology, CHU Montpellier, University of Montpellier, Montpellier, France, [31]Celgene Corporation, Summit, United States, [32]Departement de Biopathologie, Institut Paoli-Calmettes, Marseilles, [33]Hospices Civils de Lyon, Centre Hospitalier Lyon-Sud, University of Lyon, Pierre-Benite, France

Background: Standard of care for advanced stage, high tumor burden, previously untreated follicular lymphoma (FL) has been rituximab plus chemotherapy (R-chemo) followed by rituximab maintenance. Combination

immunotherapy with lenalidomide and rituximab (R^2) is a chemotherapy-free regimen that has shown promising efficacy and safety in previously untreated patients with FL.

Aims: This is the first report of the co-primary endpoints: i) complete response (CR)/CR unconfirmed (CRu) at 120 weeks and ii) interim analysis of progression-free survival (PFS; ~50% of the target of 456 events by 1999 IWG criteria) for R^2 *vs* R-chemo followed by rituximab in previously untreated patients with FL.

Methods: RELEVANCE is a global, randomized, open-label phase III trial (NCT01650701; EUDRACT2011-002792-42) in patients with grade 1-3a FL who required systemic therapy (per GELF criteria). In the R^2 arm, lenalidomide 20 mg/day on days 2-22/28 was given cycles 1 to 6-12, continued in responders at 10 mg/day for a total of 18 cycles. Rituximab was 375 mg/m^{2}weekly in cycle 1, day 1 in cycles 2-6, and continued in responders for 12 additional cycles (q8wk). R-chemo was given per investigator's choice of standard R-CHOP, R-bendamustine (R-B), or R-CVP, followed by 12 cycles of rituximab (q8wk).

Results: As of the cutoff date of 31May2017, 1030 patients with high tumor burden FL were randomized to R^2(n=513) or R-chemo (n=517; 72% R-CHOP, 23% R-B, 5% R-CVP). Both groups had similar baseline characteristics; overall median age was 59 years (range, 23-89), 49% with FLIPI score ≥3, 93% stage III/IV, and 41% bulky disease (>7 cm). The superiority of both co-primary endpoints for R^2 over R-chemo was not established after a median follow-up of 37.9 months. CR/CRu at 120 weeks was similar in the R^2 v R-chemo groups (48% v 53%, *P*=0.13 per central review; 55% v 58%, *P*=0.38 per investigator assessment). R^2 and R-chemo groups demonstrated similar 3-year PFS rates by both central review (77% v 78%) and investigator assessment (77% v 78%), respectively (Figure 1). 3-year overall survival was 94% for both arms. The toxicity profiles for R^2 *vs* R-chemo were different, with lower rates of any grade fatigue (23% *vs* 29%), nausea (20% *vs* 42%), peripheral neuropathy (11% v 22%), vomiting (7% v 19%), stomatitis (3% *vs* 7%), and alopecia (1% *vs* 9%), and higher rates of cutaneous reactions (43% *vs* 24%), diarrhea (37% *vs* 19%), and tumor flare reaction (6% *vs* 0.2%) associated with R^2. Rates of thromboembolic events were similar in both groups. Higher grade 3/4 lab neutropenia (34% *vs* 50%) and febrile neutropenia (2% *vs* 6%) were associated with R-chemo, whereas higher grade 3/4 cutaneous reactions (7% *vs* 1%) were associated with R^2. Adverse events (AEs) led to treatment discontinuation in 11% of R^2 and 3% of R-chemo patients. Grade 5 AEs were 1% for both arms, and SPMs were reported in 7% (n=38) R^2 and 9% (n=48) R-chemo patients (5% invasive SPMs for both). 69% R^2 and 71% R-chemo patients completed 30 months of treatment. For the R^2 group, 76% of patients completed all 18 cycles of lenalidomide.

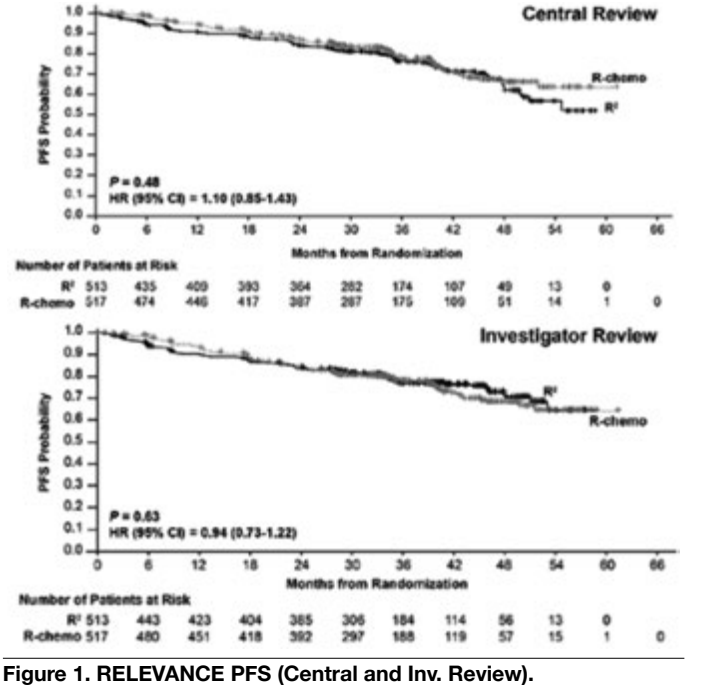


Figure 1. RELEVANCE PFS (Central and Inv. Review).

Summary and Conclusions: RELEVANCE is the first randomized phase III trial comparing the chemotherapy-free regimen R^2 v standard R-chemo followed by rituximab maintenance in previously untreated patients with FL. The superiority of R^2 over R-chemo was not demonstrated in this phase III trial, however, compared with R-chemo, R^2 appeared to show similar efficacy with a different toxicity profile.

POSTER SESSION I

Acute lymphoblastic leukemia – Biology & Translational Research

PF155

SENSITIVITY TO VENETOCLAX IN BCP-ALL IS CLOSELY CORRELATED TO HIGH FUNCTIONAL BCL-2 DEPENDENCE AND CAN BE ENHANCED BY DEPLETION OF MCL-1

F. Seyfried[1,*], S. Demir[1], R. Hörl[1], J. Ryan[2], A. Scheffold[3], M. Villalobos-Ortiz[2], S. Köhrer[1], S. Enzenmüller[1], J. Zinngrebe[1], S. Stilgenbauer[3], A. Letai[2], K.-M. Debatin[1], L.H. Meyer[1]

[1]Department of Pediatrics and Adolescent Medicine, Ulm University Medical Center, Ulm, Germany, [2]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, United States, [3]Department of Internal Medicine III, Ulm University Medical Center, Ulm, Germany

Background: Deregulated cell death pathways contribute to leukemogenesis and treatment failure in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Members of the BCL-2 family are key apoptosis regulators, therefore serving as potential targets for therapeutic intervention.
Aims: In this study, we evaluated activity and predictive models for the BCL-2 inhibitor venetoclax (VEN) in BCP-ALL and investigated co-targeting of MCL-1 to increase VEN sensitivity.
Methods: Activities of BH3-mimetics were assessed by cell viability assays analyzing half maximal effective concentrations (EC_{50}). Genetic alterations were assessed by multiplex ligation probe amplification (MLPA) and targeted sequencing. Gene expression profiling (GEP) was performed (HG 133 Plus 2.0, Affymetrix). Apoptosis signaling parameters were assessed by western blot and functional BH3-profiling. *In vivo* treatment was performed in preclinical xenograft models.
Results: In a series of N=27 BCP-ALL PDX samples different sensitivities to VEN upon *ex vivo* exposure were observed with high (EC_{50}<100 nM, n=12, 44%), intermediate (EC_{50} 100 nM - 1 µM n=8, 30%) or insensitivity (EC_{50}>1 µM, n=7, 26%). Moreover, we also assessed *in vivo* anti-leukemia activity of VEN in a preclinical trial treating ALL-bearing recipients transplanted with different BCP-ALL samples (N=8) and found distinct leukemia-free survival times reflecting the heterogeneity of VEN-sensitivity observed *ex vivo*. Interestingly, response to VEN was not associated with recurrent genetic alterations including *JAK1*, *IKZF1*, *KRAS* mutations and *CDKN2A*, *CDKN2B*, *BTG1*, *RB1*, *JAK2*, *IKZF1*, *PAX5*, *EBF1* and *ETV6* deletions, however high VEN sensitivities were identified in one sample with a *MLL-AF4* fusion, in line with previous reports, and in two *TP53* mutated cases. Importantly, no mutations in *BCL2* or *BAX* were found. Analysis of GEP comparing VEN-sensitive and -resistant ALL revealed a signature, which did not include genes coding for apoptosis regulators and which was not found to be associated with cell death pathways (gene ontology analysis). In line, transcript or protein expression of BCL-2, MCL-1 and ratios of BCL-2/MCL-1 were not or only weakly associated with *in vivo* VEN activity, indicating the necessity to globally assess apoptosis signaling. Therefore, we analyzed the impact of different apoptosis regulators on the functional interplay of mitochondrial apoptosis signaling using synthetic pro-apoptotic peptides. Importantly, mitochondrial BCL-2 dependence showed a clear significant correlation with leukemia-free survival times after *in vivo* VEN therapy (Spearman, r_s=0.955; p=0.0006). In order to overcome resistance and increase sensitivity for VEN, we evaluated the effects of co-targeting MCL-1. MCL-1 knockout in VEN-resistant ALL resulted in a clear sensitization to VEN and co-treatment with S63845 showed a clear synergism in cell line and PDX BCP-ALL samples.
Summary and Conclusions: We identified a heterogeneous sensitivity to VEN in a series of primary PDX BCP-ALL samples upon *ex vivo* and *in vivo* VEN treatment. VEN activity was not found to be associated with recurrent genetic alterations and no mutations in *BCL-2* or *BAX* were found. Importantly, mitochondrial BCL-2 dependence assessed by integrative analysis of mitochondrial apoptosis signaling (BH3-profiling) showed the most significant correlation with *in vivo* response to VEN treatment, providing a marker to identify patients who would respond to BCL-2 inhibition. Moreover, pharmacological MCL-1 depletion clearly enhanced VEN activity, pointing to co-targeting of BCL-2 and MCL-1 as effective anti-leukemia strategy.

PF156

A NEW HUMANIZED MONOCLONAL ANTIBODY AGAINST A SPECIFIC GLYCOSYLATED EPITOPE OF CD43 ANTIGEN FOR THE THERAPEUTIC TARGETING OF ACUTE LYMPHOBLASTIC LEUKEMIA T (T-ALL)

C. Botta[1,*], M.E. Gallo Cantafio[1], C. Buracchi[2], M.A. Siciliano[1], C. Riillo[1], A. Gulino[3], M. Cucè[1], K. Grillone[1], F.M. Tuccillo[4], M.T. Di Martino[1], C. Tripodo[5], M. Rossi[1], A. Biondi[6], G. Gaipa[7], P. Tagliaferri[1], P. Tassone[1]

[1]"Magna Graecia" University of Catanzaro, Catanzaro, [2]Tettamanti Research Center - Centro Maria Letizia Verga, Monza, [3]A.O.U.P " Paolo Giaccone, Palermo, [4]IRCCS Fondazione Pascale, Napoli, [5]A.O.U.P " Paolo Giaccone", Palermo, [6]University of Milano-Bicocca, [7]Tettamanti Research Center - Centro Maria Letizia Verga, Monza, Italy

Background: T-cell acute lymphoblastic leukemia (T-ALL) accounts for about 20% of ALL cases. Despite the introduction of intensive chemotherapy protocols, about 25% of children and 50% of adult patients fail to respond or relapse. The prognosis for these patients remains poor and novel treatment options are eagerly awaited. The targeting of tumor-associated antigens by monoclonal antibodies (mAb) is among the most investigated immunotherapeutic strategies. Accordingly, we previously developed a murine mAb, directed against a heavy glycosylated oncofetal epitope of CD43 (CD43/UN1) which present a high reactivity against human immature thymocytes, and T-ALL cell lines.
Aims: To investigate the therapeutic activity and mechanisms of action of a new humanized mAb directed to CD43/UN1 in experimental models of T-ALL.
Methods: The expression of CD43/UN1 was assessed on tumor cell lines, blood samples from T-ALL patients and healthy donors by flow-cytometry. Humanized mAbs were generated by combining the variable domains of the murine antibody to the corresponding human IgG1 constant domains. A tissue microarray of 30 different human normal tissues was screened by immunohistochemistry according to FDA/CE guidelines. Complement-mediated cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) and cellular phagocytosis (ADCP) on primary T-ALL cells T-ALL cell lines were evaluated by flow cytometry. 6 different *in vivo* models on NSG mice (3 with administration of NK-92-CD16+ effectors) have been performed to evaluate mAbs activity in different disease settings: orthotopic, subcutaneous advanced disease (treatments started after tumor reached 100 mmc) and subcutaneous limited disease (treatments started the day after injection).
Results: By screening different cancer cell lines, we observed CD43/UN1 to be highly expressed on malignant T-ALL cells. We then tested a series of 43 T-ALL patients-derived blasts and we observed that CD43/UN1 was specifically expressed on a subset of patients (about 80%) belonging to the cortical T-ALL (EGIL T3) group. A humanized mAb, named UMG1, and an afucosylated engineered version of this mAb, named a-UMG1, were then developed. To investigate CDC, ADCC or ADCP, T-ALL cells were cultured in the presence of complement, peripheral blood mononuclear cells (PBMCs) or macrophages, at increasing concentrations of both antibodies. Neither UMG1 nor a-UMG1 were able to induce CDC on target cells. Conversely, both mAbs induced CD16 downregulation, IFN-g production and degranulation (evaluated as CD107+ cells increase) on NK cells (more evident with a-UMG1) and T-ALL cell lines or primary blasts cytotoxicity. Additionally, both mAbs induced ADCP. Furthermore, we demonstrated potent activity of both mAbs in different T-ALL models *in vivo*. Specifically, in an orthotopic model we observed 5 out of 20 treated mice free of disease after 100 days from injection as compared to none of the control group. In both subcutaneous models, we observed a strong ability of our antibody to delay tumor growth and to increase mice survival. Of note, the addition of NK-92-CD16+ strongly improved the activity of a-UMG1. Furthermore, to explore the possibility of combination therapy, we investigated the modulation of CD43/UN1 expression by several chemotherapeutics. Interestingly, methotrexate and doxorubicin, alone or in combination, increased antigen expression, thus resulting in improved ADCC.
Summary and Conclusions: On these bases, we demonstrated that UMG1 and a-UMG1 represent novel promising immune-therapeutic tools for the treatment T-ALL patients.

PF157

CDK8 DEGRADATION - PREPARING THE GROUND FOR MTOR NHIBITION IN BCR/ABL+ ALL

I. Menzl[1,*], T. Zhang[2], R. Grausenburger[1], K. Bauer[3], F. Grebien[4,5], S. Kubicek[6], P. Valent[7], J. Zuber[8], N. Gray[2], V. Sexl[1]

[1]Department for Biomedical Science, Institute of Pharmacology and Toxicology VetMed university Vienna, Vienna, Austria, [2]Department of Cancer Biology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, United States, [3]Department of Internal Medicine I, Devision of Hematology & Hemostaseology and Ludwig Boltzmann Cluster Oncology, Medical University of Vienna, [4]Ludwig Boltzmann Institute for Cancer Research, [5]Department for Biomedical Science, Institute of Medical Biochemistry, [6]Research Center for Molecular Medicine of the Austrian Academy of Sciences (CeMM), [7]Devision of Hematology & Hemostaseology and Ludwig Boltzmann Cluster Oncology, Medical University of Vienna, [8]Research Institute of Molecular Pathology (IMP), Vienna, Austria

Background: Cyclin-dependent kinases (CDKs) trigger important cellular processes including cell cycle progression, transcriptional regulation and gene expression. It is therefore not surprising that CDKs are frequently deregulated in various types of cancer.
Aims: Investigate the role of CDK8 as a new potential therapeutic target in BCR/ABL^{p185+} leukemia.
Methods: We established an inducible knock-down system for CDK6, CDK7, CDK8 and CDK9 in murine BCR/ABL^{p185+}cells. Knock-out mouse models were generated and used to validate our findings including *Cdk8$^{fl/fl}$ Vav-Cre* or inducibly *Cdk8$^{fl/fl}$ Mx1Cre* mice. RNASeq was performed to reveal transcriptional changes upon kinase-inhibition or loss of CDK8 in murine BCR/ABL^{p185+} cells. Pathways that were identified to be differentially regulated were blocked with specific inhibitors *in vitro*. Human relevance was studied in *in vitro* experiments using different human leukemic cell lines and primary patient samples. Degradation of CDK8 and dual inhibition of mTOR was accomplished with a new compound named YKL-101-06 in human leukemic cells and patient primary samples.
Results: Inducible knock-down of various CDKs revealed a dependency of murine BCR/ABL^{p185+} cells on CDK8 expression. BCR/ABL^{p185+} *Cdk8$^{\Delta/\Delta}$* cells induced leukemia with significantly enhanced disease latency *in vivo*. Most importantly, when we deleted *Cdk8 in vivo* after the establishment of leukemia to mimic a therapeutic situation, the inducible deletion of *Cdk8* significantly prolonged survival of the affected animals. In contrast to our expectations, inhibitors targeting CDK8 kinase activity failed to mimic the effects of CDK8 knock-down. RNASeq validated these findings; whereas CDK8 kinase inhibition has minimal effects deletion of the protein induced major transcriptional changes including alteration in the mTOR, PI3K and NF-kB pathway. Accordingly, CDK8-deficient cells were 20-fold more sensitive to mTOR inhibition when compared to wildtype. These findings were reflected in human patient Ph$^+$ samples where the simultaneous degradation of CDK8 and inhibition of the mTOR pathway significantly decreased cell viability. This was achieved by a single molecule degrading CDK8 and targeting mTOR.
Summary and Conclusions: CDK8 degradation prepares the ground for mTOR inhibition in Ph+ ALL which opens novel therapeutic opportunities.

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CD123 X CD3 BISPECIFIC DART® MOLECULE EFFICIENTLY ACTIVATES T CELLS TO KILL PRIMARY B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA CELLS

D. Slavkovic Lukic[1,*], J. Duell[1], J. Davidson-Moncada[2], A. Schlagowsky[1], H. Einsele[1], M. Szczepanowski[3], D. Schewe[4], M. Brüggemann[3], M.S. Topp[1]

[1]Medizinische Klinik und Poliklinik II, Universitätsklinikum Würzburg, Würzburg, Germany, [2]MacroGenics, Rockville, United States, [3]Medizinische Klinik und Poliklinik II, [4]Klinik für Kinder- und Jugendmedizin I, Universitätsklinikum Schleswig-Holstein Campus Kiel, Kiel, Germany

Background: Targeting tumor antigens and recruiting T cells by bispecific antibodies has a great potential in cancer immunotherapy. Flotetuzumab, a CD123 X CD3 bispecific DART molecule, is currently being evaluated in a Phase 1 clinical study (NCT02152956) for treatment of acute myeloid leukemia (AML). In addition to AML, CD123 antigen is expressed in B-cell precursor cell acute lymphoblastic leukemia (B-ALL) blasts and therefore is a promising antigen for treatment of B-cell precursor ALL.
Aims: To evaluate *in vitro* activation, proliferation, and cytotoxicity of T cells by flotetuzumab in the presence of B-ALL cell lines or primary B-cell precursor ALL.
Methods: Human T cells were isolated from healthy donors and incubated with flotetuzumab (0.01 to 100 ng/ml) or control DART molecule (100 ng/ml) and ALL cell lines (E:T ratio 1:1) without exogenous cytokines. T-cell activation and cytokine production were measured after 24h. Proliferation was examined by CFSE dilution and cytotoxicity by 7AAD-AnnexinV FACS staining. To test the potential of patient T cells to kill primary tumor cells we incubated bone marrow material of 7 B-ALL patients with flotetuzumab for 6 days without addition of cytokines. The number of T cells and blasts was determined on day 2, 4 and 6. To evaluate the efficacy of flotetuzumab in the presence of drugs commonly used to treat or described to control cytokine release syndrome, T cells or bone marrow samples were preincubated with dexamethasone, tocilizumab or JAK1 and JAK2 inhibitor ruxolitinib (1h, 37°C).
Results: Flotetuzumab induced dose-dependent T-cell activation, proliferation and cytotoxicity. In the presence of ALL cell lines maximal T-cell activation was achieved with 1ng/ml flotetuzumab (Kasumi-2: 82.6±9.1% CD3$^+$CD25$^+$, *vs* 4.1±2.1% with control DART, p=0,00014, n=3). This amount of flotetuzumab resulted in significant proliferation of T cells 96h after incubation with Kasumi-2 (90.2±3.7, p=0,000002) or Nalm6 (84.9±7.6, p=0,00049) cell lines. At E:T ratio 1:1 and 1ng/ml flotetuzumab 51,6±9,7% Kasumi-2 cells were 7AAD+Annexin+, while no killing of K562 cell line was observed under the same conditions. Incubation of ALL patients' bone marrow samples with flotetuzumab resulted in response in 5 of 7 tested samples (up to E:T ratio of 0.025:1). After 6 days T cell number significantly increased (22.3±14.9 fold *vs* control group 0.6±0.3, p=0.01, n=5) and the number of blasts decreased for 99.2±0.4% (*vs* 49.4±33.1% in control group, p=0.01, n=5). Since one of the most common side effects of bispecific antibodies is cytokine release syndrome (CRS) we tested the efficacy of flotetuzumab in the presence of anti-CRS agents. 300 nM ruxolitinib significantly reduced cytotoxic potential of T cells 48h after their incubation with Kasumi-2 cell line and flotetuzumab compared to the same amounts of dexamethasone or tocilizumab (p=0.02). Similarly, dexamethasone and tocilizumab didn't influence the capacity of patients' T cells to kill their own tumor cells. However, ruxolitinib resulted in increased percentages of tumor cells due to reduced proliferation capacity and/or cytotoxic activity of T cells. Both ruxolitinib and dexamethasone reduced production of IL-6 while the effect on other cytokines was inconsistent. As expected, tocilizumab didn't change the production of cytokines.
Summary and Conclusions: These results demonstrate that targeting CD123 antigen in ALL tumors with flotetuzumab may be a promising strategy for ALL treatment. In addition, we show that dexamethasone and tocilizumab do not reduce the efficacy of flotetuzumab.

PF159

POTENT PRECLINICAL ACTIVITIES OF CD9 ANTIBODIES AGAINST HIGH-RISK PEDIATRIC B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

K.T. Leung[1,*], C. Zhang[1], K.Y.Y. Chan[1], J.T.K. Cheung[1], K. Li[1], C.K. Li[1]

[1]Paediatrics, The Chinese University of Hong Kong, Shatin, Hong Kong

Background: B-precursor acute lymphoblastic leukemia (B-ALL) is the most common malignancy and the leading cause of cancer-related death in children. Despite advances in risk-adapted multi-agent chemotherapy, specific subtypes of high-risk and relapsed/refractory B-ALL are still associated with dismal outcomes, underscoring the need for development of novel and effective therapeutic strategies.
Aims: CD9, a tetraspanin family protein, regulates multiple physiologic processes and has been associated with metastasis and disease progression in various types of cancers. We recently reported that CD9 expression was associated with inferior 5-year overall and relapse-free survival in a cohort of pediatric B-ALL patients (Leung *et al.*, EHA 2017). In this study, we aim to comprehensively evaluate the preclinical efficacy and mechanisms of targeting CD9 with neutralizing antibodies as a new treatment strategy for childhood B-ALL.
Methods: NOD/SCID mice were transplanted with patient-derived B-ALL blasts or cell lines, and randomized to receive CD9 antibody or IgG control, in addition to conventional chemotherapy consisting of vincristine, dexamethasone and L-asparaginase. Leukemic burden was assessed by bioluminescence imaging or flow cytometric identification of human CD45+CD19+ cells in murine bone marrow, spleen, blood and liver. Animals transplanted with cord blood CD34+ cells were used to evaluate the effect of CD9 antibody on normal hematopoiesis. Leukemic cell proliferation and apoptosis were measured in cultures with or without bone marrow stroma by Trypan blue exclusion and Annexin V-7AAD staining, respectively. Co-immunoprecipitation assay was performed in B-ALL cell lines to identify CD9-binding proteins.
Results: Administration of CD9 antibody as a single agent substantially reduced multi-organ leukemic burden by >90% and significantly prolonged survival of animals xenografted with the intermediate-risk *TCF3-PBX1*+ 697 and high-risk *MLL-AF4*+ RS4;11, but not the standard-risk *ETV6-RUNX1*+ Reh cell lines. In addition, CD9 blockade effectively suppressed disease progression of patient-derived xenografts of high-risk and relapsed/refractory B-ALL with distinct cytogenetic features, including *MLL*-rearrangements, *TCF3-HLF* translocation and hypodiploidy. We also showed that CD9 antibody did not affect overall and multilineage engraftment of cord blood hematopoietic stem cells, which could be attributed to low expression of CD9 in the primitive CD34+CD38- subpopulation. Combining CD9 antibody with conventional chemotherapy further reduced leukemic burden and prolonged animal survival, when compared with animals treated with CD9 antibody or chemotherapy alone. *In vitro*, CD9 blockade significantly decreased proliferation of leukemic cells and enhanced vincristine-, dexamethasone- and L-asparaginase-induced apoptosis in both mono- and stromal co-cultures. Mechanistic studies revealed that CD9 was functionally involved in leukemia-stroma interaction through binding and modulating the affinity of integrin very late antigen-4.

Summary and Conclusions: Our results collectively suggest that CD9 blockade, in adjunct to chemotherapy, could be a novel and promising strategy for treatment of high-risk and relapsed/refractory pediatric B-ALL, possibly mediated by disruption of leukemia-stroma interaction in the bone marrow microenvironment.

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PRECLINICAL DEMONSTRATION OF INTRACELLULAR ACTIVITY OF ASPARAGINASE ENCAPSULATED IN RED BLOOD CELLS BOTH IN THE ABSENCE AND IN THE PRESENCE OF NEUTRALIZING ANTI-ASPARAGINASE ANTIBODIES

K. Aguera[1,*], A. Scheer[1], F. Gallix[1], F. Tavernier[1], F. Gay[1], F. Horand[1], O. Leveneur[2], T. Mezzasalma[3], K. Swart[4], I. El Hariri[1]

[1]Erytech Pharma, [2]Institut Claude Bourgelat (ICLB), Lyon, [3]Amatsigroup, Fontenilles, France, [4]PAREXEL, Durham, United States

Background: L-asparaginase (ASNase) is an integral component of chemotherapy for acute lymphoblastic leukemia (ALL) in children and adults. ASNase is an enzyme, usually derived from the bacteria *E. coli*, that hydrolyzes asparagine (ASN) to aspartic acid. This lowers the plasma levels of ASN required for protein synthesis but no critical minimum value for efficacy has yet been established. Eryaspase is an ASNase encapsulated in human red blood cells (RBCs). Following infusion of eryaspase, ASN is actively transported into RBCs where it is hydrolyzed by the encapsulated ASNase (confirmed by previous *in vitro* studies). An open-label Phase 2/3 clinical study in 80 children and adults with relapsed or refractory ALL assessed the safety and efficacy of eryaspase compared with native ASNase. Results showed prolonged ASNase activity for eryaspase compared with native ASNase. This prolongation activity is believed to be attributable to the intracellular ASNase.
Aims: The aim of preclinical studies was to further validate the mechanism of action of eryaspase to evaluate hypothesis that ASNase activity of eryaspase is protected against degradation by encapsulation. The further objective was to elucidate the relative contribution of the intracellular and the extracellular ASNase to the depletion of ASN to low or undetectable levels.
Methods: Extracellular ASNase activity provided by eryaspase was silenced using neutralizing anti-ASNase antibodies (nAbs) *in vitro* and *in vivo.* An *in vivo* experiment was first performed in mice. The pharmacokinetic and pharmacodynamic properties of mouse RBCs (mRBCs) encapsulating ASNase were assessed in the presence or absence of nAbs (15 mice per arm). In addition, the level of ASNase activity and ASN depletion were measured in both whole blood and plasma. Mice welfare was also assessed. During the *in vitro* experiments, human RBCs encapsulating ASNase (eryaspase) were exposed to nAbs under conditions providing long-term stability of RBCs for up to 4 hours. Hemoglobin level, ASNase activity and amino-acid concentrations were measured.
Results: *In vivo* results showed that after 6 hours of exposure to free ASNase, only 12% of ASNase activity could be detected and, after 24 hours, no significant ASNase activity was detectable. In the presence of nAbs, no significant ASNase activity could be measured at any time point. In contrast, mRBCs encapsulating ASNase displayed a sustained ASN depletion (≥74%) for at least 9 days, independent of the presence or absence of nAbs. In a previous *in vivo* experiment, similar levels of ASN depletion were observed and maintained for up to 19 days. The data demonstrate that sustained ASN depletion was associated with the encapsulated ASNase, as any free ASNase activity was inhibited using nAbs. These findings were later confirmed by the *in vitro* experiments using eryaspase.
Summary and Conclusions: Eryaspase was demonstrated to effectively reduce ASN levels, also in the presence of nAbs. Silencing of free ASNase using nAbs demonstrated that the activity of eryaspase is attributed to intracellular ASNase, which must therefore be responsible for the sustained effect on ASN reduction. Eryaspase may provide a more effective approach for the delivery of ASNase in patients with ALL, especially in patients who have developed nAbs.

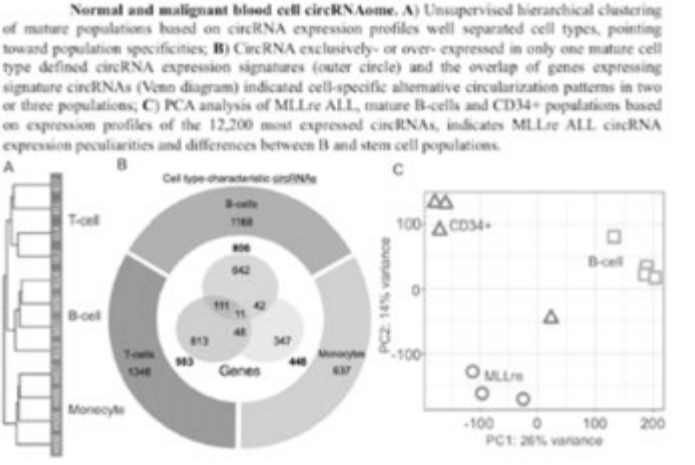
PF161

CIRCULAR RNAOME VARIATION IN NORMAL HEMATOPOIESIS AND MLL REARRANGED ACUTE LYMPHOBLASTIC LEUKEMIA

S. Bortoluzzi[1,*], E. Gaffo[1], E. Boldrin[2], A. Dal Molin[1], S. Bresolin[3], L. Trentin[3], C. Frasson[3], P. Guglielmelli[4], A. Vannucchi[4], K.-M. Debatin[2], G. Basso[3], L.H. Meyer[2], G. te Kronnie[3]

[1]Department of Molecular Medicine, University of Padova, Padova, Italy, [2]Department of Pediatrics and Adolescent Medicine, Ulm University Medical Center, Ulm, Germany, [3]Department of Women's and Children's Health, University of Padova, Padova, [4]CRIMM, Center for Research and Innovation of Myeloproliferative Neoplasms, AOU Careggi, Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

Background: Circular RNAs (circRNAs) are transcripts in which the splice donor site is covalently bound to an upstream acceptor site. Functionally, circRNAs can act as microRNA sponges controlling key microRNA-involving axes, interact with RNA-binding proteins, and regulate cellular processes. CircRNAs can be translated into biologically active peptides not encoded by linear transcripts. CircRNAs are expressed in all human tissues, including the hematopoietic compartment (*Bonizzato et al. BCJ 2016*). Even if investigation of circRNA expression in hematopoietic malignancies is in its infancy, there is evidence of circRNA deregulation in acute myeloid leukemia and of oncogenic potential of fusion-derived circRNAs in cells with chromosomal translocations.
Aims: We aimed to describe circRNAome variations in normal haematopoiesis and to disclose how circRNAs are affected in MLL rearranged (MLLre) acute lymphoblastic leukemia (ALL), an aggressive malignancy of childhood.
Methods: High depth RNA-seq was performed in B-cells, T-cells, monocytes and CD34+ cells from healthy donors (four replicates per population), and in three MLLre specimens. The CirComPara pipeline (*Gaffo et al. ncRNA J. 2017*) was used to detect circRNAs applying four algorithms, and to compare circular abundance to linear gene expression. Expression of selected circRNAs was validated by RT-PCR in PBMCs from healthy donors and in BCP-ALL samples; circularity and backsplice junctions were confirmed by RNase R treatment and Sanger sequencing.
Results: Considering all the mature blood cell populations, over 36,000 circRNAs were identified, 34% of which were novel. Notably, new circRNA-genes were uncovered. Alternative circularization was frequent: over 67% of circRNA host genes expressed multiple circular isoforms, and 33% produced 5 up to 78 circRNAs. CircRNA and linear gene expression were in general poorly correlated. CircRNA expression was sufficient to discriminate the populations (Figure 1A). Over 5,000 circRNAs differentially expressed between mature cell types were uncovered, mostly (88%) not deriving from differentially expressed genes. CircRNA expression signatures (1,168 circRNAs of B-cells, 1,346 of T-cells, and 637 of monocytes) were defined and revealed cell population specific patterns of alternative circularization (Figure 1B). Several circRNAs were validated, including newly uncovered ones and others expressed by the leukemia-associated genes *IKZF1* and *PAX5*. To identify specificities of leukemic cells, we then analysed the circRNAs expressed in MLLre ALL, mature B-cell and CD34+ populations. MLLre samples clustered together and apart from both the B- and CD34+ cell samples (Figure 1C). Of 12,200 highly expressed circRNAs, 15% were specific of MLLre samples and conversely, 9% were specific of B-cells (8%), CD34+ cells (0.3%), or expressed in both (0.7%) but not in malignant cells. Considering circRNAs expressed in both normal and malignant cells (76%), striking differences of circRNA expression in MLLre samples emerged. CircRNAs with MLLre-characteristic expression belonged to known leukemia-associated loci, non-coding RNA genes, and newly detected circRNA genes that were validated in this study.



Normal and malignant blood cell circRNAome. A) Unsupervised hierarchical clustering of mature populations based on circRNA expression profiles well separated cell types, pointing toward population specificities; **B)** CircRNA exclusively- or over- expressed in only one mature cell type defined circRNA expression signatures (outer circle) and the overlap of genes expressing signature circRNAs (Venn diagram) indicated cell-specific alternative circularization patterns in two or three populations; **C)** PCA analysis of MLLre ALL, mature B-cells and CD34+ populations based on expression profiles of the 12,200 most expressed circRNAs, indicates MLLre ALL circRNA expression peculiarities and differences between B and stem cell populations.

Figure 1.

Summary and Conclusions: We provide here an unprecedented view of the circRNAome complexity in different blood cell populations, uncovering new circRNAs potentially involved in leukemogenesis.

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TARGETING G9A/EHMT2 IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

A. Montanaro[1,*], S. Kitara[2], R. Fioretzaki[1], A. Su[2], A. Gherli[1], J. Jin[3], F. Quani[14], F. Aversa[14], B. Knoechel[5], K. Stegmaier[2], G. Roti[14]

[1]University of Parma, Parma, Italy, [2]Dana-Farber Cancer Institute, Boston, [3]Department of Pharmacological Science, Icahn School of Medicine at Mt. Sinai, New York, United States, [4]Hematology, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy, [5]Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, United States

Background: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive subgroup of genetically heterogeneous lymphoid neoplasms caused by the oncogenic transformation of immature T-cell progenitors. Although T-ALL is potentially curable in children, in adults the outcome is dismal especially for relapsed and refractory disease suggesting the urgent need for novel agents and cell therapies for poor responders. Landscape genomic studies have identified somatic alterations in genes involved in DNA methylation and post-translational histone modifications in ALL, suggesting emerging opportunities for novel therapeutic strategies.
Aims: In this study, we identified G9a/*EHMT2* as a potential target in T-ALL. G9a/*EHMT2* and GLP/*EHMT1* are conserved protein lysine methyltransferases that localize in euchromatin regions and control transcriptional repression. Despite recent evidence that G9a is involved in several tumor types, the role of the G9a/GLP complex has not been systematically studied in T-ALL.
Methods: To date several inhibitors of protein methyltransferase (PMTs), histone methyltransferases (HMTs) and demethylases have been reported, including those that target G9a and GLP with high specificity. In our initial experiments, the G9a inhibitors BIX01294 and UNC0638 demonstrated an enhanced activity in a panel of T-ALL cell lines compared to other tumors. Next, the dependency of T-ALL on *EHMT2* was validated by the intersection of an epigenome-centered shRNA screen and low throughput sgRNA CRISPR studies targeting the enzymatic domain of G9a (SET). Finally, we focused on the phenotypic consequences of G9a loss at cellular and metabolic level.
Results: Because the response to targeted therapies correlates with the transcriptional activation of cancer driver genes, we analyzed several gene expression databases including the Differentiation Map, the Cancer Cell Line Encyclopedia, and primary T-ALL dataset. Collectively these results suggest that *EHMT2* is a marker of lymphoid differentiation and it is differentially expressed in T-ALL compared to other cancer subtypes or normal bone marrow cells. Furthermore, T-ALL was the most dependent cancer on G9a suppression in the Genomic of Drug Sensitivity Database supporting our initial observation. Competitive inhibition of G9a with G9a suppressors BIX01294, UNC0638, and UNC0642 impaired T-ALL proliferation and induced apoptosis. Consistent with this observation, sgRNA-CRISPR guided loss of G9a resulted in altered cellular proliferation and induction of cell death. Interestingly G9a suppression triggered autophagy as measured by LC3-II immunostaining and cytoplasmic vacuoles formation. Because autophagic vacuoles frequently entrapped glycoproteins, we speculated as to whether vacuoles contained glycogen. We next stained T-ALL treated cells with the periodic acid-Schiff and demonstrated the accumulation of the insoluble magenta complex (PAS reaction) in the cytoplasm of treated T-ALL cell lines. Electron microscopy analysis confirmed the deposition of intracellular glycogen granules. Next, we hypothesized that G9a might control glycogen synthesis. Thus we measured the activity of Glycogen Synthase Kinase-3 (GSK3) by western blotting and observed an increase of the inhibitory site phosphorylated Ser9-GSK3β upon drug treatment consistent with the inhibition of GSK kinase activity.
Summary and Conclusions: In this study, the intersection of multiple "omics" approaches led to the identification of *EHMT2*/G9a as a new druggable target in T-ALL. Our data support the notion that G9a controls GSK kinases, suggesting an epigenetic control of glycogen metabolism in T-ALL.

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INTEGRATED GENETIC AND EPIGENETIC ANALYSIS ELUCIDATED EXPRESSION AND METHYLATION PROFILES OF ACUTE LYMPHOBLASTIC LEUKEMIA IN DOWN SYNDROME

Y. Kubota[1,*], K. Uryu[1], T. Ito[2], T. Kawai[3], M. Seki[1], T. Isobe[1], T. Toki[2], K. Yoshida[4], K. Kataoka[4], Y. Shiraishi[5], K. Chiba[5], H. Tanaka[5], S. Miyano[5], A. Oka[1], Y. Hayashi[6], S. Ogawa[47], K. Terui[2], A. Sato[8], K. Hata[3], E. Ito[2], J. Takita[1]

[1]Department of Pediatrics, The University of Tokyo, Tokyo, [2]Department of Pediatrics, Hirosaki University Graduate School of Medicine, Hirosaki, [3]Department of Maternal-Fetal Biology, National Research Institute for Child Health and Development, Tokyo, [4]Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, [5]Laboratory of DNA Information Analysis, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, [6]Department of Hematology and Oncology, Gunma Children's Medical Center, Maebashi, Japan, [7]Department of Medicine Center for Hematology and Regenerative Medicine, Karolinska Institutet, Huddinge, Sweden, [8]Department of Hematology and Oncology, Miyagi Children's Hospital, Sendai, Japan

Background: Children with Down syndrome (DS), which caused by an extra copy of chromosome 21, have a 20-fold increased risk for acute lymphoblastic leukemia (ALL) compared with non-DS children. Recent genome-wide studies have revealed that DS-ALL has uncommon genetic alterations such as mutations in *JAK2*, *NRAS*, and *KRAS*, mutations or overexpression of *CRLF2*, suggesting the unique biological features of DS-ALL distinct from non-DS ALL. However, most of previous studies regarding of molecular analysis of DS-ALL have been limited to genomic and transcriptomic characterizations, and no study showed comprehensive genetic/epigenetic overview in DS-ALL.
Aims: To get better understanding of the molecular pathogenesis of DS-ALL, we conducted integrated genetic/epigenetic analyses.
Methods: Our cohort included 179 pediatric B-cell precursor ALL samples (61 DS-ALL and 118 non-DS-ALL). We performed targeted deep sequencing for seven ALL-related genes in 61 DS-ALL, SNP array analysis in 43 DS-ALL, and whole transcriptome sequencing (WTS) in 25 DS-ALL and 118 non-DS-ALL samples. Additionally, methylation array analysis was performed in 36 DS-ALL, 17 non-DS-ALL, and 20 DS patients without ALL.
Results: Targeted deep sequencing revealed frequent gene mutations of *JAK2* (23%) and *RAS* (31%) pathway. SNP array analysis elucidated several recurrent chromosomal deletions affecting actionable gene loci; *IKZF1* (33%), *PAX5* (30%), *EBF1* (15%), *CDKN2A* (34%), and *RB1* (7%). Gains of whole chromosome X (18%) and 8 (10%) were also recurrently identified. Expression profiling stratified 143 samples into 6 clusters (E1-E6 clusters), which were well correlated with biological subtypes of ALL. Intriguingly, DS-ALL cases were clustered into four clusters, suggesting the heterogeneity of DS-ALL. Cluster E3 included one DS-ALL, which was clustered into the branch of *PAX5*-rearranged cases. SNP array analysis revealed that this case had focal amplification of *PAX5*. All DS-ALL cases with *ETV6-RUNX1* fusion were classified into cluster E4, which also included all *ETV6-RUNX1* fusion cases of non-DS-ALL. This cluster included one DS-ALL case without *ETV6-RUNX1* fusion. This particular case had a homozygous deletion of *ETV6*, implicating *ETV6-RUNX1*-like signature. DS-ALL with high hyperdiploidy (HeH) were classified into cluster E5, which also included most of HeH samples of non-DS-ALL. Among other 5 DS-ALL cases in E5, 2 cases showed *IGH-CEBPA* fusion and *IGH-CEBPD* fusion, respectively. Cluster E6 was characterized by *BCR-ABL1* fusion and Ph-like expression profiles. In our cohort, we identified 7 DS-ALL cases with Ph-like signature, which included all *JAK2* mutated samples. In E6, *PAX5* mutation and *IKZF1* mutation were detected in 2 DS-ALL cases, respectively. DNA methylation array analysis suggested DS-ALL samples were also heterogeneous. Same as expression analysis, DNA methylation analysis revealed DS-ALL samples were clustered corresponding to each subtype same as non-DS-ALL. In addition, DNA methylation array analysis revealed unique hypermethylation of promoter regions of *RUNX1* in DS-ALL. DNA hyper methylation of this region was confirmed in normal B-cell of DS without ALL, but not in non-DS-ALL.
Summary and Conclusions: We confirmed DS-ALL was highly heterogeneous due to inclusion of many subtypes, and each subtype had similar expression and methylation profiles as non-DS-ALL. Hypermethylation of *RUNX1* in chromosome 21 was a unique characteristic in DS patients and may be associated with an increasing incidence of ALL in DS patients.

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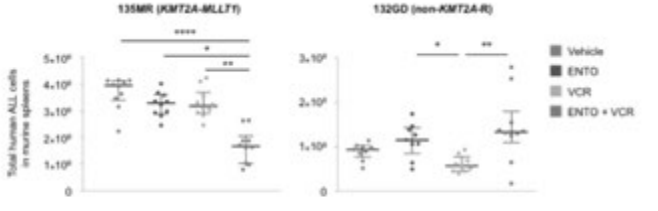
ENHANCED EFFICACY OF THE SYK INHIBITOR ENTOSPLETINIB AND VINCRISTINE IN KMT2A-REARRANGED ACUTE LYMPHOBLASTIC LEUKEMIA

J.P. Loftus[1], A. Yahiaoui[2], F. Shen[1], C. Hurtz[3], P.A. Brown[4], S. Tannheimer[2], A. Forslund[2], S. Tasian[56,*]

[1]Division of Oncology and Center for Childhood Cancer Research, Children's Hospital of Philadelphia, Philadelphia, [2]Gilead Sciences, Foster City, [3]Department of Medicine, Division of Hematology/Oncology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, [4]Pediatrics, Division of Oncology, John Hopkins University and Sidney Kimmel Comprehensive Cancer Center, Baltimore, [5]Division of Oncology and Center for Childhood Cancer Research, Children's Hospital of Philadelphia, [6]Pediatrics, Perelman School of Medicine at the University of Pennsylvania and Abramson Cancer Center, Philadelphia, United States

Background: Survival of infants with *KMT2A*-rearranged (R) B-cell acute lymphoblastic leukemia (ALL) remains dismal despite intensive chemotherapy. We previously reported constitutive phosphorylation of spleen tyrosine kinase (SYK) in infant ALL specimens (Yahiaoui EHA 2017, Loftus ASH 2017) and hypothesized that the selective SYK inhibitor (i) Entospletinib (ENTO) would inhibit activated kinase signaling *in vitro* and leukemia burden *in vivo*. We further predicted that combination treatment with ENTO and chemotherapy would have greater activity against *KMT2A*-R ALL.
Aims: (**1**) Determine effect of combined ENTO and vincristine (VCR) treatment of *KMT2A*-R and non-R ALL patient-derived xenograft (PDX) models *versus* monotherapy and (**2**) Identify constitutive signaling activation in infant ALL cells and potential biomarkers of ENTO sensitivity.
Methods: We first measured total and phosphorylated (p) SYK and SRC family kinases (SFK), FLT3, Ras/MAPK, JAK/STAT, PI3K/mTOR, and pre-BCR-associated proteins via Simple Western (SW) analysis of murine splenic lysates from infant *KMT2A*-R (n=8), infant non-*KMT2A*-R (n=3), and adult *KMT2A*-R (n=1) ALL PDX models. We then incubated ALL PDX cells *in vitro* with clinically-relevant concentrations of ENTO to assess abrogation of kinase signaling via SW and alterations in gene expression by NanoString analysis compared to non-ENTO-treated ALL cells. Finally, we treated ALL PDX models *in vivo* with control or ENTO chow *ad libitum*, VCR intraperitoneally once weekly, or both ENTO and VCR for 14-28 days and quantified human ALL burden in harvested murine tissues. Additional ALL PDX mice were treated with ENTO for 3 days to assess pharmacokinetic and pharmacodynamic effects of SYK inhibition.
Results: Basal kinase signaling activation differed among *KMT2A*-R and non-R ALLs and stratified by genetic subgroup (*KMT2A-AFF1* from t(4;11), *KMT2A-MLLT3* from t(9;11), *KMT2A-MLLT1* from t(11;19), other). *In vitro* incubation of *KMT2A*-R ALL cells with ENTO inhibited SYK pathway signaling, pAKT, and pERK. *In vivo* treatment of PDX models with ENTO showed sensitivity in 4 of 6 *KMT2A*-R and no activity in non-*KMT2A*-R ALL models. ENTO responses occurred in 1 RAS-wild-type *KMT2A-AFF1*, 1 *KMT2A-MLLT3*, 2 *KMT2A-MLLT1* ALL PDX models, but not in 2 RAS-mutant *KMT2A-AFF1* models. Greater inhibition of ALL burden with combined ENTO+VCR treatment was further observed in 2 of the 4 ENTO-sensitive *KMT2A*-R models *versus* single-agent ENTO or VCR (Figure 1). Enhanced sensitivity to ENTO/VCR appeared to occur in ALL PDXs with some surface Igm expression (pre-B lymphoblasts) and increased *HOXA9* and *MEIS1* gene expression, while mimimal response to combination treatment occurred in leukemias with concomitant *NRAS* or *KRAS* mutations. Gene expression analyses of *KMT2A*-R ALL cells from control and ENTO-treated mice also showed SYKi-induced downregulation of genes involved in DNA repair.



***In vivo* activity of entospletinib and vincristine in *KMT2A*-R and non-*KMT2A*-R ALL PDX models.** Human CD45+ CD19+ ALL cells were quantified by flow cytometry in murine spleens after 14 days of treatment. *p <0.05, **p <0.01, ****p <0.0001 by 1-way ANOVA Kruskal-Wallis with Dunn's multiple comparison test.

Figure 1.

Summary and Conclusions: Constitutive activation of SYK signaling occurs in multiple genetic subtypes of *KMT2A*-R ALL. We observed decreased SYK and other kinase signaling in *KMT2A*-R ALL treated with ENTO *in vitro* and, as well as potent ENTO-induced inhibition of leukemic burden *in vivo* in *KMT2A*-R R ALL PDX models. Combined ENTO and VCR treatment further enhanced anti-leukemia effects in some models. Clinical study of entospletinib and chemotherapy specifically in patients with *KMT2A*-R leukemias may be warranted.

PF165

NOVEL TARGETED THERAPIES FOR RESISTANT CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

S. Bhatia[1,*], V. Krieger[2], H. Ahlert[1], D. Diedrich[3], B. Frieg[3], N. Ressing[2], A. Herrlich[1], A. Borkhardt[1], H. Gohlke[3], T. Kurz[3], F.K. Hansen[2], J. Hauer[1]

[1]Department of Pediatric Oncology, Hematology and Clinical Immunology, Heinrich Heine University, Duesseldorf, [2]Institute for Pharmaceutical and Medicinal Chemistry, Leipzig University, Leipzig, [3]Institute for Pharmaceutical and Medicinal Chemistry, Heinrich Heine University, Duesseldorf, Germany

Background: During recent decade, the event-free survival of childhood acute lymphoblastic leukemia (ALL) has improved up to 80%, but there are still subgroups with a dismal prognosis. This potentiates the need to develop novel therapeutic compounds, with a low toxicity profile. We, therefore, aimed to develop and characterize novel precision compounds, which target oncogene stabilization by HSP90 and by the proteasome-HDAC6 axis.
Aims: We developed an HSP90 inhibitor (aminoxyrone) which is active as a pan-leukemia inhibitor against LSCs without the induction of any HSR and a dual proteasome-HDAC6 hybrid inhibitor (RTS-V), with sensitivity in BCR-ABL1+ TKI resistant BCP-ALL.
Methods: The specificity of aminoxyrone was evaluated by microscale thermophoresis (MST), cell-based luciferase refolding assay, 2D NMR spectroscopy, circular dichroism (CD) spectroscopy, analytical ultracentrifugation and molecular dynamics simulations.
Co-crystal structure determination was performed to analyze the binding mode of RTS-V into the β5/β6 active site of 20S yeast proteasome.
Results: HSP90 act as molecular chaperone and is highly expressed in several therapy-resistant leukemia subtypes thereby ensuring correct protein folding of several oncogenic proteins such as BCR-ABL1, FLT3-ITD and AKT. Therefore, targeting HSP90 could be a promising option in the treatment of therapy-refractory leukemia. Majority of available HSP90 inhibitors target the N-terminal domain thereby induce a protective mechanism called heat shock response (HSR), which potentially weakens the cytotoxic effect of HSP90 inhibitors and induce toxicity. We have developed first in class HSP90 inhibitor 'aminoxyrone' through structure-based molecular design and chemical synthesis which specifically targets C-terminal dimerization of HSP90. Aminoxyrone is effective in preclinical TKI (2nd and 3rd generation) resistant cell line models *in vitro* and *in vivo*, induces apoptosis in primary BCR-ABL1+ BCP-ALL and in Ph-like BCP-ALL patient-derived LSCs, without inducing any HSR. We furthermore developed a novel dual proteasome-HDAC6 hybrid inhibitor 'RTS-V'. Aggresomes are used by many malignant cells as an alternative route to degrade proteins that accumulate after proteasome inhibition. However, this mechanism depends on HDAC6 to transport ubiquitinated proteins by microtubules. Thus, inhibition of the proteasome and HDAC6 results in synergistic anticancer activity by induction of apoptosis in cancer cells. We found out that RTS-V specifically blocks chymotrypsin-like proteasome activity and at the same time causes preferential inhibition of HDAC6. The screening of RTS-V in more than 20 cell lines (including TKI-resistant BCP-ALL) and in primary relapse BCP-ALL samples revealed >20-fold higher specificity of RTS-V against leukemic cells as opposed to healthy PBMNCs. Besides that, we could show that in selected leukemic cell lines RTS-V specifically inhibits cell proliferation, induces apoptosis, accumulates cells in S phase, induces early differentiation and inhibits colony formation. Our next aim would be to test its efficacy in *in vivo* studies.
Summary and Conclusions: Taken together, 'aminoxyrone' and 'RTS-V' represents a promising starting point for future efforts towards the development of novel targeted inhibitors to overcome drug resistance and reduce toxicity, especially for the treatment of relapsed/refractory ALL.

PF166

DEHYDROPEPTIDASE 1 ENHANCED THE GROWTH AND SURVIVAL OF LEUKEMIA CELLS *IN VIVO* AND IDENTIFIED A HIGH RISK OF RELAPSE IN PATIENTS WITH COMMON B-CELL ALL

J.-M. Zhang[1,*], S.-J. Wang[1], J. Zhang[1], Y. Xu[1], L.-X. Wu[1], X.-J. Huang[1], K.-Y. Liu[1], G.-R. Ruan[#1]

[1]Peking University People's Hospital, Beijing, China

Background: Relapse remains the major cause of therapy-failure in adults with B-cell acute lymphoblastic leukaemia (ALL). Many pre-therapy variables correlate with relapse risk but a substantial part of the uncertainty in predicting relapse is unknown and new variables independently-correlated to relapse risk are needed. Dehydropeptidase-1 (DPEP1) is a zinc-dependent metalloproteinase. Abnormal DPEP1 expression is implicated in many neoplasms. High DPEP1 transcript levels are reported in persons with B-cell ALL compared with normal, but its role, if any, in adults B-cell ALL is unclear.
Aims: To investigate the role of DPEP1 in leukemia growth *in vivo* and compare the outcome of common B-cell ALL depending on the transcription level of DPEP1.
Methods: A xeno-transplant mouse model was used to assess effects of DPEP1 on tumor development. Bone marrow samples were obtained from

adults with Common B-cell ALL (N=87) and normal individuals (N=28) recruited at the Hematology Department of Peking University People's Hospital from March, 2009 to March, 2016. Subjects achieving complete remission were analyzed for cumulative incidence of relapse (CIR) and relapse free survival (RFS).
Results: In subjects with common-B-cell ALL, high transcript levels of DPEP1 was associated with a higher 5-year CIR (55%, [40, 69%] *vs* 23% [10, 35%]; P=0.021) and worse 5-year RFS (39% [24, 53%] *vs* 73% [60–86%]; P=0.037) compared with subjects with low transcript levels. This correlation was detected in subjects receiving chemotherapy (N=32; CIR: HR=5.09 [1.546, 16.78]; P=0.007) but not in those who also received an allotransplant (N=55; CIR: HR=1.52 [0.42, 5.41]; P=0.517). In subjects with common B-cell ALL, a high DPEP1 transcript level was independently-associated with CIR (HR=3.17 [1.41–7.14]; P=0.005) and RFS (HR=2.84 [1.34–6.02]; P=0.006) in multivariate analyses. Knockdown (KD) of DPEP1 in BV173, a B-cell ALL cell line reduced proliferation and survival in the presence of chemotherapy. In contrast, DPEP1 over-expression (OE) had a converse effect. Xeno-transplants were done with DPEP1-OE and DPEP1-KD cells to further investigate *in vivo* effects of DPEP1 expression. Knockdown of DPEP1 markedly decreased tumor growth compared with controls. In contrast, tumors were larger in mice bearing DPEP1-OE cells compared with controls. Histological examination showed more abundant blood sinuses in DPEP1-OE cell-derived tumors but a larger necrotic area in tumors derived from DPEP1-KD cells. TUNEL staining confirmed the increased apoptosis in specimens from the DPEP1-KD group compared with those from the vector controls.
Summary and Conclusions: In summary, our data support a proliferation and pro-survival role of DPEP1 in B-cell ALL cells *in vivo* and provide the first demonstration that in adults with common-B-cell ALL, high DPEP1 transcript levels is correlated with a higher CIR and worse RFS compared with subjects with low transcript levels. This correlation is observed in patients treated with chemotherapy only but not those with an allotransplant, which give an indication that adults with common B-cell ALL and high DPEP1 expression might benefit more from an allotransplant, although this indication needs further randomized validation.

PF167

TARGETING WNT10B*R-MEDIATED AUTOCRINE WNT SIGNALING ACTIVATION IN ACUTE LYMPHOBLASTIC LEUKEMIA

A. Cassaro[1,2,*], F. Lazzaroni[1,3], I. Esposito[1], G. Reda[4], R. Cairoli[2], A. Beghini[1]

[1]Department of Health Sciences, University of Milan, [2]Hematology Unit, Niguarda Cancer Center, Milan, Italy, [3]Department of Biochemistry, University of Losanna, Losanna, Switzerland, [4]Hematology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

Background: Acute lymphoblastic leukemia (ALL) is an aggressive malignancy of hematopoietic progenitors with poor clinical outcomes. Wnt signaling is linked to many malignancies, including ALL, dysregulation of Wnt pathway has been implicated in the rising and maintenance of leukemic initiating stem cells (LICs), a subpopulation of blast cells responsible of refractory to, or relapse after chemotherapy in acute leukemia (AL) patients. Our lab has previously showed a ligand-dependent activation of Wnt pathway through a diffuse expression and release of the hematopoietic regeneration-associated molecule WNT10B in acute myeloid leukemia. The analysis of WNT10B locus revealed the presence of a recurrent rearrangement (WNT10B*R), leading to expression of the transcript variant WNT10B*IVS1 in a cohort of acute myeloid leukemia (AML) patients with intermediate/unfavourable risk. Although these evidences suggest that aberrant Wnt pathway activation may promote the development and persistence of LICs in acute leukemia, it is still unclear the mechanism of WNT10B*Rmediated activation in acute leukemic cells.
Aims: In this study, we investigated the occurrence of WNT10B*R in T- and B-ALL patients, and determined the Frizzled (FZD) receptor component/s involved in the WNT10B-mediated activation.Moreover, we evaluated if the small molecule-mediated interference of WNTs secretion by the porcupine acyltransferase inhibitor WNT974, may represent a new potential strategy to interfere with the ligand-dependent autocrine Wnt signaling.
Methods: Detection of WNT10B*R has been evaluated in 34 primary acute lymphoblastic leukemia (5 T-ALL and 29 B-ALL) cells samples. The interaction between WNT10B*IVS1 and FZD receptors was evaluated in MOLT4 model cell line, expressing the WNT10B*R as unique Wnt ligand, by proximity ligation assay (PLA) at single cell level. MOLT4 cell line was subsequently treated with increased concentration of WNT974 inhibitor and cell viability was measured by MTT assay.
Results: We detected the WNT10B*R rearrangement in 19/34 ALL patients analyzed, supporting our hypothesis of its broad involvement in the acute leukemogenesis. In order to characterize WNT10B*IVS1molecule we selected MOLT4 cell line as cell line model, since the expression analysis by RT-PCR on several myeloid and lymphoid cell lines revealed the exclusive expression of rearrangement by MOLT4. We also confirmed the expression of WNT10B*IVS1 in MOLT4 through *in situ*-mRNA detection method. In order to determine which FZD receptor/s binds WNT10B in the T-ALL cell model MOLT4 we performed specific PLA for FZD4/5/6. Interestingly, we detected high intensity signals only for the WNT10B/FZD6 ligand/receptor-interaction, the subsequent expression analysis confirms the univocal expression of FZD6 in the MOLT4. The treatment with increasing concentration of WNT974 porcupine inhibitor decreased the MOLT4 cell viability in a dose-dependent manner, suggesting the autocrine mechanism of Wnt signaling activation.
Summary and Conclusions: Overall, these preliminary data confirm the WNT10B*R -mediated autocrine Wnt signaling activation also in ALL, and identified FZD6 as the potential receptor functionally involved in the interaction with WNT10B. Thus, our studies suggest possible molecular elements in ALL cells that may need to be targeted for more durable remissions.

PF168

MIR-451 IS A NEW BIOMARKER THAT IDENTIFIES ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS WHO MAY BENEFIT FROM TREATMENT WITH NAMPT INHIBITORS

K. Shichrur[1,2,*], I. Yaniv[1,2], J. Yarden[3], S. Avigad[1,2]

[1]Pediatric Hematology Oncology, Schneider Children's Medical Center of Israel, Petah Tikva, [2]Felsenstein Medical Research Center, Tel Aviv University, Tel Aviv, [3]Curewize Health Ltd., Yokneam, Israel

Background: MicroRNAs (miRs) are a group of small noncoding RNAs that post-transcriptionally regulate gene expression by direct binding to their corresponding targets. Aberrant miR expression has been identified in various types of cancer playing essential roles in cancer initiation and progression. Thus, miRs represent a great potential as novel diagnostic and prognostic biomarkers of cancer as well as novel targets for cancer treatment. Previously we have demonstrated that low expression levels of miR-451 could act as a biomarker that could predict high risk of relapse in pediatric acute lymphoblastic leukemia (ALL) patients (Avigad *et al.* 2016).
Aims: Our aim in this study was to explore the role of miR-451 and its target in ALL.
Methods: The relevance of various miR-451 expression levels on the growth rate of leukemic cells was evaluated using a xenograft mice model. A novel target of miR-451, identified by bioinformatic analysis, was validated for direct binding and regulation by miR-451 utilizing several putative assays. The correlation between miR-451 and it's novel target was studied also in other malignancies. Using this model we explored miR-451 as a biomarker for detecting the sensitivity to a specific pathway inhibitor of its target.
Results: We demonstrated that the growth rate of leukemic cells over expressing miR-451 was significantly reduced compared to miR-451 silenced cells in the mice (p=0.03). In this study, we identified an association between miR-451 and a putative target, NAMPT (Nicotinamide Phosphoribosyltransferase) which is a key enzyme in the NAD^+ pathway. We detected a significant inverse correlation between miR-451 expression levels and NAMPT protein levels and provided evidence for the direct binding of miR-451 to NAMPT in ALL cell line. This correlation was also evident in other malignant cell lines such as breast, colon and prostate cancer cell lines. We used a mice xenograft ALL model to demonstrate the inverse correlation between miR-451 expression levels and the sensitivity to a potent NAMPT inhibitor (FK866). ALL cells expressing low levels of miR-451 demonstrated significantly increased sensitivity to treatment with the NAMPT inhibitor compared to high expressing cells. The sensitivity of each line to FK866 treatment was examined on a group of 89 mice. At the end of the experiments the mean tumor volume of the FK866 treated group injected with low miR-451 cells was 117 ±81mm^3 compared to 1104 ±134mm^3 in the non-treated group (p = 0.000118) while in the treated mice injected with high expressing cells the mean tumor volume was 544 ±64mm^3 compared to 851 ±110mm^3 in the non-treated group (p =0.028). This specific sensitivity was also evident in a prostate cancer xenograft model.
Summary and Conclusions: NAMPT was identified as a novel target of miR-451. miR-451 may play an important role in ALL progression via NAMPT regulation. Thus, miR-451 expression levels could be used as a biomarker for the identification of patients who may benefit from treatment with NAMPT inhibitors.

PF169

NOVEL PROGNOSTIC MARKER ARID5B LOW EXPRESSION IN ACUTE LYMPHOBLASTIC LEUKEMIA

Z. Ge[1], Y. Gu[1], Q. Han[1], S. Dovat[2], C. Song[2,*]

[1]Hematology, Zhongda Hospital, Southeast University Medical School, Nanjing, China, [2]Pediatrics, Penn State University College of Medicine, Hershey, United States

Background: AT-rich interactive domain-containing protein 5B (ARID5B) forms a complex with PHD finger protein 2 (PHF2) and the ARID5B-PHF2 complex then induces the demethylation of di-methylated 'Lys-9' of histone H3 (H3K9me2) leading to transcription activation target genes. Mutations and single nucleotide polymorphisms of *ARID5B* are associated with the development of acute lymphoblastic leukemia (ALL) and treatment outcome. So far there have been no reports about *ARID5B* expression in ALL and the clinical significance of *ARID5B*^{low} and *ARID5B*^{low}*PHF2*^{low}expression in ALL patients. ChIP-seq data show the obvious binding peaks of Ikaros, the leukemia suppressor in promoter of *ARID5B*.
Aims: This study is to examine clinical significance of *ARID5B*^{low} and *ARID5B*^{low}*PHF2*^{low}expression in ALL patients and the regulatory role of Ikaros on *ARID5B* expression in ALL cells.
Methods: 164 Subjects with newly-diagnosed ALL (107 B-cell and 57 T-cell ALL; 12-77 years old) were studied. The study was approved by the Ethics Committee of the institutes. Subjects were allocated in a high or low *ARID5B* expression cohort (4th quartile *vs* 1st-3rd quartiles) with a cut-off value determined by SPSS 20.0. Median and frequency differences between the cohorts were evaluated using a Mann–Whitney U-test and uni- and multivariate Cox model. Relapse-free survival (RFS) and overall survival (OS) were estimated by the Kaplan-Meier method and compared by log-rank test.
Results: We observed the *ARID5B* mRNA level is significantly down-regulated in subset of ALL patients. *ARID5B*^{low} mRNA levels were associated with leukemic cell proliferation and poor prognostic indicators. Low *ARID5B* mRNA levels were associated with a higher median percentage of bone marrow blasts (90.0% *vs* 84.6%, *P*=0.037), a higher percentage of stem cell marker CD34+(88.8% *vs* 37.5%, *P*=0.000) and myeloid marker CD33+ (48.5% *vs* 25.0%, *P*=0.046), a higher frequency of Ik6(+), the most common *IKZF1* deletion (42.5% *vs* 20.0%, *P*=0.042), and also a lower median hemoglobin and platelet count compared to patients with high *ARID5B* expression. B-ALL patients with *ARID5B*^{low} expression represented a significantly higher percentage of the cohort of patients who took more than 4 weeks to reach complete remission (CR) (51.4% *vs* 16.0%, *P*=0.002) , a poor prognostic indicator in ALL, compared to those with high *ARID5B* expression. Particularly *ARID5B* expression is positively correlated with *PHF2* expression in ALL. The *ARID5B*^{low}*PHF2*^{low} was associated with a higher percentage of bone marrow blasts (91.2% *vs* 82.4%, *P*=0.000), stem cell marker CD34+ (88.2% *vs* 55.6%, *P*=0.000), myeloid marker CD33+ (50.9% *vs* 28.6%, *P*=0.036), splenomegaly (50.0% *vs* 22.9%, *P*=0.008), a CR time ≥4 weeks (53% *vs* 21%, *P*=0.003) and higher frequency of *Ik6*(+) (49.3% *vs* 15.8%, *P*=0.001) and lower median PLT count (10^9/L) (32.0 *vs* 58.5, *P*=0.020) compared to patients with non-*ARID5B*^{low}*PHF2*^{low} cohort. The multivariable analysis confirmed the association with bone marrow blasts, *Ik6* (+) and a CR time ≥4 weeks. We also found *ARID5B* expression was significantly lower in B-ALL with Ik6(+) (0.3153±0.0938 *vs.*1.2052±0.58441, *P*=0.02439); and Ikaros directly promotes *ARID5B* expression.
Summary and Conclusions: *ARID5B*^{low}, particularly *ARID5B*^{low}*PHF2*^{low}expression is associated with leukemia proliferation and poor prognostic markers. Our results also revealed the oncogenic effect of *ARID5B*^{low}*PHF2*^{low} expression in ALL, and identified a high-risk subgroup of ALL characterized by Ikaros dysfunction and *ARID5B*^{low}*PHF2*^{low} expression.

PF170

NOVEL IKAROS TARGET RAG1 HIGH EXPRESSION IN B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

Q. Han[1], Y. Gu[1], C. Song[2], Z. Ge[1,*]

[1]Hematology, Zhongda Hospital, Medical School of Southeast University, Nanjing, China, [2]Pediatrics, Penn State University College of Medicine, Hershey, United States

Background: The recombination activating gene (RAG)-mediated recombination is the dominant mutational process and is the predominant driver of oncogenic genomic rearrangement in acute lymphoblastic leukemia (ALL). This then leads to further leukemic clonal evolution. *IKZF1* encodes a krupel-like zinc finger protein, IKAROS that is essential for normal hematopoiesis and acts as a tumor suppressor in ALL. We found Casein Kinase II (CK2) –mediated IKAROS phosphorylation is a key mechanism responsible for IKAROS dysfunction in ALL, and CK2 inhibitor CX-4945 treatment could restore IKAROS tumor suppressor function in high-risk ALL. ChIP-seg data showed obvious IKAROS binding peaks on the promoter of *RAG1* in B-ALL patients' samples, suggesting its regulation role on *RAG1* expression.
Aims: We will explore the clinical significance of *RAG1* expression in B-ALL and IKAROS regulation on *RAG1* expression in B-ALL cells.
Methods: The 131 subjects with newly-diagnosed B-ALL (age 12-77 years old) were recruited. *RAG1* mRNA level was examined by qPCR and allocated into a high or low expression cohort (1-2th quartile *vs* 3-4th quartiles) with a cut-off value determined by SPSS 20.0. Median or frequency differences between the cohorts were evaluated using a Mann–Whitney U-test or uni- and multivariate Cox mode. The retroviral gene expression, shRNA knockdown, and chromatin-immunoprecipitation are used to observe IKAROS regulation on *RAG1* transcription.
Results: We observed that *RAG1* is significantly increased in subsets of B-ALL patients. High *RAG1* expression correlates with higher percentage of white blood cell (WBC) ≥30x10^9/L (67.7% *vs* 38.5%, P<0.001), higher blasts in peripheral blood (75.0% *vs* 61.0%, P=0.026) and higher median WBC (47.9×10^9/L *vs* 17.0×10^9/L, P=0.025), the markers of poorer prognosis and proliferation in B-ALL. We found a higher incidence of *IK*6, the most common protein produced by *IKZF1* deletion in B-ALL patients with the high *RAG1* expression group (45.5% *vs* 26.2%, P=0.021); and *RAG1* mRNA level was significantly higher in patients with *IKZF1* deletion than those without. These data indicate that *IKZF1* deletion has the key role on up-regulating *RAG1* expression in primary B-ALL cells. Moreover, ChIP-seq and qChIP data showed that IKAROS directly binds to the *RAG1* promoter and regulates *RAG1* expression in leukemic cells and patients' samples. CK2 inhibitor, CX-4945 by increasing IKAROS activity significantly increases IKAROS binding in ALL cells and suppresses *RAG1* expression in an IKAROS-dependent manner. *RAG1* expression is significantly higher in patients with *IKZF1* deletion, as compared to patients without *IKZF1* deletion. Treatment with CX-4945 also results in an increase in IKZF1 binding to the *RAG1* promoter and suppression of *RAG1* expression in primary ALL cells.
Summary and Conclusions: This is the first time to demonstrate that IKAROS directly suppresses *RAG1* expression. High expression of *RAG1* correlates with high proliferation markers in B-ALL. Our data suggest *RAG1* high expression works together with IKAROS dysfunction to drive oncogenesis of B-ALL, which have significance in an integrated prognostic model for adult ALL.

PF171

S100A16 INHIBITED PROLIFERATION AND PROMOTED APOPTOSIS THROUGH REGULATION OF ERK PATHWAY IN B CELL ALL

J. Zhang[1,2,*], W.-Y. Lu[1,2], J.-M. Zhang[1,2], Y. Xu[1,2], X.-J. Huang[1,2], K.-Y. Liu[#1,2], G.-R. Ruan[#1,2]

[1]Peking University People's Hospital, Peking University Institute of Hematology, [2]Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, Beijing, China

Background: Outcome of adults with B-cell acute lymphoblastic leukemia (ALL) remains poor and relapse is the major cause of treatment-failure. Although several biomarkers of leukemia have been reported recently, the biomarker for B-cell ALL is still lack. Identifying novel biomarkers in B-cell ALL will contribute to the exploration of disease mechanisms, the refinement of prognostic stratification and the discovery of the new therapeutic targets. S100A16 is a novel member of S100 superfamily and is abnormally expressed in lots of cancers, but its role still remains controversial. In our previously conducted microarray analysis, S100A16 was identified as one of the most differentially expressed genes which were over-expressed in B-cell ALL compared with normal bone marrow, but its physiological and pathological roles in B-cell ALL are largely unclear.
Aims: To investigate the expression levels of S100A16 in adults with B-cell ALL and to explore the biological function of S100A16 in the cell lines derived from B-cell ALL.
Methods: A real-time quantitative RT-PCR was used to examine S100A16 transcript levels in bone marrow samples from 140 adults with newly diagnosed B-cell ALL, 20 adults with newly diagnosed T-cell ALL, 20 adults with newly diagnosed AML and 35 healthy donors. We constructed two B-cell lines: S100A16 over-expressing (OE) Nalm-6 cell line and S100A16 knock-down (KD) SupB15 cell line to study the biological function of S100A16 in B-cell ALL. The cell proliferation was detected by CCK-8 and the early apoptosis was analyzed by flow cytometry. Western blot was used to determine the protein level of signaling pathways.
Results: The results showed that the relative gene expression levels of

S100A16 in bone marrow from 140 newly diagnosed B-cell ALL (median 243%; range 0-10339%) were significantly higher than those from the healthy donors (1.27%; 0-5.29%; $p<0.001$), whereas no significant difference was detected in newly diagnosed T-cell ALL and AML patients compared with health donors (Figure 1A). S100A16 gene expression was higher in BCR/ABL positive B-cell ALL lines (BV173, SupB15), CML cell line (K562), but lower in EBV-LCL, BCR/ABL negative B-cell ALL lines (Nalm-6, BALL-1), T-cell ALL(6T-CEM), AML (NB4, HL-60, KG-1), NHL (Ramos, Raji, MAVER). Proliferation was decreased in S100A16-OE cells compared with cells transfected with control lentivirus (OD value: 0.131±0.003 *vs* 0.158±0.005, $p<0.05$). Conversely, silencing of S100A16 expression markedly increased the proliferation of the transfected cells (OD value: 1.534±0.032 *vs* 0.953±0.101, $p<0.05$)(Figure 1B). The early apoptotic cells in S100A16-OE cells were higher than S100A16-OE control cells (76.8±0.286% *vs* 13.54±0.165%, p<0.0001). Instead, the early apoptotic cells in S100A16-KD cells was lower than that in the control group (11.33±0.0.176% *vs* 31.29±0.478%, $p<0.0001$)(Figure 1C). In addition, we found that silencing of S100A16 obviously inhibited the expression of p-ERK1+2 compared with the control group (Figure 1D).

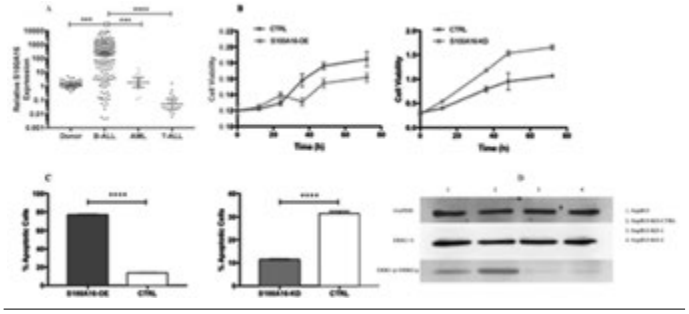


Figure 1.

Summary and Conclusions: These findings suggest that aberrant expression of S100A16 might be involved in the physiopathological mechanism of B-cell ALL. S100A16 might inhibit proliferation and promote apoptosis of B-cell ALL via the ERK signaling pathway.

PF172

MELATONIN INHIBITS MLL-REARRANGED LEUKEMIA VIA RBFOX3/HTERT AND NF-KB/COX-2 SIGNALING PATHWAYS

Y.-L. Tang[1], L.-B. Huang[1], X.-J. Lui[2,*]

[1]Department of Pediatrics, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China, [2]The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

Background: MLL-rearranged leukemia is an aggressive malignancy associated with poor outcome, which is refractory to conventional treatment.
Aims: Melatonin has been proven to exert anti-tumor activity, but the effect of melatonin on MLL-r leukemia and the underlying mechanism remain poorly understood.
Methods: Firstly, the cell viability was determined by the CCK-8 assay and the apoptosis was examined by a FACS analysis. Secondly, qRT-PCR and Western blot were used to examine the expression of RBFOX3,hTERT, NF- B and COX-2. Furthermore, streptavidin-agarose pulldown assays and ChIP assay were used to test the RBFOX3 binding to hTERT promoter and NF- B p65 subunit binding to COX-2 promoter. Moreover, We investigated the effect of melatonin on the growth in a MLL-r ALL xenograft mouse model.
Results: In this study, we showed that melatonin inhibited cell proliferation and induced apoptosis by activating the caspase-dependent apoptotic pathway in MLL-r leukemia cells. Mechanistic investigations revealed that melatonin suppressed the expression of hTERT by abrogating the binding activity of RBFOX3 to the hTERT promoter. Melatonin also blocked NF- B nuclear translocation and suppressed NF- B binding to the COX-2 promoter, thereby suppressing the expression of COX-2. In addition, clinical samples revealed that melatonin exerts anti-leukemic activity in primary MLL-r leukemia blasts *ex vivo*. *In vivo*, the mice treated with melatonin experienced a larger reduction in leukemic burden than the control group in a MLL-r leukemia xenograft mouse model.
Summary and Conclusions: These results suggested that melatonin inhibited MLL-rearranged leukemia through suppressing the RBFOX3/hTERT and NF- B/COX-2 signaling pathways. Our findings provide new insights into the role of melatonin for MLL-r leukemia treatment.

PF173

INVESTIGATING THE SYNERGISM BETWEEN CHK1 AND WEE1 PROTEINS INHIBITION IN THE TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA

A. Ghelli Luserna Di Rora'[1,*], A. Ferrari[1], V. Robustelli[1], E. Imbrogno[1], C. Papayannidis[1], M.C. Abbenante[1], G. Marconi[1], S. Paolini[1], M. Cavo[1], G. Martinelli[2]

[1]Department of Experimental, Diagnostic and Specialty Medicine,Department of Hematology and Medical Sciences "L. and A. Seràgnoli, Bologna, [2]Scientific Directorate, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy, Meldola, Italy

Background: Although several innovative therapies are today improving the outcome for adult acute lymphoblastic leukemia (ALL) patients, a large percentage frequently relapse or became refractory to treatments. Thus, there is a need to improve the efficacy of actual treatments or to identify novel therapeutic strategy. The inhibition of cell cycle checkpoints has become a promising therapeutic option for the treatment of different tumors. The preclinical data available, led the basis for the clinical evaluation of this class of compounds. On ALL only few preclinical and clinical data on the effectiveness of cell cycle checkpoint inhibitors has been reported. It has been showed that the simultaneous inhibition of CHK1 and WEE1 kinases synergizes in term of reduction of cell viability, induction of apoptosis and inhibition of proliferative capacity.
Aims: To evaluate the effectiveness of the concomitant inhibition of Wee1 and Chk1 kinases in the treatment of ALL as single agent and in combination with the S-phase specific chemotherapy agent, methotrexate.
Methods: Gene expression analysis was performed using Affymetrix GeneChip Human Transcriptome Array 2.0 on leukemic cells isolated from the bone marrow and the peripheral blood of adult B-ALL patients. B-/T-ALL cell lines and primary cells were treated with PF-00477736 and/or AZD-1775 *in vitro* and *ex vivo*. The effectiveness of the combination was evaluated in term of reduction of the cell viability, reduction of cell proliferation, cell cycle modification, induction of apoptosis and protein modification.
Results: We showed that in primary B-ALL samples at diagnosis (n=39) WEE1 and CHEK1 transcripts are highly expressed and positively co-expressed (Pearson r=0.5770, p=0.0001). Having established that the abundance of WEE1 and CHEK1 is significantly high this raised the possibility that ALL cells may be reliant on both kinases for survival. We therefore next evaluated the efficacy of the concomitant inhibition of both kinases on ALL cell lines. The two small inhibitors PF-00477736 (Chk1 inhibitor) and AZD-1775 (Wee1 inhibitor) synergized in the reduction of cell viability (CI. between 0.17-0.9), induction of DNA damages, activation of cell apoptosis and increment of S phase cells in different ALL cell lines. Interestingly the combination compromised the viability also of primary leukemic cells isolated from the bone marrow and the peripheral blood of adult B-ALL patients. To further evaluate the potential of the combination, ALL cell lines were treated with methotrexate after being exposed to PF-00477736 and/or AZD-1775. Interestingly the treatment with combination deeply sensitized different leukemic cell lines to the cytotoxicity of methotrexate. The specificity of the pharmacological schedule on S phase was confirmed using the G2/M phase specific inhibitor, doxorubicin, which showed no significant reduction of the cell viability.
Summary and Conclusions: Here we extended the already available results on this potent therapeutic strategy on ALL cell lines and primary cells. We confirmed that the concomitant inhibition of WEE1 and CHK1 mine the viability of leukemic blasts inducing DNA damages and triggering apoptosis. We demonstrated that the inhibition of WEE1 and CHK1 kinases could be a winning strategy to enhance the toxicity of conventional chemotherapy.

Acute lymphoblastic leukemia – Clinical

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AN UPDATED ANALYSIS OF TISAGENLECLEUCEL IN PEDIATRIC/YOUNG ADULT PATIENTS WITH RELAPSED/REFRACTORY (R/R) B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL) IN A US MULTICENTER CLINICAL TRIAL (ENSIGN)

S.L. Maude[1,2,*], S.A. Grupp[1,2], R. Mody[3], T. Driscoll[4], T.W. Laetsch[5,6], M. Qayed[7], S. Davies[8], C.L. Phillips[9], G.D. Myers[10], K.J. August[10], M.A. Pulsipher[11], M.W. Boyer[12], M.R. Verneris[13], K.L. Davis[14], C.M. Capitini[15], E. Nemecek[16], C.H. June[17,18], L. Yi[19], M. Cota[19], M. Leung[19], J.E. Levine[20,21]

[1]Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, [2]Division of Oncology, Center for Childhood Cancer Research and Cancer Immunotherapy Program, Children's Hospital of Philadelphia, Philadelphia, [3]Pediatric Phase-I and Experimental Therapeutics Program, Michigan Medicine, Ann Arbor, [4]Division of Pediatric Blood and Marrow Transplant, Children's Health Center, Duke University Medical Center, Durham, [5]Department of Pediatrics, The University of Texas Southwestern Medical Center, [6]Pauline Allen Gill Center for Cancer and Blood Disorders, Children's Health, Dallas, [7]Aflac Cancer and Blood Disorders Center, Emory University, Atlanta, [8]Cincinnati Children's Hospital Medical Center, Cincinnati, United States, [9]Division of Oncology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, [10]Children's Mercy Hospital Kansas City, Kansas City, [11]Division of Hematology, Oncology, and Blood and Marrow Transplantation, Children's Hospital Los Angeles, Keck School of Medicine of USC, Los Angeles, [12]Department of Pediatrics and Internal Medicine, University of Utah, Salt Lake City, [13]Division of Pediatric Blood and Marrow Transplant, University of Minnesota, Minneapolis, [14]Department of Pediatrics, Stanford University School of Medicine, Stanford, [15]Department of Pediatrics, University of Wisconsin, Madison, [16]Oregon Health & Science University, Portland, [17]Center for Cellular Immunotherapies, Perelman School of Medicine, University of Pennsylvania, [18]Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, [19]Novartis Pharmaceuticals Corporation, East Hanover, [20]University of Michigan, Ann Arbor, [21]Icahn School of Medicine at Mount Sinai, New York, United States

Background: Tisagenlecleucel, an autologous T-cell product expressing a CD19-specific chimeric antigen receptor (CAR), demonstrated a high rate of durable responses and manageable safety profile in a single-center phase 1/2a trial in pediatric/young adult patients (pts) with r/r B-ALL.
Aims: To establish safety and efficacy of tisagenlecleucel for transfer from an academic to a centralized industry-based manufacturing process, a multicenter phase 2 trial (ENSIGN; NCT02228096) was initiated.
Methods: 13 US sites participated. Leukapheresis products were shipped for centralized manufacturing by the University of Pennsylvania for the first 26 pts or Novartis for subsequent pts. Following lymphodepletion with fludarabine and cyclophosphamide, a single dose of tisagenlecleucel cells was administered (dose range: ≤50 kg, 2.0-5.0x10^6 cells/kg; >50 kg, 1.0-2.5x10^8 cells). The primary endpoint was overall remission (ORR=complete remission [CR]+ CR with incomplete blood count recovery [CRi] maintained for ≥28 d) ≤6 mo after infusion. Efficacy analyses were performed on pts who completed 6 mo follow-up or discontinued earlier; OS and safety were evaluated for all infused pts.
Results: At data cutoff (6 Oct 2017) 58/73 enrolled pts (79%) were infused with tisagenlecleucel, 42 completed 6 mo follow-up, 11 withdrew prior to infusion (5 product related issues; 6 deaths), 4 were pending infusion. 3 pts did not receive lymphodepleting chemotherapy due to leukopenia. Median time from enrollment to infusion, 41 d. Median time from infusion to data cutoff, 19.6 mo. Median infused dose was 3.55x10^6 (range, 0.2-5.0x10^6) CAR+ transduced viable T cells/kg; 49/58 (84%) infused pts were within and 9 below the dose range. The ORR was 69% (29/42 evaluable pts; 95% CI 52.9, 82.4). 13 pts did not achieve CR/CRi (6 did not achieve remission, 3 relapsed <28 days after remission onset, 2 were not evaluable [proceeded to SCT shortly after remission onset without subsequent response assessment], 2 died before day 28 [1 ALL; 1 embolic stroke]). Of 29 pts in CR/CRi, 27 (93%) were minimal residual disease-negative (<0.01%) by multiparameter flow cytometry. Relapse-free survival at 6 and 12 mo was 71% (95% CI, 48.5-85.5) and 61% (95% CI, 38-78); median duration of remission was not reached. Overall survival for all infused pts at 6 and 12 mo was 79% (95% CI, 65-88) and 63% (95% CI, 46-76); median, 23.8 mo (95% CI, 9-NE). Tisagenlecleucel was detected in peripheral blood for up to 764 d. 17 infused pts died >30 days after infusion (16 ALL; 1 complication of transplant); no deaths were attributed to tisagenlecleucel. The most common adverse event was cytokine release syndrome (CRS; all grade [G], 81%; G 3 or 4, 33%; graded on the Penn scale). For CRS treatment, 13 pts (22%) had systemic anti-cytokine therapy, 14 (24%) had high dose vasopressors for hypotension, 6 (10%) had intubation, and 4 (7%) had dialysis. No deaths were attributed to CRS. Neurological events occurred in 19 pts (32.8%; 4 G 3; no G 4; all reversible), including seizures in 3 pts; there were no cases of cerebral edema. Efficacy and safety were consistent across manufacturing processes.
Summary and Conclusions: In this first multicenter trial, tisagenlecleucel showed a high ORR with durable remissions in pediatric/young adult pts with r/r B-ALL. CRS was effectively managed with a protocol-specific algorithm by appropriately trained site staff. ENSIGN was instrumental in establishing transfer from an academic to a centralized industry-based manufacturing process, and laid the foundation for the larger global ELIANA trial.

PF175

PHASE I STUDY OF UCART19, AN ALLOGENEIC ANTI-CD19 CAR T-CELL PRODUCT, IN HIGH RISK PEDIATRIC PATIENTS WITH CD19+ RELAPSED/REFRACTORY (R/R) B-CELL ALL: PRELIMINARY RESULTS OF PALL STUDY

W. Qasim[1,*], O. Ciocarlie[1], S. Adams[1], S. Inglott[1], C. Murphy[2], K. Gilmour[2], C. Rivat[1], G. Wright[1], G. Lucchini[1], J. Silva[1], K. Rao[1], A. Zinaï[3], S. Dupouy[3], S. Balandraud[3], F. Simon[3], J. Pauly[3], C. Bermingham[4], C. Konto[4], R. Chiesa[1], S. Samarasinghe[1], H. Hara[1], A. Boyle[1], J. Chu[1], D. Pinner[1], P.J. Amrolia[1], A. Vora[1], A. Rao[1], P. Ancliffe[1], P. Veys[1]

[1]Bone Marrow Transplantation Department, [2]Clinical Immunology, Great Ormond Street hospital NHS trust, London, United Kingdom, [3]Servier, Suresnes, France, [4]Pfizer Inc, San Francisco, CA, United States

Background: UCART19 is a genetically modified CAR T-cell product (anti-CD19 scFv- 41BB- CD3) manufactured from healthy donor cells, in which *TRAC* and *CD52* genes have been disrupted to allow administration in non-HLA matched patients.
Aims: Preliminary results of the PALL trial, an open-label phase I study of UCART19 in pediatric patients (pts) with R/R B-ALL are reported.
Methods: Pediatric pts (≥6 months to <18 years) with morphological disease or minimal residual disease (MRD) level ≥1x10^{-3} and who had exhausted available treatment options were eligible for the study. Prior to UCART19 single dose infusion, a lymphodepletion regimen combining high-dose fludarabine-cyclophosphamide, with or without alemtuzumab (FCA or FC) was administered. A fixed dose of UCART19 (2x10^7 total CAR+ cells corresponding to 1.1 to 2.3x10^6 cells/kg) in 4 different weight-bands was infused on Day 0. The safety and tolerability of UCART19 and its ability to achieve molecular remission at day (D) 28 ahead of allogeneic stem cell transplantation (allo-SCT) were assessed as primary and secondary objectives, respectively.
Results: As of February 02 2018, 6 children (3 males and 3 females) between 08 months and 16.4 years have been treated. Of the 6 pts, 5 had received 3 or more previous lines of treatment and 2 had previously received allo-SCT. Prior to lymphodepletion, 5 patients had low disease burden (<10% blasts) and 1 patient had high disease burden (80% blasts). All pts experienced reversible cytokine release syndrome (CRS): 1 grade (G) 1, 4 G2, 1 G3. CRS were manageable by supportive care±tocilizumab. Time to onset of first CRS symptoms ranged between D5 and D9. One patient experienced a G1 acute skin GvHD confirmed by biopsy and recovered with topical steroids. Five out of 6 patients received FCA, viral reactivation (CMV, ADV, BK, metapneumovirus) was observed in 4/5 pts and 3/5 had persisting G4 neutropenia by D42. Out of 6 patients, 5 achieved CRi at D28-D42, with 5/5 MRD negative (<0.01%) by flow cytometry and 3/5 MRD negative by PCR. Only 1/6 pts received FC and had a refractory disease on D28. All 5 CRi pts with negative MRD underwent allo-SCT, between 50 and 63 days following UCART19 infusion. Two patients relapsed 3 months after allo-SCT (one CD19- and one CD19+; both MRD positive by PCR prior to SCT), and died from disease progression 7 and 8 months after UCART19 infusion, respectively. One patient died 2.5 months after allo-SCT from thrombotic microangiopathy in a context of severe BK virus infection. Two patients are alive with molecular remission >6 months post-transplant and continue to be monitored in the study. Out of the 5 first patients treated with FCA, levels of UCART19 vector copy number showed a peak around D14 and were detectable in blood from D7 up to D42. UCART19 was also detectable by molecular signatures of UCART19 T-cell donor chimerism up to D42 in 4 patients and up to D57 in one patient. In the last patient treated by FC without alemtuzumab, UCART19 was detectable neither by qPCR nor by chimerism.
Summary and Conclusions: UCART19 shows an acceptable safety profile and was able to induce molecular remission in 5 out of 6 patients enabling allogeneic transplantation to proceed. The study is open and recruiting at multiple sites.

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CHARACTERISTICS AND OUTCOMES OF ADULTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA RELAPSING POST-ALLOGENEIC HAEMATOPOIETIC CELL TRANSPLANTATION IN THE PROSPECTIVE UKALL14 TRIAL (ISRCTN 665421317).

A. Castleton[1,*], A.A. Kirkwood[2], L. Clifton-Hadley[2], L. Micklewright[2], P. Patrick[2], A. V. Moorman[3], C. Rowntree[4], A.K. Fielding[5], D.I. Marks[6]

[1]Haematology, The Christie NHS Foundation Trust, Manchester, [2]CRUK & UCL Cancer trials centre, University College London, London, [3]Leukaemia Research Cytogenetics Group, Wolfson Childhood Cancer Research Centre, Newcastle-upon-tyne, [4]Haematology, University Hospital of Wales, Cardiff, [5]Haematology, University College London Hospitals NHS Foundation Trust, London, [6]Haematology, University Hospitals Bristol NHS Foundation Trust, Bristol, United Kingdom

Background: The outcome of relapsed adult acute lymphoblastic leukaemia is poor. In particular, patients (pts) relapsing after allogeneic haematopoietic cell transplant (alloHCT) have limited treatment options which have recently broadened with the advent of immunotherapies.
Aims: To evaluate factors influencing post-alloHCT relapse risk and survival for pts on the UKALL14 trial.
Methods: Within UKALL14, pts ≤40 years received myeloablative conditioned (MAC) alloHCT (cyclophosphamide/TBI 13.2Gy) in first complete remission (CR1) for high risk disease (high baseline WBC count; t4;11; low hypodiploidy/near triploidy; complex karyotype; Ph+; minimal residual disease (MRD) positivity post-induction cycle 2). MAC alloHCT for standard risk disease was advised if suitable sibling donor. All >40 years were high risk and eligible for reduced intensity conditioned (RIC) (fludarabine, melphalan, alemtuzumab) alloHCT in CR1, with pre-emptive donor leucocyte infusions for mixed T-cell chimerism or positive MRD, plus 8x3-monthly intrathecal chemotherapy doses to reduce CNS relapse. No specific interventions to reduce relapse were advised for the MAC group.
Results: As of January 2018, 804 pts were enrolled, with 359 receiving alloHCT (242 RIC; 117 MAC; median age 50 (25-65) and 31 years (22-51) respectively). Of these, 325 (90.5%) had high risk disease, 301 (83.8%) were B-lineage, and 159 (44.3%) had high risk cytogenetics including 101 (28.1%) with Ph+ disease. Post-alloHCT relapses occurred in 73 pts (14/117 (11.9%) post-MAC *vs* 59/242 (24.4%) post-RIC; p=0.025). For relapsing pts, median time to relapse was 8.8 months (1.8-70.5) with no significant difference for RIC *vs* MAC. Of these, 52/73 had post-induction MRD data available, with 27/52 (51.9%) MRD+, and no difference in median time to relapse for MRD+ *vs* MRD-/positive outside quantitative range (7.43 *vs* 11.2 months respectively; HR 1.35, p=0.24). Univariable analysis showed MRD+, RIC alloHCT, age 51-65 years and lack of graft *versus* host disease (GvHD) to be associated with an increased relapse risk (Figure 1).

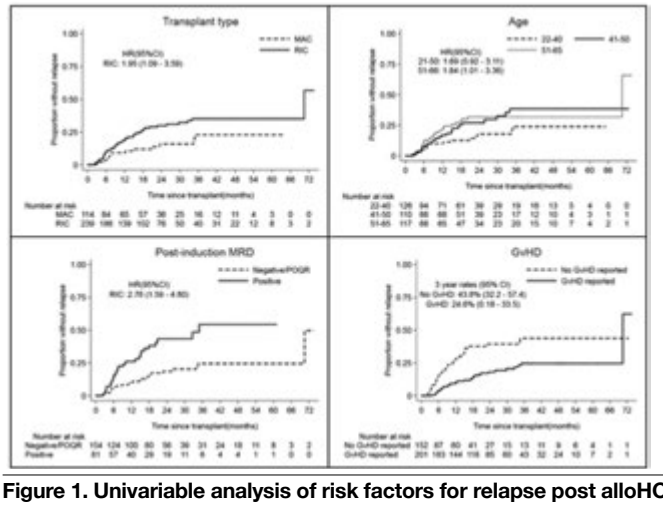

Figure 1. Univariable analysis of risk factors for relapse post alloHCT.

By transplant type, MRD+ (MAC HR 7.09, p=0.012; RIC HR 2.59, p=0.003), and lack of GvHD (MAC HR 0.33, p=0.031; RIC HR 0.45, p=0.0017) remained risk factors in both groups, whilst multivariable analysis showed MRD+ (HR 2.89, p=0.004) and sibling donor (HR 0.41, p=0.019) to independently increase risk for the RIC group. To date, 48/73 relapsed pts have died with a median survival of 6.7 months (median follow-up 5.7 months), and no survival difference between RIC and MAC. Second remission (CR2) was achieved in 22/42, with 5 remaining alive in CR2 with more than 1 year follow-up (event-free survival 5.2 months (1 day-35.2 months)). Factors influencing survival include relapse ≤*vs*.>12 months post-alloHCT (p=0.043), therapy intent (palliative *vs* CR/'cure'; p=0.007), and achievement of CR2 (p<0.001), with possible improved outcomes for those receiving targeted therapies at relapse (p=0.042).
Summary and Conclusions: MRD positivity, RIC alloHCT, age, and lack of GvHD associate with an increased risk of relapse in this series, whilst other standard prognostic factors were not significantly associated with relapse or survival. MRD negativity and unrelated donor type independently associated with a reduced relapse risk in the RIC group, the latter providing evidence for a graft *vs* leukaemia effect that may be manipulated. Novel targeted therapies should be investigated in this setting and may improve survival for pts relapsing post-alloHCT.

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INOTUZUMAB OZOGAMICIN TREATMENT IN PATIENTS WITH RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA: ANALYSIS FROM INO-VATE BY BONE MARROW BLAST PERCENTAGE

D. Marks[1,*], D. DeAngelo[2], M. Stelljes[3], M. Liedtke[4], W. Stock[5], N. Gökbuget[6], S. O'Brien[7], E. Jabbour[8], A. Merchant[9], T. Wang[10], E. Vandendries[11], A. Neuhof[12], A. Advani[13], H. Kantarjian[8]

[1]University Hospitals Bristol, Bristol, United Kingdom, [2]Dana-Farber Cancer Institute, Boston, United States, [3]Universitätsklinikum Münster, Munster, Germany, [4]Stanford Cancer Center, Stanford, [5]University of Chicago, Chicago, United States, [6]Goethe University, Frankfurt, Germany, [7]University of California, Orange, [8]University of Texas MD Anderson Cancer Center, Houston, [9]University of Southern California, Los Angeles, [10]Pfizer, Groton, [11]Pfizer, Cambridge, United States, [12]Pfizer Pharma GmbH, Berlin, Germany, [13]Cleveland Clinic Taussig Cancer Institute, Cleveland, United States

Background: Inotuzumab ozogamicin (InO) is a calicheamicin-conjugated antibody targeting CD22 on acute lymphoblastic leukemia (ALL) blast cells.
Aims: Here we report outcomes in relapsed/refractory (R/R) ALL patients receiving InO or standard of care chemotherapy (SC) in the phase 3 INO-VATE trial according to baseline bone marrow blast percentage (BMB%), an indicator of disease burden.
Methods: Adults with CD22+ ALL due to receive salvage treatment were randomized 1:1 to InO (n=164) or SC (n=162). Dosing and methods were published previously (Kantarjian *et al.*, NEJM 2016). Informed consent was obtained from all patients. BMB% was defined as low (<50%), moderate (50–90%), and high (>90%) at start of treatment.
Results: At baseline, characteristics across all groups were balanced and median BMB% was 28%, 78%, and 95% in the low, moderate, and high disease burden subgroups. Complete remission rates were significantly higher in InO *vs* SC patients, with 74% *vs* 46%, 75% *vs* 48%, and 70% *vs* 17% achieving CR/CRi in low, moderate, and high BMB% subgroups. Significantly, more patients in the InO arm achieved minimal residual disease negativity: 29/53 (55%), 52/79 (66%), and 16/30 (53%) for low, moderate, and high BMB% compared with 10/48 (21%), 11/83 (13%), and 2/30 (7%) for SC, respectively. InO-treated patients also had improved progression-free survival *vs* SC, with hazard ratios of 0.44 (97.5% CI, 0.26–0.74, 1-sided *P*=0.0001) for low, 0.50 (97.5% CI, 0.34–0.75, *P*<0.0001) for moderate, and 0.33 (97.5% CI, 0.16–0.69, *P*=0.0002) for high BMB%. Overall survival (OS) was favoured in the InO arm across groups (Table 1), with potentially the greatest difference seen in patients with high BMB% (HR=0.60 [97.5% CI 0.32–1.129, 1-sided *P*=0.03]).

Table 1.

	OS Probability, Months (95% CI)		
BMB%	**6**	**12**	**24**
Low			
InO (n=53)	56.6 (42.3–68.7)	34.0 (21.7–46.6)	30.0 (18.3–42.5)
SC (n=48)	59.5 (43.7–72.3)	38.8 (24.6–52.8)	2.4 (0.2–11.0)
Moderate			
InO (n=79)	61.7 (50.0–71.4)	35.6 (25.1–46.2)	21.1 (12.8–30.8)
SC (n=83)	51.3 (39.7–61.8)	34.0 (23.7–44.6)	15.0 (8.0–24.0)
High			
InO (n=30)	53.3 (34.3–69.1)	26.7 (12.6–43.0)	13.3 (4.2–27.8)
SC (n=30)	32.6 (16.4–49.8)	12.2 (3.2–27.8)	8.1 (1.4–22.6)

Summary and Conclusions: InO treatment resulted in superior efficacy over SC across all BMB% subgroups out to 2 years follow-up, particularly in patients with the greatest disease burden by BMB%.

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UCART19, AN ALLOGENEIC ANTI-CD19 CAR T-CELL PRODUCT, IN HIGH RISK ADULT PATIENTS WITH CD19+ RELAPSED/REFRACTORY B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: PRELIMINARY RESULTS OF PHASE I CALM STUDY

N. Jain[1], C. Graham[2,3], M. Konopleva[1], D. Yallop[2], A. Jozwik[3], P. Patten[2,3], R. Ellard[2], O. Stewart[2], K. Cuthill[2], V. Potter[2], S. Kassam[2], S. Devereux[2], A. Pagliuca[2], G. Mufti[2,3], H. Kantarjian[1], E. Jabbour[1], A. Zinai[4], S. Dupouy[4], S. Balandraud[4], C. Poirot[4], A. Philippe[4], F. Simon[4], C. Konto[5], C. Bermingham[5], R. Benjamin[2,3,*]

[1]MD Anderson Cancer Center, Houston, United States, [2]King's College Hospital NHS Foundation Trust, [3]King's College London, London, United Kingdom, [4]Institut de Recherches Internationales Servier, Suresnes, France, [5]Pfizer, San Francisco, United States

Background: UCART19 is a genetically modified CAR T-cell product (anti-CD19 scFv- 41BB- CD3) manufactured from healthy donor T cells, in which *TRAC* and *CD52* genes have been knocked out to allow its administration in non-HLA matched patients (pts) and the use of alemtuzumab as lymphodepleting agent, respectively.
Aims: Preliminary results of the CALM trial, a phase I dose finding study of UCART19 in adult patients with R/R B-ALL are reported.
Methods: Adult CD19+ R/R B-ALL pts (age ≥16 years) with morphological disease or minimal residual disease (MRD) load $\geq 1x10^{-3}$ were eligible. A lymphodepletion regimen (cyclophosphamide 1500mg/m² and fludarabine 90mg/m² with or without alemtuzumab 1mg/kg), was administered prior to UCART19 single dose infusion. The primary objective of this study is to evaluate the safety and tolerability and to determine the maximum tolerated dose of UCART19 by investigating up to four dose levels (DL). Anti-leukemic activity is assessed as a secondary objective.
Results: As of 2 February 2018, 9 pts have been treated in the dose escalation part (6 at DL1 and 3 at DL2 with $6x10^6$ and 6 or $8x10^7$ UCART19 respectively). Median age was 23 years (range 18-49). Pts received a median of 4 prior treatment lines (range 1-5). 7/9 pts had undergone a previous allogeneic stem cell transplant (allo-SCT) and had relapsed at a median of 5.9 months post transplant (range 4-11). Prior to lymphodepletion, 3 pts had low disease burden <5% blasts, 6 pts had disease burden above 5% blasts including 2 pts with very high values (88% and 95%). All pts but one experienced cytokine release syndrome (CRS): 1 G1, 6 G2 and 1 G4 (considered as a DLT). The pt with CRS G4 also developed neutropenic sepsis leading to multiple organ failure and death at D15. Tocilizumab was needed in 5/8 pts. Time to onset ranged from D5 to D12. CRS correlated with serum cytokine increase (IL-6, IL-10 and IFNg) and UCART19 expansion in blood in all pts but one. 1 pt developed G1 skin GvHD. G1 neurotoxicity was observed in 2 pts. Viral reactivations (CMV and/or adenovirus) occured in 4 pts (3 G1, 1 G3). 3/9 pts developed prolonged cytopenia defined as persistent grade 4 beyond D42 post-UCART19 infusion (after allo-SCT, 2 recovered and 1 pt died from pulmonary haemorrhage that occurred in the context of prolonged cytopenia and infection, this was considered as a DLT). 5 out of 9 pts achieved complete remission (CR) with MRD- at D28, 1 achieved CR with MRD+ at D28, 1 relapsed and 1 had refractory disease at D28 (no UCART19 expansion in both pts) and 1 died at D15. Of the 5 pts who achieved MRD negativity, 1 relapsed with CD19+ve disease at D61, received a 2nd dose of UCART19 and became MRD- again. All 5 pts who achieved MRD- proceeded to an allo-SCT with a median time of 66 days (range 51-140) post-UCART19 infusion. The pt that achieved MRD+ relapsed at D47. Post allo-SCT, 3 pts remain alive and 2 early deaths occurred at D17 and D19 secondary toinfection and pulmonary haemorrhage respectively. Out of 3 pts alive, 1 pt remains MRD- and 2 became transiently MRD+ at D52 and D97 respectively but reverted to MRD- status following withdrawal of immunosuppression. Among these 2 pts, 1 remains MRD- and 1 relapsed with CD19+ extramedullary disease at D183.
Summary and Conclusions: UCART19 shows an acceptable safety profile. 6/9 pts achieved complete remission of which 5 became MRD- enabling a second allo-SCT. Recruitment is ongoing at DL2.

PF179

LONG-TERM SURVIVAL OF ADULTS WITH B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA (BCP-ALL) AFTER TREATMENT WITH BLINATUMOMAB AND SUBSEQUENT ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

N. Goekbuget[1,*], A. Stein[2], G. Zugmaier[3], H. Dombret[4], M. Brueggemann[5], M. Bonifacio[6], X. Dong[7], H. Kantarjian[8], R. Bargou[9], M. Topp[10]

[1]Department for Internal Medicine II, Hematology/Oncology, University Hospital Frankfurt, Frankfurt, Germany, [2]City of Hope, Duarte (CA), United States, [3]Amgen Research (Munich) GmbH, Munich, Germany, [4]Hôpital Saint-Louis, University of Paris Diderot, Paris, France, [5]University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany, [6]Department of Medicine, Section of Hematology, University of Verona, Verona, Italy, [7]Global Biostatistical Science, Amgen Inc., Thousand Oaks (CA), United States, [8]Department of Leukemia, MD Anderson Cancer Center, University of Texas, Houston (TX), United States, [9]Comprehensive Cancer Center Mainfranken, Universitaetsklinikum Wuerzburg, Wuerzburg, Germany, [10]Department of Internal Medicine II, Universitaetsklinikum Wuerzburg, Wuerzburg, Germany

Background: In B-cell precursor (BCP) acute lymphoblastic leukemia (ALL), blinatumomab has demonstrated efficacy in two phase 2 trials: MT103-203 (Gökbuget *et al., Blood* 2017) in minimal residual disease (MRD) and MT103-211 in relapsed/refractory (R/R) disease (Topp *et al Lancet Oncology* 2014).
Aims: To describe the long-term outcomes after blinatumomab followed by allogeneic hematopoietic stem cell transplantation (alloHSCT).
Methods: Survival after blinatumomab and alloHSCT in continuous complete remission (CCR) was evaluated from two phase 2 trials, MT103-203 (MRD trial) and MT103-211 (R/R trial). In the MRD trial, 116 patients were treated with blinatumomab between November 2010 and February 2014. In the R/R trial, 189 patients were treated with blinatumomab between January 2012 and October 2013. For both trials, patients were followed up through 2017.
Results: Patient characteristics and outcomes are summarized in Table 1. Among blinatumomab patients in CCR, most patients who received alloHSCT in the MRD trial were >35 years of age whereas those in the R/R trial were younger (≤35 years). After a follow-up of at least 3 years, survival of blinatumomab patients with or without alloHSCT in the MRD trial according to age ≤35 years or >35 years was as follows: among patients ≤35 years, 16/26 (62%) patients with alloHSCT were alive *versus* 2/9 (22%) non-alloHSCT patients; among patients >35 yrs, 19/48 (40%) patients with alloHSCT were alive compared with 13/27 (48%) non-alloHSCT patients. Median overall survival (OS) from time of alloHSCT was not reached in patients ≤35 years in either trial (Table 1).

Table 1. Patients with allogeneic hematopoietic stem cell transplantation (HSCT) in continuous complete remission (CRR).

	MT103-203 N=74[a] n (%)	MT103-211 N=34 n (%)
Characteristics		
Age, n (%), years		
≤35	26 (35)	19 (56)
>35 to 55	29 (39)	9 (26)
>55	19 (26)	6 (18)
Median (range)	43 (18, 67)	31 (18, 65)
Donor		
Related, n (%)	19 (26)[b]	8 (23)
Unrelated, n (%)	53 (72)	23 (68)
Matched, n (%)	20 (27)	9 (26)
Unmatched	23 (31)	10 (29)
Unknown, n (%)	6 (8)	3 (9)
Cord	4 (5)	1 (3)
Unknown, n (%)	2 (3)	3 (9)
Conditioning regimen		
Myeloablative	32 (43)	15 (44)
Reduced intensity/nonmyeloablative	35 (47)	12 (35)
Unknown	7 (20)	7 (21)
100-day mortality after HST, n (%)[c]	5 (7)	4 (12)
Outcomes		
Median OS from alloHSCT, months		
Age ≤35 years	NE	NE
Age >35 years	25.7	15.9
Median RFS from alloHSCT, months		
Age ≤35 years	NE	16.4
Age >35 years	15.5	15.9

AlloHSCT: allogeneic hematopoietic stem cell transplantation; NE: not estimable; OS: overall survival; RFS: relapse-free survival.
[a]74 patients received on-study HSCT in CR; Ph+ and patients not in CR at treatment
[b]1 haploidentical start excluded
[c]No venous occlusive disease related deaths

Summary and Conclusions: These results suggest that in transplant-eligible patients in CCR, alloHSCT following blinatumomab is a potential option.

PF180

RE-THINKING THE PROGNOSTIC RELEVANCE OF A CD20 EXPRESSION CUT-OFF OF 20% IN ACUTE LYMPHOBLASTIC LEUKAEMIA: INITIAL RESULTS FROM THE UKALL14 TRIAL

K. Bailey[1,*], B. Beaton[1], A. A. Kirkwood[2], M. Aguiar[1], K. Alapi[1], L. Clifton-Hadley[2], P. Patrick[2], E. Lawrie[2], A. Douglas[2], D.I. Marks[3], A. McMillan[4], C.J. Rowntree[5], T. Menne[6], B. Wrench[7], A.V. Moorman[8], A.K. Fielding[1]

[1]Cancer Institute, University College London, [2]CRUK and UCL Cancer Trials Centre, London, [3]Bristol Haematology and Oncology Centre, United Bristol Healthcare Trust, Bristol, [4]Centre for Clinical Haematology, Nottingham City Hospital, Nottingham, [5]Cardiff and Vale UHB, Cardiff, [6]Newcastle Upon Tyne Hospitals NHS Foundation Trust, Newcastle Upon Tyne, [7]Barts Cancer Institute, Queen Mary University of London, London, [8]Northern Institute for Cancer Research, Newcastle Upon Tyne, United Kingdom

Background: The prognostic significance of CD20 expression in B-precursor acute lymphoblastic leukaemia (B-ALL) remains unclear. 'CD20 positivity' has traditionally been taken as 20% of blasts expressing the CD20 antigen. The significance of antigen density is unknown.
Aims: We examined the prognostic significance of CD20 in patients with B-ALL who had been enrolled into the rituximab randomisation of the UKALL14 (ISRCTN 66541317), irrespective of CD20 status. We determined whether measuring CD20 antigen density could improve risk stratification.
Methods: An 8-colour flow panel was employed on BD Fortessa™ cytometers with blasts gated on CD45, CD34, CD19 and CD10. Data was analysed using Flowjo® software. CD20 positivity was determined by comparison to fluorescence minus one and isotype control. CD20 antigen density was estimated by standard curves of geometric mean fluorescence intensity using BD QuantiBRITE™ beads.
Results: Of 655 patients enrolled, 145 had excess cells stored with suitable viability for flow analysis. Compared to those without samples, the patients analysed had a higher median white cell count at presentation (30.3 x10^9/l *vs* 5.9 x10^9/l, P=<0.001) and were more likely to be Philadelphia chromosome (Ph) positive (39.9% *vs* 28.3%, P=0.009). The median follow-up was 32.3 months. Seventy-three (49.6%) had CD20 expression ≥20% and 95 (65.5%) ≥10%. The percentage of CD20 positive blasts was significantly associated with inferior 3 year EFS [HR (1 log increase) 1.31(1.09 - 1.56) p=0.003] and OS [HR 1.29 (1.06 – 1.58) p=0.01] when analysed as a continuous variable. When data was analysed at 3 different CD20 percentage cut-offs of <10%, 10-19%, and ≥20%, 3 year EFS was 51.0%, 37.7%, 28.7% (p=0.014) and OS 65.1%, 35.6%, and 36.3% (p=0.012), as shown in the Kaplan Meier plots. When the data were analysed by CD20 antigen density in tertiles, the EFS and OS were similar and both remained statistically significant when adjusted for age, baseline WCC and adverse cytogenetics in a multivariable model. Percentage blasts was compared with antigen density to determine the optimal sensitivity and specificity of CD20 as a marker of outcome by logistic regression and receiver operator characteristic (ROC). The area under the curve was 0.6305 (0.52 – 0.74) for% blast method and 0.6829 (0.58-0.79) for the antigen density method. Youden's cut-off, a method which can be used to determine the best cut-off for a continuous variable, suggested a CD20% of 11.7% as optimal. Using our existing risk stratification, we calculated that the addition of CD20 >11.7% as an additional factor at baseline would include 6% more patients at high risk, increasing modestly to 7% if antigen density were included instead. In an analysis by Ph+ status, we showed impact of CD20 on survival to be confined to patients who were Ph-; there was no separation of outcome by CD20 expression in Ph+ ALL whilst the significant separation by CD20 status for patients with Ph- ALL remained by all methods of analysis (Figure 1).

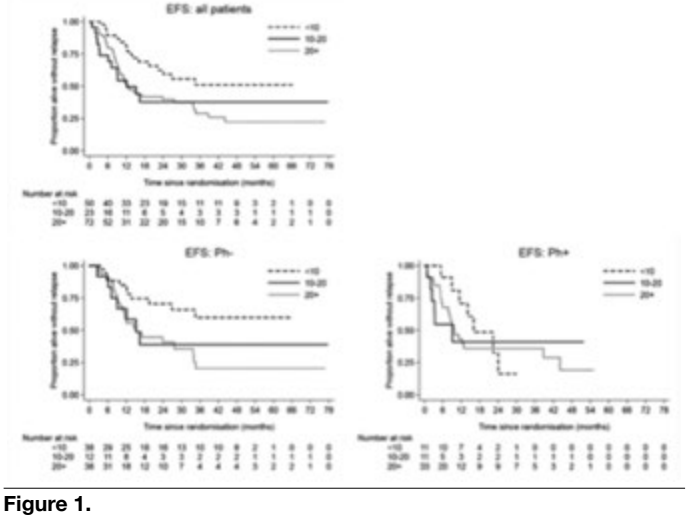

Figure 1.

Summary and Conclusions: Higher CD20 expression in Ph- (but not Ph+) adult B-ALL is associated with inferior EFS and OS. The higher risk extends to patients whose blasts express CD20 at or above 10%. Although measuring antigen density improved sensitivity and specificity over blast percentage alone, this difference was marginal, suggesting that blast percentage remains a reasonable way to assess CD20 status. Patients in this trial were randomised to receive rituximab and we anticipate presenting the un-blinded outcome results later in 2018.

PF181

IMPROVEMENT OF PATIENT-REPORTED QUALITY OF LIFE FOLLOWING TISAGENLECLEUCEL INFUSION IN PEDIATRIC AND YOUNG ADULT PATIENTS WITH RELAPSED/REFRACTORY B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

A. Baruchel[1,*], S. A. Grupp[2,3], S. Rives[4], M.W. Boyer[5], H. Bittencourt[6,7,8], P. Bader[9], J. Buechner[10], T.W. Laetsch[11,12], G. D. Myers[13], M. Qayed[14], M.A. Pulsipher[15], B. De Moerloose[16,17], H. Hiramatsu[18], G.A. Yanik[19], H.E. Stefanski[20], K.L. Davis[21], T. Driscoll[22], E. Nemecek[23], C. Peters[24], L. Yi[25], M. Cota[25], S. Shah[25], J. Zhang[25], A.C. Harris[26]

[1]Pediatric Hematology-Immunology Department, University Hospital Robert Debré (APHP), Paris, France, [2]Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, [3]Division of Oncology, Center for Childhood Cancer Research and Cancer Immunotherapy Program, Children's Hospital of Philadelphia, Philadelphia, United States, [4]Department of Pediatric Hematology and Oncology, Hospital Sant Joan de Déu de Barcelona, Barcelona, Spain, [5]Department of Pediatrics and Internal Medicine, University of Utah, Salt Lake City, United States, [6]Hematology Oncology Division, CHU Sainte-Justine, [7]CHU Sainte-Justine Research Center, Charles-Bruneau Cancer Center, [8]Department of Pediatrics, Faculty of Medicine, University of Montreal, Montreal, Canada, [9]Division for Stem Cell Transplantation and Immunology, Hospital for Children and Adolescents, University Hospital Frankfurt, Frankfurt, Germany, [10]Department of Pediatric Hematology and Oncology, Oslo University Hospital, Oslo, Norway, [11]Department of Pediatrics, The University of Texas Southwestern Medical Center, [12]Pauline Allen Gill Center for Cancer and Blood Disorders, Children's Health, Dallas, [13]Children's Mercy Hospital Kansas City, Kansas City, [14]Aflac Cancer and Blood Disorders Center, Emory University, Atlanta, [15]Division of Hematology, Oncology, and Blood and Marrow Transplantation, Children's Hospital Los Angeles, Keck School of Medicine of USC, Los Angeles, United States, [16]Department of Pediatric Hematology-Oncology and Stem Cell Transplantation, Ghent University Hospital, [17]Cancer Research Institute Ghent (CRIG), Ghent, Belgium, [18]Department of Pediatrics, Graduate School of Medicine, Kyoto University, Kyoto, Japan, [19]C.S. Mott Children's Hospital, Ann Arbor, [20]Department of Pediatric Blood and Marrow Transplant, University of Minnesota, Minneapolis, [21]Department of Pediatrics, Stanford University School of Medicine, Stanford, [22]Division of Pediatric Blood and Marrow Transplant, Children's Health Center, Duke University Medical Center, Durham, [23]Oregon Health & Science University, Portland, United States, [24]Stem Cell Transplantation Unit, St. Anna Children's Hospital, Vienna, Austria, [25]Novartis Pharmaceuticals Corporation, East Hanover, [26]Department of Pediatrics, University of Utah and Primary Children's Hospital, Salt Lake City, United States

Background: The global trial ELIANA (NCT02435849) evaluated the efficacy and safety of tisagenlecleucel, an autologous T-cell chimeric antigen receptor therapy targeting CD19+ cells, in pediatric and young adult patients with relapsed or refractory (R/R) B-cell acute lymphoblastic leukemia (B-ALL). As of the data cutoff (25 April 2017), an overall remission rate (complete remission [CR] with or without complete blood count recovery [CRi]) of 81% at ≤3 months was observed. Increasingly, patient quality of life (QOL) has become an important component in evaluating new oncology therapies.
Aims: To evaluate patient-reported QOL before and after infusion with tisagenlecleucel.
Methods: Infused patients were 3-23 years old with CD19+ B-ALL and were chemorefractory, relapsed after allogeneic stem cell transplant (SCT), or otherwise ineligible for SCT. Patients ≥8 years old completed the Pediatric Quality of Life Inventory (PedsQL) and the EuroQOL (EQ-5D/EQ-5D-Y) at baseline, day 28, and months 3, 6, 9 and 12 to evaluate QOL. Both instruments are validated measures of QOL and were summarized with descriptive statistics at the cross-sectional level and through changes from baseline. The minimal clinically important differences for the PedsQL and the EuroQOL visual analog scale (EQ-VAS) are 4.4 and 7-10, respectively. The normative mean values for healthy children are 83 and 86.2 for the PedsQL and EQ-VAS, respectively. Here we report changes in QOL in patients with a best overall response of CR/CRi.
Results: At the data cutoff, 75 of 92 enrolled patients had been infused; the median number of previous lines of therapy was 3 (range, 1 to 8). Of the infused patients, 58 patients (77%) who were ≥8 years old completed QOL instruments; 48 (83%) of whom were responders (CR/CRi). Mean baseline scores for responding patients were 58 and 68 for the PedsQL and EQ-

VAS, respectively (Figure 1A-B). Mean baseline scores for nonresponding patients (N=10) were 60 and 63 for the PedsQL and EQ-VAS scales, respectively. Both assessments demonstrated clinically meaningful improvements in the QOL of responders as observed by the mean change from baseline at day 28 (PedsQL, 6; EQ-VAS, 11), month 3 (PedsQL, 13.5; EQ-VAS, 16.5), month 6 (PedsQL, 17; EQ-VAS, 16), month 9 (PedsQL, 18; EQ-VAS, 19) and month 12 (PedsQL, 27; EQ-VAS, 25) (Figure 1A-B). 3- and 6-month assessments were completed by ≤3 nonresponders. The percentage of patients who achieved the normative mean or greater (PedsQL, ≥83; EQ-VAS, ≥86.3) increased from 12% at baseline to 64% by month 12 for the PedsQL total score (Figure 1C) and 18% at baseline to 64% by month 12 for the EQ-VAS total score (Figure 1D). Sustained improvements in patient QOL were observed for each PedsQL subscale. Notable decreases in the proportion of problems reported by responding patients in each of the EQ-5D dimensions were observed at each time point.

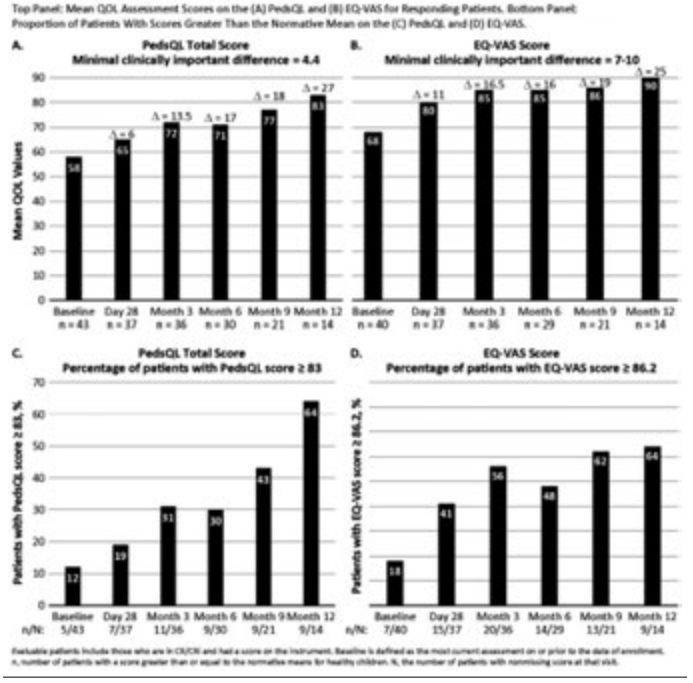

Figure 1.

Summary and Conclusions: Improvements in QOL observed at day 28 and months 3 and 6 were sustained at months 9 and 12. For pediatric and young adult patients with R/R B-ALL who achieved CR or CRi, clinically meaningful, even dramatic, improvements in QOL were observed after tisagenlecleucel infusion.

PF182

OUTCOMES WITH INOTUZUMAB OZOGAMICIN IN PATIENTS WITH PHILADELPHIA CHROMOSOME–POSITIVE RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA

G. Martinelli[1,2,*], W. Stock[3], M. Stelljes[4], D. DeAngelo[5], N. Gökbuget[6], A. Advani[7], S. O'Brien[8], M. Liedtke[9], A. Merchant[10], R. Cassaday[11], T. Wang[12], E. Vandendries[13], D. Marks[14], H. Kantarjian[15]

[1]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola (FC), [2]Institute Seragnoli, University of Bologna, Bologna, Italy, [3]University of Chicago, Chicago, [4]Universitätsklinikum Münster, Munster, [5]Dana-Farber Cancer Institute, Boston, United States, [6]Goethe University, Frankfurt, Germany, [7]Cleveland Clinic, Cleveland, [8]University of California, Orange, [9]Stanford Cancer Institute, Stanford, [10]University of Southern California, Los Angeles, [11]University of Washington School of Medicine and Fred Hutchinson Cancer Research Center, Seattle, [12]Pfizer, Groton, [13]Pfizer, Cambridge, United States, [14]University Hospitals Bristol, Bristol, United Kingdom, [15]MD Anderson Cancer Center, Houston, United States

Background: Inotuzumab ozogamicin (InO), a CD22-directed antibody-drug conjugate, is approved to treat adults with refractory/relapsed (R/R) acute lymphoblastic leukemia (ALL). Historically, patients with Philadelphia chromosome-positive (Ph+) ALL (~20–30%) have had poor prognoses compared with Ph– patients.
Aims: Here we report outcomes in Ph+ R/R ALL patients who received InO or standard of care chemotherapy (SC) as salvage therapy in two clinical studies.
Methods: Patients with R/R ALL received inotuzumab ozogamicin (InO) in a phase 1 dose-finding/phase 2 study (1010; DeAngelo *et al.*, Blood Adv 2017) and a phase 3 trial (1022; Kantarjian *et al.*, NEJM 2016) comparing InO *vs* SC. Informed consent was obtained from all patients. We analyzed outcomes in Ph+ patients (Ph chromosome or BCR-ABL gene by FISH) treated with InO (InO-1010 or InO-1022) or SC based on final data from each study.
Results: In 1010 and 1022, respectively, 16 and 22 Ph+ patients received InO; 27 Ph+ patients were randomized to SC in 1022 (22 received SC). Patients in 1010 were heavily pretreated. Among Ph+ patients in 1022, 19 (86%) InO patients and 26 (96%) SC patients had previous tyrosine kinase inhibitors (TKIs). Prior stem cell transplants (SCT) were 8 (50%) for InO-1010, 7 (32%) for InO-1022, and 9 (33%) for SC. Also, 15 (94%), 10 (45%), and 15 (56%) patients were treated in ≥2nd salvage for InO-1010, InO-1022, and SC, respectively. Efficacy outcomes are shown (Table 1). A total of 3 (19%) InO-1010 patients, 9 (41%) InO-1022 patients, and 5 (19%) SC patients proceeded to SCT after treatment. The most common nonhematologic grade 3-4 adverse events with InO in Ph+ patients were gastrointestinal disorders (31%) in 1010 and multi-organ laboratory abnormalities (41%) in 1022; 2 Ph+ patients in each InO study had veno-occlusive liver disease. Among SC patients, infections (55%) were the most common grade 3-4 nonhematologic events.

Table 1.

Efficacy Endpoints	InO-1010 (n=16)	InO-1022 (n=22)	SC (1022) (n=27)
Complete remission (CR/CRi), n (%)	9 (56)	16 (73)	15 (56)
Minimal residual disease (MRD) negativity, n (%)	10 (63)	14 (64)	5 (19)
Overall survival (mos), median (95% CI)	7.4 (4.3–11.3)	8.7 (3.6–14.1)	8.4 (5.0–14.3)
Progression-free survival (mos), median (95% CI)	4.4 (1.8–5.9)	3.9 (2.1–9.2)	3.1 (1.1–6.2)

Summary and Conclusions: In Ph+ patients with R/R ALL who failed prior TKIs +/- SCT, InO-treated patients had higher rates of CR/CRi, MRD negativity, and subsequent SCT. However, overall outcomes in 1022 InO *vs* SC were still inferior to those reported in Ph– patients; thus additional treatment combinations should be explored.

PF183

IMPACT OF MINIMAL RESIDUAL DISEASE STATUS IN CLINICAL OUTCOMES OF PATIENTS WITH RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH INOTUZUMAB OZOGAMICIN IN THE PHASE 3 INO-VATE TRIAL

N. Gökbuget[1,*], E. Jabbour[2], A. Advani[3], M. Stelljes[4], W. Stock[5], M. Liedtke[6], G. Martinelli[7,8], S. O'Brien[9], T. Wang[10], A.D. Laird[11], E. Vandendries[12], A. Neuhof[13], D. DeAngelo[14], H. Kantarjian[2]

[1]Goethe University, Frankfurt, Germany, [2]University of Texas MD Anderson Cancer Center, Houston, [3]Cleveland Clinic, Cleveland, [4]Universitätsklinikum Münster, Munster, [5]University of Chicago, Chicago, [6]Stanford Cancer Institute, Stanford, United States, [7]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola (FC), [8]Institute Seragnoli, University of Bologna, Bologna, Italy, [9]University of California, Orange, [10]Pfizer, Groton, [11]Pfizer, San Diego, [12]Pfizer, Cambridge, United States, [13]Pfizer Pharma GmbH, Berlin, Germany, [14]Dana-Farber Cancer Institute, Boston, United States

Background: Minimal residual disease (MRD) negativity is a key prognostic indicator of patient outcome in acute lymphoblastic leukemia (ALL) and is predictive of improved survival and disease-free status. In the INO-VATE ALL trial (Kantarjian, NEJM 2016), patients with relapsed refractory (R/R) ALL who received inotuzumab ozogamicin (InO) *vs* standard chemotherapy (SC) achieved greater remission (CR/CRi; 81% *vs* 29%) and MRD-negativity (78% *vs* 28%, in patients with CR/CRi) and had longer overall survival (OS): 7.7 *vs* 6.7 months, HR=0.77 (97.5% CI, 0.58–1.03, P=0.04).
Aims: This analysis was conducted to assess the prognostic value of MRD negativity by end of treatment in R/R ALL patients receiving InO as salvage therapy in the INO-VATE trial.
Methods: INO-VATE patients who received InO (n=164) were included. Informed consent was obtained from all patients. Among patients with CR/CRi, MRD status (by multiparametric flow cytometry at a central lab) was defined as negative (MRD–) if $<1 \times 10^{-4}$ blasts/nucleated cells (n=81), or as positive (MRD+; n=83), based on assessment by end of treatment. OS, progression-free survival (PFS), and predictors of MRD status (by

multivariate logistic regression) are reported from final study data as of Jan 4, 2017.
Results: MRD– status with CR/CRi was associated with significantly improved OS and PFS (Table 1) *vs* MRD+ status with CR/CRi: unstratified HR 0.512; 1-sided P=0.0009 for OS and HR 0.423; P<0.0001 for PFS. Exploratory multivariate analyses indicated that 2nd salvage compared to 1st salvage (OR 0.499, 2-sided P=0.058) was associated with lower likelihood of having MRD– status, while <1x109/L absolute circulating blast count at baseline (OR 3.231, P=0.002) and longer duration of remission (OR 1.033, P=0.005) were associated with increased likelihood of having MRD– status.

Table 1.

	CR/CRi and MRD– (n=76)	CR/CRi and MRD+* (n=45)	No CR/CRi (n=43)
Median OS, mos [95% CI]	14.1 [8.6–23.0]	7.2 [5.8–10.8]	2.6 [1.9–3.6]
Median PFS, mos [95% CI]	8.6 [6.2–11.4]	5.4 [3.9–6.2]	1.4 [1.0–1.9]
*includes 6 patients with no MRD assessment			

Summary and Conclusions: Among patients who received InO in the INO-VATE trial, having CR/CRi and MRD– status at end of treatment was associated with the greatest survival outcomes. However, patients who achieved an MRD+ CR/CRi had much greater survival than those who did not have CR/CRi. In R/R ALL, use of InO may optimize chances to attain the primary goal of complete remission and MRD– status.

PF184

EXTENSIVE SAFETY PROFILE OF INOTUZUMAB OZOGAMICIN IN RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS ENROLLED IN THE PHASE 3 INO-VATE TRIAL

D. DeAngelo[1,*], R. Cassaday[2], G. Martinelli[3,4], W. Stock[5], M. Stelljes[6], N. Gökbuget[7], A. Advani[8], S. O'Brien[9], M. Liedtke[10], A. Merchant[11], T. Wang[12], E. Vandendries[13], D. Marks[14], E. Jabbour[15], H. Kantarjian[15]

[1]Dana-Farber Cancer Institute, Boston, [2]University of Washington School of Medicine and Fred Hutchinson Cancer Research Center, Seattle, United States, [3]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola (FC), [4]University of Bologna, Bologna, Italy, [5]University of Chicago, Chicago, United States, [6]Universitätsklinikum Münster, Munster, [7]Goethe University, Frankfurt, Germany, [8]Cleveland Clinic Taussig Cancer Institute, Cleveland, [9]University of California, Orange, [10]Stanford Cancer Center, Stanford, [11]University of Southern California, Los Angeles, [12]Pfizer, Groton, [13]Pfizer, Cambridge, United States, [14]University Hospitals Bristol, Bristol, United Kingdom, [15]University of Texas MD Anderson Cancer Center, Houston, United States

Background: In INO-VATE, patients treated with inotuzumab ozogamicin (InO) *vs* standard chemotherapy (SC) had significantly greater remission rates and longer overall survival (OS), with 23% reduced risk of death (Kantarjian *et al.*, NEJM 2016).
Aims: Here we report long-term, extensive safety outcomes in relapsed or refractory acute lymphoblastic leukemia (ALL) patients receiving InO as salvage therapy in the INO-VATE trial.
Methods: Study methods were previously published. Adults with CD22+ acute lymphoblastic leukemia (ALL) in 1st or 2nd salvage were randomized 1:1 to InO (n=164) or SC (n=162). Informed consent was obtained from all patients. Data up to Jan 4, 2017 are reported.
Results: Patients who received InO had a median of 3 treatment cycles (range 1–6), while those on SC had a median of 1 cycle (range 1–4). Adverse event (AE) and serious AE rates were similar between arms even though more cycles of InO than SC were administered (Table 1). Grade 3-4 AE rates were higher with SC, while more grade 5 AEs occurred with InO *vs* SC (6% *vs* 2%); 5 cases (3%) were veno-occlusive disease (VOD). More patients taking InO discontinued due to AEs, most often from infections (10 [6%]) including pneumonia and sepsis, hepatobiliary disorders (7 [4%]), or blood/lymphatic disorders including cytopenias (5 [3%]). For SC, discontinuations were most often from infections (6 [4%]) or blood/lymphatic disorders (3 [2%]). More hepatic AEs (any grade) occurred with InO: 83 (51%) *vs* 52 (36%), including VOD (23 [14%] *vs* 3 [2%]). Five cases of VOD with Ino occurred during therapy, while all other cases for Ino and SC groups occurred following post-study stem cell transplantation. A lower percentage of death was seen with InO: 131 (80%) *vs* 126 (88%) for SC. Fewer InO patients died from ALL: 80 (49%) *vs* 100 (70%) for SC.
Summary and Conclusions: Safety data from the final report of INO-VATE are consistent with previous reports of data that also include greater efficacy (longer survival) seen with InO *vs* SC. Temporary discontinuation and dose reduction of InO were used to manage serious or severe AEs. Data suggest vigilant monitoring, treatment, and/or prevention for the most common events such as VOD and infections are needed to optimize outcomes.

Table 1.

	Treatment-emergent AEs (TEAEs)		Treatment-related TEAEs	
	InO (n=164)	SC (n=143)	InO (n=164)	SC (n=143)
Total AEs	2023	2112	764	980
Patients with AEs, n (%)	163 (99)	143 (100)	144 (88)	130 (91)
Serious AEs	85 (52)	72 (50)	52 (32)	42 (29)
Grade 3-4 AEs	147 (90)	138 (97)	114 (70)	114 (80)
Grade 5 AEs	26 (16)	16 (11)	9 (6)	3 (2)
Post AE, n (%):				
Discontinued	31 (19)	11 (8)	15 (9)	6 (4)
Dose reduced	5 (3)	3 (2)	4 (2)	1 (1)
Temporary discontinuation	72 (44)	17 (12)	51 (31)	12 (8)
Temporary discontinuation + dose reduced	3 (2)	1 (1)	3 (2)	0

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BLINATUMOMAB AND TYROSINE KINASE INHIBITORS COMBINATION IN RELAPSED/REFRACTORY ACUTE LYMPHOBALTIC LEUKEMIA: PRIMARY RESULTS AND LATE EVENTS

A. Sokolov[1,*], E. Parovichnikova[1], V. Troitskaya[1], L. Kuzmina[1], I. Galtseva[1], S. Kulikov[1], S. Bondarenko[2], I. Lukyanova[1], T. Lobanova[1], E. Usikova[1], K. Zarubina[1], O. Gavrilina[1], J. Davidova[1], N. Kapranov[1], V. Savchenko[1]

[1]National Research Center for Hematology of the Ministry of Healthcare of the Russian Federation, Moscow, [2]R. Gorbacheva memorial Institute of Oncology, Hematology and Transplantation, Saint-Petersburg, Russian Federation

Background: Bispecific monoclonal antibody blinatumomab has antileukemic activity in CD19+ relapsed/refractory both Ph-positive and Ph-negative acute lymphoblastic leukemia (R/R ALL). We established combined treatment approach with blinatumomab and tyrosine kinase inhibitors (TKI) in some targeted ALL subsets – BCR-ABL-positive, IKZF1-deleted and FLT3-ITD mutated.
Aims: To assess clinical efficacy, toxicity and long term results.
Methods: 11 patients (pts) with R/R ALL from October 2015 to February 2018 were treated with blinatumomab+TKI, 3 male and 8 female. Median age was 32 years. 8 pts had BCR-ABL-positive R/R ALL (P190), 2 pts – IKZF1-deleted R/R ALL, 1 pt – FLT3-ITD-positive R/R ALL. 2 pts with BCR-ABL-positive ALL had ABL mutation T315I. The treatment consisted of 4-5 cycles of Blinatumomab 28 mcg/day continuous infusion 28 days each cycle with 2 week intervals between cycles. All pts were treated with one of TKI from 1 day of treatment continuously. 9 pts with BCR-ABL-positive ALL and IKZF1-deleted ALL received dasatinib 140 mg/day, 1 pt with T315I mutated ABL received ponatinib 45 mg/day, 1 pt with FLT3-ITD received sorafenib 800 mg/day. 2 pts with IKZF1 deletions received ATRA 45 mg/m^2 60 days from 1 day of treatment and then 2-week cycles with 4 week intervals. Peripheral blood flow cytometry was performed weekly in each cycle of blinatumomab.
Results: Median follow up is 19 months (14-31). Neurological toxicity of 1 – 2 grade observed in 2 pts (headaches in 1 pt and ulnar neuropathy in 1 pt). Hypogammaglobulinemia was common and 8 pts received intravevous human normal immunoglobulin replacement. Hand-foot syndrome observed in 1 pt on sorafenib. The syndrome completely resolved after temporary sorafenib interruption. Diarrhea on dasatinib was observed in 3 pts. Diarrhea resolved after dasatinib replacement with bosutinib in 2 pt and nilotinib in 1 pt. CMV colitis was diagnosed in 2 pts. 1 of them has multiple intestinal ulceration with massive intestinal bleeding. Face edema and hyperemia in 1 pt on dasatinib resolved after switch to nilotinib. T-cytotoxic, NK, T -helper and T-regulatory cells were decreased during 1st blinatumomab cycle. From 2nd to 4th cycles T-cytotoxic and NK returned into normal range while T-helper and T-regulatory remained slightly decreased. 10 pts achieved complete remission (CR) and 1 pt had progressive disease after 1st blinatumomab cycle. 9 pts achieved molecular remission (MolCR) and 1 – cytogenetic remission (CyCR) on subsequent blinatumomab cycles. AlloBMT was performed in 9 pts and AutoBMT – in1 pt in MolCR. 7 pts received TKI/TKI+ATRA maintenance. 4 curable relapses observed. 1 pt on bosutinib awaiting Allo-BMT has overt hematological relapse and MolR was achieved on bortezomib-based chemotherapy+dasatinib. 1 pt with CytCR on dasatinib had cytogenetic relapse before AlloBMT. 1 pt on dasatinib after AutoBMT had molecular relapse and blinatumomab retreatment+dasatinib+ATRA was started. 1 pt on ponatinib maintenance had CNS relapse and CNS lesion regressed after intrathecal chemotherapy+ponatinib treatment and cranial irradiation is performing.
Summary and Conclusions: The combination of blinatumomab with TKI/TKI+ATRA has high MolCR rate and low toxicity profile in R/R ALL.

High incidence of relapse suggests that maintenance/preemptive treatment strategy should be revised.

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CHIMERIC ANTIGEN RECEPTOR T-CELL THERAPY IN PEDIATRIC/ADOLESCENT/YOUNG ADULT PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN FIRST RELAPSE AFTER ALLOGENEIC STEM CELL TRANSPLANT IN CR1

P. Bader[1,*], S.A. Grupp[2,3], P.L. Martin[4], M.W. Boyer[5], K. Schlis[6], L.K. Eldjerou[7], E. Bleickardt[7], L. Yi[7], S. Davies[8]

[1]Division for Stem Cell Transplantation and Immunology, Hospital for Children and Adolescents, University Hospital Frankfurt, Frankfurt, Germany, [2]Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, [3]Division of Oncology, Center for Childhood Cancer Research and Cancer Immunotherapy Program, Children's Hospital of Philadelphia, Philadelphia, [4]Division of Pediatric Blood and Marrow Transplant, Duke University Medical Center, Durham, [5]Department of Pediatrics and Internal Medicine, University of Utah, Salt Lake City, [6]Department of Pediatrics, Stanford University School of Medicine, Stanford, [7]Novartis Pharmaceuticals Corporation, East Hanover, [8]Cincinnati Children's Hospital Medical Center, Cincinnati, United States

Background: Patients with B-cell acute lymphoblastic leukemia (ALL) who relapse after first allogeneic stem cell transplant (alloSCT) have a poor prognosis; a recent study using the CIBMTR database reported a median survival of 7.4 months (95% CI, 6.0-9.6 months) (Crotta, 2017). ELIANA and ENSIGN trials of the chimeric antigen receptor (CAR) T-cell therapy tisagenlecleucel in pediatric and adolescent/young adult (AYA) patients with relapsed/refractory (R/R) ALL included patients who relapsed after alloSCT (54%). Most relapsed patients were in ≥2nd relapse. Here we evaluate the outcomes of patients who received tisagenlecleucel following their first relapse after alloSCT performed in first complete remission (CR1).
Aims: The goal of this study was to examine efficacy and safety outcomes in this subset of patients who received tisagenlecleucel for first relapse following alloSCT in CR1.
Methods: Pooled data from 2 single-arm, multicenter, phase 2 trials (ELIANA, N=75 [NCT02435849] and ENSIGN, N=58 [NCT02228096]) evaluated the efficacy and safety of tisagenlecleucel in pediatric and AYA patients with R/R ALL. Five patients received alloSCT as part of their initial therapy in CR1. After relapsing after alloSCT, they received tisagenlecleucel and were thus considered to be in their first relapse.

Table 1.

Characteristics of Patients Treated With Tisagenlecleucel in First Relapse Following AlloSCT in CR1

	Patient 1	Patient 2	Patient 3[a]	Patient 4	Patient 5
Age, years	7	10	9	4	18
Donor type	MUD	MSD	MSD	MSD	MSD
Time from transplant to relapse, months	4.5	12.4	18.6	17.5	5.8
Time from relapse to infusion, months	4.4	1.5	2.0	1.3	3.1
Best overall response	CR	CR	CR	CR	UNK[b]
MRD status	Negative	Negative	Negative	Negative	Negative
Overall survival, months	10.8+	18.8+	15.1+	33.3+	1.8+
Maximum grade CRS	-	Grade 3	-	-	-
Neurotoxicity	-	-	-	-	-

[a] Patient had Philadelphia chromosome–positive ALL.
[b] Bone marrow remission was observed, but cerebral spinal fluid assessment was not done.
AE, adverse event; CR, complete remission; CRS, cytokine release syndrome; MRD, minimal residual disease; MSD, matched sibling donor; MUD, matched unrelated donor; UNK, unknown.

Results: All 5 patients who received tisagenlecleucel in first relapse underwent chemotherapy after initial diagnosis followed by alloSCT in CR1; 1 patient received a transplant from a matched unrelated donor while the other 4 received transplants from matched siblings. Patients relapsed 4.5, 5.8, 12.4, 17.5 and 18.6 months following alloSCT with myeloablative conditioning. Blast counts at enrollment were 7%, 29%, 34%, 73%, and 79%; in the pooled ELIANA/ENSIGN cohort (N=133), bone marrow blasts ranged from 5% to 99%. Four patients were male-aged 4, 7, 9 and 10 years at enrollment. One patient was female-aged 18 years at enrollment. Infusion with tisagenlecleucel took place 1.3, 1.5, 2.0, 3.1, and 4.4 months after relapse, a range comparable to that in the ELIANA/ENSIGN cohort (1-14 months). Four of 5 patients achieved minimal residual disease (MRD)-negative CRs (overall remission rate, 80%). One patient had unknown overall response because cerebral spinal fluid assessment was not done, although MRD-negative bone marrow remission was observed. Durations of remission in patients who achieved CR starting from onset of remission were 3.2+ months (proceeded to SCT while in remission), 7.5+ months (non-SCT new therapy while in remission), 14.2+ months (ongoing remission), and 29.6+ months (non-SCT new therapy for secondary malignancy). All patients are still alive. Overall survival is 1.8+, 10.8+, 15.1+, 18.8+, and 33.3+ months after infusion. Only 1 patient experienced CRS (grade 3), and no patients experienced neurological toxicity. In the pooled cohort, 79% of patients experienced CRS (41% grade 3 or 4), and 37% experienced neurological events of any grade (Table 1).
Summary and Conclusions: Patients with ALL who received tisagenlecleucel for their first relapse following alloSCT tolerated the therapy well without increased toxicities and achieved MRD-negative and durable remissions.

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INDIRECT TREATMENT COMPARISON OF BLINATUMOMAB *VS* INOTUZUMAB OZOGAMICIN FOR TREATING ADULT PATIENTS WITH RELAPSED OR REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA RECEIVING ZERO OR ONE PRIOR SALVAGE THERAPY

J. Song[1], Q. Ma[2], W. Gao[3], Z. Cong[2,*], J. Xie[1], Z. Zimmerman[2], L. Belton[4], J. Franklin[2], S. Palmer[5]

[1]Analysis Group, Inc., Los Angeles, [2]Amgen Inc., Thousand Oaks, [3]Analysis Group, Inc., Boston, United States, [4]Amgen Inc., Uxbridge, [5]University of York, York, United Kingdom

Background: Blinatumomab (BLIN) and inotuzumab ozogamicin (INO) have demonstrated improved outcomes against standard of care (SOC) among adult patients with relapsed or refractory acute lymphoblastic leukemia (R/R ALL) in phase 3 clinical trials. In the absence of head-to-head studies, indirect comparisons provide valuable information to guide the appropriate use of alternative therapies.
Aims: This study aimed to indirectly compare the efficacy of BLIN *versus* INO among adult patients with R/R ALL.
Methods: Matching-adjusted indirect comparisons were conducted using patient-level data from the BLIN trial (TOWER) and published aggregated data from the INO trial (INO-VATE). Patients with 2+ prior salvage therapies from TOWER were excluded since these patients were not included in INO-VATE. To ensure balance in the remaining patients, TOWER population was reweighted to match the average baseline characteristics in INO-VATE, including sex, age, race, performance status, bone marrow blast, previous salvage therapy, previous allogeneic transplantation, complete remission with complete hematologic recovery (CR) to most recent induction therapy, and duration of first remission. The endpoints were overall survival (OS) and CR. Relative restricted mean survival time (RMST) at 12 months was also estimated.
Results: After excluding patients with 2+ prior salvage therapies, 310 patients in TOWER were included (BLIN: 203; SOC: 107). After reweighting, all baseline characteristics listed above were balanced between the two trials. The median OS was 9.3 months for BLIN and 7.7 months for INO (weighted log-rank test p=Not Significant [NS]). The RMST at 12 months was 1.6 months longer for BLIN than for INO (95% CI=[0.1, 3.2], p =0.042). The CR rates were similar ([BLIN-SOC in TOWER]-[INO-SOC in INO-VATE]=-2.8%, 95% CI=[-17.5%, 11.9%], p=NS).
Summary and Conclusions: After adjusting for heterogeneities in patient characteristics, BLIN demonstrated a potential survival benefit *versus* INO among adult patients with R/R ALL receiving zero or one prior salvage therapy. Our findings indicate potentially important differences in efficacy which help to inform treatment choices. Additional head-to-head studies would help to confirm our findings.

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LONG TERM OUTCOME OF 138 ALO TRANSPLANTS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA ON A SINGLE TRANSPLANT CENTER IN SPAIN

I. Ormazabal Velez[1,*], A. Figuera[1], B. González Ferrer[2], J. Cornago Navascués[1], B. Aguado Bueno[1], E. Sola[1], R. de la Cámara Llanza[1], A. García-Noblejas Moya[1], A. Alegre Amor[1], N. González Ruiz[2]

[1]Hematología y Hemoterapia, Hospital Universitario La Princesa, [2]Universidad Autónoma de Madrid, Universidad Autónoma de Madrid, Madrid, Spain

Background: Leukemia relapse remains the main cause of failure for long term survival in adult ALL, despite high rates of first complete remission (CR1) after induction and consolidation with risk adapted modern chemotherapy schemes. Further consolidation with allogeneic hematopoietic stem cell transplantation (HSCT) offers the best chance of cure for high-risk and relapsed patients. Long term follow of large patient cohorts should be evaluated for better definition of HSCT indication and timing.
Aims: The primary endpoint was disease-free survival (DFS) and overall survival (OS). Secondary endpoints refined acute graft *vs* host disease (aGvHD) and chronic GvHD (cGvHD) incidence, relapse incidence (RI), and non-relapse mortality (NRM). We also described separately the 2nd HSCT and the available cases of Minimal residual disease (MRD) by flow cytometry (FCM) before HSCT in ALL Ph-.
Methods: Between 1990 and 2016, 138 HSCT were performed in 128 patients with ALL in our hospital (10 double alloHSCT; 7,2%). Median age was 28 years (12-65). 76% of patients were younger than 40 y. 27,5% of cases (n=38) were Ph+ALL. Disease status before transplant was distributed as follows: 42% CR1, 60,5% (n=23) Ph+ALL and 35% (n=35) Ph-ALL. 58% had advanced disease (AD=≥RC2, MRD+, or active disease), 39,5% (n=15) Ph+ALL and 65% (n=65) Ph-ALL. Bone marrow (BM) progenitors were initially the preferred HSCT source (65,2%), but changed to peripheral blood (PB) after 2001. Antithymocyte globulin was administered with conditioning in case of HLA disparity (n=13; 9,4%).
Results: Focusing on the 38 cases with available MRD by FCM before HSCT in ALL Ph- , there were 11 relapses in the 18 positive cases (MRD ≥0,01%) whereas there were only 2 relapses in the 20 negative (MRD <0,01%) cases (61% *vs* 10%; p=0,03). In the case of 2nd HSCT (n=10), only 1 case was Ph+ (10%), there were 3 ALL with complex caryotype (30%) and 4 patients developed extramedular disease. Two patients remain alive. The main cause of death was relapse (n=5; 50%) followed by SOS (n=3; 30%). MRD before HSCT was available in 4 four cases, being all of them positive (1 molecular MRD+in ALL Ph+, 3 FCM ≥0,01%) (Figure 1).

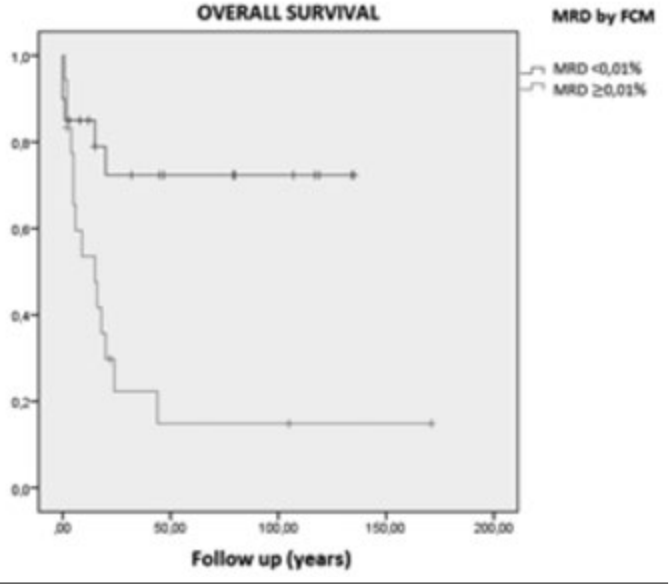

Figure 1.

Summary and Conclusions: Our serie shows a quite high, over 65%, long term DFS, for both Ph+ and Ph- ALL in patients who underwent HSCT in CR1, specially if it was MRD by FCM <0,01%. However it also shows that a fair proportion of advance disease patients could also be cured (25-30%).

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EPIGENOMIC ANALYSIS REVEALS STEM CELL-LIKE RELAPSE SIGNATURE IN PEDIATRIC ACUTE MYELOID LEUKEMIA

C. Wiggers[1,2,*], M. Baak[2], M. Creyghton[2], M. Bartels[1]

[1]Pediatrics, University Medical Center Utrecht, [2]Hubrecht Insitute, Utrecht, Netherlands

Background: Pediatric acute myeloid leukemia (AML) is characterized by a high relapse rate (~30%) and overall survival rates of 60-70%. This high relapse rate has been unchanged over the past years, illustrating the importance to increase our insights in pathophysiological mechanisms underlying AML relapse, including chemotherapy resistance and clonal selection. There is increasing evidence that epigenetic deregulation, which involves the activity of non-coding regulatory DNA elements such as promoters and enhancers, is involved in the initiation and progression of cancers, including adult AML. Since little is known concerning epigenetic (de)regulation during relapse in children, it is crucial to gain more insights into the epigenetic landscape of relapsed pediatric AML.
Aims: Identify epigenomic regulatory pathways involved in AML relapse in children.
Methods: The epigenome was investigated using ChIP-seq experiments of H3K27acetylation. This epigenetic mark is associated with active promoters and enhancers, and can therefore be used to identify active regulatory pathways. We investigated 27 (non-)relapsed pediatric AML patients, which harboured common molecular aberrations such as MLL-rearrangement, CBF-related and Flt3-ITD. Our ChIP-seq data was validated by RNA-seq experiments of several patient samples, as well as public RNA-seq data (TARGET AML study). Transcription factor motif enrichment was analysed using AME MEME Suite to evaluate potential upstream regulators.
Results: We successfully identified active promoters and enhancers marked by H3K27ac, resulting in a genome-wide epigenomic network for each pediatric AML patient. Upon sample clustering using these H3K27ac enriched regions, we observed 1) sub-clustering of samples based on molecular aberration, 2) high correlation between matched Dx-Rel samples except for 1 patient, and 3) - mainly in MLL-AF9 patient samples - a separation between relapsed (RP) and non-relapsed patients (NRP). We further investigated this separation based on relapse by linking differentially enriched (DE) regions between MLL-AF9 RP and NRP with their closest genes, resulting in RP and NRP specific gene sets. These gene sets were also differential expressed in our AML samples and publically available RNA-seq samples when analysed as one gene set. The "RP gene set" contained genes higher expressed in hematopoietic stem cells compared to monocytes and was associated with cell proliferation, proto-oncogenes as well as signalling pathways including sphingolipid signalling, a pathway involved in pro-survival and anti-apoptotic processes. Furthermore, transcription factor (TF) motif enrichment in DE regions identified motifs of which some TFs were also part of our gene sets such as GATA2, a known marker for poor prognosis in pediatric AML. At last, we were able to separate RPs from NRPs of other molecular subtypes based on a subset of regions identified in the MLL-AF9 relapse signature, suggesting that this signature also accounts for multiple pediatric AML subtypes.
Summary and Conclusions: We analysed the active chromatin landscape of pediatric AML patient samples using H3K27ac ChIP-seq experiments. Our results led to the identification of a stem cell-like gene signature associated with AML relapse, what could be used as a relapse prognostic gene set in pediatric AML. Additionally, identified signalling pathways and TFs could potentially play a role in chemotherapy resistance leading to relapse, of which sphingosine deregulation by SPHK1 inhibition is already shown to have anti-proliferation and anti-survival effects in AML cells.

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ROBUST *IN VIVO* SYNERGY BETWEEN THE ANTI-CD33 ADC, IMGN779 AND THE FLT3 INHIBITOR QUIZARTINIB IN HUMAN FLT3-ITD AML MODELS

K. Watkins[1,*], L. Flaherty[1], R. McCarthy[2], K. Francisco[2], S. Adams[2], C. Sloss[1], A. Romanelli[3]

[1]Translational Medicine, [2]Pharmacology, [3]Heme Product Development, ImmunoGen, Inc, Waltham, United States

Background: Acute myeloid leukemia (AML) patients harboring a FLT3

Internal Tandem Duplication (FLT3-ITD) have a poor prognosis and high rates of relapse following treatment with standard agents. Midostaurin has been approved recently for the treatment of newly diagnosed FLT3 mutant AML patients. Due to efficacy and toxicity concerns with Midostaurin, additional FLT3 inhibitors, such as the more potent and specific quizartinib, are currently being evaluated in clinical trials for frontline and relapsed AML patients. In pre-clinical models, FLT3 inhibitors decrease anti-apoptotic signaling and re-sensitize FLT3-ITD-positive leukemia to cytotoxic drugs. IMGN779 is a next-generation anti-CD33 antibody-drug conjugate (ADC) with a novel DNA-alkylating IGN payload and a cleavable s-SPDB linker, currently in Phase 1 development for AML. Here we show that the combination of IMGN779 with the FLT3 inhibitor quizartinib leads to increased efficacy in pre-clinical models of FLT3-ITD AML.

Aims: To investigate the anti-leukemic efficacy of IMGN779 and quizartinib in FLT3-ITD-positive AML models.

Methods: *In vitro* cell viability of FLT3-ITD AML cell lines (Molm-13 and MV4-11) treated with IMGN779, quizartinib, or the combination of both were assessed by WST-8 after 4 days of culture. Mechanistic studies were carried out by immunoblotting techniques and by flow cytometry. IMGN779 and quizartinib were evaluated *in vivo* for antitumor activity, against two disseminated AML xenografts. Mice engrafted with AML cells were treated with: vehicle control; quizartinib daily for 14 days; IMGN779 weekly for 3 weeks; or the combination of both.

Results: The *in vitro* combination of IMGN779 and quizartinib in both FLT3-ITD-positive cell lines led to dose-dependent additive or synergistic anti-leukemic activity and increases in the apoptotic proteins cleaved Caspase3 and cleaved PARP1. Cells treated with quizartinib showed decreases in the anti-apoptotic protein Mcl-1, in the pro-survival/proliferative proteins p-Stat5 and p-Erk, and in the DNA repair protein, Rad51. The combination of IMGN779 and quizartinib was also investigated *in vivo*: Molm-13-engrafted mice treated with IMGN779 and quizartinib had a 500% increased life span (ILS) over the vehicle-treated mice, superior to either single agent treatment (IMGN779=71% ILS or quizartinib=55% ILS *vs* vehicle control). Importantly, 4 out of 6 mice treated with the combination were alive at 126 days, which was twice as long as the last surviving mouse treated with either single agent (56 days with IMGN779 and 39 days with quizartinib). Similarly, MV4-11-engrafted mice treated with IMGN779 and quizartinib had a significant 134% ILS over the vehicle-treated mice. Matched dose, single agent treatment resulted in only modest increases in survival (40% ILS *vs* vehicle control) (Figure 1).

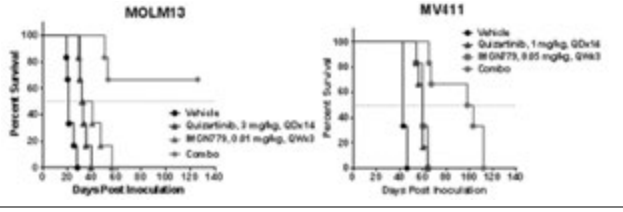

Figure 1.

Summary and Conclusions: In pre-clinical studies of FLT3-ITD AML, the combination of IMGN779 and quizartinib results in a robust anti-leukemic effect, well beyond that of either agent alone. *In vivo* studies have shown this effect in two disseminated models, suggesting cooperativity of their distinct anti-leukemic mechanisms of action without overt increases in toxicity. *In vitro* studies indicate that this effect correlates with quizartinib-driven decreases in anti-apoptotic/pro-survival signaling (Mcl-1; p-Erk; p-Stat5) and in DNA damage repair capacity (Rad51), as well as subsequent increases in apoptosis (c-PARP1; c-Caspase3). Collectively, these findings support testing the combination of IMGN779 and FLT3 inhibition in a clinical trial with FLT3-ITD mutant AML patients.

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NOVEL MUTATIONS AND TRANSLOCATIONS INVOLVING NUCLEOPHOSMIN (NPM1) GENE IN ACUTE MYELOID LEUKEMIA (AML) AND LEADING TO ABERRANT CYTOPLASMIC NPM1

M.P. Martelli[1,*], R. Rossi[1], A. Venanzi[1], M. Meggendorfer[2], V. Perriello[1], O. Spinelli[3], R. Ciurnelli[1], L. Brunetti[1], S. Ascani[1], C. Quadalti[1], G. Specchia[4], F. Di Raimondo[5], G. Avvisati[6], A. Rambaldi[7], F. Falzetti[1], E. Tiacci[1], P. Sportoletti[1], T. Haferlach[2], C. Haferlach[2], B. Falini[1]

[1]Hematology, University of Perugia, Perugia, Italy, [2]MLL, Munich Leukemia Laboratory, München, Germany, [3]ASST Papa Giovanni XXIII, Bergamo, [4]Hematology, University of Bari, Bari, [5]Hematology, University of Catania, Catania, [6]Hematology, Campus Bio-medico University of Rome, Rome, [7]Hematology, University of Milan, ASST Papa Giovanni XXIII,, Bergamo, Italy

Background: Nucleophosmin (*NPM1*) gene mutations occur in 50–60% of adult AML with normal karyotype (Falini *et al.,* NEJM 2005). About 50 *NPM1* mutations have been so far identified, all clustering in exon-12 but few sporadic cases involving either exon-9 (one) (Mariano *et al.,* Oncogene 2006) or exon-11 (two) (Albiero *et al.,* Leukemia 2007). More recently, rare NPM1 fusion proteins have been also described in AML (Paulo Vidal Campreger *et al.* Haematologica, 2016). In spite of molecular heterogeneity, all mutations cause common changes at the C-terminus of NPM1 mutants, *i.e.* loss of tryptophans 288 and 290 (or 290 alone) and creation of a new nuclear export signal (NES) motif, which binds to Exportin1/Crm1 and mediates its aberrant accumulation in the cytoplasm of leukemic cells, an event which is detectable by immunohistochemistry (Falini *et al.,* Blood 2006b).

Aims: Here, we aimed to identify novel *NPM1* genetic lesions, either mutations or NPM1 fusion proteins, leading to aberrant cytoplasmic NPM1 in AML, a critical event in leukemia development and maintenance (Brunetti, ASH Abs 877, 2017).

Methods: We applied a combinatorial approach of immunohistochemistry (IHC), western blot (WB) with specific antibodies for either the mutated or the wild-type NPM1 protein, *NPM1* gene sequencing and RNAseq to a large number (about 900) of patients from either our center or other Italian centers and the MLL laboratory in Munich, and focused on the discordant cases for IHC, WB or gene sequencing to identify novel *NPM1* genetic lesions. In the newly discovered *NPM1* mutated genes/fusion transcripts, search for acquisition of a NES motif in their protein sequence and strength of the NES were functionally evaluated in an overexpression ectopic cellular system.

Results: This research approach confirmed the rarity of mutations involving exons other that exon 12 in NPM1-mutated AML, and allowed the discovery of different novel mutations in exon 6 (n=3, Italian center) and exon 5 (n=1, MLL laboratory). An example of a mutation involving exon 6 is shown in Figure 1A. As a consequence of an in-frame 21 nucleotides insertion, a NES sequence was newly inserted whilst the rest of the protein sequence was conserved, explaining WB results. The new NES was experimentally confirmed to be active leading to cytoplasmic dislocation of the mutant (Figure 1A). In another series of patients, RNAseq analysis (MLL laboratory) lead to identification of 3 novel NPM1 gene translocation involving i) *RPP30,* t(5;10)(q35;q23); ii) *SETBP1,* t(5;18)(q34;q12); and iii) *CCDC28A,* t(5;6)(q35;q23). The latter was found also in one patient followed at our center where cytoplasmic positivity of the new fusion protein was confirmed by either IHC on patient bone marrow biopsy or immunofluorescence microscopy detection of the eGFP-NPM1/CCDC28A fusion protein (Figure 1B). Strikingly, in all cases, as shown in the representative cases in Figure 1, the predicted new protein sequence harboured a strong NES domain, either newly created or present in the partner protein sequence, which ensured its cytoplasmic accumulation, confirming the concept that *NPM1* mutation in AML are 'born to be exported'(Bolli *et al.,* Cancer Res 2007).

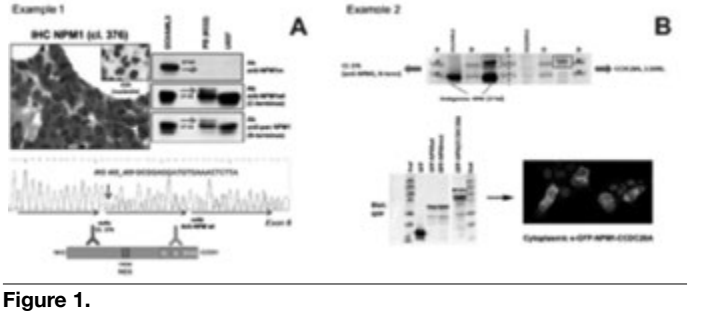

Figure 1.

Summary and Conclusions: Here, we report on the identification and functional characterization of novel *NPM1* mutations/fusion transcript in AML. Our observations further support the view that cytoplasmic NPM1 dislocation is a critical event in leukemogenesis, and that immunohistochemistry, that detects, through cytoplasmic dislocation of NPM, 'all types' of *NPM1* mutations, might be used as first step for directing further molecular studies.

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THE LSC17 SCORE ALLOWS RISK STRATIFICATION IN PEDIATRIC ACUTE MYELOID LEUKEMIA

N. Duployez[1,*], A. Marceau-Renaut[1], C. Villenet[2], A. Petit[3], S.W.K. Ng[4], F. Gonzales[5], N. Pottier[6], A. Paquet[7], B. Nelken[8], G. Michel[9], A. Baruchel[10], Y. Bertrand[11], G. Leverger[3], H. Lapillonne[12], M. Figeac[2], J.E. Dick[13], C. Preudhomme[1], M. Cheok[14]

[1]Laboratory of Hematology, UMR-S 1172, CHU Lille, Univ. Lille, INSERM, [2]Functional Genomics Platform, Univ. Lille, Lille, [3]Department of Pediatric Hematology and Oncology, GH HUEP, Trousseau Hospital, APHP, Paris, France, [4]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario M5G 1A1, Canada, [5]Department of Pediatric Hematology, UMR-S 1172, CHU Lille, Univ. Lille, INSERM, [6]Department of Biochemistry, CHU Lille, Lille, [7]Institute of Molecular and Cellular Pharmacology, Functional genomics, CNRS, University Nice Sophia Antipolis, Nice, [8]Department of Pediatric Hematology, CHU Lille, Lille, [9]Department of Pediatric Hematology, La Timone, CHU Marseille, Marseille, [10]Department of Pediatric Hematology and Immunology, Robert Debré University Hospital, APHP, Paris, [11]Institute of Hematology and Oncology Pediatrics, Hospices Civils de Lyon, Claude Bernard University, Lyon, [12]Laboratory of Hematology, GH HUEP, Trousseau Hospital, APHP, Paris, France, [13]Department of Molecular Genetics, University of Toronto, Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada, [14]UMR-S 1172, Institute for Cancer Research Lille, INSERM, Lille, France

Background: Despite major treatment improvements over the past decades, pediatric acute myeloid leukemia (AML) is still a life threatening with relapse rates up to 30% and survival rates below 75%. While strong pretherapeutic prognostic factors have emerged, especially based on cytogenetic and molecular aberrations, some patients still relapse despite the lack of adverse risk factors. Recently, we participated in a study reporting a stemness-associated 17-gene expression signature (LSC17 score) as an independent prognostic factor in adult AML, irrespective of genetic alterations (Ng *et al.*, *Nature* 2016). However, the relevance of the LSC17 score in childhood AML needs further investigations.
Aims: We retrospectively evaluated the prognostic value of the LSC17 score in a well-annotated cohort of *de novo* pediatric AML.
Methods: This study focused on 163 children (0-18 years) enrolled in the ELAM02 trial (ClinicalTrials.gov NCT00149162) with available RNA material from AML diagnosis. The Nanostring assay was performed as previously described (Ng *et al.*). Similarly, high and low scores were defined as above and below the median.

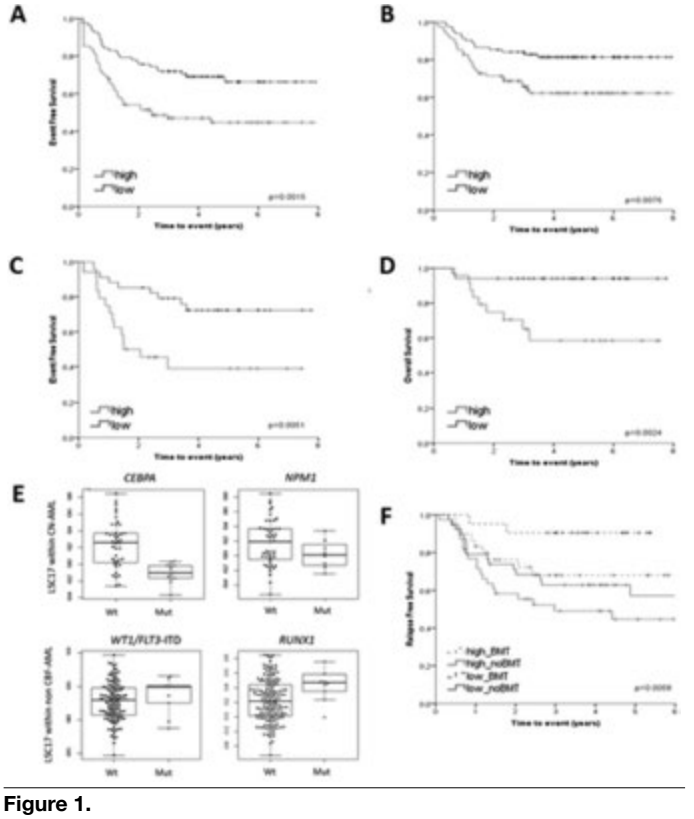

Figure 1.

Results: The distribution in the cytogenetic subgroups was: Normal karyotype (n=58), *KMT2A*-rearranged (n=48), abnormal karyotype (n=57). CBF AML were excluded from the analysis. No difference in routine clinical parameters (age, sex, WBC at diagnosis) was found between the global ELAM02 cohort and the LSC17 sub cohort, which were selected based on available RNA material. All patients received chemotherapy as induction treatment. Children with low LSC17 score had significantly better overall survival (OS) and event free survival (EFS) compared to patients with high LSC17 score. (5y-OS: 81.4% *vs* 62.2%, p=0.0034; 5y-EFS: 66.3% *vs* 44.7%, p=0.0076)(Figure 1A-B). Notably, a strong prognostic value was observed in cytogenetically normal (CN)-AML both on OS and EFS (5y-OS: 94.1% *vs* 58.5%, p=0.0024; 5y-EFS: 72.3% *vs* 39.0% p=0.0051)(Figure 1C-D). Importantly, LSC17 as a continuous variable remained a powerful prognostic factor in multivariate analysis, including WBC and genetic risk categories (accoridng to MyeChild stratification) for both EFS (hazard ratio [HR]: 5.14; IC95%: 1.97-13.40]; p=0.0008) and OS (HR: 4.88, IC95% 1.47-16.23; p=0.0098). The LSC17 score was not associated with high initial white blood cell count. High and low LSC17 scores were distributed in all cytogenetic subgroups with no significant association (Figure 1E). However, among CN-AML, a low LSC17 score was associated with favorable genotypes such as *CEBPA* biallelic mutations and *NPM1* mutations (Figure 1E). On the other hand, *RUNX1* and *WT1/FLT3*-ITD-mutated AML were associated with high LSC17 scores. Finally, allogeneic stem cell transplantation (allo-SCT) in first complete remission improved relapse free survival (RFS) in intermediate or poor genetic-risk patients with low LSC17 score (5-y RFS: 90.5% *vs* 57.1%, p=0.021) and high LSC17 score (5-y RFS: 68.0% *vs* 44.6%, p=0.101) respectively (Figure 1F).
Summary and Conclusions: As in adult AML, our study in pediatric AML shows that the LSC17 score is of comparable clinical prognostic relevance, irrespective of known adverse prognostic factors. Specifically, in AML with no cytogenetic lesion (CN-AML), which makes up 25% of all pediatric AML and forms a heterogeneous subgroup, the LSC17 score maybe a clinical useful prognostic marker. Allo-SCT still improved prognostic in patients with intermediate or poor genetic-risk although it did not reach statistical significance in patients with high LSC17 score.

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MUTATION PROFILE AND BENEFIT OF GEMTUZUMAB OZOGAMICIN IN ACUTE MYELOID LEUKEMIA PATIENTS TREATED IN THE ALFA-0701 TRIAL

E. Fournier[1,*], D. Lara[2], M. Bucci[1], N. Duployez[1], A. Marceau-Renault[1], J. Lambert[2], A. Renneville[1], D. Hervé[3], S. Castaigne[2], C. Preudhomme[1], N. Boissel[3]

[1]59, CHRU Lille, Lille, [2]78, Versailles hospital, Le Chesnay, [3]75, Saint Louis hospital, Paris, France

Background: The molecular landscape of AML has led to the discovery of a number of recurrent mutations in AML with a complex and dynamic clonal architecture. The prognostic relevance of these events has been evaluated in large cohorts of adult patients and is progressively incorporated into risk stratification (ELN-2017). However, the predictive impact of these mutations is less well known, particularly in non-targeted therapies.
Aims: We described the molecular landscape in a cohort of AML patients aged 50-70 years old randomized in the ALFA-0701 trial to receive gemtuzumab ozogamicin (GO) in combination with standard chemotherapy. We retrospectively addressed the question of the predictive value of these molecular events on the benefit of GO.
Methods: This study focus on 250 patients enrolled in the ALFA-0701 trial (NCT00927498) with available DNA at AML diagnosis. High-throughput sequencing was targeted on 43 genes recurrently mutated in myeloid malignancies. Genes were classified as follow: signaling pathway effectors (class I), transcription factors (class II), spliceosome, cohesin-complex, epigenetic regulators (including chromatin modifications and DNA methylation), tumor-suppressors and *NPM1*.
Results: The median age at AML diagnosis was 62.1 years (range 50.1-70.9). Among 250 patients, we identified 709 mutations involving 37 genes, and 233 patients (93%) had at least 1 mutation. Eleven genes were recurrently mutated in more than 10% of patients including *NPM1* (33%), *DNMT3A* (28%), *FLT3-ITD* (20%), *TET2* (20%), *RUNX1* (20%), *NRAS* (14%), *IDH2* (14%), *ASXL1* (13%), *SRSF2* (12%), *IDH1* (10%) and *TP53* (10%). Mutations involving epigenetic regulators were by far the most frequent lesions, encountered in 68% of the whole cohort. *NPM1*, *FLT3*-ITD, *DNMT3A* and *IDH2* mutations were associated with intermediate cytogenetic while *TP53* were more frequent in the poor-risk cytogenetic subgroup. The benefit of GO appeared quite heterogeneous with discrepancies according to mutational profiles. In term of event-free survival, a benefit of GO was mostly observed in patients with class I mutations (HR 0.43, 95%CI[0.29;0.66]) but also in patients with spliceosome (HR 0.47, 95%CI[0.26;0.86]), *NPM1* (HR 0.47, 95%CI[0.28;0.80]), or epigenetic mutations (HR 0.63, 95%CI[0.44;0.89]) but not in other subgroups. The benefit of GO was correlated to CD33 expression levels among the different gene mutations, with particularly high levels observed in class I mutated genes (Pearson -0.67, Figure 1A). Most importantly we observed that, among the different mutation groups that benefit from GO, this benefit was restricted to patients with cooccurring class I mutations. Indeed, a sig-

nificant interaction between class I mutation and GO administration was noted in patients with *NPM1* mutation (p=0.037) or epigenetic mutations (p=0.016, Figure 1B).

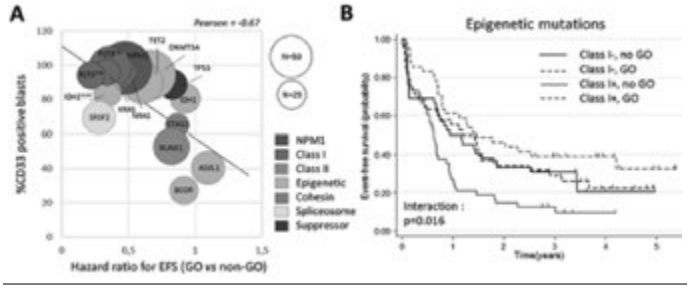

Figure 1.

Summary and Conclusions: In the present study, we showed that the benefit of GO in AML was heterogeneously distributed among the different molecular aberrations and correlated with mutation-associated CD33 expression levels. The benefit of GO was mostly restricted to the group of class I mutation whatever the associated mutation profile.

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MOLECULAR PATHOGENESIS OF DISEASE PROGRESSION IN MLL-REARRANGED AML

S. Kotani[1,2], K. Kataoka[1], A. Yoda[1,3], A. Kon[1], Y. Ochi[1,2], Y. Shiozawa[1], C. Hirsch[4], J. Takeda[1,2], H. Ueno[1], T. Yoshizato[1], K. Yoshida[1], Y. Nannya[1], T. Yamauchi[5,6], N. Kakiuchi[1], K. Aoki[1], Y. Shiraishi[7], S. Miyano[7], T. Maeda[5,8], J. Maciejewski[4], A. Takaori-Kondo[2], S. Ogawa[1,9], H. Makishima[1,4,*]

[1]Pathology and Tumor Biology, [2]Department of Hematology and Oncology, Kyoto University, Kyoto, Japan, [3]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, [4]Department of Translational Hematology and Oncology Research, Cleveland Clinic, Cleveland, [5]Division of Hematology, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, United States, [6]Department of Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, Fukuoka, [7]Laboratory of DNA Information Analysis, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, [8]Center for Cellular and Molecular Medicine, Kyushu University Hospital, Fukuoka, Japan, [9]Department of Medicine Center for Hematology and Regenerative Medicine, Karolinska Institutet, Huddinge, Sweden

Background: Despite advanced therapeutics, many leukemia patients become refractory to additional therapy, accounting for a major cause of leukemic deaths. Among major human acute myeloid leukemias (AMLs), *MLL*-rearranged AML (*MLL*-AML) is characterized by poor prognosis due to chemo-resistance and a shorter period to relapse. While many groups reported secondary somatic mutations in *MLL*-rearranged AML, their roles in disease progression and refractoriness or relapse have not fully been elucidated.
Aims: In this study, we investigated the role of secondary mutations acquired during the progression of *MLL*-AML,using a mouse model of *MLL*-AML.
Methods: We retrovirally transduced mouse stem cells with *MLL/AF9*, which were serially transplanted in C57BL/6 mice, where clonal evolution was monitored in each transplant, in terms of gene mutations using whole exome sequencing. To clarify the role of candidate driver genes, we examined their expression levels in human samples, and also performed *in vitro* functional studies using forced expression, shRNA-mediated knockdown, and CRISPR/Cas9-knockout experiments.
Results: The onset of leukemia was progressively accelerated with advanced transplants, during which increasing numbers of somatic mutations were identified with a median of 25 and 37 mutations after the first (n=2) and the fourth (n=9) transplants, respectively (P<0.001). Interestingly, these mutations included *Polr2a* and *Polr3d*, which are biologically related to Mll complex and a mutation affecting *Ptpn11* (p.G60R), which was recurrently mutated in human *MLL*-AML. Another mutation of interest was a *Gnb2* mutation affecting Gly77 that is adjacent to the residues recurrently mutated in other cancers. Allele specific quantitative PCR showed that a clonal burden of *Gnb2* mutation (p.G77R) had a significant trend to increase during serial transplantations (P=0.0012). In human *MLL*-AML cases, *GNB2* transcripts were significantly upregulated compared to *MLL*-rearrangement-negative AML cases, based on the publically available data sets for RNA sequencing (n=173) (P=0.03) and expression array (n=542) (P=0.009). Elevated *GNB2* expression was also found in human *MLL/AF9*-positive cell lines, THP1, MOLM13, and NOMO1. In functional studies, we demonstrated that overexpression of the mutant *GNB2* (p.G77R) conferred cytokine-independence to an interleukin 3 (IL-3)-dependent leukemic cell line, Ba/F3 (Figure 1a). By contrast, knock-down of *GNB2* resulted in a significantly reduced proliferation in an *MLL*-AML cell line highly expressing *GNB2* (MOLM13), compared to control (P<0.05) (Figure 1b). Furthermore, we transfected a CRISPR-Cas9 library to *MLL/AF9*-transformed mouse cells to see an enrichment or depletion of *Gnb2*-targetted sgRNA. In line with the result of the knock-down experiment, we observed a significant depletion of the *Gnb2*-targetted sgRNA after the culture (P=0.004), confirming a potential oncogenic function of *Gnb2* in *MLL*-AML.

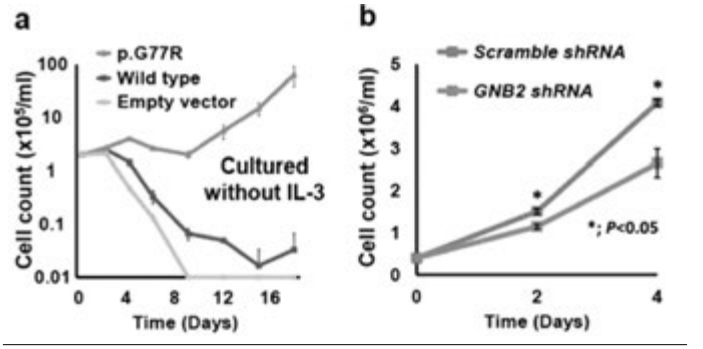

Figure 1.

Summary and Conclusions: The role of secondary mutations in the progression of *MLL*-AML was investigated using a retrovirus model, combined with whole exome sequencing and a genome-wide screen. Our findings suggest that *MLL*-fusion genes as initial drivers may cooperate subsequently with various genetic events, resulting in aggressive clinical course due to leukemic progression.

PF195

LEUKAEMIC BLASTS OUTSOURCE MITOPHAGY TO THEIR MICROENVIRONMENT

R. Horton[1,*], C. Marlein[1], T. Wileman[2], K. Bowles[3], M. Jefferson[2], S. Rushworth[1]

[1]Molecular Haematology, [2]UEA, [3]Haematology, Norfolk and Norwich University Hospital, Norwich, United Kingdom

Background: Acute myeloid leukaemia (AML) is a tumour dependant on the bone marrow microenvironment for survival and proliferation. We have recently shown that mitochondria are transferred from bone marrow stromal cells (BMSC) to leukaemic blasts to enhance leukaemic metabolic potential (1). Mitochondria, however, are highly dynamic organelles and their morphology is both tightly regulated and intimately intertwined with a cell's fate. Just as a lack of functional mitochondria leads to a metabolic deficit and stalls cellular proliferation, clearance of damaged mitochondria is equally critical for cellular fitness. Autophagy is an intracellular degradation process involved in the removal of pathogens, damaged organelles and misfolded proteins. Mitophagy is a subset of autophagy for mitochondria. Where mitophagy is inadequate, a build up of dysfunctional mitochondria leads to impaired electron transport chain function and increased oxidative stress. Here, we hypothesise that leukaemic blasts outsource their mitophagy by transferring their dysfunctional mitochondria to the bone marrow microenvironment for recycling.
Aims: To determine whether AML transfers mitochondria to its microenvironment for recycling by autophagy, and whether this response inhibits chemotherapy-induced apoptosis.
Methods: Primary acute myeloid leukaemia cells (AML) and BMSC were isolated from bone marrow aspirates following informed consent and under approval from the UK NHS Health Research Authority. Wild type and autophagy deficient mouse embryonic fibroblasts (MEF) were used. Mitochondrial membrane potential was measured by tetramethylrhodamine methyl ester (TMRM). Mitochondrial transfer was monitored using AML transduced with a rLV.EF1 mCherry-Mito-9 Lentivirus. Autophagy was measured by immunoblotting for P62 and conversion of LC3-I to LC3-II. Autophagosomes were visualised by fluorescent microscopy of BMSC transduced with adenoviral green fluorescent protein for LC-3, and this was quantified using imiris software.
Results: We report that AML is dependent on its microenvironment for the maintenance of mitochondrial membrane potential. Moreover, this protective effect of the microenvironment is seen when AML is cultured with wild-

type, but not with autophagy deficient, BMSC. In *ex-vivo* co-culture experiments, we find that AML increases the formation of LC3 puncta in BMSC, and by western blotting conversion of LC3I-LC3II. We employ a control experiment using media previously cultured with AML to demonstrate that these effects are surplus to starvation-induced autophagy. Next, we describe that AML transfers mitochondria to both wild-type and autophagy deficient MEF when in direct culture and that greater numbers of mitochondria remain in autophagy deficient MEF after 24 -hour co-culture. Furthermore, this export of mitochondria is upregulated in response to chemotherapy drugs. Finally we report that blocking the export of mitochondria increases AML apoptosis in response to chemotherapy.
Summary and Conclusions: Here, we report that AML transfers mitochondria to BMSCs where they undergo mitophagy. This response is upregulated in response to chemotherapy and may provide a novel target for overcoming chemotherapy resistant AML.

Reference:
1. Marlein CR, Zaitseva L, Piddock RE, Robinson SD, Edwards DR, Shafat MS, *et al.* NADPH oxidase-2 derived superoxide drives mitochondrial transfer from bone marrow stromal cells to leukemic blasts. Blood. 2017;130(14):1649-60.

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THERAPEUTIC TARGETING OF THE LEUKAEMIC FUSION GENE RUNX1/ETO VIA RNAI

H. Issa[1,*], H. Blair[1], N. Jyotsana[2], M. Heuser[2], O. Heidenreich[1]

[1]Wolfson Childhood Cancer Research Centre - Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom, [2]Department of Hematology, Hemostaseology, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany

Background: The t(8,21) translocation is the most prevalent chromosomal translocation in Acute Myeloid Leukaemia (AML), which leads to the expression of chimaeric RUNX1/ETO oncogene protein. Our previous work showed that RNAi-mediated *RUNX1/ETO* knockdown *in vitro* inhibits cell proliferation, reduces clonogenicity, causes G1 cell cycle arrest and impairs engraftment in immunocompromised host.
Aims: To silence *RUNX1/ETO* expression by an LNP/siRNA system *in vitro* and *in vivo* and to exploit its therapeutic potential.
Methods: We introduced several modifications on the 2'-position of the ribose and on the phosphodiester backbone. siRNA were encapsulated into Dlin-MC3-DMA lipid nanoparticles (LNP) using a microfluidic system for controlled mixing conditions on a NanoAssembr instrument. The activity of the modefied siRNA and LNP/siRNA were measured *in vitro* using t(8;21) AML cell lines: Kasumi-1, SKNO-1 and Kasumi-1 p.SLIEW stable cell line expressing EGFP and Luciferase. Patient primary t(8;21) cells were cultured on a Mesenchymal Stem Cells (MSC) feeder layer. 250,000 cells of Kasumi-1 p.SLIEW were transplanted intra-hepatically into new-born $Rag2^{-/-}$ $\gamma c^{-/-}$ mice. Engraftment was monitored by bioluminescent imaging (IVIS). LNP was functionalised with sulfo-cyanine 7.5 dye for live fluorescence imaging in the pharmacodynamic studies.
Results: *In vitro* studies demonstrated that the chemically modified siRNA substantially inhibited *RUNX1/ETO* and provided prolonged phenotypic and greater effect on AML cell lines in comparison with unmodified siRNA. Single treatment with modified LNP/siRNA induced a long-lasting inhibition of *RUNX1/ETO in vitro* and in t(8;21) AML primary patient cells as well as in t(8;21) PDX. Pharmacodynamic studies showed that a single dose of LNP/siRNA by systematic delivery routes provides global body distribution in immunocompromised mice including leukaemic tissues, liver, spleen, bone marrow and brain. Harvested human leukaemic cells from siRNA-treated $Rag2^{-/-}$ $\gamma c^{-/-}$ mice showed substantial reduction of RUNX1/ETO level with low dose of LNP/siRNA. This knockdown was associated with significant downregulation of *RUNX1/ETO* target genes such as *CCND2* and *TERT*. *RUNX1/ETO* depletion also severely impaired the clonogenic potential of the harvested leukaemic cells from LNP/siRNA treated mice and triggered senescence. Notably, only three doses of LNP/*RUNX1/ETO* siRNA entirely prevented the expansion of leukaemic cells in secondary transplanted recipients. LNP/siRNA mediated *RUNX1/ETO* depletion *in vivo* significantly enhanced the survival of immunocompromised mice. The LNP/siRNA treatment resulted in a prolonged median survival of 80 days comapred with 44 days for the control group. In line with these findings, RNA-seq indicated that *in vivo* depletion of RUNX1/ETO activates a myeloid differentiation programme.
Summary and Conclusions: Taken together, we have demonstrated that liposomal delivery of chemically modified *RUNX1/ETO* siRNA impairs RUNX1/ETO-driven transcriptional networks and therewith associated leukaemic self-renewal function and initiates differentiation, which may have a therapeutic potential.

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PREVALENCE AND CLINICAL IMPLICATIONS OF PPM1D TRUNCATING MUTATIONS IN THERAPY-RELATED MYELOID NEOPLASMS

J. Hsu[1,2,*], F. Wang[3], Y. Yan[4], J. Mathew[5], C. Gumbs[6], L. Little[3], S. Tippen[3], X. Song[3], J. Zhang[3], S. Kornblau[5], T. Kadia[5], N. Pemmaraju[5], N. Daver[5], C. DiNardo[5], M. Konopleva[5], M. Andreef[5], H. Kantarjian[5], K. Bhalla[5], G. Garcia-Manero[5], L. Donehower[7], P. A. Futreal[3], M. Goodell[8] K. Takahashi[5,6]

[1]Medical Scientist Training Program, [2]Translational Biology and Molecular Medicine, Baylor College of Medicine, [3]Department of Genomic Medicine, The University of Texas MD Anderson Cancer Center, [4]Department of Bioinformatics, The University of Texas Health Science Center at Houston, [5]Department of Leukemia, The University of Texas MD Anderson Cancer Center, [6]Department of Genomic Medicine, The University of Texas MD Anderson Cancer, [7]Molecular Virology and Microbiology, [8]Cell and Gene Therapy, Baylor College of Medicine, Houston, United States

Background: Therapy-related myeloid dysplastic syndrome (t-MDS) and therapy-related acute myeloid leukemia (t-AML), collectively termed therapy-related myeloid neoplasms (t-MNs), are rare but fatal complications of prior chemotherapy and/or radiotherapy. T-MNs have significantly worse clinical outcomes compared to *de novo* AML/MDS. However, the molecular underpinnings that distinguish t-MN from *de novo* AML/MDS are poorly understood. Truncating mutations in exon 6 of *PPM1D* (Protein Phosphatase Mg/Mn2+ 1D) have recently been identified in therapy-related clonal hematopoiesis and t-MDS (Coombs *et al.* 2017 and Lindsley *et al.* 2017), but the mutations are rarely detected in *de novo* AML/MDS. This suggests that selective pressure from prior therapy may be the key to the expansion and potential leukemogenic role of *PPM1D* mutant clones.
Aims: To understand the prevalence and clinical implications of *PPM1D* truncating mutations in t-MN.
Methods: We studied 156 patients with t-AML (N=77) or t-MDS (N=79). Diagnostic bone marrow samples from these patients were analyzed by targeted-capture deep sequencing of 295 cancer genes as well as *PPM1D* truncating mutations. The mutational landscape of t-MN was compared to that of *de novo* AML (N=120) and MDS (N=108), which were sequenced by the same platform.

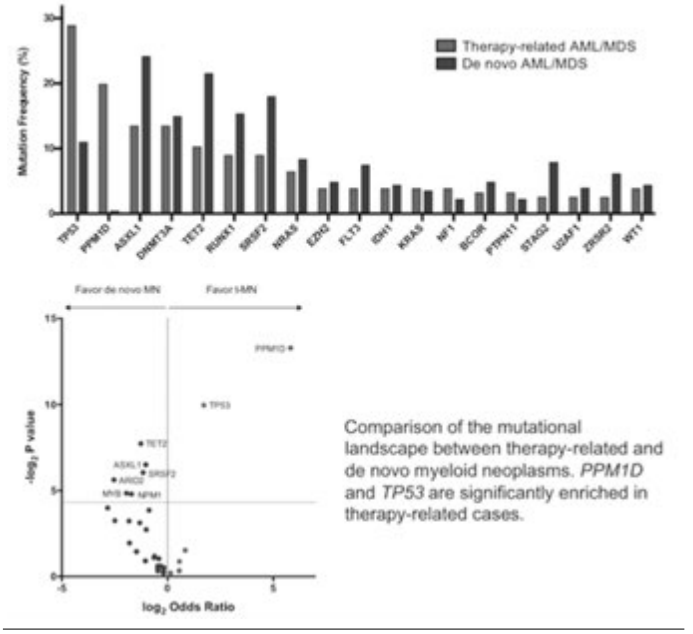


Figure 1.

Results: Of the 156 t-MN patients, *PPM1D* was mutated in 31 (20%) patients, making it the second most commonly mutated gene in t-MN after *TP53* (29%). *PPM1D* mutations were detected in only 0.5% of the matched *de novo* MDS/AML patient cohort, confirming a specific enrichment of *PPM1D* mutations in therapy-related diseases. The prevalence of *PPM1D* mutations

was similar between t-MDS and t-AML (19% *vs* 21%, p=0.904). *PPM1D* did not significantly co-mutate with other driver gene mutations or cytogenetic aberrations. All 31 *PPM1D* mutations identified in this t-MN cohort were protein-truncating variants spanning the final exon of the gene. The mean variant allele frequency (VAF) of *PPM1D* mutations was 0.105 (range 0.02-0.41). *PPM1D* VAF was higher in t-AML than t-MDS (0.148 *vs* 0.065, p=0.0065). 81% of the *PPM1D* mutations were considered sub-clonal and 19% clonal, based on VAF cutoff of 0.2. In 3 *PPM1D* mutated cases where there were available lymphoid (CD3+/CD19+) and non-lymphoid (CD3-/CD19-) fractions, *PPM1D* mutations were detected in both cellular fractions, suggesting a progenitor/stem cell origin. An analysis of prior cytotoxic exposures revealed that *PPM1D* mutations are significantly associated with prior exposure to platinum agents (p=0.004), etoposide (p=0.021), and self-reported alcohol use (p=0.015). Patients with *PPM1D* mutations did not have a statistically significant worse overall median survival compared to patients without *PPM1D* mutations (10 months *vs* 12.8 months respectively, p=0.513). Overall response rate to therapy with hypomethylating agents and cytarabine was also comparable between patients with and without *PPM1D* mutations (p=0.456 and 0.501, respectively) (Figure 1).

Summary and Conclusions: *PPM1D* is one of the top mutated genes in t-AML and t-MDS, and is specific to t-MN. The majority of the mutations were sub-clonal and *PPM1D* mutation status did not affect survival or response to therapy. *PPM1D* mutations are significantly associated with prior exposure to cisplatin and etoposide. These findings can potentially inform the choice of chemotherapeutic regimens for patients with primary cancers when clonal hematopoiesis with *PPM1D* mutations is detected.

PF198

LONGITUDINAL GENOMIC AND TRANSCRIPTOMIC TRACKING OF CORE BINDING FACTOR ACUTE MYELOID LEUKEMIA

T. Kim[1,2,*], J.H. Moon[3], J.-S. Ahn[4,5], M. Tyndel[2,6], Y.-K. Kim[4,5], S.-S. Lee[4], S.-Y. Ahn[4], S.-H. Jung[4], D.-H. Yang[4], J.-J. Lee[4], S.H. Choi[5], J.-Y. Lee[5], Y.J. Lee[3], S.K. Sohn[3], H.-J. Kim[4,5], Z. Zhang[1,2,7], D.D.H. Kim[8]

[1]Department of Computer Science, [2]Donnelly Centre, University of Toronto, Toronto, Canada, [3]Department of Hematology-Oncology, Kyungpook National University Hospital, Daegu, [4]Department of Hematology-Oncology, [5]Genomic Research Center for Hematopoietic Diseases, Chonnam National University Hwasun Hospital, Jeollanam-do, Korea, Republic Of, [6]The Edward S. Rogers Sr. Department of Electrical and Computer Engineering, [7]Department of Molecular Genetics, University of Toronto, [8]Department of Medical Oncology and Hematology, Princess Margaret Cancer Centre, Toronto, Canada

Background: Core binding factor acute myeloid leukemia (CBF-AML) is a subgroup of AML, mainly defined by two gene rearrangements affecting CBF; t(8;21)/*RUNX1-RUNX1T1* and inv(16)/*CBFB-MYH11*. Although their somatic mutation and expression profiles at diagnosis have been revealed, these profiles have not been tracked longitudinally.

Aims: To assess transcriptomic and genomic landscape of CBF-AML and identify their dynamics using serial samples

Methods: This study included bone marrow samples from 42 patients (23 t(8;21) and 19 inv(16) AML) taken at diagnosis and complete remission (CR). Using a custom myeloid gene panel covering exonic regions of 84 genes (Agilent SureSelect), we performed targeted DNA sequencing on 126 trio-samples (42 T-cell, diagnosis, and CR samples). During variant calling procedures, T-cell fraction (CD3+) was used as a control. Average on-target coverage was 1606x. Eighty-four samples (42 diagnosis and CR paired samples) were subject for RNA sequencing using Illumina TruSight Pan-Cancer panel. After read mapping, gene count was measured using HTSeq followed by DEseq2 for down-stream analyses. Gene fusion was detected using EricScript. Average number of mapped reads was 3.1M with 87% average mapping rate.

Results: At diagnosis, we detected 74 mutations from 38 patients (90%) (Figure 1A). Most frequently mutated genes were *NRAS* (n=15), *KIT* (n=15), *KRAS* (n=7) and, *ASXL2* (n=5). Chromatin modifiers and cohesin complex were exclusively mutated in t(8; 21) AML. At CR, although allelic burdens were nearly cleared (Mean VAF from 22.4% to 0.2%), residual allelic burden of 36 mutations (n=26) were still detected, commonly in *KIT* (n=11), *NRAS* (n=7), and *KRAS* (n=3) (Figure 1B-C). When projecting RNA expressions onto 2D space using principal component analyses, samples at diagnosis were distinctively clustered according to their subtype (Figure 1D). All CR samples were clustered regardless of subtypes. In addition to subtype-defining gene fusions, two additional gene fusions; *SLC45A3-ELK4* (n=3) and *CSNK1G2-JAK2* (n=2) were recurrent in t(8;21) AML. However, their presence did not result in distinct expression profile. At CR, t(8;21) was detected in 8 patients. By three-way comparisons of differentially expressed genes (DEGs), we identified 524 DEGs (Figure 1E). At diagnosis, we identified 297 DEGs, where 175 DEGs including *RUNX1T1* were highly expressed in t(8;21) AML and 122 DEGs including *MYH11* were expressed higher in inv(16) AML. When paired samples at diagnosis and CR were compared, we found 402 and 286 DEGs in t(8;21) AML and inv(16) AML, where 200 genes were shared. Interestingly, 177/200 shared DEGs showed same direction of abnormal expression. In addition, 200 shared DEGs (colored regions, Figure 1E) were enriched in KEGG terms related to immune response such as cytokine-cytokine receptor interaction (q <2.2e-5), allograft rejection (q <0.02) and, graft–*versus*–host disease (q <0.02).

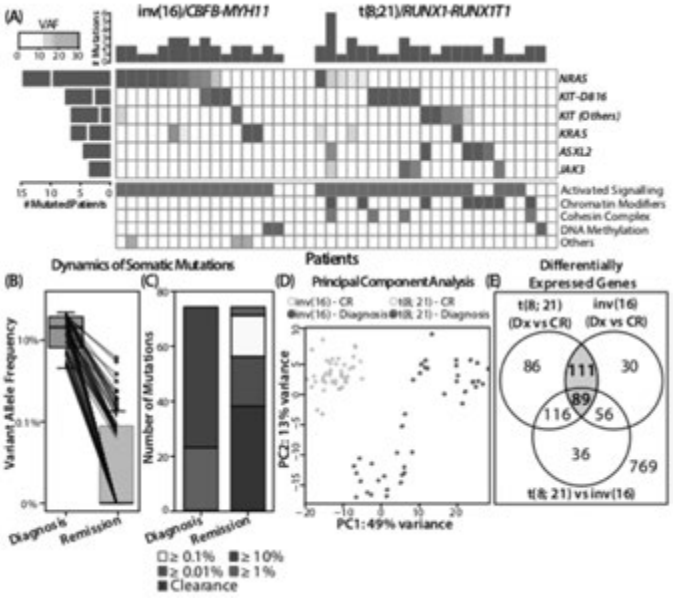


Figure 1.

Summary and Conclusions: Transcriptomic and genomic tracking of CBF AML using serial samples provided dynamics of mutation and RNA expression. With longitudinal tracking of somatic mutations, we showed residual allelic burden was detected in 62% of patients at CR. Second, RNA profiles of two subtypes of CBF-AML were distinct, but shared DEGs were enriched in immune response-related terms.

PF199

GENOMIC CHARACTERISTICS OF ACUTE ERYTHROID LEUKEMIA

J. Takeda[1,*], L.-Y. Shih[2], K. Chiba[3], Y. Shiraishi[3], Y. Shiozawa[4], H. Makishima[1], T. Yoshizato[1], Y. Nagata[5], A. Hangaishi[6], K. Ishiyama[7], Y. Miyazaki[8], H. Tsurumi[9], N. Hiramoto[10], T. Ishikawa[10], A. Takaori-Kondo[11], K. Kataoka[1], M. Sanada[12], H. Tanaka[3], K. Usuki[6], S. Miyawaki[13], S. Miyano[3], A. Ganser[14], F. Thol[14], Y. Nanya[1], K. Yoshida[1], S. Ogawa[1,15]

[1]Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto Univrsity, Kyoto, Japan, [2]Division of Haematology-Oncology, Department of Internal Medicine, Chang Gung Memorial Hospital, Chang Gung University, Taoyuan, Taiwan, Republic of China, [3]Human Genome Centre, Institute of Medical Science, [4]Department of Paediatrics Graduate School of Medicine, The University of Tokyo, Tokyo, Japan, [5]Department of Translational Haematology and Oncology Research, Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH, United States, [6]Department of Haematology, NTT Medical Centre Tokyo, Tokyo, [7]Department of Haematology, Kanazawa University, Kanazawa, [8]Department of Hematology, Atomic Bomb Disease Institute, Nagasaki University, Nagasaki, [9]Department of Hematology, Gifu University Graduate School of Medicine, Gifu, [10]Department of Hematology, Kobe City Medical Center General Hospital, Kobe, [11]Department of Haematology, Oncology, Graduate School of Medicine, Kyoto University, Kyoto, [12]Department of Advanced Diagnosis, Clinical Research Centre, National Hospital Organization Nagoya Medical Centre, Nagoya, [13]Division of Haematology, Tokyo Metropolitan Ohtsuka Hospital, Tokyo, Japan, [14]Department of Haematology, Haemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany, [15]Center for Hematology and Regenerative Medicine, Department of Medicine, Karolinska Institute, Stockholm, Sweden

Background: Acute erythroid leukemia (AEL) is a subtype of acute myeloid leukemia (AML) characterized by the predominance of erythroid proliferation as well as frequent myelodysplasia. However, it is controversial whether

or not AEL represents a discrete pathological entity; in fact, AEL has partly been integrated into a subtype of myelodysplastic syndromes (MDS) in the revised 4th edition of World Health Organization (WHO) classification. Characteristic mutational profiles of myeloid neoplasms with erythroid proliferation have not fully been elucidated except for frequent *TP53* mutations.
Aims: This study was designed to characterize the mutational profile of myeloid neoplasms with erythroid proliferation and to clarify its differences from, as well as, similarities to that of other types of AML and MDS.
Methods: We performed a comprehensive genetic study, in which paired tumor/normal DNA from 27 AMLs with increased erythroid precursors (AEL according to WHO 2000) were analyzed using whole-exome sequencing (WES). Three cases with AEL analyzed by The Cancer Genome Atlas (TCGA) were also included. We also analyzed mutations and copy number alterations in known/putative driver genes/chromosomes associated with myeloid malignancies in a total of 96 AEL cases using targeted-capture sequencing. The results were compared with those found in 1,540 AML targeted sequencing data (Papaemmanuil *et al.* NEJM. 2016).
Results: All patients were over 20 years old. The median age at diagnosis was 59 years old. Among the 59 patients whose blast count was available, 52 (88%) and 7 (12%) were classified into M6a and M6b by the French–American–British (FAB) classification, respectively. Most frequently observed in the total cohort were *TP53* mutations (n=35, 36%), of which 97% were accompanied by complex karyotype and *TP53* mutations were associated with a significantly shorter overall survival ($p < 0.001$). According to the genomic classification of AML proposed by Papaemmanuil et. al., the majority of samples (85%) were classified into 3 subgroups: those with *TP53* mutations/chromosomal aneuploidy (n=33), with *NPM1* mutation (n=15) and with mutated chromatin/RNA-splicing genes (n=34). Notably, among cases having *TP53* mutations/chromosomal aneuploidy, 12 (34%) and 14 (40%) cases had amplification in 19p and 21q, respectively, where the minimally affected regions included *EPOR* and *ERG*. In the subgroup with mutated chromatin/RNA-splicing genes, *STAG2 (53%), ASXL1* (33%) and *WT1* (20%) were enriched in AMLs with erythroid proliferation compared to other subtypes of AML. By contrast, mutations in *FLT3*, *DNMT3A* and *SF3B1* were significantly less frequent in our cohort.
Summary and Conclusions: Whole exome and targeted-capture sequencing revealed the landscape of genetic alterations in AML with erythroid proliferation. Our findings suggested that AML with erythroid proliferation is classified into 3 major subgroups having unique genetic and clinical features. Further molecular/biological characterization of each entity should be warranted.

PF200

FUNCTIONAL PROTEOMICS ANALYSIS OF AML-RELATED NUP98-FUSION PROTEIN INTERACTOMES

S. Terlecki-Zaniewicz[1,*], J. Schmöllerl[1], T. Eder[1], K. Parapatics[2], A. Müller[2], F. Grebien[1,3]

[1]Ludwig Boltzmann Institute for Cancer Research, [2]CeMM Research Center for Molecular Medicine, [3]University of Veterinary Medicine, Vienna, Austria

Background: Chromosomal translocations in cancer can result in the production of oncogenic fusion proteins (FPs). In leukemia, a particularly high number of fusion oncogenes has been identified. FPs involving the Nucleoporin 98 (*NUP98*) gene are found in ~2% of acute myeloid leukemia (AML) patients. AML patients with NUP98-rearrangements have a significant worse event-free survival compared to other AML patients and are often refractory to standard treatment. The NUP98 multi-partner translocation family (MPTF) features more than 25 different FPs, all harbouring an N-terminal fragment of the NUP98 gene fused to distinct C-terminal partners. Nup98-fusion proteins can be divided into two major categories. NUP98-homeodomain (HD) fusions link NUP98 to HD-containing transcription factors, including members of the HOXA, HOXC or PMX families. In contrast, non-HD NUP98-fusions contain plant HD (PHD) fingers or Su(var)3-9 Enhancer-of-zeste Trithorax (SET) domains, which are known to act as epigenetic modulators. Most prominently, however, coiled-coil domains are found in almost all non-HD fusion partners. Despite the heterogeneity of this MPTF, previous studies and preliminary data in our laboratory showed that different NUP98-FPs cause similar AML phenotypes in humans and mouse models.
Aims: We postulate that NUP98-FPs share molecular mechanisms that depend on conserved protein-protein interactions to modulate leukemogenic pathways. Thus, we aim to identify critical effector proteins through mass spectrometry (MS)-based profiling of the interactomes of five representative, yet distinct NUP98-fusion proteins.
Methods: Affinity-tagged variants of five selected NUP98-FPs (NUP98-HOXA9, -JARID1A, -DDX10, -NSD1 and -PSIP1) were stably expressed in a Doxycycline (Dox)-inducible fashion in human AML cells. Transgene induction was reported by expression of GFP. Routinely, 70% - 90% GFP+ cells could be detected 24 hours after Dox treatment. Protein complexes were purified from nuclear lysates and their composition was characterized by MS. Lysates from mock-transduced cells were used as negative controls. MS data were analysed using SearchGUI and PeptideShaker to identify the interactomes of NUP98-FPs.
Results: Exogenous NUP98 and known NUP98-binding partners, such as RAE1 and RAN, were highly abundant in all datasets. Upon stringent filtering, NUP98-FP interactomes revealed a network of 296 proteins, of which 103 interacted with three or more NUP98-FP. Functional annotation of conserved NUP98-FP-interactors revealed a significant enrichment for proteins involved in transcriptional regulation. In contrast, no components of the nuclear pore complex (NPC) were found, indicating that NUP98-FPs have active roles in transcriptional control but do not co-localise with the NPC.
Summary and Conclusions: In summary, this study provides the first comprehensive protein interactome of five NUP98-FPs. Together with the future functional investigation of common NUP98-FP interactors, this study will significantly enhance our understanding of the molecular mechanisms of NUP98-FP driven AML.

PF201

THE PNT DOMAIN OF ERG REGULATES HEMATOPOIETIC STEMNESS, YET IS NOT ESSENTIAL FOR ERG INDUCED ACUTE MYELOID LEUKEMIA

E. Kugler[1,*], Y. Birger[1,2], D. Yong[3,4], A. Rein[1], G. Prive[*3,4], S. Izraeli[1,2]

[1]Human Molecular Genetics and Biochemsitry, Tel Aviv University, Tel Aviv, [2]Genes Development and Cancer Childhood Research Institute, Sheba medical center, Ramat Gan, Israel, [3]Department of Biochemistry, University of Toronto, [4]Princess Margaret Cancer Centre, Toronto, Canada

Background: The ETS transcription factor ERG has a key role in hematopoiesis. It is essential for hematopoietic stem cells maintenance and megakaryopoiesis. Increased expression of ERG is associated with poor prognosis in AML. Previous studies by us and others established ERG as an oncogene in human leukemia. The PNT domain of ERG is a conserved domain that in some other ETS factors is required for protein-protein interactions. The PNT domain is deleted in ERG fusion translocations in AML, in ALL with "ERG deletion" and in Ewing Sarcoma but is conserved in prostate cancer. The function of ERG PNT domain is, however, unknown.
Aims: We aimed to establish the role of the PNT domain in ERG leukemogenic pathway.
Methods: Murine fetal liver (FL)-derived-HSPCs transduced with human WT- and deleted PNT (DelPNT) domain ERG were studied for self-renewal and differentiation in methyl cellulose cultures and for transforming ability by in C57B6 mice. To study how the PNT domain affects ERG regulation of gene expression in pre-leukemic and leukemic conditions, we performed RNA sequencing on murine fetal liver-derived-HSPC transduced with ERG variants and on blasts derived from acute myeloid leukemia induced by ERG. To characterize the ERG protein interactome and the role of its PNT, a BioID proximity ligation analysis was conducted on 293T cells transfected with ERG variants.
Results: Re-plating assays in methycellulose has shown that the PNT domain was not essential for HSPC self-renewal. Remarkably, DelPNT-*ERG* transduced cells demonstrated markedly enhanced proliferation capacity with a scattered morphology of very small colonies. Flow cytometry analysis showed decreased expression of hematopoietic stem cell markers (Ckit, Sca1) for DelPNT-ERG transduced cells. Consistent with these findings RNA sequencing demonstrated enrichment of genes associated with myeloid differentiation and loss of hematopoietic stem cell signature in HSPC transduced with DelPNT-*ERG*. Thus, *in-vitro*, the expression of DelPNT-ERG caused marked proliferation of myeloid progenitors. Transduction transplantation assays in C57B6 mice demonstrated that the PNT domain is not essential for leukemia development. However, time to leukemia development was significantly delayed for delPNT-ERG transduced mice (33.1±7.9 and 54.67±17.53 days for WT-ERG *vs* DelPNT ERG respectively, P=0.015). Further analysis of these leukemias by RNA-Sequencing demonstrated that compared with leukemia caused by DelPNT ERG, WT-ERG induced leukemia had higher expression of cell cycle and c-Myc target genes. Interestingly, DelPNT-ERG leukemia demonstrated enhanced fibroblast growth factor signaling as was described before for the ETS related oncogenic fusion, EWS-FLI1, in Ewing Sarcoma where the PNT domain of FLI1 is absent. Bio-ID analysis demonstrated that the PNT domain is essential for ERG association with chromatin remodeling complexes and epigenetic regulators.
Summary and Conclusions: These results suggest that in pre-leukemic con-

ditions, the PNT domain have a role in maintaining the stem cell phenotype associated with ERG and prevention of premature differentiation possibly through regulation of chromatin structure and accessibility. It may also be necessary for the suppression of HSC proliferation. In its absence, HSC differentiate to highly proliferative myeloid precursors that can cause leukemia as is for example in AML caused by the FUS-ERG translocation that lacks the PNT domain.

PF202

FUNCTIONAL ANALYSIS OF EPIGENETIC AND TRANSCRIPTOMIC EFFECTS OF CEBPA MUTATIONS IN ACUTE MYELOID LEUKEMIA

E. Heyes[1,*], L. Schmidt[1], T. Eder[1], G. Volpe[2], C. Nerlov[3], J. Frampton[2], F. Grebien[14]

[1]Ludwig Boltzmann Institute for Cancer Research, Vienna, Austria, [2]Institute of Cancer and Genomic Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham, [3]MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom, [4]Institute for Medical Biochemistry, University of Veterinary Medicine, Vienna, Austria

Background: The transcription factor CCAAT-enhancer-binding protein alpha (C/EBPα) is a master regulator of granulopoiesis and regulates the switch between proliferating, uncommitted progenitors and cell-cycle-arrested, differentiated myeloid cells. Usage of two alternative translation initiation sites in the *CEBPA* mRNA results in expression of a full-length C/EBPα protein p42 (42 kDa) and a shorter p30 isoform (30 kDa). *CEBPA* is mutated in 9% of adult patients with Acute Myeloid Leukemia (AML). The most predominant type of mutations represents N-terminal frameshifts, and patients harboring monoallelic N-terminal *CEBPA* mutations exhibit low overall survival rates. *CEBPA* N-terminal mutations ablate the expression of p42 and result in exclusive expression of p30, but the molecular mechanisms underlying the oncogenic effect of C/EBPα p30 are largely unknown.
Aims: We aim to understand the molecular mechanisms of C/EBPα p30-specific effects on gene expression and chromatin modulation to elucidate the role of C/EBPα p30 in oncogenic transformation.
Methods: We generated myeloid progenitor cell lines from a mouse model of C/EBPα N-terminal AML, which exclusively expresses the p30 variant of C/EBPα (*Cebpa*$^{p30/p30}$ genotype). We developed an inducible RNAi system in this cell line model to investigate the global genomic and transcriptomic effects upon C/EBPα p30 knockdown (KD). Using this model, we mapped C/EBPα p30 binding sites and promoter and enhancer regions via C/EBPα and H3K27ac ChIP-Seq, respectively. In addition, we performed RNA-Seq analysis to characterize C/EBPα p30-dependent transcriptomic programs and ATAC-Seq to profile the global landscape of accessible chromatin in *Cebpa*$^{p30/p30}$ cells.
Results: Analysis of RNA-Seq data showed that C/EBPα p30 is required for the regulation of pathways involved in proliferation and self-renewal. p30-down-regulation caused significant changes of global gene expression programs, with 2103 genes down- and 1985 genes up-regulated upon p30 KD. In particular, KD of p30 led to up-regulation of gene expression programs associated with myeloid differentiation. This effect was confirmed by increased surface expression of differentiation markers, such as Mac-1. Analysis of C/EBPα ChIP-Seq data revealed genome binding of p30 in the proximity of 43% of all genes that were differentially expressed upon p30 KD. Furthermore, we found that 28% of p30-bound regions were located in promoter regions, while 35% of p30-bound regions localized to distal intergenic regions, implicating the involvement of p30 in regulation of the enhancer landscape.
Summary and Conclusions: In summary, we have created a robust dataset for a global analysis of C/EBPα p30-dependent effects on the epigenomic and transcriptomic landscape in *CEBPA* N-terminal mutant AML. Detailed integrative bioinformatics analysis will enable the identification of specific genomic regions essential for p30-mediated leukemogenesis. This will contribute to a comprehensive understanding of the role of p30 in AML development and provide starting points for the development of novel treatments.

PF203

DETECTION OF MEASURABLE RESIDUAL DISEASE (MRD) BY ULTRADEEP NEXT GENERATION SEQUENCING (NGS) IS HIGHLY PREDICTIVE OF OUTCOME IN NPM1 MUTATED ACUTE MYELOID LEUKEMIA (AML)

N. Patkar[1,*], R. Kodgule[1], C. Kakirde[1], G. Raval[1], P. Bhanshe[1], Y. Badrinath[1], S. Ghoghale[1], S. Kadechkar[1], S.H. Khizer[2], S. Kannan[3], D. Shetty[4], A. Gokarn[2], S. Punatkar[2], H. Jain[2], B. Bagal[2], H. Menon[5], M. Sengar[2], N. Khattry[2], P. Tembhare[1], P. Subramanian[1], S. Gujral[1]

[1]Haematopathology, [2]Adult Haematolymphoid Disease Management Group, [3]Biostatistics, [4]Cytogenetics, Tata Memorial Center, Navi Mumbai, [5]Haemato-Oncology, Cytecare Cancer Hospitals, Bangalore, India

Background: Serial monitoring of *NPM1* mutations from the blood or bone marrow using mutation specific assays during chemotherapy of AML with mutated *NPM1* (*NPM1*mut AML) has been shown to be highly predictive of relapse. DNA based NGS is a promising scalable solution that has the potential to detect MRD in AML. We demonstrate that ultradeep NGS based MRD (NGS-MRD) is independently predictive of outcome for *NPM1*mut AML.
Aims: To evaluate the clinical relevance of highly sensitive NGS-MRD in NPM1 mutated AML
Methods: A total of 83 patients of *NPM1*mut AML were diagnosed over a 5-year period from October 2012 to May 2017. These patients were treated with standard 3+7 induction followed by high dose cytarabine. MRD was assessed at the end of induction (PI) from the bone marrow (BM) and the end of consolidation (PC) using BM or blood. FCM-MRD was performed using 10 colour MRD assay using a combination of difference from normal and leukemia associated immunophenotype (LAIP) based approaches. Exon 12 of NPM1 (DNA) was amplified using adapter tagged primers and a dual indexed strategy. The library was sequenced at more than 500,000x coverage to have a sensitivity of 0.001%. Based on receiver operating characteristic (ROC) and Youden analysis, we used a 1 log cut-off between paired PI and PC MRD samples to classify patients as NGS MRD positive or negative. Overall survival (OS) and relapse free survival (RFS) were calculated as per standard recommendations. FCM and NGS-MRD were factored against OS and RFS using log-rank test and displayed using the Kaplan-Meier technique. Multivariate analysis was performed for PI FCM-MRD, NGS-MRD and *FLT3*-ITD at diagnosis using cox proportional hazards regression.
Results: The median RFS and OS were 19.4 & 20.6 months respectively (median follow-up 23.5 months). We could detect and monitor 12 different types of *NPM1* mutations of which type A mutation was the commonest (69.6%). FCM-MRD could be detected in 31.7% of PI and 24.4% of PC samples. Presence of FCM-MRD at end of induction was predictive of inferior OS (p=0.006) and RFS (p=0.0098). NPM1 NGS-MRD positive patients were significantly associated with inferior OS and RFS (p=0.0009 and p<0.0001 respectively). Multivariate analysis was performed when factoring for *NPM1* NGS-MRD, FCM-MRD (PI) and *FLT3*-ITD. When factored for OS (p=0. 0018, HR:3.88[95%CI:1.66-9.04]) & RFS (p=0.0001, HR:4.9[95%CI:2.28-10.58]), NGS-MRD emerged as the most significant independent prognostic factor with a rising trend predictive of poor outcome. FCM-MRD showed a tendency to predict poor survival when factored for OS (p=0.05, HR:2.38[95%CI:1.02-5.55]) and was significantly associated with inferior outcome when factored for RFS (p=0.016, HR:2.59[95%CI:1.2-5.59]). We further analysed the impact of *NPM1* NGS-MRD in patients who were FCM-MRD negative at either time points. We detected that FCM-MRD negative group (PI &/or PC) was dichotomized by *NPM1* NGS-MRD and patients who were NGS-MRD positive had a significantly worse OS (p=0.0014) and RFS(p<0.0001) as compared to those patients who were NGS-MRD negative. Patients who were negative by both MRD assays had a significantly improved OS & RFS as compared to the rest (p=0.0014 & p=0.0001 respectively) (Figure 1).

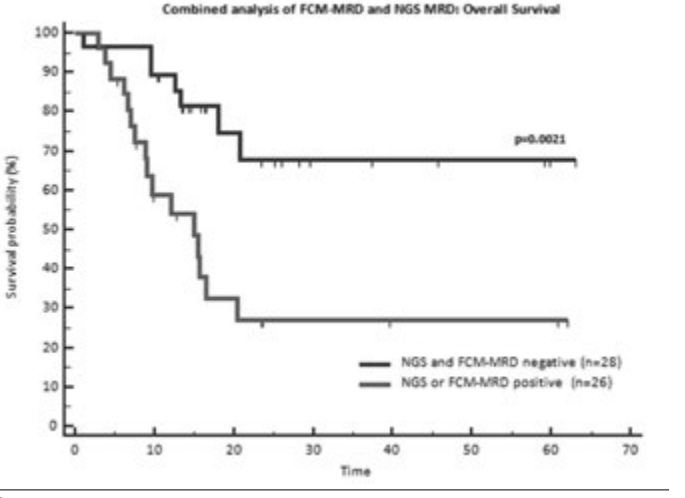


Figure 1.

Summary and Conclusions: We establish that DNA based *NPM1* NGS MRD is a highly useful test for prediction of relapse and survival in *NPM1*mut

AML. FCM and NGS-MRD for *NPM1*mut AML may be complementary in nature with patients who are negative for both FCM-MRD and NGS-MRD having excellent outcome.

PF204

PREFERENTIAL *IN VITRO* AND *IN VIVO* INDUCTION OF ENDOGENOUS RETROVIRUS TRANSCRIPTION FROM A MONOALLELIC CHROMOSOME 7Q IN DECITABINE (DAC) TREATED AML BLASTS

G. Greve[1,*], S. Bengel[1], D. Pfeifer[1], M. Lübbert[1]

[1]Hematology, Oncology and SCT, University of Freiburg Medical Center, Freiburg, Germany

Background: DNMT inhibitors (DNMTi) show an encouraging but not yet well-understood activity in AML patients (pts) with adverse cytogenetics, such as -7/7q-, (Lübbert *et al.*, Haematologica 2012). Integrative methylome/transcriptome studies have provided evidence for aberrant hypermethylation/silencing on monoallelic gene loci, including tumor suppressor genes (TSGs, Kotini *et al.*, Nat. Biotechnol. 2015). Recently, an alternative mechanism of DNMTi action was described (Roulois *et al.*, Chiappinelli et. al, Cell 2015): induction of silenced endogenous retroviral (ERV) dsRNA transcription, resulting in activation of an interferon-mediated, antitumor immune response ("viral mimicry").
Aims: We hypothesized that in AML haploinsufficient for 7q, transcriptional repression of monoallelic genes in this region may be preferentially reversed by DNMTi treatment.
Methods: The two AML cell lines ELF-153 (ELF, loss of 7p) and UCSD-AML1 (AML1, monosomy 7) were treated with DAC at equitoxic concentrations for 4 days, harvested on day 5. cDNA libraries (rRNA-depleted) were sequenced with 30 million paired-end reads, alignment to the reference genome was performed with TopHat2, read-counting with HTseq, differential expression testing with DESeq2. Selected target genes were validated by RT-qPCR. PBMCs were collected from 9 AML pts (5 pts with 7q-, 4 cytogenetically normal [CN] pts) treated with DAC (i.v. 20 mg/m^2 for 5 days) within the DECIDER trial (NCT00867672). Leukemic blasts at day 0 and day 8 were isolated using autoMACS with anti-human CD34 and CD117 microbeads. RNA-seq of these patients was performed with 60 million paired-end reads and analyzed as described for the cell lines.
Results: DAC treatment of AML1 and ELF cells resulted in massive transcriptome changes in both cell lines. Comparing DAC-induced expression changes only of genes on 7q, we identified 43 genes significantly differentially expressed only in AML1 cells, 24 only in ELF, and 19 in both. Among the genes on 7q that were selectively upregulated in AML1 were 3 with TSG features: *ZYX* (6.0-fold up), *HIPK2* (3.8-fold up), and *HBP1 (*3.1-fold up). However, the most heavily upregulated transcripts in AML1 were the endogenous retrovirus *ERV3-1* (86.4-fold up in AML1 *vs* 14.3-fold in ELF) and the neighboring *ZNF117 (*119.4-fold up in AML1 *vs* 6.5-fold in ELF). *ERV3-1* induction was accompanied by induction of the interferon response genes *RIG-I* and *IRF7* in both cell lines. Therefore, we next interrogated induction of these genes by DAC *in vivo*, utilizing sorted, matched primary AML blasts. Stronger induction of *ERV3-1* and *RIG-I* mRNA was seen in the 7q- pts compared to the CN pts: By qRT-PCR, median *ERV3-1* and *RIG-I* expression was induced 1.5- and 1.4-fold in the pts with 7q-, respectively, and 1.1- and 0.7-fold, respectively, in the CN pts. RNA-seq confirmed distinct expression changes after DAC treatment in 7q- and CN patients with 407 and 419 uniquely regulated transcripts, respectively, and only a small overlap of 22 transcripts.
Summary and Conclusions: We successfully developed an unbiased RNA-seq approach of AML cell lines either mono- or bi-allelic for 7q, demonstrating that DAC treatment preferential upregulates several monoallelic TSGs, and massively activates the *ERV3-1* gene. Induction of ERV3-1 and the dsRNA sensor RIG-I was validated *in vivo,* and preferentially seen in 7q- AML purified primary blasts. Thus, under clinically established treatment conditions, both the ERV and an interferon response gene can be activated by DAC, supporting a combination with immunotherapy.

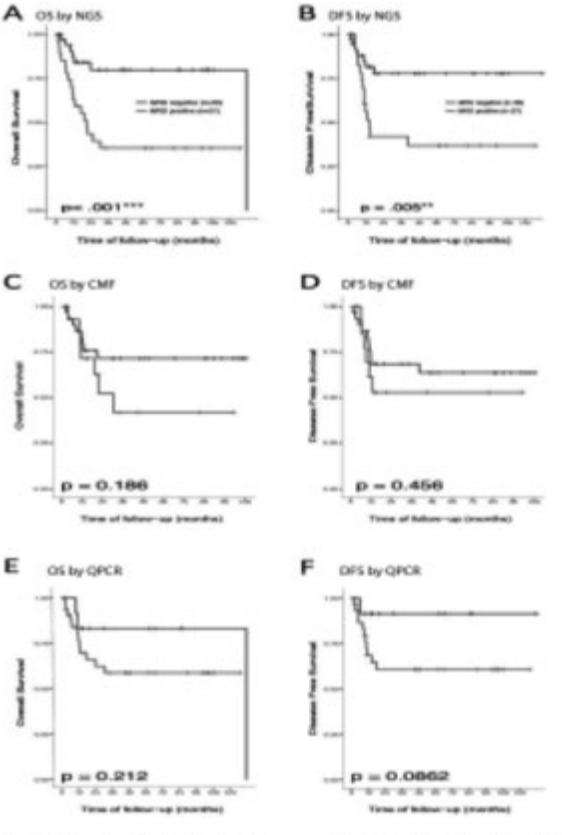
PF205

NOVEL DEEP TARGETED SEQUENCING METHOD FOR MINIMAL RESIDUAL DISEASE MONITORING IN ACUTE MYELOID LEUKEMIA

E. Onecha De La Fuente[1,*], M. Linares[1], I. Rapado[1], Y. Ruiz[1], J. Perez-Oteyza[2], P. Herrera[3], E. Barragan[4], P. Montesinos[4], J. Nomdedeu[5], M. Gallardo[1], J. Martinez-Lopez[6], R. Ayala[7]

[1]Haematological Malignancies Clinical Research Unit, H12O-CNIO, CNIO, [2]Hospital Universitario Sanchinarro,, [3]Hospital Universitario Ramon y Cajal,, Madrid, [4]Hospital Universitario La Fe, Valencia, [5]Hospital Santa Creu i Sant Pau, Barcelona, [6]Hospital Universitario 12 de Octubre,, [7]Hospital Universitario 12 de Octubre, Madrid, Spain

Background: A high percent of AML patients who achieved MRD negative status eventually relapses due a fraction of pathological clones remains undetectable by standard methods, multiparameter flow cytometry (MFC) and q-PCR, suggesting the need to improve MRD evaluation techniques.
Aims: Simplifies and standardizes MRD evaluation, with high applicability in AML, thought developing of NGS-based method.
Methods: DNA from 190 AML patients with *de novo* or secondary non-M3 AML was analyzed at diagnosis using a custom next-generation sequencing (NGS) AML panel; in addition, NPM1 was analyzed by quantitative (q)-PCR (n=233). Predictive value of MRD status by NGS, MFC, or q-PCR was determined by survival analysis.Patients were treated according to *PETHEMA* (Programa Español de Tratamientos en Hematología) or *CETLAM* (Grupo cooperativo de Estudio y Tratamiento de Leucemias Agudas y Mielodisplasias) protocols. We performed a new selection for retrospective MRD assessment using the following criteria: presence of the NPM1 type A mutation, or SNVs in FLT3, IDH1 and/or IDH2 at diagnosis, and availability of at least one follow-up genomic (g)-DNA sample. Thus, 51 (48%) follow-up samples were taken at post-induction and 55 (52%) at post-consolidation corresponding to 63 patients diagnosed between 2006 and 2016 were studied. All patients achieved CR by cytomorphological criteria after induction therapy (<5% of bone marrow blasts). Median follow-up was 73 days (range 20-596).



Analysis of OS and DFS in AML patients stratified according to MRD levels by NGS, CMF or qPCR. Kaplan-Meier plots of (A) OS and (B) DFS with respect to MRD analysis, using NGS method (A and B respectively); using MFC method (C and D respectively) and using qPCR method (E and F respectively). Only NGS method revealed better significant OS and DFS.

Figure 1.

Results: We designed and optimized a new high-throughput sequencing method for MRD assessment, measuring levels mutated clonotypes of NPM1, IDH1/2 or FLT3-SNV. The NGS-MRD workflow included one first study at diagnosis and a second study at follow-up. NGS results was contrasted with digital PCR (dPCR), for NPM1 (R2=0.98); and IDH1 or IDH2 from commercial standard (R2=0.91, R2=0.98, respectively). Clinical validation was perfomed in 2 steps. First: Study mutational profile of AML patients at diagnosis by a custom NSG AML panel of 32 genes (n=190), plus NPM1 by q-PCR and FLT3-ITD by GENESCAN (n=233). Second: Choose the best markers to follow-up the outcome of the patient

(n=106).The method achieved an applicability of 92% of AML patients and the sensitivity equates to one mutated cell per millon cells (LOQ 10-6) for InDels and one mutated cell per 10,000 cells (LOQ 10-4) for SNVs. Survival analysis showed that positive MRD status (patients with MRD levels >0.035%) was associated with a higher risks of death (37% *vs* 81%; HR:4.2; 95%CI:1.6-10.7; p<0.001) and relapse (48% *vs* 81%; HR:3.4; 95%CI:1.4-8.5; p=0.005). Multivariate analysis showed that MRD-positive status by NGS was an independent factor associated with risk of death (HR 3.91, P=0.030) and the only independent factor conferring risk of relapse (HR 4.37, P=0.015). It also improves upon MFC and q-PCR to predict AML outcome. There were not significant differences between positive and negative MRD groups of patients tested by MFC for OS (p=0.193) nor for DFS (p=0.117) (Figure 1).

Summary and Conclusions: We designed and validated a high-throughput sequencing method for MRD assessment of cell clonotypes with mutations of NPM1, IDH1/2 and/or FLT3 single nucleotide variants (SNVs). Simplifies and standardizes experimental process of MRD evaluation. MRD status measured by NGS offers prognostic clinical information and improves the power to predict AML outcome during therapy, in regard to currents techniques, MFC or q-PCR., could be incorporated in clinical settings and clinical trials.

PF206

A SYSTEMATIC EVALUATION OF PROGNOSTIC GENE EXPRESSION SIGNATURES IN 1208 ACUTE MYELOID LEUKEMIA PATIENTS REVEALS NOVEL INSIGHTS FOR FUTURE RESEARCH AND CLINICAL APPLICATION

D. Beck[1,*], A. Braytee[1], Y. Huang[1], M. Sauerland[2], S. Bohlander[3], J. Braess[4], B. Wörmann[5], W. Berdel[6], W. Hiddemann[7], K. Metzeler[8], T. Herold[7], J. Pimanda[9]

[1]Centre for Health Technologies and Lowy Cancer Centre, UTS Australia and UNSW Australia, Sydeny, Australia, [2]Institute of Biostatistics and Clinical Research, University of Muenster, Muenster, Germany, [3]University of Auckland, Auckland, New Zealand, [4]Krankenhaus Barmherzige Brüder, Regensburg, [5]Department of Medicine, Hematology, Oncology, Universitätsmedizin Berlin, Berlin, [6]Department of Medicine, Hematology and Oncology, University of Münster, Münster, [7]Department of Internal Medicine III, University Hospital Grosshadern, Munich, [8]Medizinische Klinik und Poliklinik III, Ludwig-Maximilians-Universität München, München, Germany, [9]UNSW Australia, Sydeny, Australia

Background: Prognostic gene expression signatures (pGES) have been proposed as tools to assess risk and to therapeutic options for patients with acute myeloid leukaemia (AML). However, given uncertainties about the relationship of pGES to underlying cell biology, and difficulties generalising their utility across patient populations, none have been routinely adopted in clinical trial design or practise.

Aims: We investigated 7 pGES accorss 4 independent patient cohorts.

Methods: We implemented pGES proposed by Marcucci-7, Ng-17, Li-24, Eppert-LSCR-48, Metzeler-86, Eppert-HSCR-105, Bullinger-133. We compared the identities of these pGES at the gene and pathway level and profiled their expression across 7 healthy hematopoietic stem and progenitor cell (HSPC) subtypes. Prognostic significance was evaluated in 1208 intensively treated patients from the Netherlands (HOVON), Germany (AMLCG) and United States of America (TCGA) using univariate the Kaplan-Meier estimator and Cox-regression (age, gender, white blood cell count).

Results: A comparison of the gene identities revealed that there were no common genes or enriched leukaemia associated pathways across the 7 pGES. Surprisingly, only 27/327 genes were members of at least 2 to 4 pGES (1, 8, 18 genes in 4, 3 and 2 pGES, respectively) and only 14/64 leukaemia associated pathways were enriched in at least two to four pGES (2, 5, 7 pathways in 4, 3 and 2 pGES, respectively). Similarly, interrogation of 7 HSPC populations revealed no common association with specific cell types. Interrogation of the 7 pGES across 4 independent cohorts of intensively treated patients revealed that the majority of pGES were associated with overall survival (OS) in univariate and multivariate models (Figure 1A). These included 6/7, 4/7, 5/7 and 3/7 pGES in univariate and 6/7, 4/7, 5/7 and 4/7 pGES in multivariate models when applied to the HOVON, TCGA, AMLCG-1999 and AMLCG-2008 cohorts, respectively. However, there was little agreement whether a particular patient was classified as favourable or adverse risk using the 7 pGES (Figure 1B). In the HOVON cohort, we found that 43/519 patients were classified to identical risk-groups by all 7 pGES (high consensus, CH), 189/519 and 143/519 patients were classified into the same risk-group by six and five pGES (intermediate consensus 1 and 2, CI-1 and CI-2). However, 144/512 patients were classified to opposing risk-groups by 3/4 pGES (low consensus, CL; Figure 1C). An independent re-analysis of patients in CH and CL revealed that those with CH were dichotomised into favourable or unfavourable risk-groups with significantly higher patient-consensus (Figure 1D) by twice as many pGES (Figure 1E). Interestingly, patients with CH and CL had overlapping clinical characteristics but could be distinguished based on the expression of 17 genes (Figure 1F). Therefore, we developed a 17-gene linear classifier to stratify the 43 patients with CH and 144 patients with CL (HOVON). Applied to two independent cohorts, we were able to classify 69 and 101 patients as CH, 70 and 33 patients as CL (AMLCG-2008 and TCGA, respectively). Strikingly, for patients in CH, 5/7 and 6/7 pGES and for patients with CL 1/7 pGES and 0/7 pGES were associated with patient-overall survival (Figure 1G).

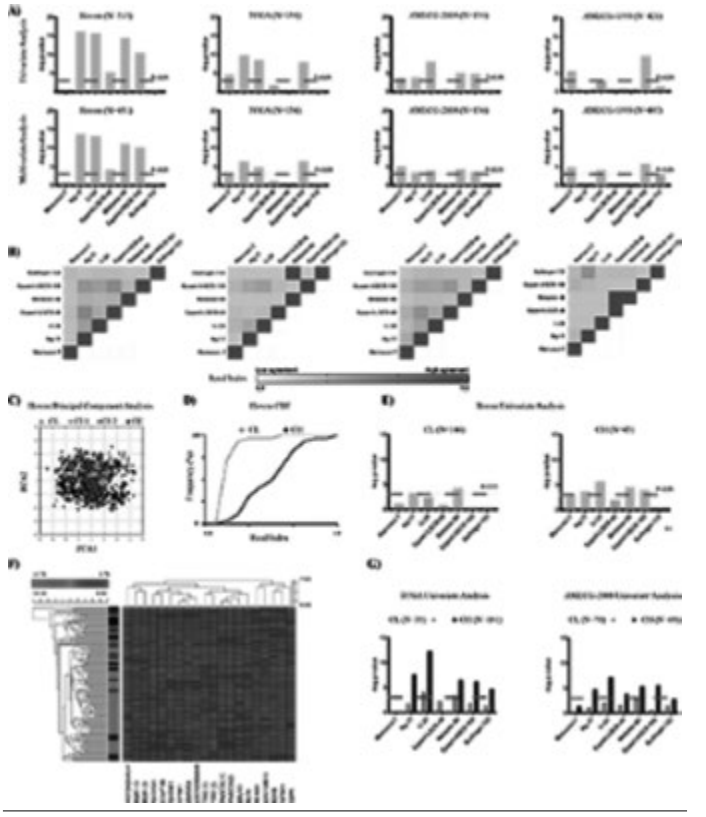


Figure 1.

Summary and Conclusions: For a subset of patients, most pGES were prognostic for overall survival despite any overt biological relationship of their constitutent genes. Importantly, these patients (30%) can be identified prospectively using a classifier of 17 genes.

PF207

AN ARTIFICIAL NEURAL NETWORK-BASED APPROACH IDENTIFIES A POWERFUL 3-GENE PREDICTOR OF ADVERSE PROGNOSIS IN ACUTE MYELOID LEUKAEMIA

S. Wagner[1,*], G. Ball[1], S. Rutella[1]

[1]John van Geest Cancer Research Centre, Nottingham Trent University, Nottingham, United Kingdom

Background: Acute myeloid leukaemia (AML) is a clonal disorder of haematopoietic stem cells with a high degree of molecular complexity. Prognosis is currently determined by response to induction chemotherapy and by cytogenetic risk features. There is an urgent need to discover better biomarkers to enable testing of investigational therapeutic strategies in clinical trials, especially for patients with high-risk AML.

Aims: We applied an artificial neural network (ANN)-based integrative data mining approach to the analysis of transcriptomic data from four cohorts of patients with newly diagnosed AML. ANNs, unlike conventional statistical approaches, are not limited by linear functionality and allow for improved representation of biological features. We compared the ranked orders of genes identified across a clinical class question (overall survival [OS] for the study period) within a given dataset.

Methods: Data sets were retrieved from publicly available sources and included a discovery AML cohort (E-MTAB-3444; n=641 patients) and three validation AML cohorts, namely, The Cancer Genome Atlas (n=145), cytogenetically normal AMLs (n=79; GSE12417) and TARGET-AML

(n=142 children with AML).
Results: ANN analysis identified the top 10 ranked genes associated with survival in the discovery AML cohort. Using Cox regression analysis, 3 genes were further investigated as they featured prominently, namely, calcitonin receptor-like receptor (*CALCRL*; a receptor for adrenomedullin), *CD109* (TGF-b co-receptor) and Leukocyte-Specific Protein 1 (*LSP1*; a gatekeeper of leukocyte trans-endothelial migration). *CALCRL*, *CD109* and *LSP1* transcript expression levels (dichotomised as median) could individually discriminate patients with longer and shorter OS (2.61 *vs* 1 year, p<0.001; 2.95 *vs* 1.01y, p<0.001; and 3.28 *vs* 1y, p<0.001, respectively). A prognostic index (PI) was then generated using b-values from Cox regression analyses together with min-max normalised gene expression levels according to the formula: (1.670×*CALCRL*)+(1.006×*LSP1*)+(0.844×*CD109*). Patients with a PI between 0 and 1 were considered to be low-risk (LR) whereas patients with a PI between 1 and 1.4 and patients with a PI >1.4 were considered to be intermediate-risk (IR) and high-risk (HR), respectively. Patients in the HR group had unfavourable cytogenetic features more often than patients in the IR and LR group. Median OS was 4.75y, 1.57y and 0.77y in LR, IR and HR patients, respectively (p<0.001). Patients in the HR group had a significantly shorter OS irrespective of their *FLT3*-ITD status, suggesting that our 3-gene PI could outperform conventional molecular prognosticators. Also, a low-risk PI identified a subgroup of *NPM1*-mutated patients and *KMT2A*-nonrearranged patients with particularly favourable clinical outcome compared with *NPM1*-mutated and *KMT2A*-rearranged patients assigned to the IR+HR score (58% *vs* 38% OS, and 50% *vs* 22% OS, respectively; p=0.0078). The clinical validity of the 3-gene PI was confirmed across the other adult AML data sets. Interestingly, 142 out of 145 childhood cases in TARGET-AML were assigned to the LR group based on the expression levels of *CALCRL*, *CD109* and *LSP1*.
Summary and Conclusions: Our 3-gene PI accurately stratifies adult patients with AML into risk categories with different OS probabilities. The functional implications of the age-related heterogeneity in *CALCRL*, *CD109* and *LSP1* expression in the AML microenvironment, and whether these surface molecules could be therapeutically targeted, remain to be determined.

PF208

PROFILING AND FUNCTIONAL ANALYSIS OF CIRCULAR RNAS IN ACUTE PROMYELOCYTIC LEUKEMIA AND THEIR DYNAMIC REGULATION DURING ALL-TRANS RETINOIC ACID TREATMENT

S. Li[1,*], Y. Ma[1], K. Wang[1]

[1]Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Background: Circular RNAs (circRNAs) are a novel class of powerful regulators in gene expression and participate in the pathogenesis of disease, including cancer. However, little is known about the roles of circRNAs in the development and treatment of acute promyelocytic leukemia (APL).
Aims: We aimed to examine the expression profiling of circRNAs in APL, including their dynamic regulation during all-*trans* retinoic acid (ATRA)-induced differentiation. Additionally, we wanted to explore the impact and mechanism of circ-HIPK2, one circRNA that was most up-regulated during ATRA-induced APL cell differentiation, in regulating APL cell function.
Methods: Three ribo-minus RNA-seq libraries (*i.e.* untreated NB4 cells, NB4 cells treated with ATRA for 24 hours, NB4 cells treated with ATRA for 48 hours) were prepared with Ribo-Zero Globin kit and sequenced on the Illumina HiSeq 2500 platform with 2x125 bp paired-end reads. STAR and DCC were used to detect circRNAs, and TopHat2 and Cufflinks were employed to calculate the FPKM value of each host gene. Cell growth and granulocytic differentiation of the NB4 cells were detected after circHIPK2 knockdown. MicroRNA sponging potential of circHIPK2 were predicted and determined by luciferase assay.
Results: We identified a total of 4,620 circRNAs, of which 1,430 were newly identified. Detailed analysis showed that circRNAs expressed in APL cells were mostly exon-derived, not by-products during splicing and could be distinguished from hematopoietic stem cells, neutrophils and lymphocytes. The true presence and stability of circRNAs were verified both in NB4 cells and primary APL patient samples. The time-series analysis of circRNAs on ATRA-treated NB4 cells demonstrated that 196 up-regulated and 138 down-regulated circRNAs were identified at 24 hours of ATRA treatment, and 188 up-regulated and 138 down-regulated circRNAs were identified at 48 hours of ATRA treatment. Among these, 95 circRNAs were constantly up-regulated and 60 were constantly down-regulated at both time points of ATRA treatment. Further evidence demonstrated that the majority of circRNAs were regulated independently of their host linear mRNAs. Specific knockdown of one of differentially expressed circRNAs, circ-HIPK2, significantly reduced ATRA-induced differentiation of APL cells, indicating that circ-HIPK2 was indispensable for the differentiation of APL. Finally, the mechanistic study revealed that circ-HIPK2 was located in cytoplasm and served as a sponge for miR-124-3p that regulates CEBPA, thereby contributing to the differentiation process of APL cells.
Summary and Conclusions: Our study identified a large number of dynamically regulated circRNAs during ATRA-induced APL cell differentiation, thus providing an important basis for further studies addressing their function and suitability as biomarkers. Moreover, we determined the biological function and mechanisms of circ-HIPK2 in the regulation of APL differentiation. Further investigation is warranted to uncover more circRNAs that participate in APL occurrence and development.

PF209

PML-RARA INTERACTS WITH NRF2, INHIBITS ITS ACTIVITY AND INCREASES THE SENSITIVITY OF ACUTE PROMYELOCITIC LEUKEMIA CELLS TO ASCORBATE

N.I. Noguera[1,2,*], G. Catalano[2], S. Travaglini[2], C. Banella[1,2], M. T. Voso[2], F. Lo Coco[1,2]

[1]Neuro-Oncohematology, Santa Lucia Foundation, [2]Biomedicine and Prevention, Tor Vergata University, Roma, Italy

Background: Nrf2 is a leucine zipper transcription factor that regulates the expression of antioxidant proteins and protects the cells against oxidative damage triggered by injury and inflammation. Nrf2 activity is controlled through a complex network that ensures its increase during redox perturbation, inflammation, growth factor stimulation and nutrient/energy fluxes. We previously showed that high doses of ascorbic acid (ASC) selectively kill leukemic cells from APL patients.
Aims: Since APL blasts are more sensitive to oxidant treatment than other AML blasts, we aimed to study the effect of PML-RARA on the cellular redox activity, in relation to Nrf2 function.
Methods: We studied primary blasts from APL, AML and normal bone marrow (NBM) patients, PR9 cells, which derive from U937 cells and are PML-RARA-inducible. By RQ-PCR and Western Blot (WB) we assessed the expression of Nrf2 and its effectors: heme-oxigenase 1 (HO-1), NQO-1 and AKR1C1 enzymes. Co-immunoprecipitation experiments, CHIP assay, confocal microscopy. MTS viability test were performed on PR9 cells after PML/RARa induction and ASC at 1mM treatment.
Results: We observed that Nrf2 protein is lower in APL cells as compared to other AML subtypes and that decreased expression is largely due to protein life span reduction, since expression at mRNA level is almost the same. Nrf2 effectors are as well down regulated (Table 1). In PR9 cells protein half-life was indeed reduced in the presence of PML/RARA (residual 15% at 1 hr and 6% at 3 hrs *versus* 58% and 48% in control cells (P=0.005). In untreated cells localization of Nrf2 protein was prevalent in the nucleus, in PML-NBs (confocal microcscopy). Eight hours following PML/RARA expression, coincident with PML NBs disruption, Nrf2 was exported to the cytoplasm and its nuclear function was abrogated, as indicated by the down regulation of HO-1 expression (PR9 cells+Zn: 1.8±0.6, *vs* Mock+Zn: 42.6±3.6 at 6 hours p=0.003). WB analysis confirmed that NRF2 protein was mainly nuclear in control cells, while mainly cytoplasmic after PML/RARA induction (p= 0.02). Co-immunoprecipitation experiments showed that NRF2 physically interacts with the PML/RARA protein. Using CHIP analysis we demonstrated that PML/RARA inhibits Nrf2 binding to antioxidant response elemente (ARE) sites on target genes. By MTS in the same system we showed that sensitivity to ASC significantly increased upon PML/RARA expression (p=0.003). HO-1 protein expression does not increase in the presence of PML/RARa upon ASC treatment, as does in control cells.

Table 1. Expression levels of Nrf-2 and its target genes.

	APL(n=15)	AML(n=12)	NBM(n=5)	p(APL vs AML)
Nrf-2	0.12 ± 0.1	0.24 ± 0.2	0.03 ± 0.01	n.s
HO-1	1.7 ± 1.8	30.6 ± 30.2	1,6 ±1.8	0.0007
NQO-1	10.5 ± 9.8	44.0 ± 46.0	1.1 ± 0.5	0.0016
AKR1C-1	0.25 ± 0.37	0.9 ± 1.8	1.0 ± 0.3	0.005

NBM: normal bone marrow; n.s.: no significative

Summary and Conclusions: Our data disclose a novel regulatory effect of the PML/RARA oncoprotein on Nrf2 function. Actually, following PML/RARA expression and NBs disruption, Nrf2 protein is clearly exported to the cytoplasm thereby deregulating cellular metabolism. In these conditions ASC treatment directly targets the cellular metabiolic abasement and causes an unredeemable stress leading to apoptosis.

PF210

A POSSIBLE FIRST TIER SCREENING TEST TO DETECT TRANSLOCATIONS IN LEUKEMIAS USING TARGETED LOCUS AMPLIFICATION

E. Van Den Berg[1,*], M.Z. Alimohamed[1], L.F. Johansson[1,2], E.N. de Boer[1], E. Splinter[3], P. Klous[3], A. Bosga[1], M. van Min[3], A.B. Mulder[4], E. Vellenga[5], R.J. Sinke[1], R.H. Sijmons[1], B. Sikkema-Raddatz[1]

[1]Genetics, [2]Genomics Coordination Center, UMCG, Groningen, [3]Cergentis BV, Utrecht, [4]Laboratory Medicine, [5]Hematology, UMCG, Groningen, Netherlands

Background: Patients with acute leukemias carry a wide range of chromosomal abnormalities, which affect their prognosis and treatment options. Currently, over 500 different translocations are reported to be involved in the disease progression. Traditional methods to detect chromosomal abnormalities involve a combination of techniques such as karyotyping, FISH, and RT-PCR. However, these methods are laborious and at times inadequate. Targeted Locus Amplification (TLA), a targeted next generation sequencing technology, can overcome these shortcomings and allows the translocation partner of a specific gene to be detected, regardless of its chromosomal origin. It is based on proximity ligation (crosslinking) of DNA and outward oriented probes for enrichment and can therefore identify chromosomal translocation partners regardless of their identities.
Aims: We aimed to develop a TLA assay as a first-tier screening test to detect translocations in acute leukemia. Here we present a comprehensive, multiplex gene panel designed to cover 17 common genes involved in acute leukemias and known to be associated with hundreds of fusion gene partners. In this proof of principle study, we compared the clinical utility of targeted translocation detection using our acute leukemia NGS gene panel with the results from current genetic diagnostic tests in a series of patient bone marrow samples.
Methods: For the panel we selected 17 genes known to be involved in many translocations present in acute leukemias. Procedures were optimized using a training set of cell line dilutions and 17 leukemia patient bone marrow samples and were then validated using a test set of cell line dilutions and bone marrow samples from a further 19 patients. Per gene we determined if its region was involved in a translocation and if so, the translocation partner. Benchmarking was performed using results from three standard diagnostic tests. A multiplex TLA panel was designed using primer sets covering known break-point regions of the 17 selected genes involved in acute leukemia's. Also, TLA was performed on five different cell lines carrying translocations detectable by our panel. [t(12;21), t(6;11), t(11;19)t(8;13), t(6;9), t(17;19)]. Various combinations of cell line mixtures in multiple dilution series were used to determine the specificity and sensitivity of the panel, and to set sample quality thresholds during analysis. Samples were processed using standard TLA protocol (de Vree *et al*., 2014). Targets were enriched by PCR amplification with the multiplex panel and subjected for sequencing on Illumina Nextseq 500. To facilitate an easy analysis workflow a semi-automated data analysis was developed.
Results: We achieved a concordance with benchmarking tests of 81% in the training set and 100% in the test set. In our sample cohort we obtained 100% specificity, with no false positives. In cell line dilution series translocations could be detected in as few as 10% aberrant cells with no false positives as no translocations other than expected for cell lines were detected.
Summary and Conclusions: This multiplex TLA proof of principle study shows promising results to be suitable as a first-tier screening test not only in acute leukemia, but also CML and lymhoma. It offers a competitive option for screening of unknown and cryptic translocations involvoing the selected genes, without prior knowledge of their translocation partners. Further optimization may make the assay suitable for diagnostic use.

PF211

FUNCTIONAL EVALUATION AND MECHANISM RESEARCH OF KEL AND CIRC-KEL IN AEL

H. Jin[1,*], H. Sun[2], Z. Sun[1], Z. Wu[1], J. Li[1]

[1]Department of Hematology, The First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital, [2]Department of Oncology Surgery, Nanjing First Hospital, Nanjing Medical University, Nanjing, China

Background: Acute erythroleukemia (AEL) is defined as an distinct subtype (<5%) of AML characterized by predominant erythropoiesis. It is associated with a poor prognosis. No recurrent cytogenetic abnormality is specific of AEL. Through "Oncomine" Database, we found KEL was significantly upregulated in AEL patients, compared with other types of AML and normal patients. It has been reported that KEL promoter exhibited a strong transcriptional erythroid activity in K562 cells, but its role in the development of AEL remains unclear. Circular RNAs (CircRNAs) are a novel type of endogenous noncoding RNAs that regulate target gene expression by interacting with microRNAs (miRNAs). Emerging evidence shows that circRNAs play important roles in biological and pathological processes. Here we found one circRNA transcripted from KEL gene (circ-KEL), which had a high expression in AEL, may play a role of coordinate regulation with KELL (a protein encoded by KEL mRNA).
Aims: Our present study aims to investigate the function of KEL and circ-KEL and enrich the potential modulation mechansim on erythroid differentiation in AEL.
Methods: In our study, we collected blood samples from 45 AEL patient, 121 other types of AML patients and 88 normal patients. Two AEL cells lines (K562 and HEL) were used as the erythroid differentiation models *in vitro*, induced with 50 M hemin from day 1 to day 7. We knocked down and/or over-expressed KEL and circ-KEL separately in AEL cell lines by using lenti-virus and siRNAs. The expression levels of γ-globin and FUT1 as well as the erythroid surface markers TER119 and GPA were examined to evaluate their function in erythroid differentiation. Initial characterization (RNase R and Actinomycin D treatment) has been performed to identify circ-KEL. Dual-luciferase reporter system, FISH assay and RIP assay have been performed to validate the interaction of circRNAs, miRNAs and mRNA. To test the protein-coding ability of circ-KEL, we took advantage of an expression vector that contained a 3×FLAG and circ-KEL sequence, which was able to produce circular transcripts. QRT-PCR and Western blot were performed to examine the expression of related RNA and protein.
Results: KEL and circ-KEL were significantly higher expressed in AEL clinical samples/cells lines, and the results were consistent with the database. When KEL was over-expressed, the relative expression of circ-KEL was increased significantly, resulted in the up-regulation of erythroid differentiation. Similar effects occured when circ-KEL was over-expressed. On the contrary, when KEL was knocked down, the relative expression of circ-KEL was significantly decreased, led to a down-regulation of erythroid differentiation. Moreover, we found that increased circ-KEL levels could rescue the erythroid differentiation inhibition when KEL was knocked down in AEL. Circ-KEL could serve as a sponge for miR-671 and up-regulate miR-671 functional target KEL mRNA, thus promote the erythroid differentiation. Combined with bioinformatics analysis, we found circ-KEL contains m6A motif in initiation codon region and an open reading frame (ORF) that could encode polypeptides (293aa). Circ-KEL translation can be modified by m6A. The function of the circ-KEL encoding protein and its influence on erythroid differentiation in AEL will be futher explored (Figure 1).

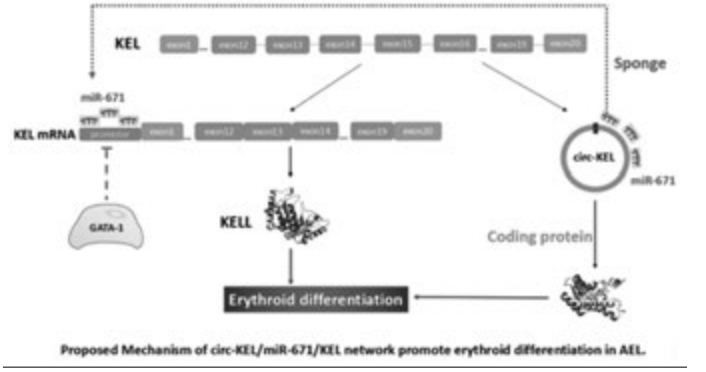


Figure 1.

Summary and Conclusions: Our study reveals a novel regulatory mechanism of KEL and circ-KEL in erythroid differentiation, suggesting that circ-KEL can be used as a potential diagnostic biomarker of AEL. This provides a promising strategy for future diagnosis, biomarker discoveries and treatment of AEL.

PF212

A NOVEL BET-BROMODOMAIN INHIBITOR, ODM-207, AS A POTENTIAL THERAPY FOR ACUTE MYELOID LEUKEMIA

J.J. Miettinen[1,*], T. Ikonen[2], T. af Hällström[2], A. Moilanen[2], K. Porkka[3], C.A. Heckman[1]

[1]Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, [2]Orion Pharma, Orion Corporation, Espoo, [3]Helsinki University Hospital and Comprehensive Cancer Center (HUS), Helsinki, Finland

Background: Bromodomain and extra-terminal (BET) family proteins are

regulators of gene transcription. Their inhibition has been shown to suppress oncogenic pathways, survival, and growth in haematological malignancies and in solid tumors. Acute myeloid leukemia (AML) is the most frequent form of myeloid cell derived haematological malignancies in adults. Currently there are only a limited number of treatments available for AML with limited efficacy. BET inhibitors have shown promise as a new targeted therapy for treating the disease. BET inhibitor ODM-207 has been shown to have anti-proliferative activity in AML cell lines, and in cell lines from other haematological malignancies (Moilanen *et al.*, Cancer Res, 2017). Its effects on AML patient samples have not been studied.
Aims: The aim of the study was to test the drug response of ODM-207 in AML patient samples, and to compare these to drug responses obtained using a set of commercially available BET inhibitors in the same samples.
Methods: Bone marrow aspirate (BM) or peripheral blood (PB) derived mononuclear cells (MNC) were enriched by Ficoll separation from 23 AML patient samples (14 diagnostic, 6 relapsed, 2 refractory, 1 relapsed/refractory) from 21 different patients, and from 4 healthy individuals. *Ex vivo* drug sensitivity and resistance testing (DSRT) (Pemovska *et al.*, Cancer Discov, 2013) was performed by treating the cells for 72 h with the desired compounds, and CellTiter-Glo (CTG) and CellTox Green (CTxG) readouts were measured. Cells were tested against ODM-207 in 9 different concentrations (3-30000 nM), and in 5 different concentrations (1-10000 nM) for the other 5 BET inhibitors (GSK525762, I-BET151, JQ1, OTX015, PFI-1) to generate dose response data. Drug sensitivity scores were calculated based on the normalized area under the dose response curve (Yadav *et al.*, Sci Rep, 2014).
Results: ODM-207 reduced cell viability in a subgroup of AML patients. There were 5 AML patients that were extremely sensitive to ODM-207, and an additional 8 sensitive patients. In 6 patients ODM-207 showed a cytotoxic effect, and in 5 a cytostatic effect. Cytostatic activity was evident in an additional 3 patients, but cytotoxicity could not be confirmed from these samples. There were also 8 AML patients not responding to ODM-207. When comparing ODM-207 response in the tested AML patient samples with responses obtained using the other 5 BET inhibitors, ODM-207 generated responses similar to JQ1 and OTX015. ODM-207 was however slightly more cytotoxic in a subgroup of patients, whereas JQ1 and OTX015 were more cytostatic in general. Other inhibitors, GSK525762, I-BET151 and PFI-1, generated a weaker drug response than ODM-207. They were not as effective in reducing cell viability in the tested AML patient samples, and their effects were mainly cytostatic.
Summary and Conclusions: ODM-207 was extremely effective in reducing cell viability *ex vivo* in 5/21 AML patients, and effective in an additional 8 patients. In 6 patients ODM-207 showed a cytotoxic effect, and in 5 a cytostatic effect. ODM-207 has a similar drug response profile as JQ1 and OTX015 in the tested AML patient samples, but ODM-207 is slightly more cytotoxic in a subgroup of patients. The current results also indicate variation in response between groups of patients whose samples were extremely sensitive or resistant to ODM-207. Therefore the molecular features defining the different ODM-207 response groups are being investigated by exome and RNA sequence analyses. Also, more AML patient samples are being DSRT tested to confirm the already analysed drug response results.

PF213

MOLECULAR PROFILES IN AML PATIENTS OLDER THAN 70 YEARS OF AGE

M. Hoyos Colell[1,2,*], A. Dolnik[1], T.J. Blätte[1], S. Skambraks[1], S. Lux[1], Z. Xia[1], V.I. Gaidzik[1], P. Paschka[1], D. Weber[1], M. Wattad[3], G. Held[4], H.A. Horst[5], F. Thol[6], M. Heuser[6], A. Ganser[6], V. Teleanu[1], J. Sierra[2], H. Döhner[1], K. Döhner[1], L. Bullinger[1]

[1]Klinik für Innere Medizin III, Universitätsklinikum Ulm, Ulm, Germany, [2]Hematology, Hospital de la Santa Creu i Sant Pau, IIB Sant Pau and Jose Carreras Research Institute. Universitat Autonoma de Barcelona, Barcelona, Spain, [3]Klinik für Hämatologie, internistische Onkologie und Stammzelltransplantation, Evangelisches Krankenhaus Essen-Werden, Essen, [4]Innere Medizin I, Universitätsklinikum des Saarlandes und Medizinische Fakultät der Universität des Saarlandes, Homburg, [5]Klinik für Innere Medizin II, Universitätsklinikum Schleswig-Holstein Campus Kiel, Kiel, [6]Klinik für Hämatologie, Hämostaseologie, Onkologie und Stammzelltransplantation, Medizinische Hochschule Hannover, Hannover, Germany

Background: Acute myeloid leukemia (AML) arises in all age groups, but it is mainly a disease of the older patients with a median age of about 70 years at diagnosis. Age has a major impact on both the management and outcome of patients with AML. Older patients frequently present with unfavorable prognostic factors that are related to patient characteristics (general health condition, specific comorbidities), but also to those related to the leukemia cells, including a higher frequency of secondary AML arising from previous myelodysplastic syndrome and adverse genetic changes. While in the past, large cohorts of younger AML patients treated within multicenter clinical trials have been extensively studied on the molecular level, the genomic aberrations underlying the AML of older patients have by far been less well characterized.
Aims: In accordance, the aim of this study was the evaluation of the mutational spectrum of a large cohort of AML patients ≥70 years (median age 77 yrs) who have been entered into the AMLSG BiO Registry (ClinicalTrials.gov Identifier: NCT01252485).
Methods: Patients included into the study were treated with low dose cytarabine, decitabine or azacitidine, or best supportive care. In total, we studied n=295 cases by an in-house targeted resequencing panel covering the entire coding region of 94 myeloid disease related genes. We used the Haloplex High Sensitivity enrichment system (Agilent), followed by Illumina sequencing and an in-house data analysis workflow. Using a respective high sensitivity molecular barcode approach, we could reliably detect low abundance mutations with a variant allele frequency (VAF) ≤1%.
Results: In 295 cases the average gene coverage was 350x(fold) for consensus reads. In line with other smaller studies, we found a very high incidence of *TP53* (30%), *ASXL1* (11%), and *RUNX1* (21%) mutations, along with a high incidence of *TET2* (31.5%) and *DNMT3A* (24%) as well as splicing factor *SRSF2* (24%) mutations (Figure 1). While we found on average three mutations per patient using our 94-gene panel, we found a broad distribution of the VAF. Most patients presented with a dominant clone displaying a VAF of ~50%, *i.e.* all tumor cells harbor the respective mutation. In agreement with findings in younger patients, these mutations often affect genes involved in DNA methylation, chromatin modification, the cohesin or spliceosome complex. Often, the subclonal mutations with VAF ≤30% involve mutations in tumor-relevant signaling pathways.

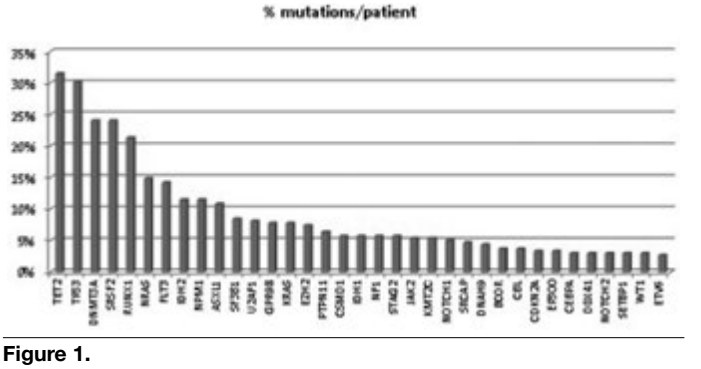


Figure 1.

Summary and Conclusions: In summary, our study demonstrates that the mutational spectrum of older AML patients differs significantly from that of young patients with a high incidence of high-risk molecular aberrations. Results from correlation of molecular with clinical data will be presented at the meeting.

PF214

COMPARISON OF WHOLE GENOME ANALYSIS APPROACHES FOR DETECTION OF MAJOR CHROMOSOMAL ABERRATIONS USING A DIVERSE SET OF LEUKEMIA PATIENTS

S. Twardziok[1,*], N. Nadarajah[1], C. Bär[1], M. Meggendorfer[1], A. Stengel[1], W. Kern[1], T. Haferlach[1], C. Haferlach[1]

[1]MLL Münchner Leukämielabor GmbH, München, Germany

Background: To date chromosome banding analysis (CBA) is the gold standard technique for the detection of structural chromosomal rearrangements and copy number changes in the diagnostic setting of hematological malignancies. However, additional techniques have been established that are capable of providing genome wide information such as genomic arrays (GA), optical mapping technologies (OM) and whole genome sequencing (WGS).
Aims: We evaluated the performance of these three whole genome analysis approaches to detect structural variations (SV) and copy number variations (CNV) using a comprehensive set of leukemia samples in comparison to CBA.
Methods: We applied WGS with 90x coverage using diagnostic peripheral blood or bone marrow samples of 129 patients from diverse leukemia entities. Library preparation was performed using Truseq DNA PCR-Free HT sample preparation kit (Illumina, San Diego, CA) according to manufacturer's protocol using fresh/frozen samples and sequenced on NovaSeq sequencing instruments. Reads were aligned by BaseSpace WGS app with

default parameters. CNV analysis was performed using software GATK4 and SVs were called using MANTA software accounting for missing matched-normal samples. For a subset of 20 samples including AML and ALL patients with a complex karyotype we also performed GA for CNV detection. Additionally, for a set of 5 samples we used data from high resolution OM provided by BioNano genomics for a cross technology comparison of SV calls and CNV calls.
Results: WGS analysis detected reciprocal translocations identified by CBA in 98/104 patients including 81/85 *BCR-ABL1* fusions, 8/9 *RUNX1-RUNX1T1* fusions and 8/10 *PML-RARA* fusions and it was also able to resolve several complex fusions not fully clarified by CBA Comparing CNV detection to CBA we found equivalent copy number changes for 59/98 chromosomes with array-CGH and for 71/98 chromosomes with WGS. Missing detections by WGS and GA were due to small subclones (<1%). Comparing WGS and GA we found 90.1% equivalence over all genomes including 11.1% aberrations undetected by CBA. Further, compared to GA WGS predicted additional 6.6% lost regions and compared to WGS GA predicted additional 3.2% gained regions. Comparing WGS and OM for SV detection with CBA set as the gold standard resulted in detection of 10/18 interchromosomal fusions by WGS and detection of 15/18 interchromosomal fusions using OM. Fusions missed by WGS were in low complexity regions or uncalled by the algorithm due to low fusion signals. For the copy number variation data, WGS identified 23/29 CNV segments and OM identified 13/29 CNV segments in accordance to orthogonal data.
Summary and Conclusions: In principle, only WGS and OM have the potential to substitute CBA as GA cannot detect balanced rearrangements. Applying WGS with current algorithms 94.2% of diagnostically significant fusions were identified. In addition, WGS found several aberrations not detected by CBA. In direct comparison, we found more fusion events using OM than with WGS although both approaches detected most fusions in specific regions of the chromosomes. Compared to CBA the resolution of GA, OM and WGS is strikingly higher leading to the identification of several additional abnormalities confirmed by at least two of the latter technologies. Such abnormalities might provide new insights into leukemia evolution and give improved sub-classification of patients for precision medicine.

PF215

SOMATIC MUTATIONS IN RELAPSED ACUTE MYELOID LEUKEMIA

S. Eldfors[1,*], M. Kontro[2], Y. Akimov[1], C. Engen[3], M. Wolf[1], B.T. Gjertsen[3,4], O. Kallioniemi[1,5,6], K. Porkka[2], C. Heckman[1]

[1]Institute for Molecular Medicine Finland, [2]Helsinki University Central Hospital Cancer Center, University of Helsinki, Helsinki, Finland, [3]Centre for Cancer Biomarkers (CCBIO), University of Bergen, [4]Department of Internal Medicine, Haukeland University Hospital, Bergen, Norway, [5]Department of Oncology-Pathology, Karolinska Institutet, [6]Science for Life Laboratory, Stockholm, Sweden

Background: Most patients with acute myeloid leukemia (AML) achieve complete remission with initial treatment, but many relapse with a therapy-resistant disease. The somatic mutations in newly diagnosed AML have been extensively characterized. However, the role of mutations in relapsed AML remains poorly understood.
Aims: We aimed to identify somatic mutations selected at relapse in AML.
Methods: We analyzed 142 bone marrow or peripheral blood samples from 124 AML patients by exome sequencing. Skin biopsy samples were sequenced as the normal tissue controls. We compared somatic mutations, copy number, and cytogenetic aberrations in newly diagnosed AML (n=81) with the mutations in relapsed AML (n=61). Matched diagnosis and relapse samples were available from 18 patients. The relapsed patients were treated with chemotherapy (n=49) or allogeneic stem cell transplantation (n=12). The acquisition and increase in variant allele frequency of mutations in matched diagnosis and relapse sample pairs provide strong evidence for mutation selection in response to therapy. Therefore, we regarded mutations that both showed enrichment in the cohort comparison and were acquired or selected for in matched diagnosis-relapse sample pairs as the most likely to contribute to therapy resistance.
Results: We found that mutations in *CBL*, *PTPN11*, *NF1* and *KRAS* were enriched in relapsed AML. Mutations in these genes have been shown to induce hypersensitivity to cytokine stimulation in hematopoietic cells. A *CBL* mutation was present in 2/81 patients (2%) with newly diagnosed AML and 6/61 patients (10%) with relapsed AML. A *CBL* and *PTPN11* mutation was acquired or increased in variant allele frequency in 2/18 patients with matched diagnosis and relapse samples (Figure 1A,B). A *PTPN11* mutation was present in 5/81 patients (6%) with newly diagnosed AML and 6/61 patients (10%) with relapsed AML. *NF1* mutations or deletions were present in 4/81 patients (5%) with newly diagnosed AML and 7/61 patients (11%) with relapsed AML. Notably, we identified biallelic *NF1* mutations or a homozygous deletion in three patients at relapse. In all, a *CBL*, *PTPN11*, *NF1* or *KRAS* mutation was present in 36% of the patients with relapsed AML and 17% of the patients with newly diagnosed AML. Our relapsed AML cohort was significantly enriched for mutations in these genes compared with the previously published TCGA AML cohort of newly diagnosed patients (Figure 1C). To determine whether *CBL*, *PTPN11*, *NF1* and *KRAS* mutations are associated with shorter survival when present at the time of diagnosis, we analyzed survival data for the TCGA patients. Patients with a *CBL*, *PTPN11*, *KRAS* or *NF1* mutation had shorter overall survival than patients who lacked mutations in these genes (Figure 1D). Other genes that were enriched for mutations at relapse and showed selection of mutations in serial samples included *TP53*, *WT1*, *PHF6* and *RUNX1*. Cytogenetic aberrations that were selected in relapsed AML included -7/-7q and +8/+8q.

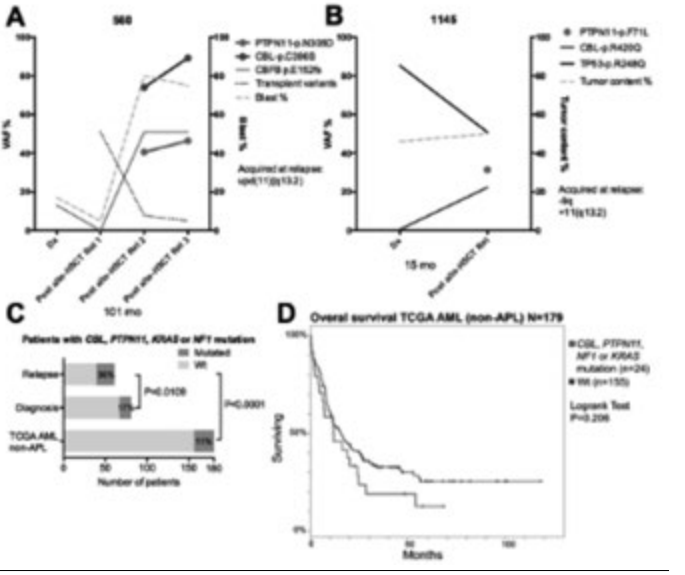


Figure 1.

Summary and Conclusions: We found that mutations in *CBL*, *PTPN11*, *NF1* and *KRAS*, which can lead to cytokine signaling activation and hypersensitivity to survival signals, are frequent and selected in relapsed AML. *TP53* mutations were present in 10% of the relapsed patients. Mutations to genes encoding transcriptional and epigenetic regulators, and monosomy 7/-7q and trisomy 8/+8q are more prevalent in relapsed AML. In contrast, mutations leading to drug resistance via impaired drug metabolism are rare. The identified genes can potentially be used to stratify patients who lack conventional prognostic markers.

PF216

EXPRESSION OF PIWI-INTERACTING RNAS (PIRNAS) AND ITS PROGNOSTIC IMPLICATION IN ACUTE MYELOID LEUKEMIA (AML): A 3-PIRNA SCORING SYSTEM PREDICTS PROGNOSIS IN AML PATIENTS

T.-C. Yu[1,*], C.-H. Tsai[1,2], M. Yao[1], Y.-Y. Kuo[3], M.-H. Tseng[1], C.-C. Li[2], S.-Y. Huang[1], B.-S. Ko[1], S.-C. Hsu[4], C.-T. Lin[2], S.-J. Wu[1], C.-Y. Chen[1], W. Tsai[1], J.-L. Tang[1,2], H.-A. Hou[1], W.-C. Chou[4], H.-F. Tien[1]

[1]Division of Hematology, National Taiwan University Hospital, [2]Tai-Cheng Stem Cell Therapy Center, [3]Graduate Institute of Oncology, College of Medicine, National Taiwan University, [4]Laboratory Medicine, National Taiwan University Hospital, Taipei City, Taiwan, Republic of China

Background: PIWI-interacting RNAs (piRNAs), a group of small RNAs with 24-31 nucleotides in length, play important roles in gene silencing, epigenetic regulations and stem-cell maintenance. The piRNAs were found dysregulated in various kinds of tumor tissues, but their roles in tumorigenesis are largely unknown. Recently, it was found that piRNAs expressions could predict prognosis in Hodgkin lymphoma. However, the biologic and prognostic relevance of piRNAs in AML remain unclear.
Aims: The aim of this study was to establish a concise scoring system based on piRNA expression and to elucidate its clinical implications.
Methods: The global gene expression profiles, including 2,041 piRNAs, of 176 adult patients with *de novo* non-M3 AML who received standard

chemotherapy were analyzed using the Affymetrix Human Transcriptome Array 2.0 chips. We randomly divided patients into the training cohort (n=88) and validation cohort (n=88). We used the multivariate Cox proportional hazards regression analysis to build the piRNA risk score system and explored its clinico-biological significances. This piRNA risk score system was further verified in the validation cohort.
Results: We identified three piRNAs, which were significantly associated with disease free survival (DFS). A robust risk scoring system composed of the sum of the piRNAs was constructed. The piRNA risk score=0.327*[TC15000168.hg.1]+0.424*[TC15000551.hg.1]+0.394*[TC15001906.hg.1]. The median of risk scores was used as the cut-off to divide patients into lower- and higher-score groups. The clinical parameters including hemoglobin, white blood cell, and platelet counts at diagnosis and the distribution of cytogenetic changes were similar between the two groups. Patients with higher scores had significantly less *CEBPA* double-mutations (*CEBPA*$^{double\text{-}mutation}$) (P=0.044). The higher-score group had a lower complete remission rate (61.4% *vs* 84.1%, P=0.030), higher relapse rate (77.8% *vs* 48.6%, P=0.022), shorter DFS (median 10.0 *vs* 78.3 months, P=0.003) and overall survival (OS) (median 17.6 months *vs* not reached, P= 0.001, Figure 1) compared with the lower-score group. In multivariate analysis, the independent poor prognostic factors for both OS and DFS included age more than 50 years, white blood cell counts at diagnosis more than 50,000 /micro-liter, unfavorable-risk cytogenetics, *RUNX1* mutations, and higher piRNA risk scores, while *CEBPA*$^{double\text{-}mutation}$, *IDH2*, and *NRAS* mutations were independent favorable prognostic factors regarding OS. The higher piRNA risk score remained to be an independent poor prognostic factor for OS and DFS (both P<0.001) in the validation cohort. We utilized Ingenuity Pathway Analysis to analyze the potential underlying pathway associated with higher piRNA risk score and constructed a network centered by *ERK* and *HOX* family genes.

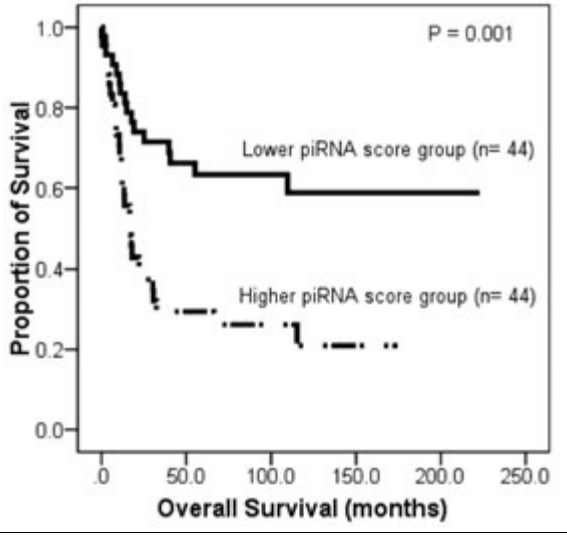


Figure 1.

Summary and Conclusions: This piRNA risk score system is a robust and easy to use risk-stratification tool. The piRNA scores were associated with distinct molecular alterations and independently correlated with clinical outcomes. Further prospective studies are warranted to validate our findings.

PF217

BASOPHIL-LINEAGE COMMITMENT IN ACUTE PROMYELOCYTIC LEUKEMIA PREDICTS FOR SEVERE BLEEDING AFTER STARTING THERAPY

S. Matarraz[1,*], P. Leoz[2], C. Fernandez[2], E. Colado[3], M.C. Chillón[4], M.B. Vidriales[4], M. González[4], D. Rivera[4], C. Salvador-Osuna[5], T. Caballero-Velázquez[6], V. van der Velden[7], M. Jongen-Lavrencic[8], O. Gutierrez[9], A. Yeguas Bermejo[10], L. García Alonso[10], M. Bourgeois García[11], C. de Ramón Sánchez[11], G. García Donas[12], A. Mayado[1], M.L. Gutiérrez[1], P. Bárcena[1], S. Barrena[1], A. López[1], J. van Dongen[13], A. Orfao[1]

[1]Medicine, Cytometry Service (NUCLEUS), Department of Medicine, IBSAL and CIBERONC, Cancer Research Center (IBMCC, University of Salamanca-CSIC), [2]Medicine, Centro de Investigación del Cáncer (IBMCC, CSIC-USAL) and IBSAL, Salamanca, [3]Hematology, Central University Hospital of Asturias, Hematology and Laboratory Medicine Department and Instituto Universitario de Oncología del Principado de Asturias (IUOPA), Oviedo, [4]Hematology, Hematology Service, University Hospital of Salamanca, IBSAL and CIBERONC, Salamanca, [5]Hematology, Miguel Servet Hospital, Zaragoza, [6]Hematology, Virgen del Rocio Hospital, Sevilla, Spain, [7]Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands, [8]Hematology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, [9]Hematology, Rio Hortega Hospital, Valladolid, [10]Hematology, Getafe University Hospital, Madrid, [11]Hematology, Valladolid University Hospital, Valladolid, [12]Hematology, Juan Ramón Jiménez Hospital, Huelva, Spain, [13]Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Netherlands

Background: Severe hemorrhagic events occur in a significant fraction of acute promyelocytic leukemia patients, either at presentation and/or early after starting therapy, leading to treatment failure and early deaths. However, identification of independent predictors for high-risk of severe bleeding at diagnosis, remains a challenge.
Aims: We investigated the immunophenotype of bone marrow leukemic cells from 109 newly-diagnosed acute promyelocytic leukemia patients, particularly focusing on the identification of basophil-related features, and their potential association with severe bleeding episodes and patient overall survival.
Methods: A total of 109 newly-diagnosed and previously untreated acute promyelocytic leukemia patients were investigated for the presence of phenotypes associated with basophil maturation on bone marrow leukemic promyelocytes, using the standardized 8-color *EuroFlow* AML/MDS antibody panel. Diagnosis was based on morphological and immunophenotypic criteria, together with the presence of *PML-RARA* gene rearrangements by iFISH and molecular techniques.
Results: From all 109 cases analyzed, 72% showed signs of bleeding. Thus, mild bleeding was observed at diagnosis and during/after starting therapy in 50% and 19% patients, respectively, while severe bleeding occurred in 14% and 21% cases, respectively. From all phenotypic markers investigated, partial expression of the CD203c and/or CD22 basophil-associated antigens on bone marrow leukemic cells showed the strongest association with both the occurrence and the severity of bleeding at diagnosis and after starting therapy. Thus, an increasing frequency of cases with CD203c$^+$ leukemic cells was observed among patients with severe hemorrhage *vs* those with mild and no signs of bleeding, both at diagnosis (87% *vs* 25% and 13%; p<0.001) and after starting therapy (80% *vs* 20% and 18%, respectively; p<0.001). Multivariate analysis of prognostic factors showed that high peripheral blood leukemic cell counts (>30 x10^9/L) was the only parameter showing an independent predictive value for severe bleeding at any time point evaluated (HR: 22.4; p=0.007), while CD203c expression on leukemic promyelocytes (HR: 26.4; p=0.003) together with an older age (HR: 5.4; p=0.03) was the best combination of prognostic factors for cumulative incidence of severe bleeding after starting therapy. In turn, low fibrinogen levels (HR: 8.8; p=0.001), age >70 years (HR: 9.0 p=0.002), high (>30 x10^9/L) leukocyte count (HR: 5.6; p=0.02) and CD203c expression on bone marrow leukemic cells (HR: 4.4; p=0.01), were the most informative independent predictors for overall survival of acute promyelocytic leukemia patients.
Summary and Conclusions: In summary, here we show for the first time that basophil maturation of leukemic promyelocytes defined on immunophenotypic grounds occurs in around one third of promyelocytic leukemia patients, providing a greater risk of severe bleeding both at diagnosis and (particularly) after starting therapy. Thus, expression of the CD203c basophil maturation-associated marker on leukemic cells showed a strong and independent predictive value for both the occurrence of severe bleeding after starting therapy and overall survival of these patients. Further studies in larger series of promyelocytic leukemia patients are required to confirm our findings, and to establish preventive and/or treatment measures for an improved management of patients at increased risk for severe bleeding, particularly among this unique subgroup of CD203c$^+$ promyelocytic leukemia cases.

PF218

RECURRENCE OF THE 8Q24 REARRANGEMENT IN BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM AND ITS ASSOCIATION WITH IMMUNOBLASTOID CYTOMORPHOLOGY, MYC EXPRESSION, AND DRUG RESPONSE

K. Sakamoto[1,*], R. Katayama[2], R. Asaka[1], S. Sakata[1], S. Baba[1], H. Nakasone[3], S. Koike[2], N. Tsuyama[4], A. Dobashi[1], M. Sasaki[5], R. Ichinohasama[6], E. Takakuwa[7], R. Yamazaki[8], J. Takizawa[9], T. Maeda[10], M. Narita[11], K. Izutsu[12], Y. Kanda[3], K. Ohshima[13], K. Takeuchi[1]

[1]Pathology Project for Molecular Targets, The Cancer Institute, Japanese Foundation for Cancer Research, [2]Division of Experimental Chemotherapy, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, [3]Division of Hematology, Jichi Medical University Saitama Medical Center, Saitama, [4]Division of Pathol-

ogy, The Cancer Institute, Japanese Foundation for Cancer Research, [5]Department of Hematology, Juntendo University School of Medicine, Tokyo, [6]Department of Hematopathology, Tohoku University Graduate School of Medicine, Sendai, [7]Department of Surgical Pathology, Hokkaido University Hospital, Sapporo, [8]Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo, [9]Department of Hematology, Endocrinology, and Metabolism, Faculty of Medicine, Niigata University, Niigata, [10]Department of Community Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, [11]Laboratory of Hematology and Oncology, Graduate School of Health Sciences, Niigata University, Niigata, [12]Department of Hematology, National Cancer Center Hospital, Tokyo, [13]Department of Pathology, Kurume University, School of Medicine, Kurume, Japan

Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare skin-tropic hematological malignancy with poor outcomes and uncertain pathogenesis. Under the microscope, classic BPDCN cells appear as medium-sized blasts containing nuclei with variable irregularities and fine chromatin. Nucleoli are either inconspicuous or one to several small nucleoli are observed. However, in 2001, we identified a case, wherein, cells morphologically resembled immunoblasts (activated B cells characterized by a round vesicular nucleus with a large centrally-located nucleolus) and have unofficially referred to cases with such cytomorphology as the "immunoblastoid" variant. Meanwhile, 8q24 (*MYC* locus) rearrangement has been sporadically reported in BPDCN but is still unclear about the positivity rate and the biological and clinical relevance. Recently, we encountered an "immunoblastoid" BPDCN case with 8q24 rearrangement and MYC expression, which suggested a genotype-phenotype correlation in BPDCN.
Aims: We aimed to elucidate the presence of subgroups in BPDCN based on such genotype-phenotype correlations and to evaluate the therapeutic significance of this subclassification.
Methods: Specimens and clinical information from 154 patients diagnosed with BPDCN or with an equivalent diagnosis were collected from 52 institutions in Japan. By histopathological analysis, 118 cases were confirmed to be BPDCN and were included in the present study. The cytomorphology of these cases was examined using the formalin-fixed paraffin-embedded tissues. Subsequently, 8q24 rearrangement and MYC expression was evaluated by fluorescence *in situ* hybridization and immunohistochemistry, respectively. We also performed *in vitro* functional analyses using BPDCN cell lines with and without 8q24 rearrangement.

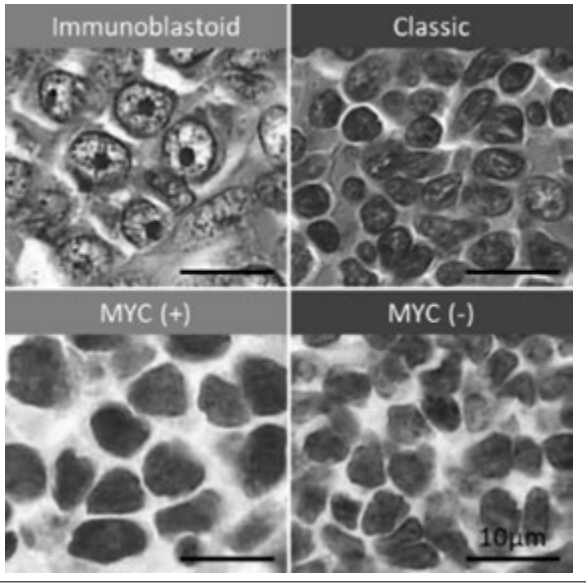


Figure 1.

Results: Among the 118 BPDCN patients, 62 (53%) and 41 (35%) exhibited the classic and immunoblastoid cytomorphology, respectively. Forty-one (38%) MYC$^+$BPDCN (positive for rearrangement and expression) and 59 (54%) MYC$^-$BPDCN (both negative) cases were identified. All immunoblastoid BPDCN cases examined were MYC$^+$BPDCN (39/39, 100%) and nearly all classic BPDCN cases were MYC$^-$BPDCN (54/56, 96%). All examined MYC$^+$BPDCN samples were negative for *MYB*/*MYBL1* rearrangement (0/36). Clinically, MYC$^+$BPDCN patients showed late onset, poor outcomes, and localized skin tumors more commonly than MYC$^-$BPDCN patients. *MYC* was demonstrated by expression profiling as one of the clearest discriminators between CAL-1 (MYC$^+$BPDCN) and PMDC05 (MYC$^-$BPDCN) cell lines, and shRNA knockdown of *MYC* suppressed CAL-1 viability. Inhibitors for bromodomain and extraterminal protein (BETis) and aurora kinases (AKis) inhibited CAL-1 growth more effectively than that of PMDC05. We further showed that a BCL2 inhibitor was effective in both CAL-1 and PMDC05, indicating that this inhibitor can be used to treat MYC$^-$BPDCN cases, for which BETis and AKis are probably less effective (Figure 1).
Summary and Conclusions: We revealed that 8q24 rearrangement was recurrent (approximately 40%) in BPDCN and was associated with immunoblastoid cytomorphology, MYC expression, and higher sensitivity to BETis and AKis. In addition, the characteristics of MYC$^+$BPDCN and MYC$^-$BPDCN patients were found to be appreciably different in clinical respects. Our data will provide a rationale for the development of new treatment strategies for patients with BPDCN in accordance with precision medicine.

PF219

COMBINATION OF FLT3 INHIBITOR QUIZARTINIB AND MDM2 INHIBITOR MILADEMETAN RESULTS IN GREATER PRE-CLINICAL ANTI-LEUKEMIC ACTIVITY IN FLT3-ITD MUTANT/P53 WILD-TYPE ACUTE MYELOID LEUKEMIA MODELS

M. Andreeff[1,*], T. Seki[2], W. Zhang[1], T. Isoyama[3], K. Iwanaga[2], N. Togashi[2], P. Kumar[4], O. Zernovak[4], N. Daver[1]

[1]MD Anderson Cancer Center, Houston, United States, [2]Daiichi Sankyo, Ltd., Tokyo, Japan, [3]Daiichi Sankyo, Ltd., Tokyo, [4]Daiichi Sanyko, Inc., Basking Ridge, United States

Background: Quizartinib, a highly selective and potent FLT3 inhibitor, and milademetan (DS-3032b), a small molecule MDM2 inhibitor, have both demonstrated single-agent treatment activity in pre-clinical and clinical studies in acute myeloid leukemia (AML). These pre-clinical studies explored the rationale and molecular basis for the combination of quizartinib and milademetan for the treatment of FLT3-ITD mutant/TP53 wild-type AML.
Aims: Evaluate pre-clinically the feasibility of combining quizartinib and milademetan for treatment of FLT3-ITD mutant/TP53 WT AML.
Methods: We investigated the effect of quizartinib and milademetan combination on cell viability and apoptosis in established AML cell lines, including MV-4-11, MOLM-13 and MOLM-14, which harbor FLT3 ITD mutations and p53 wild type. We further investigated the effect of the combination regimen by using quizartinib and murine specific MDM2 inhibitor DS-5272 in murine leukemia cell lines Ba/F3-FLT3-ITD, Ba/F3-FLT3-ITD+F691L and Ba/F3-FLT3-ITD+D835Y, which harbor FLT3 ITD, ITD plus F691L and ITD plus D835Y mutations, respectively. F691L or D835Y mutations are associated with resistance to FLT3-targeted AML therapy. Combination efficacy was investigated in subcutaneous and intravenous xenograft models generated in male NOD/SCID mice inoculated with MOLM-13 and MV-4-11 human AML cells.

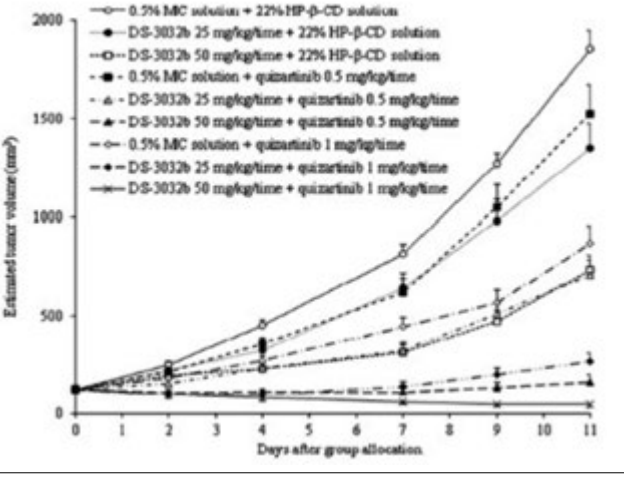


Figure 1.

Results: Combination treatment with milademetan (or DS-5272) and quizartinib demonstrated greater anti-leukemic activity compared to the respective single-agent treatments in FLT3 mutated and TP53 wild type cells. Combination indexes (CIs) were 0.25±0.06, 0.61±0.03, 0.62±0.06, 0.29±0.004 and 0.50±0.03, respectively, in MV-4-11, MOLM-13, MOLM-14, Ba/F3-FLT3-ITD+F691L and D835Y cell lines. All these cell lines harbor FLT3 ITD or ITD plus TKD point mutations. The combination regimen triggered synergistic pro-apoptotic effect in a p53 dependent manner. Mechanistically, the combination treatment triggered significant suppression of phospho-FLT3, -ERK

and -AKT compared to single agent treatments; and also up-regulated p53, p21 and the pro-apoptotic Bim proteins. In an *in vivo* study using the MOLM-13 subcutaneous xenograft model, quizartinib treatment at 0.5 and 1 mg/kg and milademetan treatment at 25 and 50 mg/kg demonstrated a significant tumor growth inhibition compared with the vehicle treatment or compared with the respective single-agent treatments. In MV-4-11 intravenous xenograft model, the combination of quizartinib plus milademetan showed a significantly prolonged survival (no mouse death during the study period) compared to their respective single agent treatments and the untreated control. Additional preclinical combination studies are ongoing (Figure 1).
Summary and Conclusions: Greater pre-clinical combination activity was observed for quizartinib plus milademetan treatment. A phase I clinical trial is planned to start in May 2018.

PF220

A GROUP OF EMA-APPROVED IMMUNOMODULATORS TARGETS AML STEM CELLS BY SABOTAGING THE CELLULAR RECYCLING MACHINERY

J.M. Cornet Masana[1,2,3,*], A. Banús-Mulet[1,4], A. Etxabe[1,4], M.C. Lara-Castillo[1], M.Á. Torrente[5], J. Esteve[3,5,6,7], R.M. Risueño[1]

[1]Leukaemia Stem Cell Lab, Josep Carreras Leukaemia Research Institute, Barcelona, [2]Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol, Badalona, [3]Faculty of Medicine, [4]Faculty of Pharmacy, University of Barcelona, [5]Department of Hematology, Hospital Clínic, [6]Josep Carreras Leukaemia Research Institute, [7]Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain

Background: A number of different genetic alterations are known to be involved in leukemogenesis and a handful of novel treatments aim at specific targeting of features related to those alterations. Nevertheless, diverse broad cellular and molecular elements are thought to be involved in sustaining Acute Myeloid Leukemia (AML) independently of specific genetic lesions. Using an AML-transformation gene signature and thanks to connectivity maps, an *in silico* screening identified several EMA-approved immunomodulators (IM) reverting that leukemogenic signature. Here, we describe the preclinical activity of those drugs and its targeting of the cellular recycling machinery.
Aims: The main objective of the study was to identify novel drugs against AML stem cells based on broad transformation-associated signatures and characterize its mechanism of action.
Methods: For the *in silico* screening, an MLL-AF9 gene signature was used in connectivity maps, and drugs were ranked according to its potential to revert the signature. To study the cytotoxic effects of IMs, cell viability was assessed by flow cytometry (7-AAD exclusion, volumetric count) at 48h using 4 human AML cell lines, cells from 9 AML patients and Mononuclear cells (MNCs) from 5 healthy-donor buffy coats. Clonogenicity assays were executed in AML cell lines and patient samples and lineage-depleted umbilical cord blood (UCB) cells by 18h IM treatment followed by 14-day culture in complete Methocult medium. *In vivo* studies were performed with busulfan-conditioned NOD-scid IL2Rg null (NSG) mice xenotransplanted with AML or UCB cells treated with IMs for 18h. Engraftment was analyzed after 8 weeks by flow cytometric determination of human CD45-expressing cells in bone marrow. To study the previously described GPCR target for the IMs, cells were cultured with IMs and high concentrations of the endogenous ligand, followed by a cytotoxicity assay as above described. Effects on lysosomes and autophagy were studied by Lysotracker and CytoID staining and by western blot detection of LC3BII.
Results: 4 structurally similar immunomodulators (IM) induced cytotoxicity in the low micromolar range in AML cell lines and patient samples, while displaying limited effects in healthy-donor and UCB MNCs. Clonogenicity studies yielded similar results, with a dramatic decrease of CFU formation in leukemic cells and mild effects in healthy HSCs, suggesting a differential effect on the more primitive AML stem cell fraction. Interestingly, cytotoxic effects were observed irrespective of specific AML subtypes and mutations. *In vivo* studies further corroborated the results, with IM treatment practically abolishing AML engraftment while sparing healthy hematopoiesis. When the mechanism of action was interrogated, the previously described target for IMs, a GPCR, was found not to be implicated in its antileukemic capacity, as the effects could not be reverted with the endogenous ligand for the receptor. Further studies concerning the mechanism of action found a disruption of lysosomal and autophagic homeostasis, with the study of specific targets currently ongoing.
Summary and Conclusions: 4 EMA-approved IMs have been identified as antileukemic candidates affecting AML stem cells independently of their immune target by affecting a broad cellular process known to be dysregulated in AML, namely the cellular recycling machinery, rather than a specific mutation-related feature.

PF221

EXPLORING ALTERNATIVE TREATMENTS TO IMPROVE OUTCOME FOR PEDIATRIC PATIENTS WITH ACUTE MYELOID LEUKEMIA

K. Lappin[1,*], J. Blayney[1], K. Matchett[1], K. Mills[1]

[1]CCRCB, Queen's University Belfast, Belfast, United Kingdom

Background: Leukemia is the most frequently (30%) occurring type of paediatric cancer. Of these, approximately 80% are acute lymphoblastic leukaemia (ALL) with acute myeloid leukemia (AML) cases making up the remaining 20%. Management of ALL has improved dramatically in recent years with around 90% of patients surviving 5 years or more. Unfortunately, children with AML do not have the same promising outlook with only 60% surviving 5 years or longer and a high risk (20%) of not responding to the standard of care induction therapy. In addition, the high rate of relapse which when combined with the side effects of the harsh chemotherapy regimen give a bleak outlook. Recent data (Bolouri *et al.*, 2017), strongly indicates the need for age specific therapies for AML patients.
Aims: The aim of this study is to generate a gene signature that can signpost pediatric AML patients who will not benefit from the standard of care therapy and identify alternative therapies that have the potential to be used in the treatment for these patients.
Methods: *In silico* analysis of RNA-sequencing data from the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) study generated by the National Cancer Institute (NCI) was performed on the gene count tables provided and gene expression analysis was determined using Galaxy DESeq2 software comparing each subgroup to the patients who survived without relapse following treatment. High throughput compound screening was performed on several cell lines, representative of pediatric AML cases with different cytogenetics and mutational status backgrounds to identify effective compounds across the different patient groups. Compounds were used at varying doses and assayed at 24, 48 and 72 hours post treatment using CellTox™ Green reagent.
Results: *In silico* analysis of RNA sequencing data collated as part of the TARGET study, identified a number of key pathways deregulated amongst non-responder patients who died within 1 year compared to those who survived without relapse, the most common pathways deregulated in non-responders were associated with DNA damage response (DDR). Thirty-seven DDR genes were deregulated with 89% up-regulated in comparison to patients who survived without relapse. Up-regulation of DDR genes has been shown to interfere with a patient's response to chemotherapy (Broustas and Lieberman, 2014), providing a possible explanation to why non-responders do not respond to Cytarabine+Daunorubicin induction therapy. Furthermore, we have developed a gene signature that can signpost patients who will not benefit from standard of care therapy. Due to the severe side effects of the standard therapy and the considerably high percentage of patients who will not respond, we also sought to identify possible alternative therapies for the treatment of these patients. In an *in vitro* paediatric setting, we have screened libraries of experimental compounds and FDA approved therapies. This screen has identified several compounds with repurposing potential for age-specific AML treatment, such as Venetoclax.
Summary and Conclusions: To improve the outcome for children and young people diagnosed with AML, it is imperative that alternative therapies are identified which when combined with a means of identifying non-responders will lead to improved outcomes for pediatric patients with AML.

PF222

INTEGRATIVE ANALYSIS OF GENOMIC DATA REPOSITIONS THE USE OF 5'-AZACYTIDINE AND DECITABINE IN BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM TREATMENT

M.R. Sapienza[1,*], F. Abate[2]

[1]Department of Experimental, Diagnostic, and Specialty Medicine, University of Bologna, Bologna, Italy, [2]Department of Systems Biology, Columbia University College of Physicians and Surgeons, New York, United States

Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare hematological malignancy derived from precursors of plasmacytoid dendritic cells (pDCs) and classified among acute myeloid leukemia. BPDCN patients show a very aggressive clinical course (median overall survival of 12 months), and, despite an initial response to chemotherapy, regularly relapse. To date, no standardized therapy has been established and the optimal treatment remains to be defined. Recent studies suggested new therapeutic options mainly derived from the investigation of the BPDCN transcriptome. On the contrary, the BPDCN DNA features were scarcely evaluated.

Aims: We aimed to design the first therapeutic strategy derived from the mutational profile of the BPDCN exome.
Methods: We analyzed by whole-exome sequencing (WES) fourteen BPDCN samples and the BPDCN patient-derived CAL-1 cell line, the largest case series sequenced so far. To evaluate the mutational impact at gene expression and epigenetic levels, we investigated by RNA sequencing all the BPDCN transcriptome and analyzed by chip-sequencing the genome-wide distribution of H3K27me3 and H3K27Ac, two epigenetic marks of transcriptional repression and induction, respectively. The integration of sequencing data led to identify a new therapeutic approach that we tested in a preclinical BPDCN-mouse model, established by the CAL-1 cell line xenografting.
Results: Mutations were accumulating in the epigenetic program, involving 25 epigenetic modifier genes, and, among them, *ASXL1* was the highest recurrently affected (28.6% of cases) (Figure 1a).The functional enrichment analysis of WES data, recognized the epigenetic process as the most undermined by mutational events (P<.0001)(Figure 1b). Gene set enrichment analysis reported the significant deregulation of gene-signatures involved in the methylation pathway and responsive to hypomethylating agents, namely Decitabine (NES>2, FDR q<.001) (Figure 1c). The BPDCN patients converged on the same H3K27-acetylated regions and the integration with transcriptomic data highlighted a set of genes marked by promoter-acetylation, aberrantly up-regulated and involved in the cell-cycle regulation Figure 1d). Globally, the integrative analysis of genomic data suggested to use a therapy based on epigenetic drugs. We tested *in vivo* the efficacy of epigenetic agents FDA approved: 5'-Azacytine, Decitabine, Romidepsine and Bortezomib, alone and in combination (Figure 1e). The combined use of 5'-Azacytine and Decitabine reached the best results demonstrating to significantly arrest the *in vivo* BPDCN tumor growth (Figure 1f,g).

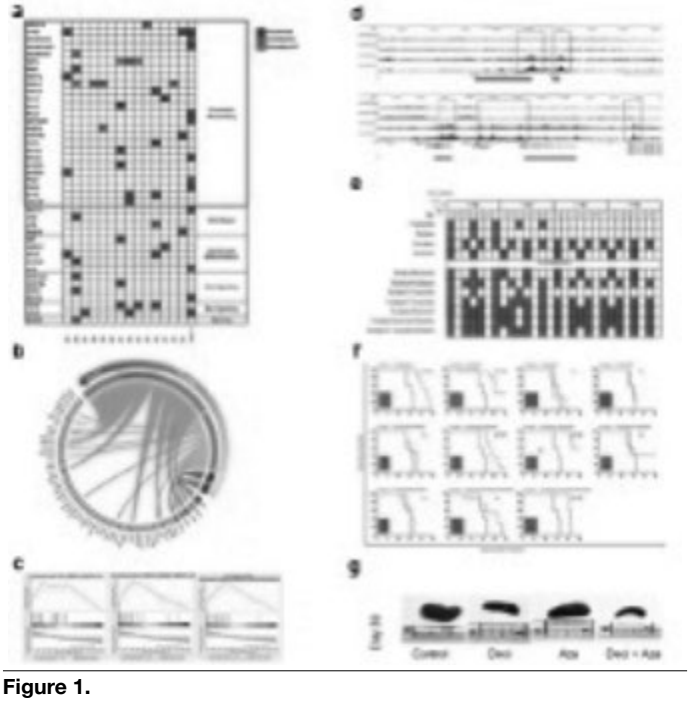


Figure 1.

Summary and Conclusions: In conclusion, we identified the deregulation of epigenetic program as a genetic hallmark of BPDCN and suggested a novel therapeutic approach based on the combination of two hypomethylating agents 5'-Azacytidine and Decitabine to be tested in future clinical trials.

PF223

LOSS OF TET2 FOLLOWED BY ACQUISITION OF ACTIVATING KRAS MUTATION LEADS TO COOPERATIVE MALIGNANT MYELOPROLIFERATION THROUGH AMPLIFICATION OF RAS SIGNALING IN A DOSE- AND TIME-DEPENDENT MANNER

M. Miller[1,*], J. Rogers[2], P. Narayanan[3], R. Gupta[2], M. Redell[1], M. Goodell[4], R. Rau[1]

[1]Cancer Center, [2]Baylor College of Medicine, Houston, United States, [3]Texas Children's Cancer and Hematology Center, [4]Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, United States

Background: Mutations of epigenetic modifiers and activated signaling mutations frequently co-occur in myeloid malignancies, suggesting cooperative leukemogenesis. In acute myeloid leukemia (AML) and chronic myelomonocytic leukemia (CMML), TET2 loss-of-function mutations non-randomly co-occur with activating RAS pathway mutations. Thus, we hypothesized that Tet2 loss and activating Ras-pathway mutations collaborate to promote the development of myeloid malignancies.
Aims: Using a novel murine model, we aimed to: 1) Determine if loss of Tet2 followed by acquisition of a Ras-pathway mutation synergistically drives the development of myeloid malignancies; and 2) Define the mechanisms by which Tet2 loss contributes to the development of Ras-driven malignancy.
Methods: We crossed Tet2 knock-out mice to mice with Cre-inducible $Kras^{G12D}$ and the ER-T2Cre driver. We transplanted Sca1-enriched stem/progenitor bone marrow cells from 6-8 week old CD45.2+ C57BL/6 mice with the genotypes of interest into lethally irradiated CD45.1+ recipients. After engraftment, we induced $Kras^{G12D}$ expression by tamoxifen treatment at either 1 or 3 months post-transplant. We tracked mice for evidence of disease, comparing mice with both Tet2 loss and Kras mutation to mice with either mutation alone. We analyzed activation of the Ras-pathway by phospho-flow and gene expression studies.
Results: All mice with $Kras^{G12D}$ had anemia, thrombocytopenia, and splenomegaly as well as myeloid expansion in the peripheral blood and spleens by 4-weeks post Ras-pathway activation; Tet2 loss increased the severity of these symptoms in a dose-dependent manner (Figure 1 and Figure 2). Tet2 dose also impacted disease phenotype, with $Tet2^{-/-}/Kras^{G12D}$ mice dying exclusively of myeloid disease whereas mice with $Kras^{G12D}$ alone and $Tet^{+/-}/Kras^{G12D}$mice developed either myeloid or T cell disease. Disease latency was dependent upon both the dose and duration of Tet2 loss. When $Kras^{G12D}$ was activated at 3 months post-transplant, $Tet2^{-/-}/Kras^{G12D}$ had the shortest survival followed by $Tet^{+/-}/Kras^{G12D}$, both significantly shorter than mice with $Kras^{G12D}$ alone. However, there was no difference in survival if tamoxifen was injected 1 month post-transplant. We found evidence that loss of Tet2 amplifies Ras-signaling with increased pErk and pS6 in $Tet2^{-/-}/ Kras^{G12D}$ bone marrow cells compared to $Kras^{G12D}$ alone.

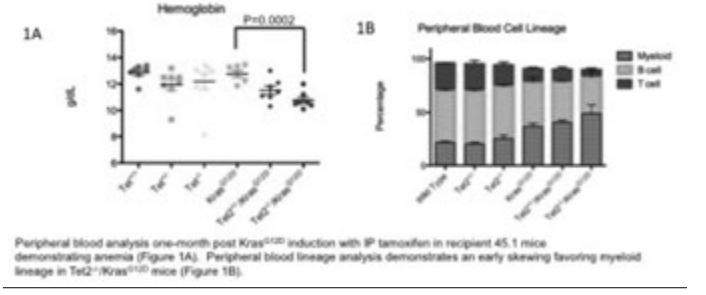


Figure 1.

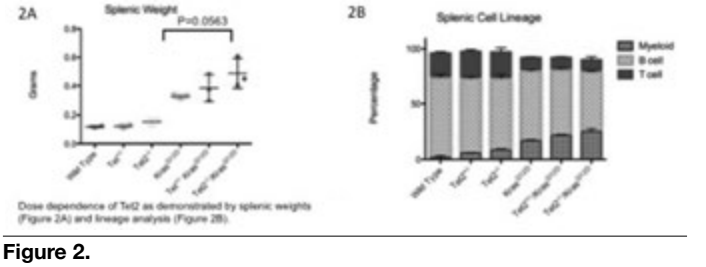


Figure 2.

Summary and Conclusions: TET2 mutations are typically founder mutations in AML and CMML and are frequent in age-related clonal hematopoiesis. This mutational pattern suggests that loss of Tet2 leads to gradual expansion of an aberrant hematopoietic stem/progenitor population that may be primed for malignant transformation. Using a novel murine model, we found that Tet2 loss impacts Kras-induced myeloproliferation in a dose-dependent manner. Further, we found that prolonged loss of Tet2 decreased disease latency after the acquisition of a Ras pathway mutation, possibly mimicking the disease process in adults with TET2 mutant myeloid malignancies arising from a clonal population of TET2-mutant cells that has expanded over time. These results suggest that the extent of aberrant DNA methylation determined by the dose and duration of Tet2 loss is critical to the development of TET2 mutant malignancies. Our mechanistic work indicates that loss of Tet2 cooperates with RAS pathway mutations by amplifying Ras signaling, consistent with recent data (Kunimoto, Cancer Cell, 2017). Thus, targeting the methylation defects with hypomethylating agents and the RAS-pathway with available inhibitors could be therapeutically effective.

PF224

5-AZACYTIDINE ENHANCES THE ANTI-LEUKEMIC ACTIVITIY OF IDH1 INHIBITOR BAY 1436032 *IN VIVO* IN IDH1 MUTANT ACUTE MYELOID LEUKEMIA

A. Chaturvedi[1,*], C. Gupta[1], R. Goparaju[1], R. Gabdoulline[1], S. Kaulfuss[2], K. Görlich[1], R. Schottmann[1], B. Othman[1], O. Panknin[2], M. Wagner[2], R. Geffers[3], A. Ganser[1], F. Thol[1], M. Jeffers[4], A. Haegebarth[2], M. Heuser[1]

[1]Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, [2]Bayer AG, Berlin, [3]Genome Analytics Research Group, Helmholtz Centre for Infection Research, Braunschweig, Germany, [4]Bayer AG, Whippany, NJ, United States

Background: Early data for IDH1 inhibitors show that 30-40% of AML patients respond to monotherapy with a median duration of response of 8 months, suggesting that IDH1 inhibitors should be combined with other agents to improve efficacy. BAY 1436032 (BAY) is an oral pan-mutant IDH1 inhibitor currently undergoing phase 1 clinical trials. 5-Azacitidine (AZA) is a hypomethylating agent and can activate key epigenetically silenced pathways in AML cells, leading to an arrest of AML cell proliferation.
Aims: To investigate the efficacy of IDH1 inhibitor BAY 1436032 in combination with AZA in a preclinical AML model.
Methods: For *in vitro* studies IDH1 wildtype (IDH1wt) or IDH1mut AML cells from patients were incubated with BAY alone or in combination with escalating doses of AZA. For *in vivo* studies leukemic cells from an AML patient with mutated IDH1 were xenografted in NSG mice and treated with either vehicle, BAY 150 mg/kg once daily p.o. continuously, and/or AZA 1 mg/kg once daily s.c. for 5 days. To quantify the stem cell frequency serial dilution transplantation with 20 to 200,000 cells was performed after four weeks of treatment (3 mice/dose).
Results: Combination of 100 nM (IC50 on IDH1mut AML cells) of BAY with 1 µM AZA reduced colony formation by 98% in mIDH1 AML cells but only by 18% in IDH1wt AML cells. IDH1mut human AML cells cultured in suspension medium *ex vivo* and treated with the combination of BAY (100 nM)+AZA (100 nM) proliferated significantly less compared to single agent treatment. For *in vivo* experiments IDH1mut xenografted mice in test groups were treated with BAY and AZA in the doses mentioned above either starting both drugs on day 1 (simultaneous group) or starting AZA on day 1 but BAY 1436032 on day 6 (sequential group). Treatment stopped at 84 days. Leukemic cells in peripheral blood constantly increased in vehicle and AZA treated mice, while leukemic cells declined from week 4 until the end of treatment at week 12 in mice treated with BAY alone as well as in the simultaneous and sequential combination groups treated with BAY and AZA. However, in week 20 (8 weeks after stopping BAY) leukemia relapsed in the BAY alone as well as the sequential BAY+AZA groups and all mice died by week 24. Interestingly, the frequency of leukemic cells remained low in peripheral blood of mice treated simultaneously with BAY+AZA and 2/6 mice from this cohort remained negative until the end of the study at 36 weeks. To assess the effect of simultaneous and sequential treatment with AZA+BAY on leukemia stem cell self-renewal we performed a limiting dilution transplantation experiment. The LSC frequency was 1 in 304 cells in AZA, 1 in 8,580 in BAY and 1 in 34,314 in BAY+AZA sequentially treated mice, respectively, while it was less than 1 in 200,000 transplanted cells in mice treated with BAY+AZA simultaneously (Table 1). Gene expression analysis showed additive suppression of MAP kinase (*ELK1, ETS1* and *CCND1*) and E2F signaling (*E2F1, CCNA2* and *CCNE1*), which are involved in cell survival and proliferation, whereas myeloid differentiation genes (*PU.1, CEBPA* and *GABPA*) were upregulated by BAY+AZA compared to single agents. This suggests synergy in induction of differentiation and inhibition of self-renewal of the combination treatment through distinct pathways.

Table 1.

Limiting dilution transplantation of bone marrow cells from PDX mice treated with vehicle, 150 mg/kg BAY 1436032 and/or 1 mg/kg azacitidine. 200,000, 20,000, 2,000, 200 or 20 cells per mouse were transplanted into 3 recipient mice per group. LSC frequencies are shown (mean ± SEM, n = 3).

Group	Stem cell frequency	± SEM	P value
Vehicle	1/74	19-287	0.2
AZA	1/304	48-1,944	0.005
BAY-1436032	1/8,580	2,440-30,176	0.2
BAY+AZA Sequential	1/34,314	5,913-199,114	0.005
BAY+AZA Simultaneous	>200,000		

Summary and Conclusions: Our study provides evidence that simultaneous combination of an IDH inhibitor with a hypomethylating agent synergistically inhibits leukemia stem cells. Clinical development is ongoing with phase 1 studies using BAY 1436032 in IDH1 mutant solid tumors and AML, and simultaneous combination of BAY with AZA is warranted.

PF225

COMBINATORIAL CSF3R AND RUNX1 MUTATIONS INVOLVED IN THE LEUKEMIC PROGRESSION OF SEVERE CONGENITAL NEUTROPENIA CONFER A G-CSF-DEPENDENT PREMALIGNANT STATE IN MICE TERMINATING IN G-CSF INDEPENDENT AML

P. Olofsen[1,*], S. Fatrai[1], P. van Strien[1], J. Obenauer[1], I. Touw[1]

[1]Hematology, Erasmus MC, Rotterdam, Netherlands

Background: Severe congenital neutropenia (SCN) is a bone marrow failure syndrome characterized by a block in neutrophil development. In the majority of patients neutrophil production is restored by sustained G-CSF treatment. During G-CSF treatment, SCN patients frequently acquire clones with truncated *CSF3R* mutations which are associated with progression to MDS or AML. At this stage the majority (~75%) acquired secondary mutations in *RUNX1*, mainly affecting the Runt homology domain (RHD). The combination of *CSF3R* and *RUNX1* mutations is unique for SCN-MDS/AML, but the consequences of having both mutations and the effect of G-CSF therapy on leukemic progression are unknown.
Aims: To determine the effects of mutations in *CSF3R* and *RUNX1,* and to assess the impact of G-CSF treatment, in the leukemic transformation of mouse bone marrow cells.
Methods: Csf3r-d715 mutant lineage depleted mouse bone marrow cells were transduced with RUNX1 RHD mutant (D171N), or empty vector control, IRES-GFP lentivirus. These cells were transplanted into 8.5 Gy irradiated wildtype recipients and the recipient mice (n=9 per group) were injected 3x a week with G-CSF or solvent control (PBS). Peripheral blood was analysed biweekly for GFP-marked engraftment, immunophenotypes and cytology. *In vitro* progenitor cell colony assays were performed, and bone marrow, spleen and liver samples were used for phenotypic analyses and secondary/tertiary transplantations.
Results: Analyses of the peripheral blood showed rapidly decreasing GFP+ numbers in all groups except for the mice transplanted with CSF3R/RUNX1 mutant cells that were treated with G-CSF, where GFP+ cells could be detected till the end of the experiment, 35 weeks after transplantation. These CSF3R/RUNX1 mutant cells did not show clonal dominance as fluctuations in GFP+ blood cells were observed. When analysing the cellular content of the blood we found an accumulation of cKit+ cells in the CSF3R/RUNX1 mutant G-CSF treated group, which had a blast morphology and colony forming potential *in vitro.* These mice also showed elevated extramedullary hematopoiesis in the spleen. However, the CSF3R/RUNX1 mutant cells were still capable of forming mature neutrophils and mice did not succumb to this pre-leukemic condition. CSF3R/RUNX1 mutant bone marrow cells could successfully engraft sub-lethally irradiated secondary recipients, again resulting in accumulation of cKit+ blast cells in the blood. The GFP+ cells were vastly accumulating and >90% myeloblast infiltration was observed in the bone marrow of these mice 10 weeks after transplantation, indicating that the mice had developed AML. Re-transplantation of these mutant bone marrow cells showed G-CSF independent accumulation of GFP+ cKit+ cells in the blood, bone marrow, spleen and liver. G-CSF independent proliferation of SCN-AML cells has been linked previously to autoactivating mutations in the already truncated CSF3R mutant. To interrogate this and the possible acquisition of other secondary mutations, we are currently completing whole exome DNA sequencing.
Summary and Conclusions: The combination of *Csf3r* and *RUNX1* mutations and sustained G-CSF treatment results in selective accumulation of blast-like cells in an *in vivo* transplant model. This pre-leukemic state is seen in primary recipients and progresses to a fully transformed AML in secondary recipients. These leukemic cells can be re-transplanted and become G-CSF independent. This model is attractive for unravelling the molecular steps of leukemic transformation in SCN-MDS/AML, and for preclinical testing of potential new therapeutic options.

PF226

IDENTIFYING NEW DISEASE GENES IN FAMILIAL MYELODYSPLASIA/ ACUTE MYELOID LEUKEMIA

A. Rio-Machin[1,*], S. Cardoso[2], A. Ellison[2], K. Tawana[1], J. Wang[3], C. Chelala[3], V. Plagnol[4], N. Pontikos[5], P. Page[6], K. Reay[6], Y. Wallis[6],

G. Ryan[6], A. Al Seraihi[1], A. Walne[2], H. Tummala[2], J. Fitzgibbon[1], I. Dokal[2], T. Vulliamy[2]

[1]Centre for Haemato-Oncology, Barts Cancer Institute, [2]Centre for Genomics and Child Health, Blizard Institute, [3]Centre for Molecular Oncology, Barts Cancer Institute, [4]UCL Genetics Institute, [5]UCL Institute of Ophthalmology, University College London, London, [6]Cancer Programme, West Midlands Regional Genetics Laboratory, Birmingham, United Kingdom

Background: Myeloid neoplasms with germ line predisposition is included in the World Health Organization (WHO) classification 2016 as a new diagnostic category, encompassing multiple inherited hematological syndromes and typically characterized by familial myelodysplasia/acute myeloid leukemia (MDS/AML) with two or more affected individuals within the same family. Although it is considered that these familial cases represent <5% of all MDS/AML cases, they constitute a high-risk population that requires unique management and genetic counselling.
Aims: To identify novel loci associated to familial MDS/AML development.
Methods: Eighty eight families where two or more members have been diagnosed with hematological disease (leukemia, MDS or bone marrow failure) including at least one individual with MDS or AML in each family, were enrolled into the study. Twelve genes (*ACD, ANKRD26, CEBPA, DDX41, ERCC6L2, ETV6, GATA2, MECOM, RUNX1, SRP72, TERC* and *TERT*) were analyzed using a targeted Next Generation Sequencing panel (Agilent SureSelect QXT target enrichment, Illumina MiSeq platform). Families where variants were not identified underwent Whole Exome Sequencing (WES) analysis (Illumina Nextera reagents, HiSeq2000, GATK v3.2).
Results: Forty four of the eighty eight families in our cohort (50%) have likely pathogenic variants in 10 of the 12 known loci analyzed by targeted sequencing: *CEBPA* (7), *DDX41* (6), *ERCC6L2* (1), *ETV6* (1), *GATA2* (7), *MECOM* (1), *RUNX1* (12), *SRP72* (2), *TERC* (3) and *TERT* (4). WES was performed in the remaining 44 families. In phase I of these studies, we focused attention on genes linked with other inherited hematological syndromes, assigning a likely causal germline variant in 4 additional families: a novel *WASP* variant (p.Lys446fs), two known pathogenic biallelic *SBDS* variants (c.258+2T>C and p.Lys62*) and novel *SAMD9L* variants (p.Ser1473Asn and p.Asn140Ser) in two families. Based on the characteristics of known germline disease causing variants in familial MDS/AML, we established the following criteria to enrich for genes with pathogenic relevance in the remaining 40 families: (i) genes with variants in at least three families; (ii) novel variants not described in ExAC or gnomAD; and (iii) variants predicted to be pathogenic with two out of four tools for functional annotation (PolyPhen2, MutationTaster, SIFT and Provean). The chosen criteria allowed us to limit the search to the two most relevant genes with germline variants in our cohort, *PRR23B* (N=3) and *CPEB2* (N=3). However when these criteria are relaxed, in particular the number of families or ExAC frequency, we observed a significant increase in credible candidates including *ADA, DNAH11, CSNK1A1L, DHX34, HELQ, LTK, PDCD4, PPM1J, PRUNE2, SLC13A2, TMIUSP31, VPS13C, VWDE or ZNF208.*
Summary and Conclusions: Our results demonstrate that the intra and inter family clinical variability is likely to be mirrored by significant genetic heterogeneity. It is notable, that our candidate genes mutated in small fractions of families are rarely detected in sporadic MDS/AML, suggesting that the identification of novel loci will provide fresh insight into the biology of AML. Our ongoing functional studies and the investigation of additional patient cohorts will establish whether these candidate genes can be assigned as true 'familial MDS/AML' disease loci. Full knowledge of the diversity of predisposing variants is critical to improve the management and counselling of these families.

Acute myeloid leukemia – Clinical

PF227

ADDITION OF CRENOLANIB TO STANDARD INDUCTION AND CONSOLIDATION THERAPIES IMPROVED LONG-TERM OUTCOMES IN NEWLY DIAGNOSED FLT3-MUTANT AML PATIENTS ≤60 YEARS OLD

R.B. Walter[1,*], R.H. Collins[2], R.M. Stone[3], M.S. Tallman[4], C. Karanes[5], C. E. Vigil[6], J.R. Eckardt[7], E.S. Wang[8]

[1]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, [2]University of Texas Southwestern Medical Center, Dallas, TX, [3]Dana-Farber Cancer Institute, Boston, MA, [4]Division of Hematologic Oncology, Leukemia Service, Memorial Sloan Kettering Cancer Center, New York, NY, [5]Gehr Family Center for Leukemia Research, City of Hope, Duarte, CA, [6]Division of Hematology, Oncology, and Blood & Marrow Transplantation, The University of Iowa, Iowa City, IA, [7]AROG Pharmaceuticals, Inc., Dallas, TX, [8]Roswell Park Comprehensive Cancer Center, Buffalo, NY, United States

Background: The multikinase inhibitor midostaurin was recently approved in combination with chemotherapy based on the RATIFY trial of younger patients with treatment-naïve *FLT3*-mutant AML demonstrating a survival benefit (2yr OS: 60% midostaurin *vs* 51% placebo). We hypothesized that a more selective *FLT3*-targeted agent such as crenolanib, a potent and specific FLT3 tyrosine kinase inhibitor (TKI) which inhibits both FLT3ITD and FLT3TKD mutations, would further improve patient outcomes when combined with chemotherapy. We here report an analysis of patients treated with crenolanib combined with chemotherapy similar to the population studied in the RATIFY trial.
Aims: To assess the outcomes of a sub-group of newly diagnosed FLT3-mutant AML patients treated with crenolanib and standard chemotherapy to be targeted in a pivotal phase III trial comparing crenolanib with midostaurin.
Methods: Twenty-seven of 29 consecutive patients ≤60 years old enrolled in a phase II study of crenolanib combined with chemotherapy in newly diagnosed FLT3-mutant AML (NCT02283177) were included in this analysis. Two patients were excluded due to 1) prior treatment for a myeloproliferative disorder and 2) pre-existing liver cirrhosis. Patients received 7+3 induction with cytarabine 100 mg/m^2 for 7 days and either daunorubicin 90 mg/m^2 (n=16) or idarubicin 12 mg/m^2 (n=11) for 3 days. Crenolanib 100 mg TID was administered continuously starting 24 hours after chemotherapy until 72 hours prior to the next chemotherapy cycle. Consolidation consisted of up to four cycles of high-dose cytarabine (HiDAC: 3 g/m^2 for <60 years and 1 g/m^2 for 60 years) q12 hours on days 1, 3, and 5 with crenolanib starting 24 hours after the final HiDAC dose in each cycle. Eligible patients proceeded to allogeneic hematopoietic stem cell transplant (HSCT). Maintenance crenolanib at 100mg TID was started after HiDAC or 30-90 days after HSCT for a maximum of 12 cycles.

Table 1.

Characteristic	n = 27
Age, median (range)	51 (19 – 60)
Baseline WBC per μL, median (range)	33,010 (2,300 – 248,800)
Baseline WBC ≥100,000 per μL	6 (22%)
FLT3 mutation status	
FLT3-ITD (± TKD)	23 (85%)
FLT3-TKD	4 (15%)
FLT3-ITD/NPM1/DNMT3A mutant	6 (26%)
Cytogenetic risk classification	
Intermediate	24 (89%)
Adverse	2 (7%)
Not available	1 (4%)
OS, median (2yr)	Not reached (81%)
EFS, median (2yr)	Not reached (62%)
CIR, median (2yr)	Not reached (17%)

Results: Patients included in the analysis were generally older (median 51 *vs* 47 years for RATIFY) and most patients (85%) had FLT3-ITD mutations. Six patients had concurrent NPM1 and DNMT3A mutations, a constellation associated with poor prognosis. Only 4 (15%) patients had more favorable FLT3-TKD mutations compared with 23% in the RATIFY study. As of February 2018, 22/27 (81%) patients are alive with a median follow-up of 20.8 months. Median overall survival (OS), event-free survival (EFS), and cumu-

lative incidence of relapse (CIR) have not been reached. Fourteen patients received HSCT of which 11 are alive free of disease. Seven patients were consolidated with HiDAC and did not undergo HSCT. Only one of these seven patients has relapsed and the other six remain alive free of disease, suggesting that standard chemotherapy plus crenolanib can provide durable remissions without HSCT. 4/6 (67%) patients with high-risk concomitant mutations in FLT3-ITD, DNMT3A and NPM1 are alive free of disease. Overall, only 3/21 patients have relapsed, none of whom received >1 week of crenolanib maintenance (Table 1).

Summary and Conclusions: This analysis suggests that outcomes in younger patients with newly diagnosed FLT3-mutant AML may be improved by adding a potent pan-FLT3 inhibitor to chemotherapy. A phase III randomized multicenter trial has been initiated to compare the efficacy of crenolanib *versus* midostaurin combined with standard chemotherapy for newly diagnosed patients with FLT3-mutant AML (NCT03258931).

Table 1. Characteristics of patients enrolled in the trail.

		Arm A (n=101)	Arm B (n=103)	Arm C (n=102)
Sex	male	52	61	61
	female	57	52	51
Median age (range)		40 (18-60)	38 (18-59)	42 (18-60)
NCCN risk stratification	favorable	33	30	35
	intermediate	35	40	26
	unfavorable	41	43	51
Median WBC counts at diagnosis (meidan, ×10E9/L)		13.4 (0.73-290.31)	11.52 (1.04-248.0)	18.95 (0.89-406.1)
blast% in BM (median,range)		42.5 (21-93.5)	50.0 (22.5-95)	51.0 (24.0-95.0)

WBC: white blood cell; BM: Bone Marrow

PF228

COMBINATION OF IDARUBICIN, CYTARABINE AND CLADRIBINE AS INDUCTION REGIMEN FOR UNTREATED ADULT AML AGED 60 OR YOUNGER: A PHASE III RANDOMIZED CLINICAL TRIAL

J. Chen[1,*], Y. Han[1], C. Chen[1], A. Sun[1], H. Qiu[1], X. Tang[1], C. Fu[1], Z. Jin[1], M. Miao[1], Y. Wang[1], C. Li[1], S. Chen[1], Y. Xu[1], Y. Fan[1], X. Zhang[1], T. Song[1], D. Wu[1]

[1]The First Affiliated Hospital of Soochow University,Jiangsu Institute of Hematology, Suzhou, China

Background: A successful treatment for AML relies on the ability of inducing long-term remission, in which the induction therapy is quite important. DA regimen has become the standard induction therapy for decades. Although great efforts have been made to improve the outcome including intensifying the dose of anthracycline agents or applying HiDAC regimens, complete remission rarely exceed 70% in younger patients or 50% in older patients. Recently the Polish Adult Leukemia Group has proved the value of adding cladribine into DA regimen (DAC regimen). Since idarubicin plus cytarabine (IA regimen) is another standard induction regimen which is well-accepted in China, we conducted a randomized clinical trial to evaluate the efficacy and safety of IAC regimen (consisted of idarubicin, cytarabine and cladribine) as induction therapy for untreated adult AML patients with age of 60 or younger.

Aims: We conducted a randomized clinical trial to evaluate the efficacy and safety of IAC regimen (consisted of idarubicin, cytarabine and cladribine) as induction therapy for untreated adult AML patients with age of 60 or younger.

Methods: We conducted a phase III randomized study (NCT02323022) to compare the efficacy of following three induction regimens: Arm A: idarubicin 10 mg/m^2 for 3 days plus cytarabine 100 mg/m^2 for 7 days; Arm B: idarubicin 12 mg/m^2 for 3 days plus cytarabine 100 mg/m^2 for 7days; Arm C: idarubicin 8 mg/m^2 for 3 days, cytarabine 100 mg/m^2 for 7 days and cladribine 5 mg/m^2 for 5 days. Patient fulfilled following criteria was recruited and randomized allocated into the three arms above: 1) diagnosed as AML (expect APL); 2) aged 18 – 60; 3) with an ECOG score of 0 – 3 and tolerated to chemotherapies. The primary endpoint in this trial was completion remission (CR) rate after induction.

Results: From December, 2014 through June, 2017, totally 306 cases were recruited in this trial (characterized in Table 1). As a result, compared to the CR rate of Arm C (80.39%), the CR rate of Arm A (67.32%) was significantly inferior (P=0.034) despite that of Arm B (73.79%) was similar. In the subgroup analyses, Arm C revealed the highest CR rate (76.27%) in AML patients with unfavorable-risk cytogenetic and molecular abnormalities (according to NCCN guidelines) compare to Arm A and Arm B (55.85% and 57.44% respectively) with statistical difference (P=0.033). In addition, Arm C also exhibited better CR rate in patients with higher WBC counts (≥30 × 10^9/L) and higher blast proportion in bone marrow (≥50%) at diagnosis, with P value of 0.039 and 0.012 respectively. Toxicity among the three groups were comparable. Multivariate risk factor analysis showed blast proportion in bone marrow at diagnosis and NCCN risk stratification were independent risk factors affecting CR, while induction regimens had a marginal significance (P=0.055) due to the inapparent difference between Arm B and Arm C.

Summary and Conclusions: We concluded that IAC regimen could decrease the dose of anthracycline agents and achieve at least comparable CR rate with standard IA regimen without additional toxicity in adult AML patients aged 60 or younger. For patients with genetic or non-genetic risk factors, IAC regimen had potential superiority to induce CR. Reducing the dose of idarubicin in IA regimen had negative impact on CR rate.

PF229

REASSESSING THE PROGNOSTIC SIGNIFICANCE OF RECURRING CHROMOSOMAL ABNORMALITIES AND FLT3-ITD IN ACUTE MYELOID LEUKEMIA PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION: AN ALWP/EBMT ANALYSIS

J. Canaani[1,*], M. Labopin[2], M. Itälä-Remes[3], D. Blaise [4], G. Socié[5], E. Forcade [6], J. Maertens[7], D. Wu[8], R. Malladi[9], J.J. Cornelissen[10], A. Huynh[11], J.H. Bourhis[12], J. Esteve[13], M. Mohty[2], A. Nagler[12]

[1]Hematology Division, Chaim Sheba Medical Center, Ramat Gan, Israel, [2]EBMT Paris study office / CEREST-TC, Saint Antoine Hospital, Paris, France, [3]Stem Cell Transplantation Unit, HUCH Comprehensive Cancer Center, Helsinki, Finland, [4]Programme de Transplantation & Thérapie Cellulaire, Institut Paoli Calmettes, Marseille, [5]Hematology, Hopital St. Louis, Paris, [6]CHU Bordeaux Hôpital Haut-Leveque, Pessac, France, [7]Hematology Department, University Hospital Gasthuisberg, Leuven, Belgium, [8]Hematology Department, First Affiliated Hospital of Soochow University, Suzhou, China, [9]Hematology Department, Queen Elizabeth Medical Centre, Birminghan, United Kingdom, [10]Hematology Department, University Medical Center Rotterdam, Rotterdam, Netherlands, [11]Institut Universitaire du Cancer Toulouse, Toulouse, [12]Hematology Division, Gustave Roussy institut de cancérologie, Villejuif, France, [13]Hematology Department, Hospital Clínic de Barcelona, Barcelona, Spain

Background: Baseline cytogenetics remain the single most important determinant of outcome in patients with acute myeloid leukemia (AML). The Medical Research Council (MRC) prognostic model is currently the benchmark for risk assessment in this patient population (Grimwade *et al.* Blood 2010). However, the prognostic role of the complete gamut of cytogenetic aberrations reported above in AML patients undergoing allogeneic stem cell transplantation (allo-HSCT) is currently undefined. In addition, their significance in conjunction with *FLT3-ITD* status has not been addressed thus far.

Aims: Using the ALWP/EBMT international database we conducted a retrospective analysis to determine the clinical outcomes of AML patients undergoing allo-HSCT with respect to specific recurring cytogenetic abnormalities complemented with *FLT3-ITD* status.

Methods: Using cox regression analyses with forward selection for each cytogenetic aberrancy allowed classification of patients into favorable, intermediate or adverse risk groups. Factoring in the presence or absence of a complex karyotype, defined as 3 or more cytogenetic abnormalities, classified patients into 4 prognostically distinct groups. We then assessed the prognostic impact of *FLT3-ITD* status in each subgroup.

Results: We analyzed a cohort consisting of 9113 adult AML patients who underwent allo-HSCT in first remission from either a matched sibling (n=4167) or a matched unrelated donor (n=4946), respectively in 2006-2016. Patients with inv(3)(q21q26)/t(3;3)(q21;q26), del(5q), del(7q), del(17p)/i(17q), and monosomies of chromosomes 4,5,7,8,17,21 as well as those patients with t(10;11) and t(6;11) experienced significantly inferior leukemia-free survival (LFS) compared to patients with a normal karyotype (P<0.0001). Del 9q [including add(9q)] and loss of chromosome X were associated with improved LFS rates (P<0.0001). Diverging from the MRC data, monosomies 4,8,21, trisomies 19,21 and abnormality of 16q [other than inv(16)] were determined to be poor prognostic factors. The presence of a complex karyotype was independently associated with outcome in the non-favorable risk groups, allowing us to identify 4 prognostic groups in the entire cohort analyzed. *FLT3-ITD* positivity (data available for 3997 patients) was also found to be an independent prognostic factor resulting in refinement of the model and the identification of 3 subgroups defined according to the presence of individual chromosomal abnormalities, complex karyotype, and *FLT3-ITD* status, with significant prognostic implications (Figure 1).

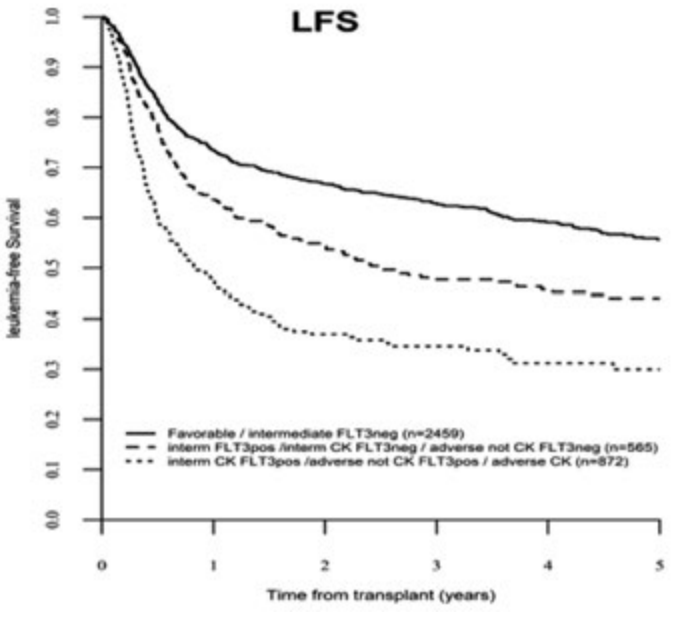


Figure 1.

Summary and Conclusions: In this analysis of a large cohort of more than 9000 AML patients, we confirm the prognostic significance of the MRC prognostic model in patients undergoing allo-HSCT in CR1 with some specific modifications. Furthermore, consideration of *FLT3-ITD* and complex karyotype improved the predictive capacity of the MRC model in transplanted patients.

PF230

Abstract withdrawn.

PF231

DECITABINE IN DAY TO DAY PRACTICE IN UNTREATED AML, RISK FACTORS AND COMPARISON WITH DACO16

A. De La Fuente[1,*], P. Beneit[2], A. Muñoz Gama[3], A. Sampol Mayol[4], K. Javier González[5], L. Cañas de Dios[6], J. Dávila Valls[7], A. Roldán Pérez[8], J. Cornago Navascues[9], C. Barrenetxea Lekue[10], I. Oiartzabal[11], S. Suarez Ordoñez[12], S. Vives Polo[13], C. Martinez Chamorro[14], R. Coll Jorda[15], S. Brunet[16], M. Arnan Sangerman[17], A. García de Coca[18], L.M. Gomez García[19], M.A. Foncillas[20], M.J. Otero[21], J.M. Grasa Ullrich[22], B. González González[23], J. González Campos[24], J.A. Vera Goñi[25], E. Sola Aparicio[9], G. Barranco Lampon[1], A. Diaz Lopez[26], P. Montesinos[27]

[1]Hematology, MD Anderson CC Madrid Spain, Madrid, [2]Hematology, Hospital San Juan, Alicante, [3]Hematology, Hospital Virgen de la Salud, Toledo, [4]Hematology, Hospital Son Espases, Palma de Mallorca, [5]Hematology, Hospital General Universitario de Valencia, Valencia, [6]Hematology, H.U. Príncipe de Asturias, Madrid, [7]Hematology, Complejo Asistencial de Ávila, Ávila, [8]Hematology, H. U. Infanta Sofía, [9]Hematology, H.U. La Princesa, Madrid, [10]Hematology, Organización Sanitaria Integrada Bilbao-Basurto, Vizcaya, [11]Hematology, H.U. Araba Txagorritxu, Vitoria, [12]Hematology, H. Alvaro Cunqueiro, Vigo, [13]Hematology, ICO H. Germans Trias i Pujol, Barcelona, [14]Hematology, H. Quiron Salud, Madrid, [15]Hematology, ICO Girona, Girona, [16]Hematology, Hospital Sant Pau, [17]Hematology, ICO Duran i Reynals, Barcelona, [18]Hematology, H.C.U. de Valladolid, Valladolid, [19]Hematology, H. Nuestra Señora de Prado, Toledo, [20]Hematology, H.U. Infanta Leonor, [21]Hematology, H. Central de la defensa, Madrid, [22]Hematology, Quiron Salud, Zaragoza, [23]Hematology, Hospital Universitario de Canarias, Tenerife, [24]Hematology, H.U. Virgen del Rocío, [25]Hematology, H. Virgen de la Macarena, Sevilla, [26]Hematology, Medicina Traslacional Fundación MD Anderson, Madrid, [27]Hematology, H.U. La Fe, Valencia, Spain

Background: Outcome for elderly AML patients is disappointing (median OS <6 months). The MD Anderson CC has identified as OS risk factors for elderly AML patients treated with intensive chemotherapy: age >80 yrs, ECOG ≥2, creatinine >1.3mg/dL and adverse cytogenetic (0 as opposed to 4 risk factors OS: 11.3 and 0.5 months respectively); *Kantarjian et al Blood 2010*. Decitabine (Dec) has been EMA approved for first line AML patients not fit for intensive chemotherapy, ECOG, cytogenetic and high WBC has been suggested as risk factors (Daco16) *Kantarjian et al JCO 2012*.

Aims: The aim of this study is to analyze effectiveness and tolerance of Dec as first line in AML in day to day practice and to identify risk factors.
Methods: We carried out the analysis on patients with previously untreated AML included in the MDA-AML-2017-05 study on 25 Spanish sites. Inclusion criteria as follows Age >18, diagnosis of AML under WHO criteria, treated with Dec during the period 01/09/2014 to 31/12/2016. We evaluated effectiveness as ELN-2010 criteria, toxicity as CTCAE v3.0 scale, OS by Kaplan-Meier and the mortality within the first 8 weeks (M8wks). This study has been approved by the Spanish Medicines Agency AEMPS code MDA-AML-2017-05.
Results: Of the 228 patients included in the MDA-AML-2017-05 study 126p (77M, 49F) received Dec as first line. Average age 75.7 (48-91), 80 yrs and above 57p, ECOG≥2: 38p, Creatinine>1.3 mg/dL: 23p, adverse cytogenetic: 39p, WBC pre-Dec >15.000/µL: 21p. A total of 716 cycles were analyzed, median 4 (1-31) per patient, 26 cases of hospitalization due to adverse events. No cases of treatment related mortality. One hundred and three patients were studied for effectiveness ORR 49% (CR 20p, PR 21p, ED 28p). With a mean follow up of 8 mths 77 died. The M8wks was 24.5% and the median OS 8 months. OS for patients who met the Daco16 inclusion criteria (83p) was 11 montrhs. In this study WBC pre-Dec >15.000/µL (p <0.01) creatinine >1.3 mg/dL (p0.02), ECOG≥2 (p<0.01) and adverse cytogenetics (p<0.01) resulted in statistical differences for OS. Age >80 revealed no differences. We observed OS benefit in patients with CR 20 mths (p <0.01) and for PR+ED *vs* PD 11.5 *vs* 3 mths (p<0.01). The DecLAM scale (WBC pre-Dec >15.000/µL, creatinine >1.3 mg/dL, ECOG≥2 and adverse cytogenetics) allows us to identify risk groups with 0 *vs* 1-2 *vs* 3-4 risk factors with differences for OS (19 *vs* 5.5 *vs* 3.5 m p<0.01) (Figure 1).

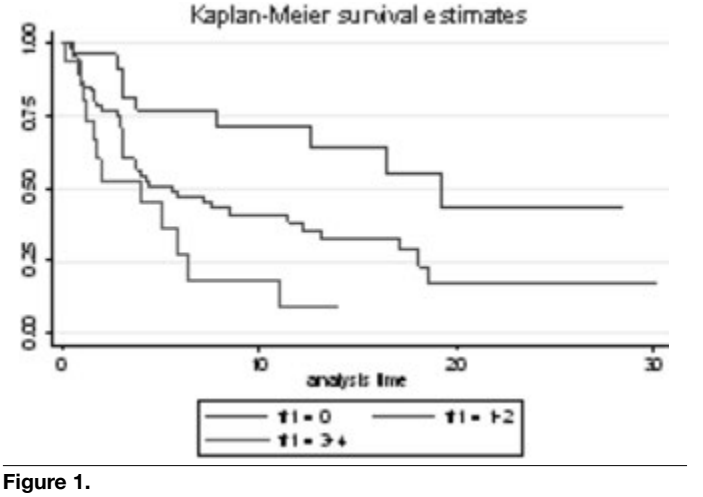


Figure 1.

Summary and Conclusions: The results of this study confirm Dec as an effective and well tolerated first line treatment in AML patients not candidates for intensive chemo. The OS observed in our study seems better that previously reported. The DecLAM scale identifies risk groups with differences in OS.

PF232

INITIAL PHASE 1 RESULTS OF THE FIRST-IN-CLASS ANTI-CD47 ANTIBODY HU5F9-G4 IN RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA PATIENTS

P. Vyas[1,*], S. Knapper[2], R. Kelly[3], R. Salim[4], M. Lubowiecki[1], D. Royston[1], H. Johnson[5], C. Roberts[6], J. Chen[7], B. Agoram[7], J. Liu[7], J.-P. Volkmer[7], R. Majeti[8], I. Weissman[8], S. Goudar[7], C. Takimoto[7], M. Chao[7], M. Dennis[9]

[1]University of Oxford and Oxford University Hospitals, Oxford, [2]Cardiff University School of Medicine, Cardiff, [3]Leeds Teaching Hospital NHS Trust, Leeds, [4]Royal Liverpool University Hospital, Liverpool, [5]Oncology Clinical Trials Office, [6]Centre for Statistics in Medicine, Nuffield Department of Orthopaedics, Rhuematology and Muscoloskeletal Sciences, University of Oxford, Oxford, United Kingdom, [7]Forty Seven, Inc., Menlo Park, [8]Stanford University, Stanford, United States, [9]The Christie NHS Foundation Trust, Manchester, United Kingdom

Background: Novel and well tolerated therapies are needed in relapsed/refractory (r/r) acute myeloid leukemia (AML). Hu5F9-G4 (5F9) is a first-in-class humanized antibody targeting CD47, a protective "don't eat me" signal on cancers, that stimulates tumor cell phagocytosis and an

anti-tumor T cell response. Pre-clinically, 5F9 eliminates leukemic disease and induces durable remissions in patient-derived xenograft mouse models. This trial is the first to investigate an anti-CD47 antibody in AML patients.
Aims: The main objectives were to determine the safety, tolerability and recommended Phase 2 dose of 5F9 in r/r AML.
Methods: This Phase 1 trial enrolled r/r AML patients in a 3+3 dose escalation design (NCT02678338). An intra-patient dose escalation design was used with escalation from 0.1 to 30 mg/kg twice weekly dosing of 5F9. Safety, preliminary efficacy, pharmacokinetic and pharmacodynamic parameters were measured.
Results: 15 r/r AML patients were enrolled across 5 dose cohorts as of 19 January 2018. Median age was 71 years (range 31-78) with a median of 2 prior therapies (range 1-5). 47% were relapsed, 47% relapsed/refractory and 7% primary refractory. 80% were intermediate to poor cytogenetic risk (20% not performed). 5F9 was well tolerated. Common treatment-related AEs were anemia (93%), hemagglutination (87%), pyrexia (27%), and headache (27%). All AEs were grade 1 or 2 (except anemia, a known on target effect of CD47 blockade). No patient developed a grade 4 or 5 treatment-related AE. 79% of these patients had grade 3 anemia prior to study. RBC transfusions were successfully administered and well tolerated with treatment despite interference with cross-matching in some patients due to 5F9 RBC binding. Hemagglutination was observed on peripheral smear in most patients with no significant clinical sequelae. No other significant myelosuppression was observed. No DLTs were observed with no patients discontinuing therapy due to AEs. The maximum tolerated dose (MTD) has not yet been reached up to 30 mg/kg twice weekly of 5F9 dosing. CD47 receptor occupancy >90% was achieved on blood and bone marrow WBCs, indicating near-maximal leukemic cell target saturation. A Phase 2 dose of 30 mg/kg 5F9 weekly after two weeks was selected. 73% of patients achieved stable disease with no objective responses. 40% of patients had a reduction in bone marrow blast count (mean decrease of 27%, range 5% - 67%). Two patients had biologic activity, defined as significant reduction in marrow cellularity observed similarly in pre-clinical models. Somatic mutation sequencing demonstrated that the first patient with an ASXL1 mutation had a 92% decrease in variant allele frequency (VAF) on treatment. Significant reduction in myeloid mutation VAFs was observed in several patients (range 7.4 – 95% decrease in VAF). The 2nd patient achieved a >50% blast count reduction and was on therapy for 11.8 months. This patient had a significant increase in T cell infiltrate in the bone marrow during treatment, suggesting activation of the adaptive immune system by Hu5F9-G4.
Summary and Conclusions: 5F9 is a novel immunotherapy inhibiting a key macrophage/cancer checkpoint. It is well tolerated in r/r AML with no DLTs or an MTD observed. Biologic activity was seen with T cell bone marrow infiltration in a long-term treated patient and reduction in somatic myeloid mutations in several patients. A Phase 1b trial of 5F9 in combination with azacitidine has been initiated based on these initial Phase 1 results (NCT03248479).

PF233

IMPACT OF CONCOMITANT EXPRESSION OF CD200 AND BCL2 ON OUTCOME OF ACUTE MYELOID LEUKEMIA

M. Tiribelli[1,*], A. Geromin[1], D. Raspadori[2], A. Michelutti[1], M. Cavallin[1], S. Sirianni[2], S. Di Giusto[1], E. Toffoletti[1], R. Fanin[1], M. Bocchia[2], D. Damiani[1]

[1]Division of Hematology and BMT, Azienda Sanitaria Universitaria Integrata di Udine, Udine, [2]Division of Hematology, University of Siena, Siena, Italy

Background: In the last years, evidence emerged that CD200 overexpression is associated with poor prognosis acute myeloid leukemia (AML) and that inhibition of apoptotic pathways mediated by bcl2 proteins has a role in myeloid malignancies. Moreover, we have observed a frequent association of bcl2 positivity and CD200 overexpression in AML patients; however, no data are available on the role of concomitant aberrant CD200 and bcl2 expression on outcomes of patients.
Aims: We aimed to elucidate the prognostic role of CD200/bcl2 co-expression and the association with specific leukemia subsets, to identify patients suitable for new target treatments.
Methods: .Two hundred and ninety-one patients diagnosed with AML and treated at our Institutions between 2009 and 2015 were included in this analysis. Median age was 60 (range: 18-84) years, and 183/291 (63%) patients were older than 55 years. Ninety-one patients (27%) had secondary leukemia, 109 (37%) had WBC count ≥30x109/L, 81 (28%) had unfavorable karyotype and 82 (28%) had an unfavorable cytogenetic/molecular risk. CD200 was evaluated by multi-parametric flow cytometry (MCF), with high intensity of expression defined by a MFI >11; bcl2 was evaluated by MFC, with a positivity defined by a MFI >17
Results: CD200 was expressed in 172/291 patients (59%) and bcl2 was overexpressed in 136/291 (45%) cases. CD200 positivity and concomitant bcl2 overexpression (double-positive, DP) was found in 93/291 (32%) patients, while 77 (26%) were double negative (DN) and 120 (41%) expressed only CD200 or bcl2. CD200/bcl2 DP cases were more frequent in patients with secondary leukemia (43% *vs* 27%, p=0.008), in CD34+ AML (39% *vs* 24%,p=0.006) and in CD56- patients (38% *vs* 20%, p=0.002). Overall, 274 patients were evaluable for response to induction therapy, and 175 (64%) attained a complete remission (CR). CR rate was lower in CD200+ patients (60% *vs* 75%, p=0.007); co-expression of CD200 and bcl2 (*i.e.* DP cases) did not worsen CR probability, but DN patients have significantly higher CR rate compared to all the other groups (77% *vs* 59% p=0.01). At the time of analysis 60/175 patients have relapsed and 115/175 remained in CR, with a 3-year DFS of 59%. Neither CD200 nor bcl2, alone or in association, influenced DFS. Of 291 patients included in the study, 174 (60%) have died, with a 3-years OS in the whole population of 36%. CD200 and bcl2 DP patients had a 3-year OS of 23%, compared to 35% in patients with isolated CD200 or bcl2 overexpression and 54% in DN ones (p=0.004). In multivariate analysis (MVA) statistical significance was found for age ≥55, CD34 positivity, high WBC count and CD200/bcl2 DP. Combining the four variables resulting from MVA, we designed a score predicting very different OS probability: 3-year OS was 91% in patients without risk factors compared to 51%, 29%, 13% and 0% in those with 1, 2, 3 or 4 risk factors, respectively (p<0.00001) (Figure 1).

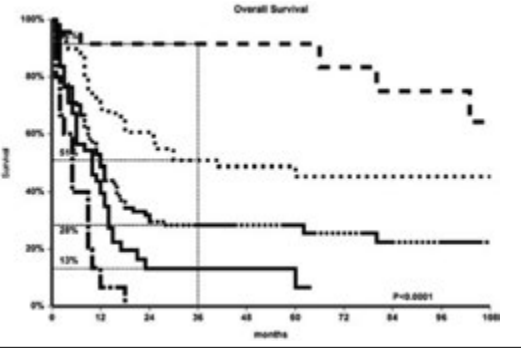


Figure 1.

Summary and Conclusions: CD200 and bcl2 concomitant expression was associated with lower survival probability compared to that of isolated bcl2 or CD200 expression or double negativity, a negative impact maintained also in MVA, along with known prognostic factors such as age, WBC count and CD34 positivity. The combination of these four factors in a risk score based on their presence or absence defined five subgroups with very different survival probabilities. These data may foster the use of bcl2 inhibitors and anti-CD200 antibodies in DP AML patients.

PF234

TP53 MUTATIONS NEGATIVELY IMPACT SURVIVAL OF ACUTE MYELOID LEUKEMIA PATIENTS TREATED WITH STANDARD DOSES OF HYPOMETHYLATING AGENTS

P. Bories[1,2,*], N. Prade[2], S. Lagarde[2], B. Cabarrou[3], I. Luquet[2], V. De Mas[2], J. Plenecassagnes[4], M. Cassou[4], A. Sarry[5], L.-M. Fornecker[6], C. Simand[6], S. Bertoli[5], C. Recher[5], E. Delabesse[2]

[1]Onco-occitanie regional cancer network, [2]Department of Haematology, [3]Biostatistic Unit, Institut Claudius Regaud, [4]Bioinformatics Unit, Institut Claudius Regaud, [5]Department of Hematology, Toulouse University Institute of Cancer-Oncopole, Toulouse, [6]Department of Hematology, Strasbourg University Hospital, Strasbourg, France

Background: Despite little improvement in their overall survival (OS) over the last decade, older patients (pts) with acute myeloid leukemia (AML) still harbor dismal prognosis and validated therapeutic options are limited. Patient selection for these options remains controversial. The AZA-AML-001 and DACO-16 phase III studies failed to demonstrate the superiority of hypomethylating agents (HMA, azacitidine and decitabine, respectively) over intensive chemotherapy (ICT) for pts over 65 years with non-prolif-

erative AML, which increases the uncertainty regarding the optimum strategy for any individual pt. There is currently no validated molecular biomarker which can been used to guide therapeutic decision. *TP53* mutations which are known to negatively impact AML pts outcome when treated with ICT, have been recently described as a positive prognosis factor for blast clearance with a 10-days regimen of decitabine (N England J Med 2016;375:2023-36).
Aims: To evaluate the impact of *TP53* mutational status on outcome of a real-world, well characterized cohort of pts, treated frontline with standard schemes of HMA.
Methods: Characteristics of non-M3 AML patients, consecutively enrolled in the regional cancer network ONCOMIP registry between 2007 and 2016 and treated frontline with HMA were reviewed. Cytogenetic risk was assessed according to the MRC classification and karyotype were all reviewed for the presence of a chr. 17 monosomy (-17) or deletion chr.17p (del17p). For pts with an available bone marrow baseline DNA sample, *TP53* mutations were screened with next-generation sequencing (NGS) on an Illumina® MiSeq sequencer. Sequencing results were filtered with the IARC *TP53* mutations database and a variant allele frequency (VAF) >10%, strengthening the specificity of the data of this cohort. Response to HMA and overall survival (OS) were analyzed for all pts and according to *TP53* mutational status and cytogenetics.
Results: From January 1st 2007 to December 31st 2016, we identified 289 AML pts treated frontline with HMA (azacitidine n=279, decitabine n=6; guadecitabine n=4). Median age was 75 years (IQR: 71-81), karyotype was adverse in 135 pts (47%), including 54 pts with -17 or del17p (23.7%). Seventy-five pts had secondary AML to myelodysplastic syndrome (MDS, 25.9%) and 24 pts to myeloproliferative neoplasm (MPN, 8.3%). Forty-nine pts had therapy-related AML (t-AML, 17%). Pts received a median of 6 cycles (range 1-67). Fifty-five pts obtained CR or CRi (19%) and median OS was 10.3 months (95%CI: 9.3-11.9). Over the 228 pts with a baseline DNA sample, 55 pts (24.1%) had a *TP53* mutation (*TP53*mut). Of those, 53pts had adverse cytogenetics (96.4%), 16 pts had secondary AML to MDS or MPN (29.1%) and 13 pts had t-AML (23.6%). Response rates did not differ between *TP53*mut (21.8% CR/CRi) and *TP53*wt (24.2% CR/CRi, p=0.92), nor between pts with *TP53*mut and/or -17/del17p (20.5%CR/CRi) and pts without *TP53* abnormality (25.6%CR/CRi, p=0.41). Median OS was 7.9 months in pts with *TP53*mut and 12.6 months in TP53wt (p<.0001). With regards to the group of 109 pts with adverse karyotype, response rates did not differ between *TP53*mut pts (20.7% CR/CRi) and *TP53*wt (14.3%, p=0.37) and median OS was 7.9 months for *TP53*mut pts *versus* 9.6 for *TP53*wt pts (p=0.01).
Summary and Conclusions: Overall the response rate was not influenced by the *TP53*mut status, but median OS was negatively impacted by the *TP53*mut status in the entire cohort and in the sub-group of pts with adverse karyotype.

PF235

CHARACTERISTICS AND OUTCOME OF PATIENTS WITH ACUTE MYELOID LEUKEMIA AND T(8;16)(P11;P13): RESULTS FROM AN INTERNATIONAL COLLABORATION

S. Kayser[1,*], R.K. Hills[2], M. Kramer[3], Z. Sustkova[4], E.H. Estey[5], C. Shaw[5], J. Mayer[4], P. Zak[6], T. Szotkowski[7], P. Cetkovsky[8], D. Grimwade[9], U. Platzbecker[3], A. Krämer[1], A.K. Burnett[10], A.D. Ho[1], R.B. Walter[5], C. Thiede[3], G. Ehninger[3], C. Röllig[3], C. Müller-Tidow[1], N.H. Russell[11], R.F. Schlenk[12], M. Levis[13]

[1]University Hospital of Heidelberg, Heidelberg, Germany, [2]Cardiff University School of Medicine, Cardiff, United Kingdom, [3]University Hospital Carl-Gustav-Carus, Dresden, Germany, [4]Masaryk University and University Hospital Brno, Brno, Czech Republic, [5]Fred Hutchinson Cancer Research Center, Seattle, United States, [6]Charles University and University Hospital Hradec Králové, Hradec Kralove, [7]Palacky University Olomouc and University Hospital Olomouc, Olomouc, [8]Institute of Hematology and Blood Transfusion, Prague, Czech Republic, [9]King's College London, London, United Kingdom, [10]Cardiff University School of Medicine, Cardiff, Germany, [11]Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom, [12]National Center for Tumor Diseases, Heidelberg, Germany, [13]Johns Hopkins University, Baltimore, United States

Background: In adult patients (pts) with acute myeloid leukemia (AML), the balanced translocation t(8;16)(p11;p13) is a very rare abnormality resulting in the fusion of *MYST3* and *CREBBP*. Both genes encode proteins with histone acetyltransferase activity and are involved in transcriptional regulation. Previous small series suggested a poor outcome but neither prospective nor larger retrospective cohort studies are available to support these results.
Aims: To characterize AML with t(8;16) and compare outcomes with different treatment strategies.
Methods: We retrospectively studied 48 AML pts with t(8;16) (median age at diagnosis, 52 yrs; range, 16-72 yrs) diagnosed between 1992 and 2016 within five study groups/institutions of the US and Europe. Pts with CBF-AML or APL were excluded.
Results: Median white blood cell and platelet counts at diagnosis were 8.7/nl (range, 1.79-235.9/nl) and 58.5/nl (range, 10-388/nl), respectively. Type of AML was *de novo* in 31 (65%), secondary after MDS/MPN (sAML) in 3 (6%), and therapy-related (tAML) in 14 (29%) of the pts. Thirty-three pts (69%) were female. Cytogenetic analysis revealed additional abnormalities (abn) in 34 (71%) pts, most frequently ≥3 abn (n=23) including further balanced (n=2) or unbalanced translocations (n=13). Breakpoint of t(8;16) was typical in n=33 pts. None of 32 pts tested harbored a *NPM1* mutation and only one of 35 was *FLT3*-ITD positive. Two pts died prior to initiation of therapy due to severe bleeding or rapid progressive disease and one patient died 5 days after initiation of therapy with pazopanib. CR after intensive anthracycline-based induction therapy was achieved in 76% (n=34/45), additionally 4 pts responded to salvage treatment. Five pts were primary refractory and additionally 2 pts experienced early death after therapy initiation, one due to CNS hemorrhage. Seventeen (38%) pts underwent allogeneic hematopoietic cell transplantation (allo-HCT). Of those, 12 pts were transplanted in 1st CR, 2 pts in 2nd CR and 3 with refractory disease, respectively. Type of donor was matched-related in 5, matched-unrelated in 8 and haplo-identical in 4 pts, respectively. Median follow-up for the entire cohort was 55.4 months (95%>CI, 26.2 months-not reached). Median and 4-year overall survival (OS) were 6.7 months (95%>CI, 5.0-9.0 months) and 14.7% (95%>CI, 7.1-30.2%). Median and 4-year relapse-free survival were 5.7 months (95%>CI, 3.9-9.5 months) and 14.4% (95%>CI, 6.4-32.4%). Median and 4-year OS in pts achieving a 1st CR and proceeding to allo-HCT were 15.8 months and 36.4% (95%>CI, 14.5-79.5%) as compared to 6.7 months and 12.5% (95%>CI, 4.4-36.1%) after consolidation with chemotherapy. There was no difference in OS if a typical or atypical breakpoint of t(8;16) was present (p=0.43). Of note, pts proceeding to allo-HCT in 1st CR with a typical breakpoint (n=8) had a favorable OS at 4 years with 57%, whereas none of the pts (n=4) with an atypical breakpoint survived beyond 2 years. Neither additional chromosomal abn nor presence of a complex karyotype (≥3 abn) had a prognostic impact on OS (p=0.44 and p=0.32; respectively). Pts with *de novo* AML had better outcome as compared to those with s-/tAML. (p=0.04).
Summary and Conclusions: Our cohort of AML pts with t(8;16) showed a high CR rate after intensive induction therapy; however, most pts rapidly relapsed and succumbed of their disease. Those pts with a typical breakpoint who proceeded to allo-HCT in 1st CR achieved encouraging OS rates, suggesting that an early transplant in 1st CR should be standard of care when possible for these pts.

PF236

A PHASE 1 DOSE ESCALATION STUDY OF THE IDH1M INHIBITOR, FT-2102, IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) OR MYELODYSPLASTIC SYNDROME (MDS)

M. Baer[1,*], J. Watts[2], S. Lee[3], J. Yang[4], S. Dinner[5], T. Prebet[6], G. Schiller[7], K. Seiter[8], P.B. Ferrell[9], P. Kelly[10], P. Li[10], J. Sweeney[10], C. Watson[10], H. Mohamed[10], J. Cortes[11]

[1]University of Maryland Greenebaum Comprehensive Cancer Center, Baltimore, [2]University of Miami, Miami, [3]Cornell University, New York, [4]Karmanos Cancer Center, Detroit, [5]Northwestern University, Chicago, [6]Yale University, New Haven, [7]Ronald Reagan UCLA Medical Center, Los Angeles, [8]New York Medical College, Valhalla, [9]Vanderbilt University, Nashville, [10]FORMA Therapeutics, Inc., Watertown, [11]MD Anderson Cancer Center, Houston, United States

Background: *Isocitrate dehydrogenase 1* mutations (*IDH1*m) occur in 7-14% of AML patients (pts) and 3% of MDS pts. FT-2102 is a highly potent, selective small molecule inhibitor of *IDH1*m without anticipated CYP or QTc liabilities at the recommended phase 2 dose.
Aims: A Phase 1/2 study to evaluate the safety, pharmacokinetics and pharmacodynamics (PK/PD), and clinical activity of FT-2102 alone or in combination with azacitidine (AZA) in *IDH1*m AML/MDS pts.
Methods: In the phase 1 portion of the study, FT-2102 was dose escalated in a 3+3 design to define the maximum tolerated doses (MTDs) or maximum evaluated doses (MEDs) as a single-agent (SA) and in combination with AZA (CO) followed by expansion cohorts. Doses evaluated were 150 mg QD (SA, CO), 300 mg QD (SA), and 150 mg BID (SA, CO). Safety was assessed by incidence and severity of treatment-emergent adverse events (TEAEs) for all pts and efficacy derived by IWG criteria (2003 AML and 2006 MDS) based on investigator assessment for evaluable pts.
Results: At the data cutoff, 35 pts with a median of 2 prior treatments (range

0-9) had received FT-2102 in dose-escalation, including 22 SA pts and 13 CO pts. Sixteen pts remain on treatment (SA, n=10; CO, n=6) with a median of 2 treatment cycles (range 1-16); 4 pts discontinued for transplant. FT-2102 has been well tolerated both as SA and in combination with AZA. Overall, regardless of causality, most TEAEs were grade (gr) 1/2; most common (>20%) TEAEs were fatigue (34%), nausea (29%), and febrile neutropenia (23%). The most common (>15%) gr 3/4 TEAEs were febrile neutropenia (23%), anemia (20%), and pneumonia (17%). Five (14%) pts had gr 3 differentiation syndrome that was manageable and did not result in discontinuation. Administration of FT-2102, both as SA and in CO at 150 mg BID, enabled all pts to achieve the target steady-state exposure (Css) that exceeded the IC90 of *IDH1*m while staying below the exposures projected from the monkey toxicology studies to possibly be associated with QTc prolongation. PK data were available through Cycle 6; steady-state plasma drug levels were maintained at target Css over the evaluated period. A reduction of 2-HG was observed across all dose levels, with pts receiving 150 mg BID having a median within the normal 2-HG limits. Best response in all evaluable SA pts (n=16) included: 2 (13%) complete remissions (CR), 4 (25%) complete remissions with incomplete hematologic recovery (CRi), and 5 (31%) clinical benefit (CB; stable disease lasting ≥8 weeks), including 2 with >50% reduction of marrow blasts (MB). Best response in evaluable CO pts (n=11) included: 2 (18%) CR, 1 (9%) CRi, and 5 (45%) CB, including 2 with >50% reduction of MB.

Summary and Conclusions: FT-2102 has shown favorable safety, PK/PD, and clinical activity in *IDH1*m AML/MDS pts with a single agent complete response rate (CR/CRi) of 38% and a complete response rate of 27% in combination with AZA. Current data support the continued evaluation of 150 mg BID in the Phase 1 expansion and Phase 2 stages of the study.

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RISK FACTORS IN RELAPSE/REFRACTORY AML TREATED WITH DECITABINE

A. De La Fuente[1,*], A. Muñoz Gama[2], A. Sampol Mayol[3], P. Beneit[4], K. Javier González[5], L. Cañas de Dios[6], J. Dávila Valls[7], A. Roldán Pérez[8], J. Cornago Navascues[9], C. Barrenetxea Lekue[10], I. Oiartzabal[11], S. Suarez Ordoñez[12], S. Vives Polo[13], C. Martinez Chamorro[14], R. Coll Jorda[15], S. Brunet[16], M. Arnan Sangerman[17], A. García de Coca[18], L.M. Gomez García[19], M.A. Foncillas[20], M.J. Otero[21], J.M. Grasa Ullrich[22], B. González González[23], J. González Campos[24], J.A. Vera Goñi[25], P. Lopez[9], A. Diaz Lopez[26], A. Figuera[9], P. Montesinos[27]

[1]Hematology, MD Anderson CC Madrid Spain, Madrid, [2]Hematology, Hospital Virgen de la Salud, Toledo, [3]Hematology, Hospital Son Espases, Palma de Mallorca, [4]Hematology, Hospital San Juan, Alicante, [5]Hematology, Hospital General Universitario de Valencia, Valencia, [6]Hematology, H.U. Príncipe de Asturias, Madrid, [7]Hematology, Complejo Asistencial de Ávila, Ávila, [8]Hematology, H.U. Infanta Sofía, [9]Hematology, H.U. La Princesa, Madrid, [10]Hematology, Organización Sanitaria Integrada Bilbao-Basurto, Vizcaya, [11]Hematology, H.U. Araba Txagorritxu, Vitoria, [12]Hematology, H. Alvaro Cunqueiro, Vigo, [13]Hematology, ICO H. Germans Trias i Pujol, Barcelona, [14]Hematology, H. Quiron Salud, Madrid, [15]Hematology, ICO Girona, Girona, [16]Hematology, Hospital Sant Pau, [17]Hematology, ICO Duran i Reynals, Barcelona, [18]Hematology, H.C.U. de Valladolid, Valladolid, [19]Hematology, H. Nuestra Señora de Prado, Talavera de la Reina Toledo, [20]Hematology, H.U. Infanta Leonor, [21]Hematology, H. Central de la defensa, Madrid, [22]Hematology, Quiron Salud, Zaragoza, [23]Hematology, Hospital Universitario de Canarias, Tenerife, [24]Hematology, H.U. Virgen del Rocío, [25]Hematology, H. Virgen de la Macarena, Sevilla, [26]Hematology, Medicina Traslacional Fundación MD Anderson, Madrid, [27]Hematology, H.U. La Fe, Valencia, Spain

Background: Over 60% of patients with AML require salvage therapy. The following risk factors have been described, age, cytogenetics, length of relapse-free interval and previous SCT *Breems et al JCO2005*. Decitabine (Dec) has been EMA approved for first line AML patients not fit for intensive chemotherapy in base on the results of phase 3 study *Kantarjian et al JCO 2012*.

Aims: The aim of this study is to analyze effectiveness and tolerance of Dec as salvage therapy in AML and to identify risk factors.

Methods: We carried out the analysis on patients with relapse/refractory (R/R) included in the MDA-AML-2017-05 study on 25 Spanish sites. Inclusion criteria as follows Age >18 yrs, diagnosis of AML under WHO criteria, treated with Dec during the period 01/09/2014 to 31/12/2016. We evaluated effectiveness as ELN-2010 criteria, toxicity as CTCAE v3.0 scale, OS by Kaplan-Meier and the mortality within the first 8 weeks (M8wks). This study has been approved by the Spanish Medicines Agency AEMPS code MDA-AML-2017-05.

Results: Of the 228 patients included in the MDA-AML-2017-05 study 61p (36M, 25F) received Dec as salvage therapy. Average age 68.6 (34-86), ECOG≥2: 11p, Creatinine>1.3 mg/dL: 3p, adverse cytogenetics: 13p, WBC pre-Dec >15.000/µL: 13p, previous azacitidine 26p, relapse-free interval <12m: 47p. A total of 292 cycles were administered, median 5 (1-23) per patient, 5 cases of hospitalization due to adverse events. No cases of treatment related mortality. Fifty one patients were studied for effectiveness ORR 39% (CR 10p, PR 14p, ED 10p). With a median follow up of 5mths 53p progressed and 39 died. The M8wks was 22% and the median OS 6.9 months (1yr OS 28%). In this study WBC pre-Dec >15.000/µL (11.1 *vs* 4 ms p 0.02) and previous azacitidine (9 *vs* 4 ms p0.05) resulted in statistical differences for OS, ECOG≥2, adverse cytogenetics and relapse-free interval <12m showed differences but with no statistical significance. Neither Age 65/70/75/80 nor creatinine >1.3 mg/dL revealed differences. No differences were observed comparing Dec as second line or later (8.7 *vs* 5.5 ms p0.37). We observed OS benefit in patients with CR 14.4 mths (p 0.05), as for PR+ED *vs* PD 11.4 *vs* 3.8 mths (p<0.01). The DecLAMRR scale (WBC pre-Dec >15.000/µL, previous azacitidine, ECOG≥2 and adverse cytogenetics) allows us to identify risk groups with 0 *vs* 1-2 *vs* 3-4 risk factors with differences for OS (21 *vs* 6.2 *vs* 3m p0.015) (Figure 1).

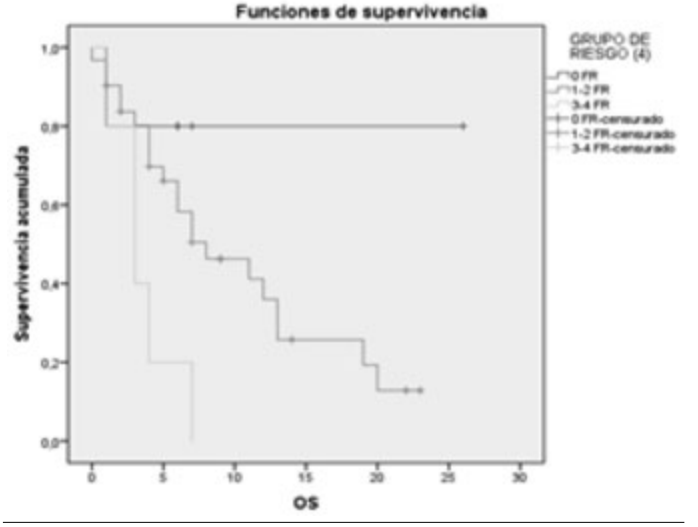

Figure 1.

Summary and Conclusions: The results of this study suggest that Dec is feasible as salvage therapy in AML patients who are not candidates for intensive chemo even in those patients that do not reach a CR. The DecLAMRR scale identifies risk groups with differences in OS

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SAFETY AND PHARMACOKINETICS OF A NOVEL ORAL CAPSULE FORMULATION OF ARSENIC TRIOXIDE, ORH-2014, IN PATIENTS WITH ADVANCED HEMATOLOGIC DISORDERS

F. Ravandi-Kashani[1,*], K. Vaddi[2], A. Johri[3], M. Pudipeddi[4], S. Nidamarthy[5], G. Garcia-Manero[6], G. Borthakur[6], K. Naqvi[6], M. Meyer[6], I. Koumenis[5], H. Kantarjian[6]

[1]Dept of Leukemia, MD Anderson Cancer Center, Houston, [2]Founder Orsenix, Wilmington, [3]Consultant, N Jersey, [4]Consultant, [5]Orsenix, Wilmington, [6]MD Anderson, Houston, United States

Background: The management of Acute Promyelocytic Leukemia (APL) has evolved significantly with the concurrent use of ATRA and ATO for induction, considered as standard of care, as proven by large randomized studies. These studies have demonstrated that non-high-risk APL patients (WBC<$10*10^9$/L) can achieve sustained and prolonged clinical and molecular remission using ATRA and intravenous (IV) Arsenic Trioxide (ATO), without chemotherapy. Similar results using oral arsenic formulations have been reported by Chinese investigators. Given the need for prolonged daily administration of intravenous arsenic trioxide, oral arsenic derivatives represent an important advance in the treatment of APL.

Aims: Orsenix set out to develop a simple, scalable and orally bioavailable capsule formulation manufactured by a unique lyophilization process that results in a drug substance, ORH-2014, that demonstrates high surface area and rapid dissolution.

Methods: A multicenter Phase I study, started in Dec 2016, to identify the

recommended dose of ORH-2014 in patients with relapsed and/or refractory hematologic disorders. ORH-2014 was administered orally, once a day in the fasted state. A total of seven patients were enrolled in two cohorts: 3 patients in Cohort 1 at the 5 mg dose and 4 in cohort 2, at 10mg dose. The primary endpoint was to assess safety and tolerability of ORH-2014 and identify the recommended Phase II dose. Secondary endpoints were: pharmacokinetics (PK), effect on QTc, and efficacy. Dose Limiting Toxicities (DLT) were monitored for Days 1-29 of dosing, and plasma samples were collected for PK between Days 1-22 of dosing. Total arsenic concentrations in plasma were measured by a validated method using inductively coupled plasma mass spectrometry (ICP-MS).

Results: Patients with advanced myelodysplastic syndrome or acute myeloid leukemia were enrolled. The median age of patients was 76 (45-78) years. There were no significant safety issues with no drug related severe adverse events including no significant QT prolongation and no DLTs. Two patients were replaced due to progression of AML and early withdrawal from study (25 days and 22 days). Following oral administration of 10 mg QD ORH-2014, for 15 days, geometric mean (geo CV% [min, max]) C_{max} and AUC_{0-24} values of total arsenic were were 64.5 (43.2% [43.0, 115]) ng/mL and 1340 (43.9% [839, 2330]) h*ng/mL, respectively. The measured plasma exposure at Day 15 (AUC_{0-24}) following 10 mg QD oral dosing was similar to that reported for the approved dose of IV ATO. Total Arsenic plasma concentrations after 5 and 10 mg ORH-2014 QD ranged 21.8-36.9 ng/mL and 30.5-83.4 ng/mL, respectively, and these levels were similar to the Day 8 trough levels of 21.0-35.0 ng/mL following IV administration of 0.15 mg/kg Trisenox.

Summary and Conclusions: The 10 mg QD oral dose of ORH2014 provides similar exposure of total arsenic, measured in plasma, to that of the approved dose of IV ATO (0.15mg/kg). This safe and well tolerated dose of ORH104 is recommended for a future randomized trial for frontline therapy in standard risk APL patients.

PF239

OUTCOMES BY NUMBER OF INDUCTION CYCLES WITH CPX-351 *VERSUS* 7+3 CHEMOTHERAPY IN OLDER ADULTS WITH NEWLY DIAGNOSED, HIGH-RISK/SECONDARY ACUTE MYELOID LEUKEMIA (SAML)

J.E. Cortes[1,*], B.C. Medeiros[2], G.L. Uy[3], L.F. Newell[4], T.L. Lin[5], E.K. Ritchie[6], R.K. Stuart[7], S.A. Strickland[8], D. Hogge[9], S.R. Solomon[10], R.M. Stone[11], D.L. Bixby[12], J.E. Kolitz[13], G.J. Schiller[14], M.J. Wieduwilt[15], D.H. Ryan[16], R.J. Ryan[17], M. Chiarella[17], A.C. Louie[17], J.E. Lancet[18]

[1]The University of Texas MD Anderson Cancer Center, Houston, TX, [2]Stanford University School of Medicine, Stanford, CA, [3]Washington University School of Medicine, St Louis, MO, [4]Oregon Health & Science University, Portland, OR, [5]University of Kansas Medical Center, Kansas City, KS, [6]Weill Cornell Medical College of Cornell University, New York, NY, [7]Hollings Cancer Center, Medical University of South Carolina, Charleston, SC, [8]Vanderbilt-Ingram Cancer Center, Nashville, TN, United States, [9]Leukemia/BMT Program of British Columbia, Vancouver, BC, Canada, [10]The Leukemia Program at Northside Hospital Cancer Center Institute, Atlanta, GA, [11]Dana-Farber Cancer Institute, Boston, MA, [12]Comprehensive Cancer Center, University of Michigan, Grass Lake, MI, [13]Monter Cancer Center, Northwell Health System, Lake Success, NY, [14]David Geffen School of Medicine/UCLA, Los Angeles, CA, [15]University of California – San Diego Moores Cancer Center, La Jolla, CA, [16]University of Rochester, Rochester, NY, [17]Jazz Pharmaceuticals, Palo Alto, CA, [18]H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL, United States

Background: CPX-351, a liposomal co-encapsulation of cytarabine and daunorubicin at a synergistic 5:1 molar ratio, is approved in the United States for the treatment of adults with newly diagnosed therapy-related AML or AML with myelodysplasia-related changes. In a randomized, phase 3 study, CPX-351 significantly improved overall survival and remission rates compared with conventional cytarabine/daunorubicin (7+3) in patients aged 60-75 years with newly diagnosed high-risk or sAML.

Aims: This exploratory analysis of the phase 3 data compared efficacy and safety outcomes in patients treated with CPX-351 *versus* 7+3 who received 1 or 2 induction cycles.

Methods: In the phase 3 study (NCT01696084), patients were randomized 1:1 to receive 1-2 induction cycles with CPX-351 (100 units/m^2 [cytarabine 100 mg/m^2+daunorubicin 44 mg/m^2] on Days 1, 3, and 5 [2nd induction: Days 1 and 3]) or 7+3 (cytarabine 100 mg/m^2/day continuously for 7 days [2nd induction: 5 days]+daunorubicin 60 mg/m^2 on Days 1-3 [2nd induction: Days 1-2]). Patients who achieved complete remission (CR) or CR with incomplete platelet or neutrophil recovery (CRi) could receive up to 2 consolidation cycles. All patients provided informed consent.

Results: In total, 304 patients were treated with CPX-351 (n=153) or 7+3 (n=151). A greater proportion of patients treated with CPX-351 achieved remission after 1 induction cycle than with 7+3 (CR: 47/105 [45%] *vs* 28/100 [28%]; CR+CRi: 58/105 [55%] *vs* 34/100 [34%], respectively; Table 1). Remission rates after 2 induction cycles were comparable between treatment arms (CR: 10/48 [21%] *vs* 12/51 [24%]; CR+CRi: 15/48 [31%] *vs* 18/51 [35%], respectively; Table 1). The frequency of grade 3-5 TEAEs in patients with 1 induction cycle was similar for CPX-351 (75/105 [71%]) and 7+3 (74/100 [74%]; Table 2). Among patients who received 2 induction cycles, fewer patients treated with CPX-351 experienced grade 3-5 TEAEs (38/48 [79%]) compared with those treated with 7+3 (48/51 [94%]). Febrile neutropenia was the most common grade 3-5 TEAE in both treatment arms (Table 2). The incidence of serious TEAEs was similar in the CPX-351 and 7+3 treatment arms (1 induction: 35/105 [33%] *vs* 33/100 [33%]; 2 inductions: 14/48 [29%] *vs* 13/51 [25%], respectively).

Table 1.

	1 induction			2 inductions		
	CPX-351 (n = 105)	7+3 (n = 100)	Odds ratio (95% CI)	CPX-351 (n = 48)	7+3 (n = 51)	Odds ratio (95% CI)
CR, n (%)	47 (45)	28 (28)	2.08 (1.17, 3.73)	10 (21)	12 (24)	0.86 (0.33, 2.21)
CR+CRi, n (%)	58 (55)	34 (34)	2.40 (1.36, 4.21)	15 (31)	18 (35)	0.83 (0.36, 1.93)

Table 2.

	1 induction		2 inductions	
	CPX-351 (n = 105)	7+3 (n = 100)	CPX-351 (n = 48)	7+3 (n = 51)
Any TEAE, n (%)	83 (79)	88 (88)	44 (92)	48 (94)
Grade 3-5 TEAE, n (%)	75 (71)	74 (74)	38 (79)	48 (94)
Febrile neutropenia	61 (58)	57 (57)	29 (60)	41 (80)
Hypoxia	11 (11)	15 (15)	7 (15)	8 (16)
Pneumonia	14 (13)	14 (14)	7 (15)	5 (10)
Hypertension	10 (10)	4 (4)	5 (10)	4 (8)
Bacteremia	7 (7)	1 (1)	5 (10)	1 (2)
Enterococcal bacteremia	3 (3)	0	1 (2)	6 (12)
Serious TEAE, n (%)	35 (33)	33 (33)	14 (29)	13 (25)

Summary and Conclusions: In this population of older adults with newly diagnosed high-risk/sAML, the majority of patients who responded to CPX-351 achieved remission after 1 induction cycle and CPX-351-treated patients were more likely to achieve remission after 1 induction cycle than those treated with conventional 7+3; remission rates were similar between treatment arms among patients who received 2 induction cycles. Patients who received 1 induction cycle of CPX-351 had a similar frequency of grade 3-5 TEAEs compared with 7+3, while those who received 2 induction cycles of CPX-351 experienced fewer grade 3-5 TEAEs compared with 7+3.

PF240

POST HOC EXPLORATORY ANALYSIS OF TWO PHASE 2 TRIALS OF QUIZARTINIB MONOTHERAPY IN PATIENTS WITH FLT3-ITD MUTATED RELAPSED/REFRACTORY AML AND PRIOR FLT3 TYROSINE KINASE INHIBITOR TREATMENT

J. Cortes[1,*], C. Smith[2], K. Ishizuka[3], K. Kobayashi[3], M. Arunachalam[3], Y. Wang[3], D. Lazzaretto[4], M. Levis[5]

[1]MD Anderson Cancer Center, Houston, [2]University of California, San Francisco, San Francisco, [3]Daiichi Sankyo, Inc., Basking Ridge, [4]Daiichi Sankyo, Inc., San Diego, [5]The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, Baltimore, United States

Background: First-generation tyrosine kinase inhibitors (TKIs) such as sorafenib and midostaurin target FMS-like tyrosine kinase 3 (FLT3) in addition to other kinases, and are increasingly used for treatment of *FLT3* mutated acute myeloid leukemia (AML). Quizartinib is a highly potent and selective next-generation FLT3 inhibitor with strong clinical anti-leukemic activity in patients with *FLT3*-internal tandem duplication (ITD) mutated relapsed/refractory (R/R) AML.

Aims: To analyze the clinical activity of quizartinib in patients with prior exposure to first-generation FLT3 TKIs and gain early clinical insights into the potential benefit of agents with varying kinase and safety profiles.

Methods: This post hoc exploratory analysis was performed using data from two phase 2 trials of quizartinib monotherapy in patients with *FLT3* mutated

R/R AML (Studies (A) NCT01565668, (B) NCT00989261) to assess quizartinib activity in patients with *versus* patients without prior therapy with first-generation FLT3 TKIs. Patients with *FLT3*-ITD allelic frequency ≥3% were considered *FLT3*-ITD positive for this analysis. In both studies, patients received quizartinib for 28-day cycles until relapse, intolerance, or proceeding to hematopoietic stem cell transplant (HSCT). In Study A, patients received 90, 135, or 200 mg of quizartinib daily, administered as quizartinib dihydrochloride and equivalent to 79.5, 119.3, or 176.7 mg free-base, respectively. In Study B, patients received 30 or 60 mg of quizartinib daily, administered as quizartinib dihydrochloride and equivalent to 26.5 or 53 mg free-base, respectively.
Results: In Study A, 27 of 261 patients with *FLT3*-ITD mutations received prior sorafenib and/or midostaurin (24 sorafenib, 1 sorafenib and midostaurin, 2 midostaurin). In Study B, 11 of 72 patients with *FLT3*-ITD mutations received prior TKIs (10 sorafenib, 1 sorafenib and midostaurin). Clinical activity of quizartinib, as demonstrated by composite complete remission (CRc, defined as complete response with or without hematologic and platelet recovery), overall response rates (ORR, defined as CRc+partial responses [PR]), and median survival duration, was meaningful and similar in patients with or without prior TKI treatment (Table 1).

Table 1.

CRc, ORR, and survival in quizartinib-treated patients with *FLT3*-ITD mutated R/R AML and prior exposure to first-generation FLT3 TKIs

	Study A NCT01565668		Study B NCT00989261	
	With prior TKI N = 27	Without prior TKI N = 221	With prior TKI N = 11	Without prior TKI N = 61
CRc, % (n)	33 (9)	53 (117)	36 (4)	48 (29)
ORR, % (n)	67 (18)	75 (165)	45 (5)	69 (42)
Median survival, weeks	24.6	24.7	20.9	23.7

Summary and Conclusions: This analysis demonstrates meaningful clinical activity of quizartinib in patients with *FLT3*-ITD mutated R/R AML who have prior exposure to first-generation FLT3 TKIs. Limitations are small sample size and post hoc analysis. Additional studies, including mutational analyses, are warranted to further characterize the potential mechanism(s) of response to quizartinib in patients who have failed prior FLT3 TKI.

PF241

Abstract withdrawn.

PF242

RISK FACTORS OF EARLY DEATH FOR PATIENTS WITH NEWLY DIAGNOSED ACUTE PROMYELOCYTIC LEUKEMIA UNDER THE TREATMENT OF ATRA, ATO WITH OR WITHOUT CHEMOTHERAPY

L. Chen[1,*], H. Zhu[1], F. Dong[1], L. Wang[1], S. Li[2], H. Zhao[1], Q. Chen[1], Y. Chen[1], Z. Shen[1], K. Wang[2], J. Li[1]

[1]Hematology, Shanghai Institute of Hematology, Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, [2]State Key Laboratory of Medical Genomics and Shanghai Institute of Hematology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Background: The combination of all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) has converted acute promyelocytic leukemia (APL) from highly fatal to nearly curable. Yet, early death remains a formidable problem of treatment failure in APL, which strongly affects the prognosis of patients with APL. Mounting evidence from epidemiological studies suggests that the early death rate for APL is underestimated in clinical trials, and most of these studies are focused on patients treated with ATRA plus chemotherapy. Early death for patients treated with ATRA and ATO needs to be addressed.
Aims: We aimed to investigate the risk factors of early death for patients with APL receiving the treatment of ATRA and ATO plus chemotherapy.
Methods: This study retrospectively analyzed 291 patients with newly diagnosed APL from 2001 to 2012. The clinical manifestations, blood test, coagulation index between early death and non-early-death patients were compared.
Results: This study included 149 males and 142 females and the median age was 38 years (13 to 79 years). The early death rate (EDR) was 10.0% (29/291) in all patients: 2.6% (2/77), 8.5% (12/142) and 20.8% (15/72) were in low-, intermediate- and high-risk patients (P<0.001), respectively. Most of the early death occurred within the first week of diagnosis. Hemorrhage, especially intracranial hemorrhage, was the most common reason. Among 29 early death patients, 18 cases suffered from hemorrhage, accounting for 62.1% of early death, in which intracranial hemorrhage was the most common cause (13 cases), and the remaining 5 cases suffered from disseminated intravascular coagulation (DIC) (2 cases), gastrointestinal bleeding (2 cases) and pulmonary hemorrhage (1 case). In addition, 4 cases suffered from severe pulmonary infection, 2 from differentiation syndrome, 2 from cardiac events (1 case of arrhythmia and the other of acute heart failure), 1 from cerebral infarction, 1 from acute renal failure and one patient died of unknown cause (Figure 1). Multivariate analysis showed that the age more than 60, the high white blood cell (WBC), the decreased prothrombin time (PT) and the reduced fibrinogen (Fg) levels were independent risk factors of early death (*P*=0.013, 0.004, 0.013 and 0.033, respectively).

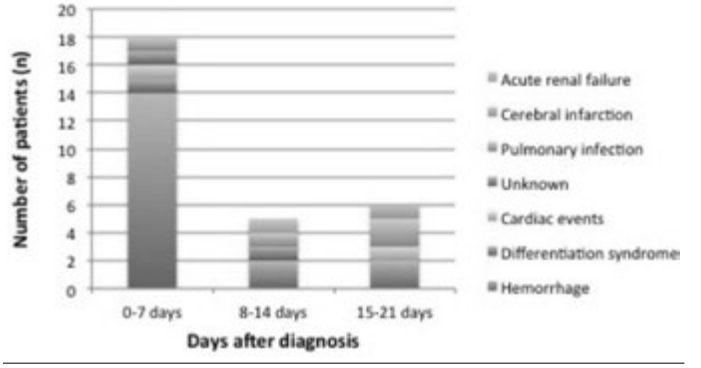


Figure 1. Causes of APL early death and distribution of death time.

Summary and Conclusions: Collectively, this study suggests that early death is still the greatest contributor to treatment failure in APL under the treatment of ATRA and ATO, and highlight a need of timely control of white blood cells and the correction of clotting disorders to reduce the early death rate of APL.

PF243

PHASE II STUDY INCORPORATING A NOVEL BH3-PROFILING BIOMARKER APPROACH OF ALVOCIDIB FOLLOWED BY CYTARABINE AND MITOXANTRONE IN RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA (AML)

J.F. Zeidner[1,*], C.E. Vigil[2], T. Lin[3], M.Y. Levy[4], M. Frattini[5], A. Dalovisio[6], S. Anthony[7], D. Bearss[7], S. Weitman[7], B.D. Smith[8]

[1]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC, [2]University of Iowa, Iowa City, IA, [3]University of Kansas Medical Center, Westwood, KS, [4]Baylor Sammons Cancer Center, Dallas, TX, [5]Columbia University Medical Center, New York, NY, [6]Ocshner Clinic Foundation, New Orleans, LA, [7]Tolero Pharmaceuticals, Inc, Lehi, UT, [8]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins Hospital, Baltimore, MD, United States

Background: Serial studies have shown the activity of alvocidib, followed by cytarabine and mitoxantrone, in newly diagnosed and relapsed/refractory (R/R) AML. Alvocidib's anti-leukemic pharmacologic activity appears to be predominantly due to the inhibition of transcriptional regulator, CDK9, resulting in suppression of CDK9-regulated genes, such as the BCL-2 family member, MCL-1. Retrospective correlative analyses showed that leukemic cells dependent on MCL-1 have heightened sensitivity to alvocidib-containing regimens.
Aims: To evaluate the efficacy and safety of alvocidib, in combination with cytarabine and mitoxantrone, in R/R AML patients (pts) dependent on MCL-1.
Methods: We are conducting a multicenter, international, open-label, phase II clinical study of alvocidib given as timed sequential therapy prior to cytarabine and mitoxantrone for adults with R/R AML. The key eligibility criteria are: ages 18-65 years; refractory to 1-2 cycles of induction therapy, or in first

relapse AML with complete remission (CR) duration ≤2 years; ≥40% myeloblast MCL-1 dependency determined by BH3 profiling; ECOG PS 0-2; and no major organ dysfunction. Pts who received prior allogeneic stem cell transplant (alloSCT) were not excluded. All pts gave appropriate informed consent prior to enrollment. Treatment consisted of alvocidib 30 mg/m^{2}as a 30-minute IV bolus followed by 60 mg/m^2 over 4 hours on Days 1-3, cytarabine 667 mg/m^2/day by continuous IV infusion days 6-8, and mitoxantrone 40 mg/m^2 IV on day 9 starting 12 hours after completing cytarabine. Up to 3 additional cycles of the same regimen (with or without mitoxantrone) were permitted in responders. The primary endpoint is the rate of CR+CR with incomplete recovery (CRi). The null hypothesis that the CR rate=50% will be tested against a one-sided alternative hypothesis=70% CR rate, yielding a type 1 error rate and power=5% and 80%, respectively. Stage I of this study was considered positive if ≥13 CRs were seen in first 23 enrolled pts. Key secondary endpoints are overall survival, event free survival, combined response rate and safety assessed by adverse events and laboratory results.

Results: A total of 17 pts have been enrolled to date (Table 1). The median MCL-1 dependency was 61% (range 41%>98%). The overall CR rate is 59% (n=10). Six out of eight (75%) pts with refractory (no response to induction therapy or CR1 duration <90 days) AML achieved an overall CR and 5 of these pts proceeded to an alloSCT. The most common NCI CTCAE ≥Grade 3 treatment-emergent nonhematologic AEs noted in >1 pt in the safety population were hypophosphatemia (41%); tumor lysis syndrome (35%; 5-Grade 3 and 1-Grade 4); hypokalemia (29%); increased AST and diarrhea (23%); hyponatremia, sepsis and increased ALT (18%); acute kidney injury, syncope and hypoalbuminemia (12%).

Table 1.

Patient Characteristics	N=17
Age (Median; Range)	47 (26-65)
Male/Female	5 (29%)/12 (71%)
ECOG PS (0/1/2)	10 (59%)/6 (35%)/1 (6%)
Response to First-Line Therapy (n=patients)	
Refractory (Persistent Disease or CR <90 days)	8 (47%)
Early Relapse (90 days to 1 year)	5 (29%)
Late Relapse (>1 year but <18 months)	1 (6%)
Baseline WBC Prior to Treatment (Median; Range)	2.8 (1.2-34.5)
2017 ELN Risk Stratification	
Favorable	1 (6%)
Intermediate	14 (82%)
Adverse	2 (12%)
Secondary AML	4 (24%)
Prior alloSCT	3 (18%)
Responses	
CR	9 (53%)
CRi	1 (6%)
CR + CRi	10 (59%)
Morphologic Leukemia Free State (MLFS)	1 (6%)
PR	0
No Response	3 (18%)
Non-Evaluable	3 (18%)
60 Day Mortality	3 (17%)

Summary and Conclusions: Alvocidib given as time sequential therapy prior to cytarabine and mitoxantrone in MCL-1 dependent AML has shown encouraging activity, with the majority of patients treated achieving CR. Future directions include a randomized phase 2 expansion comparing cytarabine and mitoxantrone with or without preceding timed sequential alvocidib, and a phase I study of alvocidib followed by 7+3 induction in newly diagnosed AML with prospective BH3 profiling assessed.

PF244

OUTCOME OF A REAL-LIFE POPULATION OF ACUTE PROMYELOCYTIC LEUKEMIA TREATED ACCORDING TO PETHEMA LPA 2005 PROTOCOL: THE POLISH ADULT LEUKEMIA GROUP (PALG) EXPERIENCE

M. Sobas[1,*], A. Holowiecka[2], P. Montesinos[3], W. Miśkiewicz[1], E. Zarzycka[4], A. Pluta[5], M. Paluszewska[6], M. Podhorecka[7], M. Majcherek[8], B. Piątkowska-Jakubas[9], A. Armatys[2], G. Helbig[2], M. Markiewicz[2], A. Gołos[10], A. Ejduk[11], A. Skręt[12], G. Charliński[13], M. Calbecka[13], E. Guzicka[14], E. Cichocka[15], J. Oleksiuk[15], M. Watek[16], T. Gromek[17], S. Grosicki[18], E. Bodzenta[18], J. Holojda[19], H. Dorota[20], M. A. Sanz[3], S. Giebel[21]

[1]Department of Hematology, Blood Neoplasms and Bone Marrow Transplantation, Medical University, Wroclaw, [2]Medical University of Silesia, Katowice, Poland, [3]Hospital Universitario La Fe, Valencia, Spain, [4]Medical University School, Gdańsk, [5]Medical University, Lodz, [6]Medical University, Warsaw, [7]Medical University, Lublin, [8]University of Medical Science, Poznan, [9]Collegium Medicum Jagiellonian University, Krakow, [10]Institute of Hematology, [11]Institute of Hematology Warsaw, Warsaw, [12]City Hospital, Rzeszow, [13]Nicolaus Copernicus Hospital, Torun, [14]Pomeranian Medical University, Szczecin, [15]Medical University, Bialystok, [16]Holy Cross Cancer Center, Kielce, [17]Medical University, Lublin, [18]City Hospital, Chorzow, [19]City Hospital, Legnica, [20]Hospital Brzozow, Brzozow, [21]Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland

Background: Acute promyelocytic leukemia (APL) is associated with favourable prognosis. According to results of clinical trials based on the use of all-*trans* retinoic acid (ATRA) in combination with chemotherapy the cure rates may exceed 80%. However, prospective studies may not include the whole population of APL patients and therefore real-life results may not necessarily be as favourable.

Aims: To analyze the outcome and prognostic factors of unselected Polish patients with APL intended for the treatment according to PETHEMA LPA 2005 protocol.

Methods: Between 2005 and 2017, 289 Polish patients were diagnosed APL. The diagnosis was confirmed either by t(15;17) or PML/RARA. Clinical and analytical data were collected at diagnosis and during the follow-up. Sanz risk score was calculated. Treatment was based on PETHEMA LPA 2005 which consisted of Induction therapy with oral ATRA (45 mg/m2/d) and intravenous idarubicin (12 mg/m2/d x4 days) followed by three courses of consolidation with anthracyclines. Ara-C was added in consolidation for high-risk patients. The maintenance therapy consisted of intermittent ATRA and low dose chemotherapy with methotrexate and 6-mercaptopurine.

Results: Out of 289 registered patients, 129 (44.6%) were men. The median age at diagnosis was 45 years (range, 14 – 90 years). Sanz risk score was calculated in 279 patients and assessed as low (n=64), intermediate (n=128) or high (n=87). Fourteen (4.8%) patients died before start of the treatment mainly because of coagulopathy (50%). Data concerning response were available for 270 patients: complete remission (CR) was achieved in 217 (80.4%) cases, no response (NR) in 3 (1.1%) while 50 (18.5%) patients died early during induction: 16 (32%) because of coagulopathy, 15 (30%) of differentiation syndrome (DS) and 13 (26%) of infection, there were no data in 6 (12%) patients. Differentiation syndrome was observed in 25% patients. With the median follow-up of 4.1 years the probability of the overall survival at 4 years was 69% (+/-3%). In a univariate analysis, the OS rate was higher in patients with white blood count (WBC) ≤10G/l *vs* WBC >10G/l (76% *vs* 52%; p=0.0001), in patients with no history of bleeding at diagnosis (72 *vs* 65%; p=0.04), with lower Sanz risk score (low *vs* intermediate *vs* high: 80 *vs* 75 *vs* 52%, respectively; p<0.0001), with lower ECOG at diagnosis (level 0 *vs* 1 *vs* 2 *vs* 3 *vs* 4: 97 *vs* 80 *vs* 57 *vs* 32 *vs* 0%, respectively; p<0.0001; Figure 1). The 4-years OS rate was higher in CD2(+) APL *vs* CD2(-) APL (93 *vs* 62%; p=0.02) while was lower in CD56(+) APL *vs* CD56(-) APL (46 *vs* 74%; p=0.04). In a multivariate analysis, ECOG (for each grade) retained the only independent predictive factor with HR=2.3 (95% CI, 1.74-2.61); p<0.000001. The incidence of relapse at 4-years was 14% (+/-3%). The relapse rate was lower for APL with WBC count ≤10 G/l *vs* >10 G/l (8 *vs* 30%; p=0.01), for a lower Sanz risk grups (p=0.04) and lower ECOG grade (p=0.005). In a multivariate analysis, WBC (>10G/L) retained the only independent predictive for the risk of relapse: HR=6.69 (1.59-8.54); p=0.002.

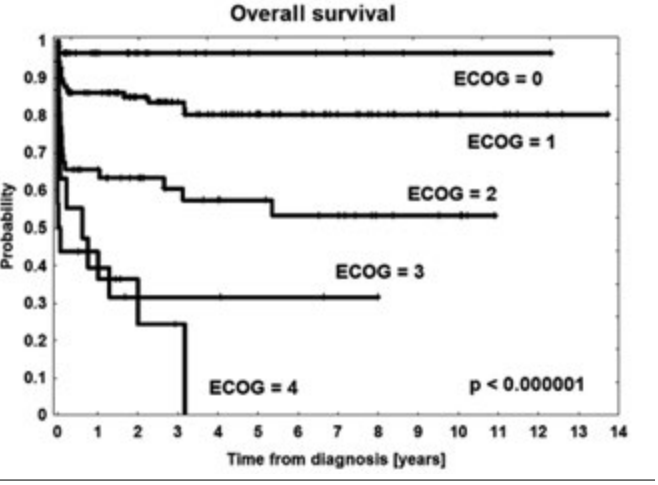


Figure 1.

Summary and Conclusions: In a real–life population there is a high proportion of patients who die before start of the treatment or during induction. Therefore, despite high response rate the OS rate is lower than previously reported in clinical trials. The performance status at diagnosis is the strongest predictor of survival.

PF245

GLASDEGIB IMPROVED OVERALL SURVIVAL IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) OR MYELODYSPLASTIC SYNDROME (MDS) WHO ACHIEVED COMPLETE REMISSION (CR) AND THOSE WHO DID NOT ACHIEVE CR

J. Cortes[1,*], F. Heidel[2,3], W. Fiedler[4], B.D. Smith[5], T. Robak[6], P. Montesinos[7,8], A. Candoni[9], B. Leber[10], M. Sekeres[11], D. Pollyea[12], M. Zeremski[13], W.W. Ma[13], T. O'Brien[13], A. O'Connell[13], G. Chan[13], M. Heuser[14]

[1]MD Anderson Cancer Center, University of Texas, Houston, United States, [2]Otto-von-Guericke University Medical Center Magdeburg, Magdeburg, [3]Internal Medicine II, University Hospital Jena, Jena, [4]Department of Hematology and Oncology, University Hospital Hamburg-Eppendorf, Hamburg, Germany, [5]Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, United States, [6]Department of Hematology, Medical University of Lodz, Lodz, Poland, [7]Hospital Universitari i Politècnic La Fe, Valencia, [8]CIBERONC, Instituto Carlos III, Madrid, Spain, [9]Universitaria Integrata di Udine, Udine, Italy, [10]Juravinski Hospital at Hamilton Health Sciences, Hamilton, Canada, [11]Leukemia Program, Cleveland Clinic, Cleveland, [12]Division of Hematology, University of Colorado School of Medicine, Aurora, [13]Pfizer Oncology, New York, United States, [14]Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany

Background: Glasdegib is a potent and selective oral inhibitor of the Hedgehog signaling pathway. In a phase 2 randomized trial in patients with AML or high-risk MDS, the addition of glasdegib to low-dose cytarabine (LDAC) improved median overall survival (OS) *versus* LDAC alone (8.8 *vs* 4.9 months; hazard ratio [HR] 0.51; 80% confidence interval [CI], 0.39–0.67; *P*=0.0004), with benefit consistent across subgroups by risk and disease.
Aims: We evaluated the efficacy of glasdegib+LDAC *versus* LDAC alone by disease response (CR *vs* no CR) and baseline mutational analyses.
Methods: Patients randomized to receive glasdegib+LDAC (n=88) or LDAC alone (n=44) were classified into 2 subgroups: those who achieved CR (defined as neutrophils ≥1000 µL, platelets ≥100000 µL, hemoglobin ≥11 g/dL [MDS only], with <5% bone marrow blasts), and those that did not achieve CR. Biomarker assessments included mutational status of the following genes: CEBPA, DNMT3A, FLT3, IDH1, IDH2, KIT, KRAS, NPM1, NRAS, RUNX1, TET2, and WT1. OS in CR-defined subgroups was summarized using the Kaplan–Meier (K-M) method; OS in biomarker-defined subgroups was summarized by K-M method and Cox proportional hazards model.
Results: 15 out of 88 patients treated with glasdegib+LDAC and 1 out of 44 patients treated with LDAC alone achieved CR at any point during the study. Demographics of the two CR-defined subgroups were balanced with respect to age, comorbidities, and European LeukemiaNet cytogenetic risk group. Median duration of treatment was 16.5 months (range, 0.9–31.9) in patients who achieved CR with glasdegib+LDAC, and 7.3 months in the one patient who achieved CR with LDAC alone. In patients without CR, median duration of treatment was 2.0 months (range, 0.1–27.8) with glasdegib+LDAC, and 1.5 months (range, 0.2–7.9) with LDAC alone. In patients who achieved CR with glasdegib+LDAC, median duration of response was 9.9 months (range, 0.03–28.8). In patients who achieved CR, median OS was 26.8 months (95% CI, 12.3–not reached) with glasdegib+LDAC *versus* 12.9 months for the one patient who achieved CR with LDAC alone; HR and *P* value were not estimated due to small sample size. In patients without CR, median OS was 6.5 months (95% CI, 3.7–9.1) with glasdegib+LDAC *versus* 4.8 months (95% CI, 2.3–6.4) with LDAC alone (HR 0.65; 95%CI 0.43–0.98; *P*=0.018). Responses were observed across all mutations assessed, and of the 4 genes with a mutation frequency of ≥5 mutations in each arm (DNMT3A, IDH2, RUNX1, and TET2), the HR of median OS for glasdegib+LDAC *versus* LDAC alone was similar in mutated and non-mutated subgroups.
Summary and Conclusions: The addition of glasdegib to LDAC *versus* LDAC alone improved OS in the entire population, and even in those patients not achieving CR. Baseline mutations did not affect response or survival benefit, although data for each mutation are limited by the small sample size. Together these data suggests that glasdegib improves OS by preventing or delaying disease relapse or progression regardless of baseline mutation status. Randomized studies with glasdegib in combination with standard therapies are underway.

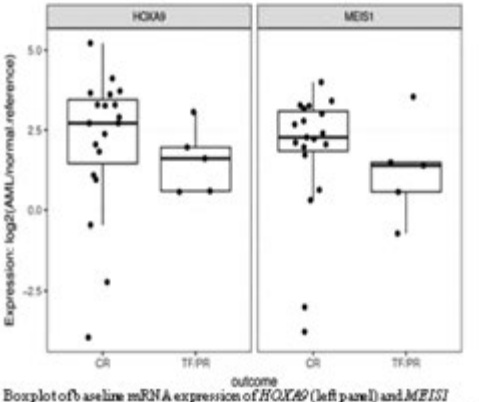
PF246

HIGH COMPLETE REMISSION RATES WITH ORAL SYK INHIBITOR ENTOSPLETINIB (GS-9973) IN ACUTE MYELOID LEUKEMIA PATIENTS ASSOCIATED WITH HIGH HOXA9/MEIS1 EXPRESSION

J. Liu[1], V. Munugalavadla[1,*], T. Oellerich[2345], H. Serve[23], J.C. Byrd[6], A.R. Walker [6], M. Peach[1], S.D. Patterson[1], A. . Rao[1], Y. Pan[1]

[1]Gilead Sciences, Inc., Foster City, United States, [2]Department of Medicine II, Hematology/Oncology, Goethe University, Frankfurt am Main, [3]German Cancer Research Center and German Cancer Consortium, Heidelberg, Germany, [4]Department of Haematology, University of Cambridge, [5]Cambridge Institute for Medical Research, Wellcome Trust/MRC Stem Cell Institute, Cambridge, United Kingdom, [6]The Ohio State University, Columbus, United States

Background: Acute myeloid leukemia (AML) is an aggressive hematopoietic malignancy characterized by diverse genetic and molecular abnormalities which contribute to poor response and survival rates. Spleen tyrosine kinase (SYK) signaling pathway induces cell survival and proliferation in AML by activating multiple signaling pathways including STATs and MYC. Entospletinib (ENTO), an oral selective SYK inhibitor, is currently in clinical trials in AML. Recently, a novel signaling pathway was identified linking *HOXA9* and *MEIS1* (*H/M*) overexpression to addiction to SYK pathway in AML. This analysis aims at exploring the relationship between clinical response and baseline *H/M* levels in the myeloid blasts of newly diagnosed AML patients treated with ENTO plus induction chemotherapy.
Aims: (1) Retrospective analysis of baseline *H/M* gene expression levels from subjects enrolled in NCT02343939 and their correlation with clinical response (2) Correlate *H/M* expression with baseline common cytogenetic and molecular AML subgroups.
Methods: Baseline bone marrow mono-nuclear samples from 34 AML patients treated with ENTO monotherapy for up to 14 days and subsequently in combination with induction chemotherapy (7+3*;* cytarabine 100 mg/m^2 for 7 days plus daunorubicin 60 mg/m^2 for 3 days) were analyzed for mRNA expression of *H/M* using a custom NanoString assay. *H/M* expression level was evaluated in the context of clinical outcome, event free survival (EFS), and overall survival (OS). Key molecular mutations including *NPM1*, *FLT3-ITD/TKD* and *KMT2A/*mixed lineage leukemia *[MLL]* gene rearrangements were utilized for potential associations with the expression of *H/M.*
Results: Median *H/M* expression in patients with CR (n=24) was approximately two-fold higher than in patients with treatment failure (TF, n=4) or partial response (n=1) (Figure 1). Significantly (p<0.05) higher *H/M* expression was observed in AML patients with *KMT2A/MLL* gene rearrangements (n=6), and *NPM1* mutations (n=10, 3 with concomitant *FLT3-ITD*). Among patients with high *H/M* expression (*i.e.* ≥3-fold higher than a pooled (n=20) healthy donor sample), 84% (16/19) achieved a CR with ENTO plus induction chemotherapy, compared with 53% (8/15) of the patients with lower *H/M* expression. Furthermore, we explored the performance of key molecular, cytogenetic factors along with baseline *H/M* expression levels and disease burden to distinguish CR from TF/PR. Among the best predictors for CR were *H/M* expression (PPV=0.94, NPV=0.33) and alterations in either *NPM1* or *MLL* (PPV=0.96, NPV=0.32). Analysis of the preliminary survival data showed trends of better EFS and OS in patients with high *H/M* expression. Data from non-evaluable (n=2) and early treatment assessed (n=3) subjects and an updated analysis of EFS/OS will be presented.



Boxplot of baseline mRNA expression of *HOXA9* (left panel) and *MEIS1* (right panel) in AML patients from clinical trial NCT02343939. Plotted values on Y- axis are normalized to expression from pooled healthy BM-MNCs (0 indicates expression values in healthy BM-MNCs). CR: complete remission (n=24), PR: partial remission(n=1), TF: treatment failure (n=4)

Figure 1.

Summary and Conclusions: Response rates to ENTO are enriched in biomarker defined sub-groups, *i.e.* patients with high *H/M* expression including *MLL* rearrangement, and *NPM1* mutation. Increased response rates in the high baseline *H/M* expression population are consistent with pre-clinical findings suggesting that AML patients addictive to SYK signaling may be more sensitive to ENTO treatment. The predictive utility of *H/M* expression should be tested further in a large study.
First two authors contributed equally.

PF247

WEEKEND *VERSUS* WEEKDAY DIAGNOSIS AND EARLY MORTALITY IN ACUTE PROMYELOCYTIC LEUKEMIA: A POPULATION-BASED ANALYSIS IN THE NETHERLANDS

A. Dinmohamed[1,2,3,*], R. Damhuis[1], O. Visser[4], J. Janssen[5], G. Huls[6], A. van de Losodrecht[5]

[1]Research, Netherlands Comprehensive Cancer Organisation (IKNL), Utrecht, [2]Public Health, Erasmus University Medical Center, [3]Hematology, Erasmus MC Cancer Institute, Rotterdam, [4]Registration, Netherlands Comprehensive Cancer Organisation (IKNL), Utrecht, [5]Hematology, VU University Medical Center, Amsterdam, [6]Hematology, University Medical Center Groningen, Groningen, Netherlands

Background: Acute promyelocytic leukemia (APL) represents a serious medical emergency associated with complications that may lead to early death (ED) if not promptly recognized and subsequently treated. Despite the widespread availability of all-*trans* retinoic acid since the late 1980s, data from the few available population-based studies demonstrated that ED rates in APL remain high. Prior studies in a wide range of diseases have demonstrated that hospital admissions on the weekend, as compared with weekdays, are associated with ED (also known as "the weekend effect"). However, the weekend effect has never been explored in APL at the population level.
Aims: In this nationwide, population-based study, we set out to assess the weekend effect in APL during a 27-year period in the Netherlands.
Methods: We selected all adult (≥18 years) APL patients diagnosed between 1989-2015 from the nationwide Netherlands Cancer Registry (NCR) with survival follow-up through February 1, 2017. The date of diagnosis was defined as the date of diagnostic bone marrow sampling. Patients were categorized into two age groups (18-60 and >60 years). The primary endpoint was the risk of death within 30 days after diagnosis (ie, ED). We used multivariable Cox regression to assess the risk of ED associated with weekend *versus* weekday diagnosis, adjusted for age, sex, year and hospital type of diagnosis, and receipt of anti-leukemic therapy. A $P<0.05$ indicated statistical significance.
Results: Our analytical cohort included 675 APL patients (median age 53 years; 36.6% age >60; 47.8% males), of whom 46 (6.8%) were diagnosed on weekends. No significant differences were noted in the aforementioned characteristics between patients diagnosed on weekends and those diagnosed on weekdays for the overall cohort and the two age groups (*P* for Fisher's exact test >0.05 for all comparisons). The rate (18.8% *v* 28.3%; *P* for Fisher's exact test=0.123) and risk of ED (hazard ratio [HR], 2.59; 95% confidence interval [CI], 1.43-4.66; *P*=0.002) was higher for patients diagnosed on weekends (Figure 1A). Subgroup analysis by age group showed for patients aged 18-60 diagnosed on weekends that there were no significant differences in the rate (12.1 *v* 15.6; *P*=0.575) and risk of ED (HR, 1.99; 95% CI: 0.76-5.22; *P*=0.163; Figure 1B). However, for patients aged >60, the rate (30.0% *v* 57.1%; *P* for Fisher's exact test=0.042) and risk of ED (HR, 3.42; 95% CI, 1.59-7.37; *P*=0.002) were significantly higher for those diagnosed on weekends (Figure 1C).

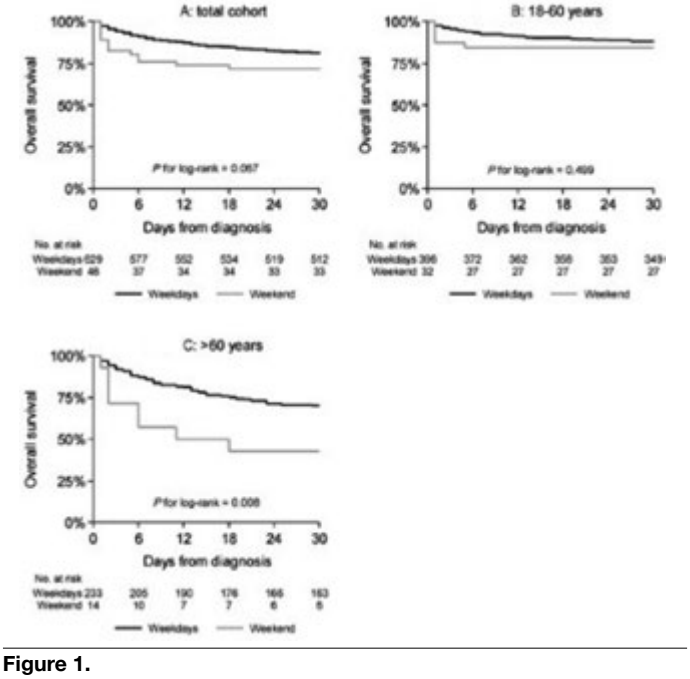


Figure 1.

Summary and Conclusions: In this nationwide, population-based study, we demonstrated that higher ED was confined to elderly (aged >60) APL patients diagnosed on weekends. Delayed institution of therapy among elderly patients diagnosed on weekends might explain the weekend effect. However, assessment of time from diagnosis to therapy is only possible in the NCR for patients diagnosed from 2014 onwards. Although our analysis is somewhat limited by relatively small patients numbers, it generally remains of vital importance that medical providers should act promptly when APL is suspected—especially among elderly patients admitted on the weekends—which in turn may prevent ED. Future research should center on validating our population-based findings and effective education strategies to increase the level of vigilance among a broad range of medical providers regarding this diagnosis in a contemporary era with well-established APL management.

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DECITABINE IN PATIENTS WITH NEWLY DIAGNOSED AND RELAPSED ACUTE MYELOID LEUKEMIA (AML): THE REAL LIFE EXPERIENCE FROM THE "ITALIAN TRIVENETO REGISTRY"

C. Fili'[1,*], A. Candoni[1], S. Imbergamo[2], G. Festini[3], G. Nadali[4], E. Di Bona[5], A. Ermacora[6], M. Gottardi[7], M. Michieli[8], M. Caizzi[3], D. Facchinelli[4], R. Ciancia[8], D. Lazzarotto[1], M.E. Zannier[1], M.V. Dubbini[1], F. Gherlinzoni[7], G. Semenzato[2], R. Fanin[1]

[1]SOC Clinica Ematologica ASUIUD Udine, Udine, [2]Ematologia e Immunologia Clinica Azienda Ospedaliera, Padova, [3]SC Ematologia Clinica ASUITS, Trieste, [4]UOC Ematologia Policlinico GB Rossi, Verona, [5]Unità Operativa Ematologia, Ospedale S.Bortolo, Vicenza, [6]SC Medicina Interna Azienda Ospedaliera S.Maria Angeli, Pordenone, [7]Struttura Complessa Ematologia Ospedale Cà Foncello, Treviso, [8]SOSDTerapia Cellulare e Chemioterapia alte dosi (CRO), Aviano, Italy

Background: The hypomethylating agent Decitabine (DAC) has emerged as an alternative to first line and salvage therapy in acute myeloid leukemia (AML), particularly in elderly patients (pts) which may not benefit from intensive chemotherapy (CHT).The use of hypomethilating agents for the treatment of AML is currently evolving however no many data are available regarding efficacy and safety of DAC in daily clinical practice.
Aims: We retrospectively reviewed data of 104 AML pts treated with DAC in 8 Italian Hematological Centers (Udine, Padua, Trieste, Verona, Vicenza, Pordenone, Treviso, Aviano) from February 2015 to August 2017.The objective of this study was to review the treatment outcomes of newly diagnosed older pts with AML and pts with relapsed AML aged ≥18 years treated with DAC outside of clinical trial.
Methods: Seventy-five (75%) pts received DAC as first line treatment (Cohort 1) and 29 pts as salvage therapy (Cohort 2).All pts received the same DAC schedule, 20 mg/mq intravenously over 1 h for repeated 5-day cycles approximately every 28 days.In the total population the median age was 72,5 years (74 in cohort 1 and 66 in cohort 2) and 16,3% of pts had a ECOG scale >2 at the moment of DAC treatment (with non significant difference in the two cohorts).The cumulative illness rating scale (CIRS) was >6 in the 27% of pts.Forty-five pts (43,2%) had secondary AML. Bone marrow blast count was >30% in 67 patients (64,4%).In the relapsed cohort 17/29 (59%) patients were treated with DAC after conventional chemotherapy, 5/29 (17%) after allogeneic stem cell transplantation and 7/29 (24%) after azacitidine therapy.
Results: A total of 469 DAC cycles were given to the 104 pts with a median of 3 cycles (range 1-21) in cohort 1 and 2 cycles (range 1-7) in cohort 2; 45/104 (43,2%) pts received ≥4 cycles.The Overall Response Rate (ORR=CR+PR) was 32,9%, significantly higher in Cohort 1 (41,5%) compared to Cohort 2 (13,8%) (p=0.009).CR was achieved in 30,8% pts of newly diagnosed AML whereas in the salvage AML group no CR was obtained.The median duration of response was 6 months (range 1-20).In Cohort 1 time to best response (70% of CR and 74% ORR) was obtained between 3 and 6 cycles.In multivariate Cox regression analysis, CR or PR (HR=0.78; p=0,0004), CIRS <6 (HR=0,9; p=0,04) and complex karyotype (HR=0,8; p=0,03) represent significant predictors of improved overall survival.After a median follow up of 4,5 months (range 0,3-22) median overall survival (OS) from the start of DAC therapy was 11 months for the whole population with a significant OS advantage in Cohort 1 (median OS 12,7 mths *vs* 6,3 mths-p=0,003).Not surprisingly median OS was significantly longer among responders, 22,6 mths *vs* 5,7 mths of non- responders (p<0,0001).At the moment of this analysis, 56 patients (53,8%) are still alive and 48 (46,2%) are dead.The main cause of death was disease progression (70,8%).The most common toxicities

were myelosuppression and documented infectious complications, mainly in the first 4 cycles.
Summary and Conclusions: These data show the efficacy and the emerging role of DAC in the real life management of AML, with a significant better performance in first line therapy, even in elderly and unfit pts unsuitable for intensive CHT (ORR 41,5%, median OS 12,7 mths).The efficacy of DAC, as a single agent for salvage therapy, may probably be improved with combined treatment strategies and/or with different DAC schedules that increase its anti-leukemic effect.

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PROGNOSTIC IMPACT OF SKELETAL MUSCLE ASSESSED BY COMPUTED TOMOGRAPHY SCAN IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

N. Nakamura[1,*], S. Ninomiya[1], H. Nakamura[1], J. Kitagawa[1], T. Hara[1], K. Saito[2], M. Shimizu[1], H. Tsurumi[13]

[1]Department of Hematology, Gifu University Graduate School of Medicine, [2]Department of Internal Medicine, Gihoku Kosei Hospital, Gifu, [3]Department of Hematology, Matsunami General Hospital, Hashima, Japan

Background: Skeletal muscle atrophy, referred to as a sarcopenia, is often observed in cancer patients, especially elderly patients. Sarcopenia has been demonstrated to be associated with the risk of morbidity and mortality in some kinds of cancer patients, including hematological malignancies. We previously reported that sarcopenia was an independent prognostic factor in patients with diffuse large B-cell lymphoma. Fat tissue is also one of the important components which is an energy store reserve. However, the clinical values of sarcopenia and adipopenia in adult AML have not been studied and remain unclear.
Aims: The aim of this study was to reveal the impact of sarcopenia and adipopenia on clinical outcomes in patients with AML.
Methods: We retrospectively analyzed 90 patients (age ≥18 years) with *de novo* AML who received chemotherapy in Gifu University Hospital between December 2004 and October 2016. Sixty-five patients underwent standard intensive chemotherapy consisting of anthracycline and cytarabine (Ara-C). Twenty-eight patients received HSCT. The patients who received only palliative care were excluded. Skeletal muscles and visceral and subcutaneous fat areas were assessed from a single axial slice at the third lumbar (L3) level by using CT scans performed prior to any treatment.. This value was normalized for stature to calculate the lumbar L3 skeletal muscle index (SMI, in cm^2/m^2), the lumbar L3 adipose tissue index (ATI, in cm^2/m^2).
Results: The median SMI was 42.5 cm^2/m^2 (26.8-61.8 cm^2/m^2) and the median ATI was 73.9 cm^2/m^2 (2.1-188.9 cm^2/m^2). Thirty-nine (43%) patients were defined as sarcopenia and 35(39%) patients were adipopenia. 39% of patients with PS ≤1were sarcopenia, whereas 67% of patients with PS ≥2 were sarcopenia (P <0.05). Of the 90 patients, 62 (78%) achieved CR within 2 cycles of induction chemotherapy. The CRs rate in the patients with adverse chromosome, less intensive chemotherapy and BMI under 25, were significantly low. Patients with sarcopenia or adipopenia tended to have a lower CR rate, but were not significant. OS, EFS and DFS at 3 years in all patients were 53%, 35% and 52%, respectively. OS at 3 years was 35% and 67% for sarcopenia and non-sarcopenia groups, respectively (P <0.001), and DFS at 3 years was 25% and 58% (P <0.0001). OS at 3 years was 33% and 67% for adipopenia and non-adipopenia groups, respectively (P <0.005), and DFS at 3 years was 31% and 64% (P <0.05). Multivariate analysis showed an association between sarcopenia and lower OS (hazard ratio 2.27, 95% confidence interval 1.11-4.79, P <0.05), with other prognostic factors being worse PS (>2) (P <0.05) and adverse cytogenetic risk (P <0.05). Next, we investigated the impact of sarcopenia in the elderly patients over age 60, who need the careful assessment to decide their treatment strategies. Nine (20%) patients were then defined as sarcopenia. 12% of patients with PS ≤1 were sarcopenia, whereas 50% in PS ≥2 were sarcopenia (P <0.05). OS at 3 years was 0% and 49% for sarcopenia and non-sarcopenia groups, respectively (P <0.0005), DFS at 3 years was 0% and 61% (P <0.0001), and EFS at 3 years was 0% and 46% (P<0.005).
Summary and Conclusions: Our data illustrates a marked clinical impact of sarcopenia and adipopenia in the patients with AML. Evaluation of skeletal muscle and adipose tissue depletion by CT imaging is a useful objective tool for predicting AML patient outcomes. Moreover in the elderly patients, presence of sarcopenia before treatment may be useful factor for avoiding intensive therapeutic strategies for AML.

PF250

INCIDENCE OF MARROW NEOPLASMS IN BREAST CANCER SURVIVORS: A NATIONAL POPULATION-BASED COHORT STUDY

M.J. Jabagi[1,*], N. Vey[2], A. Goncalves [2], M. Zureik[1], R. Dray-Spira[1]

[1]ANSM, Agence Nationale de la Securité du Medicament ANSM, Paris, [2]IPC, Institut Paoli Calmettes, Marseille, France

Background: Patients with a history of cancer are at a higher risk of certain types of Marrow neoplasms (MN) including Acute Myeloid Leukemia (AML) and Myelodysplastic Syndrome (MDS). However, information on the frequency of these MN after cancer in real life are scarce, especially in the recent context of increased cancer survival. In addition, little is known about the incidence of other MN types after cancer.
Aims: The aim of this study was to estimate the incidence of the various types of marrow neoplasms in breast cancer (BC) survivors in France.
Methods: Data from the French National Health Data System (SNDS) were used. All female patients covered by the general health insurance scheme (*i.e.* about 75% of the French population), aged 20 to 85 years and with an incident primary breast cancer between 2006 and 2015 were included and followed until MN occurrence, death or December 31, 2016. The main outcomes were cases of MN occurring at least 6 months after breast cancer diagnosis and identified in the database using ICD-10 codes of hospital discharge and/or long-term disease diagnoses. Incidence rates of the various types of MN in the breast cancer cohort were estimated and compared to those in the general population using direct age-standardization.
Results: 439 704 female patients were included (mean age, 59.3 years) and followed for a mean of 5.2 years. Overall, 2 991 cases of MN occurred: 509 Acute Myeloid Leukemia (AML), 832 Myelodysplastic syndrome (MDS), 365 Multiple Myeloma (MM), 912 Hodgkin and Non Hodgkin Lymphoma (HL/NHL), 106 lymphocytic Leukemia/Lymphoma (LLL), and 267 Myeloproliferatif Neoplasm (MPN). Mean time between BC diagnosis and MN occurrence was 3.9 years overall, ranging from 3.5 years for AML to 4.2 years for MPN. Crude incidence rates per 100 000 person-years of the different types of MN were: 22.2 (95% CI 20.3-24.2) for AML; 36.3 (95% CI, 33.9-38.9) for MDS; 15.9 (95% CI, 14.3-17.6) for MM; 39.9 (95% CI, 37.3-42.5) HL/NHL; 4.6 (95% CI, 3.7-5.6) for LLL; 11.7 (95% CI, 10.3-13.1) for MPN. Compared to the general population, these incidence rates were higher for AML (Standardized Incidence Rate Ratio, SIRR 2.5; 95% CI, 2.2-2.9), MDS (SIRR 4.5; 95% CI, 3.9-5.1), MM (SIRR 1.1; 95% CI, 1.0-1.3) and LLL (SIRR 1.8; 95% CI, 1.2-2.7), while they did not differ for the other types of MN.
Summary and Conclusions: Incidences of AML and MDS and, to a lower extent, MM and LLL are higher among breast cancer survivors than in the general population. Further investigations are necessary to assess the role of cancer therapies in explaining these increases.

PF251

WILMS TUMOR 1 EXPRESSION IN ACUTE MYELOID LEUKEMIA CORRELATES WITH CYTOGENETICS OR COLLABORATIVE MUTATIONS: HOKKAIDO LEUKEMIA NET STUDY

D. Hidaka[1,*], O. Masahiro[1], J. Hashiguchi[1], N. Miyashita[1], K. Kasahara[1], S. Fujisawa[2], E. Hayase[12], K. Okada[1], S. Shiratori[1], H. Goto[1], J. Sugita[1], M. Nakagawa[1], K. Kahata[1], D. Hashimoto[1], T. Endo[1], S. Yamamoto[3], Y. Tsutsumi[4], Y. Haseyama[5], T. Nagashima[6], A. Mori[7], S. Ota[8], K. Imai[8], T. Miyagishima[9], Y. Kakinoki[10], M. Kurosawa[11], H. Iwasaki[12], C. Shimizu[2], T. Kondo[1], T. Teshima[1]

[1]Hematology, Hokkaido University, [2]Laboratory and Transfusion Medicine, Hokkaido University Hospital, [3]Sapporo City General Hospital, Sapporo, [4]Hakodate Municipal Hospital, Hakodate, [5]Tonan Hospital, Sapporo, [6]Japanese Red Cross Kitami Hospital, Kitami, [7]Aiiku Hospital, [8]Sapporo Hokuyu Hospital, Sapporo, [9]Kushiro Rosai Hospital, Kushiro, [10]Asahikawa City Hospital, Asahikawa, [11]Hokkaido Cancer Center, [12]Sapporo Kosei General Hospital, Sapporo, Japan

Background: The prognostic impact of WT1 expression at diagnosis of acute myeloid leukemia (AML) has been controversial. Some studies showed a correlation of higher expression of WT1 with poor prognosis, whereas other studies showed lower expression of WT1 was related to poor prognosis. The relationship between WT1 expression and each cytogenetic category has not been fully determined. Further, prognostic impact of WT1 expression has not been elucidated in each cytogenetic category.
Aims: To determine the relationship between WT1 expression at diagnosis of AML and established prognostic cytogenetics and molecular abnormalities.

Methods: Diagnostic bone marrow samples of AML patients were collected from all over Hokkaido district and studied for relevant molecular markers. Total 252 patients (17 – 89 years old; median 57) who were diagnosed with AML from 2007 to 2017 were enrolled in this study. WT1 expression and Flt3-ITD mutation were analyzed for all patients. The WT1 transcript levels in samples were determined by reference to the corresponding transcript levels of K562 cells (/ 10^4 x K562 RNA). NPM1 mutation and CEBPA double mutation were analyzed for cytogenetically normal (CN)-AML. KIT mutation was analyzed for core-binding factor (CBF)-AML.
Results: Expression of WT1 at diagnosis of AML varied from <1 to 25118.9 (median, 1156.15). WT1 levels were not associated with overall survival (OS) in our entire AML cohort (p=0.393). The patients were divided into six groups depending on cytogenetic abnormalities, including t(8;21) (N=34), inv(16) (N=23), t(15;17) (N=27), cytogenetically normal (CN) (N=93), poor cytogenetics (N=34), and others (N=41). WT1 expression was correlated with certain cytogenetics or molecular mutations that were already known prognostic factors. Within the cytogenetically favorable prognosis group, WT1 expression in AML with inv(16) or t(15;17) was significantly higher than that in AML with t(8;21) (median WT1 1778.3, 3388.4 *vs* 468.95, respectively, p=0.001, p<0.001). Patients with KIT mutation in the t(8;21) group tended to show lower WT1 expression (median WT1 117.5 *vs* 575.4, p=0.073) and poor prognosis (p=0.409), but the tendency was not statistically significant. In cases with CN-AML, Flt3-ITD and NPM1 mutations were both correlated with higher expression of WT1 (respectively, median WT1 3706.8 *vs* 600.6, 3351.65 *vs* 653.1, p<0.001, p<0.001), whereas CEBPA double mutation was related to lower WT1 expression (WT1 median 524.8 *vs* 1520.0, p=0.023). The existence of both Flt3-ITD and NPM1 mutations showed synergistically higher expression of WT1 in CN-AML (WT-1 median 4466.8 *vs* 436.5, p<0.001). After divided into cytogenetic category, the expression levels of WT1 still did not show a significant prognostic impact in each cytogenetic category (Figure 1).

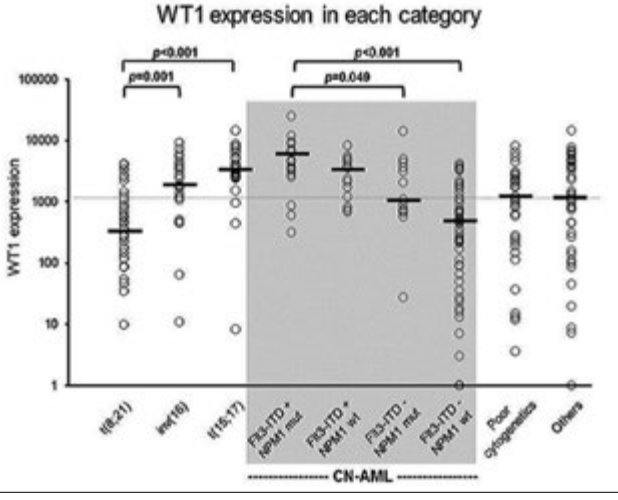

Figure 1.

Summary and Conclusions: Some favorable prognostic markers (inv(16), t(15;17), NPM1 mutation) and poor prognostic marker (Flt3-ITD mutation) were independently associated with higher WT1 expression. In each cytogenetic category, WT1 expression did not show clear prognostic significance in our series. So the prognostic significance of WT1 at diagnosis of AML was weak compared to other established prognostic factors. Inconsistency of previous reports describing both favorable and poor prognostic impact of higher WT1 expression at AML diagnosis could be due to the correlation of WT1 expression with these cytogenetic or molecular abnormalities. Distribution of the patients' cytogenetics could affect interpretation of WT1 expression as a prognostic marker.

PF252

OUTCOMES FOR T(9;11) PATIENTS ARE NO BETTER THAN FOR OTHER REARRANGEMENT PARTNERS IN 11Q23-REARRANGED ADULT AML: A COMPREHENSIVE REVIEW OF MLL-R AML TREATED AT MEMORIAL SLOAN KETTERING CANCER CENTER

K. Menghrajani[1,*], Y. Zhang[2], C. Famulare[3], S. Devlin[4], M. Tallman[1,5]

[1]Leukemia, [2]Pathology, [3]Sloan Kettering Institute, [4]Epidemiology-Biostatistics, Memorial Sloan Kettering Cancer Center, [5]Medicine, Weill Cornell Medical College, New York, United States

Background: Cytogenetic abnormalities involving 11q23, the site of the mixed-lineage leukemia (*MLL*) gene, are seen in approximately 5-10% of adult AML. These rearrangements commonly occur in patients previously treated with a topoisomerase II inhibitor, such as an anthracycline. Previous studies have shown that t(9;11) has an intermediate prognosis by OS whereas other 11q23 translocations have a poor prognosis [Chen *et al. Leukemia* 2013]. There are limited data on why such a difference may exist.
Aims: 1. Identify translocation partners in 11q23-rearranged adult AML; 2. Classify the most common translocations in therapy-related disease; 3. Assess if achieving CR with a single induction is predictive of one-year OS; 4. Identify the frequency of post-transplant relapse.
Methods: Patients seen at MSKCC between 1997 and 2017 were included in the analysis. 11q23-rearranged AML was identified centrally by the cytogenetics core. Additional patients were found through a search of the electronic medical record for all malignancies with 11q23 alterations. Manual review and selection of 11q23 rearrangements in AML patients ≥18 years of age at diagnosis was performed. Data on patient characteristics, diagnosis, disease characteristics, treatment, response, and transplantation were collected from the EMR. "Therapy-related disease" was noted in patients who had achieved anthracycline-based chemotherapy prior to AML diagnosis. Given that CRi, CRp, and CRh were not defined at the time of diagnosis for many patients, composite CR was used.
Results: We evaluated the clinical characteristics of 65 adult patients with AML and a translocation involving 11q23 who were seen at MSKCC between 1997 and 2017. Data on the translocation partner for 11q23 was available for 61 patients. Translocations of 11q23 with 9p22 occurred in 48% of patients; with 19p13 in 25% of patients; and with other partners in 27% of patients (including 6q27 in 10% of patients). The 23 patients with therapy-related disease were significantly more likely to have a translocation involving 9p22 than any other partner (p=0.01). There was no difference in CR rate between translocations involving 9p22 and translocations with other partners (p=0.66). For those who received their initial induction therapy at MSKCC (31 patients) and achieved a CR, the one-year overall survival was 50%, as opposed to 12% in those who did not achieve a CR (p=0.008). Thirty-two patients were able to undergo allogeneic HSCT; of these, 10 relapsed after transplant (31%) (Figure 1).

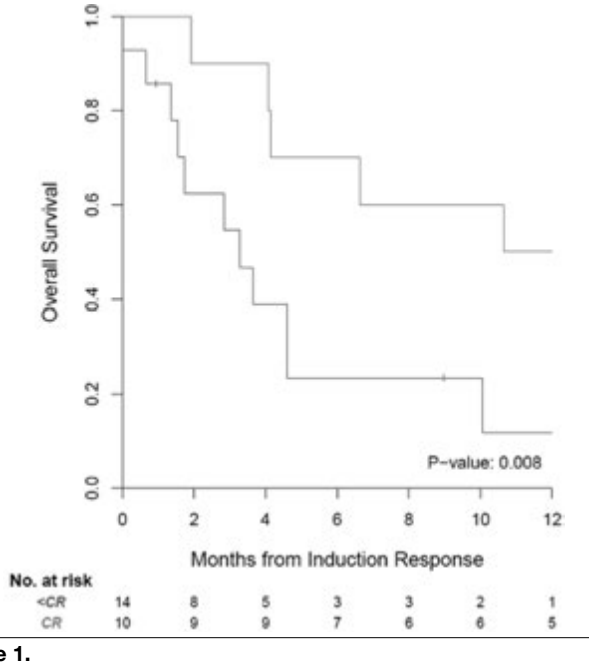

Figure 1.

Summary and Conclusions: - Therapy-related 11q23-rearranged AML was most likely to occur with 9p22 as the translocation partner. - The presence of a 9p22 translocation did not predict a higher likelihood of achieving CR despite being classified as intermediate risk by ELN. - Factors which may drive a difference in OS despite no difference in CR rate, such as relapse rate or the presence of molecular alterations which modify disease biology, require further study. - Achievement of CR after first induction appears to be a strong predictor of survival at one year. - Post-transplant relapse remains a significant challenge in this disease. - Prospective studies evaluating novel treatment options for these patients will be critical to meeting a clear unmet need.

PF253

HYPOMETILATING AGENTS (HMA) AS SALVAGE THERAPY IN RELAPSED OR REFRACTORY AML: AN ITALIAN 9-CENTER RETROSPECTIVE STUDY

M. Laurino[1,*], F. Lessi[1], A. Candoni[2], D. Lazzarotto[2], C. Papayannidis[3], M. Gottardi[4], O. Vitagliano[5], G. Reda[6], M. Riva[7], E. Crisà[8], M. Ermani[9], G. Semenzato[1], F. Ferrara[10]

[1]Department of Medicine, Hematology and Clinical Immunology Unit, University of Padua, Padua, [2]Hematology, University of Udine, Udine, [3] Institute of Hematology and Oncology L. and A. Seràgnoli, University of Bologna, Bologna, [4]Division of Hematology, Hospital of Treviso, Treviso, [5]Department of Clinical Medicine and Surgery, Federico II University Medical School, Naples, [6]Hematology, Fondazione IRCCS Ca'Granda Ospedale Maggiore Policlinico, [7]Niguarda Cancer Center, ASST Grande Ospedale Metropolitano Niguarda, Milan, [8]Hematology, Ospedale Maggiore della Carità, Novara, [9]Department of Neurosciences, Statistics and Informatics Unit, University of Padua, Padua, [10]Hematology, Cardarelli Hospital, Naples, Italy

Background: 5-azacytidine and decitabine have been widely studied as first line chemotherapy in acute myeloid leukemia (AML) patients not eligible for allogenic stem cell transplantation, but data on their use as salvage chemotherapy are limited.
Aims: We tried to define efficacy and feasibility of hypometilating agents (HMA) as salvage chemotherapy in patients refractory or relapsed after one or more lines of therapy, with or without previous allogenic stem cell transplantation.
Methods: We retrospectively reviewed clinical records of 79 patients treated with HMA as salvage therapy in our 9 institutions since their introduction in clinical practice for AML patients.
Results: Median age was 64 years. 53 patients were men and 26 women. One patient was AML with t(9;11), 2 were AML with t(8;21),one was AML with inv(3), one patient was AML with inv(16), 23 were AML MRC, 2 were therapy related AML, 49 were AML NOS. 10 patients were favorable risk sec ELN 2017, 35 were intermediate, 30 were adverse risk. Cytogenetic risk was not available for 4 patients. 29% of patients were secondary AML. 61% of patients received HMA as second line therapy for their disease, 26% as third line and 13% were beyond the third line. 18% of patients underwent allogenic stem cell transplantation before HMA. 64 patients were treated with azacitidine and 15 with decitabine. All patients underwent intensive chemotherapy (*i.e.* FLAI or 3+7 like) as first line induction, and we excluded patients who had an HMA as first line chemotherapy and another one as second line. Median number of hospitalization days during HMA therapy was 0.5 (0-112). 42 patients showed no response to HMA, 14 patients showed response to HMA (CR, PR, or CRi) and 23 patients showed stable disease (SD). Median OS from HMA in patients with *de novo* AML was 5,3 months, while OS in patients with secondary AML was 43,4 months (p=0,012). This extremely good OS is surprising, it could be due to the small number of secondary AML, and it should be confirmed. Median OS in patients with refractory disease before HMA therapy was 5,1 months, while OS in patients with relapsed disease was 14,9 months (p=0,033). Median OS after HMA in patients with SD as best response to HMA was similar to median OS in patients with response to HMA (26,1 months *vs* 24,88 months), while OS in non responsive patients was 3,6 months (p<0,00001). 50% of patients with adverse cytogenetic risk and 42% of patients with favorable/intermediate cytogenetic risk reached a response or a stable disease with HMA, but we did not find significant difference in median OS between these two groups of patients. On multivariate analysis, OS differences between the SD+response group and the progressive disease group and between the secondary AML and *de novo* AML remained significant. We did not find significant differences between packed red blood cells transfusion needs before and after best response to therapy, and we find a worsen in platelets transfusion needs after best response to HMA (mean 1,5 platelet concentrates before HMA, mean 2,4 after HMA, p=0,0013).
Summary and Conclusions: HMA showed efficacy and a considerable OS in a subgroup of patiets (relapsed patients and secondary AMLs). Hospitalization was limited, and we can argue that this could lead to costs saving and improve quality of life, but further studies are needed. HMA could be a good clinical option in a selected population of relapsed patients, apparently regardless of cytogenetic risk, especially in those not suitable for allogenic bone marrow transplantation, in whom the prognosis is generally extremely poor.

PF254

COMPREHENSIVE ANALYSIS OF THE CORE-BINDING FACTOR ACUTE MYELOID LEUKEMIA WITH KOREAN AML REGISTRY

H.-J. Shin[1,*], W.-S. Min[2], Y. H. Min[3], J.-W. Cheong[3], J.-H. Lee[4], I.-H. Kim[5], D. S. Hong[6], J.-S. Ahn[7], H.-J. Kim[7], W.-S. Lee[8], C. W. Jung[9], J.-H. Jang[9], Y. Park[10], H.-J. Kim[2]

[1]Pusan National University Hospital, Busan, [2]The Catholic Blood and Marrow Transplantation Centre, [3]Yonsei University College of Medicine, [4]Asan Medical Center, [5]Seoul National University Hospital, [6]Soonchunhyang University Bucheon Hospital, Seoul, [7]Chonnam National University Hwasun Hospital, Jeollanam-do, [8]Inje University Busan Paik Hospital, Busan, [9]Samsung Medical Center, [10]Korea University Medical Center, Seoul, Korea, Republic Of

Background: The registry data for core-binding factor acute myeloid leukemia (CBF-AML) in Asian countries have rarely been reported.
Aims: The aims of this study were to evaluate clinical characteristics, treatment outcomes including SCT, and prognostic factors for CBF-AML using Korean AML registry.
Methods: We analyzed data of 392 patients with CBF-AML from among data for 3,041 AML patients in the Korean AML Registry.
Results: Interestingly, del(9q) was less frequently detected in Korean patients with t(8;21) than German patients (7.5% *vs* 17%), and del(7q) was more frequently detected (9.9%) in Korean patients with inv(16). Overall survival (OS) was similar between patients in first complete remission (CR) who received allogeneic stem cell transplantation (alloSCT) and those who received autologous SCT (ASCT) for both t(8;21) and inv(16) AML. Three-year OS of t(8;21) patients was poor when undergoing alloSCT in second or third CR, while OS of inv(16) patients in second or third CR was similar with that in first CR. Patients with a more than 3-log reduction of RUNX1/RUNX1T1 qPCR had improved 3-year event-free survival (EFS) than those without 3-log reduction (73.2% *vs* 50.3%). Poor outcome of <3-log reduction of qPCR can be overcome by alloSCT/ASCT as post-remission treatment. The t(8;21) AML patients with D816 mutation showed inferior EFS and OS and this poor outcome might be overcome by alloSCT. Multivariate analysis for OS in patients with t(8;21) revealed older age, >1 course of induction chemotherapy to achieve CR, loss of sex chromosome, del(7q), and second/third CR or not in CR before SCT as the independent prognostic variables. Especially del(7q) in t(8;21) patients showed significantly poor OS and all patients with del(7q) died less than 2 years after diagnosis (P <0.001). On the contrary, inv(16) patients with del(7q) had better OS than those without del(7q).
Summary and Conclusions: The prognostic significance of del(7q) in different subtypes of CBF-AML greatly varies and should be focused on future studies.

PF255

REAL WORLD EVIDENCE ON THE BURDEN OF ILLNESS EXPERIENCED BY PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AND SYSTEMIC MYCOSES

A. Forsythe[1,*], J. Hearnden[1], G. Lewis[2], R. Schreiber[2]

[1]Purple Squirrel Economics, New York, [2]Mayne Pharma, Raleigh, United States

Background: Invasive fungal infections, also called systemic mycoses, affect immunocompromised populations disproportionately.
Aims: This investigation focused on the burden of illness experienced by patients with hematological malignancies and concurrent systemic mycoses.
Methods: Patients with systemic mycoses including aspergillosis, histoplasmosis, and blastomycosis were identified by strategically querying a database of electronic medical records *via* ICD-10 codes. The database features information on over 34 million patients in 30 US hospital institutions. Two cohorts were established consisting of patients with systemic mycoses and 1) Neither cancers nor another form of immunosuppression (immunosuppressant treatment, transplants, HIV), or 2) Cancer diagnoses. A third control cohort (3) consisting of patients with cancer diagnoses but without systemic mycoses was also established. A range of constitutional, respiratory, metabolic, and gastrointestinal symptoms were analyzed within six months of: 1&2) Initial treatment with antifungal medications (primarily itraconazole, fluconazole, nystatin, amphotericin, and voriconazole) and 3) Cancer diagnosis. The proportion of patients in each cohort who experienced a given symptom was determined. Comparisons were performed using two-tailed t-tests (95% percent confidence limits). Comparison of Cohorts 1 and 2 addressed the added burden experienced by patients with both systemic mycoses and cancers. Comparison of Cohorts 2 and 3 isolated the burden of systemic mycoses opposed to a concurrent cancer.
Results: A search conducted on October 26th, 2017 spanning five years identified 11,619 patients with systemic mycoses who were treated with antifungals. Cohorts 1 (N=2370), 2 (N=3284), and 3 (N=1139) were established. Among cancer patients with systemic mycoses, 59% had hematological malignancies (35% myeloid leukemia, 22% lymphoid leukemia,

22% leukemia of unspecified cell type). Across cohorts, systemic mycoses patients had consistent mean age (no cancer: 52, cancer: 57) and similar proportions of male (no cancer: 51%, cancer: 53%) and white (no cancer: 65%, cancer: 78%) patients. Cancer patients without systemic mycoses were assigned to Cohort 3 (N=774313). Cancer patients with systemic mycoses experienced the highest rates of all constitutional, respiratory, metabolic, and gastrointestinal symptoms *versus* mycoses patients without cancer (>100% increase in all symptoms, p<0.001 for all comparisons). The symptomatic burden experienced by cancer patients with systemic mycoses also significantly exceeded the burden on cancer patients without systemic mycoses (>330% increase in all symptoms, p <0.001) (Figure 1).
Summary and Conclusions: Patients with cancers and systemic mycoses experienced far greater rates of all symptoms than patients with either condition alone. This study served to illustrate the high symptomatic burden experienced by patients with cancers, primarily hematological malignancies, due to systemic mycoses.

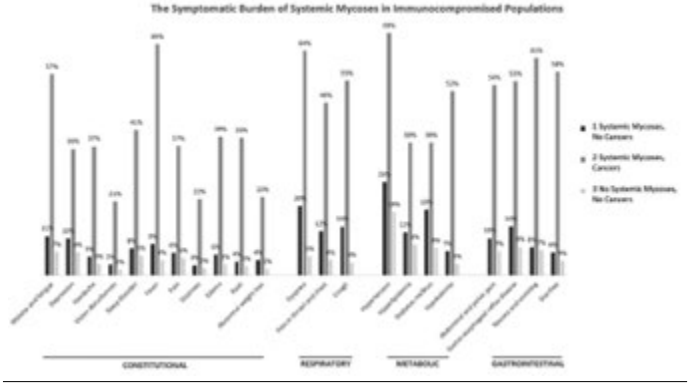

Figure 1.

PF256

EVALUATION OF FOOD EFFECT ON PHARMACOKINETICS OF IVOSIDENIB (AG-120), AN ORAL, POTENT, TARGETED, SMALL MOLECULE INHIBITOR OF MUTANT IDH1, IN HEALTHY SUBJECTS

B. Fan[1,*], D. Dai[1], G. Connor[1], H. Liu[1], G. Liu[1], S. V. Agresta[1], H. Yang[1]

[1]*Agios Pharmaceuticals, Inc., Cambridge, United States*

Background: Ivosidenib (IVO; AG-120) is an oral, potent, targeted, small molecule inhibitor of mutant isocitrate dehydrogenase 1 (mIDH1). Somatic mutations in IDH1 result in gain-of-function activity, catalyzing the reduction of α-ketoglutarate to the oncometabolite D-2-hydroxyglutarate (2-HG), leading to oncogenesis. IVO is a highly targeted therapeutic candidate for the treatment of mIDH1-driven cancers, including relapsed or refractory acute myeloid leukemia, which is currently being investigated in clinical trials. As IVO is orally administered, it is important to determine the relative bioavailability in relation to meal consumption.
Aims: To investigate the effect of a high-fat, high-calorie meal on the pharmacokinetics (PK) of IVO in healthy subjects.
Methods: In this randomized, two-period, two-way crossover design study (Clinicaltrials.gov NCT02579707), a single 500 mg dose (two 250 mg tablets) of IVO was administered under fasting conditions (10-hr overnight fast before IVO dosing) and after a high-fat meal (standardized FDA high fat content breakfast [approximately 900–1000 calories; 56%–60% fat] consumed within 30 minutes prior to IVO dosing) to 30 healthy subjects (aged 18 55 years). Of the 30 subjects enrolled, 15 were randomized to sequence 1 (fasted, then fed) and 15 were randomized to sequence 2 (fed, then fasted). There was a washout period of at least 25 days between the two treatments in each sequence. Blood samples for the analysis of IVO concentrations were collected predose and up to 504 hr postdose. The plasma PK parameters in the fed (test) and fasted (reference) groups were analyzed using analysis of variance.
Results: Following administration of IVO 500 mg in the high-fat fed and fasted states, IVO was readily absorbed, with similar median T_{max} values of 3.00 and 3.03 hr postdose, respectively (Table 1). After reaching C_{max}, plasma concentrations slowly declined in a multiphasic manner, with similar mean $t_{1/2}$ values of 53.2 hr in the fed state and 55.4 hr in the fasted state. An approximately 2-fold increase in mean C_{max} was observed, and mean AUC was 25% greater, when IVO was administered following a high-fat meal compared with fasted conditions. Following administration of single doses of IVO 500 mg, intersubject variability (assessed by geometric coefficient of variation for AUC and C_{max}) was similar and moderate for both fed and fasted states.

Table 1. Plasma PK parameters of IVO under fasted and fed conditions.

PK parameter	500 mg IVO (fasted)	500 mg IVO (fed)
$AUC_{0\text{-}t}$ (hr·ng/mL)[a]	136,000 (31.6)	166,000 (31.2)
$AUC_{0\text{-}\infty}$ (hr·ng/mL)[a]	143,000 (31.1)	174,000 (31.2)
C_{max} (ng/mL)[a]	2270 (21.3)	4490 (24.3)
T_{max} (hr)[b]	3.03 (1.00–24.0)	3.00 (1.00–6.00)
$t_{1/2}$ (hr)[c]	55.4 (20.5)	53.2 (18.3)

[a]Geometric mean (coefficient of variation, %)
[b]Median (min–max)
[c]Arithmetic mean (standard deviation)

Summary and Conclusions: There was an effect of a high-fat meal on IVO exposure, with an increase in C_{max} of approximately 98%.

PF257

AZACITIDINE FRONTLINE AND SALVAGE THERAPY FOR ACUTE MYELOID LEUKEMIA PATIENTS. CLINICAL EXPERIENCE AND DEVELOPMENT OF RISK SCORE

S. Imbergamo[1,*], A. Visentin[1], I. Kostic[1], S. Pravato[1], E. Scomazzon[1], M. Laurino[1], L. Ammirati[1], M. Riva[1], F. Lessi[1], G. Binotto[1], T. Berno[1], C. Gurrieri[1], G. Semenzato[1], L. Trentin[1]

[1]*Department of Medicine, Hematology and Clinical Immunology Unit, Padua University School of Medicine, Padova, Italy*

Background: Acute myeloid leukemia (AML) is an aggressive hematological malignancy characterized by a poor prognosis. Azacitidine (AZA) therapy has shown significant response and increased survival in clinical trial in patients with treatment naive (TN) or relapsed/refractory (R/R) AML.
Aims: The aim of this study was to evaluate the overall survival (OS) of AZA in patients with TN, not eligible for intensive chemotherapy, and R/R AML and to identify clinical and biological variables associated with increase survival.
Methods: A retrospective analysis was conducted on 39 patients with AML treated with AZA followed at Padua University Hospital from April 2012 to January 2018. The diagnosis was made according to 2016 WHO criteria. All patients received s.c. 5-Azacitidine 75 mg/m^2 for 7 days every 4 weeks until disease progression.
Results: The median age at diagnosis was 64 years (range 49-81). 30 (76%) patients were TN and 9 (24%) were R/R. Eighteen patients (46%) were considered at high, 19 (48%) intermediate and 2 (6%) low-cytogenetic risk. The complete remission was in 47% patients. The median OS for the whole cohort was 10.7 months, without any difference between TN and R/R patients (p=0.2743). In univariate analysis, variables associated with increased OS were age >70y (p=0.036), adverse cytogenetic-risk (p=0.021), reaching a partial remission (p=0.0037). All these variables were confirmed in multivariate analysis. There were no differences in OS for the percentage of peripheral and bone marrow blast cells. Based on hazard ratios obtained by multivariate analysis we assigned a risk-values to each significant variables. A risk score was then calculated as the sum of each risk values, ranging from 0 to 10. We found that median OS were 16.9 and 6.9 months for patients with ≤4 points and those with 5-10 score, respectivelly (p<0.0001).
Summary and Conclusions: We herein provide evidence that 5-Azacitidine is feasible and effective in patients with acute myeloid leukemia, regardless the lines of treatment. Moreover, we developed a scoring system able to identify patients with long lasting response and improved survival.

PF258

MYELOID SARCOMAS IN AML TRANSLOCATION (8;21) IS IT STILL A FAVOURABLE RISK GROUP?

R. Halder[1,*], M. Aggarwal[1], M. Mahapatra[1], T. Seth[1], U. Kumar[1], R. Dhawan[1], H.P. Pati[1], S. Tyagi[1], R. Saxena[1], S. Mirgh[1], S. Kothari[1], R. Chauhan[1]

[1]*Department of Hematology, All India Institute of Medical Sciences, New delhi, India*

Background: Acute Myeloid Leukemia(AML) with translocation (8;21) is a favourable disease. Prognostic impact of myeloid sarcomas on aml t(8;21) is not much studied.
Aims: To study and compare the outcome of patients of acute myeloid

leukemia with translocation (8;21) with and without myeloid sarcoma at presentation.
Methods: It was a retrospective analytical study of patients of acute myeloid leukemia with translocation (8;21) with and without myeloid sarcomas that were treated from 2014 to 2017 in our hospital.
Results: During the study period we treated 24 cases were of aml with t(8;21), 14 in the adult age group and 10 in pediatric(<18 years) age group. 9 out of 24 cases had myeloid sarcoma. C-kit mutation for D816V was done for 11 patients, none of whom were positive.
All patients were treated with standard Daunorubicin and Cytarabine (3+7) induction chemotherapy followed by 3 cycles of high dose cytarabine (@3gram/m2) once every 28 days as per standard of care.
4 out of the 9 cases(44.4%) of myeloid sarcoma with aml t(8;21) had either relapsed or died. Where as in the aml t(8;21) without myeloid sarcoma group 6 out of 15 patients (40%) relapsed or died. (p=0.2)
Summary and Conclusions: Presence of myeloid sarcomas in patients with aml t(8;21) does not seem to have an adverse prognostic impact on the course of disease.

Aggressive Non-Hodgkin lymphoma – Clinical

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AXICABTAGENE CILOLEUCEL (AXI-CEL) IN PATIENTS WITH REFRACTORY LARGE B CELL LYMPHOMA: DURABILITY OF RESPONSE IN ZUMA-1

F.L. Locke[1], A. Ghobadi[2], C.A. Jacobson[3], E. Jacobsen[3], D.B. Miklos[4], L.J. Lekakis[5], I. Braunschweig[6], O.O. Oluwole[7,*], Y. Lin[8], T. Siddiqi[9], A. Deol[10], P.M. Reagan[11], U. Farooq[12], A. Bot[13], Y. Jiang[13], J.M. Rossi[13], A. Xue[13], W. Go[13], S.S. Neelapu[14]

[1]Moffitt Cancer Center, Tampa, [2]Washington University School of Medicine, St. Louis, [3]Dana-Farber Cancer Institute, Boston, [4]Stanford University, Stanford, [5]University of Miami Health System, Sylvester Comprehensive Care Center, Miami, [6]Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, [7]Vanderbilt-Ingram Cancer Center, Nashville, [8]Mayo Clinic, Rochester, [9]City of Hope National Medical Center, Duarte, [10]Karmanos Cancer Center, Wayne State University, Detroit, [11]University of Rochester School of Medicine, Rochester, [12]University of Iowa, Iowa City, [13]Kite, a Gilead company, Santa Monica, [14]The University of Texas MD Anderson Cancer Center, Houston, United States

Background: Axi-cel, an anti-CD19 CAR T cell therapy, demonstrated significant clinical benefit and a manageable safety profile for patients with refractory large B cell lymphoma in ZUMA-1 (Neelapu & Locke *et al. NEJM.* 2017). These results led to its approval by the US FDA for the treatment of adult patients with relapsed or refractory large B cell lymphoma after ≥2 prior lines of therapy.
Aims: To examine responses over time in Phase 2 of ZUMA-1.
Methods: All patients provided written informed consent. Patients with refractory large B cell lymphoma received 2×10^6 CAR T cells/kg after low-dose conditioning chemotherapy (Neelapu & Locke *et al. NEJM.* 2017). Best objective response rates (BORs) were analyzed locally by investigators and centrally by independent review committee (IRC; Cheson *et al. J Clin Oncol.* 2007); concordance was measured as the percentage of patients whose IRC matched local assessment.
Results: As of 8/11/17, median follow-up was 15.1 months for the 101 patients treated with axi-cel. While the BOR of 82% at the primary analysis (median follow-up 8.7 months) by local assessment remained consistent (83%) at long-term follow-up (LTFU; median of 15.1 months), complete response (CR) rates increased from 54% to 58% (Table 1). Out of 34 patients with partial response (PR) at 1 month, 11 (32%) converted to CR by the LTFU. High concordance (77%–79%) was observed for objective response rates (ORR [CR+PR]) between local assessment and IRC at all times assessed. Landmark analysis of progression-free survival (PFS) by response status (per local assessment) revealed that most of the 60 patients with disease control (stable disease or better) at 3 months had prolonged disease control with a 73% 12-month PFS rate. Of the 42 patients with CR and 9 with PR at 3 months, the 12-month PFS rates were 79% and 78%, respectively.

Table 1.

Data-cut; median follow-up, months N = 101	BOR, n (%)				ORR Concordance, %
	Local Assessment		IRC		
	ORR	CR	ORR	CR	
Primary analysis; 8.7	83 (82)	55 (54)	72 (71)	52 (51)	77
Axicabtagene ciloleucel US prescribing information; 11.6	84 (83)	55 (54)	73 (72)	52 (51)	79
LTFU; 15.1	84 (83)	59 (58)	73 (72)	52 (51)	79

Summary and Conclusions: Treatment with axi-cel induces high response rates in patients with refractory large B cell lymphoma. CR rates increased through the LTFU, suggesting that responses deepen over time and that patients with PR can achieve CR as late as a year post-infusion. ORR at 3 months may be prognostic for prolonged PFS.

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IMPACT OF COMORBIDITY ON DISEASE CHARACTERISTICS, TREATMENT INTENT AND OUTCOME IN DIFFUSE LARGE B-CELL LYMPHOMA – A SWEDISH LYMPHOMA REGISTER STUDY

T. Wästerlid[1,*], K. Ekström Smedby[1], I. Glimelius[2], M. Jerkeman[3], S. Eloranta[1]

[1]Department of Medicine Solna, Division of Clinical Epidemiology, Karolinska Institutet, Stockholm, [2]Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, [3]Department of Oncology, Skåne University Hospital, Lund, Sweden

Background: Presence of comorbid disease is known to impact overall survival among patients with diffuse large B-cell lymphoma (DLBCL). Thus far, studies that have evaluated the influence of comorbidity in DLBCL have been heterogeneous in study design and have often been restricted in size and to an elderly population. Therefore, associations of comorbidity with lymphoma characteristics, treatment selection and lymphoma-specific mortality in a real-world setting remain to be established.
Aims: We aimed to examine the relationship between history of comorbid disease and lymphoma characteristics, treatment intent and overall- and lymphoma-specific survival among adult patients with DLBCL in Sweden.
Methods: A total of 3905 adult patients diagnosed with DLBCL 2007-2013 were identified through the Swedish Lymphoma Register, with detailed clinical data available. Comorbid disease was defined according to the Charlson comorbidity index (CCI), with addition of psychiatric disorders. Data regarding comorbidities was collected from the Swedish National Patient register. To assess lymphoma-specific mortality, the study cohort was linked to the Cause-of-Death register. Associations of comorbid disease history with lymphoma characteristics and treatment intent were investigated using Relative Risk Ratios (RRR) from multinomial logistic regression models. Hazard ratios (HR) for all-cause and lymphoma-specific survival was estimated using flexible parametric survival models. These models were also used to estimate adjusted lymphoma-specific survival curves, as a complement to survival estimates obtained using the Kaplan-Meier method.
Results: Forty-five percent of patients (n=1737) had at least one comorbidity as classified according to the CCI, of which 997 (26% of the total population) had a CCI score of ≥2. As expected, presence of comorbidity increased with age (CCI>0 among 15% *vs* 65% of patients aged <50 *vs* ≥80, respectively). The relative probability of presenting with poor performance status *versus* being asymptomatic (PS=0) was higher among comorbid patients compared to those without a history of comorbidities ($RRR_{PS>2}$:2.02, 95% CI:1.63-2.51). No corresponding association was observed with stage, bulky disease, elevated levels of lactate dehydrogenase or extranodal involvement. Comorbid patients had a lower relative probability of receiving curative treatment (RRR:0.48, 95% CI:0.38-0.61). Comorbid patients selected for palliative treatment had an increased risk of both all-cause death (HR:2.12, 95% CI:1.53-2.94) and lymphoma-specific mortality (HR:1.74, 95% CI:1.21-2.51). In contrast, among comorbid patients selected for curative treatment, comorbidity was associated with an increased risk of all-cause death (HR:1.54, 95% CI:1.32-1.80), but not with inferior lymphoma-specific survival (HR:1.05, 95% CI:0.86-1.28, Figure 1).

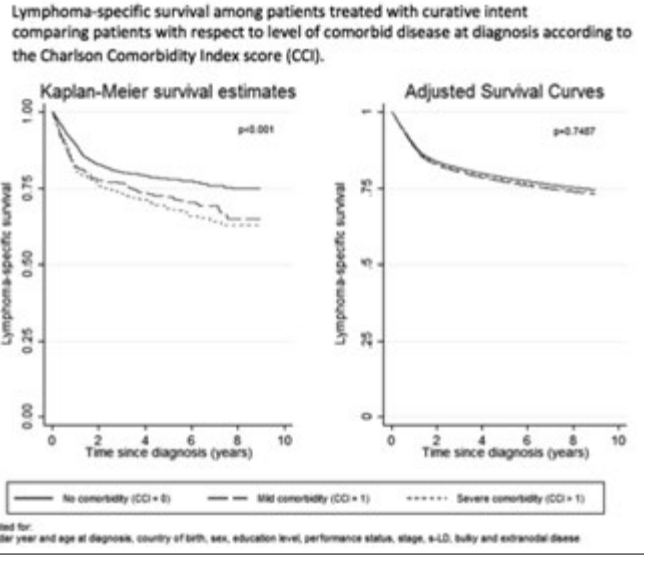

Figure 1.

Summary and Conclusions: In this population-based study we show that comorbid DLBCL patients more often present with poor performance status, but not more frequently with other disease characteristics associated with advanced disease. Moreover, we demonstrate that comorbidity is associated with lower likelihood of receiving treatment with curative

intent, leading to inferior lymphoma-specific outcome. Among comorbid patients treated with curative intent, inferior overall but not lymphoma-specific survival indicate a need for reduction of toxicity and optimization of comorbid conditions in parallel with DLBCL treatment.

PF261

THE NOVEL SYK/JAK INHIBITOR CERDULATINIB DEMONSTRATES GOOD TOLERABILITY AND CLINICAL RESPONSE IN A PHASE 2A STUDY IN RELAPSED/REFRACTORY PERIPHERAL T CELL LYMPHOMA

S. Horwitz[1,*], P.A. Hamlin[1], T.A. Feldman[2], B.T. Hess[3], A.R. Mato[4], J. Munoz[5], M.R. Patel[6], S.D. Smith[7], J.C. Ye[8], A.J. Steele[9], A. Pandey[9], M.R. Birrell[9], J.M. Leeds[9], G.P. Coffey[9], J.T. Curnutte[9]

[1]Department of Medical Oncology, Memorial Sloan-Kettering Cancer Ctr, New York, NY, [2]Hackensack University Medical Center, Hackensack, NJ, [3]Division of Hematology/Oncology; Hollings Medical Cancer Center, Medical University of South Carolina, Charleston, SC, [4]Hospital of the University of Pennsylvania, PA Cancer Immunotherapy, Philadelphia, PA, [5]Cancer Immunotherapy Program, Banner MD Anderson Cancer Center, Gilbert, AZ, [6]Drug Development Unit, Florida Cancer Specialists/Sarah Cannon Research Institute, Sarasota, FL, [7]University of Washington/Fred Hutchinson Cancer Research Center, Seattle Cancer Care Alliance, Seattle, WA, [8]University of Michigan Cancer Center, Ann Arbor, MI, [9]R&D, Portola Pharmaceuticals, South San Francisco, CA, United States

Background: Pre-clinical data suggest a role for SYK and JAK signaling pathways as oncogenic drivers in peripheral T cell lymphoma (PTCL). A published study described a unique translocation in 17% of PTCL specimens (t(5;9)(q33;q22)) resulting in a SYK-ITK fusion protein, in which the kinase domain of SYK was constitutively active. This fusion protein was expressed transgenically in CD4 positive T cells in mice, resulting in a lethal T cell proliferative disease. Transgenic expression of this fusion protein in CD19 positive B cells in mice did not result in clonal expansion. In an independent set of 141 PTCL specimens, wildtype SYK was expressed in 93% of all PTCL cases, including 100% of 35 AITL cases and 94% of 66 PTCL-NOS cases. This was independently confirmed, although SYK expression was observed at lower frequencies (45% of 71 systemic ALCL cases, and 0% of 20 PTCL-NOS cases). Additionally, gene expression profiling revealed two dominant sub-clusters implicating JAK/STAT signaling in this disease; one enriched for GATA-3 and its cytokine gene targets (eg, IL-4, IL-5, IL-13), while the other is enriched for T-bet and its cytokine gene targets (eg, IFN-γ and IFN-γ-inducible genes). Frequent activating mutations to common γ chain, JAK1, JAK3, or STAT5b are also observed. Overall, the data suggest that dual inhibition of SYK and JAK may perturb multiple and independent survival mechanisms in PTCL. Cerdulatinib is a small molecule reversible ATP competitive inhibitor of SYK and JAK family members. Phase 1 studies in patents with B cell malignancies demonstrated good tolerability and initial evidence of clinical activity, and the phase 2a extension study in patients with specific subtypes of B cell or T cell NHL or CLL/SLL is ongoing.
Aims: This is an interim analysis of an ongoing phase 2a study in a subpopulation of patients with relapsed/refractory PTCL.
Methods: Eighteen relapsed/refractory PTCL patients received 30 mg cerdulatinib orally BID. Response was assessed by Lugano classification criteria at the end of 2 months and every 3 cycles thereafter.
Results: Patents included PTCL-NOS (7), AITL (6), ALCL (2), HSTCL (1), Gamma-delta TCL (1), and MEITL (1). Median (range) age: 70 (48-84]); median prior therapies: 3 (1-9); 28% had prior transplant; and 44% was refractory to last therapy. Eleven patients were evaluated for clinical response, 3 discontinued prior to evaluation (2 due to progression; 1 withdrew consent), and 4 patients have yet to be evaluated. Six patients have responded (ORR 43%): 4 patients achieved a CR after 2 cycles, 2 achieved a PR, and 2 SD. Four responding patients remain on drug: 1 for 11+ months and the remaining for 3-7 months. A patient with CR was referred to allogeneic transplant and censored after cycle 2. An additional patient achieved complete remission of target lesions, but discontinued therapy due to a new lesion. The majority of responses were observed in PTCL-NOS and AITL. Importantly, CRs and PRs occurred in patients who failed multiple lines of therapy, including pralatrexate, romidepsin, belinostat, and an investigational PI3K inhibitor. The most common AEs of any grade were diarrhea (33%), fatigue (22%), lipase increase (17%), and nausea (17%). Grade 3+ AEs occurring in ≥2 patients are neutropenia (4), diarrhea (3), lipase increase (2), and pneumonia (2). The target PK range was achieved with an average SSCmin of ~0.8 μM.
Summary and Conclusions: These data suggest that cerdulatinib is well tolerated and capable of generating durable complete responses in heavily pretreated PTCL.

PF262

THE COMBINATION OF IBRUTINIB, LENALIDOMIDE, AND RITUXIMAB (IR2) IS ACTIVE IN RELAPSED/REFRACTORY (R/R) NON–GERMINAL CENTER-LIKE (NON-GCB) DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL): INTERIM ANALYSIS

R. Ramchandren[1,*], N. Ghosh[2], P. Johnson[3], D. Cunningham[3], K.M. Ardeshna[4], G. Verhoef[5], R. Johnson[6], E. Van Den Neste[7], S. Kassam[8], F. Offner[9], J. Radford[10], J. Ping[11], M. Co[11], J.K. Neuenburg[11], A. Goy[12]

[1]Karmanos Cancer Institute, Wayne State University, Detroit, MI, [2]Levine Cancer Institute/Carolinas Healthcare System, Charlotte, NC, United States, [3]The Royal Marsden NHS Foundation Trust, London and Surrey, [4]University College Hospital, London, United Kingdom, [5]UZ Leuven - Campus Gasthuisberg, Leuven, Belgium, [6]Leeds Cancer Centre, St. James University Hospital, Leeds, United Kingdom, [7]Cliniques Universitaires UCL Saint-Luc, Brussels, Belgium, [8]Kings College Hospital NHS Foundation Trust, London, United Kingdom, [9]Universitair Ziekenhuis Gent, Ghent, Belgium, [10]University of Manchester and the Christie NHS Foundation Trust, Manchester, United Kingdom, [11]Pharmacyclics LLC, an AbbVie Company, Sunnyvale, CA, [12]John Theurer Cancer Center at Hackensack University Medical Center, Hackensack, NJ, United States

Background: Treatment outcomes for patients (pts) with R/R DLBCL are poor, particularly for those ineligible for hematopoietic stem cell transplantation (SCT). Ibrutinib (ibr), a first-in-class, once-daily inhibitor of Bruton's tyrosine kinase, is approved for the treatment of various B-cell malignancies. An ongoing phase 1b/2, open-label, multicenter study is evaluating the safety and efficacy of ibr in combination with lenalidomide (LEN) and rituximab (RTX) in SCT-ineligible pts with R/R DLBCL. During the dose escalation in phase 1b, a LEN dose of 20 mg on Days 1–21 in combination with once-daily 560 mg ibr and 375 mg/m^2 IV RTX on Day 1 of Cycles 1–6 in 28-day cycles (iR^2) was considered the recommended phase 2 dose (RP2D). Across phase 1b dose cohorts, iR^2 demonstrated promising activity, with an overall response rate (ORR) of 43% (all) and 61% (non-GCB) in response-evaluable pts; the median duration of response (DOR) was 16 months. Results from the phase 2 interim analysis are reported here.
Aims: To evaluate the safety and activity of the iR^2 regimenat the RP2D in SCT-ineligible pts ≥18 years with histologically confirmed R/R non-GCB DLBCL after ≥1 prior therapy.
Methods: All pts provided written informed consent. Ibr, LEN, and RTX were administered in 28-day cycles at the RP2D (Figure 1). The primary efficacy endpoint in phase 2 was ORR. The null hypothesis of an ORR of 40% will be tested against the alternative hypothesis of an ORR of >60%. An interim analysis including 28 evaluable pts with adequate tumor assessment could be performed per protocol. If only 11 or fewer responders (≤11/28) are observed, discontinuation of the study could be considered by the Sponsor as per the statistical framework of Simon's minimax 2-stage design (Simon 1989). Enrollment continued during the interim analysis. The secondary endpoints included complete response rate, DOR, progression-free survival (PFS), overall survival, and safety. For severe rash and neutropenia, study medication was withheld until improvement to grade ≥1 or resolution and managed with oral corticosteroids and antihistamines (rash) and hematopoietic growth factors (neutropenia), as applicable.
Results: At the interim analysis, 42 pts were enrolled and treated in phase 2. Median age was 63 years; 62% were male; 62% had stage IV disease; 26% had primary refractory disease; median prior DLBCL systemic therapies was 2 (range: 1–5). The iR^2 regimen demonstrated promising activity: among the initially enrolled 28 response-evaluable pts for the interim analysis, 17 had a response (9 complete [32%]; 8 [29%] partial), with an ORR of 61% (95% CI: 41%–79%) and a median DOR of 10 months. Two (7%) pts had stable disease. The study passed the interim analysis criteria, as the number of responders (n=17 of the first 28 evaluable pts) exceeded the 11 responders needed to continue study enrollment. Among all 42 pts, the median PFS was 5 months (95% CI: 3–12) as of this interim analysis, with 57% of pts still receiving treatment. Grade ≥3 TEAEs were experienced by 79% of pts, and 57% of pts experienced a grade ≥3 TEAE considered related to study drug. Grade 3–4 TEAEs in >5% of pts were neutropenia (19%), anemia (14%), and maculopapular rash (12%).

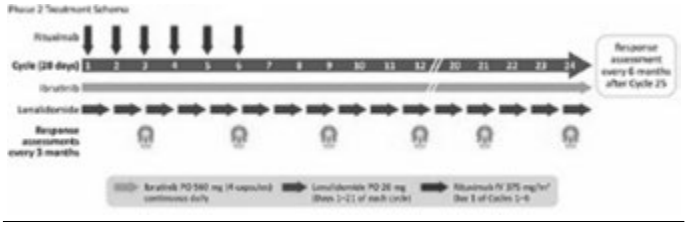

Figure 1.

Summary and Conclusions: The combination of ibr, LEN, and RTX demonstrated promising activity and a manageable safety profile at the RP2D in SCT-ineligible pts with R/R non-GCB DLBCL. Further evaluation of this combination, including a cohort with 25 mg LEN, is currently ongoing.

PF263

OVERWEIGHT LYMPHOMA PATIENTS TREATED BY ACTUAL BODY WEIGHT HAD NO EXCESS OF CHEMOTHERAPY TOXICITY: EXPERIENCE FROM TWO LARGE TRIALS

B. Merron[1,*], T. Maishman[1], A. Preston[2], J. Northey[1], A.A. Kirkwood[3], J. Caddy[1], L. Stevens[3], G. Griffiths[1], L. Clifton-Hadley[3], A. Davies[4], P.W. Johnson[4]

[1]Southampton Clinical Trials Unit, University of Southampton, Southampton, [2] Pharmacy Department, University Hospitals Bristol, Bristol, [3]Cancer Research UK & UCL Cancer Trials Centre, University College London, London, [4]Cancer Research UK Centre, University of Southampton, Southampton, United Kingdom

Background: The incidence of obesity in the western world has increased dramatically, with 1.9 billion overweight adults in 2016. ASCO guidelines on chemotherapy dosing in the obese population advocate the use of actual body weight but dose reductions (DRs) are often made owing to the perceived risk of greater toxicity. For diseases treated with curative intent such as Hodgkin lymphoma (HL) and diffuse large B-cell lymphoma (DLBL) DRs in the obese might result in reduced survival.
Aims: Using data from two large trials in HL (RATHL) and DLBL (REMoDL-B), we examined chemotherapy toxicity in the obese *versus* non-obese populations. We also studied survival outcomes in relation to BMI and dose reductions.
Methods: In RATHL, patients with newly diagnosed HL underwent paired baseline and interim PET-CT scans after 2 cycles of ABVD (PET2). Patients with a negative (Deauville 1-3) PET2 were randomized to ABVD/AVD for 4 more cycles. If the PET2 remained positive (Deauville 4-5), patients proceeded to intensification with BEACOPP-14/escalated BEACOPP. In REMoDL-B, patients newly diagnosed with DLBL commenced conventional R-CHOP chemotherapy whilst whole transcriptome profiling (GEP) was performed on their tumour sample. After successful GEP, patients were randomised to R-CHOP +/- bortezomib days 1+8 for cycles 2-6. In both studies baseline height and weight was recorded along with drug doses delivered and toxicities recorded.
Results: BMI data was available for 1199 eligible patients in RATHL and 1062 patients in REMoDL-B (Table 1). Within RATHL, 25.4% were overweight (BMI 25-29.9) and 16.2% obese (BMI≥30). In REMoDL-B, 38% were overweight, 25% obese. There was no difference in prognostic scores or stage in either study. In RATHL, obese patients were older, less likely to have 'B' symptoms and more likely to be female. In REMoDL-B, obese patients were younger than the non-obese. In both studies, obese patients were significantly more likely to undergo elective dose reductions (below 90% of dose based on actual body weight) of at least one drug in each cycle than the non-obese population. In both studies, obese patients who received full dose chemotherapy had no higher incidence of grade ≥3 haematological AEs or neuropathy in comparison to full dose, non-obese counterparts in both studies. In RATHL, there was no significant difference in the use of granulocyte colony-stimulating factor (GCSF) between obese and non-obese groups. In REMoDL-B non-obese patients were significantly more likely to receive GCSF than the obese population, in particular as secondary prophylaxis. This may reflect the older population and higher frequency of full dose chemotherapy in this BMI. In neither study was there a difference in PFS or OS in the obese population between those dose reduced and those not dose reduced. Across BMI groups there was no difference in PFS or OS.

Table 1. Table of baseline characteristics.

	REMoDL-B		RATHL	
Characteristic	**Non-obese**	**Obese**	**Non-obese**	**Obese**
BMI, N (%)	795	267	1004	195
Age, Median (range)	65 (20-86)	62.0 (28-83)*	31.0 (18-78)	39.0 (18-79)*
Male, N (%)	444 (55.8)	143 (53.6)	565 (56.3)	89 (45.6)*
B Symptoms present, N (%)	379 (48)	113 (42.3)	638 (63.5)	96 (49.2)*

* $p < 0.05$

Summary and Conclusions: Elective dose reductions were significantly more common in patients with BMI≥30 in both studies. In obese patients who were dosed according to actual body weight there was no increase in haematological toxicity. Those undergoing modest dose reductions did not experience inferior PFS or OS.

PF264

QUALITY OF LIFE IN CUTANEOUS T-CELL LYMPHOMA SUBJECTS TREATED WITH THE ANTI-CCR4 MONOCLONAL ANTIBODY MOGAMULIZUMAB *VERSUS* VORINOSTAT: RESULTS FROM THE PHASE 3 MAVORIC TRIAL

P. Quaglino[1,*], S. Hudgens[2], P. Porcu[3], R. Cowan[4], L. Floden[2], A. Tsianakas[5], M. Leoni[6], S. Dale[6], M. Duvic[7]

[1]University of Turin, Turin, Italy, [2]Clinical Outcomes Solutions, Tucson, AZ, [3]Thomas Jefferson University, Philadelphia, PA, United States, [4]Christie Hospital Foundation, Manchester, United Kingdom, [5]Specialist Clinic Bad Bentheim, Bad Bentheim, Germany, [6]Kyowa Kirin Pharmaceutical Development, Inc., Princeton, NJ, [7]MD Anderson Cancer Center, Houston, TX, United States

Background: Cutaneous T-cell lymphomas (CTCL) are rare non-Hodgkin's lymphomas that cause significant morbidity and adversely affect quality of life (QoL), most severely in Sezary syndrome (SS) patients.
Aims: To assess meaningful change on patient reported outcomes (PRO) for mogamulizumab *versus* vorinostat.
Methods: A large, multicenter Phase 3 trial compared mogamulizumab (MOGA) *vs* vorinostat (VOR) in patients with Stage IB-IV CTCL who had failed ≥1 systemic therapy. Progression free survival was the primary endpoint. Validated QoL measurements included the Skindex-29, Functional Assessment of Cancer Therapy-General (FACT-G) and EuroQol-5D-3L (EQ-5D-3L). Skindex-29 and FACT-G are reported here. Longitudinal modeling of symptoms, function, and QoL subdomains were evaluated using longitudinal mixed models on prespecified covariates. Meaningful change threshold (MCT) was evaluated and categorical change analyzed by group over time. Time to clinically meaningful symptom worsening was defined using distribution-based minimally important difference thresholds.
Results: 372 patients were randomized (186 in each arm). MOGA resulted in symptomatic and functional improvement with differences in Skindex-29 Symptoms (Cycles 3, 5, and 7; $p<0.05$) and Functional (Cycles 3 and 5; $p<0.05$) scales. The proportion of patients who improved by at least the MCT from baseline was significantly greater for MOGA *vs* VOR on Skindex-29 Symptoms at Cycle 3 (61.1% *vs* 45.3%), Cycle 5 (64.5% *vs* 42.4%), Cycle 7 (67.1% *vs* 47.5%), and Cycle 11 (84.1% *vs* 50.0%) and Skindex-29 Functioning domain at Cycle 5 (54.3% *vs* 28.8%). Significant difference in the FACT-G Physical Well-Being scale (Cycles 1, 3, and 5; $p<0.05$) were observed in favor of MOGA and a greater proportion of patients declined by at least the MCT in favor of MOGA *vs* VOR at Cycle 1 (19.3% *vs* 34.7%), Cycle 3 (17.4% *vs* 42.9%), Cycle 5 (13.1% *vs* 43.3%), and Cycle 7 (15.9% *vs* 37.5%). The median time to worsening of symptoms on Skindex-29 was 27.4 m for MOGA *vs* 6.6 m for Vor. In SS patients, the median time to worsening varied in favor of MOGA ($P<.005$) on all Skindex-29 domains. In mycosis fungoides (MF) patients, time to worsening on Skindex-29 did not vary between arms.
Summary and Conclusions: Symptoms, function, and overall QoL of CTCL patients favored MOGA over VOR across study time points in the MAVORIC trial. Patients with the highest symptom burden and functional impairment derived the most QoL benefit from MOGA.

PF265

ANALYSIS OF CNS RELAPSES RISK FACTORS IN A POPULATION OF 1615 UPFRONT DLBCL PATIENTS TREATED IN LYSA PROTOCOLS LNH03 AND LNH07-3B

S. Bernard[1,*], N. Mounier[2], N. Ketterer[3], C. Recher [4], O. Fitoussi[5], R. Delarue[6], O. Casanovas[7], F. Morschhauser[8], T. Petrella[9], C. Copie [10], A. Bosly[11], C. Gisselbrecht[12], C. Haioun[13], T.-J. Molina[14], H. Tilly[15], C. Thieblemont[16]

[1]Hemato-oncology, Hôpital Saint Louis - APHP, PARIS, [2]Hemato-oncology, CHU Nice, Nice, France, [3]Oncology, Clinique Bois Cerf, Lausanne, Switzerland, [4]IUCT Oncopole, Toulouse, [5]Polyclinique Bordeaux Nord, Bordeaux, [6]Hematology, Hopital Necker, Paris, [7]Hematology, CHU, Dijon, [8]Hematology, CHU, Lille, [9]Pathology, CHU, Dijon, [10]Pathology, Hopital Mondor, Créteil, France, [11]Hematology, UCL Mont Godinne, Yvoir, Belgium, [12]LYSARC, LYON, [13]Hematolgy, CHU Mondor, Créteil, [14]Pathology, Hopital Necker, Paris, [15]Hematology, Centre Henri Bequerel, Rouen, [16]Hematology, Hopital Saint Louis, Paris, France

Background: Central Nervous system (CNS) relapse is a rare event in diffuse large B-cell lymphoma population (DLBCL), and is associated with a very poor prognosis. Indentification of CNS relapse risk factors has long been a subject of clinical research because of the fatal issue of these relapses. A better identification of high risk patients allow clinicians to stratify DLBCL patients and to propose optimized CNS relapses prophylaxis and thus, potentially, decrease the relapse rate.
Aims: The main objective of our study was to analyse CNS relapse risk factors in a population of 1615 *de novo* DLBCL patients, aged 18-80, and included in 6 prospective LYSA phase III studies. Secondary objectives were overall survival and progression-free survival datas of patients with CNS relapses.
Methods: Clinical, biological and histological data at diagnosis, treatments and prophylaxis received were collected and analyzed. The primary objective was the analysis of risk factors associated with CNS relapse in a cohort of patients treated in controlled trials. A statistical analysis was performed.
Results: Among the 1615 patients analyzed, 28 patients presented a CNS relapse (1.7%) and 330 presented another relapse (20.4%). Risk factors at diagnosis time significantly associated with CNS relapse were: age>60 years (p <.001), Performans Status 2-3 (p <.006), extranodal involvement>2 (p <.001), disseminated satge III / IV (p <.005), elevated LDH (p <.018), IPIaa 2-3 (p <.001), SNC-IPI score high risk(p <.001), renal involvment (p <.017), and protein expression of Cmyc>40% (p=0.003). In multi-variate analysis, high IPI, age>60 years, extranodal involvement >2 and a high CNS-IPI score (4-6) had an independent prognostic value. A high CNS-IPI score (>4) was associated with an independent risk of relapse or cerebral relapse in competitive models with HR=3.00 [1.28-7.01], P=0.011 for CNS and HR relapses=1.40 [1.07-1.84], P=0.014 for death due to cerebral relapse. The onset of CNS relapse occurred in 6.2% of patients with a high CNS-IPI score *vs* 2.3% of intermediate-risk patients and 0.2% of low-risk patients. The median OS of patients with CNS relapse was 11 months (not reached in the population without relapse), and the median PFS was 6.6 months.
Summary and Conclusions: This study confirms the predictive value of the CNS-IPI score and defines a population of patients with a high risk of CNS relapse.The poor prognosis of this population and the identification of a high risk population must lead to an optimization of CNS prophylaxis for high risk patients.

PF266

SUBSTITUTION OF CISPLATIN FOR CARBOPLATIN OR OXALIPLATIN IN THE DHAP REGIMEN IS ASSOCIATED WITH IMPROVED SURVIVAL IN RELAPSED/REFRACTORY NON-HODGKIN'S LYMPHOMAS

E. Martin[1,*]

[1]Department of Hematology, University Hospital of Tours, Tours, France

Background: Salvage of relapsed/refractory non-Hodgkin's lymphomas (NHL) is a therapeutic challenge, the multiple chemotherapy regimens published contrasting with the few comparative studies available. The DHAP (Dexamethasone, High-dose cytArabine and cisPlatine) regimen is commonly used in such cases. However, cisplatin nephrotoxicity is a major issue, which has been circumvented by the use of other platinum-related drugs such as carboplatin or oxaliplatin. However, evidence for a comparable efficacy and tolerance is lacking.
Aims: The aim of this retrospective multicenter study was to compare the effectiveness of cisplatin with that of oxaliplatin and carboplatin, in terms of complete response (CR), progression free-survival (PFS) and overall survival (OS).
Methods: Twenty-four volunteering centers of the LYSA group retrieved the medical records of patients treated between January 1, 2007 and December 31, 2012, with R-DHAP 100mg/m^2, R-DHAOx (Oxaliplatin) 130mg/m^2 or R-DHAC (Carboplatin) 5 Area Under Curve at day 1, with 21-day intervals. The study retrospectively included patients over 18 years old who received platinum salt for relapsed or refractory follicular lymphoma, transformed follicular lymphoma, other transformed indolent lymphoma or diffuse large B-cells lymphoma. A standardized electronic case report form (CRF) was set up and completed by a single clinical research assistant (CRA) who visited the centers and controlled the accuracy of all data. Comorbidities were retrieved according to medical records. An analysis adjusted on comorbidities, age, sex, number of previous treatment lines, FLIPI or IPI and histology, was planned.
Results: After exclusion of 235 observations with missing data, 462 patients were evaluable. Of them, 173 were treated with R-DHAP, 75 with R-DHAC, and 214 with R-DHAOx. Because of the specific toxicity profile of cisplatin, patients treated with R-DHAC and R-DHAOx were merged in a "non-cisplatin" group. Patients in this "non-cisplatin" group were significantly older (58.5y *vs* 54.8y, p<0.01). There was no statistical difference between the two groups for sex, IPI or FLIPI severity, comorbidities, histology and number of previous lines. The CR rate was similar, 43% in the both group (p=0.96). The PFS for the cisplatin and non-cisplatin groups was 16,8 and 18,9 months respectively (p=0.46). However, in multivariate analysis, with adjustment on age, FLIPI or IPI group, number of previous lines and comorbidities, the median OS was shorter in the cisplatin group compared to the non-cisplatin group (5,38y *vs* not reached, p=0.005). Other factors associated with death were number of previous treatment lines >1 (HR: 1.53, p=0.02), and FLIPI/IPI of 3 or above (HR: 1.56; p=0.007). In the FL group (n=140), there was no difference in term of PFS, but a statistically significant difference for OS (HR=3.2, p=0.03) in favour of non-cisplatin therapy. Similarly, in the DLBCL (n=238) group, there was no difference for PFS but a statistically significant difference in OS (HR=1.8, p=0,007) in favour of non-cisplatin therapy. However, there was no difference for PFS nor OS for transformed follicular (n=64) and other indolent transformed lymphomas (n=20). No statistical difference in the causes of death was noted (Figure 1).

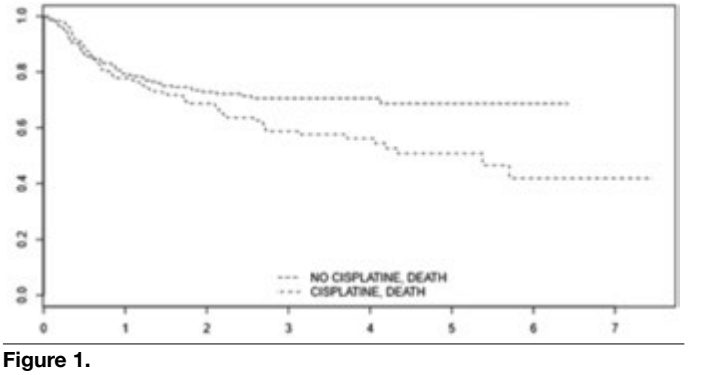

Figure 1.

Summary and Conclusions: This study suggests that when compared to cisplatin, the use of oxaliplatin and carboplatin yields a prolonged OS without differences in CR nor PFS, especially in FL and DLBCL. A corollary is that cisplatin can efficiently be replaced by other platinum salts in the treatment of NHL.

PF267

VCAP-AMP-VECP *VERSUS* CHOP AS FIRST-LINE CHEMOTHERAPY FOR AGGRESSIVE ADULT T-CELL LEUKEMIA-LYMPHOMA: A PROPENSITY SCORE ANALYSIS

S. Fuji[1,*], T. Yamaguchi[2], Y. Inoue[3], A. Utsunomiya[4], Y. Moriuchi[5], S. Owatari[6], T. Miyagi[7], Y. Sawayama[8], E. Otsuka[9], S.-I. Yoshida[10], T. Fukuda[11]

[1]Department of Hematolgoy, Osaka International Cancer Institute, Osaka, [2]Division of Biostatistics, Tohoku University Graduate School of Medicine, Sendai, [3]Department of Hematology, Kumamoto University Hospital, Kumamoto, [4]Department of Hematology, Imamura General Hospital, Kagoshima, [5]Department of Hematology, Sasebo City General Hospital, Sasebo, [6]Department of Hematology, National Hospital Organization Kagoshima Medical Center, Kagoshima, [7]Department of Hematology, Heart-Life Hospital, Okinawa, [8]Department of Hematology, Nagasaki University Hospital, [9]Department of Hematology, Oita Prefectural Hospital, Oita, [10]Department of Hematology, National Hospital Organization Nagasaki Medical Center, Nagasaki, [11]Department of Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan

Background: Adult T-cell leukemia-lymphoma (ATL) is a distinct type of peripheral T-cell lymphoma (PTCL) caused by human T-cell lymphotropic virus type I. Patients with aggressive ATL have poor outcomes with intensive chemotherapy. To improve the clinical outcome in patients with aggressive ATL, dose-intensified multiagent chemotherapy called VCAP-AMP-VECP as first-line therapy was investigated in Japan, and a prospective randomized controlled study comparing biweekly CHOP with VCAP-AMP-VECP regimen demonstrated that the probability of overall survival (OS) at 3 years and CR rate with VCAP-AMP-VECP were higher compared with biweekly CHOP. However, the previous RCT was rather small, and there was no subsequent study to confirm the benefit of VCAP-AMP-VECP over CHOP.
Aims: The objective of the current study was to compare clinical outcomes of patients with aggressive ATL who were treated with front-line VCAP-AMP-VECP with those who were treated with front-line CHOP.
Methods: Using a database of a nationwide survey of patients with aggres-

sive ATL aged 70 years or younger, we conducted a retrospective analysis including patients only who received front-line VCAP-AMP-VECP or front-line CHOP. A propensity score analysis was used to balance patient characteristics.
Results: Overall, 947 patients and 513 patients were treated with VCAP-AMP-VECP and CHOP, respectively. The median follow-up of surviving patients was 1006 days. The probabilities of 2-year OS for patients in the VCAP-AMP-VECP and CHOP groups were 31.2% (95% CI, 28.2–34.3%) and 24.6% (95% CI, 20.7–28.7%), respectively (P <0.001). Using the IPTW method to compare the probability of OS, treatment with VCAP-AMP-VECP was associated with a significantly higher OS rate (HR 0.82; 95% CI 0.71–-0.94, P=0.003). Stratified by risk group according to modified ATL-PI score at diagnosis, the probabilities of 2-year OS in the VCAP-AMP-VECP and CHOP groups were 39.8% (95% CI, 33.0–46.5%) and 45.0% (95% CI, 35.5–54.0%) in the low-risk group (P=0.69), 32.2% (95% CI, 28.2–36.3%) and 21.6% (95% CI, 16.8–26.7%) in the intermediate-risk group (P <0.001), and 17.2% (95% CI, 11.8–23.4%) and 6.2% (95% CI, 2.2–13.4) in the high-risk group (P=0.005). Using the IPTW method, treatment with VCAP-AMP-VECP was associated with a favorable OS in the intermediate- and high-risk groups, but not in the low-risk group (low-risk group, HR 0.95, 95% CI 0.72–1.24, P=0.690; intermediate-risk group, HR 0.71, 95% CI 0.61–0.84, P <0.001; high-risk group, HR 0.67, 95% CI 0.51–0.89, P=0.005).
Summary and Conclusions: In conclusion, our current analysis incorporating propensity score analysis reinforced the recommendation of VCAP-AMP-VECP regimen as first-line therapy in patients aged up to 70 years with aggressive ATL in the intermediate- and high-risk groups.

PF268

POSSIBLE FACTORS ON EFFICACY AND SAFETY OF CAR–T THERAPY IN RELAPSED OR REFRACTORY AGGRESSIVE B-CELL LYMPHOMA

H. Huang[1,*], Y. Jiang[1], Z. Jln[1], C. Li[1], D. Wu[1]

[1]Department of Hematology, The First Affiliated Hospital of Soochow University, Suzhou, China

Background: Recent advances have improved the treatment of B-cell malignancies, but patients who have disease resistant to primary or salvage treatment or who relapse after transplantation have an extremely poor prognosis. Studies of chimeric antigen receptor T-cell (CAR-T) therapy have shown high response rates and long response duration in refractory B-cell lymphomas after the failure of conventional therapy, which suggest that this therapy may be potentially curative.
Aims: To explore the possible factors on efficacy and safety of CAR T-Cell therapy in relapsed or refractory aggressive B-cell lymphomas.
Methods: From March 2017 to January 2018, 20 patients were enrolled into our clinical trialNCT03196830). Different targets of CAR T-cells were infused, including only anti-CD19 (n=8), only anti-CD20 (n=2), combined anti-CD20 and anti-CD19 (n=7), combined anti-CD19 and anti-CD22 (n=3). Patients received conditioning treatment (low-dose cyclophosphamide, 300 mg/m² per day, and fludarabine, 30 mg/m² per day) on days -5, -4, and -3 before the administration of autologous CAR T-cells. The primary endpoint was the proportion of patients with an objective response.
Results: Among the 20 patients who were enrolled, the median age was 56 years (range, 27 to 68). Diffuse large B-cell lymphoma was the underlying disease in 16 patients, Burkitt lymphoma in 1, mantle cell lymphoma in 1, Richter's syndrome in 1, and transformed follicular lymphoma in 1. All of the patients received at least three lines of therapy.Before receive CAR-T therapy, 4/20 patients were in complete remission(CR), 4/20 were in partial remission(PR), and 12/20 were in stable disease(SD) or progressive disease(PD). At the time of the primary analysis (February 15, 2018), 6 of 9 evaluable patients who were in SD or PD had an objective response, with 3 (33%) achieving CR a and 3 (33%) achieving a PR, of which 4 patients had an ongoing response (from 2 to 10 months). 7 of 7 evaluable patients in CR or PR had an ongoing response. With a median follow-up of 4 months, including 16 evaluable patients showed a median progression-free survival of 4 months. The most common adverse events during treatment were pyrexia in 70% patients, hypogammaglobulin in 65% patients, neuropenia in 40% patients, and hypotension in 25% patients. The cytokine release syndrome(CRS) occurred in 14 patients (70%). 57% patients were of low grade, and 43% were of grade 3 or higher (21% of grade 3, 7% of grade 4, and 14% of grade 5). Grade 3 or higher neurologic events occurred in 15% of the patients. The total number of infused CAR-T cells and single or double target of CAR-T cells had no significant correlation with survival, efficacy or grade of cytokine release syndrome. Serum biochemical index analysis confirmed associations of interleukin-2, and -4 with the grade 3 or higher neurologic events, and the level of lactate dehydrogenase(LDH) before therapy with grade 3 or higher CRS.
Summary and Conclusions: Our study demonstrates the efficacy and safety of CAR-T therapy in relapsed or refractory aggressive B-cell lymphoma. The level of LDH before therapy was higher in patients who developed grade 3 or serious CRS, which suggest that we should improve safety by reducing tumor burden before CAR T-cells infusion. Due to the small number of enrolled cases, no significant improvement of efficacy was observed when anti-CD19 CAR T-cells was combined with anti-CD20 or anti-CD22 CAR T-cells. This result needs to be further confirmed by expanding the number of study cases.

PF269

TITLE: LENALIDOMIDE PLUS R-CHOP THERAPY PROVIDES DURABLE LONG-TERM REMISSIONS IN PATIENTS WITH NEWLY DIAGNOSED DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL): COMBINED ANALYSIS FROM TWO PHASE 2 TRIALS

A. Castellino[1,2,*], B.R. LaPlant[2], L. Pederson[2], A. Chiappella[1], G. Gaidano[3], W.R. Macon[4], A. Tucci[5], R.L. King[4], A. Congiu[6], T.E. Witzig[2], U. Vitolo[1], G.S. Nowakowski[2]

[1]Hematology, Citta' della salute e della Scienza, Torino, Italy, [2]Hematology, Mayo Clinic, Rochester, United States, [3]Hematology, Hospital and University of Novara, Novara, Italy, [4]Pathology, Mayo Clinic, Rochester, United States, [5]Hematology, Ospedali Civili, Brescia, [6]Hematology, Ospedale San Martino, Genova, Italy

Background: The combination of lenalidomide (Len) with Rituximab-CHOP (R2CHOP21) has been shown to be safe and effective. These early results [Nowakowski *et al.* JCO 2014, Vitolo *et al.* Lancet Oncol 2014] led to two currently ongoing randomized trials. However, long term efficacy and safety have not been reported.
Aims: The aim of the present analysis is to present the long-term FU of efficacy and safety in DLBCL patients (pts) who received R2CHOP21 in two independent phase 2 studies.
Methods: We included newly diagnosed histologically-confirmed *de-novo* DLBCL pts enrolled in two R2CHOP21 phase 2 trials, conducted by Mayo Clinic (MC) and Fondazione Italiana LinfomiItalian Lymphoma Foundation (FIL). All pts received R-CHOP21 plus Len at 25 mg/day for 10 days/cycle and 15 mg/day for 14 days/cycle in MC and FIL trial respectively. We analyzed the long term FU outcome in terms of progression-free survival (PFS), time to progression (TTP), overall survival (OS) and the cumulative incidence of late toxicities and second tumors.
Results: 108 DLBCL pts (59 MC, 49 FIL) were included. Median age was 69 years (y) (range 22-87), with 48 (44.4%) and 4 (3.7%) pts over 70 y and over 80 y respectively. Main characteristics were: male 65 (60.2%) pts, advanced stage III-IV in 94 (87.0%) pts, B symptoms in 38 (35.2%), International Prognostic Index (IPI) intermediate-low in 42 (38.5%), intermediate-high/high in 60 (61.5%), central nervous system (CNS)-IPI intermediate risk in 79 (73.1%) and high risk in 22 (20.4%). As for Cell of origin (COO), germinal center (GCB) phenotype *vs* non-GCB were 45 (41.7%) *vs* 41 (38%) pts respectively; 22 (20.4%) pts were not evaluable for COO. At a median FU of 5.1 years (y), 5y PFS was 65.4%, 5y TTP 69.9% and 5y OS 77.4% (Figure 1). In total 31 relapses were observed, with only 2 cases of CNS recurrences and only 4 relapses occurring beyond 3y. Outcome results in a subgroup analysis performed stratifying patients according to COO phenotype were: 5y PFS 55.8% *vs* 65.7%, 5y TTP 62.3 *vs* 68.0 and 5y OS 71.7% *vs* 75.3% in germinal center (GCB) phenotype *vs* non-GCB respectively. Only 4 pts experienced grade (gr) 4-5 late toxicities (one toxic death because of a gr 5 sepsis and 3 cases of gr 4 persistent neutropenia). Milder toxicities were infections (N 5, only 1 gr 3), thrombosis (N 1, gr 2) and persistent neuropathy (N 3 (2.8%), all gr 1-2). 3 cases of cardiovascular disease gr 3 were reported. Secondary malignancies were observed in 8 pts (6.4%): 1 (0.9%) acute myeloid leukemia, 2 (1.8%) second lymphoma (T-cell) and 5 (4.5%) other solid tumors.
Summary and Conclusions: Long term FU shows that R2CHOP21 efficacy was maintained over time with high rate of PFS, TTP and OS, considering high risk features of patients included. The addition of len to RCHOP appears to mitigate the negative prognostic impact of non-GCB phenotype. The incidence of second tumors was low and no new worrisome long-term safety signals were seen. Despite a large number of intermediate/high CNS-IPI pts, CNS recurrences were less than expected, suggesting a role of len combination with R-CHOP in decrease risk of CNS involvement. These long -term efficacy and safety data will aid interpretation of early results from randomized clinical trials, expected to be reported in near future.

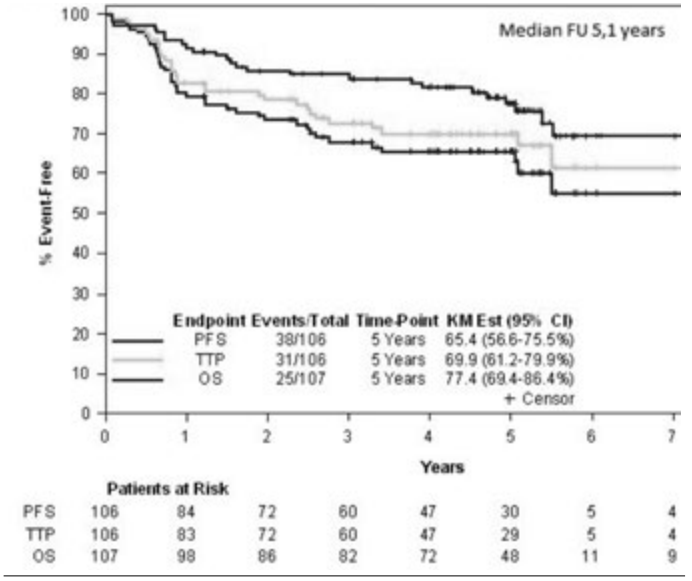

Figure 1. 5y PFS, TTP, OS in whole cohort DLBCL patients treated with R2CHOP.

PF270

DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) PRESENTING WITH SYNCRHONOUS CNS AND SYSTEMIC DISEASE AT DIAGNOSIS: RESULTS FROM AN INTERNATIONAL COLLABORATIVE STUDY

J. Wight[1,*], L. Churilov[2], M. Yue[3], C. Keane[3], A. Johnston[45], K. Linton [6], C. Chin[7], S.H. Wai[8,9], C. Coombes[10], C. Cheah[11], G. Gregory[9], D. Talaulikar[10], E. Hawkes[8,12,13]

[1]Haematology, Austin Health, Heidleberg, [2]University of Melbourne, Melbourne, [3]Princess Alexandra Hospital, Brisbane, Australia, [4]The Christie Hospital, Manchester, United Kingdom, [5]Royal Hobart Hospital, Hobart, Australia, [6]Division of Cancer Sciences, University of Manchester Manchester Cancer Research Research Centre, Manchester, United Kingdom, [7]Haematology, Sir Charles Gairdner Hospital, Perth, [8]Austin Health, Heidleberg, [9]Monash Health, Clayton, [10]Canberra Hospital, Canberra, [11]Sir Charles Gairdner Hospital, Perth, [12]Box Hill Hospital, Box Hill, [13]La Trobe University, Melbourne, Australia

Background: DLBCL presenting with both CNS and systemic disease at first diagnosis is rare. Such patients are excluded from clinical trials; thus, the optimal treatment is unknown and outcomes are poorly described.
Aims: To describe treatment outcomes of patients with synchronous CNS and systemic DLBCL at first diagnosis.
Methods: Multicentre retrospective international study (6 Australian & UK sites). Cases were identified from clinical and pharmacy records. Eligible patients had histologically proven DLBCL, with radiological, histological, or CSF evidence of synchronous systemic & CNS disease, treated with combination chemotherapy and rituximab. Patients with relapsed disease were excluded. Primary Endpoint: OS. Secondary endpoints: CR rate, PFS, toxicity. P values of <0.05 were considered significant.
Results: Of 59 patients, 71% were male and the median age was 66yrs (range 17-86). 45 (76%) had NCCN-IPI ≥4. Median number of extranodal sites outside the CNS was 2 (range 0-8). 10% were double-hit by FISH, and 35% of those with data available were double-expressors of MYC and BCL2 protein. CNS disease was leptomeningeal only in 24 (41%); 35 (59%) had parenchymal disease, 8 (14%) had both. 34 (58%) received systemic therapy (predominantly R-CHOP, n=31) plus a CNS-directed treatment (group A). 25 (42%) underwent intensified MTX and/or Ara-C containing therapy: hyper-CVAD n=14, CODOX-M/IVAC n=10, DHAC=1 (group B). CNS-directed therapy in group A included: IV HD-MTX in 19 (56%), HD-MTX+Ara-C in 2 (6%), intrathecal therapy (IT) only in 10 (29%), radiotherapy (RT) only in 2 (6%). Specific CNS therapy was omitted in one patient due to early PD. Additional consolidative therapy included CNS RT in 18 (31%) (whole brain in 8, site-specific in 10), and autologous SCT in CR1 in 8 (13%) using BEAM (n=4) or BCNU+thiotepa (n=4) conditioning. All SCT patients were from group B. 23 (39%) required dose reductions and 23 (39%) required early cessation of therapy. Treatment-related mortality was 14% for the whole group (4 in each group, including 2 during transplant). End of treatment CR rate was 58%; 62% for group A and 57% for group B (p=0.69). 25% were primary refractory; 26% in group A and 20% in group B (p=0.68). Site of relapse was: CNS only in 14, systemic only in 8, both in 6. Incidence rate of CNS progression at 2 years was 40%. 19/20 patients with CNS relapse died with median OS of 7.2 months (mo). Of the CNS relapses, 12 occurred in group A and 8 in group B (p=0.79). The estimated 2yr OS for those without CNS relapse was 74%. Median OS: Whole cohort=11.1 mo, group A=11 mo, group B=11.8 mo (p=0.82). Median PFS: Whole cohort=10.1 mo, Group A=9.6 mo, Group B=10.6 mo B (p=0.65). The OS of transplanted *vs* non-transplanted was similar (p=0.18); 5/8 transplanted patients remain disease free with >1 year of follow-up. There was no survival difference between parenchymal *vs* leptomeningeal disease (p=0.56). The estimated 2yr PFS and OS was 41%, and 52% respectively, with no significant differences between groups (p=0.64, Figure 1).

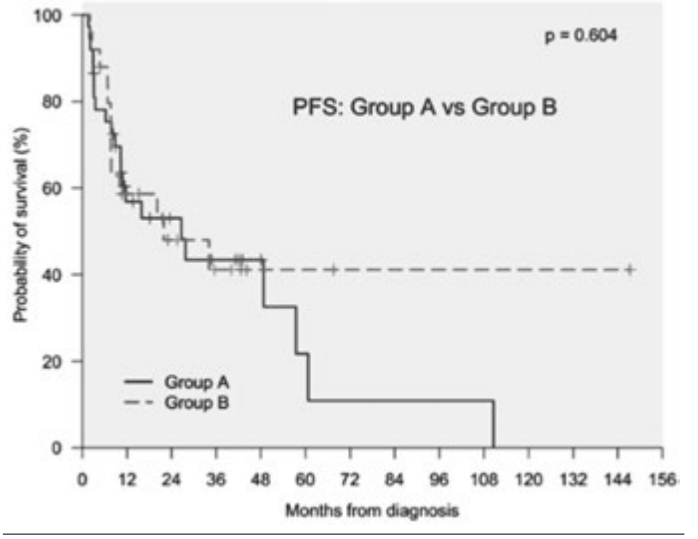

Figure 1.

Summary and Conclusions: Among this large cohort of synchronous+systemic DLBCL, intensified regimens and additional consolidation therapy were not associated with clinical benefit. The CNS is the most common site of treatment failure, which is associated with a dismal prognosis. OS is comparable to PCNSL (Ferreri, 2009), but those who achieve CNS control have 2yr outcomes similar to systemic DLBCL (Cunningham, 2013). Future efforts should focus on preventing CNS relapse. Biomarker tissue analysis is underway.

PF271

FRONT-LINE TREATMENT OF DLBCL WITH 6 *VERSUS* 8 CYCLES OF R-CHOP IS NOT ASSOCIATED WITH INFERIOR SURVIVAL IN ELDERLY PATIENTS: RESULTS FROM A RETROSPECTIVE ANALYSIS OF THE SEER-MEDICARE DATABASE

A. Shang[1,*], J. Black[1], A. Surinach[2], A. Kotb[1]

[1]F. Hoffmann-La Roche Ltd, Basel, Switzerland, [2]Genesis Research, Hoboken, New Jersey, United States

Background: Diffuse large B-cell lymphoma (DLBCL) is the most common histologic subtype of non-Hodgkin lymphoma (NHL). Immunochemotherapy with R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisone) is standard of care treatment for previously untreated DLBCL pts. Clinical practice guidelines recommend 6 to 8 cycles of R-CHOP for first-line treatment. In the United States (US) and Europe, many physicians prescribe 6 cycles, and elderly pts may benefit from this less intensive therapy. In this retrospective analysis, real-world use of R-CHOP as front-line DLBCL treatment for elderly pts in the US was evaluated using the linked Surveillance, Epidemiology and End Results (SEER)-Medicare database.
Aims: To compare overall survival (OS) with 8 *versus* 6 cycles of R-CHOP in previously untreated elderly pts with DLBCL.
Methods: SEER-Medicare data (2004 to 2011) for pts aged >65 years with a primary diagnosis of DLBCL who received R-CHOP after their initial diagnosis were analysed. Descriptive analyses were performed for R-CHOP and baseline characteristics. Multivariable Cox Regression (MCR) was used to estimate the HR of OS between 8 and 6 cycles of R-CHOP by adjusting for factors that may affect pts' health status (*e.g.* age, National Cancer Insti-

tute [NCI] comorbidity index, Ann Arbor stage, previous G-CSF treatment). To obtain better estimates of the treatment effect on survival, we also adjusted for occurrence of emergency department (ED) visits and inpatient stays prior to treatment and use of durable medical equipment (DME; *e.g.* wheelchairs). Multiple sensitivity analyses were performed.

Results: A total of 3953 pts received R-CHOP. Most pts (70.1%; n=2773) completed <6 cycles; only 955 pts (24.2%; median age 74 years) completed 6 cycles and 112 pts (2.8%; median age 72 years) completed 8 cycles. Of those pts with available baseline data who completed 8 cycles, a slightly higher proportion had stage III/IV disease (57%, 63/111) compared with 6 cycles (49%, 463/948). The median NCI comorbidity score was zero in both groups. Prior to treatment, more pts who completed 8 cycles had an inpatient stay (41%, 45/111) and/or ED visit (38%, 42/111), or required the use of DME (56%, 62/111), than pts who completed 6 cycles (35%, 328/948; 29%, 274/948; and 38%, 364/948, respectively). Previous G-CSF treatment was infrequent in both groups (8 cycles: 1%, 1/111; 6 cycles: 1%, 11/948). Median (interquartile range) duration of follow up was 1531 days (914, 2280) in the 8 cycle group and 1481 days (857, 2267) in the 6 cycle group. The Kaplan-Meier curves of OS for 8 cycles and 6 cycles overlap with each other (Figure 1). In a multivariate analysis, OS rates for pts receiving 8 cycles were comparable to those of pts receiving 6 cycles (HR: 1.12; 95% CI: 0.81–1.57; p=0.38). Sensitivity analyses using the inverse probability treatment weighting method confirmed the primary analyses.

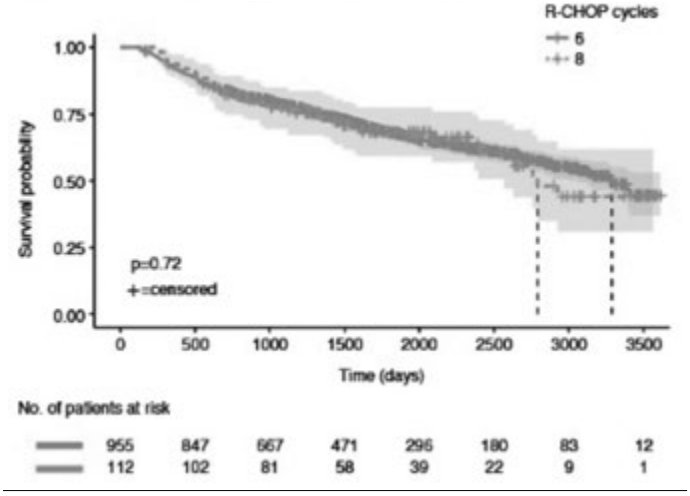

Figure 1. Overall survival probability over time by number of cycles of R-CHOP received.

Summary and Conclusions: Real-world data indicate that front-line DLBCL treatment with 6 *versus* 8 cycles of R-CHOP is not associated with inferior survival in elderly pts. Compared with 8 cycles, more elderly pts completed 6 cycles of R-CHOP as front-line treatment. Pts receiving 8 cycles tended to have more comorbidities than those receiving 6 cycles of R-CHOP. MCR suggests that pts receiving 8 cycles of R-CHOP may not benefit from longer OS *versus* pts receiving 6 cycles only. These findings confirm those recently reported from a similar analysis of real-world data using the Swedish and Danish lymphoma registries.

PF272

PRECLINICAL VALIDATION OF VECABRUTINIB (SNS-062) EFFICIENCY AGAINST BTK-C481S MUTATED LYMPHOMAS

C. Libre[1,*], L. Moro-Sibilot[1], P. Taverna[2], N. Bissay[1], G. Salles[1], L. Genestier[1], P. Sujobert[1]

[1]Centre de recherche en cancérologie de Lyon, Lyon, France, [2]Sunesis Pharmaceuticals, South San Francisco, United States

Background: The B cell receptor signaling pathway, and especially the Bruton Tyrosine Kinase (BTK), has become a therapeutic target in lymphoid malignancies. Ibrutinib, the first in class BTK inhibitor has proven to be highly efficient in chronic lymphocytic leukemia or mantle cell lymphoma, and is thought to be of potential interest in the Activated B cell subtype of diffuse large B cell lymphomas (ABC-DLBCL). However, the emergence of resistant clones has been described, especially through the p.C481S mutation of BTK that disrupts the covalent binding of ibrutinib to BTK. Given the very poor prognosis of ibrutinib resistant patients, the development of drugs able to inhibit BTK even in case of C481S mutation is of utmost importance. Vecabrutinib (formerly known as SNS-062) is a noncovalent, reversible inhibitor of BTK that retains activity against the C481S mutation at least in kinase assays.

Aims: The aim of this work was to assess the activity of vecabrutinib in models of ibrutinib resistant lymphoid malignancies.

Methods: First, we have tested *in vitro* the sensitivity of a panel of 6 lymphoma cell lines to ibrutinib and vecabrutinib (determination of the IC50 for apoptosis induction). Second, we have genetically modified 2 ibrutinib sensitive lymphoma cell lines (TMD8 and REC1) to overexpress either BTK WT and mCherry or BTK C481S and GFP. We performed 7 days co-culture competitive assays in the presence or absence of ibrutinib or vecabrutinib, and assessed by flow cytometry (GFP and mCherry) the clonal composition of the survivng cells.

Results: Lymphoma cell lines addicted to BTK signaling for survival (TMD8, REC1 and HBL1) were sensitive to vecabrutinib (IC 50 50-100nM), whereas cell lines not addicted to BTK signaling (RL, SUDHL4, Raji) were totally resistant to vecabrutinib (Figure 1). These results confirm that vecabrutinib is a highly specific BTK inhibitor. In competitive co-culture assays, we observed as expected that cell lines harboring the BTK C481S mutation were strongly selected under ibrutinib. Interestingly, this was not the case when we treated the cells with vecabrutinib, suggesting that vecabrutinib is able to overcome the resistance due to BTK C481S mutation. The validation of these results in the *in vivo* setting is ongoing and the results will be presented at the EHA congress. We will also present our mechanistic analysis of the modulation of the signaling pathways by vecabrutinib and ibrutinib in the different models of lymphoma.

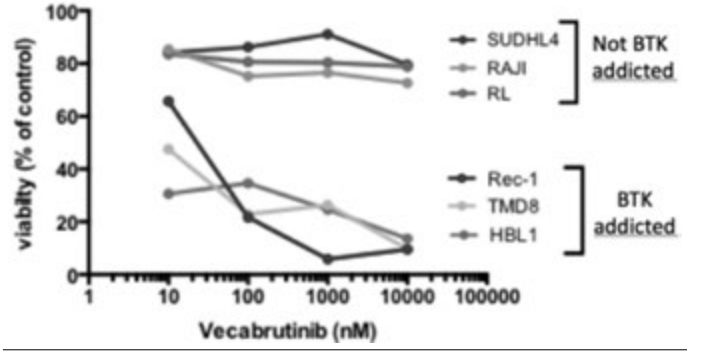

Figure 1.

Summary and Conclusions: These preclinical data suggest that vecabrutinib is a highly specific and potent drug that is able to overcome the resistance due to BTK C481S mutations. Given its very favorable pharmacokinetic and safety profile, a phase 1b/2 dose escalation and cohort expansion clinical trial has been recently launched in patients with previously treated B-lymphoid malignancies (NCT03037645).

PF273

MONITORING OF RHOA G17V MUTATION DURING THE COURSE OF THERAPY IN PATIENTS WITH ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA BY QUANTITATIVE ALLELE-SPECIFIC PCR WITH LNA-MODIFIED PRIMERS

Y. Sidorova[1,*], N. Chernova[1], M. Sinitsyna[1], M. Aleksenko[1], O. Glinshchikova[1], S. Smirnova[1], N. Ryzhikova[1], E. Nikulina[1], E. Zakharko[1], E. Rybkina[1], V. Dvirnyk[1], A. Kovrigina[1], E. Zvonkov[1], A. Sudarikov[1]

[1]National Research Center for Hematology, Moscow, Russian Federation

Background: Angioimmunoblastic T-cell lymphoma (AITL)characterized by a generalized tumor process and frequent extranodal involvement. In addition, approximately 2/3 of patients develop relapses in the first 2-3 years after therapy completion. Recently discovered point somatic RHOA Gly17Val mutation is present in 53-71% of angioimmunoblastic T-cell lymphomas and allows to quantify the tumor cells. We used allele-specific PCR with LNA (locked nucleotide acid) modified primers, which increase the sensitivity of the determination. LNA nucleotide has a greater binding ability in the complementary interaction and its presence at the 3 'end of the primer leads to an increase in the specificity of allele-specific amplification.

Aims: Development of a sensitive method for detection of RHOA Gly17Val mutation for the estimation of minimal residual disease (MRD).

Methods: RHOA Gly17Val mutation was analyzed by quantitative TaqMan allele-specific PCR with LNA-modified primers (qAS-PCR-LNA). Bone marrow (BM) and peripheral blood (PB) samples from 14 patients with AITL who achieved clinical and hematologic remission were studied at the diag-

nosis and during the therapy. Control points: before the start of therapy, after completion of induction chemotherapy, after 1, 2 years of maintenance therapy with small doses of cytotoxic drugs or immunomodulatory therapy, and also in some patients 3-4 years after the onset of the disease.
Results: The standard curve method based on serial dilutions showed that developed qAS-PCR-LNA quantitation method has 0.01% (10^{-4}) sensitivity (Figure 1A). The number of detectable cells with the mutation before and during therapy in blood (Figure 1C) was higher than in BM (Figure 1B). All examined patients achieved clinical remission after induction chemotherapy and they all had complete or partial molecular response. However, 10 of 14 patients had a tumor clone (0.02-2% of the total number of cells) after completion of induction chemotherapy in the blood and/or BM. During the maintenance therapy the number of tumor cells in PB/BM remained above 0.05% in two patients, and in three patients tumor cell growth was up to 0.15-0.3%. In patients No. 4 and No. 8 flow cytometry of blood sample confirmed the presence of tumor cells with a specific immunophenotype in the amount of 2.3% of lymphocytes (0.3% of all events) and 4.8% of lymphocytes (0.4% of all events). An increasing number of cells with a RHOA Gly17Val mutation may be a predictor of disease recurrence. The data obtained may be beneficial for the correction of antitumor therapy.

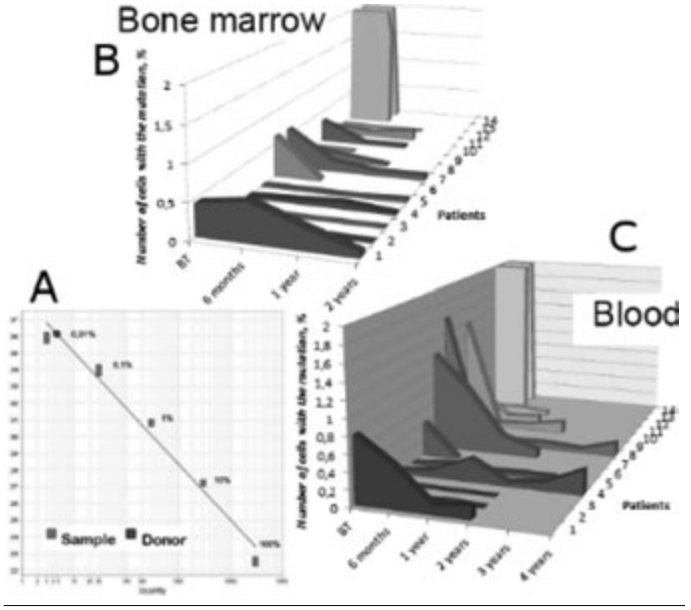

Figure 1.

Summary and Conclusions: The sensitivity of RHOA Gly17Val mutation quantification by LNA-modified primers is sufficient for MRD detection. The persistence of tumor cells with RHOA Gly17Val mutation was shown in most patients with AITL after the induction chemotherapy. This method should be included into the standard protocols for management of patients with AITL to monitor therapy efficacy and remission duration.

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CHARACTERISTICS, TREATMENT PATTERNS AND OUTCOME OF ELDERLY SYSTEMIC T/NK CELL LYMPHOMA IN A RESOURCE-LIMITED COUNTRY: THE RESULT OF THAILAND NATIONWIDE LYMPHOMA REGISTRY

K. Wudhikarn[1,*], U. Bunworasate[1], J. Julamanee[2], A. Lekhakula[2], S. Ekwattanakit[3], A. Khuhapinant[3], S. Chuncharunee[4], P. Niparuck[4], T. Numbenjapon[5], K. Prayongratana[5], N. Kanitsap[6], S. Wongkhantee[7], N. Makruasi[8], P. Wong[9], L. Norasetthada[10], W. Nawarawong[10], C. Sirijerachai[11], K. Chansung[11], T. Suwanban[12], P. Praditsuktavorn[13], T. Intragumtornchai[1]

[1]Medicine, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Bangkok, [2]Medicine, Prince of Songkla University, Songkhla, [3]Medicine, Siriraj Hospital, Mahidol University, [4]Medicine, Ramathibodi Hospital, Mahidol University, [5]Medicine, Phramongkutklao Hospital and College of Medicine, [6]Medicine, Thammasart University, Bangkok, [7]Medicine, Khonkaen Regional Hospital, Khon Kaen, [8]Medicine, Srinakharinwirot University, Nakhon Nayok, [9]Medicine, Naresuan University, Phitsanulok, [10]Medicine, Chiang Mai University, Chiang Mai, [11]Medicine, Khon Kaen University, Khon Kaen, [12]Medicine, Rajavithi Hospital, [13]Medicine, Chulabhorn Hospital, Bangkok, Thailand

Background: Systemic T/NK cell non-Hodgkin lymphoma (NHL) is an uncommon lymphoma subtypes but relatively more prevalent in Asia. Its natural history and prognosis generally fare worse compare to B cell NHL. However, data on systemic T/NK cell NHL especially in older patients was limitedly described. Proper management of this subgroup is not known and remains an unmet need. Herein, we reported clinical data, treatment delivery pattern and outcome of systemic T/NK cell NHL in the elderly from Thailand nationwide lymphoma registry.
Aims: To better characterize clinical data, treatment pattern, prognostic determinants and outcome of T/NK cell NHL in the eldery from real world setting.
Methods: From the nationwide multicenter registry of 4,371 newly diagnosed lymphoma patients in Thailand between 2007 and 2014, after excluding primary cutaneous T cell lymphoma, there were a total of 464 systemic T/NK cell lymphoma patients. We described clinical characteristics, treatment patterns and outcomes of PTCL in the elderly including exploring the effect of age and other clinical parameters affecting outcome in this group of patients.
Results: Of 464 systemic T/NK cell NHL, 127 (27.4%) were older than 60 years old. The median age was 67 years old (range; 60-91 years old) including 98 patients in 60–74 years and 29 patients in 75 years or older age group. Patients aged older than 75 years old had similar characteristics to 60-74 years old group except higher median score of Charlson's comorbidity index in patients aged older than 75 years. A total of 98 patients (77.2%) received chemotherapy. Of these, 79 patients (62.2%) received intensive multi-agent chemotherapy. The proportion of patients receiving intensive chemotherapy was lower in patients aged older than 75 years old (34.5% *vs* 70.4%, p<0.001). Among patients who received intensive chemotherapy, overall response rate was 58.2% (48% complete remission and 10.2% partial remission). After a median follow up of 17.3 months, 83 patients had died. Two-year progression free survival and overall survival (OS) for the entire cohort were 38.1% and 48.5% respectively. Univariate and multivariable analysis revealed that older age, poor performance status, high comorbidity index and absence of definite multi-agent chemotherapy were associated with inferior survival. Intensive multi-agent chemotherapy was independent prognostic factor for OS after adjusted for age, comorbidity index, performance status and prognostic index for T-cell lymphoma (p=0.04; Figure 1).

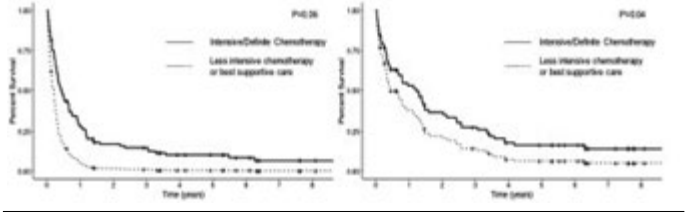

Figure 1.

Summary and Conclusions: Prognosis of T/NK cell lymphoma in elderly was dismal. Age, performance status and comorbidities were independent prognostic factors for survival. However, despite poor prognosis of elderly systemic T/NK cell NHL, chemotherapy could result in objective response and long term survival in selected patients thus emphasizing the importance of comprehensive geriatric evaluations. Incorporating geriatric assessment into management plan of elderly PTCL patients would be warranted.

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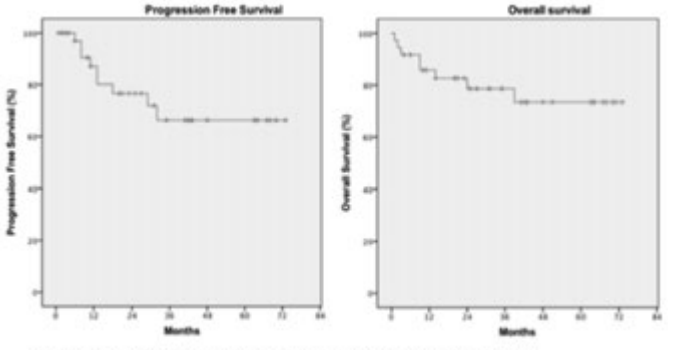
RETROSPECTIVE ANALYSIS OF PATIENTS WITH PRIMARY CENTRAL NERVOUS LYMPHOMAS TREATED WITH METHOTREXATE-BASED REGIMEN FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANT AND TEMOZOLOMIDE MAINTENANCE

M. Ljungqvist[1,2,*], F. Celsing[1], B. Wahlin[1,3], K. E. Smedby[1,2]

[1]Division of Hematology, Department of Medicine, Karolinska University Hospital, [2]Division of Clinical Epidemiology, Department of Medicine, Solna, [3]Division of Haematology, Department of Medicine, Huddinge, Karolinska Institutet, Stockholm, Sweden

Background: Primary central nervous lymphoma (PCNSL) is an uncommon lymphoma subtype with poor overall survival (OS). In clinical trials, long-term remissions have been achieved using high-dose methotrexate (HD-MTX)-based chemotherapy followed by intensive consolidation treatment. However, outcome of this regimen has seldom been evaluated in the population-based setting.
Aims: To retrospectively analyse outcome of patients with PCNSL treated,

in clinicla routine, with HD-MTX and Cytarabin followed by consolidation with high-dose chemotherapy and autologous stem-cell transplant (HDT-ASCT) and maintenance with temozolomide.
Methods: Patients aged 70 years or younger with newly diagnosed PCNSL at Karolinska University Hospital (Sweden) between 2011 and 2017 were eligible for treatment according to Arm B of IELSG32 trial. One cycle consisted of: MTX 3.5 mg/m² on day 1, Cytarabin 2 g/m² twice daily on days 2 and 3 and Rituximab 375mg/m². Patients with at least partial remission (PR) after 4 cycles of induction therapy received consolidation with HDT-ASC followed by maintenance with temozolomide 150mg/m² for five days, repeated every four weeks, for one year. During the study period, 46 patients aged ≤70 were diagnosed. Ten patients were not treated according to Arm B of IELSG32 trial; three patients were above the age of 65 and had an ECOG performance status of 3, two patients were above 65 years and had severe comorbidity, and two patients were below the age of 65 but had poor performance status (ECOG 3-4) and comorbidities. Main study endpoints were feasibility, progression-free survival (PFS) and OS.
Results: Altogether, 36 patients started treatment according to protocol. The median age at diagnosis was 59 years (range 35-70), with 6 (17%) being above 65 years of age. Twenty-three (64%) were men. Of the 36 patients with intention to treat according to IELSG32 trial, 26 patients (72%) received all four induction courses and 22 (61%) underwent HDT-ASCT. After a median follow-up time of 29 months (range 1-71 months), eight (22%) patients had died and nine (25%) had recurrent disease. One patient (3%) died from treatment complications, two (6%) from primary refractory lymphoma and five (14%) from recurrent disease. Median OS and PFS ware not reached during follow-up. The mean overall survival (OS) was 58 months (95% CI 48-67 months) and the mean PFS was 54 months (95% CI 44-64). The 3-year OS and PFS was 81% and 75% respectively (Figure 1).



Progression Free Survival and Overall Survival in 36 patients with Primary CNS-lymphoma starting treatment according to Arm B of IELSG32 trial.

Figure 1.

Summary and Conclusions: Intensive treatment with HD-MTX, Cytarabin and Rituximab followed by HDT-ASCT and maintenance with temozolomide is feasible and effective in clinical routine for fit patients with PCNSL diagnosed at 70 years or younger.

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RITUXIMAB, GEMCITABINE AND OXALIPLATIN (R-GEMOX) IN REFRACTORY/RELAPSED PATIENTS NOT CANDIDATES FOR HIGH-DOSE THERAPY WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL). A SINGLE CENTER STUDY OF 115 PATIENTS

C. Cazelles[1,*], K. Belhadj[2], N. Mounier[3], C. Copie-Bergman[4], A. Nicolae[4], F. Pirenne[5], A. Rahmouni[6], E. Itti[6], M. Carvalho[7], J. Dupuis[2], F. Lebras[2], F. Lemonnier[2], C. Salvado[2], E. Scherman[2], L. Roulin[2], T. El Gnaoui[2], P. Gaulard[4], C. Haioun[2]

[1]Hematology, Necker Hospital Paris, [2]Hematology, Henri Mondor Hospital, Paris, [3]Hematology, CHU Nice, Nice, [4]Pathology, [5]EFS, [6]Radiology, [7]Pharmacy, Henri Mondor Hospital, PARIS, France

Background: R-GemOx is one of the most common regimen used in France in relapsed/refractory patients with DLBCL non candidate for high dose therapy (HDT).
Aims: We here report the results of a single center retrospective study conducted, at Henri Mondor University Hospital, in order to evaluate the efficacy and good safety profile of this regimen in consecutive patients with DLBCL (*de novo* or transformed).
Methods: Between May 2002 and May 2017, 115 patients (pts) with refractory or relapsed DLBCL were treated. Forty-four pts received R-GemOx in first, 38 in second or subsequent relapses and 33 pts were refractory to the previous therapy. Treatment was given every 2 weeks as previously described (Mounier N. *et al.*[1]). Pts were planned to receive 8 cycles if at least a partial response (PR) was observed after 4 cycles. Median age was 71 years (range, 20-89). All pts have been previously treated with anthracyclines and rituximab and 17 (14.7%) were previously treated with consolidative HDT, 63 (53.9%) had an anteriority of indolent lymphoma. Age-adjusted international prognostic index at enrolment was >2 in 71 pts (62.7%). Median delay from last treatment to R-GemOx was 8 months (range 0.3-82). Median duration between initial diagnosis and R-GemOx was 12.8 months (range 0-185).
Results: 632 cycles were given. Median received dose intensities of gemcitabine and oxaliplatin were 68.9% and 67.8% of the theoretical dose, respectively. After 4 cycles, according to IWC (Cheson 1999 or 2007), the overall response rate (ORR) defined as the complete response (CR) plus PR was 60% and the CR rate was 28.6%. At the end of treatment, the ORR and CR rates were 29.6 and 26.9%, respectively. The median duration of response was 13 months (range 0-184 months). The CR rate at the end of treatment was significantly better for patients with a previous history of indolent lymphoma (32,3% *vs* 24,5%, p=0.0270). With a median follow-up of 17 months, progression-free survival and overall survival at 2 years were 36% (CI 95%=26.5-45.5%) and 17% (CI 95%=9.8-24%), respectively. Grade 3-4 toxicities occured in 35% of pts with 52 hospitalizations (8.2% of 632 cycles), including 16 hospitalizations for febrile neutropenia. The most common toxicities during treatment were hematological and were manageable. Thirty-four percent of pts required at least one red blood cell transfusion and 42% at least one platelet transfusion. Grade 1-2 peripheral neuropathy attributed to oxaliplatin was observed in 31% of pts, one patient had a grade 3 and dose reductions were required for 8% of pts. Two pts died during treatment from infection.
Summary and Conclusions: Given the patient characteristics in this real life study, results of R-GemOx show an ORR, a duration of response and a safety profile in line with published data. Improving outcome of pts with relapsed or refractory patients with DLBCL represents an unmet medical need. In the current context of development of more targeted therapies, such results could be improved by addition of new molecules, as it is proposed in the ongoing NIVEAU trial (NCT03366272).

Reference:

(1) Mounier N *et al.* Rituximab plus gemcitabine and oxaliplatin in patients with refractory/relapsed diffuse large B-cell lymphoma w ho are not candidates for high-dose therapy. A phase II LYSA trial. Haematologica. 2013 Nov;98(11):1726-31.

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THE HIGH EXPRESSION OF CD38 IS ASSOCIATED WITH POOR PROGNOSIS IN *DE NOVO* DIFFUSE LARGE B-CELL LYMPHOMA

K. Sueoka[1,*], T. Yabushita[2], H. Maruoka[1], Y. Shimomura[2], Y. Ono[2], N. Hiramoto[2], S. Yoshioka[2], N. Yonetani[2], A. Matsushita[2], H. Hashimoto[2], T. Ishikawa[2]

[1]Clinical Laboratory, [2]Hematology, Kobe City Medical Center General Hospital, Kobe, Japan

Background: Diffuse large B-cell lymphoma (DLBCL) is a heterogenous group of non-Hodgkin lymphomas which accounts for 30-40%. After the advent of rituximab, the survival of patients with DLBCL has apparently improved, but approximately one-third still have refractory or relapsed clinical course after standard R-CHOP. The progress of molecular technologies and targeted drugs has nearly shifted to subtype-specific treatment strategies for DLBCL. The surface antigen is one of the most important target in development of novel drugs for DLBCL. CD38 is one of the drugable surface antigens expressed on the surface of lymphoma cells with variable frequency in patients with DLBCL. On the other hand, the prognostic significance of CD38 for DLBCL remains elucidated in the immunochemotherapy era.
Aims: The purpose of this study was to estimate the prognostic impact of the high expression of CD38 in patients with DLBCL.
Methods: We retrospectively identified 109 consecutive patients diagnosed with *de novo* DLBCL at our institution and eligible for R-CHOP from January 2005 to June 2015. We excluded 28 patients with no available data of flow cytometry. The expression of CD38 on gated lymphoma cells (CD45brightCD19+FSC^{HIGH}) was evaluated using 6-color flow cytometry. The mean fluorescence intensity (MFI) for CD38 was calculated as the ratio of the fluorescence intensity of the test sample to that of the isotypic control. By the survival classification and regression tree (CART) analysis, we stratified *de novo* DLBCL patients into two groups: CD38^{HIGH} and CD38^{LOW}.The primary endpoint was 3-year overall survival (3-yr OS). The secondary end-

point was 3-year progression-free survival (3-yr PFS). OS and PFS were estimated using the log-rank test. Multivariate analysis using Cox proportional hazard ratio was performed for 3-yr OS. This study was approved by the institutional review board of the Ethics Committee and complied with the Declaration of Helsinki.

Results: Out of the 81 patients enrolled (median age: 69 years old), 46 patients (56.7%) had an advanced stage disease and 40 (49.3%) had a high-intermediate or high International Prognostic Index (IPI) score. The median follow-up was 37.8 months (2.0 – 58.8 months). The median MFI of CD38 were 5.41 (range: 0.97-78.2). The survival CART analysis revealed that the cutoff value for discrimination in 3-year OS was 6.307.We defined patients with MFI of CD38 over 6.307 as $CD38^{HIGH}$ group, and patients with MFI of CD38 less than this cutoff as $CD38^{LOW}$group. There was no statistical significance between $CD38^{HIGH}$ group (n=36) and $CD38^{LOW}$ group (n=45) in gender, age, laboratory data, number of extranodal lesions, bone involvement, stage or the presence of bulky lesions. Three-year OS and PFS were significantly better in the $CD38^{LOW}$group than in $CD38^{HIGH}$ group (Figure 1) (OS: 91.7% *vs* 66.3%, p=0.002; PFS: 70.3% *vs* 48.7%, p =0.014). In multivariate analysis adjusted by standard IPI risk, the high expression of CD38 was an independent prognostic factor for 3-yr OS (hazard ratio: 3.51; 95% CI: 1.06-11.62; p=0.04).

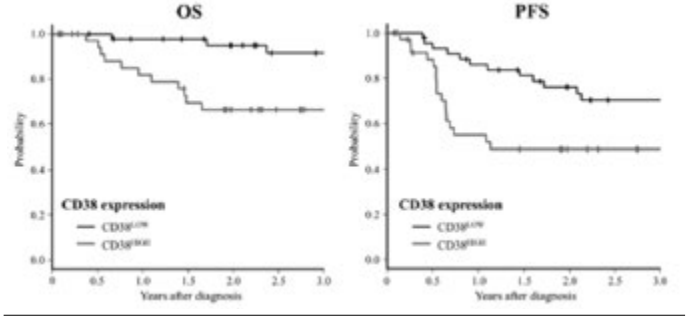

Figure 1.

Summary and Conclusions: In the present study, we demonstrated that the high expression of CD38 predicts poor clinical outcomes in newly diagnosed *de novo* DLBCL patients.

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CLINICOPATHOLOGIC ANALYSIS OF PROGRAMMED CELL DEATH 1 AND ITS LIGANDS PD-L1/PD-L2 EXPRESSIONS IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

C.-L. Cheng[1,*], S.-C. Yu[2,3], C.-W. Lin[3], H.-F. Tien[1]

[1]Internal Medicine, [2]National Taiwan University Hospital, Taipei, Taiwan, Republic of China, [3]Pathology, National Taiwan University Hospital, Taipei, Taiwan, Republic of China

Background: Primary central nervous system lymphoma (PCNSL) is a rare non-Hodgkin lymphoma confined to the CNS. PCNSL frequently exhibited copy number alterations and/or translocations involving 9p24.1, the locus containing the genes encoding programmed cell death-ligands 1 and 2 (PD-L1 and PD-L2). Besides, recent studies showed the effectiveness of programmed cell death-1 (PD-1) blockade therapy in relapsed/refractory PCNSL. The PD-1/PD-L1 and PD-L2 axis probably plays an important role in the tumoral biology of PCNSL. Up to now, little is known about the clinicopathologic features and prognostic implications of tumoral expressions of PD-1 and its ligands PD-L1/PD-L2 in patients with PCNSL.

Aims: To investigate the clinicopathologic characteristics of tumoral expressions of PD-1 and its ligands PD-L1/PD-L2, as well as their prognostic implications in immunocompetent patients with PCNSL.

Methods: We conducted a retrospective study in a tertiary referral hospital in Taiwan. From January 1996 to December 2016, patients with newly diagnosed CD20+ large B-cell PCNSL and had received high-dose methotrexate-based chemotherapy as the initial treatment were reviewed. Those without adequate samples for study or with HIV infection were excluded. We performed immunohistochemical analyses of tumoral PD-L1 and PD-L2 expression using a double-staining technique (PD-L1/PAX5 and PD-L2/PAX5, respectively). Except for PD-L1(+) or PD-L2(+) PCNSL, we defined an entity for microenvironmental PD-L1(+) (mPD-L1(+)) or mPD-L2(+) PCNSL which means PD-L1(-) or PD-L2(-) PCNSL in which PD-L1(+) or PD-L2(+) non-malignant cells are abundant in the tumor microenvironment. The number of PD-1(+) tumor-infiltrating lymphocytes (TILs) was also accessed. The cohort was followed up until the end of December 2017.

Results: Sixty-five patients were analyzed in our cohort, with a median age of 62.1 years and an equal sex ratio. The median overall survival and progression-free survival was 31.1 months and 12.7 months after a median follow-up time of 45 months. The prevalence rates of PD-L1(+) and mPD-L1(+) PCNSL were 15.4% and 27.7%, respectively. Interestingly, almost all of cases were PD-L2(+) PCNSL (61/65, 93.8%). Patients with PD-L1(+) PCNSL had a trend of poor ECOG performance status (P=0.303) compared to patients with mPD-L1(+) and PD-L1(-) PCNSL. Nevertheless, no significant difference was found in other clinical background comparisons. The tumoral expression of PD-L2 did not impact the clinical features. The number of PD-1(+) TILs was significantly higher in patients with age >60 years and in patients with CD79B exon 5 mutation. The quantity of PD-1 (+) TILs did not correlate with the level of PD-L1 or PD-L2 expression, respectively. Regarding to outcome, there were no significant differences in overall survival or progression-free survival according to the expression of PD-L1 or PD-L2, whether in tumor cells or in non-malignant cells, as well as the quantity of PD-1(+) TILs.

Summary and Conclusions: This is the first report to demonstrate the exact percentage of PD-L1(+) and PD-L2(+) tumor cells in PCNSL by PD-L1/PAX5 and PD-L2/PAX5 double immunostainings. The percentage of PD-L1(+) PCNSL is below the ratio we expected. In contrast, almost all patients were PD-L2(+) PCNSL. The tumoral expression of PD-1, PD-L1 or PD-L2 did not have prognostic implication in our cohort. Nevertheless, our results suggest that PD-L2 other than PD-L1 may be a more relevant biomarker and a potential therapeutic target in PCNSL due to its extensive expression in tumor cells.

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PROGNOSTIC FACTORS IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA (PMLBCL) UNDER RITUXIMAB-CHOP (R-CHOP) CHEMOTHERAPY WITH OR WITHOUT RADIOTHERAPY (RT)

T. Vassilakopoulos[1,*], S. Papageorgiou[2], M. Angelopoulou[1], Z. Galani[1], G. Kourti[3], C. Kalpadakis[4], M. Kotsopoulou[5], T. Leonidopoulou[6], P. Konstantinidou[7], I. Kotsianidis [8], M. Moschgiannis[9], D. Boutsis[10], E. Michali[11], M. Michail[12], E. Terpos[13], G. Karianakis[14], C. Poziopoulos[15], T. Ikonomopoulos[16], E. Vrakidou[14], K. Anargyrou[17], E. Vervessou[18], A. Simeonidis[19], E. Stefanoudaki[20], E. Hadjiharissi[21], M. Papaioannou[21], P. Repoussis[5], E. Megalakaki[5], T. Marinakis[11], T. Assimakopoulou[6], S. Sachanas[9], E. Katodritou[7], T. Karmiris[3], M. Siakantari[22], E. Variami[22], V. Pappa[2], E. Papadaki[4], M. A. Dimopoulos[13], K. Konstantopoulos[1], P. Panagiotidis[23], G. Pangalis[9], P. Roussou[24]

[1]Department of Haematology and Bone Morrow Transplantation, Laikon General Hospital, National and Kapodistrian University of Athens, [2]2nd Propedeutic Department of Internal Medicine, Attikon University Hospital, National & Kapodistrian University of Athens, [3]Department of Haematology, Evangelismos General Hospital, Athens, [4]Department of Haematology, Heraklion University Hospital, University of Crete, Heraklion, [5]Department of Haematology, METAXA Anticancer Hospital, Piraeus, [6]Department of Haematology, Sismanoglion Hospital, Athens, [7]Department of Haematology, Theagenion Anticancer Hospital, Thessaloniki, [8]Department of Haematology, Democritus University of Thrace, Alexandroupolis, [9]Department of Haematology, Athens Medical Center, Psychikon Branch, [10]Department of Haematology, Navy Hospital, [11]Department of Haematology, Athens General Hospital, Athens, Greece, [12]Department of Haematology, Nicosia General Hospital, Nicosia, Cyprus, [13]Department of Therapeutics, Alexandra Hospital, National and Kapodistrian University of Athens, [14]Department of Haematology, HYGEIA Hospital, Athens, [15]Department of Haematology, Metropolitan Hospital, Piraeus, [16]Department of Haematology, Athens Medical Center, [17]Department of Haematology, Air Force Hospital, [18]Department of Haematology, Er. Dynan Hospital, Athens, [19]Department of Haematology, University of Patras, Rio, [20]Department of Haematology, Al. Fleming Hospital, Athens, [21]Department of Haematology, AHEPA University Hospital, Thessaloniki, [22]1st Department of Internal Medicine, [23]1st Propaedeutic Department of Internal Medicine, Laikon General Hospital, National and Kapodistrian University of Athens, [24]3rd Department of Internal Medicine, Sotiria Chest Hospital, National and Kapodistrian University of Athens, Athens, Greece

Background: Prognostic factors (PFs) have not been extensively studied and prognostic models specifically applicable to PMLBCL have not been developed, mainly because PMLBCL is a rare entity. The International Prognostic Index (IPI) is applied based on the projection of DLBCL data, but its performance is questionable. R-CHOP provides satisfactory results in PMLBCL, minimizing failure rates. Only 2 moderately-sized studies have appeared regarding prognostic factors in PMLBCL after R-CHOP (96 and 123 patients from British Columbia and Japan). Given that more intensive

chemotherapy (R-da-EPOCH) might be better than R-CHOP, the applicability of various PFs, including the laboratory ones, needs to be urgently evaluated in the rituximab era in order to define subgroups of patients at high- or very low-risk for treatment failure and death

Aims: The identification of PFs for the outcome of patients with PMLBCL treated with RCHOP±RT.

Methods: 262 patients with PMLBCL were treated with RCHOP±RT (usually 6-8 cycles) in a multicenter setting in Greece and Cyprus (1 Center). The following potential prognostic factors were evaluated: Age (median 32; range 16-82; >60 years only 4%), gender (female 66%), B-symptoms (29%), stage III/IV (11%), infradiaphragmatic disease (6%), extranodal involvement (E or IV, 38%), pleuritis (32%), pericarditis (28%), any serositis (44%), bulky disease (≥10 cm; 60%), performance status (PS) ≥2 (15%), LDH levels (83%), anemia (39%), leukocytosis ≥10x10^9/L (25%), ESR ≥50 mm/h (40%), albumin <4 g/dL (45%), age-adjusted IPI (aaIPI; ≥2 in 20%).

Results: The median follow-up of currently alive patients was ~5 years (59 months, range 2-198). Among 61 failures, 58 occurred within 2 years (all within 4 years) from diagnosis. The 5-year freedom from progression (FFP) was 75%. With 30 deaths recorded (including 2 unrelated deaths), the 5-year overall survival (OS) was 87%. The aaIPI identified a very small minority of patients (aaIPI=3; 3% of total) with a 5-year FFP of 38% *vs* 74, 77% and 87%% for those with aaIPI 2,1 and 0 (p=0.02), being therefore of questionable value. On univariate analysis, any extranodal involvement (E/IV), elevated LDH, any serositis, bulky disease, infradiaphragmatic disease, leukocytosis and albumin <4 g/dL were significantly associated with inferior FFP. In multivariate analysis of FFP, any extranodal involvement and serositis were independent PFs (hazard ratios 2.2 and 1.9; p=0.01 and p=0.04 respectively). None, 1 or 2 of these factors were present in 41%, 36% and 24% of the patients with 5-year FFP of 90%, 69% and 60% (p=0.0001). OS (disease specific) at 5 years was 98%, 84% and 75% respectively (p=0.0005).

Summary and Conclusions: In the largest patient series reported so far, RCHOP±RT provided long-term disease control in 75% of patients with PMLBCL with an excellent OS of 87%. The aaIPI was moderately predictive of the outcome. The combination of any extranodal involvement (E/IV) and serositis defined a subgroup, comprising 1/4 of the patients, with ~40% risk of failure and 25% risk of death, who can be suitable for treatment intensification or incorporation novel agents in the 1st line. More importantly, the absence of both factors defined a subgroup comprising >40% of patients with only 10% failure rate and minimal disease-specific mortality (2%), who might not benefit from any treatment intensification, such as R-da-EPOCH.

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SOLUBLE INTERLEUKIN-2 RECEPTOR AS A SURROGATE BIOMARKER OF METABOLIC TUMOR VOLUME MEASURED BY 18F-FDG PET/CT IN DIFFUSE LARGE B-CELL LYMPHOMA

H. Senjo[1,*], M. Kanaya[1], K. Izumiyama[1], K. Hirata[2], A. Mori[1], M. Saito[1], T. Irie[1], M. Tanaka[1], H. Iijima[3], E. Tsukamoto[4], D. Hashimoto[5], T. Teshima[5], M. Morioka[1]

[1]Hematology Department, Aiiku Hospital, [2]Nuclear Medicine Deparment, [3]Clinical Research and Medical Innovation Center, Hokkaido University, [4]Radiology Department, Central CI Clinic, [5]Hematology Department, Hokkaido University, Sapporo, Japan

Background: Diffuse large B cell lymphoma (DLBCL) is a heterogeneous disease and more than 30% of the patients treated with R-CHOP would not respond or relapse. The significant value of serum sIL-2R as a prognostic indicator of DLBCL had been actively reported (Goto *et al*., Annals of Hematology 2012; 91: 705). However, it remains to be clarified whether the serum level of sIL-2R directly correlates with tumor burden or is affected by activities of bystander immune cells in DLBCL patients.

Aims: In this work, we aimed to evaluate the correlation between serum level of sIL-2R at diagnosis and total metabolic tumor volume (TMTV) based on PET-CT scan images at initial staging in DLBCL patients.

Methods: We conducted a retrospective study of 64 patients with newly diagnosed DLBCL treated between 2008 and 2014 in our institute. All patients were measured baseline serum levels of sIL-2R and they underwent PET-CT scan before introduction of chemotherapy. SUV Computer-aided analysis of PET-CT images for TMTV calculations was performed using Metavol® (Hirata *et al*., PLoS ONE 2014; 9: e105682). TMTV was defined as the volume of lymphoma visualized on PET-CT scans with Standardized uptake value (SUV) greater than or equal to an absolute threshold of 4.0 as previously described (Kurtz *et al*., Blood 2015; 125: 3679). The correlation between sIL-2R and TMTV is assessed by using Pearson's product-moment correlation coefficient. Written informed consent was obtained from all patients.

Results: The median age was 73 years-old and 81% of the patients had advanced stage. With a median follow-up of 2.7 years in survivors, the estimated overall survival (OS) rate and event-free survival (EFS) rate at 5 years were 53.1% and 45.4%, respectively. Median TMTV and sIL-2R at diagnosis were 236 cm^3, and 1735 U/ml, respectively. Pearson's correlation tests demonstrated that serum levels of sIL-2R were significantly correlated with TMTV (r=0.490, P<0.001) (Figure 1A). We found TMTV ≥150 cm^3 and sIL-2R ≥1300 U/ml are associated with worse 5-year OS and 2-year EFS. In a multivariate analysis that included sIL-2R and all factors in NCCN-IPI, it was evident that age and sIL-2R were independently associated with a worse prognosis in terms of 5-year OS (age; HR, 4.44; 95% CI, 1.05 to 18.7; log-rank, P=0.0424, sIL-2R; HR, 4.45; 95% CI, 1.04 to 19.1; log-rank, P=0.0444). Another multivariate analysis that included TMTV and all factors for NCCN-IPI demonstrated that TMTV was an only independent prognostic factor for 5-year OS (HR,3.87; 95% CI, 1.08 to 13.8; log-rank, P=0.0373). Subgroup analyses included the 49 patients with NCCN-IPI High-int to High demonstrated that TMTV <150 cm^3 could find the patients with favorable OS and EFS from this poor prognostic group [OS; 75.0% *vs* 27.7%, P=0.0355 (Figure 1B), EFS; 66.7% *vs* 29.7%, P=0.0493]. Similar results were obtained using sIL-2R; sIL-2R <1300 U/mL could find the patients with better prognosis in this group [OS; 75.0% *vs* 25.9%, P=0.0182 (Figure 1C), EFS; 58.3% *vs* 29.7%, P=0.0499].

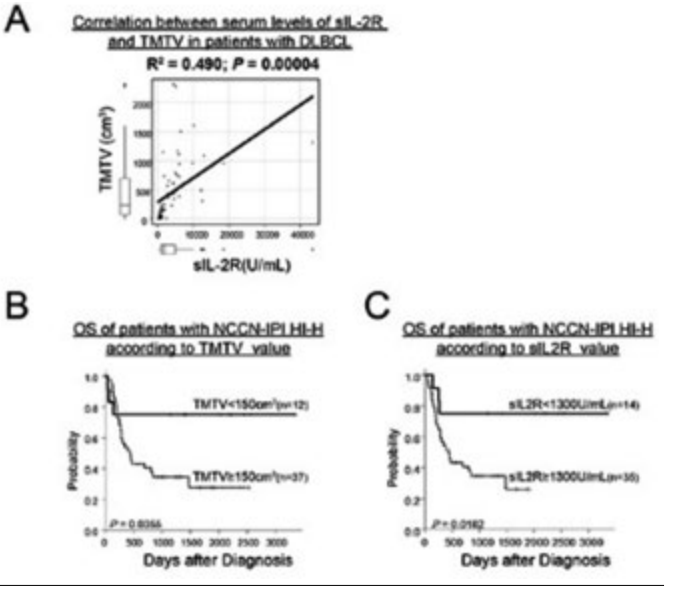

Figure 1.

Summary and Conclusions: We found that there was positive correlation between serum level of sIL-2R and TMTV in DLBCL patients. Our study demonstrated that serum sIL-2R, which measured easily in the clinical practice, can be used as a surrogate biomarker to assess tumor burden of DLBCL patients. Furthermore, we found that both sIL-2R and TMTV could predict treatment outcomes, independently of NCCN-IPI.

PF281

TAK-659 PLUS BENDAMUSTINE (+/-RITUXIMAB), GEMCITABINE, LENALIDOMIDE, OR IBRUTINIB IN PATIENTS (PTS) WITH ADVANCED NON-HODGKIN LYMPHOMA (NHL)

S. Assouline[1,*], M.-M. Keating[2], P.F. Caimi[3], A. Mattour[4], J. Zhu[5], S. Wang[5], Y. Shou[5], Y. Rong[5], J. Redman[5], C. Diefenbach[6]

[1]Jewish General Hospital, Montreal, Quebec, [2]Queen Elizabeth II Health Sciences Center, Halifax, Canada, [3]University Hospitals of Cleveland, Cleveland, OH, [4]Henry Ford Cancer Institute, Detroit, MI, [5]Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, MA, [6]New York University Cancer Institute, New York, NY, United States

Background: TAK-659 is an investigational, reversible, potent dual inhibitor of SYK and FLT-3. In preclinical studies using diffuse large B-cell lymphoma (DLBCL) models, TAK-659 in combination with bendamustine (BEND) or lenalidomide (LEN) demonstrated synergistic antitumor activity, and TAK-659 with gemcitabine (GEM) or ibrutinib (IBRU) demonstrated additive antitumor activity.

Aims: The primary objective of this phase 1b study (NCT02954406) was

to determine the maximum tolerated dose (MTD) of TAK-659 when combined with BEND (+/-rituximab [RITU]), GEM, LEN, or IBRU. Secondary objectives included pharmacokinetics (PK) of TAK-659 and preliminary efficacy.
Methods: This multicenter open-label, dose escalation (3+3) study enrolled adults with NHL who were refractory or relapsed after ≥1 prior line of therapy and for whom no effective standard therapy was available. Informed consent was received. For all treatment groups, pts received oral TAK-659 QD at a starting dose of 60 mg plus one of the following: intravenous (IV) BEND 90 mg/m^2 days 1 and 2 +/- IV RITU 375 mg/m^2 day 1 of a 21-day cycle; IV GEM 1000 mg/m^2 days 1 and 8 of a 21-day cycle; oral LEN 25 mg QD days 1–21 of a 28-day cycle; or oral IBRU 560 mg QD of a 28-day cycle. Treatment was given until progressive disease (PD) or unacceptable toxicity. PK samples were collected pre- and post-dose on days 1, 8 and 15 of cycle 1. Response was by investigator assessment per the International Working Group revised criteria for malignant lymphoma.
Results: As of 17-Nov-2017, 19 pts with advanced NHL (DLBCL n=14; mantle cell lymphoma [MCL] n=2; composite lymphoma [CL] n=2; and follicular lymphoma [FL] n=1) were enrolled to receive TAK-659+BEND (+/-RITU [n=6 each]), TAK-659+GEM (n=3), TAK-659+LEN (n=3), or TAK-659+IBRU (n=1). Median age was 64 years (range 44–92), 13 pts (68%) were male, 8 pts (42%) had ≥4 prior therapies, and 13 pts (68%) had an ECOG PS of 1. Across all cohorts, pts received a median of 2 treatment cycles (range 1–4); 17 pts discontinued (13 due to PD). Two pts who received TAK-659 60 mg+LEN had DLTs: one pt with grade 3 skin rash and one pt with grade 4 neutropenia for >7 days. The TAK-659 dose was escalated from 60 mg to 80 mg in three pts for each dose of the TAK-659+BEND and TAK-659+BEND+RITU treatment groups; dose escalation for the remaining cohorts is ongoing. Overall, 18 pts (95%) experienced any-grade adverse events (AEs), 8 pts (42%) had AEs related to TAK-659 and 6 pts (32%) had AEs related to the combination drug(s). Thirteen pts (68%) reported grade ≥3 AEs, 8 pts (42%) and 6 pts (32%) had AEs related to TAK-659 and combinations drug(s), respectively. Serious AEs were reported in 10 pts (53%). The most frequent any-grade and grade ≥3 AEs overall and by treatment groups are shown in the Table 1. There was one on-study death due to a cardiac event (TAK-659+IBRU) that was considered unrelated to treatment by the investigator. Of the 17 response-evaluable pts, 3 pts (18%) had complete remissions (CRs) (TAK-659 60 mg+BEND in DLBCL, TAK-659 60 mg+BEND+RITU in FL, TAK-659 60 mg+IBRU in MCL) and 2 pts had partial remissions (PRs) (TAK-659 80 mg+BEND in FL and TAK-659 80 mg+BEND+RITU in CL), giving an overall response rate (CR+PR) of 29%. Following co-administration of TAK-659 60 mg+BEND (+/-RITU), preliminary TAK-659 PK profiles were generally comparable with those from single-agent TAK-659 in lymphoma or solid tumor pts.

Table 1. Safety.

	TAK-659 plus:					All pts
Incidence ≥15% overall, n (%)	BEND n=6	BEND+RITU n=6	GEM n=3	LEN n=3	IBRU n=1	N=19
Any-grade AEs						
Overall	6 (100)	6 (100)	3 (100)	2 (67)	1 (100)	18 (95)
Anemia	4 (67)	1 (17)	1 (33)	1 (33)	-	7 (37)
Chills	2 (33)	4 (67)	-	-	1 (100)	7 (37)
Hypophosphatemia	4 (67)	1 (17)	-	1 (33)	-	6 (32)
Pyrexia	3 (50)	2 (33)	-	-	1 (100)	6 (32)
Fatigue	2 (33)	1 (17)	-	-	1 (100)	4 (21)
Headache	1 (17)	2 (33)	-	-	1 (100)	4 (21)
Hypokalemia	2 (33)	-	-	1 (33)	1 (100)	4 (21)
Nausea	1 (17)	2 (33)	1 (33)	-	-	4 (21)
Amylase increased	1 (17)	1 (17)	1 (33)	-	-	3 (16)
Blood creatine phosphokinase increased	1 (17)	1 (17)	-	1 (33)	-	3 (16)
Decreased appetite	1 (17)	1 (17)	-	-	1 (100)	3 (16)
Febrile neutropenia	2 (33)	1 (17)	-	-	-	3 (16)
Lipase increased	1 (17)	1 (17)	1 (33)	-	-	3 (16)
Platelet count decreased	-	1 (17)	-	2 (67)	-	3 (16)
Pneumonia	1 (17)	-	1 (33)	-	1 (100)	3 (16)
Rash	-	1 (17)	-	1 (33)	1 (100)	3 (16)
White blood cell count decreased	-	-	1 (33)	2 (67)	-	3 (16)
Grade ≥3 AEs						
Overall	5 (83)	4 (66.7)	1 (33)	2 (67)	1 (100)	13 (68)
Hypophosphatemia	4 (67)	1 (16.7)	-	1 (33)	-	6 (32)
Anemia	2 (33)	1 (16.7)	1 (33)	-	-	4 (21)
Febrile neutropenia	2 (33)	1 (16.7)	-	-	-	3 (16)

BEND, bendamustine; GEM, gemcitabine; IBRU, ibrutinib; LEN, lenalidomide; pts, patients; RITU, rituximab; AE, adverse event

Summary and Conclusions: The MTDs were not reached and dose escalation is ongoing for each treatment group. Updated safety and efficacy data will be presented.

PF282

82RB PET FOR EARLY DETECTION OF DOXORUBICIN-INDUCED CARDIOTOXICITY

A.H. Laursen[1,*], M. Bayer Elming[2], R. Sejersten Ripa[3], P. Hasbak[3], A. Kjær[3], J. J. Thune[4], M. Hutchings[1]

[1]Department of Haematology, [2]Department of Cardiology, [3]Department of Clinical Physiology, Nuclear Medicine & PET, Rigshospitalet, [4]Department of Cardiology, Bispebjerg and Frederiksberg Hospitalt, Copenhagen, Denmark

Background: Doxorubicin is the most frequently used anthracycline and a cornerstone in lymphoma treatment. Doxorubicin treatment is limited by dose-dependent cardiotoxicity that is often recognized too late to avoid high mortality heart failure (HF). Several different pathophysiological mechanisms have been proposed including injury to non-cardiomyocyte cardiac cells, *e.g.* the endothelial cells of the coronary microcirculation. Myocardial perfusion imaging (MPI) with 82 Rubidium positron emission tomography (^{82}Rb PET) represents a novel imaging modality for assessment of coronary microvascular function. Acute doxorubicin-induced microvascular injury represents a potential early marker of cardiotoxicity.
Aims: To investigate the value of ^{82}Rb PET myocardial perfusion imaging for early detection of subclinical doxorubicin-induced cardiotoxicity.
Methods: We prospectively included 70 doxorubicin-naive lymphoma patients aged 19 to 86 years with normal cardiac function. All subjects provided written informed consent. ^{82}Rb PET MPI was performed prior to chemotherapy (baseline) and shortly thereafter (acute). The median time from chemotherapy to imaging was 3 days. 60 patients completed both baseline and acute imaging. Absolute myocardial perfusion (ml/g/min) was measured during rest and adenosine stress testing. The coronary flow reserve (CFR) was calculated as the ratio between maximal coronary blood flow and resting flow. Perfusion was also graded in a semiquantitative manner using a 17 segment model of the left ventricle and a 5-point scale ranging from normal (=0) to absent perfusion (=4) with intermediate scores representing mild (=1), moderate (=2) and severe (=3) perfusion defects. The summed rest score (SRS) and summed stress score (SSS) are the cumulative scores of all 17 segments during rest and stress, respectively. The SRS represents fixed perfusion defects while the SSS incorporates both myocardial scarring and stress-induced ischemia. The summed difference score (SDS=SSS-SRS) represents the ischemic burden. Left ventricular ejection fraction (LVEF) was also measured.
Results: CFR was significantly lower after the initial doxorubicin exposure (2.69 *vs* 2.51 ml/g/min, p=0.026, Figure 1). We also registered a non-significant decline in stress flow (3.18 ml/g/min *vs* 3.02 ml/g/min, p=0.084). Resting myocardial perfusion did not change. All results are shown in Table 1.

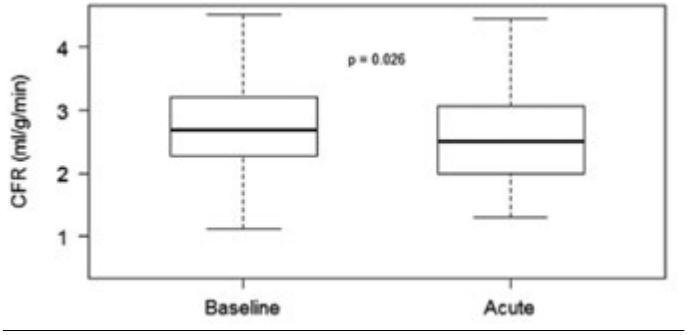

Figure 1. Coronary flow reserve before and after doxorubicin exposure.

Table 1.

	^{82}Rb PET results		
Variable, unit	Baseline	Acute	P-value
CFR, ml/g/min	2.69 (2.29-3.22)	2.51 (1.99-3.05)	0.026
Rest flow, ml/g/min	1.21 (0.96-1.38)	1.23 (0.99-1.48)	0.16
Stress flow, ml/g/min	3.18 (2.80-3.52)	3.02 (2.57-3.43)	0.084
SRS	0 (0-7)	0 (0-6)	0.38
SSS	1 (0-10)	2 (0-12)	0.80
SDS	1 (0-9)	1 (0-9)	0.81
LVEF rest, %	67 (63-71)	68 (63-71)	0.23
LVEF stress, %	74 (69-77)	75 (69-78)	0.32

Summary and Conclusions: - Using ^{82}Rb PET, we found a significant decline in CFR after initial doxorubicin exposure. - Decreases in CFR may represent injury to the myocardial microcirculation, a potential marker of doxorubicin-induced cardiotoxicity. - Future studies will elucidate whether acute decreases in CFR translate into an increased risk of later onset of myocardial fibrosis and HF

PF283

IMPACT OF OMISSION/REDUCTION OF VINCRISTINE FROM R-CHOP THERAPY IN TREATMENT OF GCB TYPE DLBCL

S. Marshall[1,*], N. Nishimura[2], A. Nishihara[2], N. Inoue[2], H. Yamauchi[2], Y. Kusano[2], N. Tsuyama[3], K. Takeuchi[3], Y. Mashima[2], M. Yokoyama[2], Y. Terui[2]

[1]Medical Oncology, [2]hematology, [3]Division of Pathology, The Cancer Institute Hospital of JFCR, Tokyo, Japan

Background: R-CHOP regimen (rituximab, cyclophosphamide, doxorubicin, vincristine, predonisolone) is the standard therapy for the patients with diffuse large B cell lymphoma (DLBCL). However, vincristine is sometimes omitted or reduced due to side effects.
Aims: The purpose of this study was to investigate whether omission/reduction of vincristine from the R-CHOP regimen reduced the efficacy of R-CHOP on DLBCL. We also aimed to illustrate the difference of the efficacy regarding GCB type (germinal center B-cell-like type) and non-GCB type.
Methods: We retrospectively reviewed patients with newly diagnosed DLBCL who received R-CHOP like chemotherapy in our institute from January 2005 to February 2018. We compared the overall survival (OS) and the progression free survival (PFS) with and without the omission/reduction of vincristine from the R-CHOP regimen. Clinical outcomes of R-CHOP with and without omission/reduction of vincristine regarding to GCB type and non GCB type were also evaluated.
Results: A total of 663 cases were reviewed. R-CHOP regimen was administered in all except 5 cases in which the R-CEOP regimen was used instead. The Median age was 66 (range 17-90). 25% had stage or , and 8% had PS 2-4. 25% had more than 2 extranodal sites involvement. Patients with IPI score of 0, 1, 2, 3, 4, and 5 were 15%, 30%, 23%, 18%, 11%, and 4%, respectively. 51% had GCB type, while 45% had non GCB type, but 4% of the patients' tumor type was unknown. Among the patients who were treated with R-CHOP like regimen, 56 patients (8%) omitted or reduced vincristine. The reason for omission/reduction was peripheral neuropathy (19 patients), ileus (18), constipation (9), SIADH (5), post operation (3), and others (4). Of the patients with vincristine omission/reduction, 32 patients had relative dose intensity (RDI) of less than 50%. Median observation time was 4.5 years for the survival patients. Four-year OS and PFS for all the patients was 80% and 72%. Patients with omission/reduction (RDI <50%) of vincristine had significantly worse OS compared to patients without omission/reduction of vincristine (4-year OS: 66% *vs* 80%, p=0.028), as well as PFS (56% *vs* 73%, p=0.037). In regard to GCB and non GCB type, 15 patients and 17 patients had less than 50% RDI of vincristine respectively. Among patients with GCB type, OS was significantly worse in patients with vincristine omission/reduction, with 4-year OS of 67% *vs* 85% (p=0.005). However, OS did not show the significance between with and without omission/reduction of vincristine.
Summary and Conclusions: The omission/reduction of vincristine from R-CHOP like regimen might lead to a substantial loss of efficacy especially in GCB type. High intensity of R-CHOP like therapy should be maintained when treating DLBCL with GCB type.

PF284

EPIDEMIOLOGY AND TREATMENT PATTERNS IN LATIN AMERICAN PATIENTS WITH NON-HODGKIN'S LYMPHOMA: FINDINGS FROM THE HEMATO-ONCOLOGY IN LATIN AMERICA (HOLA) OBSERVATIONAL REGISTRY STUDY

M. Pavlovsky[1,*], D. Cubero[2], G. Vásquez[3], A. Enrico[4], M. Osorio[1], J. Armenta[5], L. Foggliatto[6], R. Ovilla[7], O. Flores[8], T. Santos[9], P. Barreyro [10]

[1]Servicio de Hematología e Investigación Clínica, Fundación para Combatir la Leucemia (FUNDALEU), Buenos Aires, Argentina, [2]CEPHO/ABC School of Medicine, Santo André, Brazil, [3]Instituto Nacional de Ciências Médicas e Nutrição, Salvador Zubirán, Mexico City, Mexico, [4]Hospital Italiano La Plata, Buenos Aires, Argentina, [5]Centro Oncológico Estatal ISSEMYM, Toluca, Mexico, [6]Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil, [7]Hospital Angeles Lomas, Huixquilucan, Mexico, [8]Medical Solutions S.A. , Guatemala City, Guatemala, [9]Janssen-Cilag Farmacêutica Ltda, São Paulo, Brazil, [10]Janssen-Cilag Farmacêutica Ltda, Buenos Aires, Argentina

Background: Real-world evidence concerning the epidemiologic characteristics and treatment patterns in Latin American patients with non-Hodgkin lymphoma (NHL) is limited.
Aims: Objectives of this study were to evaluate: 1) proportion of subtype presentation among all NHL's, 2) patient characteristics, and 3) treatment patterns.
Methods: This multicenter, observational, retrospective chart review included patients with T-cell or B-cell lymphomas, including diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle-cell lymphoma (MCL), and mucosa-associated lymphoid tissue (MALT) lymphoma. Eligible for analysis were adults with NHL who received care at 30 tertiary-care centers (oncology concentration) in Argentina (n=5 sites), Brazil (n=9), Chile (n=1), Colombia (n=5), Mexico (n=6), Panama (n=3), and Guatemala (n=1). Patients who had ≥1 year of follow up from January 1, 2008, through December 31, 2015 were included. Chart abstractors were centrally trained in case ascertainment.
Results: Across the seven countries, 2,967 patients had a diagnosis of NHL. A total of 2,948 (99.3%) patients with subtype information were included in the analysis by subtype. Mexico, Argentina, Colombia, and Brazil contributed large proportions of patients with most forms of NHL (80.9%). Majorities of patients with NHL (2,518/2,948=85.4%) had B-cell subtypes, including 1,457/2,518 (57.9%) individuals with DLBCL; 578/2,518 (23.0%) with FL; 183/2,518 (7.3%) with MCL; 90/2,518 (3.6%) with chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma; 84/2,518 (3.3%) with MALT; 60/2,518 (2.4%) with Burkitt lymphoma; and <1.5% each with lymphoplasmacytic lymphoma (35/2,518; 1.4%) or B-lymphoblastic lymphoma (n=31/2,518; 1.2%). A further 250/2,948 (8.5%) patients had T-cell NHL, and 180/2,948 (6.1%) patients with all other types of lymphoma. MCL had the highest percentage of male patients (128/183; 69.9%), and MALT had the lowest percentage (36/84; 42/9%). Patients with DLBCL, CLL/small lymphocytic lymphoma, and B-lymphoblastic lymphoma were more gender balanced. The median age at diagnosis ranged from 32 years for B lymphoblastic lymphoma to 64 years for CLL/small lymphocytic lymphoma or lymphoplasmacytic lymphoma. The median age at diagnosis was 58 years for DLBCL. Most patients (54.0% FL; 59.1% DLBCL; 59.5% MCL; 65.0% other types of lymphomas; 65.5% MALT; 67.7% CLL/small lymphocytic lymphoma; 68.6% lymphoplasmacytic lymphoma) had prior comorbidities at the time of diagnosis, while 67.7% with B-lymphoblastic lymphoma and 50.4% with T-cell lymphoma had no previous comorbidities. For patients with most NHL subtypes, the most frequent chemotherapy was R-CHOP (rituximab–cyclophosphamide-doxorubicin-vincristine-prednisolone). Among patients receiving chemotherapy, R-CHOP was used in 998/1,420 (70.3%) patients with DLBCL, 280/546 (51.3%) with FL; 71/172 (41.3%) with MCL; and 24/67 (35.8%) with MALT. Other forms of chemotherapy included CHOP and RCVP (rituximab-cyclophosphamide-vincristine-prednisolone). Most patients (23/30; 76.7%) with B-lymphoblastic lymphoma treated using chemotherapy received hyperCVAD (hyperfractionated cyclophosphamide-vincristine-doxorubicin-dexamethasone).
Summary and Conclusions: Most Latin American residents with NHL had DLBCL, FL, T-cell lymphoma or MCL, and the median age at diagnosis ranged from 32 to 64 years. Most patients had prior comorbidities at diagnosis, and R-CHOP was the most frequent chemotherapy for most NHL subtypes.

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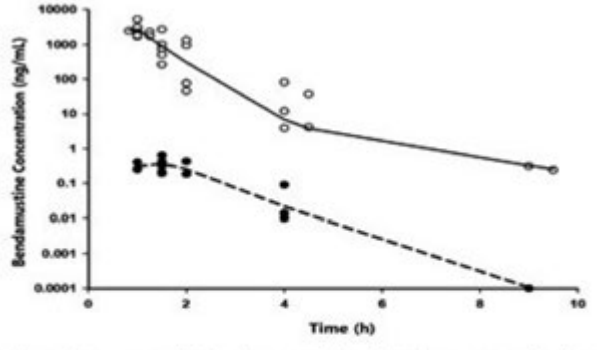
CLINICAL RESPONSE AND PHARMACOKINETICS OF BENDAMUSTINE AS A COMPONENT OF SALVAGE R-B(O)AD THERAPY FOR THE TREATMENT OF PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA (PCNSL)

D.-H. Yang[1,*], S.-Y. Ahn[1], S.-H. Jung[2], H.-J. Kim[3], J.-J. Lee[1], Y. R. Do[4]

[1]Hemato-Oncology, [2]Chonnam National University Hwasun Hospital, Jeollanam-do, Korea, Republic Of, [3]Hemato-oncololgy, Chonnam National University Hwasun Hospital, Jeollanam-do, [4]Hemato-oncology, Dongsan Medical Center, Daegu, Korea, Republic Of

Background: A relatively high proportion of patients diagnosed with primary CNS lymphoma will experience recurrent disease, yet therapy options are limited in salvage therapy. This was the first study to evaluate a bendamustine-based combination regimen for the treatment of relapsed/refractory (R/R) PCNSL and to characterize bendamustine pharmacokinetics in the human CSF.
Aims: This was a prospective, open-label, pilot study investigating the safety and efficacy of the bendamustine-based combination regimen R-B(O)AD, designed to define CSF and plasma PK profiles of bendamustine in R/R patients.

Methods: All patients received either R-BOAD or R-BAD intravenously (rituximab 375 mg/m^2 on day 1; vincristine 1.4 mg/m^2 on day 1, omitted in patients ≥70 years of age due to risk of neurotoxicity; bendamustine 75 mg/m^2 over 1 h on days 2 and 3; cytarabine 1000 mg/m^2 over 3 h on days 2-4; dexamethasone 20 mg on days 1-4), every 4 weeks up to 4 cycles. Response, survival outcomes, and adverse events of the regimen were assessed. A sparse sampling strategy and population based modeling approach was utilized for evaluation of plasma and CSF levels of bendamustine. CSF exposure was estimated as $C_{max,}$CSF/$C_{max,}$plasma and AUC_{CSF}/AUC_{plasma} ratios. Non-compartmental methods were used to calculate the area under the concentration–time curve ($AUC_{0\text{-}inf}$) in WinNonlin, version 5.2 (Pharsight, St. Louis, MO, USA).
Results: Ten patients were enrolled into study of whom 70% were of refractory disease and with high IELSG prognostic risk scores. Twenty-seven cycles of R-B(O)AD were administered, at a median of three cycles per patient, and vincristine was omitted in four patients. The ORR of R-BOAD was 50% (95% CI, 0.24 to 0.76) with one patient achieving CR and four PR. Primary toxicity of the regimen was reversible myelosuppression, mostly grade 3 or 4 neutropenia. Time of maximum concentration (t_{max}) was found at the end of infusion ($t_{max,plasma}$=1 h) for plasma and at 0.5 h after end of infusion ($t_{max,csf}$=1.5 h) for CSF. The mean maximum peak concentrations for plasma and CSF were 2669 ng/mL and 0.397 ng/mL, respectively, and patients with response at deep tumor sites displayed higher trends in peak exposure. Pharmacokinetic data was best described by a four-compartment model with first-order elimination of drug from central plasma and CSF compartments (Figure 1).



Bendamustine concentration-time profiles. Circles represent observed values for plasma (•) and CSF (◦) drug levels. Best-fit curves from the final population PK model are shown for plasma (–) and CSF (----).

Figure 1.

Summary and Conclusions: R-BOAD is an effective salvage option for PCNSL, but with significant hematologic toxicity. Bendamustine CSF levels are minimal; however correspond to plasma exposure and response.

PF286

OUTPATIENT SYSTEMIC HIGH-DOSE METHOTREXATE AS CNS PROPHYLAXIS IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

R. Fernández-Alvarez[1,*], S. Sánchez-Matías[1], A. Rubio-Castro[1], C. de Brabandere[1], M. Sarasa Valdés[1], J. Torres Varona[1], A. Silva Soto[1], E. González-García[1], A.-P. Gonzalez[2], C.-R. Fernández-Alvarez[1]

[1]Hematology, Hospital Universitario de Cabueñes, Gijon, [2]Hospital Universitario Central de Asturias, Oviedo, Spain

Background: Systemic high-dose methotrexate (HD-MTX) is increasingly used as CNS prophylaxis in high-risk patients with diffuse large B-cell lymphoma (DLBCL). To ensure safe administration and to prevent nephrotoxicity, conventional schedules for HD-MTX delivery require hospitalization for inpatient hydration and urine alkalinization. To improve patients' quality of life and reduce healthcare costs, we developed an institutional protocol for HD-MTX administration in the outpatient setting.
Aims: To analyze the safety and feasibility of an ambulatory HD-MTX regimen for CNS prophylaxis in DLBCL patients.
Methods: This single-center retrospective study included all DLBCL patients who received outpatient HD-MTX as CNS prophylaxis between 2015 and 2017. Patients were planned to receive 3 doses of HD-MTX after alternate R-CHOP courses. Requirements included: high-risk criteria for CNS involvement, good performance status, creatinine clearance (CrCl) >60 mL/min, absence of fluid retention and serum bilirubin ≤1.5xULN. Patients were instructed to keep an adequate oral hydration (2 l/m^2/day) and urine alkalinization by using sodium bicarbonate (2 grams QID) and acetazolamide (250 mg QID) from 72 hours previous to HD-MTX infusion. Comedications were reviewed to minimize the risk of interactions. HD-MTX (3.5 g/m^2) was infused over 4 hours, together with 1 liter of hydration and sodium bicarbonate 50 mEq. Urine pH was checked before HD-MTX administration, and for any value less than 7 a sodium bicarbonate bolus (1 mEq/kg) was given. Leucovorin at a standard dose (30 mg orally every 6 hours) was begun 24 hours after HD-MTX. MTX serum concentrations were monitored daily from 24 hours after administration until clearance (level ≤0.1 micromol/L). Examined parameters included: urine pH, MTX levels and toxicity according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.
Results: A total of 37 ambulatory HD-MTX courses were given to 13 patients (median age 54, range 21-76), after a median of 14 days from R-CHOP administration (range 10-19). Initial dose of MTX was 3.5 g/m^2 in all cases and no dose reduction was required afterwards. All patients completed succesfully the planned 3 doses in an outpatient basis, except for one patient who received only one dose due to pneumonitis. Previous to MTX infusion, urinary pH >7 was achieved in 33 (89%) cycles. Median MTX levels at 24, 48 and 72 hours after administration were 1.40, 0.18 and 0.08 micromol/L, respectively. MTX clearance (≤0.1 micromol/L) was achieved by 72 hours in 27 courses (70%), and by 96 hours in 100%. There were no cases of delayed MTX elimination. Creatinine level increases grades I and II were observed in 29 (78%) and 4 (11%) cycles, respectively. No episode of severe (grade 3-4) nephrotoxicity was observed. The main hematological toxicity was neutropenia (grade 3 in 19%). Extra-hematological toxicities were mild and included: mucositis (grades 1-2, 24%), nausea and vomiting (grades 1-2, 8%) and hypokalemia (grades 1-2, 8%). Admission was required only in one case of pneumonitis.
Summary and Conclusions: Outpatient administration of HD-MTX as CNS prophylaxis is safe and feasible following the described approach. Emphasis should be put on careful selection of patients and strict adherence to ambulatory oral hydration and urine alkalinization. An inter-professional team approach including nurses, medical staff and pharmacists was key to the successful implementation of this ambulatory regimen.

PF287

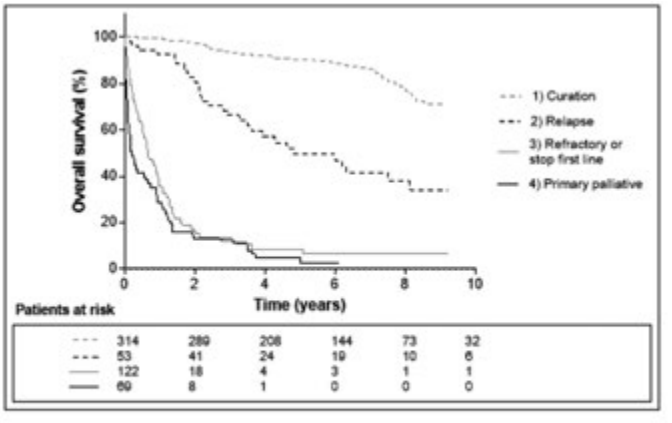
OUTCOME OF A 10-YEAR POPULATION-BASED COHORT OF DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS IN THE FIRST-LINE, REFRACTORY AND RELAPSED SETTING: 'REAL-WORLD' DATA IDENTIFY SUBGROUPS IN NEED OF NEW THERAPIES

H. van der Galien[1,*], H. Kooistra[1], N. Veeger[2], R. Kibbelaar[3], H. Storm[4], E. de Waal[1], M. Hoogendoorn[1], H. van Kamp[5], S. Hovenga[5], L. Schipper[5], G. Veldhuis[6], G. Woolthuis[6], B. van Rees[7], T. van Meerten[8], R. van Rijn[1]

[1]Department of Hematology, Medical Center Leeuwarden, [2]Department of Epidemiology, MCL Academy, [3]Department of Pathology, Pathology Friesland, [4]Department of Clinical Chemistry, Certe, Leeuwarden, [5]Department of Internal Medicine, Nij Smellinghe Hospital, Drachten, [6]Department of Internal Medicine, Antonius Hospital, Sneek, [7]Department of Internal Medicine, Tjongerschans Hospital, Heerenveen, [8]Department of Hematology, University Medical Center Groningen, Groningen, Netherlands

Background: In diffuse large B-cell lymphoma (DLBCL), with standard chemo-immunotherapy a high treatment failure rate remains of about 40%. For relapsed or refractory (R/R) patients who are ineligible for salvage therapy with autologous stem cell transplantation (ASCT) outcome is poor. Recently, several novel agents have demonstrated promising results in R/R DLBCL patients. However, the applicability of these results to 'real-world' patients is unclear as patient details from population-based cohorts in DLBCL are scarce.
Aims: To obtain 'real-world' prognostic factors, treatment patterns and outcomes of DLBCL patients in the rituximab era in first-line, refractory and relapsed setting. Hereby, we aim to distinguish subgroups that may benefit most from new treatments.
Methods: An observational study was performed using the population-based 'HemoBase' registry from the province of Friesland, the Netherlands. All DLBCL patients diagnosed between 2005 and 2015 were included with a follow-up to early 2018. Patients had free access to health care. Fully detailed information of patient and disease characteristics and outcomes for all lines of treatment were collected. Survival analysis was performed by Kaplan-Meier and multivariate by Cox proportional hazard models.
Results: 568 patients (median age 70 years, 53,8% male) were included.

Median follow up was 6.3 years. Progression free survival (PFS) and overall survival (OS) at 4 years were 55% and 60%, respectively. The International Prognostic Index (IPI) score at diagnosis was calculated for 96% of patients and was 0 in 43 (8%), 1-2 in 278 (52%) and 3-5 in 214 patients (40%) with corresponding 4-year OS of 94%, 70% and 46%. 69 patients (12%) received no treatment or palliative treatment only. These patients were older (median age 83 years, p<0.01) and had a worse WHO performance status (84%≥2, p<0.01) compared to the 489 patients treated with curative intent. 314 patients (65%) achieved continuous complete remission after first-line chemo-immunotherapy. 61 patients (12%) did not complete treatment due to toxicity or death. In 61 patients (12%) refractory disease (primary refractory or early relapse <6 months after completing first-line therapy) and in 53 patients (11%) relapse was found. Second-line palliative therapy was offered to 45% of refractory (median OS 0.9 years) and to 59% of relapsed patients (median OS 2.9 years) and consisted of chemotherapy, radiotherapy, steroids or supportive care. Salvage therapy was initiated in 55% of refractory (median OS 1.3 years) and 41% of relapsed DLBCL (median OS 6.0 years). 17% of R/R patients underwent ASCT with a continuous curation rate of 63% and 4-year OS of 58%. Outcome in relapse was favorable over refractory disease (HR3.26, p<0.01). Multivariate survival analysis will be presented for age, sex, lower performance, Ann Arbor stage, B-symptoms, IPI score, LDH, Charlson Comorbidity Index and subtypes based on immunohistochemistry and histology (Figure 1).



Overall survival of patients with diffuse large B-cel lymphoma divided into four subgroups: 1) continuous complete remission after first line treatment; 2) relapse (>6 months) after first line treatment; 3) refractory disease or not completing first line treatment; 4) primary palliative or no treatment in first-line

Figure 1.

Summary and Conclusions: In this detailed population-based DLBCL cohort, primary palliative patients, patients unable to complete first-line treatment and patients with refractory disease were identified as subgroups with exceptional poor prognosis. This substantial group of 34% of all DLBCL patients had a lower performance and a higher age and might benefit from less toxic (up-front) novel therapies. In addition, given the response rates to palliative second-line therapy in patients with relapse, this group should be considered for participation in clinical trials investigating novel approaches.

PF288

LOW SUNLIGHT EXPOSURE AT TIME OF DLBCL DIAGNOSIS MAY BE ASSOCIATED WITH INFERIOR SURVIVAL IN ELDERLY WOMEN

A. Elinder Camburn[1,*], H. Chan[1]

[1]*Haematology, North Shore Hospital, Auckland, New Zealand*

Background: Data from RICOVER60 demonstrates that vitamin D deficiency is a risk factor for elderly patients with Diffuse Large B-cell Lymphoma (DLBCL) treated with R-CHOP chemotherapy due to postulated impaired rituximab-mediated cellular cytotoxicity. The primary source of vitamin D for most people is from sun exposure of epidermal tissue, and this varies with the amount of daylight hours in different seasons.
Aims: The purpose of this restrospective study was to analyse the long term survival of elderly patients with DLBCL diagnosed in different seasons of the year.
Methods: Cases were extracted from the New Zealand Cancer Registry, and all patients diagnosed with DLBCL between 1 January 2010 and 31 December 2016 were included. Overall survival was calculated from the date of diagnosis to the date of death by any cause. All cases that were not known to be dead at the time of analysis were presumed alive and censored. Southern hemispher summer season was defined as the months between November and February, and winter season was defined as the months between May and August.
Results: Between 1 January 2010 and 31 December 2016, a total of 2263 cases of DLBCL were reported in patients aged 18 or over. 755 were diagnosed in the winter months and 761 were diagnosed in the summer months. The remaining 747 patients were diagnosed between the two seasons. Female patients aged 50 or above, diagnosed in the winter months had a significant inferior survival compared to those diagnosed during the summer months (55.9 *vs* 87.6 months, p=0.039). For men aged 50 or above, a difference in overall survival relating to season at time of diagnosis was not observed (median OS 62.6 *vs* 48.8 months, p=0.297) (Figure 1).

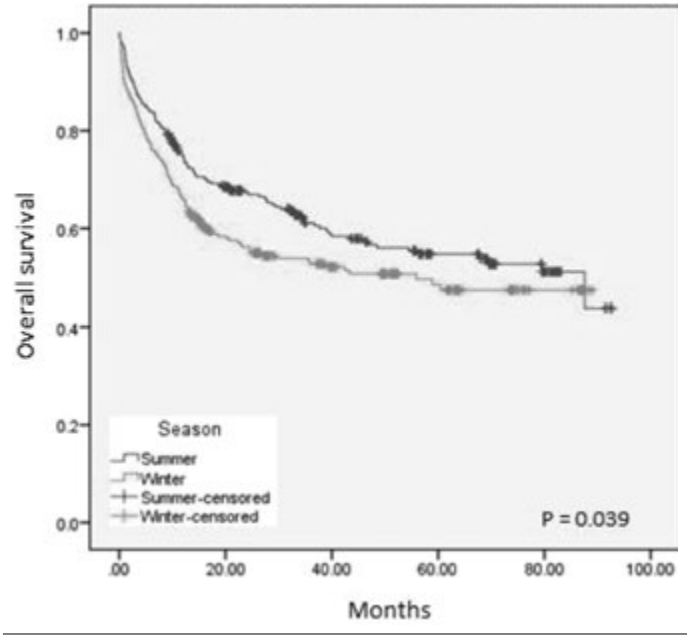


Figure 1.

Summary and Conclusions: Elderly female patients diagnosed with DLBCL in the winter months had inferior survival compared to those diagnosed in the summer months. This difference in survival may be partly due to the increased prevalence of vitamin D deficiency in elderly women relating to reduced sunlight exposure during the winter months.

PF289

THE PROGNOSTIC ROLE OF 18FDG-PET/TAC AT THE END OF FIRST LINE CHEMO-IMMUNOTHERAPY IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA: A MONOCENTRIC STUDY ON 308 PATIENTS

E. Maiolo[1,*], F. D'Alò[1], S. Bellesi[1], A. Cuccaro[1], E. Alma[1], V. Rufini[2], L. Leccisotti[2], S. Annunziata[2], A. Rizzo[2], G. Macis[3], L. Calandriello[3], M. Balducci[4], S. Chiesa[4], V. De Stefano[1], S. Hohaus[1]

[1]*Institute of Hematology, Policlinico Gemelli Foundation, Catholic University of the Sacred Heart,* [2]*Institute of Nuclear Medicine, Policlinico Gemelli Foundation, Catholic University of the Sacred Heart,* [3]*Institute of Radiology, Policlininico Gemelli Foundation, Catholic University of the Sacred Heart,* [4]*Radiation Therapy Department, Policlinico Gemelli Foundation, Catholic University of the Sacred Heart, Rome, Italy*

Background: Although ^{18}FDG-PET/TAC is the gold standard for definition of the final response to chemio-immunotherapy in patients with diffuse large B cell lymphoma (DLBCL), data about its prognostic role are few and sometimes conflicting. PET scan evaluation in DLBCL is often performed using either the International Harmonized Project (IHP) or Deauville score (DS) visual criteria.
Aims: Aim of our study was to retrospectively assess the prognostic role of PET scan evaluation in patients with DLBCL and to analyse for patient characteristics that associate with PET response.
Methods: ^{18}FDG-PET/CT performed at the end of first line chemo-immunotherapy were retrospectively but blindly evaluated using visual methods according to available literature (both IHP and DS) by a physician

with experience in nuclear medicine and lymphoproliferative disease. The prognostic power of visual scores was evaluated considering lack of complete remission, relapse or progression as adverse events. Progression-free survival (PFS) curves were estimated using the Kaplan-Meier method and compared to each other by the log-rank test.
Results: We analysed PET/CT scans of 308 patients with DLBCL treated with chemo-immunotherapy (rituximab+CHOP) between 2009 and January 2017 at our centre were included. Median follow up was 33 months. Scans were negative according to IHP criteria in 212 (69%) patients, positive in 96 (31%) patients. Deauville scoring classified scans as DS1 in 163 (53%) patients, DS2 in 44 (14%), DS3 in 36 (12%), DS4 in 43 (14%), and DS5 in 22 (7%) patients. Variables significantly associated with PET negativity were ECOG <2 *(p=0.004),* albumin>4 g/dL *(p<0.0001),* absence of bulky disease *(p<0.0001),* limited stage (*p<0.0001),* and the anthracycline dose *(p=0.012).* End of chemo-immunotherapy PET predicted for outcome. The 3-years PFS was 96% for patients with negative PET according to IHP and 69% for patients with a positive PET, respectively *(p<0.0001);* Deauville scoring was even more powerful to predict 3-year PFS: 95% for patients with DS 1-3 and 61% for patients with DS 4-5. The survival difference according to PET was confirmed also when analysing patients at low and high risk according to IPIaa (0-1 *vs* 2-3) separately. Anthracycline dose was the most important risk factor for disease recurrence in patients with DS 1-3 *(p=0.02).* In patients with DS4, consolidative radiotherapy appeared to be associated with better outcome *(p=0.02).*
Summary and Conclusions: ^{18}FDG-PET/CT is a prognostic factor in patients with DLBCL at the end of first line chemo-immunotherapy in a large monocentric cohort. Patients obtaining PET Deauville Score 1-3 had an very good prognosis. Still classical risk factors as IPI and dose-intensity of anthracyclines impacted on prognosis. Prognosis of patients with DS4 was significantly worse, and consolidation with radiotherapy might improve prognosis. We are currently evaluating semi-quantitative parameters in this cohort in a attempt to further improve the predictive power of end of chemo-immunotherapy PET.

PF290

CLINICAL CHARACTERISTICS AND OUTCOMES OF BURKITT LYMPHOMA IN A DEVELOPING COUNTRY

W. Fernandes Da Silva Junior[1,*], L. Inês da Rosa[1], L. A. de Pádua Covas Lage[1], V. Rocha[1], J. Pereira[1]

[1]Internal Medicine, Institute of Cancer of São Paulo (ICESP) / University of São Paulo (USP), São Paulo, Brazil

Background: Burkitt lymphoma (BL) is a highly aggressive but curable disease characterized by high proliferation rate and chromosomal translocation involving the oncogene c-MYC and an immunoglobulin locus. Despite its first description in a developing country, few studies have been performed in this world region, particularly in adults and in the Human Immunodeficiency Virus (HIV) infection setting. Likewise, in Brazil the data on adult BL are yet scarce.
Aims: To analyze clinical features and to evaluate response and survival of adults with BL in HIV(+) and HIV(-) patients treated in our institution.
Methods: Retrospective observational study involving patients older than 15 years diagnosed with BL treated in ICESP from 1999 to 2017. BL diagnosis was performed according to WHO criterion.
Results: Seventy patients met the eligibility criteria. Median age was 35 y (15–66 y), 48/70(69%) were men and 30/70(43%) HIV(+). Median serum lactate dehydrogenase (LDH) was 828 IU/L (range 135–9314), with abnormal level in 90%(62/70) patients. CNS involvement was found in 18/70(26%) and bone marrow (BM) and gastrointestinal tract (GIT) involvement was noticed in 19/70(27%) and 34/70 (49%), respectively. 59/70(84%) were in advanced stage. HIV-associated BL showed a higher rate of CNS involvement (15% *vs* 40%, p=0.027) and 9/30(32%) HIV(+) patients were using combined anti-retroviral therapy (cART). 17/30 (68%) HIV(+) patients showed CD4$^+$ lower than 200 cells/mL with a median of 123 cells/mL (15–800). Median HIV viral load was $10.2x10^3$ copies/mL (0–$6639.5x10^3$). Adapted LMB regimen and Hyper-CVAD were the most common regimen used for HIV(-) (n=35) and HIV(+) (n=23) patients, respectively, and 12 patients were treated with CHOP-like due to poor performance status (PS). When possible, all these regimens were adapted to outpatient setting. Only one HIV(-) patient was referred to autologous hematopoietic stem-cell transplantation in first partial remission. For HIV(+) patients, the cART was given along with chemotherapy. Rituximab was not used since it is not yet reimbursed for BL in our public health system. 50/70 (71.4%) patients achieved complete response (CR) and early death (during the first 30 days of the start of chemotherapy) occurred in 20/70(28%) patients, mostly HIV(+) patients (80%). Similarly to literature, CR was more frequent in HIV(-) than in HIV(+) (90 *vs* 47%, p<0.001). Age (>35 years) was also a statistically significant factor for CR (p=0.022) in both groups. In a median follow-up of 5.6 years (3.6–7.5 y) the relapse rate was 21.4% for HIV(+) *vs* 8.3% for HIV(-) (p=0.075). CNS infiltration was found as risk factor for relapse (p=0.002) and other factors as age, clinical stage, LDH, BM and GIT involvement, CD4+ cell and viral load were not significant. 5-year overall survival (OS) was 85% for HIV(-) and 34% for HIV(+) (p<0.001) (Figure 1).

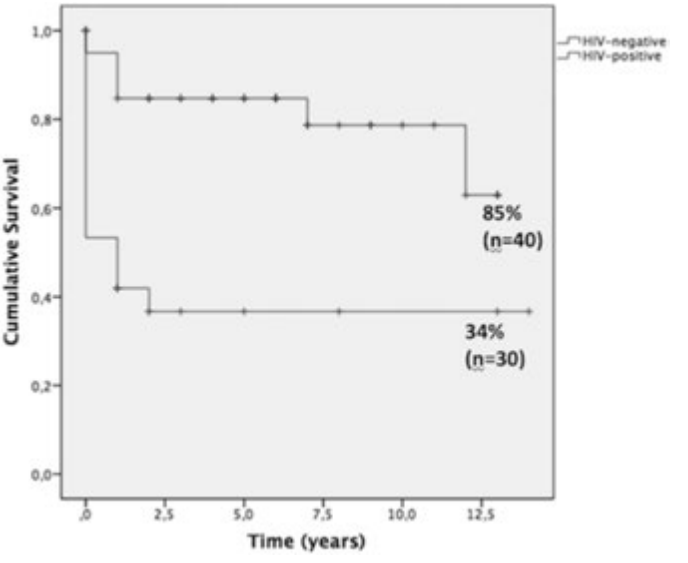


Figure 1.

Summary and Conclusions: In this study, results of HIV(-) BL patients were similar to the literature, despite of unavailability of rituximab. However, HIV(+) BL patients had a decreased PS, with a lower level of CD4$^+$ cells, advanced disease and higher CNS involvement in comparison to literature, that may explain the unsatisfactory outcome found in this group. It seems that, at least in developing countries, optimizing regimens for HIV(+) patients providing early access to treatment and improving supportive care are still an unmet need.

PF291

AN INCREASED RISK OF POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER IN CHILDREN AFTER CARDIAC TRANSPLANTATION FOR CONGENITAL HEART DISEASE

U. Offor[1,*], S. Bomken[1], C. Bacon[1], A. Gennery[2], Z. Reinhardt[3]

[1]Northern Institute for Cancer Research, [2]Institute of Cellular Medicine, Newcastle University, [3]Department of Cardiopulmonary Transplantation, Newcastle Hospitals NHS Trust, Newcastle Upon Tyne, United Kingdom

Background: Patients subjected to lifelong immunosuppressant therapy for solid organ transplantation are at an increased risk of developing malignancies. Post-transplant lymphoproliferative disorder (PTLD) represents the most common cause of malignancy seen in children after cardiac transplantation. The significance of pre-transplant cardiac diagnosis as a risk factor for PTLD in this population has not been previously investigated in depth.
Aims: This study aimed to examine the risk factors for development of PTLD specific to children undergoing cardiac transplantation.
Methods: We retrospectively reviewed the demographics, clinical features and outcomes of all 203 children (<18 years), who received a heart transplant in our institution between January 2000 and December 2015. Kaplan-Meier method and Cox proportional hazards were used to assess the impact of prognostic factors including pre-transplant cardiac diagnosis on survival and freedom from PTLD.
Results: The study cohort consisted of 75 patients transplanted for congenital heart disease (CHD) and 128 patients for idiopathic cardiomyopathy (IC). The median follow up was 5.5 years. There was a significant difference in age at first invasive cardiac procedure via median sternotomy between children with CHD and IC (4 months *vs* 5 years, p <0.001) but no difference in the age at transplantation between both groups (p= 0.78). PTLD was diagnosed in 15 (7.4%) children transplanted for underlying CHD (10/15)

and IC (5/15) with a median age at diagnosis of 10.4 years (range; 6.6-14.0) and 11.5 years (range; 4.8-13.5) respectively. The median time to PTLD was 24 months (range; 10 - 50 months) with no significant difference in time to PTLD between patients with CHD and IC (p= 0.09). All tumour samples were Epstein Barr Virus (EBV) positive and of B-cell lineage. The histological subtypes consisted of 10 (67%) monomorphic lesions, 3 (20%) polymorphic, and 2 (13%) classical Hodgkin lymphoma. Early stage disease (stage I-II) was observed in 5 (33%) patients while advanced tumour (stage III-IV) was seen in the remaining 10 (67%) children. Overall freedom from PTLD was 96% at 1-year, 92% at 5-years and 90% at 10-years. The risk of PTLD was not significantly associated with age at transplantation, gender, ethnicity or type and number of immunosuppressants used (p >0.05). Recipient EBV seronegativity prior to transplantation increased the risk of PTLD (p= 0.04) on multivariate Cox regression, while children with CHD had a significantly higher risk of developing PTLD compared to those with IC (Hazard Ratio= 5.3; 95% Confidence Interval= 1.5–18.9).

Summary and Conclusions: The identification of congenital heart disease as an important risk factor in the development of PTLD after paediatric heart transplantation is a novel finding. We posit that the underlying diagnosis of CHD is a potential proxy marker for early dysregulation of T cell mediated immunity occuring as a consequence of earlier thymectomies during invasive cardiac procedures in these patients compared to children with IC, predisposing them to primary EBV infection and aberrant proliferation of lymphoid cells. This hypothesis is supported by our study finding of a significant age disparity at the time of first invasive cardiac procedure via median sternotomy between children with CHD and IC, which warrants further investigation.

Bleeding disorders (congenital and acquired)

PF292

IDENTIFICATION OF NOVEL MECHANISMS UNDERLYING FUNCTIONAL RESPONSE TO DRUG-INDUCED READTHROUGH OF HAEMOPHILIA B NONSENSE MUTATIONS

M. Ferrarese[1,*], F. Bernardi[1], M. Pinotti[1], A. Branchini[1]

[1]Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy

Background: Drugs inducing ribosome readthrough over premature stop codons, with synthesis of full-length proteins, are proposed as potential therapy for genetic disorders caused by nonsense mutations. This is particularly relevant for coagulation disorders, in which even small increases in functional levels would significantly ameliorate patients bleeding phenotypes. However, the poor responses reported in several patients underlies high variability in the efficacy of readthrough-inducing drugs. The functional rescue results from the interplay between mRNA (nucleotide context) and protein (impact of the amino acid inserted at the nonsense codon) components.

Aims: To investigate the responsiveness to the readthrough-inducing drug geneticin of a wide panel of factor IX (FIX) nonsense mutations, accounting for ~80% of Haemophilia B patients with this mutation type.

Methods: Transient expression of FIX variants in HEK293 cells and evaluation of protein levels (ELISA), full-length/truncated forms (Western Blotting) and activity (chromogenic/coagulant assays).

Results: Among the 11 investigated nonsense mutations, only the p.W240X and p.R384X responded with a remarkable rescue of activity. The amount of rescued FIX protein and activity was comparable for the p.W240X. Strikingly, the activity for the p.R384X (7.5±0.7% of wt) remarkably exceeded secreted protein levels (2.0±0.3%). Data indicate specific favourable mechanisms underlying the observed response: i) re-insertion of the original residue (tryptophan) for the p.W240X and ii) hyperactive features for the p.R384X (Padua position), further confirmed by the 4-fold increased specific activity of the readthrough-deriving R384W missense variant. For most nonsense mutations, an impaired secretion/function prevented a significant functional rescue due to readthrough-mediated amino acid substitutions, as experimentally demonstrated by expression of the most probable missense variants arising from readthrough. We further hypothesized that mutations falling in regions such as pre-peptides would be favoured upon readthrough. Indeed, since these regions are poorly conserved and intracellularly removed, the impact of missense changes on the secreted protein could be vanished. Investigation on paradigmatic nonsense mutations of FIX pre-pro-peptide demonstrated that the p.G21X at the variable hydrophobic core appreciably responded (4±0.3%) to geneticin treatment and the position tolerated all the predicted readthrough-deriving missense changes (G21W/C/R). Specific activity of the secreted proteins was not affected, pointing towards the secretion of wild-type FIX upon pre-pro-peptide removal. As expected, induction of two mutations facing constraints of adjacent cleavage sites was virtually ineffective.

Summary and Conclusions: Data obtained from the optimized FIX expression platform help interpreting the variable response to induced readthrough and lead to the identification of mechanisms underlying a therapeutically-relevant output: i) re-insertion of the authentic residue; ii) rare gain-of-function effects; iii) favourable localization in regions removed during biosynthesis (*i.e.* pre-peptide, B-domain). Taken together, these data support a rational approach to identify a few nonsense mutations, and thus patients, potentially eligible for therapeutic options based on readthrough.

PF293

ASSESSMENT OF IMMUNE FUNCTION BEFORE AND AFTER TREATMENT WITH RECOMBINANT FACTOR VIII FUSED WITH FC FRAGMENTS

S. P'ng[1,*], C. Almeida[1]

[1]Haematology, Fiona Stanley Hospital, Perth, Australia

Background: Up to 30% of Haemophilia A patients develop of inhibitors or alloantibodies to Factor VIII. Modifications to the Factor VIII product to increase their half life are speculated affect their immunogenicity. In particular Elocate which contains the Fc fragment of immunoglobulin (FVIIIFc). This Fc fragment contains regulatory T cell (Treg) epitopes which protect the FVIII molecules from immune responses generated by T cells. There is

evidence in a mouse model that repeated administration of FVIIIFc resulted in significantly lower antibody responses to the rFVIII compared with standard recombinant products. The immune response was characterized by higher percentage of Treg cells, lower percentage of pro-inflammatory splenic T cells and upregulation of tolerogenic cytokines and markers compared with exposure to standard rFVIII.
Aims: This study examines the potential change in immune responses after repeated exposure to FVIIIFc in patients with severe haemophilia A.
Methods: Peripheral blood mononuclear cells (PBMCs) from two individuals were collected pre- and post-treatment with Eloctate and cultured *ex vivo* with both drugs. The frequency of various CD4 and CD8 T cell subsets was measured along with the activation and cytokine-producing capabilities of these subsets. Flow cytometry was used to measure the frequency of various CD4+ and CD8+ T cellular populations. Intracellular levels of TNF-α were measured by flow cytometry.IFNγ was measured by enzyme linked immunosorbent assays (ELISAs, BD) in culture supernatants. Inhibitor FVIII levels were measured in plasma samples collected at the three time-points using the Bethesda assay.
Results: The frequencies of CD4+ and CD8+ T cells were not significantly affected by treatment with Xyntha or Eloctate. The frequency of naïve CD4+ and CD8+ T cells was the highest at each time point, irrespective of drug treatment. Both the CD4+ and CD8+ terminally differentiated cells (CD45Ra+/CD27-) were present at higher frequencies in cells cultured with 100 U/mL of drug. The expression of PD-1 markedly increased in PBMCs collected at the three and six month time points and cultured in the presence of 100 U/mL of Xyntha. PD-1 expression on PBMCs collected at these same time points and cultured in the presence of Eloctate did not vary with drug concentration but were higher in comparison to cells collected before the two subjects' commenced treatment with Eloctate. The levels of TNFα and IFNγ did not vary with time or drug treatment. Neither did the expression of the activation marker CD137 and the frequency of CD4+TRegs.
Summary and Conclusions: Our study demonstrated increased levels of PD-1 expression after 6 months of Eloctate exposure.There was an increase in terminally differentiated T cells after this period suggesting a relative suppression of the immune response and development of T cell anergy. The lack of cytokine production and absence of T cell activation could potentially be attributed to the up-regulation of PD-1, which has previously been associated with T cell exhaustion. This was most noticeable at the 6 month mark suggesting exposure to the Fc fused FVIII product Eloctate may be contributory to a T cell anergy. This is the first human clinical study to support the theory that the Fc fused product maybe less immunogenic than unaltered recombinant FVIII. Further studies are required to support and expand this theory in the clinical setting.

PF294

Abstract withdrawn.

PF295

FACTOR VIII RECOVERY TEST IN PERIOPERATIVE MANAGEMENT OF ORTHOPEDIC SURGERY IN ADULT PATIENTS WITH SEVERE HEMOPHILIA A

J.J. Han[1,*], J. Kong[1], G. Shin[1], Y.J. Cho[2], H.-J. Yoon[1]

[1]Internal Medicine, [2]Orthopaedic surgery, Kyung-Hee University Medical Center, Seoul, Korea, Republic Of

Background: Hemophilic arthropathy caused by recurrent bleeding inside joints has been the most serious complication of hemophilia. Outcome of orthopedic surgery in adult patients with severe hemophilia A may be influenced by many factors, including concentration of factor VIII (FVIII) and type of blood group. Perioperative dosing of FVIII concentrate is challenging and vary between individuals.
Aims: To evaluate clinical characteristics of adult patients with severe hemophilia A those who underwent orthopedic surgery and preoperative test of FVIII recovery in association with the outcome of surgery.
Methods: This retrospective analysis included adult patients with severe hemophilia A those who underwent orthopedic surgery at a single center from January 2000 to January 2018. Preoperative test of FVIII recovery was done before the surgery. FVIII recovery rate (%/IU^{-1}/kg^{-1}) was calculated using the following formula: [Post-test FVIII level (%) – Pre-test FVIII level (%)]/Test unit of FVIII concentrate (IU)/Body weight (kg). Blood sampling for post-test FVIII level was taken after 30 – 60 minutes from FVIII concentrate infusion. Patients with high titer inhibitors who received bypassing agents were excluded in this study. All patients received VIII concentrate replacement as intermittent bolus infusion during the perioperative hospital stay. Blood sampling for trough FVIII levels were collected during the hospital stay. Target range plasma levels of FVIII during postoperative day (POD) 1 to 3 were defined according to the WFH guideline on management of hemophilia. Complications were defined as reoperation, more than 2 units of red blood cell transfusion, and death.
Results: 98 severe hemophilia A patients with median age of 36 (range, 19 – 64) were included. 84 (86%) of them received surgery for hemophilic arthropathy, including knee (n=41), ankle (n=20), and hip joints (n=16). 69 patients received plasma derived FVIII concentrate and 29 patients received recombinant product during perioperative factor replacement. 20 patients (20%) had low titer inhibitors at the time of admission (median=0.7 BU/ml; range 0.6 – 1.69). The median FVIII recovery rate was 1.7%/IU^{-1}/kg^{-1} (range, 0.3 – 7.7). FVIII recovery rate of lower 25% and upper 25% was 1.3 and 2.1%/IU^{-1}/kg^{-1}, respectively. FVIII recovery rate were significantly lower in patients who received plasma derived product than those who received recombinant product (1.6 *vs* 2.1%/IU^{-1}/kg^{-1}, p=0.004). Patients with blood group O also showed lower FVIII recovery rate than those with other blood groups (1.3 *vs* 1.9%/IU^{-1}/kg^{-1}, p=0.002). FVIII recovery rate showed a significant positive correlation with body mass index (r=0.22, p=0.029). Patients who had complications were older than those without complications (mean age: 45 *vs* 34 years old, p<0.001). More patients in blood group O could not reached target FVIII levels during POD 1-3 than those in other blood groups (58% *vs* 28%, p=0.013). FVIII recovery rate was significantly lower in patients who could not reached target FVIII levels during POD 1-3 (1.4 *vs* 1.8%/IU^{-1}/kg^{-1}, p=0.029). Patients who had complications had significantly lower FVIII recovery rate than those without complications (1.4 *vs* 1.8%/IU^{-1}/kg^{-1}, p=0.044).
Summary and Conclusions: FVIII recovery rate, in addition to blood group O, could predict the achievement of postoperative target FVIII levels during POD 1-3 and complications after elective orthopedic surgery in adult patients with severe hemophilia A.

PF296

PREDICTION OF INHIBITORS IN HAEMOPHILIA PATIENTS BY SCORE IN ONE CENTER

O. Stasyshyn[1,*], V. Krasisvska[1]

[1]General and Haematological Surgery, SI "Institute of Blood Pathology and Transfusion Medicine of UNAMS, Lviv, Ukraine

Background: Replacement therapy in hemophilia patients is complicated by the formation of inhibitors among 30% of hemophilia A (HA) patients and 3–5% of hemophilia B (HB) patients. The treatment of bleeding and elimination of inhibitors is complicated, costly and not always successful.
Aims: To develop a simple score that stratifies patients with hemophilia according to their risk of developing inhibitory antibodies.to develop a simple score that stratifies patients with hemophilia according to their risk of developing inhibitory antibodies.
Methods: 135 patients with HA and HB divided into two groups: Group I – 74 patients with inhibitors and group II – 61patients without inhibitors. We analyzed 16 factors affecting the tendency to develop inhibitor: type of hemophilia (A or B), severity, age, age at diagnosis, hereditary or sporadic, family history of inhibitors, intensive treatment at initial treatment (ED/1 episode), age at first exposure to FVIII/IX, reason for first treatment with FVIII, FVIII/IX product type, prophylaxis or "on demand" treatment, clotting factor concentrate switching, significant and life-threatening bleeding localization; surgery: I – the urgency (urgent and selective); II – the type (large and small), and the presence of purulent complications. To examine the relationship between risk factors and the likelihood of inhibitor development, we used regression models of discrete choice. Specifically, we estimate the range of one factor binomial choice models to study the individual effects of each factor on the probability of inhibitor development. In addition, we analyze the joint impact of risk factors using multiple logit regression that allows exploring the effects and importance of each factor, controlling for the presence of other factors.
Results: To build a multivariate logit model we used stepwise forward regression with 1%, 5% and 10% significance levels. The first model includes the following factors: age, , switching, FVIII/IX product type, purulent complications and the number of (ED)/1 episode. At 5% significance level, the type of surgery (large and small) as well as positive "inhibitory" history are added. At the 10% significance level, the model additionally contains a variable characterizing the urgency of surgery (urgent or planned). The number ED/1 episode has the highest impact on the probability of inhibitor development: if it increases, the likelihood of

inhibitor development increases by 27%. For patients who switch the type of concentrates, the likelihood of inhibitor development increases by 23%. The effect of the FVIII/IX product type, the age and the type of the surgery - is negative and significant, while purulent complications and burdened "inhibitory" history result in the increase in the likelihood of inhibitor development by 21% and 12%, respectively.
Summary and Conclusions: The prediction based on the multiple logit regression allows to identificate patients at high risk of inhibitor development. According to our model, the factors associated with treatment have the highest impact on the probability of inhibitor development. Based on the results, reducing the frequency of inhibitor development can be achieved by changing the approaches to the treatment of patients with hemophilia.

PF297

REAL WORLD SWITCHING FROM CONVENTIONAL FVIII PRODUCTS TO RFVIIIFC IN FRANCE, GERMANY, ITALY AND THE UK

K.-J. Myren[1], M. van der Sluijs[1], P. Kritikou[1,*], S. Lethagen[1]

[1]Swedish Orphan Biovitrum AB, Stockholm, Sweden

Background: Recombinant factor VIII Fc (rFVIIIFc), an extended half-life product for the treatment of haemophilia A, was first approved by EMA in November 2015. Since then a growing number of patients have been treated with rFVIIIFc in the real world setting. However, limited data are available on physicians' switching behaviour from conventional FVIII products to rFVIIIFc in clinical practice.
Aims: The aim of this physician survey of patient records was to describe the characteristics of patients who switched to rFVIIIFc. The aim was further to analyse the impact of rFVIIIFc on factor consumption and injection frequency in prophylactically treated patients who had switched from conventional FVIII products.
Methods: A random sample of physicians in France, Germany, Italy and the UK, who had initiated treatment with rFVIIIFc during the last 12 months were asked to collect and review patient level information for up to 5 of their most recent rFVIIIFc patients and report the requested information in an on-line electronic survey. The patient record survey was performed during July to September 2017. Data on factor consumption, treatment regimen, duration on treatment, reason for switch and age were some of the key variables collected. Physicians excluded records of patients currently involved in a clinical trial or who currently had an inhibitor to FVIII. The results were reported as descriptive statistics.
Results: The physician survey of patient records included 139 patients who switched to or from rFVIIIFc during the last 12 months. The mean age was 33 years. 53% had severe, 37% moderate and 10% mild haemophilia A. 74% of the rFVIIIFc patients were on long term prophylaxis (LTP), whereas 26% were treated on-demand (OD) or with intermittent prophylaxis (IP). The most common reason for switch was to improve quality of life (40%). In the 87 patients who switched from conventional FVIII prophylactic treatment to rFVIIIFc prophylactic treatment the annualised mean factor consumption decreased from 515,610IU to 378,849IU (27%). Further, the mean number of injections per week was reduced from 3 to 2.1 per week (30% reduction). Patient switch trends to or from rFVIIIFc are outlined below to further describe survey results (Table 1).

Table 1.

	Patients switched to rFVIIIFc			Patients switched from rFVIIIFc		
Regimen before switch	Regimen after switch			Regimen after switch		
	LTP	OD	IP	LTP	OD	IP
LTP	87		1	5		
OD	13	26		3	8	
IP	4	1	7			2

Summary and Conclusions: Patients on prophylactic regimens reduced their mean factor consumption by 27% and their injection frequency by 30%. The most common reason for switching to rFVIIIFc was to improve patients' quality of life. These results are in line with current published real world prophylactic switching data on rFVIIIFc. Further studies are warranted to gather deeper insights into patients' rationale for switching in Haemophilia A, as only the physician perspective was captured in this study.

PF298

APPLICATION OF THE ISTH BLEEDING SCORE IN HEMOPHILIA

N. Fatima[1,*], M. Borhany[2], M. Abid[1], M. Othman[3], T. Shamsi[4]

[1]Research and Development, [2]Hematology, National Institute of Blood Diseases and Bone Marrow Transplantation, Karachi, Pakistan, [3]Biomedical and Molecular Sciences, Queen's University, Ontario, Canada, [4]Hematology, National Institute of Blood disease and Bone Marrow Transplantation, Karachi, Pakistan

Background: Hemophilia is an inherited bleeding disorder. With proper treatment and self-care, patients with hemophilia can maintain an active and productive lifestyle. Hemophilia can be mild, moderate, or severe, depending on the degree of plasma clotting factor deficiency. The ISTH/SSC joint committee established a bleeding assessment tool (BAT), to standardize the reporting of bleeding symptoms. To date, the majority of research on bleeding questionnaires has been focused on von Willebrand disease (VWD) and to a lesser extent on platelet disorders with very little if any on hemophilia.
Aims: The objective of this study was to evaluate the use of ISTH-BAT in hemophilia patients of varying severity at higher hemorrhagic risk, compare the bleeding score (BS) in adult and pediatric groups and to investigate its correlation with plasma factor levels. In developed countries, the diagnosis of hemophilia is usually made during childhood. Due to the limited healthcare resources hemophilia patients in Pakistan are often only recognized after childhood age. This provided the unique chance to compare the association of the BAT and factor levels obtained in previously unknown hemophilia patients (before the factor levels became available) with the BAT in known hemophilia patients within the study.
Methods: This cross sectional analytical study was conducted at the National Institute of Blood Disease & Bone Marrow Transplantation, Karachi, Pakistan after getting approval from institutional ethics committee. One hundred and fifteen patients of Hemophilia A (HA) and Hemophilia B (HB) and 100 healthy male controls comprised of relatives of patients and blood donors were included from 2014 to 2016. Written informed consent was obtained from all participating. A team of hematologist and a trained clinical research officer in the outpatient clinics conducted these assessments and administered the ISTH BAT. The data was analyzed by using SPSS version 23.
Results: A total of 115 patients including 78 HA and 37 HB patients and 100 healthy controls were investigated using the ISTH-BAT. The mean age was 11.8 years for HA patients, 18.1 years for HB patients. Bleeding scores were higher in hemophilia patients compared to controls (P-value= 0.001), but did not differentiate between HA and HB patients (P-value=0.738). BS was lower in the pediatric group compared to the adult group (P-value =0.02) in HA patients, while there was no difference in the BS of pediatric and adult HB patients (P-value=0.3). The BS was very similar in newly diagnosed compared to known hemophilia patients and higher in severe compared to mild HA patients (P-value= 0.004). Muscle hematoma and hemarthrosis were found to be the most common symptoms in HA and HB.
Summary and Conclusions: The ISTH BAT gives comparable results whether the factor levels are known or not. For the first time we generated data on the score in newly diagnosed adult hemophilia patients. Our ongoing study will show, whether the score can predict the risk for bleeding and pediatric and/or adult hemophilia patients.

PF299

MYPKFIT HEMOPHILIA EXPERIENCE IN PEDIATRIC CASES

S. Aytac[1,*], I. Yaman Bajin[1], M. Cetin[1], F. Gumruk[1]

[1]Pediatric Hematology, Hacettepe University, Ankara, Turkey

Background: Prophylaxis is the gold standart treatment of severe Hemophilia A. However prophylaxis regimens in patients with severe Hemophilia A can be individualized according to the age, bleeding phenotype, presence of hemophilic arthropathy, life-style, physical activity and PK. MyPKFIT hemophilia dosing tool let us to estimate indivudial half life with 2 sample using Bayesian method and based on the PK prophile it can easily help to determine dosing and dosing interval.
Aims: Our aim is to show MyPKFIT experince in a small group of patient with severe Hemophilia A.
Methods: 9 patient (FVIII<1% n=7, FVIII≤2% n=2) with a median age of 11 years (2-17 years) were analysed. All were on prophylaxis without inhibitor at the time of analysis. 3 blood samples were drawn at pre-infusion and post infusion (+4 hour and +24 hours) for each case. All were on prophylaxis without inhibitor at the time of analysis. They were receiving prophlylaxis with a 25-40IU/kg adminestered at a frequency of 2 days / a week so FVIII throgh levels were taken in between 48- 72 hours after the last injection. 3 patients had a history of annual 1/2/4 joint bleeding and

the rest no joint bleeding during the last year. 4 had a history of radionuclide synovectomy in 7 joints.
Results: The mean FVIII trough levels for the cases with an initial FVIII <1% and ≤2% were 2.48±0.97%(1.48-3.75%), 5.2±0.5%(4.8-5.6%) respectively. Mean t1/2 for the cases with an initial FVIII<1% and ≤2% were 8.7±1.1 hours(7.2-10.4h), 12±0.4(11.7-12.4h), respectively. Mean t1/2 was found to be longer in patients with an initial FVIII level≤2%. When we match the patients with a bleeding event during the last year and/or HJHS >4 point (n=4 patient) with the MyPKFIT recommendation of increase dose+decrease intervals(n=3) only one patient find to be present in both of these groups.On the other hand rest of these patients(n=5)(patients without any joint bleeding during the last year and/or HJHS<4 point(n=4 patients), patients without any bleeding during the last year and/or HJHS>4 points(n=1)) myPKFIT recommend to increase dose interval without changing dose or decrease dose +increase dose interval.
Summary and Conclusions: According to our experience with the help of MyPKFIT, clinic evaluation and HJHS we can easily find high risk of patients and individualize their dose and intervals in a proper manner in developping countries.

PF300

CHALLENGES OF MANAGEMENT OF REFUGEE CHILDREN WITH CONGENITAL FACTOR DEFICIENCIES

T.N. Güçer[1], Z. Şalcıoğlu[1], E. Türkkan[2], C. Bayram[1], E.P. Uysalol[1], N.N. Ozdemir[1,*]

[1]Pediatric Hematology Oncology, Kanuni Sultan Suleyman Education and Research Hospital, [2]Pediatric Hematology Oncology, Okmeydani Research and Training Hospital, Istanbul, Turkey

Background: Refugee patients with congenital factor deficiencies (CFD) are faced with problems such as delayed diagnosis, inability to access specialist care, inadequate treatment and associated joint damage, more often than other patients. Here, we present the demographic data, treatment, outcome and problems in management of refugee children in Istanbul, Turkey.
Aims: Hemophilia and rare factor deficiency patients from two centers caring refugee patients between 2011 and 2017 were included.
Methods: A retrospective review of patients' files was performed.
Results: Of a total 526 congenital factor deficiency patients, on follow-up, 20 refugee patients were included (18 male, 2 female). Mean age of the patients was 12±10.5 years (Range 1.3-40 yrs). Nineteen were from Syria and one was from Egypt. Fifteen had history of coagulation disorder in the family. Nine had HA, 5 had HB, 2 had FVII deficiency, 2 had afibrinogenemia and 2 had vWD. Two HA, 3 HB, 2 vWD and 2 afibrinogenemia patients were consanguineous. Three hemophilia patients were diagnosed due to excess bleeding at circumcision, 11 were diagnosed after joint bleeding or muscle haematoma. 6 (2 vWFD, 2 FVII, 2 afibrinogenemia) were diagnosed after mucocutaneous bleeding. Mean age of the diagnosis was 2.2 yrs (Range 2 days-17 yrs). Only one patient from Egypt was treated with specific factor concentrate on demand before migration. Rest of the patients were treated with fresh frozen plasma (FFP) on demand in their country. No information on exposure days was available. In Turkey, all patients were treated with factor concentrate after diagnosis if required. No inhibitor was detected in any of the patients. Six patients had target joints.
Summary and Conclusions: The number of refugees is increasing worldwide due to conflicts and war which affect many public health issues. Over 2 million refugees are estimated to migrate to Turkey in recent years. Chronic diseases in refugees cause a substantial burden to the health systems of the host countries. Patients are confronted with language barriers, problems in compliance to treatment, shelter and psychosocial problems. Medical records, factor exposure days, previous treatment, inhibitor history or viral infections are unknown to health care staff caring factor deficiency patients. Analysis of care of refugee patients with CFD is needed to inform future responses.

Bone marrow failure syndromes incl. PNH – Biology & Translational Research

PF301

PROTEIN EXPLORER IN THE CONTEXT OF OVERLAP OF BONE MARROW FAILURE DISORDERS

A. Alaiya[1,*], Z. Shinwari[1], H. Alzahrani[2], T. Owaidah[3], F. Al Mohareb[2], F. Al Sharif[2], R. Al Nounou[3], W. Rasheed[2], R. El Fakih[2], A. Hanbali[2], M. Shaheen[2], F. Alfraih[2], H. Shahrukh[2], M. Dasouki[4], N. Chaudhri[2], S.O. Ahmed [2], M. Aljurf[2]

[1]Stem Cell & Tissue Re-Engineering Program, Research Center, [2]2Adult Hematology/HSCT Section, Cancer Center, [3]Hematopathology Section, Department of Pathology & Laboratory Medicine, [4]Department of Genetics, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

Background: There is considerable overlap among BMFs entities impacting on accurate diagnostic pitfalls and caveats. The lack of surveillance tools for disease progression and possible cancer development poses significant implications on the management and care of these patients. It is therefore fundamental to understand the indications and limitations of the emerging number of analysis platforms that requires interdisciplinary interactions to overcome this difficulty.
Aims: We have aimed to identify and validate panels of disease specific/disease associated proteomics based biomarkers of potential clinical value in patients presenting with various forms of bone marrow failure syndromes including Severe Aplastic Anemia (SAA), Paroxysmal Nocturnal Hemoglobinuria (PNH) and Hypoplastic Myelodysplastic Syndrome (MDS). Others are Inherited BM Failure Syndromes (IBMFS), Fanconi Anemia (FA), Cytopenia-Severe Congenital Neutropenia (CSCN), and Thrombocytopenia (TCP).
Methods: Peripheral blood plasma (PBP) samples from 49 subjects diagnosed with bone marrow failure; including AA/ MDS/ PNH/FA/IBMFS/CSCN/TCP as well as PBP from 10 healthy subjects and 2 family unaffected family members of two affected subjects were analyzed using liquid chromatography tandem mass spectrometry (LC-MS/MS).
Results: Well over 400 unique protein species were identified from PBP of which 106 were significantly differentially expressed (≥2 to ∞ - fold change & p <0.05) between SAA/MDS/PNH/ Normal control subjects. These protein fingerprints independently discriminates all patients and control subjects into four distinct clusters using principal component analysis.
The expression changes of the 106 proteins were tested on four additional samples groups (Fanconi/CSCN/TCP/SAA+IBMF) and all samples were classified distinctively into 8 clusters using PCA. Furthermore, the diagnostic accuracy of the 106 protein signatures was tested on three additional samples without prior knowledge of their proven or presumptive diagnosis. Remarkably, these new samples stood out as outliers from the 8 other distinct cohorts. These samples ultimately turned out to be Dyskeratosis Congenita (DKC). Furthermore; analysis of two unaffected family members shares similar protein signatures as their corresponding affected siblings suggesting the likelihood of being carriers or having the risk of developing the disease. Some of our identified potential biomarker proteins were implicated in hematological diseases and multiple signaling networks associated with known bone marrow failure syndrome genes using Ingenuity Pathway Analysis (Figure 1).

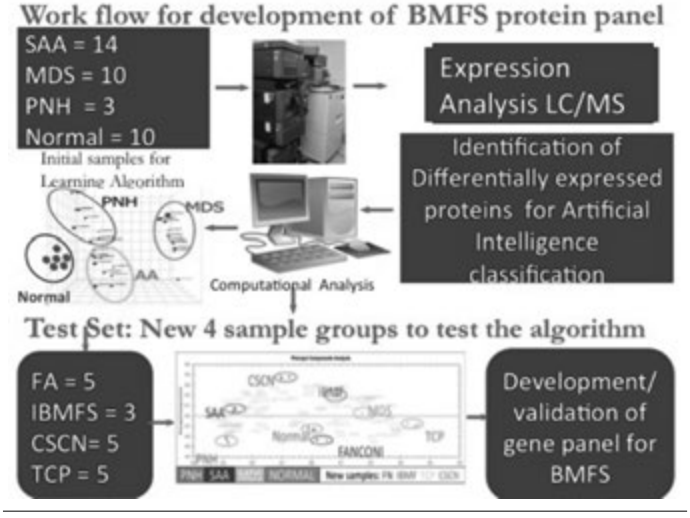


Figure 1.

Summary and Conclusions: We have identified 106 proteins panel as potential biomarker protein signatures capable for objective molecular diagnosis of bone marrow failure entities. It is anticipated that this study might reveal sets of proteins that are unique to each of the BMF entities. These proteins once validated, on a larger cohort of patients, might be valuable to complement the currently existing genetic alterations parameters for reliable and objective disease diagnosis, prognostic indicators in the management of and clinical outcome of bone marrow failure syndrome patients.

PF302

NOVEL MUTATIONS IN PATIENTS WITH TELOMEROPATHIES

M. Haubitz[1,*], I. Helsen[1], E. Oppliger Leibundgut[1,2], G.M. Baerlocher[1,2]

[1]Department for BioMedical Research, Bern University, [2]Department of Hematology, University Hospital Bern, Bern, Switzerland

Background: Telomeropathies are characterized by insufficient telomere maintenance resulting in short telomeres due to defects in the telomerase, shelterin, CST or Cajal body complex. The phenotypes of patients (pts) with such defects are very heterogeneous ranging from mild cytopenias to severe bone marrow failure (BMF), classical dyskeratosis congenita (DC, triad of leukoplakia, hyper- and hypopigmentation, nail dystrophy), lung or liver fibrosis and described are a shorter life span and predisposition of cancer. In part of the pts mutations are found in 12 genes involved in telomere maintenance, however, consequences of novel mutations are often unknown.
Aims: Our aims were to screen suspected individuals and pts for telomere length (TL) and in case of short telomeres to search for telomeropathy-relevant variants as well as functionally characterize novel mutations.
Methods: TL in leukocyte subsets was measured using combined fluorescence *in situ* hybridization and flow cytometry (flow FISH) and compared to percentiles (P) of reference ranges. Mutation detection was performed in 12 genes (*ACD*, *CTC1*, *DKC1*, *NHP2*, *NOP10*, *PARN*, *RTEL1*, *TCAB1*, *TERC*, *TERT*, *TINF2* and *USB1*) by high throughput sequencing. Enzyme activity and altered protein function are investigated by TRAP, Western blot, D-loop recombination assay and immunostaining.
Results: We assessed the TL in leukocyte subsets of 349 blood samples from 235 index pts (IP) (median age 17 years (y), range 0.1-77 y) and 114 relatives (RL) (median age 32 y, range 0.9-69 y) sent for the TL screening due to a suspected telomeropathy. 226/235 IP and 10/114 RL were described with a clinical phenotype; 2% had DC, 59% cytopenias and/or BMF of unknown origin, 11% myeloid neoplasia, 4% other organs involved and 25% suspected telomeropathies. 60% of IP and 38% of RL had TL <1st P and 27% of IP and 34% of RL between the 1st-10th P in all subsets of leukocytes. 82 IP and 42 RL had a mutation analysis. In this cohort 72% of IP, 75% of the 9 affected RL and 45% of non-affected RL had TL <1st P. 34% of IP, 33% of non-affected RL and 78% of affected RL had variants in the genes *TERT*, *TERC*, *DKC1*, *NPH2*, *TCAB1*, *TINF2*, *ACD*, *RTEL1* and *CTC1*. 18 novel and 6 known mutations were identified in IP; recurrent mutations included a known mutation in *TERT* in 3 IP and 2 novel variants in *RTEL1* and *CTC1* in 2 and 4 IP, respectively. In IP of 5 unrelated families with histories of bone marrow failure and with TL <1st P, but no signs for defects in other organs, we found novel heterozygous variants in *TERC* and *TERT* leading to reduced telomerase enzyme activity. Two novel heterozygous *RTEL1* variants, not affecting telomerase activity, but binding of the telomerase to telomeres, were detected in 3 unrelated families with histories of bone marrow failure, head and neck cancer and liver fibrosis and TL <10th P. In 5 IP with variable clinical signs and TL <10th P two or three heterozygous variants each in the genes *TERT*, *TERC*, *NHP2*, *ACD*, *CTC1* and *RTEL1* were detected.
Summary and Conclusions: We found mutations in 39% of IP and affected RL, a high proportion of novel variants and 2 recurrent in 6 families. 82% of IP or RL with mutations present TL values in leukocyte subsets <1st P but 13% fall in the range 1st-10th P. The investigation of the functional consequences of important mutations in genes of the telomerase, shelterin, CST or Cajal body complex either alone or in combination is ongoing. Since telomerase activity can be increased by hormonal stimulation as a treatment option, uncovering the pathophysiologic mechanisms of these mutations is of great importance.

PF303

LOW PROPORTION OF MYELOID DERIVED SUPPRESSOR CELL POPULATIONS IN THE PERIPHERAL BLOOD OF PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA

N. Bizymi[1,*], M. Velegraki[1], A. Damianaki[1], H. Koutala[1], V. Kaliafentaki[1], M. Ximeri[1], P. Kanellou[1], I. Mavroudi[1], C. Pontikoglou[1], H. Papadaki[1]

[1]Haemopoiesis Research Laboratory, School of Medicine, University of Crete and Department of Haematology, University Hospital of Heraklion, Heraklion, Greece

Background: Chronic Idiopathic Neutropenia (CIN) is a mild bone marrow (BM) failure syndrome characterized by presence of oligoclonal/monoclonal T-cell populations with myelosuppressive properties. The triggering event leading to T-cell activation remains unknown. The myeloid derived suppressor cells (MDSCs) are cells of myeloid origin capable of suppressing T-cell responses mainly through activation of arginase 1 and nitric oxide synthase 2 and production of reactive oxygen species, modulation of macrophage polarization and induction of T-regulatory cells. MDSCs are divided in two subpopulations, namely the polymorphonuclear (PMN)-MDSCs and monocytic (M)-MDSCs characterized by the immunophenotype HLA-DR$^{low/-}$CD11b^{+}CD33^{+}CD15^{+} and HLA-DR$^{low/-}$CD11b^{+}CD33^{+}CD14^{+}, respectively. The possible involvement of MDSCs in the aberrant T-cell responses associated with CIN has not been studied before.
Aims: The aim of the present study is to investigate the quantitative characteristics of MDSCs in the peripheral blood (PB) of CIN patients in comparison to healthy subjects.
Methods: We have studied 34 patients fulfilling the previously reported diagnostic criteria for CIN and 23 age- and sex-matched with the patients, healthy subjects. According to the criteria, CIN patients had absolute neutrophil counts (ANC) below 1800 x 10^6/L for a period at least 3 months, had no clinical, serological or ultrasonic evidence of any underlying disease associated with neutropenia, no history of exposure to irradiation, use of chemical compounds or intake of drugs to which neutropenia might be ascribed, normal BM karyotype and negative antineutrophil antibodies. Cyclic cases were excluded by serial neutrophil enumerations. The proportion of PB MDSCs was evaluated by flow cytometry. According to the current recommendations (*Bronte et al., Nature Communications 2016*), MDSCs should be studied in the fraction of PB mononuclear cells (PBMCs). Thus, we have changed our previously reported (EHA 2017) protocol that included staining of total PB and performed flow cytometric analysis of PMN-MDSCs and M-MDSCs in the PBMC fraction.
Results: Surprisingly, in contrast to our previous preliminary results (EHA 2017) from total PB, CIN patients displayed decreased proportion of M-MDSCs in the PBMC fraction (0.657±0.664) compared to the healthy controls (2.085±2.496; P=0.0228). No statistically significant difference was identified between CIN patients and controls in the proportion of PMN-MDSCs. The proportion of M-MDSCs correlated with the number of ANC (r=0.2976, P=0.0273) suggesting that patients with more severe neutropenia display lower M-MDSCs numbers. A positive correlation was also found between the proportion of M-MDSCs and the number of PB monocytes (r=0.2672, P=0.0486).
Summary and Conclusions: CIN patients display low proportions of M-MDSCs compared to the healthy subjects. These cells normally protect from uncontrolled immune responses; therefore the low number of these cells in CIN patients may contribute to the sustained chronic inflammation. The results of this study contribute to the better understanding of the pathogenesis of CIN and underlie the importance of the methodology used to study this population of cells.

Bone marrow failure syndromes incl. PNH – Clinical

PF304

CLINICAL SIGNIFICANCE OF PNH CLONES IN 3085 PATIENTS WITH CYTOPENIA: A LARGE SINGLE-CENTER EXPERIENCE

B. Fattizzo[1,*], A. Dunlop[1], R. Ireland[1], S. Kassam[1], D. Yallop[1], G. Mufti[1], J. Marsh[1], A. Kulasekararaj[1]

[1]Hematology, King's College Hospital, London, United Kingdom

Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare clonal disorder due to GPI anchored proteins deficiency on blood cells surface, resulting in complement activation and chronic intravascular hemolysis. Along with classic one, PNH in the setting of bone marrow disorder [aplastic anemia (AA)/myelodysplastic syndrome (MDS)], and subclinical PNH, with a small PNH population and no evidence of hemolysis, have been described. The prevalence and clinical significance of PNH clones, especially small ones, detected by high sensitive FLAER are still under debate.
Aims: To evaluate the prevalence of PNH clone in 3085 patients with cytopenia tested at a single tertiary center, and to assess their relationship with disease severity and outcome.
Methods: We collected clinical (diagnosis, stage, therapy, complications and outcome) and laboratory features (complete blood counts, LDH, PNH clone) of 3085 patients tested from March 1998 till October 2017.
Results: Main baseline clinical and laboratory characteristics of patients, divided according to presence or absence of PNH clones, are shown in Table 1.

Table 1. Patients screened for PNH clones at our Institution.

N 3085	PNH neg	PNH pos
Study period	Mar 1998 to Oct 2017	
Number of patients, N (%)	2311 (75)	774 (25)
Male/Female ratio	1.17	1.05
median age years (range)	55 (0-91)	47 (1-89)*
	N=2160	N=744
MDS N(%)	693 (32)	176 (23.6)*
AA N(%)	204 (9.4)	327 (43.9)*
MDS/AA N(%)	5 (0.2)	22 (2.9)*
Acute leukemia N(%)	209 (9.6)	29 (3.9)*
Haemolytic PNH N(%)	0 (0)	97 (13)
MPN N(%)	76 (3.5)	16 (2.15)
MDS/MPN N(%)	92 (4.2)	9 (1.2)*
Isolated cytopenia N(%)	512 (23.7)	39 (5.2)*
Isolated thrombosis N(%)	284 (13)	17 (2.3)*
Other reason N(%)	85 (3.9)	12 (1.6)*
Thrombosis occurrence N(%)	370 (17.12)	96 (12.9)
Death N(%)	725 (33.6)	141 (18.9)*
Lost to follow-up N(%)	146 (6.75)	75 (10)
Haematological parameters	N=1027	N=744
Hb<100 g/L, N(%)	409 (40)	351 (47)**
PLT<100x10^3/mmc, N(%)	463 (45)	423 (57)*
ANC<1.5x10^3/mmc, N(%)	478 (46.5)	342 (46)
Pancytopenia N(%)	160 (16)	183 (24.5)*
Median LDH U/L (range)	212 (92-1520)	245 (70-4614)*

*p≤0.0001, **p=0.01

PNH clone (PNH+) was found in 774 cases (25%), mostly AA (44%), MDS (24%), and florid hemolytic PNH (13%). Clone size, evaluated on granulocyte in 468 cases, was <1% in 224, 1-50% in 120, and >50% in 124, and correlated with LDH levels (p<0.0001). Considering diagnosis, PNH+ MDS displayed smaller clones compared to AA and haemolytic PNH ones (60% of cases with PNH clone size >50%). Serial PNH clone evaluation (n=230), showed mean clone size increase along time, particularly in haemolytic PNH cases treated with eculizumab. Among PNH- cases, the most frequent reason for testing were MDS (32%), idiopathic cytopenia (23.7%), and isolated thrombosis (13%). PNH+ cases were younger (p<0.0001), more frequently anaemic (p=0.01), thrombocytopenic (p=0.0003), or pancytopenic (p<0.0001), with higher LDH (p<0.0001). PNH+ patients also showed longer OS from first test [mean 14.24±0.35 years (95%CI 13.56-14.93) *versus* 8.16±0.26 years (7.64-8.68), p<0.0001]. PNH+ MDS patients (N=176, 20.3%) were significantly younger, more hypoplastic (p<0.001), and less frequently showed excess of blast (p=0.01); they also showed deeper cytopenias

(p=0.04 for Hb, and p<0.0001 for PLT), and had higher LDH levels (p<0.0001). Moreover, they had more frequently received cyclosporine and ATG (p=0.0001), less frequently chemotherapy or azacytidine (p<0.0001 and p=0.002), and 7 cases had been treated with eculizumab. PNH+ MDS showed lower rate of higher risk progression (p=0.003), AML evolution (p=0.01), and death (p<0.0001), but had higher incidence of thrombotic events (p=0.05). Survival analysis also showed a longer OS for PNH+ MDS [mean 11.9±0.7 years (10.5-13.3) *vs* 7.3±0.3 (6.6-7.9), p<0.0001] compared to PNH- ones. PNH+ AA (61%) showed deeper thrombocytopenia (p<0.0001), higher reticulocyte counts (p=0.0004) and LDH values (p<0.0001). PNH+ AA were more frequently treated (p<0.0001), and showed lower MDS progression and deaths (p=0.01 and p<0.0001), and longer OS [mean 15.8±0.43 years (14.9-16.7) *vs* 6.5±0.35 (5.8-7.21), p<0.0001].

Summary and Conclusions: Prevalence of PNH clones of any size is high in patients with bone marrow failure and carries prognostic significance. In this largest reported retrospective series, even the presence of small clones correlates with lower blood counts, increased LDH, and occurrence of thrombosis. Finally, PNH positivity seems to be more frequent in patients of younger age and to predict a better survival

PF305

RA101495, A SUBCUTANEOUSLY-ADMINISTERED PEPTIDE INHIBITOR OF COMPLEMENT COMPONENT C5, FOR THE TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA: PHASE 2 RESULTS

A. Hill[1,*], H. Schrezenmeier[2], P. Hillmen[1], J. Szer[3], H. Pullon[4], R. Spearing[5], C. Forsyth[6], E. Armstrong[7], C. Patriquin[8], I. Weitz[9], Y.-M. Shen[10], T. Munir[1], M. Griffin[1], M. Hoarty[11], A. Ricardo[11], R. Farzaneh-Far[11], A. Kulasekararaj[12]

[1]Department of Haematology, St. James' Institute of Oncology, Leeds, United Kingdom, [2]Center for Transfusion Medicine and Immunogenetics, Ulm University, Ulm, Germany, [3]Royal Melbourne Hospital, Melbourne, Australia, [4]Waikato Hospital, Hamilton, [5]Christchurch Hospital, Christchurch, New Zealand, [6]Jarrett Street Specialist Center, Gosford, Australia, [7]Helsinki University Cancer Hospital, Helsinki, Finland, [8]Toronto General Hospital, Toronto, Canada, [9]University of Southern California, Los Angeles, [10]University of Texas Southwestern Medical Center, Dallas, [11]Ra Pharmaceuticals, Inc., Cambridge, United States, [12]King's College School of Medicine, United Kingdom, United Kingdom

Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, clonal, hematopoietic stem cell disorder caused by a deficiency of glycosylphosphatidylinositol-anchored proteins (GPI-AP) on cell surfaces. Patients with acquired mutations in the phosphatidylinositol glycan class A gene lack functional complement-regulatory proteins, resulting in abnormal accumulation of complement fragments on the surface of erythrocytes, and subsequent intravascular hemolysis by the membrane attack complex (MAC). RA101495 is a synthetic macrocyclic peptide that binds with high affinity to C5 and prevents its cleavage into C5a and C5b, thereby preventing the assembly and cytolytic activity of MAC on GPI-AP-deficient erythrocytes. In a completed Phase 1 study in healthy volunteers, subcutaneously-administered RA101495 was safe and well-tolerated, and achieved rapid, complete, and sustained inhibition of complement activity.

Aims: Study RA101495-01.201 and RA101495-01.203 are international, multicenter, open-label, Phase 2 dose-finding studies designed to evaluate the safety, tolerability, and efficacy of RA101495 in patients with PNH.

Methods: Study RA101495-01.201 enrolled separate cohorts based on prior eculizumab treatment history: the treatment naïve cohort recruited 10 patients who had not previously received eculizumab; the eculizumab switch cohort recruited 16 patients who had received treatment with eculizumab for at least 6 months prior to Screening. Study RA101495-01.203 recruited 3 patients with an inadequate response to eculizumab. All patients received an initial loading dose of 0.3 mg/kg of RA101495 administered subcutaneously (SC) at the Day 1 visit. Thereafter, patients self-administered once daily SC doses of 0.1 mg/kg or, upon dose escalation, 0.3 mg/kg for 12 weeks. The primary endpoint was the change in lactate dehydrogenase (LDH) from baseline to the mean of the Week 6-12 values. Patients completing 12-weeks of dosing were eligible to enter a long-term extension study RA101495-01.202.

Results: In treatment naïve patients, the pre-specified primary endpoint was met for LDH reduction from baseline to the mean of Weeks 6-12 (p=0.002). All 10 treatment-naïve patients exhibited LDH reductions on RA101495, and all successfully completed 12 weeks of dosing. The reduction in LDH levels after initiation of RA101495 was rapid and robust, and has been sustained in the long-term extension study for up to 36 weeks of dosing (Figure 1). The effect of RA101495 to reduce LDH in treatment-naïve patients was accompanied by consistent and complete (95-98%) suppression of complement activity in an *ex-vivo* antibody-sensitized sheep red blood cell (sRBC) direct hemolysis assay. LDH reductions in treatment-naïve patients were associated with reductions in transfusion dependence and improvement in quality of life measures. In patients switching from eculizumab to RA101495 a divergent LDH response was observed, with prior transfusion-dependence on eculizumab associated with breakthrough hemolysis after switching. Overall, the majority (16/21, 76%) of patients completing the 12-week study continue to receive RA101495 in the longterm extension.

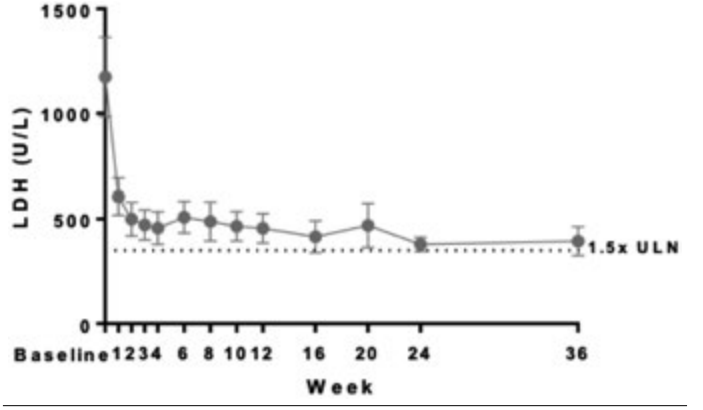


Figure 1.

Summary and Conclusions: RA10495, self-administered daily by subcutaneous injection, appears safe and well-tolerated in patients with PNH. RA101495 rapidly and robustly reduces LDH to the levels seen in patients receiving eculizumab, and which are associated with improved long-term outcomes in PNH. These Phase 2 findings support a phase 3 confirmatory study, and indicate that RA101495 may provide a more convenient and cost-effective treatment for PNH patients.

PF306

DIFFICULTIES IN DIAGNOSIS AND MANAGEMENT IN PATIENTS WITH CYCLIC NEUTROPENIA

S. Mellor-Heineke[1], F. Froemling[1], M. Klimiankou[2], J. Skokowa[2], K. Welte[3], C. Zeidler[1,*]

[1]Department of Hematology, Hemostasis,Oncology, Stem Cell Transplantation, Hannover Medical School, Hannover, [2]Department of Oncology,Hematology, Immunology, [3]Pediatric Hematology, Oncology, University of Tuebingen, Tuebingen, Germany

Background: Cyclic neutropenia (CyN) is a rare hematological condition in which underlying ELANE mutations can be detected in approximately 50% of patients. The Diagnosis of CyN is based on the cycling course of neutrophils which decreases to <0.5 x 109/L, at 21 day intervals. Patients suffer from recurrent bacterial infections during the neutrophil nadir. Typical infections include recurrent aphthae, tonsillitis or skin abscesses as well as early loss of teeth.

Aims: Depending on the time of the cycle bone marrow morphology can vary between normal or severe maturation arrest. Normal bone marrow morphology may be misleading towards autoimmune neutropenia. Therefore patients do not receive adequate therapy. Here we describe the difficulties of making the diagnosis already in the first months of life.

Methods: We analyzed the clinical data of 100 patients with CyN reported to the Severe Chronic Neutropenia International Registry (SCNIR) Europe.

Results: Out of the 100 patients with CyN 58/100 (28 female, 30 male) patients revealed a mutation in the ELANE gene, 9/100 patients have been tested negative and 33/100 patients have not been tested so far. The age at diagnosis ranges from 0 to 35.5 years in the 58 ELANE positive patients, including 11 families with 2 or more affected family members. 53/58 patients are receiving G-CSF treatment with a median dose of 2 µg/kg/day (range 0.15–17.86 µg/kg/d). Under G-CSF therapy infectious episodes are significantly reduced. Intriguingly, treatment with G-CSF in patients with CyN does not abrogate cycling, but increases the absolute neutrophil count, shortens the cycle periodicity from the usual 21 days to about 14 days and prevents serious infections. CyN has a lower risk of malignant evolution as compared to other patients with ELANE mutation positive congenital neutropenias. To document the difficulties of making the diagnosis already in the first months of life, we will present seven young adult patients who have been diagnosed with CyN at the age of 12-30 years. All 7 patients have a history of recurrent bacterial infections starting in early childhood. In 3 patients bone marrow aspirations showed normal maturation. 2 patients

presented with severe parodontitis and imminent loss of teeth. Complete blood counts (CBC) were performed sporadically before G-CSF treatment showing fluctuating absolute neutrophil counts ranging from 0-3400/µl. CyN diagnosis was finally established with the collection of CBC 2-3x/week over a period of 4-6 weeks. In all 7 patients genetic analysis revealed an ELANE mutation. G-CSF therapy was initiated on a daily basis and it is well tolerated. However the cycling of neutrophils is still present under G-CSF treatment.

Summary and Conclusions: Cyclic neutropenia still remains difficult to discriminate from other types of congenital neutropenia. The delay of diagnosis is mainly due to milder morbidity and insufficient number of blood counts available to document the cycle and the heterogeneity of bone marrow morphology. There might be a dark digit of undiagnosed patients suffering from severe infections who have not been diagnosed so far. ELANE-CyN Patients with affected family members are often diagnosed earlier than patients with *de-novo* mutations due to the awareness of the disease. To diagnose CyN it is most important to collect a sufficient number of CBCs (3x/week over a period of 6 weeks). The presence of ELANE mutations does not discriminate between CyN and other congenital neutropenias. G-CSF therapy given at an individual dose on a daily basis is beneficial to protect from infections.

PF307

CLINICAL AND MORPHOLOGIC PREDICTORS OF OUTCOME IN APLASTIC ANEMIA PATIENTS TREATED WITH ELTROMBOPAG

B. Fattizzo[1,*], N. Benson-Quarm[2], A. Hill[3], L. Arnold[4], K. Riley[5], T. Munir[4], M. Griffin[4], R. Ireland[2], H. De Lavallade[2], D. Mc Lornan[2], K. Raj[2], G. Mufti[2], J. Marsh[2], A. Kulasekararaj[2]

[1]Haematology, [2]Hematology, King's College Hospital, London, [3]Haematology, [4]Hematology, Leeds Teaching Hospital, [5]Hematology, St James's University Hospital, Leeds, United Kingdom

Background: Aplastic anemia (AA) is a rare autoimmune disease with high morbidity and mortality mainly due to bleeding and infections. Although immunosuppressive therapy (IST) is a highly effective first line treatment, up to 30-40% of cases would be refractory to or relapse after IST. TPO mimetic eltrombopag (EPAG) has demonstrated 40% response in refractory AA, however little is known about predictors of response.

Aims: To evaluate clinical/morphologic predictors of response and outcome in a cohort of EPAG treated patients.

Methods: 49 AA patients treated with EPAG in a compassionate use program from January 2012 to July 2017 were evaluated. Clinical and hematologic parameters including treatment response (PR=transfusion independence, CR=hematologic normalization) and marrow characteristics were collected.

Results: Table 1 shows baseline clinical and morphologic characteristics: all cases were transfusion dependent and 4 showed cytogenetic aberrations (+8, delY, and del13q in 2 cases). 43 cases had received a previous treatment (IST or androgen). Patients received EPAG for a median of 4 months (range 3.5-30), and 11 cases (22%) responded, of whom 4 PR and 7 CR. Median time to best response was 3 months. Compared to baseline, 21% of cases became RBC and 14% PLT transfusion independent. Hematologic improvement was observed in all three lineages (mean Hb, PLT and ANC increase was 34±12.4 g/L p<0.001, 80±53x10^3/mcL p<0.001, and 1.25±0.95x10^3/mcL, p=0.02, respectively). The presence of moderate disease was associated with a better response (73% in responders *vs* 24 in non-responders, p=0.004), together with a lower lymphoid marrow percentage (9.1 *vs* 18.1%, p=0.04), and with a smaller PNH clone (0.69±1.1% *versus* 11.22±24%, p=0.01). Patients who relapsed after IST showed a better response to EPAG (p=0.05). Marrow cellularity significantly improved after EPAG therapy in responders (p=0.004), whereas PNH clone dynamic changes did not show any relationship with response. 29% of cases experienced an adverse event including nausea (N=2, grade I and II), diarrhea (2, grade II and III), liver enzyme elevation (3, grade II), rash (grade II), muscle cramps (grade I), pneumonia (grade III), sepsis (grade III), and bone marrow fibrosis (2). Eleven patients (9 responders and 2 non-responders) are still on treatment, whereas 38 discontinued because of death (11), lack of response (21), loss of response (2), evolution to MDS (1), treatment intolerance (1), no response with increase in marrow fibrosis (1), and longstanding CR with bone marrow fibrosis (1). Three cases relapsed after 7, 15 and 34 months, respectively. One received danazol, another cyclosporin and tacrolimus, and the latter was re-challenged with EPAG with a new CR. Among non-responders, 10 cases received supportive care, 6 androgens, 1 ATG, 3 cyclosporin, and 7 underwent HSCT. One patient with large PNH clone was treated with eculizumab. Median overall survival (OS) calculated from the beginning of eltrombopag was 9 months (range 4-54 months), and was significantly longer in patients responding to eltrombopag therapy (34 *versus* 7 months, p=0.003), with 100% OS in responders *versus* 50% in non-responders at 12 months.

Table 1. Baseline clinical and laboratory characteristic of AA patients enrolled.

	Patients N=49
Age years mean±SD	62±16.4
Gender F/M	22/27
AA type	
acquired N(%)	48 (98)
inherited N(%)	1 (2)
AA severity	
moderate N(%)	22 (44)
severe N(%)	14 (29)
very severe N(%)	13 (27)
Overlapping MDS N(%)	6 (12)
EPO levels (N=32,65%) mean±SD	695.32±599
TPO levels (N=10,20%) mean±SD	1892.04±925.99
Hb g/L, mean±SD	89±16
PLT x10 ^{9}/L mean±SD	21±16*
ANC x10 ^{9}/L mean±SD	1±0.88*
Transfusion dependence	
RBC N(%)	43 (88)
PLT N(%)	42 (86)
BM features (N=46, 94%)	
cellularity % mean±SD	16.08±13.5
MGK	
absent N(%)	22 (48)
reduced N(%)	18 (39)
dysplastic N(%)	4 (9)
normal N(%)	2 (4)
Lymphoid infiltrate % mean±SD	16.15±19.19
PNH clones positivity N(%)	32 (65)
Cytogenetic abnormalities N(%)	4 (8)
Normal	37 (76)
Failed	8 (16)
Abnormal	4 (8)

*Mean PLT were 34±7x10^9/L and mean Hb 110±13 g/L in transfusion independent patients.

Summary and Conclusions: EPAG is effective and safe in the real world setting, although with a lower response rate (22%). Non-severe AA, relapsed disease rather than refractory AA, smaller PNH clone, and lower marrow lymphoid percentage seem to correlate with better response to treatment. Finally, response to treatment significantly predicts better survival.

PF308

A COMPARATIVE STUDY OF INTENSIVE IMMUNOSUPPRESSIVE THERAPY AND HAPLOIDENTICAL TRANSPLANTATION FOR YOUNG SEVERE APLASTIC ANEMIA

M. Chen[1,*], Y. Wu[2], W. Xiaoxiong[2], B. Han[1]

[1]Department of Hematology, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Sciences, [2]Department of Hematology, The First Affiliated Hospital, Chinese PLA General Hospital, Beijing, China

Background: Intensive immunosuppressive therapy of anti-thymocyte immunoglobulin(ATG) combined with CsA is the first choice for young severe aplastic anemia patients without matched sibling donor.Haploidendical donor hematopoietic stem cell transplantation (HID-HSCT) has been improved greatly and showed similar efficiency as matched sibling donor transplantation.

Aims: This study aimed to compare the differences of intensive immunosuppressive therapy(IST) and HID-HSCT for young patients with severe aplastic anemia.

Methods: A retrospective study of 55 severe aplastic anemia(SAA) patients aged 14-30 years old was done. They were treated with anti-thymocyte immunoglobulin(ATG) combined with CsA (29 cases) or HID-HSCT (26 cases). The efficacy, survival, side effects and costs of two groups were compared.

Results: The median follow-up of survival patients was 30 (12-71) months. Complete remission (CR) in IST group and HID-HSCT group were 58.6% *vs* 84.6% (P=0.034), and overall reaction (OR) was 86.2% *vs* 84.6%(P=1.000), respectively. There was no significant difference in estimated overall survival for 5 years (IST 77.9±11.7% *vs* HID-HSCT 82.1±8.4%) and event free survival (IST 67.6±11.7% *vs* HID-HSCT 69.2±9.1%). The relapse rate of IST group was 3.4% and the clonal transformation was 13.8%. In the HID-HSCT group, both relapse and clonal

transformation was 0, but the incidence of grade II-IV aGVHD and extensive and limited cGVHD was 19.2% and 15.4%. The hospitalization expense in IST group was significantly lower than that in HID-HSCT group (median $ 24 thousands *vs* $ 54 thousands, P=0.000) (Figure 1).

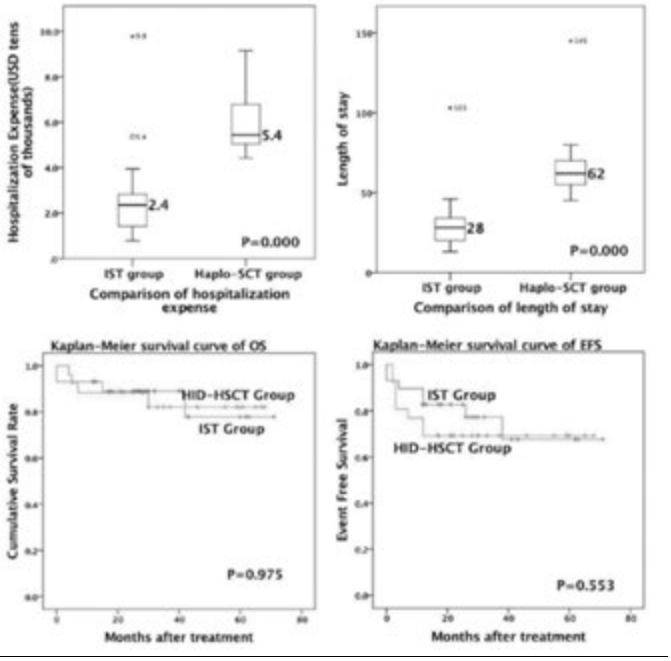


Figure 1.

Summary and Conclusions: IST should still be the first choice for young SAA without matched sibling donors. HID-HSCT can improve the outcome of patients who failed by IST, relapsed or clonal transformed.

PF309

EFFECTIVENESS OF ECULIZUMAB IN PNH PATIENTS RECEIVING CONCOMITANT IMMUNOSUPPRESSIVE THERAPY

A. Hill[1,*], R. Peffault de Latour[2], A.G. Kulasekararaj[3], M. Griffin[1], R.A. Brodsky[4], J.P. Maciejewski[5], A. Wilson[6], J.L. Marantz[6], H. Schrezenmeier[7]

[1]Leeds Teaching Hospitals, Leeds, United Kingdom, [2]Hôpital Saint-Louis AP-HP, Paris, France, [3]King's College Hospital, London, United Kingdom, [4]Johns Hopkins University School of Medicine, Baltimore, [5]Taussig Cancer Institute, Cleveland Clinic, Cleveland, [6]Alexion Pharmaceuticals, Inc., Lexington, United States, [7]Institute of Transfusion Medicine, University of Ulm and Institute of Clinical Transfusion Medicine and Immunogenetics, German Red Cross Blood Transfusion Service Baden-Württemberg-Hessen and University Hospital Ulm, Ulm, Germany

Background: Paroxysmal nocturnal hemoglobinuria (PNH) often occurs concurrently with aplastic anemia (AA), and immunosuppressive therapy (IST) is commonly used in the management of AA. Data regarding concomitant use of eculizumab and IST in patients with PNH and AA are limited. The International PNH Registry (NCT01374360) is a prospective, multinational, observational study to collect data on the long-term efficacy and safety of eculizumab treatment.
Aims: To evaluate real-world effectiveness of eculizumab in PNH patients receiving concomitant IST.
Methods: Patients enrolled in the PNH Registry on or before January 1, 2018, with known demographics and enrollment date, who (1) ever reported a diagnosis of AA, (2) were ever treated with eculizumab, and (3) were ever treated with IST (defined as cyclosporine and/or anti-thymocyte globulin). Patients were categorized into 3 cohorts: (I) IST before eculizumab, no overlap; (II) IST before eculizumab, with overlap; (III) eculizumab before IST, with overlap. Baseline was defined as initiation of eculizumab for all patients. The numbers of units of red blood cells (RBCs) during follow-up were calculated and summarized as rates with 95% confidence intervals (CIs) using Poisson regression for each cohort. Lactate dehydrogenase (LDH) ratios (LDH/LDH upper limit of normal) within 6 months of baseline and last follow-up were assessed, mean change was adjusted for baseline and cohort, and the following pairwise comparisons were made: cohort I *vs* cohort II; cohort I *vs* cohort III; and cohort II *vs* cohort III.
Results: Results of interest are shown in the Table 1. Patients receiving IST before eculizumab with no overlap (cohort I) had the highest platelet count at baseline (mean [SD], 141.3 [88.07] x 10^9/L) with slightly improved platelet count and the lowest rate (95% CI) of RBC transfusions during follow-up (2.1 [2.0, 2.2]). In contrast, patients receiving IST before concomitant eculizumab (cohort II) and those receiving eculizumab prior to concomitant IST (cohort III) had lower mean (SD) platelet counts at baseline (108.1 [75.32] x 10^9/L and 82.3 [71.26] x 10^9/L, respectively) with platelet count minimally improving or declining and higher transfusion rates (95% CI) during follow-up (3.9 [3.8, 4.1] and 7.3 [6.9, 7.7], respectively). Despite these differences, all 3 cohorts had elevated mean (SD) LDH ratio at baseline: 5.4 (3.23) in cohort I, 4.8 (3.33) in cohort II, and 4.2 (2.80) in cohort III. LDH ratios were reduced during follow-up to 1.5 (1.69), 1.4 (1.40), and 1.2 (0.85), respectively. No significant between-group differences were observed in pairwise comparisons of cohort I *vs* II, cohort I *vs* III, or cohort II *vs* III for adjusted mean change from baseline in LDH or PNH granulocyte clone size.

Table 1.

	Cohort I IST before ecu, no overlap of treatment n=199	Cohort II IST before ecu, with overlap of treatment n=182	Cohort III Ecu before IST, with overlap of treatment n=47
RBC transfusions between baseline and last follow-up	n=192	n=138	n=46
Total units transfused, n	1823	2391	1206
Patient-years of follow-up	862.1	607.5	166.0
Rate of transfusions per year (95% CI)	2.1 (2.0, 2.2)	3.9 (3.8, 4.1)	7.3 (6.9, 7.7)
LDH ratio (LDH/LDH ULN)	n=95	n=75	n=22
Baseline, mean (SD)	5.4 (3.23)	4.8 (3.33)	4.2 (2.80)
Last follow-up, mean (SD)	1.5 (1.69)	1.4 (1.40)	1.2 (0.85)
Change from baseline to last follow-up, adjusted mean (SE)	-3.6 (0.15)	-3.6 (0.17)	-3.7 (0.33)
Percent GPI-deficient granulocytes	n=43	n=33	n=11
Baseline, mean (SD)	78.8 (24.58)	73.1 (26.06)	69.1 (26.95)
Last follow-up, mean (SD)	77.3 (29.20)	78.5 (29.25)	82.9 (19.42)
Change from baseline to last follow-up, adjusted mean (SE)	-1.0 (3.57)	3.8 (4.05)	11.1 (7.26)
Platelets (x10^9/L)	n=123	n=90	n=26
Baseline, mean (SD)	141.3 (88.07)	108.1 (75.32)	82.3 (71.26)
Last follow-up, mean (SD)	152.7 (75.76)	120.4 (69.90)	79.0 (74.72)
Change from baseline to last follow-up, adjusted mean (SE)	19.3 (5.28)	6.4 (6.14)	-30.5 (12.94)*

CI, confidence interval; ecu, eculizumab; eGFR, estimated glomerular filtration rate; GPI, glycosylphosphatidylinositol; IST, immunosuppressive therapy; LDH, lactate dehydrogenase; RBC, red blood cell; SD, standard deviation; SE, standard error; ULN, upper limit of normal.
*P=0.0004 vs cohort I; P=0.0105 vs cohort II.

Summary and Conclusions: This analysis of patients with PNH in a real-world setting confirms the effectiveness of eculizumab during concomitant treatment with IST, which did not differ with the sequence of use.

PF310

EFFECT OF ECULIZUMAB ON TRANSFUSION NEEDS IN PNH PATIENTS WITH AND WITHOUT TRANSFUSION HISTORY

A. Röth[1,*], D. Araten[2], L. Larratt[3], A. Kulasekararaj[4], J. Maciejewski[5], A. Wilson[6], P. Gustovic[7], Y. Kanakura[8]

[1]West German Cancer Center, University Hospital Essen, Essen, Germany, [2]NYU Langone Medical Center, New York, United States, [3]University of Alberta, Edmonton, Canada, [4]King's College Hospital, London, United Kingdom, [5]Taussig Cancer Institute, Cleveland Clinic, Cleveland, [6]Alexion Pharmaceuticals, Inc., Lexington, United States, [7]Alexion Pharma GmbH, Zürich, Switzerland, [8]Osaka University Graduate School of Medicine, Suita, Japan

Background: Eculizumab, a humanized monoclonal antibody that blocks terminal complement activation at C5, prevents complement-mediated hemolysis and reduces need for transfusions in paroxysmal nocturnal hemoglobinuria (PNH) patients who were transfusion-dependent before treatment initiation; it has also been shown to reduce lactate dehydrogenase (LDH) irrespective of transfusion history. The International PNH Registry (NCT01374360) is an ongoing prospective, multinational, observational study to record the natural history of PNH and evaluate the long-term efficacy and safety of eculizumab.
Aims: Evaluate, in a real-world setting, effectiveness of eculizumab on transfusion needs and other parameters in PNH patients with and without history of transfusion at initiation of eculizumab.
Methods: Patients enrolled in the PNH Registry on or before January 1, 2018, with known demographics and enrollment date, who (1) were treated with eculizumab, (2) had known red blood cell (RBC) transfusion status for 12 months before initiation of eculizumab, and (3) had ≥12 months of follow-up after initiation of eculizumab, were included. Numbers of units

of packed RBCs received in the year before initiation of eculizumab and during follow-up were calculated and summarized as rates (units/patient-year). Event rates for thromboembolism (TE) and all major adverse vascular events (MAVE; including TE) were calculated from disease start until eculizumab initiation and from eculizumab initiation to last follow-up. Transfusion rates were further stratified by history of bone marrow disease (BMD). LDH ratio (LDH/LDH upper limit of normal [ULN]) was assessed and change from initiation of eculizumab to last treated follow-up was analyzed using general linear mixed models, adjusted for baseline and subpopulation.

Results: 596/543 patients had/did not have history of RBC transfusion in the 12 months before initiation of eculizumab, and were included. Mean (SD) age at eculizumab initiation was 44 (17) years in both groups. Both cohorts had >2600 patient-years of follow-up. Results for outcomes of interest are in the Table 1. In patients with a history of transfusion, the transfusion rate (95% CI) decreased from 10.6 (10.3, 10.9) units/year in the year before initiation of eculizumab to 3.3 (3.3, 3.4) for a 70% reduction in transfusions (Table 1). This reduction was more pronounced in patients without history of BMD (80% reduction) *versus* those with history of BMD (60% reduction). In the overall group with no history of transfusions, the rate (95% CI) of transfusions during follow-up was 1.5 (1.4, 1.5) units/year; the rate in patients with a history of BMD was 2.2 (2.1, 2.3) units/year, as compared with the rate in patients with no history of BMD of 1.1 (1.1, 1.2) units/year. Patients with and without a history of transfusion at initiation of eculizumab showed a significant reduction in hemolysis after initiation of eculizumab (mean change in LDH ratio –5.4 [P<0.0001] and –3.9 [P<0.0001]), and mean LDH was <1.5 xULN in both groups. Rates of TE and MAVE declined by 70% after initiation of eculizumab regardless of transfusion history.

Table 1.

Outcomes Overall	History of RBC Transfusion n=596		No History of RBC Transfusion n=543	
RBC Transfusions				
During 12 months prior to initiation of ecu	n=592		N/A	
Transfusion rate/patient-years (95% CI)	10.6 (10.3, 10.9)		N/A	
Between initiation of ecu and last treated follow-up	n=592		n=540	
Transfusion rate/patient-years (95% CI)	3.3 (3.3, 3.4)		1.5 (1.4, 1.5)	
Transfusion rate ratio (95% CI)	0.3 (0.3, 0.3)		N/A	
LDH Ratio	n=378		n=273	
Initiation of ecu (xULN), mean (SD)	6.8 (4.1)		5.1 (3.6)	
Last treated follow-up (xULN), mean (SD)	1.4 (1.5)		1.2 (0.9)	
Change from baseline to last treated follow-up, mean (SD)	−5.4 (4.2) P<0.0001		−3.9 (3.7) P<0.0001	
TE*	n=595		n=541	
Disease start to initiation of ecu				
Incidence rate/100 patient-years (95% CI)	3.9 (3.4, 4.6)		4.3 (3.7, 5.1)	
Initiation of ecu to last treated follow-up				
Incidence rate/100 patient-years (95% CI)	1.1 (0.8, 1.6)		1.1 (0.8, 1.6)	
Incidence rate ratio (95% CI)	0.3 (0.2, 0.4)		0.3 (0.2, 0.4)	
MAVE†	n=595		n=541	
Disease start to initiation of ecu				
Incidence rate/100 patient-years (95% CI)	5.7 (5.0, 6.4)		6.3 (5.5, 7.2)	
Initiation of ecu to last treated follow-up				
Incidence rate/100 patient-years (95% CI)	1.5 (1.1, 2.0)		1.7 (1.3, 2.3)	
Incidence rate ratio (95% CI)	0.3 (0.2, 0.4)		0.3 (0.2, 0.4)	
RBC Transfusions Stratified by BMD‡ History	**BMD n=247**	**No BMD n=327**	**BMD n=198**	**No BMD n=331**
During 12 months prior to initiation of ecu	n=246	n=324	N/A	N/A
Transfusion rate/patient-years (95% CI)	12.1 (11.7, 12.6)	9.6 (9.3, 9.9)	N/A	N/A
Between initiation of ecu and last treated follow-up	n=246	n=324	n=195	n=331
Transfusion rate/patient-years (95% CI)	4.8 (4.7, 5.0)	2.3 (2.2, 2.4)	2.2 (2.1, 2.3)	1.1 (1.1, 1.2)
Transfusion rate ratio (95% CI)	0.4 (0.4, 0.4)	0.2 (0.2, 0.3)	N/A	N/A

BMD, bone marrow disease; CI, confidence interval; ecu, eculizumab; LDH, lactate dehydrogenase; MAVE, major adverse vascular event; N/A, not applicable; RBC, red blood cell; SD, standard deviation; TE, thromboembolism; ULN, upper limit of normal.
*TE includes thrombophlebitis/deep vein thrombosis, renal vein thrombosis, renal arterial thrombosis, mesenteric/visceral vein thrombosis, mesenteric/visceral arterial thrombosis, hepatic/portal vein thrombosis, dermal thrombosis, acute peripheral vascular disease occlusion, cerebral arterial occlusion/cerebrovascular accident, cerebral venous occlusion, and pulmonary embolus.
†MAVE includes TE (as defined above), amputation (nontraumatic, nondiabetic), myocardial infarction, transient ischemic attack, unstable angina, gangrene (nontraumatic, nondiabetic), and other MAVE.
‡BMD includes aplastic or hypoplastic anemia, acute myelogenous leukemia, myelodysplastic syndrome, myelofibrosis, and other bone marrow diseases.

Summary and Conclusions: In this analysis of real-world data from the International PNH Registry in patients with PNH, eculizumab was efficacious in reducing transfusion rates, complement-mediated hemolysis, TE, and MAVE, irrespective of prior transfusion history or BMD status. Transfusion rates in patients with no history of transfusion in the year before eculizumab initiation remained low after initiation of eculizumab, regardless of BMD status.

PF311

PROGNOSTIC SIGNIFICANCE OF RENAL DYSFUNCTION DURING THE CLINICAL COURSE IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA: A KOREAN MULTICENTER STUDY

Y.-C. Mun[1,*], J.S. Kim[2], J.H. Jang[3], D.-Y. Jo[4], J.W. Lee[5]

[1]Hematology and Oncology, Ewha Womans University Hospital, College of Medicine, Ewha Womans University, [2]Hematology, Severance Hospital, Yonsei University College of Medicine, [3]Hematology-Oncology, Samsung Medical Center, School of Medicine, Sungkyunkwan University, Seoul, [4]Internal Medicine, Chungnam National University Hospital, School of Medicine, Chungnam National University, Daejeon, [5]Hematology, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic Of

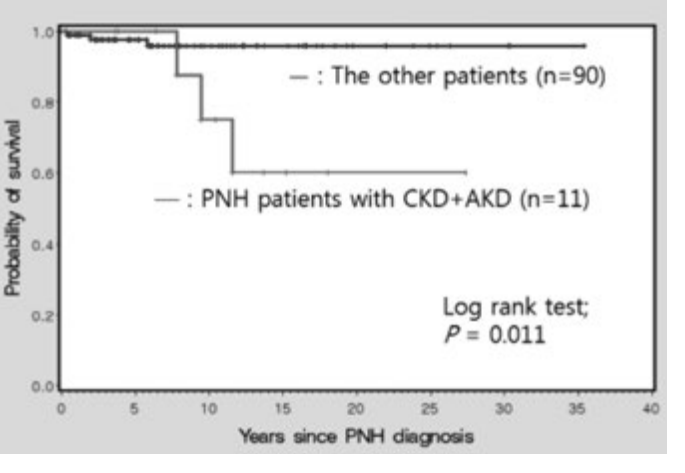
Background: Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hematopoietic stem cell disease characterized by the intravascular lysis of red blood cells, thromboembolism (TE), and bone marrow failure. Previous study from Korean PNH registry reported that renal dysfunction defined as history of acute renal failure or reported glomerular filtration rate (GFR) <60 mL/min/1.73m^2 at the time of diagnosis of PNH was 16%. However, renal events may occur repeatedly during the clinical course of PNH. Therefore, the clinical characteristics and dynamics of renal dysfunction during PNH natural disease course should be systematically evaluated.

Aims: We investigated the clinical characteristics and dynamics of renal dysfunction during the natural course of PNH to affect the survival of PNH patients.

Methods: We conducted a multicenter, retrospective study of 101 Korean PNH patients with PNH granulocyte clone size >10% and serum LDH >1.5 fold of upper limit of normal at the time of PNH diagnosis for identifying an association between renal dysfunction and clinical outcomes during the disease course. Renal events such as acute kidney disease (AKD) or chronic kidney disease (CKD) were defined according to the definition of KDIGO (Kidney Disease Improving Global Outcomes). Medical records were reviewed until eculizumab was administered.

Results: During the 94.2 months of median follow up duration, the renal events were observed in 55 patients (55/101; 54.5%). Eleven patients (10.9%) experienced both AKD and CKD. Median time to first renal event from diagnosis of PNH was 79.3 months. The elevated levels of LDH at the time of first renal event were observed compared to the level of LDH at the time of diagnosis (Pearson correlation coefficient r=-0.58, P=0.022 in the patients with 1 renal event and r=-0.75, P=0.0002 in the patients with renal events ≥2). The rate of TE was a higher trend in the patients with renal events ≥2 (10/32, 31.3%) compared to those with renal events ≤1 (15/69, 21.7%). (P=0.303). The rate of recurrent TE was significantly higher in patients with renal events ≥2 (6/32, 18.8%) compared to those with renal events ≤1 (2/69, 2.9%) (P=0.012). The patients with renal events ≥2 showed a trend toward inferior overall survival (OS) compared to those with renal events ≤1 (86.4% *vs* 96.0% at 10 years OS; P=0.124). The rate of TE was a higher trend in the patients with the patients with CKD+AKD compared to the other patients (5/11, 45.5% *vs* 20/90, 22.2%, P=0.134). The rate of recurrent TE was significantly higher in patients with CKD+AKD (3/11, 27.3%) compared to the other patients (5/90, 5.6%) (P=0.040). Of note, the OS was significantly inferior in the patients with CKD+AKD compared to the other patients (75.0% *vs* 95.8% at 10 years OS; P=0.011) (Table 1).

Table 1.



Summary and Conclusions: These data indicate that the rate of recurrent TE was higher in PNH patients with recurrent renal events, especially in patients with CKD+AKD group. The OS of PNH patients with recurrent renal events including CKD+AKD group has been shown to be lower than those of other patients. Therefore, physician should pay attention to evaluate the renal events during follow-up period. The monitoring of renal function is essential for identifying the high risk patients and making a decision for appropriate management.

PF312

COMPLEMENT ACTIVATION AND EXACERBATION OF HAEMOLYSIS SECONDARY TO RESPIRATORY VIRAL INFECTIONS IN PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA (PNH) PATIENTS TREATED WITH ECULIZUMAB

I. Lazana[1,*], S. Apap Mangion[1], S. Babiker[1], B. Fattizzo[1], K. Bell[1], J. Large[1], M. Sudhanva[1], J. Marsh[1], S. Gandhi[1], M. Zuckerman[2], A. Kulasekararaj[1]

[1]Haematology, [2]Virology, King's College Hospital, London, United Kingdom

Background: Paroxysmal Nocturnal Haemoglobinuria (PNH) is a clonal stem cell disorder disorder, with haemolysis being a major clinical and diagnostic feature. Precipitation or exacerbation of haemolysis can be induced by stress conditions, which activate the complement system, including infections, surgical procedures and vaccinations. Early diagnosis and treatment of an acute haemolytic process is critical, as it may lead to thrombosis and organ damage. Case reports have suggested that viral infections may have a role in inducing acute haemolysis. However, there are no studies investigating the possible role of respiratory virus infections in inducing/exacerbating haemolysis.
Aims: A retrospective study was performed, analysing PNH patients, presenting with coryzal symptoms during the influenza season, between September 2016 and April 2017, for acute haemolysis.
Methods: 33 of 110 patients presented with fever and coryzal symptoms and had a combined nose and throat swab (NTS) collected and tested for 10 respiratory virus infections, using a multiplex PCR. 10 of 33 NTS were positive (1 parainfluenza, 6 rhinovirus, 2 influenza B and 1 influenza A (H3N2) virus). The median age and the number of patients on prophylactic penicillin V and/or EPO, were similar between the two groups (NTS+ and NTS-).
Results: Patients in the NTS+ group had been on eculizumab treatment for a significantly longer period compared to NTS- patients (83.1 *vs* 52.7 months, p 0.023). A CRP rise was noted in 5/10 of NTS+ patients compared with only 8/23 NPA- patients. 5/10 of NTS+ patients required antibiotics and 4/10 received oseltamaivir and 1/10 patients required GCSF for neutropenia, in contrast to the NTS- group, where only 5/23 patients were given antibiotics and none of them required GCSF. Furthermore, 3/10 NTS+ patients required hospitalisation, compared with only 2/23 of NTS- patients. It is of note that two patients, suffered from recurrent viral infections (three and two episodes, respectively) and that in all cases a significant haemolysis, requiring red cell transfusion, was noted. In one case an extra dose of eculizumab had to be given to control the degree of haemolysis.
With regards to the haemolysis markers, a significantly higher increase in LDH and a significantly higher% increase in bilirubin was noted in the NTS+ group, compared to the NTS- group, (%LDH increase: 81.21 *vs* 51.6, p 0.03,%bilirubin increase: 73.1 *vs* 22.6, p 0.02, respectively). Similarly a significantly higher number of NTS+ patients exhibited a drop in their Hb, compared to the NTS- group (70 *vs* 34%), and again the% decrease in Hb was significantly higher in the NTS+ group. Furthermore, 3/10 NTS+ patients required urgent red cell transfusion. The reticulocyte count was increased (from baseline) in more patients in the NTS+ group, compared to the NTS- group, but this was not significant. None of the NTS- group required an extra dose of eculizumab, whereas 2/10 NTS+ patients did, to control haemolysis. The time from the last dose of eculizumab to a positive NTS, appeared longer in the NTS+ group (10.1 *vs* 2.8 days), but this difference was not significant. No difference in vaccination for seasonal influenza uptake was seen in the two groups, although the overall uptake was low.
Summary and Conclusions: Overall, our data indicate that respiratory virus infections may pose a significant risk of acute haemolysis to PNH patients, underlying the need for regular screening and close monitoring of symptomatic or high risk PNH patients.

PF313

RESULTS OF COBALT, A PHASE II CLINICAL TRIAL OF COVERSIN IN PNH

A. Hill[1,*], J. Windyga[2], T. Robak[3], A. Hellman[4], A. Kulasekararaj[5], W. Weston-Davies[6], M. Griffin[1], T. Munir[1], A. Szmigielska-Kaplon[3], A. Piekarska[4], M. Nunn[67]

[1]Haematology, St James's University Hospital, Leeds, United Kingdom, [2]Department of disorders of hemostasis and Internal Medicine, Institute of Hematology and Transfusology, Warsaw, [3]Haematology, Medical University of Lodz, Lodz, [4]Haematology and Transplantology, Medical University of Gdansk, Gdansk, Poland, [5]Haematology, Kings College Hospital, [6]Akari Therapeutics plc, [7]Haemostasis Research Unit, UCL, London, United Kingdom

Background: The advent of eculizumab, a monoclonal antibody which prevents the cleavage of C5 to C5a and C5b transformed the prognosis of patients with PNH and led to a marked improvement in their quality of life. However, eculizumab is administered by health care professionals by intravenous infusion which may interfere with the life-styles, occupations and personal privacy of patients. Interval dosing has led to concerns by some clinicians related to breakthrough haemolysis. Coversin is a protein suitable for small-volume subcutaneous (SC) injection and can be self-administered by patients.
Aims: The aims of this study were to assess the safety and tolerability of Coversin, the efficacy of the dosing regime and whether self-injection by patients is well-accepted.
Methods: In a Phase 2 single arm open label trial, PNH patients who had not previously had access to complement inhibitors were treated with Coversin for 90 days. The aim of the trial was to recruit 8 adult patients with a diagnosis of PNH confirmed by flow cytometry. For the first 5 patients, following an initial ablating regime (AR) of 60mg followed by 1 - 3 doses of 30mg q12 hours delivered by SC injection, a dose of 15 or 22.5mg q12 hours was given. On day 28, they switched to 30 or 45mg q24 hours for the remainder of the trial. All patients were encouraged to self-inject after suitable instruction.Those patients not satisfactorily controlled could be up dosed or switched to the same dose divided into 2 doses administered q12 hours. After admission of the first 5 patients an amendment to the protocol was made under which all patients were started on 22.5mg 12 hourly after the AR, switching to 45mg daily at day 28. Patients could revert to 22.5mg 12 hourly in the event of unsatisfactory control. All patients who completed the trial and wished to do so, could continue to receive Coversin under a long-term safety and efficacy protocol. The primary end point was LDH <1.8X ULN at the local laboratory at day 28. Secondary endpoints included LDH (xULN) at 60 and 90 days, terminal complement activity assessed by CH50 ELISA (Quidel®), haemoglobin stabilisation, reduction in requirement for blood transfusions, sheep erythrocyte haemolysis assay, PK (free and bound Coversin levels), anti-drug antibodies (ADA) and quality of life.
Results: Eight patients were enrolled in the trial in the UK and Poland. One patient was withdrawn at Day 43 because of a suspected co-morbidity unrelated to the study drug. Seven patients completed the study and all opted to continue to receive Coversin in the long-term follow-up study, CONSERVE. The trial met its primary endpoint with a median LDH at Day 28 of 1.5X ULN (n=8). At the conclusion of the trial 6 patients were on 45mg/day and 1 was on 30mg/day. There were no serious adverse events related to Coversin, patients tolerated the drug well, and for those experiencing them, the injection site reactions observed were mild to moderate, self-limiting and diminished with time. There has been no evidence of the formation of neutralising antibodies (Figure 1).

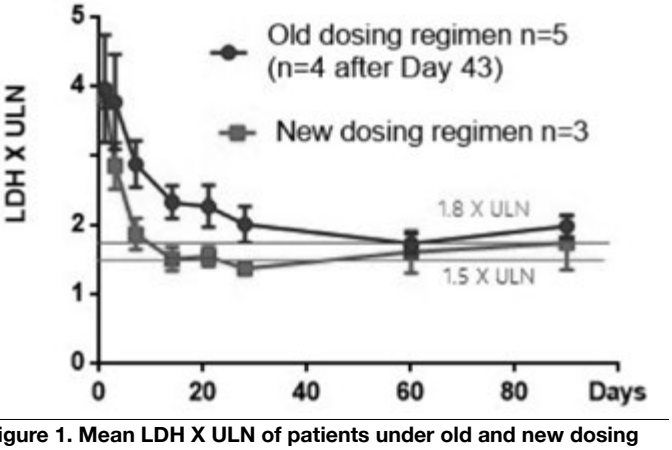


Figure 1. Mean LDH X ULN of patients under old and new dosing regimen.

Summary and Conclusions: Coversin may be an effective alternative for patients with PNH who prefer the independence of self- administration. The relatively short dose interval may also help to reduce breakthrough events.

PF314

THE INCIDENCE OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA CELL CLONES IN THE NORDIC COUNTRIES

E.-S. Korkama[1], U. Overgaard[2], E. Tjønnfjord[3], M. Vikman[4], A. Ahmadi[4], T.-T. Pelliniemi[5], H. Jarva[16], S. Meri[16], E. Armstrong[7], C. Kjellander[8,*]

[1]Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Fin-

land, [2] Department of Hematology, Rigshospitalet, Copenhagen, Denmark, [3]Department of Hematology, Oslo University Hospital, Oslo, Norway, [4]Department of Clinical Pathology and Cytology, Karolinska University Hospital Huddinge, Stockholm, Sweden, [5]Tampere University Hospital, Fimlab, Tampere, [6]Helsinki University Hospital Laboratory (HUSLAB), [7]Coagulation Disorders Unit, Helsinki University Central Hospital Comprehensive Cancer Center, Helsinki, Finland, [8]Department of Hematology, Karolinska University Hospital Solna, Stockholm, Sweden

Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired hematopoietic stem cell disorder characterized by the triad of intravascular hemolysis, thrombosis and bone marrow failure. The symptoms are caused by complement-mediated damage of cells of hematopoietic origin. A mutation on the *PIG-A* gene leads to the lack of glycosylphosphatidylinositol (GPI) -anchored complement regulators, CD55 and CD59, on the cell surface, which allows the complement system to attack host cells. The detection of a PNH clone is made by analyzing GPI-anchored molecules on blood cells by flow cytometry. PNH is known to be a rare chronic disorder. Well conducted studies on prevalence and incidence are lacking. Probably the best estimates come from Yorkshire (UK) and Spain, reporting an incidence of 1.3/1 million/year (1991-2006) and 2.5/million/year (2011-2014), respectively. The development of modern flow cytometric techniques has improved the diagnostic accuracy and created possibilities for the follow-up of small clones.
Aims: The Nordic countries have unique systems of database records and centralization of diagnostics to a few centers, which allows accurate characterization of incidence and prevalence values. Our aim is to describe PNH clones detected over a period of 6 years by flow cytometry in Denmark (Den), Finland (Fin), Norway (Nor), and Sweden (Swe).
Methods: Data was collected from the Stockholm and Gotland regions in Sweden, Copenhagen region in Denmark, Oslo region in Norway and from the whole of Finland. The total population of the study regions is 13.2 millions. Diagnostics were done in six different laboratories. PNH clones were detected by flow cytometry. For gating and detection of GPI deficient cells CD45, CD15, CD64 (or CD157), CD24, CD14 antibodies, FLAER reagent and CD235a, CD59 antibodies were used in leukocytes (neutrophils and monocytes) and red blood cells, respectively. All laboratories used accredited methods. The study population included all patient samples referred to the laboratories for PNH testing between 2011 and 2016. We included all newly detected PNH clones in neutrophils between 0.1-100% in the study.
Results: The mean incidence of newly detected PNH clones between 0.1 and 100% was 2.33 per year and million inhabitants in the Nordic countries (Den 2.05/million, Fin 2.98/million, Nor 2.53/million, and Swe 1.74/million). Of all newly diagnosed PNH clones, 41% were <1% and 17% >50%. The mean age at detection of the clone was 52 years (range from 6 to 90 years). PNH clones were evenly distributed between age groups, and there was no gender difference.
Summary and Conclusions: This is the first study to report the annual incidence (2.33/million) and distribution of PNH clones in the Nordic countries. PNH clone incidence was similar to the reported Spanish data where PNH clone distribution was missing. In the Yorkshire data 18% of the PNH clones were ≤1% and 20% >50%, compared to 41% and 17% respectively in our material. This may reflect an increase in the testing for PNH clones in bone marrow failure syndromes or other conditions associated with PNH, or more sensitive modern laboratory methods. In the Finnish data 49% of the patients had a clone <1% compared to 28%, 38% and 39% in Denmark, Norway and Sweden, respectively. This may explain the seemingly higher incidence of PNH clones in Finland. Our study has given an estimate of PNH incidence in the Nordic countries and important information for further health economical studies.

Chronic lymphocytic leukemia and related disorders – Biology & Translational Research

PF315

USP28 LOCALIZED IN 11Q23 REGULATES C-MYC, NOTCH1 AND FBXW7 IN CLL

V. Meyer-Pannwitt[1,2,*], J. Bloehdorn[1], S. J. Kugler[1,2], F. Oswald[3], J. Edelmann[4], H. Döhner[1], S. Stilgenbauer[1], D. Mertens[1,2]

[1]Internal Medicine III, Ulm University, Ulm, [2]Department of Molecular Genetics (B061), Cooperation Unit "Mechanisms of Leukemogenesis" DKFZ, Heidelberg, [3]Internal Medicine I, Ulm University, Ulm, Germany, [4]Barts Cancer Institute, Queen Mary University of London, London, United Kingdom

Background: FBXW7, c-MYC and NOTCH1 are of central relevance in the pathomechanism of chronic lymphocytic leukemia (CLL). Mutations of *FBXW7* occur in 2.5-4% of CLL cases, dysregulation of c-MYC coincides with disease progression and CLL patients with a mutation of *NOTCH1* (4-13% of cases) do not respond appropriately to chemoimmunotherapy. FBXW7 regulates the stability of oncoproteins such as c-MYC and NOTCH1 via ubiquitination. FBXW7-mediated ubiquitination can be antagonized by the deubiquitinase USP28, which is frequently dysregulated in cancer. Interestingly, *USP28* is located in 11q23, a region commonly deleted in CLL. We speculate that c-MYC, NOTCH1 and FBXW7 are regulated via USP28 in CLL.
Aims: To delineate the function of USP28 in CLL with regard to c-MYC, NOTCH1 and FBXW7 stability and activity.
Methods: Our high-resolution SNP-array data (Edelmann, Blood 2012) allowed to assess the frequency of *USP28* deletion in 96 untreated CLL cases with del(11q) and to correlate deletion with *USP28* expression levels. The consequence of USP28 dysregulation on its direct substrates c-MYC, NOTCH1 and FBXW7, as well as NOTCH1 target gene expression was investigated in primary CLL cells with and without del(11q).
Results: In line with our hypothesis for a role of USP28 in the pathomechanism of CLL, we found that USP28 protein levels varied in CLL, strongly correlated with c-MYC and NOTCH1 and anti-correlated with FBXW7 levels. USP28 levels were independent of the del(11q) status. Monoallelic deletion of *USP28* was detectable in 90% (86/96) of untreated del(11q) CLL patients, which resulted in a significant decrease of *USP28* expression in comparison to non-del(11q) cases. In the same cohort, deletion of *ATM* was detectable in 100% of cases (96/96), while deletion of *BIRC3* occurred in only 61% (59/96) of cases. Low *USP28* expression in del(11q) patient cells correlated with reduced NOTCH1 target gene expression of *HES1*, *HEY1* and *HES2* in comparison to non-del(11q) patients, which is in line with reduced NOTCH1 activity in del(11q) patients. ATM has been shown to regulate USP28 by phosphorylation, and we postulate that this has an effect also on NOTCH1 target gene expression. To disentangle effects of ATM, we selected only non-del(11q) patient samples and separated them into groups with *USP28* high or low expression. Strikingly, in CLL cases with low *USP28* expression and no del(11q), 12/17 NOTCH1 target genes were significantly upregulated. In line with elevated NOTCH1 target gene expression, significantly reduced *MS4A1* (CD20) expression was detectable in *USP28*-low expressing CLL cells.
Summary and Conclusions: Deletion of *USP28* was detected in del(11q) CLL patients more commonly than *BIRC3* disruption and coincided with decreased *USP28* expression. Therefore, we propose *USP28* as a novel oncogenic candidate of relevance in CLL. In CLL, USP28 not only regulates levels of FBXW7 and NOTCH1, which are frequently mutated, but also stabilizes c-MYC. USP28 levels correlate with protein levels of c-MYC, NOTCH1 and FBXW7 and provide another mechanism of NOTCH1 dysregulation in CLL. Furthermore, low expression of *USP28* correlated with increased NOTCH1 target gene expression and reduced *MS4A1* (CD20) expression, which coincides with reduced response to anti-CD20 treatment.

PF316

INTERROGATION OF HAEMATOPOIETIC BONE MARRROW PROGENITORS FOR DRIVER MUTATIONS OF CHRONIC LYMPHOCYTIC LEUKAEMIA

D. Bruce[1,*], A. Burns[2], P. Robbe[2], N. Appleby[2], J. Klintmann[2], D.R.A. Silveira[3], A. Rodriguez-Meira[4], A.J. Mead[4], A. Schuh[2]

[1]Department of Oncology, University of Oxford, Oxford,, [2]Department of Oncology, University of Oxford, Oxford, United Kingdom, [3]Haematology, University of Sao

Paulo, Sao Paulo, Brazil, [4]Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom

Background: Chronic lymphocytic leukaemia (CLL) is generally considered a disease derived from mature B-cells. Novel agents targeting B-cell receptor signalling are highly effective in controlling the disease, but do not cure. One hypothesis is that leukaemia is maintained by cells that have not yet undergone immunoglobulin rearrangement.
Aims: To phenotypically and genotypically characterise pre and post-treatment sort-purified bone marrow derived progenitors from patients with CLL treated with either chemo-immunotherapy or with novel agents.
Methods: A flow cytometry panel was developed to assess populations of haematopoietic and lymphoid progenitors. Mutations in peripheral blood CLL were detected either from whole genomes generated as part of the Genomics England CLL Pilot or using a 21 gene NGS panel which covers all major driver genes in CLL. Ethical approval for the study was via the Oxford Radcliffe Biobank Haem-Subprotocol. Twenty-four bone marrow (BM) and peripheral blood (PB) samples from patients taking part in clinical trials involving the novel small molecule inhibitors ibrutinib and venetoclax in which pre- and post-treatment bone marrow collection was mandated were collected. FACS was used to isolate: CD34+38- haematopoietic stem cell compartment, CD34+38+10-19- multipotent progenitor compartment, Pro B-cells, CD34+38+10+19- lymphoid progenitors and CD5+CD19+ CLL. Sort purity was at least 98%. Whole genome amplification (WGA) was performed using Repli-G mini kit (Qiagen). We used a modified Nextera (Illumina) approach and performed PCR using mutation specific primers with Nextera tags. A second PCR added indexes before the library was sequenced on an Illumina MiSeq and aligned using BWA v2.1.0.
Results: Preliminary results of 6 patients (3 treated with FCR, 3 with ibrutinib-rituximab in treatment naïve patients) show stable levels of haematopoietic stem cell and multipotent progenitor compartments, but a trend in reduction in pro B-cells post-treatment compared to pre-treatment (p=0.06). We determined the sensitivity of our approach by FACS sorting a total of 500 cells made up of two patient samples of PB CLL, one of which had a clonal MYD88 p.L265P mutation, the other was wild-type. In this artificial mix clonal MYD88 mutant CLL was present at 0, 1, 2, 5, 10 and 20% in 4 replicates each except for the 0% level where 6 replicates were used to establish a baseline sequencing variability. Using this method we could reliably detect samples with 5% mutation and in 2/4 samples down to a level of 2%.FACS was performed on 6 pre-treatment samples carrying 8 driver mutations, seven of which were clonal in PB CLL (ATMx2, SF3B1, EGR2, RPS15, MYD88, FBXW7 and subclonal TP53). Samples were sorted and analysed as above. A control of DNA from another sample (processed in the same way) that lacked the driver mutation was used to establish the baseline sequencing variation rate at that individual base (median read depth 1676, range 533-6872).In one case a clonal ATM "mutation" was present in all fractions with a variant allele frequency of between 30-60%, and it is likely this is a private SNP. In five other samples, no clear evidence of mutation in any progenitor fraction was detected.
Summary and Conclusions: Using a stringent cell purification approach and internal controls to exclude sequencing errors or contamination by CLL cells, we have so far not identified any CLL driver mutations in bone marrow progenitors. Further experiments, including interrogation of early events (del13q; XPO1) and single cell analyses are on-going.

PF317

SMG1 LOSS ENHANCES MTOR SIGNALLING IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

P. Rebeiro[1], A. James[1], S. Ling[2], N. Caixeiro[1], T. Roberts[1,*]

[1]Ingham Institute for Applied Medical Research, Western Sydney University, [2]Haematology, Liverpool Hospital, Sydney, Australia

Background: SMG1 (suppressor of morphogenesis in genitalia) is a member of the PI-3 kinase like kinase (PIKK) family of proteins which includes ATM, ATR, DNA-PK and mTOR. Members of this family have well-characterised roles in responses to cellular stress including DNA damage and nutrient deprivation. SMG1 is central to nonsense-mediated decay (NMD), a process that degrades mRNA containing premature stop codons thus prevening the production of truncated proteins. NMD is particularly active in B and T cells to prevent expression of PTC containing B and T cell receptor transcripts as such B and T cells normally have high levels of SMG1 expression. In our mouse model loss of one SMG1 allele resulted in increased development of B cell cancers. mTOR is the kinase core of two complexes: mTOR complex (mTORC) 1 and 2. Signalling via these complexes is often enhanced in cancers including lymphomas and leukemias.
Aims: We aimed to understand how loss of SMG1 enhanced cancer cell growth and whether loss of SMG1 in patients alters their responses to therapy.
Methods: We combined the study of cell lines, animal models and isolated patient tumour cells to examine how loss of SMG1 altered cellular biology and to identify key dysregulated signaling pathways.
Results: Here we show that PIKK family member, SMG1, is a novel negative regulator of mTORC2 signalling. SMG1 constitutively interacts with the mTORC2. Immunoprecipitation experiments show that SMG1 interacts specifically with mTORC2 and not mTORC1. SMG1 knockdown in cell lines leads to increased activation of mTORC2 and increased phosphorylation of the mTORC2 substrates Akt and Protein kinase C. We examined patients with chronic lymphocytic leukaemia and showed that >30% of patients lack detectable SMG1 protein. Further, the level of SMG1 expression in CLL patients B cells inversely correlated with Akt phosphorylation indicating that in these tumours loss of SMG1 resulted in increased mTORC2 signalling. Preliminary *ex vivo* assays suggest that CLL patient cells lacking SMG1 have altered susceptibility to mTOR inhibitor.
Summary and Conclusions: SMG1 loss correlates with increased mTORC2 signalling in CLL patient cells and that SMG1 interacts with the mTORC2 complex to negatively regulate its activity. Loss of SMG1 may identify a sub-group of patients with altered susceptibility to mTOR inhibition.

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TP53 CLONAL AND SUBCLONAL ARCHITECTURE IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS UNDER CHEMOIMMUNOTHERAPY AND IBRUTINIB

L.V. Cappelli[1,*], L. Cafforio[1], I. Del Giudice[1], C. Ilari[1], S. Raponi[1], P. Mariglia[1], M.S. De Propris[1], M. Filetti[1], F.R. Mauro[1], A. Guarini[2], R. Foà[1]

[1]Department of Cellular Biotechnologies and Hematology, [2]Department of Molecular Medicine, Hematology, Sapienza University, Rome, Italy

Background: In patients (pts) with chronic lymphocytic leukemia (CLL), analysis of *TP53* defects is mandatory before each line of treatment.
Aims: To analyze and compare the *TP53* clonal and subclonal architecture in CLL pts under multiple lines of chemoimmunotherapy (CIT) and ibrutinib (IBR).
Methods: Twenty-two pts (Table 1), undergoing a longitudinal *TP53* monitoring by Sanger sequencing (SS) before each line of CIT, developed a clonal *TP53* mutation over time; 8 pts also received IBR after a median of 2 CIT lines (range: 1-4) and were studied both before and after the drug intake (IBR exposition: 2.1 to 4 years in 7/8 pts). A total of 70 samples were retrospectively analyzed by ultra-deep sequencing. Libraries, prepared by TruSeq Custom Amplicon Low Input Library Prep kit (Illumina, Inc., San Diego, CA), were run on Illumina MiSeq sequencer (Illumina, Inc.) to obtain a ~5000x coverage/amplicon. For variant calling, the MiSeq Reporter software and an in-house bioinformatics pipeline were applied. Variants identified were checked on the IARC *TP53* database. The variant allele frequency (VAF) was corrected to cancer cell fraction (CCF) by the proportion of CD19+/CD5+ cells in each sample.
Results: Overall, 15/22 pts (68%) had at least 3 available time points. The mean number of clonal/subclonal *TP53* mutations per patient was 3.4 (range 1-11). In the 70 samples, 136 mutations were detected. According to SS sensibility, 41/136 (30%) mutations were clonal (mean VAF: 31.9%; range 10.3%>80.8%) and 95/136 (70%) were subclonal (mean VAF: 2.94%; 0.46%>9.89%). Of these, 83/95 (87%) showed a VAF≤5%. In the 22 pts receiving CIT, we identified 3 patterns of *TP53* mutation kinetics: 1) clonal mutations present from the first time point (n=2); 2) subclonal mutations evolving to clonal with or without additional minor subclones (n=10); 3) clonal mutations emerging with additional subclones not predicted by a previous subclone (n=10). In group 2, the mean CCF of the evolving *TP53* mutations increased from 5% to 43.5% (p<0.0001). In 14 pts with available pretreatment samples (n=21), 8 mutations (6 subclonal, 2 clonal) were detected, whilst the posttreatment samples (n=28) showed 71 mutations (41 subclonal, 30 clonal) (p<0.0001). The mean CCF was 5.1% (0.9%>17.2%) *vs* 16.8% (0.9%>80.7%) (p=0.002), respectively. Furthermore, we longitudinally followed the 8 IBR pts during the CIT and the subsequent IBR phases. During the CIT phase (mean duration: 3.1 years), 11 novel clonal/subclonal mutations developed, 2 subclonal evolved to clonal, 14 remained stable and none was lost. On the contrary, during the IBR phase (mean duration: 2.6 years), only 2 subclonal novel mutations emerged, one clonal decreased to subclonal, 17 remained stable and 9 -all subclonal- were lost (p<0.0001). Notably, the mean CCF of the existing clonal mutations remained stable before and after IBR treatment (CCF means: 32.2% *vs* 31.3%; p=NS), although the lymphocyte count significantly decreased (ly: 44.7 x 10^9/L *vs* 8 x 10^9/L; p=0.027).

Table 1.

Clinical and biological characteristics of 22 patients with *TP53* mutated CLL.	
Gender	16 M (73%) / 6 F (27%)
Median age (range)	55.5 years (38-71)
Median follow-up from diagnosis	10.8 years (3.5-22.2)
Median time from diagnosis to clonal *TP53* mutation	9.4 years (2.7-21.3)
Germline *IGHV*	15/21 (71.4%)
FISH del 17p	8/19 (42%)
TP53 mutations	59
	41/59 (69%): missense
	11/59 (19%): indels
	5/59 (8.5%): splicing
	2/59 (3.5%): nonsense
Mean lymphocyte count (x10^9/L):	
at first time point (n= 22 pts)	56.6 (1-207.5)
Pre-ibrutinib (n=8 pts)	44.7 (1.5-137.8)
Post-ibrutinib (n=8 pts)	8 (2.7-18.9)

Summary and Conclusions: Clonal *TP53* mutations emerging under CIT can be anticipated in at least half of the cases by subclones carrying the same mutation, according to the positive selection of resistant clones. The major clone can be accompanied by several other subclones which often remain under the SS detection limits, likely having a lower functional relevance. Contrariwise, IBR appears to lead to a decrease of the numerosity and complexity of *TP53* mutations. However, despite a marked reduction in lymphocytosis, the dominant clones survive the effect of the drug and may give rise to subsequent relapses.

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PAPTP LEADS TO NEOPLASTIC CELL APOPTOSIS IN THE EM-TCL1 CHRONIC LYMPHOCYTIC LEUKEMIA MOUSE MODEL

F. Severin[1,2,*], V. Martini[1,2], L. Leanza[3], M. Azzolini[4,5], F. Frezzato[1,2], F. Raggi[1,2], V. Trimarco[1], M. Facco[1,2], A. Mattarei[6], L. Biasutto[4,5], C. Paradisi[7], M. Zoratti[4,5], G. Semenzato[1,2], I. Szabò[3], L. Trentin[1,2]

[1]Department of Medicine, University of Padua, [2]Venetian Institute of Molecular Medicine (VIMM), [3]Department of Biology, [4]Department of Biomedical Sciences, University of Padua, [5]CNR Institute of Neuroscience, [6]Department of Pharmaceutical and Pharmacological Sciences, [7]Department of Chemical Sciences, University of Padua, Padova, Italy

Background: The potassium channel Kv1.3 is highly expressed in CLL cell mitochondria. We previously demonstrated that direct inhibition of Kv1.3 using mitochondria-targeted inhibitors alters mitochondrial function and leads to ROS mediated death of even chemoresistant cells. The inhibitor PAPTP killed 98% of *ex vivo* primary CLL cells while sparing healthy B cells. E -TCL1 CLL murine model is characterized by a high expression of TCL1 protein in B cells leading to the development of a CLL-like lymphoproliferative disease.
Aims: We were aimed to evaluate the toxicity and the efficacy in killing pathological B cells of PAPTP in the E -TCL1 mouse model.
Methods: Since the CLL phenotype is arising late in mouse age (around 10-18 months), we used only animals at that age presenting at least 50% of clonal expansion of CD19+/CD5+ in the peripheral blood. Furthermore, we isolated B CLL cells in order to confirm both surface antigen expression and clonality. Indeed, cells were stained with antibodies specific for murine CD45, CD5, CD19, Igk, Igl, CD3, CD4 and CD8 and evaluated by flow cytometry (FC). Once assessed the presence of disease, we cultured *ex-vivo* mouse leukemic lymphocytes from mice for 24h with or without PAPTP (10mmol/l) and evaluated cell viability by FC. 11 mice were treated with an injection of PAPTP 5 nmol/gbw diluted in DMSO and 8 control mice with only DMSO (5 days/week for 2 weeks). At the end of treatment, mice were sacrificed and blood, spleen, bone marrow and intraperitoneal wash were collected stained and evaluated either for the antigen distribution by FC as well as by histological stain with Hematoxylin and eosin. Splenomegaly was also assessed by spleen volume measurements.
Results: We confirmed the efficacy of PAPTP also in the murine neoplastic clone in *ex vivo* experiments: Kv1.3 protein is over-expressed in pathological B cells and its mitochondria targeted inhibitor was able to reduce E -TCL1 B CLL cell viability. Furthermore, we performed *in vivo* experiments. After 2 weeks of therapy, we observed an improvement of treated mice in term of appearance (posture and weight gain) with respect to controls. We also demonstrated a decrease in absolute total lymphocyte number after PAPTP administration and a higher reduction of pathological B cells (CD19+/CD5+) in spleen and bone marrow of treated, with respect to untreated, mice. Moreover, hematoxylin and eosin stain showned that untreated mice had enlarged spleen and liver, with evidence of CLL infiltration in both organs. Treated mice had smaller spleens and livers, with minimal (if any) CLL infiltration. In particular, the spleens disclosed well-preserved withe pulp architecture with clear-cut follicles and marginal zones.
Summary and Conclusions: The high selectivity of PAPTP and its capability to induce selectively apoptosis in B CLL cells also *in vivo* in E -TCL1 mouse model, may suggest the use of this inhibitor for designing new therapeutic strategies.

PF320

OVERTIME ASSESSMENT OF EPIGENETIC PROFILES IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS TREATED WITH CHEMOIMMUNOTHERAPY

M. Tsagiopoulou[1,2,*], N. Papakonstantinou[1], T. Moysiadis[1], V. Ljungström[3], N. Chalkidis[1], M. Duran-Ferrer[4], A.C. Queirós[4], A. Malousi[1], S. Bhoi[3], K. Plevova[5], L. Mansouri[3], P. Kollia[2], D. Oscier[6], A. Anagnostopoulos[7], L. Trentin[8], M. Ritgen[9], S. Pospisilova[5], N. Stavroyianni[7], P. Ghia[10], J.I. Martin-Subero[4], C. Pott[9], R. Rosenquist[3,11], K. Stamatopoulos[1,3]

[1]Institute of Applied Biosciences, Centre for Research and Technology Hellas (CERTH), Thessaloniki, Greece, [2]Department of Biology, National and Kapodistrian University of Athens, Athens, Greece, [3]Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden, [4]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Universitat de Barcelona, Barcelona, Spain, [5]Center of Molecular Biology and Gene Therapy, Department of Internal Medicine–Hematology and Oncology, University Hospital Brno and Medical Faculty of the Masaryk University, Brno, Czech Republic, [6]Department of Haematology, Royal Bournemouth Hospital, Bournemouth, United Kingdom, [7]Hematology Department and HCT Unit, G. Papanicolaou Hospital, Thessaloniki, Greece, [8]Department of Medicine, Hematology and Clinical Immunology Branch, Padua University School of Medicine, Padua, Italy, [9]Second Medical Department, University Hospital Schleswig-Holstein, Kiel, Germany, [10]Division of Experimental Oncology and Department of Onco-Hematology, IRCCS San Raffaele Scientific Institute and Università Vita-Salute San Raffaele, Milan, Italy, [11]Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden

Background: Aberrant DNA methylation is implicated in the pathogenesis of chronic lymphocytic leukemia (CLL), however its contribution to disease progression has not been conclusively defined.
Aims: The purpose of this study was to investigate the role of DNA methylation changes overtime in a group of CLL patients homogeneously treated with fludarabine, cyclophosphamide and rituximab (FCR).
Methods: Using 450K Illumina arrays, we determined methylation profiles in 34 CLL patients before treatment and at relapse; corresponding whole exome-seq data was available for 26/34 cases. We also analysed by 450K arrays 2 samples of memory B cells (MBC) from healthy donors. ChIP-seq data for histone modifications of MBC was also available.
Results: Differential methylation analysis did not identify reccurent changes at group level (all pre-treatment samples *vs* all relapse samples). However, analysis of each individual patient separately, in two axes, revealed significant differences in the number of differentially methylated CpGs (DMCpGs). More specifically, we compared: (i) MBC *vs* pre-treatment CLL sample, with the number of DMCpGs, referred as epigenetic burden (EB), ranging from 32,380 to 54,257; and, (ii) pre-treatment *vs* relapse sample, with the number of DMCpGs, referred as relapse changes (RC), ranging from 45 to 81,383. We found a correlation between EB and time to first treatment (TTFT) (rho=-0.41, p=0.01) and also between RC and time to relapse (TTR) (rho=0.39, p=0.02). Comparing low-EB (n=25, EB=32,380-45,372; median=40,669) *versus* high-EB cases (n=9, EB=45,383-60,526; median=50,443), the latter showed very early need for treatment (median TTFT=0.1 *vs* 2 years, p-value<0.001) and a higher frequency of *TP53* mutations after relapse (p=0.04). Focusing on the low-EB group, we found a strong negative correlation (rho=-0.58, p=0.002) between EB and RC, an association not observed amongst high-EB cases.

Despite the large heterogeneity between cases, DNA methylation changes were not fully stochastic since we found that hypomethylating events, regarding both EB and RC, mainly occurred within gene bodies, introns and 3' UTRs in heterochromatin and polycomb repressed regions. Hypermethylation events targeted enhancers, promoters and polycomb repressed regions, while at relapse these were mainly restricted to poised promoters and polycomb regions. In both analyses (EB and RC), we found enrichment for pathways common between patients (*e.g.* signaling pathways of Calcium, Rap1, cAMP) and strong association with binding sites of common transcription factors, mainly on hypermethylated CpGs (*e.g.* EB analysis:, MEF2A, POU2F1, ZIC2, AP-1, JUN -B/-D, c-Fos| RC analysis: MEF2A, POU2F1, E2F, p53). Additionally, using a machine learning approach, we identified an epigenetic signature of 10 CpG sites that was capable of accurately predicting the early relapse cases (TTR<2 years) (OOB error=10.53%, ROC-AUC= 0.978).
Summary and Conclusions: In conclusion, this study highlights the active role of DNA methylation changes in CLL evolution, particularly in response to chemoimmunotherapy, involving several transcription factors and pathways and displaying association with particular genetic events. Moreover, it raises the possibility of using the DNA methylation levels of specific CpGs as prognostic biomarkers.

PF321

JAK/SYK INHIBITION IS VITAL TO PREVENT B CELL RECEPTOR SIGNALING AND ITS REGULATION BY THE TUMOUR MICROENVIRONMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA

R. Dobson[1], M.D. Blunt[1], K.-R. Rogers Broadway[1], L.D. Smith[1], L. . Karydis[1], A. Minton[1], J.C. Strefford[1], F.K. Stevenson[1], M.S. Cragg[1], F. Forconi[1], G.P. Coffey[2], P.B. Conley[2], G. Packham[1], A.J. Steele[1,2,*]

[1]Cancer Sciences Unit, Somers Building (MP824), Tremona Rd, University of Southampton, Southampton, United Kingdom, [2]Portola Pharmaceuticals, Inc., South San Francisco, CA, United States

Background: Understanding the role of the B cell receptor (BCR) in chronic lymphocytic leukemia (CLL) pathology is critical for the identification of effective treatment strategies for this currently incurable disease. We previously demonstrated that the tumour microenvironment is pivotal to BCR signaling. Indeed, IL-4 treatment increased surface (s)IgM (but not sIgD) expression and augmented downstream signaling. These effects were restricted to IL-4 and observed down to 0.1ng/ml, with maximal effects seen between 4 and 10ng/ml IL-4 for all cases. Pivotally we demonstrated that IL-4 treatment reduced the effectiveness of ibrutinib and idelalisib to inhibit the BCR signaling pathway using calcium flux and phosphorylated ERK as readouts. IL-4 induced BCR signaling and resistance to BCR-kinase inhibitors was greater within CLL cases with progressive (U-CLL) compared to indolent (M-CLL) disease, suggesting that IL-4 effects may contribute to BCR induced tumour pathogenesis and therapy resistance.
Aims: To investigate the effect of SYK/JAK inhibition on BCR signaling and its regulation via IL-4 in CLL
Methods: Primary CLL cases were treated with anti-IgM in the presence or absence of IL-4 following pre-incubation with or without ibrutinib, idelalisib, tofacitinib, fostamatinib, PRT062607 or cerdulatinib (all 1μM). Drug effects on BCR-induced signaling was performed by calcium flux using flow cytometry or immunoblotting.
Results: Ibrutinib and idelalisib inhibited anti-IgM induced signaling at 1μM as previously demonstrated, however, this inhibition was variable and signaling was only inhibited 80-90% *in vitro*. The ability of ibrutinib and idelalisib to block BCR signaling was further reduced following IL-4 treatment (n=20). However importantly, the addition of the JAK1/3 inhibitor tofacitinib restored complete inhibition of the BCR pathway by ibrutinib and idelalisib (n=10). To determine whether these effects were restricted to BTK or PI3K, we subsequently treated the same CLL samples with the SYK inhibitors fostamatinib (R406), PRT062607 or the dual SYK/JAK inhibitor cerdulatinib. In contrast to ibrutinib and idelalisib, PRT062607, cerdulatinib or R406 alone completely inhibited anti-IgM induced signaling at equivalent concentrations in the absence of IL-4 in the majority of patient samples. However, in contrast to ibrutinib and idelalisib, IL-4 treatment did not reduce the effectiveness of PRT062607 or cerdulatinib to inhibit anti-IgM signaling, suggesting that SYK was involved in the IL-4 induced BCR signaling axis. Next we subsequently explored protein expression associated with positive BCR signaling (GAB1, FOXP1) and suppressors of cytokine (SOCS1, SOCS3) signaling following treatment with IL-4. IL-4 treatment significantly induced expression of FOXP1, GAB1, SOCS1 and SOCS3 within 24h. Interestingly these IL-4-mediated changes in protein expression were greater in U-CLL cases compared to M-CLL cases but were suppressed by the SYK/JAK inhibitor cerdulatinib. Cerdulatinib unlike PRT062607, R406, ibrutinib and idelalisib can simultaneously inhibit IL-4 and BCR signaling and may therefore have a dual role in reducing BCR signaling in CLL cases.
Summary and Conclusions: These data suggest that targeting SYK/JAK disrupts both BCR signaling directly as well as cross-talk via the IL-4 receptor. Therefore combined SYK/JAK inhibition appears to be a rational strategy to control important survival signals in CLL and potentially follicular lymphoma where the microenvironment plays a role in disease pathogenesis.

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CLL-DERIVED EXOSOMES REPROGRAM THE PHOSPHOPRTEOMICS LANDSCAPE OF ENDOTHELIAL CELLS

L. Lipshtein[1,*], O. Uziel[1], E. Beery[1], P. Raanani[2], M. Lahav[1], U. Rozovski[2]

[1]FMRC, Rabin Medical Center and Tel Aviv University, Petah-Tikva, Israel, [2]Hematological Institute, FMRC, Rabin Medical Center and Tel Aviv University, Petah-Tikva, Israel

Background: CLL cells travel between blood, bone marrow and lymphatic tissues. In the lymph nodes CLL cells receive pro-survival signals coming from neighboring cells. For example, the interaction of CLL cells with vascular endothelial cells enhances the survival of the neoplastic cells. How endothelial cells provide CLL cells with a survival advantage is unknown. We hypothesized that CLL cells shape their own fate by sending "floating" massages that are taken up by endothelial cells. These massages subsequently reprogram the protein repertoire of the recipient cells to support the survival of the neoplastic cells. Exosomes are cellular-derived nano-sized particles, secreted by different type of cells including tumor cells, carrying a cargo of proteins and nucleic acids. They are up taken by other cells and alter their phenotype. Here we asked whether CLL derived exosomes are taken up by endothelial cells, modify their phenotype and transforming them to CLL-supportive cells.
Aims: 1) To quantify the uptake of CLL-exosomes by vascular endothelial cells. 2) To determine whether CLL-exosomes alter the phosphoproteomics makeup of recipient endothelial cells. 3) To determine whether and how exosomal-treated endothelial cells support the survival of CLL cells.
Methods: CLL cells were isolated from patients and cultured in exosome free medium for 72h. Thereafter, exosomes were isolated from the cultured media by ultracentrifugation and quantified by NanoSight tracking system. Human Umbilical Vein Endothelial Cells (HUVEC) were exposed to CLL-derived exosomes. The uptake of exosomes was quantified by flow cytometry and by fluorescent microscopy. The phosphoprotein profiling of exposed and unexposed cells was analyzed by Mass spectrometry. RT-PCR was conducted to validate the array results and Western blotting was employed to quantify levels of beta catenin protein. IL-6 levels in culture media were quantified by ELISA. To annotate the proteomics data we used DAVID (Database for Annotation, Visualization and Integrated Discovery) and ANAT (Advanced Network Analysis Tool).
Results: We found that HUVECs uptake exosomes in a dose and time dependent manner, the pick uptake being 24 h from exposure. 52 peptides were significantly more phosphorylated after the cells were exposed to CLL-derived exosomes. Intriguingly, none of the peptides tested had reduced phosphorylation status in exosomal-treated cells. The phosphoproteomics results were validated in 3 of 52 top altered peptides. These proteins are involved in "cell to cell adhesion", operative for trafficking and retention of CLL cells in the bone marrow and lymphoid organ, "mRNA splicing" and "phosphorylation of the PDZ motif" which facilitate the anchoring of receptor proteins to the actin cytoskeleton. Among these 52 proteins is beta catenin for which phosphorylation levels increased 3.5-fold after CLL exosomal exposure. It was shown to induce secretion of IL-6. Furthermore, IL-6 induces STAT3 phosphorylation, thus increasing CLL cells' viability. We confirmed that phosphor-beta catenin is several fold higher in exosomal-treated cells and these cells secrete IL-6 to the cultured media.
Summary and Conclusions: By secreting exosomes which carry a cargo of mRNAs and proteins CLL cells reprogram endothelial cells to activate various pathways. In this way the crosstalk between CLL cells and endothelial cells may contribute to CLL cells' survival at lymph nodes where CLL cells survive and proliferate.

PF323

DYNAMIC REGULATION OF THE HS1/CXCR4 AXIS IN CHRONIC LYMPHOCYTIC LEUKAEMIA CELLS

L. Pavesi[1,2,*], F. Barbaglio[1], L. Scarfò[12], E. Ferrero[1], D. Belloni[1], M. Ponzoni[2,3], L. Bongiovanni[3], D. Zambroni[4], V. Caiolfa[4,5], K. Stamatopoulos[6], F. Caligaris-Cappio[7], P. Ghia[1,2], C. Scielzo[1]

[1]Division of Experimental Oncology, IRCCS San Raffaele Scientific Institute, [2]Università Vita-Salute San Raffaele, [3]Pathology Unit, [4]Centre for Experimental Imaging, IRCCS San Raffaele Scientific Institute, Milan, Italy, [5]Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain, [6]Institute of Applied Biosciences, Center for Research and Technology Hellas, Thessaloniki, Greece, [7]Associazione Italiana per la Ricerca sul Cancro (AIRC), Milan, Italy

Background: Chronic Lymphocytic Leukaemia (CLL) cells traffic and home to specific hemato-lymphoid tissues where they interact with the surrounding microenvironment, proliferate and accumulate. We previously identified Hematopoietic Cell-Specific Lyn Substrate 1 (HS1) protein, a central cytoskeletal regulator, as a strong prognostic factor in CLL patients and found that HS1 activity and expression significantly impact on the migration, adhesion and bone marrow (BM) homing of CLL cells. We here report the identification of a novel HS1/CXCR4 axis that is heterogeneously expressed (in a dynamic fashion) in the leukemic clone especially in the BM and is possibly involved in the regulation of trafficking, homing and tissue retention of CLL cells.
Aims: The aim of the present study was to dissect: (1) HS1 and CXCR4 expression in different hemato-lymphoid tissues infiltrated by CLL cells; (2) CLL cell dynamics thanks to novel 3D models that better mimic the complexity of the *in vivo* microenvironment.
Methods: We utilized: (1) primary cells derived from CLL patients' peripheral blood (PB) or BM, (2) CLL cell lines (MEC1, Knocked-Down or WT for HS1 expression), (3) human BM derived stromal cells (HS-5) to perform co-cultures. To mimic the BM setting, we optimized a 3D co-culture model (CLL+HS-5 cells seeded on spongostan scaffolds) coupled with a dynamic growth in a bioreactor under microgravity, interestingly also suitable to analyse cell mobilization from the scaffold after kinase inhibitor treatment. We evaluated changes in HS1/CXCR4 axis expression by RT-qPCR and confocal microscopy. We concomitantly evaluated at single cell level the intra-clonal expression of HS1/CXCR4 by ImageStream analysis (capable to combine flow cytometry and imaging).
Results: By analysing BM and PB CLL cells isolated from 9 CLL patients we found that both *HS1* and *CXCR4* were significantly down-regulated in the BM (p=0,0498). At single cell level, HS1/CXCR4 expression appeared to be heterogeneous and we observed a population negative for HS1 expression mainly represented in the BM, as confirmed by ImageStream. Considering the possibility of microenvironmentally-mediated regulation of the *HS1/CXCR4* axis, we co-cultured in 2D CLL cells in the presence of HS-5 cells and observed significant down-regulation of *HS1* (p=0,0062) and *CXCR4* (p=0,0537) in the leukemic clones. Accordingly, our 3D model allowed to detect strong down-regulation of the *HS1/CXCR4 axis* in the scaffold, while the expression remained unchanged in the cells outside the scaffold, confirming the dynamic expression of the genes.
Taking advantage of our 3D model, we tested Ibrutinib effect on primary CLL cells and observed a strong mobilization effect on all patients analysed (n=8, p=0,0047), and that cells retained in the scaffold maintained HS1/CXCR4 axis downregulation.
Summary and Conclusions: The present findings support our hypothesis that tissue homing and migration of CLL cells *in vivo* are tightly regulated by a complex interplay between cytoskeleton regulators (HS1) and chemokine receptors (CXCR4), that changes over time. Our novel 3D *in vitro* model appears to closely mimic the *in vivo* setting, allowing to observe and follow dynamic changes occurring in tissues, especially in the BM, including the pharmacological effect of novel inhibitors that cannot be thoroughly explored in conventional 2D co-culture models.

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CHRONIC LYMPHOCYTE LEUKEMIA CELLS SHARE A UNIQUE CIRCULAR RNA EXPRESSION PATTERN

O. Raz[1,*], G. Granot[1], M. Pasmanik-Chor[2], P. Raanani[3,4], U. Rozovski[3,4]

[1]Felsenstein Medical Research Center (FMRC), Beilinson Hospital, Rabin Medical Center, Petah Tikva, [2]Faculty of Life Sciences, Tel-Aviv University, Tel Aviv, [3]Institute of Hematology, Davidoff Cancer Center, Beilinson Hospital, Rabin Medical Center, Petah Tikva, [4]Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

Background: MicroRNAs are known to be dysregulated in CLL and have been shown to be involved in the initiation and progression of the disease. The role of other non-coding RNAs in the pathobiology of CLL is less clear. Circular RNAs (circRNAs) are endogenous noncoding RNAs that represent approximately 10% of the human transcriptome. They result from noncanonical alternative splicing which generates a stable circular form through a covalent bond between their 3' and 5' ends. The expression profile of circRNAs has been shown to have a unique pattern in various pathological conditions. Yet, their expression has not been tested in CLL. Although the exact mechanism of action of circRNAs remains to be clarified, one suggested mode of action is regulation of gene expression via an interaction with miRNAs thus functioning as miRNA sponges. Other alternative modes of action include protein binding or regulation of transcription and post-transcriptional modifications. Through these functions, circRNAs may act as tumor suppressors or as oncogenes and contribute to the pathogenesis and/or progression of CLL
Aims: To decipher the circRNA expression profile in CLL cells and compare it to that of normal B-lymphocytes and to evaluate the potential impact of the CLL specific circRNA profile on the pathogenesis and/or progression of CLL.
Methods: Lymphocytes from 6 CLL patient and 3 healthy volunteers were separated on a ficoll gradient and B cells were sorted with the aid of magnetic anti-CD19+ microbeads. circRNA expression in CLL lymphocytes was identified and quantified using a microarray based platform, provided by Arraystar Inc. Real-time PCR was used to validate the microarray results. Extensive bioinformatics analysis was performed in order to construct circRNA-miRNA-mRNA-protein networks and to identify pathways that may be influenced by these differently expressed circRNAs.
Results: Of the 13,438 circRNAs transcripts that are represented in the array, 13,195 (98%) were present in at least 3 of 9 sample tested, indicating that circRNAs are abundant in both normal and neoplastic lymphocytes. Overall, 397 circRNAs were upregulated in CLL cells compared to normal B cells and 688 were downregulated. This downregulation of circRNA expression is 2 folds more than is expected by chance ($P<0.01$), suggesting a possible global downregulation of cirRNA transcription activity in CLL cells. Next, in an attempt to model a possible sponge effect, we constructed four circRNA-miRNA-mRNA networks. Two networks represent up regulated circRNAs (hsa_circRNA_104424, hsa_circRNA_100251) and two represent downregulated circRNAs (hsa_circRNA_102680, hsa_circRNA_001430). Each network consists of one circRNA, five miRNAs and over 1000 target genes. Deregulated expression of all of the circRNAs was verified by real-time PCR. Multiple fundamental biological processes that may be associated with the deregulated circRNAs including; apoptosis, cell cycle regulation, B cell activation and RNA transcription and processing were revealed by the DAVID and WebGestalt database resource and by GO enrichment analysis.
Summary and Conclusions: Our study demonstrates that the circRNA profiling of CLL cells is significantly different from that of normal B-cells and revealed that the deregulated circRNAs may have profound impact on the pathogenesis of CLL. This is the first study publishing circRNA profiling in CLL thus paving the way towards an understanding of whether a specific expression pattern is associated with prognosis and whether targeting circRNAs may have therapeutic benefit in CLL.

PF325

T CELL CLONAL DYNAMICS ARE ASSOCIATED WITH CLINICAL RESPONSE AFTER LENALIDOMIDE CONSOLIDATION TREATMENT IN CLL

A. Vardi[1], E. Vlachonikola[2], C. Galigalidou[2], K. Gemenetzi[2], K. Pasentsis[2], N.E. Kay[3], A. Anagnostopoulos[1], A.G. Ramsay[4], T.D. Shanafelt[5], K. Stamatopoulos[2], A. Hadzidimitriou[2,*]

[1]Hematology Department & HCT Unit, G. Papanikolaou Hospital, Thessaloniki, Greece, [2]Institute of Applied Biosciences, Centre for Research and Technology, Thessaloniki, Greece, [3]Division of Hematology, Department of Medicine, Mayo Clinic, Rochester, MN, United States, [4]Department of Haemato-Oncology, School of Cancer and Pharmaceutical Sciences, Faculty of Life Sciences and Medicine, King's College London, London, United Kingdom, [5]Division of Hematology, Stanford University School of Medicine, Stanford, CA, United States

Background: The T cell repertoire in chronic lymphocytic leukemia (CLL) is characterized by the existence of clones which persist and expand over time in untreated patients, appear to be disease-specific and may be shared by different cases, alluding to selection by CLL-related antigens. In preliminary NGS immunoprofiling studies investigating the impact of treatment on T cell repertoire dynamics in CLL, we suggested that while chemoimmunotherapy increases T cell clonality through an ablative mechanism, signaling inhibitors retain T cell clones that may have developed in response to tumor antigens. In order to further explore the differential impact of various treatment types, we evaluated the *in vivo* impact of an IMID (lenalidomide [len]) on T-cell dynamics. *Ex vivo* studies suggest len may repair the B-T cell immune synapse defect in CLL.
Aims: To investigate T cell dynamics overtime in a phase II trial for previously untreated CLL patients where len was administered as consolidation following pentostatin-cyclophospamide-rituximab (PCR).
Methods: We analyzed paired samples from 14 CLL patients (i) after com-

pleting 6 cycles of PCR (post-PCR, pre-len) and (ii) following 6 months of continuous lenalidomide administration (post-len). TRBV-TRBD-TRBJ gene rearrangements were PCR-amplified on DNA from PBMCs, subjected to paired-end NGS and characterized in detail by a standardized bioinformatics pipeline.

Results: Only productive TRBV-TRBD-TRBJ rearrangements were evaluated (n=2,916,367; median 99,468/sample). For repertoire analysis, clonotypes (*i.e.* rearrangements with identical TRBV gene usage and TRB CDR3 amino acid sequence) were considered (median: 7006 distinct clonotypes/sample). All cases displayed significant clonal expansions both pre- and post-len (clonality, measured as median cumulative frequency of the 10 most expanded T cell clonotypes/sample, 20.1% *vs* 27.0%, respectively), with post-PCR major T cell clones persisting over len treatment (median of 5/10 most expanded post-PCR clonotypes). However, there was no clear trend in clonal dynamics post len-treatment, with clonality increasing in 9 patients and decreasing in the remaining 5 patients (p=0.43). Therefore, we evaluated the association between clonality changes and clinical response among cases with available staging data (n=10). Deepening clinical response [from partial remission (PR) to complete remission (CR), n=2] and sustained CR status (n=2) were associated with decrease in T cell clonality (24.5% pre-len *versus* 14.3% post-len), while stable PR status (n=5) or progressive disease (n=2) were associated with increased T cell clonality (18.3% pre-len *versus* 31.2% post-len). Despite small sample size, the clonality fold-change difference between these two groups was statistically significant (median fold change 0.66 *versus* 1.48, respectively, p=0.004).

Summary and Conclusions: Differential T cell clonal dynamics were observed with respect to clinical response following len consolidation in treatment-naïve CLL patients who had received induction chemoimmunotherapy. Clonality increase in stable PR or progressive disease recalls previous findings of T cell clones expanding overtime in untreated CLL patients, possibly in response to CLL-related antigens, albeit unable to mount effective anti-tumor responses. On the other hand, eradication of the malignant clone in patients achieving sustained CR or deepening response may explain the decrease in T cell clonality in the context of gradual reconstitution of a "normal" T cell repertoire after the ablative effect of chemoimmunotherapy.

PF326

PREVALENCE AND PROGNOSTIC IMPACT OF THE IGLV3-21 G110R MUTATION IN CLL – A PATHOBIOLOGICAL REDEFINITION OF THE UNFAVORABLE CLL SUBTYPE II

R.S. Barendse[1], W. Ismailzada[1], M.T. Koning[1], E. Quinten[1], H. Jumaa[2], C. A. van Bergen[1], H. Veelken[1,*]

[1]Hematology, Leiden University Medical Center, Leiden, Netherlands, [2]Immunology, University of Ulm, Ulm, Germany

Background: One-third of CLL cases can be classified into stereotypes by sequence similarities of their B-cell receptors (BCR). CLL stereotypy has been interpreted as consequence of selection for antigen binding. Since antigen-independent, autonomous BCR signaling has been discovered as an indispensable, dominant oncogenic CLL driver, stereotypy likely reflects BCR sequences that structurally facilitate autonomous BCR signaling. CLL subtype II has a relatively poor prognosis and is conventionally defined by expression of a mutated IGHV3-21/IGHJ06-containing BCR heavy chain (HC) with a CDR3 of 9 amino acids and a D or E residue at position 3, and coexpression of an IGLV3-21-containing BCR light chain (LC). A recurrent G110R mutation of the IGLV3-21 allele causes autonomous BCR signaling in subtype II.

Aims: 1. To determine the prevalence of the IGLV3-21^{G110R} mutation in CLL. 2. To assess its impact on the natural CLL history, irrespective of its occurrence in conventionally defined subtype II.

Methods: BCR HC and LC sequences were determined by ARTISAN PCR on 132 cases of biobanked CLL (diagnosis confirmed by immunophenotyping). Time from diagnosis to first treatment (TTFT) and overall survival (OS) were obtained from patients' records and compared by log-rank test.

Results: Six CLL cases (4.5%) were conventional subtype II, all indeed expressing the mutated IGLV3-21^{G110R} allele. Four CLL (3.0%) expressed a LC containing wild-type IGLV3-21. Twenty-six CLL expressed IGLV3-21 that carried the G110R mutation (19.7%; 95% CI: 12.9-26.5%). In 122 CLL with sufficient clinical information, age, Rai stage, and chromosomal aberrations were not significantly different between IGLV3-21^{G110R}-expressing and remaining mutated CLL. Conventional subtype II cases had a non-significantly different TTFT (median: 22 months) from unmutated CLL (n=65; median 13 months; p=0.91) and an inferior TTFT compared to the remaining mutated CLL (n=51, median 121 months; p=0.016). TTFT of IGLV3-21^{G110R}-expressing CLL (median: 28 months) was similar to unmutated CLL (p=0.23) and strikingly inferior to the remaining mutated CLL (median: 147 months; p<0.0001). TTFT of IGLV3-21^{G110R}-expressing CLL not belonging to conventional subtype II (*i.e.* expressing a HC chain not containing IGHV3-21; n=20) likewise had an inferior TTFT (median: 28 months; p=0.001) than mutated CLL not expressing IGLV3-21^{G110R}. The median OS of patients with conventionally defined subtype II CLL (146 months) was not statistically different from the remaining mutated CLL (211 months) or unmutated CLL (102 months). Patients with a IGLV3-21^{G110R}-expressing CLL had inferior median OS (143 months) compared to mutated CLL (247 months; p=0.03); their survival was not significantly different form unmutated CLL (101 months; p=0.08). When conventional subtype II cases were excluded, OS of IGLV3-21^{G110R}-expressing CLL (132 months) remained inferior to mutated CLL (p=0.04) but was not significantly different from unmutated CLL (p=0.12) (Figure 1).

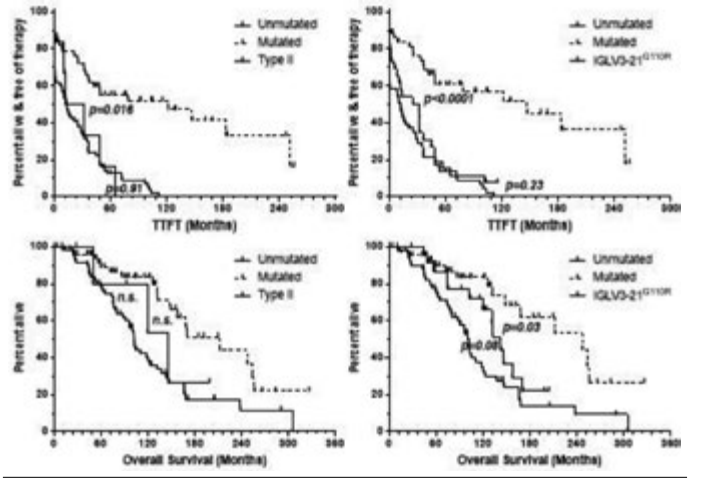

Figure 1.

Summary and Conclusions: The IGLV3-21^{G110R} mutation has an unfavorable prognostic impact in accordance with its role in causing antigen-independent, autonomously active BCR signaling. The IGLV3-21^{G110R} mutation rather than BCR HC criteria should serve as a new definition of the prognostically unfavorable CLL subtype II, thereby creating the largest (app. 20%) immunologically defined subgroup of CLL.

PF327

EVOLUTION OF GENOMIC ABNORMALITIES DURING CLL DISEASE COURSE IS ASSOCIATED WITH TELOMERE LENGTH CHANGES

H. Olbertova[1,2,*], K. Plevova[1,2], J. Malcikova[1], J. Kotaskova[2], S. Pavlova[1], M. Spunarova[2], M. Trbusek[2], V. Navrkalova[1], B. Kantorova[2], Y. Brychtova[2], A. Panovska[2], M. Doubek[2], S. Pospisilova[1,2]

[1]Central European Institute of Technology, Masaryk University, [2]Department of Internal Medicine – Hematology and Oncology, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic

Background: Telomeres are structures protecting the ends of chromosomes, which are getting shorter with each cell division. In CLL a telomere length is considered to be stable during disease course, even despite a treatment administration. It was also shown that short telomeres predict for an early need of treatment and reduced overall survival, and associate with the unmutated status of IGHV locus and higher genomic complexity.

Aims: To study telomere length in the context of genomic architecture of CLL clones and their evolution during disease course.

Methods: Telomere length was measured in 198 CLL patients using established qPCR protocol. Relative telomere length (RTL) was assessed by comparing to β-globin (HBB) as a single-copy gene and Human Genomic DNA (Promega) as a reference DNA. Genes recurrently mutated in CLL were sequenced by amplicon next-generation sequencing. Following mutation frequencies were found using 5% VAF cut-off: *TP53* (82/244; 33.6%), *ATM* (45/233; 19.3%), *NOTCH1* (36/242; 14.9%), *SF3B1* (59/238; 24.8%), *BIRC3* (26/232; 11.2%). In 36 patients with clonal evolution detected in relapse, samples from two (29 cases), or more than two (7 cases) timepoints were analyzed. Relative expression of hTERT telomerase subunit was assessed by reverse transcription TaqMan qPCR assay, using GAPDH as a housekeeping gene and RAMOS cell line as a positive control.

Results: In the cohort of 198 CLL patients, significant associations with time to first treatment, IGHV mutation status, 11q deletion, complex karyotype and mutations in *TP53* and *ATM* genes were found in concordance with published results. In 36 patients that manifested CLL cells clonal evolution, telomere length of the major leukemic clone was different between pre-therapy and corresponding relapse sample. This specific cohort consisted primarily of patients with therapy-driven *TP53* mutation expansion (26/36; 72.2%). Changes in other tested genes were less frequent in this subgroup, which precluded their more detailed evaluation. Thus, focusing only on the cases with *TP53* evolution, we observed that telomeres in relapse became shorter, remained stable, or became longer in 12/26 (46.1%), 8/26 (30.8%), and 5/26 (19.2%) of patients, respectively. Additionally, in 1/26 cases studied in three timepoints, telomeres initially lengthened and subsequently shortened following exchange of dominating TP53 mutation clone (Figure 1). Altogether, in 4/7 cases with TP53 mutation proportion change tested in >2 timepoints, telomere length changed according to alteration in TP53 mutation proportion. Since the observed changes of telomere length could potentially be caused by deregulated telomerase, we tested also the hTERT gene expression in all 36 patients who underwent clonal evolution. However, only weak or null hTERT expression was detected, showing no apparent correlation with telomere length or its change.

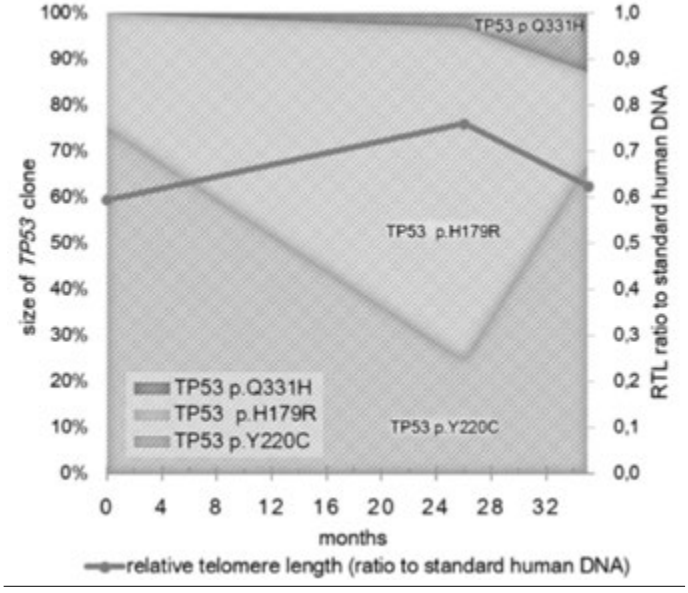

Figure 1. Example case for telomere length change associated with changing dominant clone of TP53.

Summary and Conclusions: Although telomere length is generally considered to be stable in CLL, our findings suggest that in patients undergoing clonal evolution significant changes may occur. This telomere length evolution can be likely attributed to selective pressure of therapy favoring some clones over the others. Our observation concerns primarily an acquisition of *TP53* defects, thus further investigation also in the context of other CLL-related genes is warranted.

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PF328

NOTCH1, TP53, SF3B1, ATM AND BIRC3 GENE MUTATIONS ARE ASSOCIATED WITH A WORSE OUTCOME IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH 13Q LOSSES

C. Pérez Carretero[1,2], A.E. Rodríguez-Vicente[1,2,*], M. Hernández-Sánchez[1,2], M. Quijada-Álamo[1,2], R. Benito[1,2], J. Hernández-Sánchez[1,2], M. Martín-Izquierdo[1,2], T. González[3], M.J. Vidal-Manceñido[4], A. García de Coca[5], M.P. Delgado-Beltrán[6], J.N. Rodríguez[7], M. Vargas[8], J.A. Queizán[9], I. de la Fuente[10], J. Labrador[11], B. Albarrán Servero[12], J.-Á. Hernández[13], J.-M. Hernández Rivas[1,2]

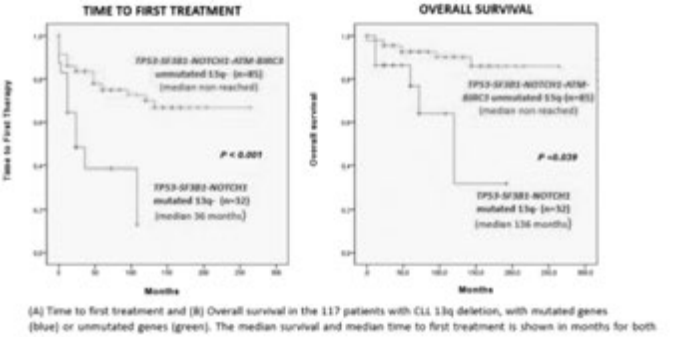
[1]IBSAL, IBMCC, Universidad de Salamanca, CSIC, Centro de Investigación del Cáncer, [2]Servicio de hematología, Hospital Universitario de Salamanca, [3]Servicio de hematología, Hospital Universitario de Salamanca, Salamanca, [4]Servicio de hematología, Hospital Virgen Blanca, León, [5]Servicio de hematología, Hospital Clínico Universitario, Valladolid, [6]Servicio de hematología, Hospital Miguel Servet, Zaragoza, [7]Servicio de hematología, Hospital Juan Ramón Jiménez, Huelva, [8]Servicio de hematología, Hospital de Jarrio, Asturias, [9]Servicio de hematología, Hospital General, Segovia, [10]Servicio de hematología, Hospital Río Hortega, Valladolid, [11]Servicio de hematología, Hospital Universitario, Burgos, [12]Servicio de hematología, Hospital Río Carrión, Palencia, [13]Servicio de hematología, Hospital Universitario Infanta Leonor, Madrid, Spain

Background: 13q deletion (13q-) is the most common cytogenetic aberration in chronic lymphocytic leukemia (CLL) associated with a favorable prognosis. However, CLL patients showing 13q- can have a variable outcome. Although the use of next-generation sequencing (NGS) has expanded our knowledge of the genomic alterations in CLL, the mutational background of 13q- patients has not been analyzed in detail so far.

Aims: To analyze the mutational status of CLL patients with 13q- by NGS in order to improve our understanding of the genetic underpinnings of this subgroup of CLL.

Methods: A total of 213 untreated CLL patients were selected for the study. Clinical and biological data were recorded. CD19 positive B cells were isolated and DNA extracted to perform NGS. The mutational status of 54 genes (8,952 probes, 416 Mb) was evaluated using a custom-designed gene panel (MiSeq, Illumina) including recurrent mutated genes in CLL associated with CLL pathogenesis. 95% of regions were sufficiently covered (>100X) and a mean depth of 343 reads/base within the regions of interest was obtained, allowing us to identify variants at low allele frequencies (down to 3%).

Results: A total of 117 patients (54.9%) had 13q- as a sole abnormality. Most patients (82.9%) were in Binet's stage A. The median follow-up for the CLL patients was 60 months. A total of 172 mutations were detected in 39 genes in seventy-three 13q- CLL patients (13q- mut). The median of mutations detected per patient was 1 (0-7). 63% of the 13q- mut cases presented more than one mutation. Surprisingly, the most frequently mutated genes were *NOTCH1* (16.7%), *TP53* (14.3%), *SF3B1* (11.9%) *ATM* (7.1%) and *BIRC3* (4.7%), genes previously associated with bad prognosis. The presence of mutations was associated with Binet B/C (p<0.001), unmutated *IGHV* gene (p=0.001), CD38 positivity (p=0.05) and need for treatment (p=0.004). Of note, in 13q- patients with mutations in *NOTCH, TP53, SF3B1, ATM or BIRC3* (32 cases, 27.35%), OS was 136 months (CI95% 98–174) whereas in the group without any mutation, OS has not been reached (CI95% 216–254) (p=0.039). In the univariate analysis, Binet B/C (p<0.001), unmutated *IGHV* status (p<0.001), B symptoms (p=0.002), hepatomegaly (p<0.0001), splenomegaly (p=0.01), lymphocyte count>20·10^9/L (p<0.001) and b2M high levels (p=0.048) were significant associated with a short OS. In the multivariate analysis, only unmutated *IGVH* status resulted significant in predicting OS (HR 5.89, CI95% 1.15–30.13, p=0.033). Regarding TFT, in 13q- mut patients the median TFT was 36 months (CI95% 0–87.7), whereas in the group without any mutation TFT has not been reached (p<0.001). In the univariate analysis, significant variables were Binet B/C (p<0.001), unmutated *IGHV* status (p<0.001), b2M levels (p<0.001), splenomegaly (p=0.015), lymphocyte count>2020·10^9/L (p<0.001), the percentage of cells >80% with 13q- (p=0.008) and B symptoms (p<0.001). In the Cox analysis, Binet stage (HR 10.1, CI95% 2.83–26.89,p<0.0001), B symptoms (HR 0.17, CI95% 0.05–0.54, p=0.003), lymphocytes count >20·10^9/L (HR 0.38, CI95% 0.18–0.81,p=0.012) and the presence of mutations in *NOTCH, TP53, SF3B1, ATM or BIRC3* (HR 0.45, CI95% 0.21–0.96,p=0.039) resulted significant in predicting TFT (Figure 1).



(A) Time to first treatment and (B) Overall survival in the 117 patients with CLL 13q deletion, with mutated genes (blue) or unmutated genes (green). The median survival and median time to first treatment is shown in months for both groups.

Figure 1.

Summary and Conclusions: 13q- CLL patients with mutations in *NOTCH1, TP53, SF3B1, ATM* or *BIRC3* have shorter overall survival (OS) and time to first treatment (TFT).

PF329

CHARACTERIZATION OF A NOVEL ENTITY IN CLL: SYMPTOMATIC BRONCHIAL INVOLVEMENT; A STUDY OF THE FRENCH INNOVATIVE LEUKEMIA ORGANIZATION (FILO) GROUP

M. Nudel[1,*], F. Baran-Marszak[2], R. Dubois[3], H. Dapvril[4], J. Dupuis[5], K. Laribi[6], J.O. Bay[7], C. Tomowiak[8], B. Dreyfus[8], S. Lepretre[9], H. Demarquette[10], F. Wallyn[11], L. Wemeau[11], M. Wemeau[12], S. Poulain[13], F. Morschhauser[10], F. Cymbalista[14], C. Herbaux[10]

[1]Hématologie, CHRU Lille, Lille Cedex, [2]Laboratoire hématologie, Hopital Avicenne, Bobigny, [3]anatomopathologie, [4]Radiologie, CHRU Lille, Lille, [5]Hématologie, Hopital H Mondor, Créteil, [6]Hématologie, CH Le Mans, Le Mans, [7]Hématologie, CHU Clermont Ferrand, Clermont Ferrand, [8]Hématologie, CHU Poitiers, Poitiers, [9]Hématologie, CHU H Becquerel, Rouen, [10]Hématologie, [11]Pneumologie, CHRU Lille, Lille, [12]Hématologie, CH Victor Provo, Roubaix, [13]Laboratoire hématologie, CHRU Lille, Lille, [14]Hématologie, Hopital Avicenne, Bobigny, France

Background: Chronic lymphocytic leukemia (CLL) infiltration is mostly described in peripheral blood, lymph nodes and bone marrow. Large autopsy reviews revealed that other organs can be infiltrated by CLL. Nevertheless, extra-medullary infiltration is rarely responsible of symptoms. Leukemic pulmonary infiltration (LPI) is a well-known process with a development of the disease in the lung parenchyma. Bronchial involvement is a distinct infiltration that has been rarely described in few case reports.
Aims: The aim of this study is to better characterize symptomatic infiltration of the bronchial tree by CLL, using clinical, radiological, biological and therapeutic tools.
Methods: We retrospectively collected 19 cases of symptomatic bronchial involvement of CLL. A histological evidence of bronchial involvement by CLL lymphocytes was available for 17 patients (90%). We disposed of lung function tests for 13 patients. Airflow obstruction was defined by a ratio of Forced Expiratory Volume in one second on Forced Volume Capacity less than 0.7. A thoracic CT scan was performed for 18 patients. A central reanalysis of thoracic CT scan was performed for 13 patients. Molecular analysis by NGS was performed on 15 patients.

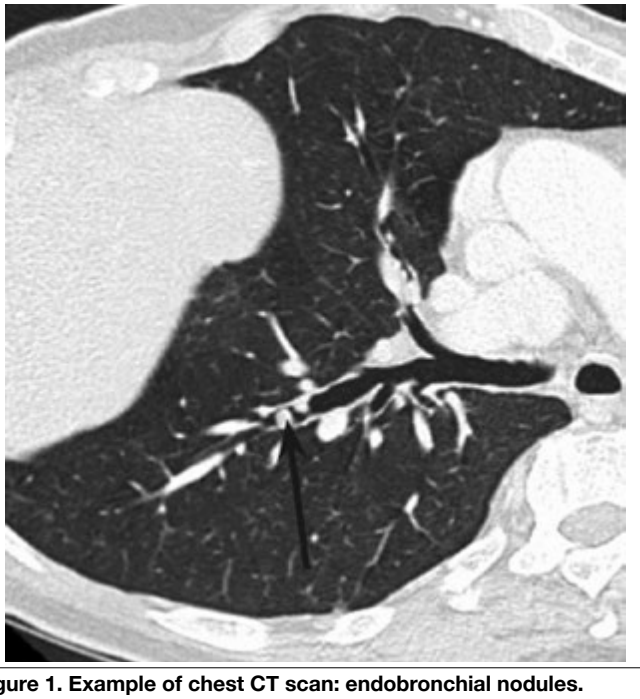


Figure 1. Example of chest CT scan: endobronchial nodules.

Results: During the course of the disease, all patients presented bronchial symptoms: cough, recurrent bronchitis, dyspnea. Bronchial spur biopsies showed a diffuse mucosal infiltration by small B lymphocytes with a cytoplasmic expression of CD79a, CD5 and CD23. In the remaining 2 patients, a body of evidence strongly suggested a bronchial involvement (symptoms, imaging and analysis of bronchoalveolar lavage fluid). Lung function tests revealed an airflow obstruction in 9/13 (69%) evaluated patients. The presence of bronchial involvement was not correlated with the importance of lymphocytosis. Karyotype or FISH were available for all patients. Of note, a high incidence of trisomy 12 was observed: 7/19 cases (36.8%) concordant with a low incidence of del13q (4/19: 21%). Molecular analysis found different mutations: 5 *TP53*, 4 *NOTCH1*, 2 *SF3B1*, 2 *RPS15*, 1 *FBXW7* and 1 *ATM* mutations. Thoracic CT scan analysis revealed a radiological anomaly of the bronchial tree for all patients. The presence of multiple endobronchial nodules was observed; these lesions are extremely rare and seem pathognomonic of the bronchial involvement by CLL. Symptomatic bronchial involvement had an impact on treatment initiation (first line or more) for 13 patients (76%). It was the only treatment criteria for 9 patients (53%). Other signs of CLL proliferation were not always noted. Indeed, Binet stage was A for 6 patients (32%), B for 11 (52%) and C for 2 patients (16%). Treatments included classic immunochemotherapy and BCR inhibitors. Twelve patients (66%) presented an improvement of bronchial symptoms (clinical, lung function test and/or CT improvement) after CLL treatment (Figure 1).
Summary and Conclusions: To our knowledge, this is the first extensive description of this type of extra medullary involvement in CLL. Symptomatic bronchial involvement by CLL may occur in the absence of other signs of progression. Airway obstruction symptoms in CLL should rapidly lead to an anatomopathologic analysis by bronchial spur biopsy if lung function tests and CT scan are compatible. In particular, the presence of multiple endobronchial nodules is very evocative. This study establishes a novel entity in CLL, the specific bronchial involvement. It should be suspected in case of unexplained bronchial symptoms and can indicate the initiation of CLL therapy, even in the absence of other classical treatment criteria.

PF330

GENETIC ABERRATIONS AND PROGRESSION-FREE SURVIVAL IN CLL PATIENTS TREATED WITH FRONT LINE RITUXIMAB-BASED CHEMOIMMUNOTHERAPY (FCR/BR): CLINICAL PRACTICE EXPERIENCE

M. Spunarova[1,*], J. Malcikova[1], N. Tom[2], M. Borsky[1], Y. Brychtova[1], M. Doubek[1], A. Panovska[1], H. Skuhrova Francova[1], K. Durechova[1], A. Brzobohata[1], A. Freibergerova[1], M. Jarosova[1], M. Mraz[1], S. Pospisilova[1], J. Mayer[1], M. Trbusek[1]

[1]University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, [2]CEITEC, Masaryk University, Brno, Czech Republic

Background: Fludarabine with cyclophosphamide and rituximab (FCR) constitute a front-line therapy for younger and fit chronic lymphocytic leukemia (CLL) patients. Elderly patients should be provided with alternative regimen consisting of bendamustine with rituximab (BR). Neither of these regimens is suitable for CLL patients harboring *TP53* defects. Inferior prognosis after FCR treatment has also been reported for patients with unmutated status of the immunoglobulin heavy chain variable region (*IGHV*), 11q deletion (11q-) or *NOTCH1* gene mutations.
Aims: To analyze progression-free survival (PFS) according to the presence of genetic defects in CLL patients treated with front line FCR or BR regimen in routine clinical practice at a large university hospital.
Methods: Mutation analysis of *ATM* (exons 2-63), *SF3B1* (exons 14-16), *NOTCH1* (part of exon 34 a 3 UTR), *BIRC3* (exons 7-10) and *TP53* (exons 2-11) genes was performed by the next-generation sequencing using MiSeq (Illumina) in pre-therapy samples. The cut-off was set at 10% VAF to consider clinicaly relevant mutated clones. *ATM* variants were verified by online tools (SIFT/PolyPhen), and by western blot (WB) of ATM level. Notch1 protein level (ICN domain) was assessed by WB in *NOTCH1*-mutated patients. Cytogenetic aberrations and *IGHV* status were determined by karyotyping, FISH and sequencing. Complex karyotype (CK) presence (analyzed at least 15 mitoses) was defined as three or more aberrations in two or more mitoses. The PFS analysis was done by log-rank (Mantel-Cox) test using interval from therapy initiation to clinical progression (according to the iwCLL recommendations) or the last follow up. Patients were provided with 2-6 cycles (median 4) of FCR (n=77 patients), FCR-Lite (reduced doses; n=30) or BR (n=43) according to clinical protocols.
Results: For PFS analysis, we included only patients with intact *TP53* gene (n=150). Further, 8 patients experienced progression or stable disease after the therapy and were also excluded from the PFS analysis; they did not manifest a predominant genetic trait. The PFS was assessed on the final cohort of 142 patients; 100 of them (70%) harbored unmutated *IGHV*. We observed the following outputs: (a) similar effect of FCR and BR regimens (median PFS 30 and 28 months (m), respectively); this justifies the pooling of the regimens to one analysis; (b) clear impact of *IGHV* mutation status (27 *vs* 52 m in unmut-*IGHV* and mut-*IGHV* patients; P 0.001); based on this result, we performed subsequent analyses separately according to the *IGHV* status; (c) clear negative impact of *ATM* mutations in patients with mut-*IGHV* (25 *vs* 56 m in *ATM*-wt patients; P=0.017); (d) no negative impact of *ATM* mutations in patients with unmut-*IGHV* (30 *vs* 24 m in *ATM*-wt patients); (e) very short PFS in patients with sole 11q- (other *ATM* allele intact) (15 m *vs* 28 m in *ATM*-wt patients and 30 m in *ATM*-mut

patients; P=0.052); the analysis was done in *IGHV*-unmut patients (f) no impact of *SF3B1* mutations; (g) no impact of *NOTCH1* mutations or high Notch1 protein level; (h) no impact of CK; (i) trend to shorter PFS in patients with *BIRC3* mutations (19 *vs* 28 m in *BIRC3*-wt patients; P=0.056). Note: analyses (g)–(i) were performed in *IGHV*-unmut patients.
Summary and Conclusions: We show predictive impact of *IGHV* status after the first-line FCR/BR. The shortest PFS was recorded for patients with unmutated *IGHV* and sole 11q-. We did not observe negative impact of Notch1 activation, *SF3B1* mutations or CK on the PFS. Supported by projects AZV 16-32743A, FNBr 65269705, MUNI/A/0968/2017.

PF331

CIRCULATING SEX HORMONES AND NUCLEAR HORMONE RECEPTOR EXPRESSION ARE ASSOCIATED WITH TREATMENT-FREE SURVIVAL IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

E.P. Allain[1], K. Venzl[2], P. Caron[1], V. Turcotte[1], D. Simonyan[3], M. Gruber[2], T. Le[2], E. Lévesque[1], C. Guillmette[1,4], K. Vanura[2,*]

[1]Laval University, Quebec, Canada, [2]Medical University of Vienna, Vienna, Austria, [3]CHU de Québec Research Center, [4]Canada Research Chair in Pharmacogenomics, Quebec, Canada

Background: CLL is the most prevalent adult leukemia in the western world. While it is not considered a hormone-regulated cancer, sex is a known risk factor with a male/female incidence ratio of 2:1 and male patients also developing progressive disease more frequently. In spite of these significant clinical observations, a detailed analysis of sex steroids and pituitary hormones, and hormone receptor expressions in CLL patients is still lacking.
Aims: This study aimed to provide a first insight into sex steroids in CLL and explore possible relationships between these hormones, their receptors and disease progression in CLL. We hypothesized that variable hormonal exposure may have a sexually dimorphic effect on CLL progression.
Methods: We quantified 15 circulating sex steroids (androgens, estrogens and progesterone) by sensitive and specific mass spectrometry and measured two pituitary hormones (luteinizing hormone (LH) and follicular secreting hormone (FSH)) by immunoassay in 156 samples from mostly early-stage, treatment-free CLL patients. Nuclear receptor expression levels were measured by RT-qPCR. Data were analyzed separately by sex and in relation to treatment-free survival (TFS). Univariate and multivariate analyses of TFS were performed using Cox's proportional hazard model.
Results: Median age of CLL patients was 59.8 and 62.9 years for men and postmenopausal women, respectively. Expression-based and cytogenetic CLL prognostic markers had very similar frequencies between male and female cases. Median TFS was shorter for male patients than for women (80.7 *vs* 135.0 months, P=0.033). Hormonal profiles of CLL patients differed significantly from those of healthy donors whereas male CLL cases had higher levels of circulating steroids than female CLL patients, confirming the relevance of analyzing them separately. In male CLL cases, no association was noted between steroid levels and TFS; however, higher LH levels were associated with shorter TFS in multivariate analyses with an adjusted hazard ratio (HRadj) of 2.11 (P=0.004). In female CLL cases, high levels of potent ligands of the androgen receptor (AR), testosterone (T) and dihydrotestosterone (DHT), were associated with improved TFS with HRadj values of 0.24 (P=0.007) and 0.53 (P=0.023), respectively. In line, in univariate analyses, high AR expression was associated with improved outcome in female CLL cases (HR=0.70, P=0.009). A trend for a similar association with AR expression in male patients was also noted (HR=0.84, P=0.081), along with a trend for improved survival with high estrogen receptor α expression (HR=0.76, P=0.056).
Summary and Conclusions: This study is the first to comprehensively profile steroids and pituitary hormones in CLL patients and to establish a link between outcome of CLL patients and circulating hormones. It reveals a sex-specific hormonal imbalance associated with disease progression, and suggests a possible role for nuclear steroid hormone receptor signaling in CLL cases.

PF332

DESIGN AND MINION TESTING OF A NANOPORE SEQUENCING SPECIFIC GENE PANEL FOR CHRONIC LYMPHOCYTIC LEUKEMIA

P. Orsini[1], C. Minervini[1], C. Cumbo[1], L. Anelli[1], A. Zagaria[1], A. Minervini[1], N. Coccaro[1], G. Tota[1], P. Casieri[1], L. Impera[1], C. Brunetti[1], E. Parciante[1], A. Giordano[1], G. Specchia[1], F. Albano[1,*]

[1]D.E.T.O. - Hematology Section, University of Bari, Bari - Italy, Bari, Italy

Background: MinION is a single-molecule nanopore sequencer from Oxford Nanopore Technologies connected to a laptop through a USB 3.0 interface. Sequencing is performed by the moving of individual DNA strands through biologic nanopores on a chip, where an electric field is applied and electrical signal variations are recorded. In the last years, next generation sequencing (NGS) methods have identified a wide range of gene mutations which have improved our knowledge about chronic lymphocytic leukemia (CLL) development, allowing to refine both the prognostic subgroups and better therapeutic strategies. Consequently, it is reasonable to assert that integration of the newly discovered genetic lesions into a comprehensive prognostic model based on both chromosomal abnormalities and gene mutations would help to improve prognostication of CLL patients.
Aims: In this study, we report a custom gene panel assay based on multiplex long-PCR followed by sequencing on MinION to identify single nucleotide variations (SNV) and insertions/deletions (indels) in 5 prognostically relevant genes in CLL: TP53, NOTCH1, BIRC3, SF3B1 and MYD88.
Methods: We designed a custom gene panel consisting of 7 primers pairs in 2 pools, with a total panel size of 15kb. Twelve patients were selected according to specific cytogenetic and molecular features significantly associated with the mutational status of these genes. For each DNA sample, 2 multiplex long-PCRs were prepared. MinION library preparation, sequencing and data analysis were performed. All cases included in the study were analyzed by Sanger Sequencing (SS) or other molecular assays for all the targets included in the gene panel, in blinded manner.
Results: Read depth analysis showed that the range of sequencing depth was inversely related to the amplicon size, with the smaller amplicons having a higher coverage (up to about 2100x). Anyway, the minimum coverage value was never below 50x. The error rate calculated was on average 6% and 2% for indels and SNV respectively. Except for the known mutation hotspots of NOTCH1 and MYD88 and the polymorphisms identified, considering the low chance to find a rare variant simultaneously in a small cohort, we decided to exclude the variants occurring in multiple samples from further validation analyses; excluding these critical positions from the analysis, the actual coverage of the custom panel was 94,7%. These data are closely related to the chemistry and basecalling algorithms used and are probably intended to improve with the progress of nanopore technology. Overall, 8 pathogenic mutations were detected in 6 patients, with 2 patients harboring concurrently 2 mutations: 6 SNV and 2 indels. The lowest mutation allelic ratio was around 10%. These mutations were simultaneously identified and confirmed with SS or other molecular assays.
Summary and Conclusions: Our assay allows a rapid analysis of the prognostically relevant genes in CLL, with just 2 PCRs per patient. This is the first report of targeted gene sequencing based on a custom panel of prepooled multiplexed primers on MinION. This approach offers a rapid, easy and affordable workflow of analysis compared to SS or the common NGS platforms, even if it is still not ready to substitute the other NGS platforms because of MinION error proneness. Anyway, the rapid and constant improvements of nanopore technology promise an exclusive and convenient use of MinION in the next future.

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LOW CATALASE EXPRESSION CONFERS REDOX HYPERSENSITIVITY AND IDENTIFIES AN INDOLENT CLINICAL BEHAVIOR IN CHRONIC LYMPHOCYTIC LEUKEMIA

M.T. Scupoli[1,*], C. Cavallini[1], R. Chignola[2], O. Perbellini[3], E. Mimiola[4], O. Lovato[1], C. Laudanna[5], G. Pizzolo[4], M. Donadelli[6]

[1]Research Center LURM, [2]Department of Biotechnology, University of Verona, Verona, [3]Department of Cell Therapy and Hematology, San Bortolo Hospital, Vicenza, [4]Department of Medicine, Section of Hematology, [5]Department of Medicine, Section of General Pathology, [6]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy

Background: B-cell receptor (BCR) signaling is a key determinant of variable clinical behavior and a target for therapeutic interventions in chronic lymphocytic leukemia (CLL). Endogenously produced H_2O_2 is thought to fine-tune the BCR signaling by reversibly inhibiting phosphatases. However, little is known about how CLL cells sense and respond to such redox cues and what impact they have on CLL.
Aims: In this study, we aimed at characterizing signaling sensitivity to exogenous H_2O_2 in prognostic subsets of leukemic cells and investigating possible mechanisms regulating response of leukemic cells to redox cues in CLL.
Methods: We analyzed peripheral blood mononuclear cell (PBMC) samples from 42 untreated CLL patients and from 9 age-matched healthy donors. To characterize signaling response of BCR proteins to exogenous H_2O_2, we used phospho-specific flow cytometry. Antioxidant enzymes were analyzed

at mRNA level using real-time PCR quantification and at a protein level using monoclonal antibodies and flow cytometry.
Results: CLL cells exhibit a specific redox sensitivity pattern with an overall higher and more heterogeneous redox response than healthy-donor B cells. Moreover, exogenous H_2O_2 in the absence of BCR engagement induced a signaling response of BCR proteins that was higher in CLL with favorable prognostic parameters (M-*IGHV*, CD38 and ZAP70 negative expression) and an indolent clinical course, measured as a longer time to first treatment (TTFT). We hypothesized that redox hypersensitivity could be due to an increased accumulation of exogenous H_2O_2 within the cells –possibly linked to a reduced antioxidant capacity- which would induce a higher inhibition of phosphatases with the consequent shift of the enzymatic balance towards phosphorylation. To test this hypothesis, we analyzed major cellular antioxidant systems -enzymatic and nonenzymatic- and compared them between the two CLL subsets characterized by different redox sensitivity. We identified low *catalase* expression as a possible mechanism accounting for redox signaling hypersensitivity: inhibiting catalase activity resulted in a further increase of protein phosphorylation whereas incubation of cells with exogenous catalase before addition of H_2O_2 completely reversed protein phosphorylation induced by H_2O_2. These findings establish a functional link between catalase and redox sensitivity and support the hypothesis that a lower catalase activity could cause an escalated accumulation of exogenous H_2O_2 in leukemic cells, with a consequent greater inhibition of phosphatases and an increase in redox signaling sensitivity. Moreover, lower levels of *catalase* were significantly associated with a slower progression of the disease. In leukemic cells characterized by redox hypersensitivity, we also documented an elevated accumulation of reactive oxygen species (ROS) and an increased mitochondrial amount.
Summary and Conclusions: In conclusion, this study shows that differential redox profiles in CLL are associated with divergent clinical behaviors and advances our understanding of the redox and signaling heterogeneity of CLL. Future challenges are to design therapeutic strategies targeting redox pathways that could implement the effectiveness of current therapies and overcome drug resistance in CLL.

PF334

STAT3 AND STAT5B MUTATIONS IN LARGE GRANULAR LYMPHOCYTES LYMPHOCYTOSIS/LEUKEMIAS: DIAGNOSTIC AND PROGNOSTIC VALUE

N. Muñoz-García[1,*], M. Jara-Acevedo[1], C. Caldas[1], P. Bárcena[1], A. López[1], N. Puig[2], M. Alcoceba[2], N. Villamor[3], P. Fernandez[4], M. González[2], A. Orfao[1], J. Almeida[1]

[1]Department of Medicine and Service of Cytometry, Cancer Research Center [IBMCC, CSIC-USAL], University of Salamanca, IBSAL & CIBERONC, Salamanca, Spain, [2]Department of Hematology, University Hospital & IBSAL, Salamanca, [3]Department of Pathology, Hematopathology Unit, Hospital Clínic, IDIBAPS, University of Barcelona, Barcelona, Spain, [4]Department of FACS/Stammzell, Institut für Labormedizin, Kantonsspital, Aarau, Switzerland

Background: T-cell large granular lymphocytic leukemia (T-LGLL) and chronic lymphoproliferative disorders of natural killer cells (CLPD-NK) are rare entities usually showing an indolent clinical course; despite this, these diseases are often associated with autoimmune disorders and even in some cases may evolve into aggressive forms of leukemia. Diagnosis is usually difficult, due to the absence of clear phenotypic clonality/malignant-related markers, the difficulty of distinguishing T clonal expansions from reactive oligoclonal process of T cells and, in CLPD-NK, the absence of a universal molecular marker of clonality. The presence of somatic *STAT3* and *STAT5b* mutations has recently been found in a subgroup of T-LGLL (particularly in CD8+ T-LGLL) and CLPD-NK; however, its systematic utility in clinical settings remains unknown.
Aims: The aim of this study was to analyze the frequency and type of somatic mutations in the *STAT3* and *STAT5b* genes in all subgroups of clonal large granular lymphocytes (LGL) expansions, and relate these findings with the phenotype of the expanded cells, as well as with the clinical characteristics of patients with T-LGLL and CLPD-NK, in order to better establish its diagnostic and prognostic utility.
Methods: *STAT3* and *STAT5b* genes were sequenced in a total of 132 populations of FACS sorted lymphoid cells (previously phenotyped by flow cytometry), from 78 patients with monoclonal LGL expansions (35 TabCD8$^+$, 12 TabCD4$^+$CD8$^{-/+d}$, 1 TabCD4$^+$CD8$^+$, 2 TabCD4$^-$CD8$^-$, 12 Tgd and 16 CLPD-NK), 6 patients with oligoclonal LGL expansions (4 TabCD8$^+$ and 2 Tgd), 13 patients with non-cytotoxic T-CLPD and 35 polyclonal T-cell populations.
Results: Somatic mutations in *STAT3* and *STAT5b* genes were detected in 25/78 populations from patients with clonal LGL expansions (32%), all but one in the *STAT3* gene (Y640F mutation was the most frequent, among others previously described). Furthermore, two mutations – not previously described - were found (K658F and Gly656_Tyr657insPhe). According to the cell lineage involved in the expansion, mutations were present in 37% of TabCD8$^+$-LGLL, 20% of CLPD-NK, 8% of TabCD4$^+$CD8$^{-/+d}$-LGLL, 100% of TabCD4$^+$CD8$^+$-LGLL, 50% of TabCD4$^-$CD8$^-$LGLL and 50% of Tgd-LGLL. *STAT3* mutations were not only found in T-LGLL and CLPD-NK cases, but also in 1/14 population from a patient with non-cytotoxic T-CLPD. None of the polyclonal cytotoxic T-cell populations showed *STAT3* or *STAT5b* mutations. Two patients who had two or more clonal LGL expansions from different cell lineages (*i.e.* one of them from NK cells and the other(s) from T cells) showed that at least two of them had *STAT3* mutations, which would support the monoclonal diagnosis. In 15 out of 22 mutated cases (68%) in which clinical information could be available, patients showed a significantly higher incidence of autoimmune disorders (*vs* non-mutated monoclonal LGL expansions), further supporting the prognostic interest.
Summary and Conclusions: Our results support the utility of the *STAT3* and *STAT5b* mutations study for clonality assessment and prognostic evaluation in T-LGLL and CLPD-NK; additionally, these findings suggest that in LGLL, similar or identical activation pathways would be involved in the pathogenesis of the disease, regardless of the cytotoxic cell lineage involved.

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T-PLL CELLS RESEMBLE MEMORY-TYPE T-CELLS WITH ABERRANT EFFECTOR FUNCTIONS IMPLICATING A LEUKEMOGENIC COOPERATION OF TCL1A WITH TCR SIGNALING

S. Oberbeck[1,*], K. Warner[2], A. Schrader[1], G. Crispatzu[1], P. Mayer[1], S. Pützer[1], H. Diebner[3], S. Stilgenbauer[4], G. Hopfinger[5], J. Dürig[6], T. Haferlach[7], M. Lanasa[8], I. Roeder[3], M. Hallek[1], D. Mougiakakos[9], M. von Bergwelt-Baildon[10], M. Brüggemann[11], S. Newrzela[2], H. Abken[1], M. Herling[1]

[1]Department I of Internal Medicine, Center for Integrated Oncology (CIO) Köln-Bonn, University Hospital of Cologne, Cologne, [2]Senckenberg Institute of Pathology, Goethe-University, Frankfurt/M., Frankfurt, [3]Institute for Medical Informatics and Biometry, Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, Dresden, [4]Department of Internal Medicine III, Ulm University, Ulm, Germany, [5]Department of Internal Medicine I, Bone Marrow Transplantation Unit, Medical University of Vienna, Salzburg, Austria, [6]Clinic for Hematology, University Hospital Essen, Essen, [7]MLL Munich Leukemia Laboratory, MLL Munich Leukemia Laboratory, Munich, Germany, [8]Duke University Medical Center, Durham, NC, Durham, United States, [9]Department of Medicine 5, Haematology and Oncology, University Hospital Erlangen, Erlangen, [10]Medizinische Klinik und Poliklinik III, Ludwig-Maximilians-University Munich, Munich, [11]Medical Department II of Hematology and Oncology, University Hospitals of Schleswig Holstein, Campus Kiel, Kiel, Germany

Background: The pathogenesis of the rare and aggressive T-cell prolymphocytic leukemia (T-PLL) is poorly understood, which particularly applies to a mechanistic concept around its hallmark oncogene TCL1A. Existing data implicate TCL1A as a catalytic enhancer of the oncogenic kinase AKT, a central node in a T-cell's antigen receptor (TCR) signaling cascade, which mediates proliferation and differentiation. The levels and role of TCR activation in T-PLL's pathogenesis are not known.
Aims: The aim of our study was to describe the T-PLL cell phenotypically, by gene expression, and their effector functions compared to healthy donor T-cells. Furthermore, we wanted to dissect TCL1As impact on T-cell receptor signaling and its functional relevance in leukemia development.
Methods: We performed immunophenotyping and gene expression profiling of 79 T-PLL samples and compared these to healthy donor derived T-cells. In functional experiments we used T-cell lines with or without TCL1A overexpression. Different mouse models were used for the investigation of TCL1As impact on leukemia development with or without *in vivo* stimulation.
Results: To first clarify which physiological T-cell subset T-PLL cells most resemble, we performed comprehensive global gene expression profiling & immunophenotyping of primary T-PLL (n=79) in comparison to healthy-donor derived T-cell populations. Principle component analyses and gene signature alignments revealed a high similarity of T-PLL cells to (central) memory T-lymphocytes over naïve T-cells. Surface markers revealed a spectrum of memory-type differentiation (n=69/79; 87%) with predominant central-memory stages (n=35/79; 44%). The usually TCR and/or CD28-coreceptor positive T-PLL cells revealed no restrictions to genetic or surface TCR-clonotypes. The abnormally high basal activation

levels (surface CD25, CD38, CD69) correlated in their degree with inferior clinical outcomes (med. survival 20.8 *vs* 58.3 mo.). In parallel, T-PLL cells lost expression of negative-regulatory TCR-co-receptors (*e.g.* CTLA-4, LAG3). Fittingly, TCR engagement of primary T-PLL cells revealed a trend to hyperactive intracellular responses and interleukin(IL)-2 release alongside a prominent Th1-cytokine program. T-PLL cells also showed a robust resistance to stimulation-induced cell death and agonistic CD95 ligation. TCR-derived signals (phospho-kinase induction, IL-2 release) were enhanced *in vitro* by the modulated presence of TCL1A with kinetics indicative of a sensitizer relationship, mainly in the CD3 axis as opposed to the CD28 branch.

A mouse model with TCL1A-initiated protracted development of T-PLL (*Lck*^{pr}*-TCL1A*^{tg}) revealed congruent findings with the aberrant T-cell phenotype of human T-PLL. TCL1A expressing T-cells of this model, that were further equipped with monoclonal epitope-defined TCRs against ovalbumine or a chimeric-antigen-receptor (CAR) against carcinoembryonic antigen, gained a pre-leukemic growth advantage in scenarios of pulsed or continuous low-level receptor stimulation.

Summary and Conclusions: Overall, we establish that T-PLL cells resemble antigen-experienced memory T-cells. Retention of functional effector responses to TCR stimulation and loss of restricting activation regulators underlie a highly activated phenotype and a marked resistance to death-inducing signals. TCL1A proactively enhances TCR responses and we postulate that this leukemogenic cooperation drives accumulation of memory-type cells that utilize amplified, hence permissive, low-level cognate antigen input.

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INTEGRATED GENETIC PROFILES OF T-PLL IMPLICATE A TCL1/ATM-CENTERED MODEL OF ABERRANT DNA DAMAGE RESPONSES

A. Schrader[1,2,3,*], G. Crispatzu[1,2,3], S. Oberbeck[1,2,3], P. Mayer[1,2,3], S. Pützer[1,2,3], J. von Jan[1,2,3], E. Vasyutina[1,2,3], K. Warner[4], N. Weit[1,2], N. Pflug[1], T. Braun[1,2,3], E.I. Andersson[5], B. Yadav[5], A. Riabinska[1], B. Maurer[6], M. Ventura Ferreira[7], F. Beier[7], J. Altmüller[8], M. Lanasa[9], C.D. Herling[1,2], T. Haferlach[10], S. Stilgenbauer[11], G. Hopfinger[12], M. Pfeifer[13], T. Brümmendorf[7], P. Nürnberg[8], K. Elenitoba-Johnson[14], S. Zha[15], M. Hallek[1,2], R. Moriggl[16], C. Reinhardt[1,2], M.-H. Stern[17], S. Mustjoki[5], S. Newrzela[4], P. Frommolt[18], M. Herling[1,2,3]

[1]Department I of Internal Medicine, Center for Integrated Oncology (CIO) Köln-Bonn, University of Cologne (UoC), [2]Excellence Cluster for Cellular Stress Response and Aging-Associated Diseases (CECAD), UoC, [3]CMMC, Center for Molecular Medicine University of Cologne (UoC), Cologne, [4]Senckenberg Institute of Pathology, Goethe-University, Frankfurt, Germany, [5]Hematology Research Unit Helsinki, Department of Medicine and Clinical Chemistry, University of Helsinki and Helsinki University Central Hospital (HUCH), Helsinki, Finland, [6]Institute of Animal Breeding and Genetics, University of Veterinary Medicine; Ludwig Boltzmann Institute for Cancer Research, Medical University of Vienna, Vienna, Vienna, Austria, [7]Department of Hematology, Oncology, and Stem Cell Transplantation, RWTH Aachen University Medical School, Aachen, [8]Cologne Center for Genomics, UoC, Institute of Human Genetics, University of Cologne (UoC), Cologne, Germany, [9]Duke University Medical Center, Durham, NC, Durham, United States, [10]MLL Munich Leukemia Laboratory, Munich, [11]Department III of Internal Medicine, University Hospital Ulm, Ulm, [12]Department of Internal Medicine I, Bone Marrow Transplantation Unit, Medical University of Vienna, Vienna, [13]Department of Translational Genomics, UoC, Cologne, Germany, [14]Department of Pathology and Laboratory Medicine, University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA, Philadelphia, [15]Institute for Cancer Genetics, Department of Pathology and Cell Biology, Herbert Irving Comprehensive Cancer Center, Division of Pediatric Oncology, Department of Pediatrics, Columbia University Medical Center, Columbia University, New York, United States, [16]Institute of Animal Breeding and Genetics, University of Veterinary Medicine; Ludwig Boltzmann Institute for Cancer Research, Medical University of Vienna, Vienna, Austria, [17]INSERM U830, Institut Curie, PSL Research University, Paris, France, [18]Bioinformatics Core Facility, CECAD, UoC, Cologne, Germany

Background: T-cell prolymphocytic leukemia (T-PLL) is a rare disorder with an aggressive clinical course and a generally fatal outcome (median OS ~20-36 months). Limited therapeutic options warrant the need for more efficient approaches that address the specific biology of the transformed mature T-cell. The usually complex karyotypes of T-PLL most recurrently (80%) harbor rearrangements of the T-cell leukemia 1 (TCL1) locus at 14q32.1. Supporting the initiating capacity of TCL1 are transgenic mice (lck-TCL1), which develop T-PLL-like expansions. However, a refined mechanistic disease concept of T-PLL is not established.

Aims: Our aim was to genetically characterize a large T-PLL cohort and to drwa functional conclusions.

Methods: To address its incomplete molecular concept, we integrated large-scale profiling data of alterations in gene expression, allelic copy number (CN), and nucleotide sequences in 111 well-characterized patients.

Results: The dominant alteration of T-PLL's molecular make-up is a unique and functionally synergistic combination of TCL1-overexpression and damaging ATM lesions. We also identified novel tumor-specific hot-spots for CN variability, fusion molecules, transcript variants, and progression-associated dynamics. The overall lesional spectrum of T-PLL is mainly annotated to axes of DNA damage responses, of cytokine signaling, and histone modulation. The chromosomal complexity of T-PLL is determined by a specific phenotype of impaired proximal DNA damage processing, telomere attrition, and abrogated cell death execution. Despite frequently identified ATM mutations and genomic losses, specific targeting of factors in potentially synthetic lethal relationships to ATM through small molecule inhibitors did not affect T-PLL cell viability in the context of DNA damage.

Summary and Conclusions: Based on the most current lesions, we established a model of T-PLL evolution resolved for pivotal genetic alterations integrated with landmarks of cellular dysfunctions.

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CG'806, A NON-COVALENT PAN-FLT3/PAN-BTK INHIBITOR, EXHIBITS UNIQUE BINDING TO WILD TYPE AND C481S MUTANT BTK AND GREATER POTENCY THAN IBRUTINIB AGAINST MALIGNANT B CELLS

H. Zhang[1,*], A. Local[1], K. Benbatoul[1], P. Folger[1], S. Sheng[1], S.E. Kurtz[2], J.W. Tyner[3], S.B. Howell[4], W.G. Rice[1]

[1]Aptose Biosciences, Inc, San Diego, CA, [2]Knight Cancer Institute, Division of Hematology and Medical Oncology, Oregon Health & Science University, [3]Department of Cell, Developmental & Cancer Biology, Oregon Health & Science University, Knight Cancer Institute, Portland, OR, [4]Moores Cancer Center, Department of Medicine, University of California, San Diego, La Jolla, United States

Background: Bruton's tyrosine kinase (BTK) is a validated drug target due to its role in B-cell malignancy development. Ibrutinib, an irreversible BTK inhibitor that covalently binds to cysteine residue 481 (C481) and is approved for chronic lymphocytic leukemia (CLL) and other B-cell malignancies, is limited by its adverse effects and resistance resulting from C481S or other mutations. A safe and potent inhibitor against all forms of BTK is needed for patients intolerant, refractory and resistant to ibrutinib. CG'806 is an oral small molecule pan-FLT3/pan-BTK inhibitor, designed to solve ibrutinib's shortcomings. It is in development for acute myeloid leukemia (AML) and B-cell malignancies.

Aims: We compared CG'806 and ibrutinib with respect to BTK binding mode, kinase inhibition profiles and cytotoxic activity against cultured and patient-derived malignant B-cells. CG'806 was also screened for potential safety issues using *in vitro* biomarkers.

Methods: CG'806 was co-crystallized with the kinase domain of wild type (WT) and C481S mutant forms of BTK. CG'806 was tested at 1 μM for biochemical inhibition of 583 kinases, and IC_{50}s were determined on the most sensitive kinases. The Safety44 panel (DiscoverX) was screened to identify potential off-target activities. CG'806 was evaluated in a cytotoxicity assay on cultured malignant B-cell lines or freshly isolated mononuclear cells from patients. The effect of CG'806 on cell signaling was assessed by Western blotting.

Results: The co-crystal complex of CG'806 bound to BTK-WT or -C481S at resolution of 1.84Å and 1.63Å, respectively, revealed a binding mode of an atypical type II inhibitor. The DFG motif occupies an atypical conformation whereby the Phe540 is rotated out of the ATP binding pocket and the Asp539 side chain is tilted to hydrogen bond with CG'806. Moreover, CG'806 interacts with the hinge region and the αC-helix is in a partial out position. No electron density interaction was observed between CG'806 and the C481 residue. Kinase profiling revealed that CG'806 most potently inhibits kinases from the BTK, FLT3, TRK, and AURK clusters with IC_{50}s <25 nM. CG'806 had similar potency against BTK-WT (IC_{50}=8.4 nM) and C481S mutant (IC_{50}=2.5 nM) as opposed to ibrutinib that was >60-fold less potent against the C481S mutant. Importantly, CG'806 did not inhibit TEC, EGFR or ERBB2/4, which are related to ibrutinib's side effects. CG'806 inhibited cell proliferation 50-6,000 times more potently than ibrutinib in 11 tested malignant B-cell lines; it also had greater activity on primary CLL samples than ibrutinib. CG'806 inhibited signaling from BCR, AKT/mTOR and NF B as demonstrated by decreasing phosphorylation of BTK, PLCγ2, AKT, PI3K and ERK in a cell line dependent manner. CG'806 demonstrated a desirable safety profile when tested against the hERG ion

channel (IC_{50} >10 μM) and CYP450 enzymes (IC_{50}s >50 μM on Cyp3A4, 2C19, 2D6, 1A2; IC_{50}=8.6 μM on Cyp2C9). At 10 μM, CG'806 had no significant effect on the 44 common side-effect related GPCRs, nuclear receptors, transporters or ion channels.
Summary and Conclusions: CG'806 is a potent, non-covalent, oral inhibitor of WT and C481S BTK with a favorable *in vitro* safety profile. CG'806 killed cultured and primary malignant B-cells more potently than ibrutinib and was equipotent against WT and C481S BTK without affecting TEC, EGFR, ERBB2/4 or other safety-related targets. The data support clinical development of CG'806 in patients with CLL and other B-cell malignancies intolerant, resistant, or refractory to ibrutinib.

PF338

EPIGENETIC DRUG SCREEN ON RITUXIMAB-RESISTANT CELLS REVEALED AN UNEXPECTED ROLE OF AURORA KINASE INHIBITORS IN CD20 EXPRESSION

V. Kozlova[1,*], A. Ledererova[1], M. Doubek[1,2], J. Mayer[1,2], S. Pospisilova[1,2], M. Smida[1,2]

[1]Molecular Medicine, CEITEC MU, [2]Department of Internal Medicine - Hematology and Oncology, Medical Faculty of Masaryk University and University Hospital Brno, Brno, Czech Republic

Background: CD20 antigen is the prime target of monoclonal antibody (mAb) therapy, a standard of care for B-lymphoid malignancies. Although effective at first, repeated cycles of anti-CD20 treatment (*e.g.* rituximab (RTX)) often result in the loss of CD20 from the surface of malignant B cells and consequently in therapy resistance and therapy failure. Interestingly, mechanisms regulating CD20 expression are largely unknown. Yet, the possibility to modulate CD20 levels seems an appealing strategy to enhance the efficacy of CD20 mAbs.
Aims: RTX was suggested to induce epigenetic changes within the CD20 promotor and few epigenetic inhibitors were proposed to increase CD20 expression in some lymphoma cell lines. Our aim was to use an unbiased screening approach with a broad panel of epigenetic inhibitors to search for drugs that are able to increase CD20 expression and thereby improve the effectiveness of CD20 mAbs.
Methods: We have generated RTX-resistant cells by chronic exposure of B-lymphoid cell lines to gradually increasing doses of rituximab in multiple cycles. Thereby we have created cell lines that have permanently strongly downregulated CD20 protein on the cell surface as well as reduced CD20 gene transcription. These cells are fully resistant to additional treatment with CD20 antibodies, which is maintained long-term. We then screened these cells against a library consisting of 182 small-molecule compounds targeting various epigenetic modifying and related enzymes (histone deacetylases, methyltransferases, etc.) to determine surface CD20 expression changes by flow cytometry.
Results: We have applied the epigenetic drug library on our resistant CD20-low cells to identify drugs enhancing CD20 expression. Surprisingly, the most significant increase in CD20 surface density was reproducibly detected using multiple diverse Aurora kinase inhibitors. We then modified the screen and added RTX with the human serum (source of complement) together with the epigenetic drug panel to assess which inhibitors are able to enhance the response to RTX in a viability assay. Multiple diverse targets were identified, among them also Aurora kinases. We then selected few of the most significant aurora kinase inhibitors to validate in follow-up experiments. By performing multiple-point dose-response curves on resistant CD20-low cells, we could confirm that these compounds are able to increase CD20 surface levels in a dose-dependent manner. Importantly, such increase was not observed when using wildtype cells. In addition, we could confirm these results on both MEC1 and Ramos cell lines and on cells rendered resistant by chronic exposures to another CD20 mAb Ofatumumab. Aurora kinase inhibitors triggered CD20 increase was sustained for extended time period. Finally, when we first pretreated resistant cells with the aurora kinase inhibitors and then added RTX with the human serum, we could observe a profound shift in a viability curve and hence a strong sensitization towards RTX.
Summary and Conclusions: Our results indicate an unexpected role of Aurora kinases in CD20 regulation and their possible use for combination with CD20 mAbs. Molecular analysis is underway in order to understand the mechanistic effect of Aurora kinase inhibitors upon CD20 expression, as their co-administration could lead to the improvement in the efficacy of CD20 mAbs.
This research has been financially supported by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601) and by the research grant AZV-MZ-CR 15-33561AA-4/2015 and grant MUNI/A/0968/2017.

PF339

DISTINCT IMMUNE SIGNATURES IN CHRONIC LYMPHOCYTIC LEUKEMIA AND RICHTER'S SYNDROME

Y. Wang[1,*], S. Sinha[1], H. Dong[1], C. Secreto[1], T. Call[1], S. Parikh[1], J. Leis[2], K. Rech[1], A. Feldman[1], S. Kenderian[1], N. Kay[1], S. Ansell[1], R. He[1], W. Ding[1]

[1]Mayo Clinic, Rochester, [2]Mayo Clinic, Phoenix, United States

Background: Immune checkpoint blockade with the anti-PD-1 monoclonal antibody pembrolizumab was effective in Richter's Syndrome (RS) but not Chronic Lymphocytic Leukemia (CLL) in a phase 2 clinical trial (NCT02332980). Analyzing the immune signatures including PD-L1 expression and T cell diversity is important for understanding the differential responses.
Aims: To compare the tissue PD-L1 expression and peripheral blood T cell diversity in CLL and RS patients enrolled in the NCT02332980 trial, and to correlate the results with clinical response to pembrolizumab.
Methods: 15 CLL and 14 RS patients in the NCT02332980 trial were included in this study. Expression of PD-L1 in the lymph node was analyzed by immunohistochemistry staining. Peripheral blood and lymph node T cell diversity was analyzed using the ImmunoSEQ platform (Adaptive Biotechnology), which quantifies the clonailty of T cells by deep sequencing of the CDR3 region of the T cell receptor (TCR). The calculated clonality ranges from 0 to 1, and a higher clonality indicates a more diverse T cell population. Data analysis (Student's t-test and Mann-Whiney U test) was done by GraphPad Prism (v7).
Results: PD-L1 expression was significantly lower in CLL (n=6) *vs* RS (n=12) patients (mean 5.5% *vs* 22.9%, *P*=0.003). A control CLL cohort (n=11, Mayo CLL tissue registry) also had lower PD-L1 expression compared with the RS cohort (7.7% *vs* 22.9%, *P*=0.002). RS Patients progressed on ibrutinib or chemotherapy had similar PD-L1 expression (25.3% *vs* 19.6%, *P*=0.416). Peripheral blood TCR clonality at trial baseline was significantly lower in RS (n=13) *vs* CLL (n=13) patients (median 0.098 *vs* 0.342, *P*=0.026). Patients progressed on ibrutinib or chemotherapy had similar TCR clonality (CLL: 0.34 *vs* 0.24, *P*=0.524; RS: 0.11 *vs* 0.09, *P*=0.534). Five RS patients achieved an objective response (1 complete response [CR], 2 partial response [PR], 1 complete metabolic response [CMR] and 1 partial metabolic response [PMR]), 4 of which were after progression on ibrutinib. For CLL patients, only 1 nodal reduction was seen. Four RS patients had prior CLL state samples available for TCR analysis. Two patients had a notable decrease of TCR clonality (0.21 to 0.04 and 0.17 to 0.03) from CLL (peripheral blood) to RS state (lymph node), and achieved CR and PR, respectively. Two other patients with stable clonality (0.04 to 0.05 and 0.03 to 0.08) had stable disease (SD) and PMR, respectively. Peripheral blood TCR clonality did not change significantly after treatment with pembrolizumab (CLL [n=11]: *P*=0.775; RS [n=11]: *P*=0.830; paired t-test).
Summary and Conclusions: RS patients had higher expression of PD-L1 and lower TCR clonality (more diverse T cells) compared with CLL patients. The distinct immune signatures may explain their differential responses to PD-1 blockade. The dynamic changes of TCR clonality during Richter's transformation may predict a response to PD-1 blockade.

Chronic lymphocytic leukemia and related disorders – Clinical

PF340

DURABILITY OF RESPONSE TO VENETOCLAX (VEN) IN PATIENTS WITH CLL RELAPSED/REFRACTORY TO IBRUTINIB AND/OR IDELALISIB

J.C. Byrd[1,*], W.G. Wierda[2], M.S. Davids[3], M. Choi[4], R.R. Furman[5], A. Mato[6], L. Zhou[7], M. Verdugo[7], J. Potluri[7], S. Coutre[8]

[1]The Ohio State University, Columbus, [2]University of Texas MD Anderson Cancer Center, Houston, [3]Dana-Farber Cancer Institute, Boston, [4]UCSD Moores Cancer Center, San Diego, [5]Weill Cornell Medicine, [6]Memorial Sloan Kettering Cancer Center, New York, [7]AbbVie, Inc., North Chicago, [8]Stanford Cancer Center, Stanford University School of Medicine, Stanford, United States

Background: Therapies are urgently needed for patients (pts) with CLL relapsed/refractory (R/R) to BCRi.
Aims: We report outcomes for the full trial population from an ongoing phase 2 trial with VEN monotherapy, including the impact on detectable minimal residual disease (MRD).
Methods: Pts with CLL R/R to ibrutinib and/or idelalisib received 400 mg daily VEN after initial dose ramp up. Results are from a data cut on 28 Nov 2017.
Results: Pts (N=127) had received a median of 4 prior therapies (1 – 15). Del(17p) was noted in 40% (50/126) and 28% (34/122) of pts had mutated *TP53*. After a median of 17 (.1 – 36) months on VEN, the best overall response rate was 66% (84/127; CR/CRi – 10%, nPR/PR – 56%) per investigators (INV) and 70% (89/127) by independent review committee (IRC). Per INV (median follow up, 16 [.03 – 33] months), estimated median progression-free survival (PFS) was 25 months (18-month rate, 66%); neither median duration of response (18-month rate: 75%) nor median overall survival (18-month rate: 88%) was reached. 36/77 pts assessed (47%; 28% [36/127] by intent to treat) had undetectable blood MRD, 9/26 assessed were also undetectable in marrow (2 CR/CRi, 7 PR). Median PFS was longer for pts with undetectable MRD in blood *vs* positive (not reached *vs* 21.9 months; HR, .148 [.04 – .49], *p*=.0019). 64 pts discontinued VEN, most commonly for CLL progression (n=35; median time, 10 months [.1 – 29]), AEs (n=8), and Richter's transformation (n=6; median time, 13 months [4 – 19]). Common any-grade AEs were GI AEs (diarrhea [50%], nausea [49%]) and cytopenias (anemia [43%], neutropenia [41%], thrombocytopenia [28%], decreased white blood cell count [28%]) (Table 1).

Table 1.

n (%)	Last prior BCRi				N=127	
	Ibrutinib n=91		Idelalisib n=36			
	INV	IRC	INV	IRC	INV	IRC
ORR	59 (65)	64 (70)	25 (69)	25 (69)	84 (66)	89 (70)
CR	5 (6)	0	2 (6)	0	7 (6)	0
CRi	4 (4)	1 (1)	2 (6)	0	6 (5)	1 (1)
nPR	3 (3)	0	0	0	3 (3)	0
PR	47 (52)	63 (69)	21 (58)	25 (69)	68 (54)	88 (69)
SD	21 (23)	27 (30)	9 (25)	11 (31)	30 (24)	38 (30)
PD	5 (6)		2 (6)		7 (6)	
Early discontinuation	6 (7)		0		6 (5)	

Summary and Conclusions: Based on longer follow up, VEN monotherapy demonstrates robust activity, with good tolerability in pts with CLL R/R to ibrutinib and/or idelalisib. Though most pts achieved PR, outcomes appear durable with undetectable MRD.

PF341

SALVAGE USE OF IBRUTINIB AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION FOR CHRONIC LYMPHOCYTIC LEUKEMIA OR MANTLE CELL LYMPHOMA: A STUDY ON BEHALF OF THE SFGM-TC AND THE EBMT

M. Michallet[1,*], P. Dreger[2], M. Sobh[1], J. Hoek[3], A. Boumendil[4], L. Muller[5], P. Corradini[6], W. Bethge[7], D. Russo[8], N. Durakovic[9], M. Van Gelder[10], N. Schaap[11], N. Kroger[12], J. Schetelig[13]

[1]Hematology, Centre Léon Bérard, Lyon, France, [2]Internal Medicine V, University of Heidelberg, Heidelberg, Germany, [3]EBMT Data Office, Leiden, Netherlands, [4]EBMT Data Office, Paris, France, [5]Hematology, Martin-Luther-Univeristy, Halle, Germany, [6]Hematology, University hospital of Milano, Milan, Italy, [7]Hematology, Eberhard Karls University Tuebingen, Tuebingen, Germany, [8]Hematology, University of Brescia, Brescia, Italy, [9]Hematology, Clinical Hospital Center Zagreb, Zagreb, Croatia, [10]Hematology, University Medical Center, Maastricht, [11]Hematology, Radboud University Medical Centre, Nijmegen, Netherlands, [12]Hematology, University Hospital Eppendorf, Hamburg, [13]Hematology, University Hospital Dresden, Dresden, Germany

Background: Allo-HSCT is an accepted treatment option for patients with relapsed and refractory mantle cell lymphoma (MCL) or high-risk chronic lymphocytic leukemia (CLL). This treatment option is currently reshaped by the introduction of pathway inhibitors (PWI). In case of disease relapse after transplantation, the outcome is a major concern and the prognosis is dismal. PWI could be very promising when used before and/or after transplantation.
Aims: The purpose of our study is to provide information on the safety and efficacy of ibrutinib when administered as salvage treatment after allo-HSCT for CLL or MCL.
Methods: A total of 62 patients (56 CLL and 6 MCL), were included, 41 (63%) males; median age at transplantation was 49 (range: 35-64) years and the median number of treatment lines prior to transplantation was 3 (1-10). Before transplantation, del17p was present in 30% of patients and del11q was present in 18% of other cases. Disease status at allo-HSCT was sensitive in 45/60 (75%) patients. Conditioning was reduced-intensity in 68% of patients.
Results: In all patients, Ibrutinib was initiated for disease progression after allo-HSCT. The median time between allo-HSCT and Ibrutinib administration was 30 months (range: 1-140) and the median time between post-allo-HSCT relapse and Ibrutinib initiation was 3 months. Overall, 44/62 (71%) patients responded to Ibrutinib; 24 (39%) patients with a PR, and 20 (32%) patients reached CR. Of these, 11 were evaluated for MRD by flow cytometry, 5 were MRD negative and 6 were MRD positive . All of the 44 responding patients were still on Ibrutinib at the last follow-up with a median exposure of 407 days (104-937). The 18 other patients failed to respond after Ibrutinib, 4 did not obtain any change in response while 14 patients progressed, among them 5 received idelalisib and failed to respond. At time of ibruinib initiation, 10 patients had still an active chronic GVHD, all of these patients had their GVHD resolved after receiving Ibrutinib. Ibrutinib was generally well tolerated, 14 (22%) patients discontinued ibrutinib, 4 because of toxicity and 10 because of disease progression. Overall, 16 patients relapsed (median PFS=24 months), 9 died (all CLL) only from disease progression. Two-year OS and PFS probabilities from start of ibrutinib were 72% and 49% respectively. OS and PFS after ibrutinib were not influenced by del17p/del11q while patients with late relapse after allo-HSCT (>24 months) had a better PFS after ibrutinib.
Summary and Conclusions: We showed in this largest series of patients described to date in this indication, that ibrutinib can be safely administered for CLL/MCL relapse after allo-HSCT, with an efficacy at least similar to non-transplanted patients with high-risk disease.

PF342

MANAGEMENT, ADVERSE EVENTS, AND OUTCOMES OF 282 CLL PATIENTS (PTS) TREATED WITH VENETOCLAX (VEN) IN THE REAL WORLD

C. Nabhan[1,*], A. Klink[2], J. Samp[3], J. Pauff[3], J. Nielsen[3], B. Meissner[3], H. Keim[3], K. Kennard[4], A. Mato[5]

[1]Cardinal Health, Dublin, [2]Cardinal Health, Dallas, [3]AbbVie, Chicago, [4]Hematology and Oncology, University of Pennsylvania, Philadelphia, [5]Memorial Sloan Kettering Cancer Center, New York, United States

Background: Ven is an oral bioavailable BCL-2 inhibitor with demonstrated activity in clinical trials for relapse/refractory (R/R) CLL pts including those with del17p or failing a kinase inhibitor (KI). Ven was initially approved in the US and EU in 2016. As expanded indications for Ven in CLL are under investigation, additional data are needed to understand use and outcomes in clinical practice.
Aims: Describe initiation, management and outcomes of Ven-treated CLL pts in the real world.
Methods: This is a retrospective cohort study of CLL pts initiating Ven. Investigators from 33 community centers provided pt-level data from medical records including demographics, clinical characteristics, ramp up management and outcomes. Tumor burden was assessed per FDA label, tumor lysis syndrome (TLS) was defined by Howard criteria and response was

based on iwCLL criteria. The primary endpoints were progression free survival (PFS) and overall survival (OS). The secondary endpoint was overall response rate (ORR: complete or partial response [CR or PR]). Characteristics and outcomes were summarized by descriptive statistics, and comparisons of ORR by subgroups were assessed on univariate analyses.

Results: 282 Ven pts were included, of whom 22% used Ven in combination with ibrutinib (7%) or an anti-CD20 (15%). Median age was 67 years (range 60-73); 83% had *TP53* interruption; 64% had 1 prior therapy and 23% had ≥2 prior therapies (median 1; range 0-6); 61% and 3% had prior use of 1 and 2 KIs, respectively. At baseline, 12%, 54% and 34% had low, medium and high tumor burden, respectively; 32% initiated Ven as inpatient. During ramp up, 9% had a dose interruption; 4 of 6 dose reductions were due to TLS or hematologic abnormalities. TLS events occurred in 8% of pts (n=22) with 5 pts experiencing clinical TLS. With a median follow up of 5.8 months, ORR was 82% (CR: 29%). Responses were not negatively affected by pt, disease or treatment factors that were assessed with the exception of TP53 status (Table 1; response not assessed in 27(9.6%) pts). The 12-month PFS and OS were 83% and 91%, respectively (Figure 1). Among 48 pts assessed for minimal residual disease (MRD) during Ven treatment, 27 (63%) were MRD negative. Resolution of baseline lymphadenopathy, lymphocytosis, or B symptoms were reported in 94%, 95% and 95%, respectively. Among 48 pts (17%) who discontinued Ven, median time to discontinuation was 6.1 months. Discontinuation was mainly due to response to therapy (n=12), refractoriness (n=11), pt request (n=10) or relapse (n=5).

Table 1.

	All Ven pts	Age		Prior lines			TP53 interruption		Prior KI use		Lymph node size		TLS during ramp-up		Max Ven dose	
		<65 years	≥65 years	0	1	≥2	Present	Absent	Yes	No	All LN <5cm	Any LN ≥5cm	Yes	No	400 mg	<400 mg
ORR, n (%)	208 (82%)	76 (83%)	132 (81%)	32 (91%)	130 (80%)	46 (81%)	176 (84%)	32 (70%)	117 (82%)	91 (81%)	95 (80%)	105 (83%)	16 (80%)	192 (82%)	108 (81%)	100 (83%)
CR, n (%)	73 (29%)	30 (33%)	43 (26%)	13 (37%)	44 (27%)	16 (28%)	61 (29%)	12 (26%)	38 (27%)	35 (31%)	28 (24%)	45 (36%)	8 (40%)	65 (28%)	41 (31%)	32 (26%)
ORR across subgroups, *p*-value	-	0.87		0.28			0.03		0.75		0.62		0.77		0.75	

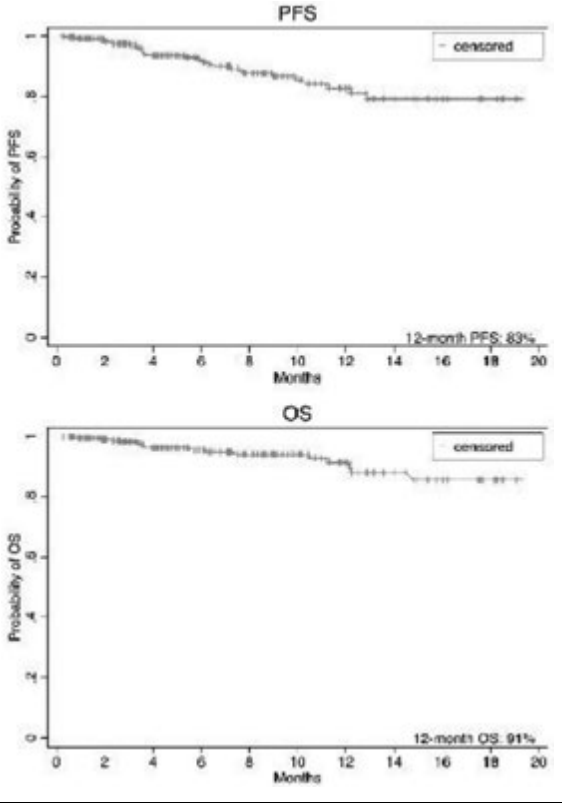

Figure 1.

Summary and Conclusions: In the largest study of Ven-treated CLL pts in community practice, PFS, OS and response rates were comparable to clinical trials with most clinical factors not impacting response to therapy. Ven was well tolerated and few experienced a TLS/ hematologic event. Taken together, these results support the generalizability of Ven use in community settings. Further analyses on ramp up management and outcomes will be presented.

PF343

IBRUTINIB FOR FIRST-LINE TREATMENT OF OLDER PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOMA (CLL/SLL): A 4-YEAR EXPERIENCE FROM THE RESONATE-2 STUDY

J. Burger[1,*], P. Barr[2], T. Robak[3], C. Owen[4], P. Ghia[5], A. Tedeschi[6], O. Bairey[7], P. Hillmen[8], S. Coutre[9], S. Devereux[10], S. Grosicki[11], H. McCarthy[12], J. Li[13], D. Simpson[14], F. Offner[15], C. Moreno[16], S. Dai[17], J. Dean[17], D. James[17], T. Kipps[18]

[1]University of Texas MD Anderson Cancer Center, Houston, TX, [2]University of Rochester, Rochester, NY, United States, [3]Medical University of Łódź, Łódź, Poland, [4]Tom Baker Cancer Centre, Calgary, AB, Canada, [5]Università Vita-Salute San Raffaele and IRCCS Istituto Scientifico San Raffaele, [6]ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy, [7]Rabin Medical Center, Beilinson Campus, Petah Tikva, Israel, [8]The Leeds Teaching Hospitals, St. James Institute of Oncology, Leeds, United Kingdom, [9]Stanford University School of Medicine, Stanford, CA, United States, [10]Kings College Hospital, NHS Foundation Trust, London, United Kingdom, [11]School of Public Health, Silesian Medical University, Katowice, Poland, [12]Royal Bournemouth Hospital, Bournemouth, United Kingdom, [13]Jiangsu Province Hospital, Nanjing, China, [14]North Shore Hospital, Auckland, New Zealand, [15]Universitair Ziekenhuis Gent, Gent, Belgium, [16]Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, [17]Pharmacyclics LLC, an AbbVie Company, Sunnyvale, CA, [18]University of California, San Diego, Moores Cancer Center, La Jolla, CA, United States

Background: Ibrutinib (ibr), a first-in-class, once-daily inhibitor of Bruton's tyrosine kinase, is approved in the EU and other regions for the initial treatment of CLL. RESONATE-2 is a phase 3 study designed to compare efficacy and safety of first-line ibr *vs* chlorambucil (clb) in patients (pts) with CLL/SLL. Primary results (assessed by independent review committee [IRC]) with a median follow-up of 18.4 mo demonstrated that ibr reduced the risk of progressive disease (PD) or death by 84% (P<0.001) (Burger, *N Engl J Med* 2015).

Aims: This extension study updates long-term ibr efficacy and safety from RESONATE-2.

Methods: Pts with treatment-naïve CLL/SLL aged ≥65 y (ITT n=269) were randomized 1:1 to receive 420 mg ibr once-daily continuously or clb for up to 12 cycles. Pt informed consent was obtained. The primary endpoint was PFS by IRC. Secondary endpoints included ORR, rate of hematologic improvement, and safety; long-term follow-up safety focused on ibr. Pt-reported outcomes included FACIT-Fatigue (F). Pts with PD on clb could crossover to ibr. IRC was discontinued after primary analysis. Long-term efficacy is reported per the investigator. Additional review for CR was undertaken by the sponsor to assure alignment with iwCLL criteria.

Results: With median follow-up of 4 yrs (max 55 mo), prolongation of PFS benefit for ibr *vs* clb was sustained (HR 0.137; 95% CI 0.090-0.210; Figure 1), including in high-risk subgroups (del11q: HR 0.034; 95% CI 0.011-0.110; unmutated (UM)-*IGHV*: HR 0.088; 95% CI 0.046-0.169). 48-mo PFS rates for ibr *vs* clb overall were 74% *vs* 16%, in pts with del11q were 79% *vs* 0% (72% with ibr in pts without del11q) and in UM-*IGHV* were 75% *vs* 4% (79% with ibr in pts with mutated *IGHV*). ORR was 91% with ibr *vs* 37% with clb; sponsor-confirmed CR with ibr was 18% (increased from 4% [IRC-confirmed] at primary analysis). Ibr led to sustained hematologic improvement in 80% of pts with baseline anemia *vs* 24% with clb (P<0.0001) and in 54% of pts with thrombocytopenia *vs* 25% with clb (P=0.0229). Ibr led to significantly greater improvements over time *vs* clb in FACIT-F (P=0.0014; repeated measure analysis). Grade (Gr) ≥3 adverse events (AEs) in ≥5% of pts over the 4-yr follow-up included neutropenia (13%), pneumonia (12%), anemia (7%), hypertension (HTN; 7%) and hyponatremia (5%) terms. Gr ≥3 hematologic and infectious toxicities were generally highest in the first yr and then decreased, while HTN remained stable. Of interest, Gr ≥3 atrial fibrillation occurred in 4% of ibr-treated pts and Gr ≥3 major hemorrhage in 7%. The most common reason for discontinuation in ibr-treated pts was AEs (19%, n=26), which decreased over time (yr 0-1, n=9; yr 1-2, n=7; yr 2-3, n=6; yr 3-4, n=4). AEs leading to discontinuation in >1 pt were atrial fibrillation (n=4), palpitations, and pneumonia (n=2 each). With a median ibr treatment duration of 46.9 mo (range 0.7-55.2), 89/136 (65%) pts remain on first-line ibr; 7/136 pts discontinued ibr due to PD. 11/136 pts received subsequent CLL therapy after stopping ibr (most commonly FCR [n=3], BR [n=2], acalabrutinib [n=2]).

Summary and Conclusions: With 4 yrs of follow-up, efficacy of single-agent ibr continues to endure with 86% risk reduction of PD or death. Ibr substantially improved PFS in pts with traditional high-risk features del11q or

UM-*IGHV* (risk reduction 97% and 91% *vs* clb, respectively). PFS with ibr was preserved in pts with or without del11q or UM-*IGHV*. CR rates improved with prolonged follow-up. Discontinuation due to AEs decreased over time, with 65% of ibr pts continuing daily treatment.

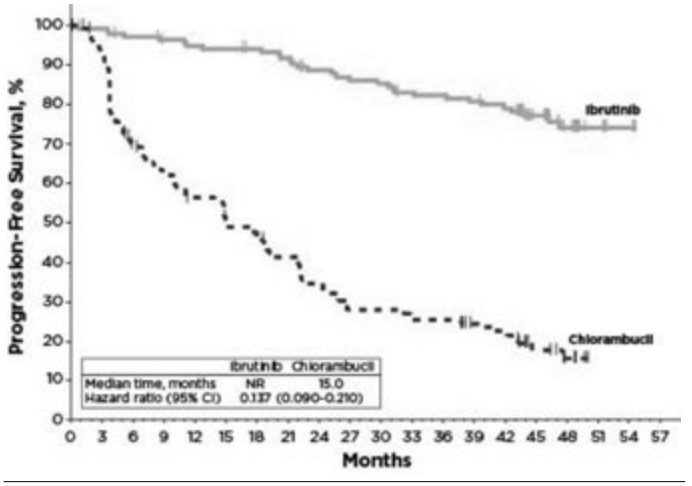

Figure 1.

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IMPACT OF TP53-MUTATED CLONE SIZE ON OUTCOME OF RELAPSED/REFRACTORY (R/R) CLL PATIENTS TREATED WITH VENETOCLAX PLUS RITUXIMAB WITHIN THE PHASE 3 MURANO STUDY

A.P. Kater[1,*], E.A. Punnoose[2], M. Andre[3], G. Cartron[4], B. Eichhorst[5], S. Grosicki[6], P. Hillmen[7], A. Illes[8], U. Jaeger[9], T.J. Kipps[10], H. Mocikova[11], M. Montillo[12], A. Tempescul[13], B. Chyla[14], M. Boyer[15], K. Humphrey[15], J. Wang[2], J. Wu[2], M. Mobasher[2], J.F. Seymour[16]

[1]Department of Hematology, Academic Medical Center Amsterdam, Amsterdam, Netherlands, [2]Genentech, Inc., South San Francisco, United States, [3]CHU UCL Namur, Yvoir, Belgium, [4]Department of Hematology, Centre Hospitalier Universitaire Montpellier, Montpellier, France, [5]University Hospital Cologne and the Center for Integrated Oncology Cologne-Bonn, Cologne, Germany, [6]Community of Hospitals Chorzow, Katowice, Poland, [7]St. James's University Hospital, Leeds, United Kingdom, [8]University of Debrecen, Debrecen, Hungary, [9]Dept. of Medicine I, Division of Hematology and Hemostaseology, Medical University of Vienna, Vienna, Austria, [10]University of California School of Medicine, San Diego, United States, [11]Charles University, Prague, Czech Republic, [12]Niguarda Cancer Center, Niguarda Hospital, Milan, Italy, [13]Centre Hospitalier Universitaire Brest, Brest, France, [14]AbbVie, North Chicago, United States, [15]F. Hoffmann–La Roche, Welwyn Garden City, United Kingdom, [16]Peter MacCallum Cancer Centre, Royal Melbourne Hospital, and University of Melbourne, Melbourne, Australia

Background: CLL pts with alterations in the *TP53* pathway (including del(17p) and *TP53* mutations) are a high-risk population in need of novel therapies. Retrospective studies suggest that pts with low clone size (subclones) of del(17p) and/or *TP53* mutations have similar poor survival as pts with high clone size following immunochemotherapy, but remains to be confirmed. We recently reported on improved efficacy with venetoclax plus rituximab (VenR) *vs* bendamustine plus rituximab (BR) in R/R CLL for ORR, PFS and MRD negativity (MRD–) (MURANO phase 3 study).
Aims: Assess the impact of clone size of *TP53* alterations on prognosis and MRD negativity with both regimens in MURANO.
Methods: *TP53* mutation status assessed centrally by targeted next-generation sequencing spanning exons 2–11 (entire *TP53* coding region). Cutoff was ≥5% allele frequencies (AF). del(17p) status was assessed centrally by Vysis CLL FISH probe kit, with cutoff of 7% del(17p) nuclei used to define abnormality. Low clone size *vs* high clone size was defined for del(17p) by 7%–≤20% del(17p) nuclei *vs* >20%; and *TP53* mutations by 5%–≤20% mutant AF *vs* >20% AF; definitions enabled subsets of adequate sample size to assess correlation with outcome.
Results: By central assessment 27% of pts had only del(17p), 26% only *TP53* mutations, and 13% del(17p) and *TP53* mutations; equally balanced between treatment arms. Among 92 pts with del(17p), clone size was highly variable (median=15%; range, 7.5%–94% of del(17p) nuclei). Among del(17p) pts, high del(17p) clone size (n=36, 10.5%) was associated with higher incidence of *TP53* mutations *vs* low clone size (69% *vs* 34%), and in pts treated with BR, high del(17p) clone size was also associated with inferior PFS. At median follow-up of 23.8 mo., median PFS for VenR *vs* BR was not reached (NR) *vs* 8 mo. (HR 0.1; 0.02–0.47) in high clone size del(17p) pts, NR *vs* 21 mo. (HR 0.14; 0.04–0.43) in low clone size, and NR *vs* 21 months (HR 0.19; 0.12–0.32) in non-deleted pts. MRD negativity in VenR *vs* BR was 60% *vs* 10% in high clone size del(17p) pts, 94% *vs* 28% in low clone size and 87% *vs* 26% in non-deleted pts. Similarly for *TP53* mutations, high clone size was associated with worse PFS *vs* low clone size in pts treated with BR. Patients with both del(17p) and *TP53* mutations, indicative of loss of TP53 on both alleles, had inferior outcomes to pts with either del(17p) or *TP53* mutation alone, with BR. Importantly, PFS was superior for VenR *vs* BR across subgroups with del(17p) and/or *TP53* mutations including high and low clone size (Table 1 and Figure 1). 2-year PFS rates between subsets by treatment arm will be presented.

Table 1.

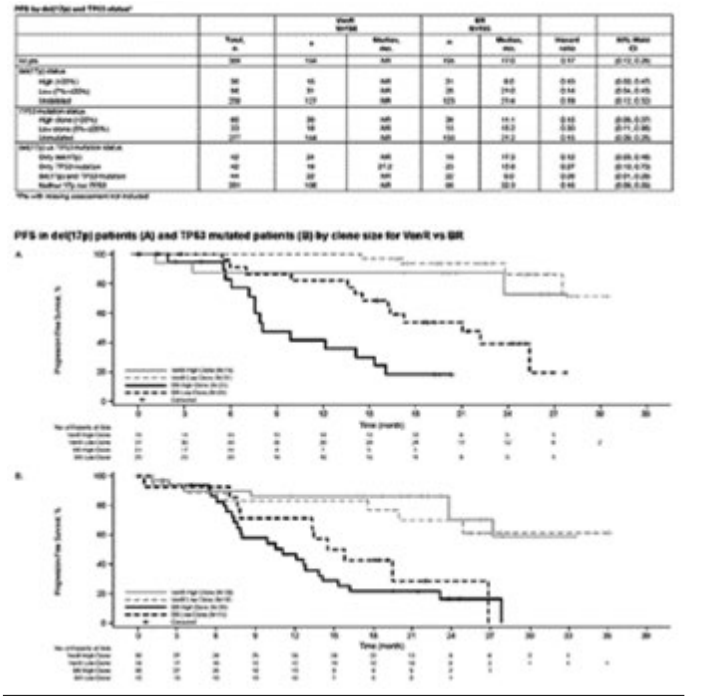

Figure 1.

Summary and Conclusions: A clear prognostic difference was found between low and high clone size with respect to chemoimmunotherapy. High-risk prognostication based on *TP53* alterations is minimized by treatment with VenR; VenR is superior to BR in these biological subsets.

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B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL) IN CLL PATIENTS TREATED WITH LENALIDOMIDE

M. Fürstenau[1,*], A.-M. Fink[1], C. Herling[1], C. Maurer[1], N. Kutsch[1], J. de la Serna[2], M. Coscia[3], R. Eckert[4], M. Crespo[5], F. Bosch[5], T. Noesslinger[6], P. Ghia[7], A. P. Kater[8], M. Ritgen[9], A. Schilhabel[9], S. Stilgenbauer[10], E. Tausch[10], K. Fischer[1], M. Hallek[1], B. Eichhorst[1]

[1]Department I of Internal Medicine and Center of Integrated Oncology Cologne-Bonn, German CLL Study Group, University of Cologne, Cologne, Germany, [2]University Hospital 12 De Octubre, Madrid, Spain, [3] Division of Hematology, A.O.U. Città della Salute e della Scienza, University of Torino, Torino, Italy, [4]Onkologische Schwerpunktpraxis Esslingen, Esslingen, Germany, [5]Department of Hematology, Vall d'Hebron Institute of Oncology, Vall d'Hebron University Hospital, Universitat Autònoma de Barcelona, Barcelona, Spain, [6]3rd Department for Hematology and Oncology, Hanusch Hospital, Vienna, Austria, [7]Universita Vita-Salute San Raffaele and IRCCS Instituto Scientifico San Raffaele, Milano, Italy, [8]Department of Hematology, Academic Medical Center Amsterdam, University of Amsterdam, on behalf of the HOVON CLL Working Group, Amsterdam, Netherlands, [9]2nd Department of Internal Medicine, University of Schleswig-Holstein, Campus Kiel, Kiel, [10]Department of Internal Medicine III, University of Ulm, Ulm, Germany

Background: The immunomodulatory drug lenalidomide has shown clinical activity in CLL. It has been associated with an increased rate of second pri-

mary malignancies (SPM) in multiple myeloma (MM). In MM degradation of transcription factors Ikaros and Aiolos leads to its anti-tumor activity and T-cell activation; in CLL, its mechanism of action has not been determined yet. In our CLLM1 trial, 3 of 56 pts developed precursor B-ALL during or after lenalidomide maintenance while there were no cases in the placebo group.
Aims: To evaluate an association between exposure to lenalidomide and the occurrence of B-ALL in subjects with CLL receiving lenalidomide maintenance.
Methods: First, we screened all phase III trials using lenalidomide in CLL patients for reported ALL cases. Second, we obtained reports from the FDA Adverse Event Reporting System (FAERS) to identify additional cases of B-ALL in CLL patients treated with lenalidomide. Next, we analysed available data for B-ALL cases in five non-lenalidomide GCLLSG trials in order to estimate the occurrence of B-ALL in CLL patients not exposed to lenalidomide. Finally, in the 2 BCR-ABL positive B-ALL cases identified in CLLM1, RQ-PCR was performed for detection of BCR-ABL fusion transcripts at different time points before B-ALL diagnosis; NGS-based clonality testing of IGHV will be done to assess clonal relationships of B-ALL and underlying CLL.
Results: In 3 published phase III trials (CLLM1, CONTINUUM, ORIGIN) that evaluated the use of lenalidomide in CLL, a total of 846 patients were enrolled, with 438 receiving lenalidomide monotherapy. Six out of 438 patients (1.4%) developed B-ALL either during treatment (n=1) or after discontinuation (n=5). No B-ALL cases were reported in the control groups including 408 patients. Of the 6 patients (3M, 3F), 2 received lenalidomide as firstline treatment and 4 as maintenance after firstline therapy, median length of exposure was 33,5 months (range 15-47) and median age at B-ALL diagnosis was 70 years (range 60-82). All evaluable patients were categorized as either 'high risk' or 'very high risk' according to CLL-IPI, 1 patient featuring a TP53 mutation, 1 patient with a 17p-deletion, all patients showed unmutated IGHV. In the two BCR-ABL positive patients, BCR-ABL fusion transcripts could not be detected at any time point before B-ALL diagnosis. Clonal relationship of B-ALL and underlying CLL is currently being analysed. In order to estimate the occurrence of B-ALL in CLL patients not exposed to lenalidomide, data of patients treated in GCLLSG trials were evaluated. In 2015 patients evaluable for second primary malignancies, 2 cases of B-ALL were identified (0.1% of all patients). Cytogenetic abnormalities were del(17p) in one patient and del(11q) in the other, both patients exhibited unmutated IGHV. This rate of B-ALL occurrence in CLL patients is consistent with findings of other groupsand underscores the assumption that secondary B-ALL is usually a rare event in CLL.
Summary and Conclusions: According to a large GCLLSG cohort and in accordance with published data on second primary malignancies in CLL, B-ALL is a rare event in CLL patients. In CLL patients treated with lenalidomide as firstline therapy or maintenance following chemotherapy, an increased number of B-ALL was observed, the reason for this increase is unknown. Further investigation of the evolution of different lymphoid populations during treatment is warranted to better understand the aetiology of secondary B-ALL in CLL.

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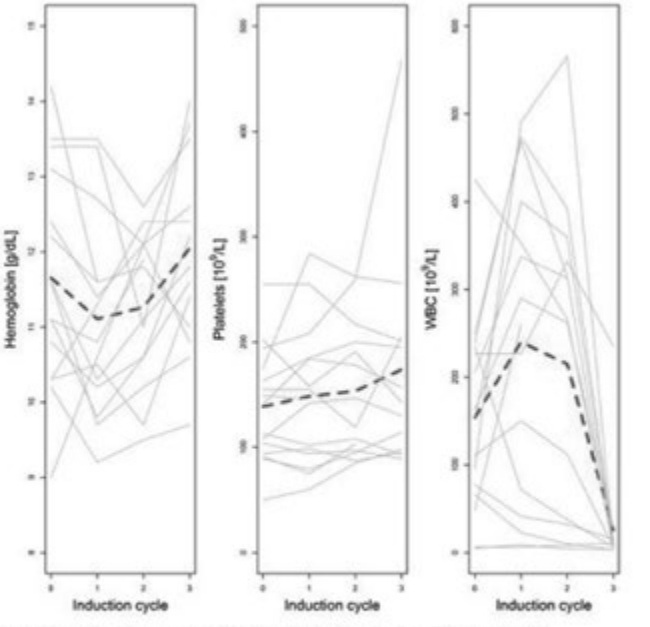
SAFETY ANALYSIS OF VENETOCLAX AND IBRUTINIB FOR PREVIOUSLY TREATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): FIRST INTERIM ANALYSIS FROM THE PHASE II VISION HO141 TRIAL

C.U. Niemann[1,*], M.-D. Levin[2], K. Nasserinejad[3], A.M. Janssens[4], L. Enggaard[5], S. Kersting[6], G.J. Veldhuis[7], R. Mous[8], C.H. Melink[9], J.A. Dobber[9], I. Schjødt[1], E. Dompeling[10], C.B. Poulsen[11], H.T. Tran[12], T. Salmi[13], M. Mattsson[14], A.P. Kater[9]

[1]Department of Hematology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark, [2]Department of Internal Medicine, Albert Schweitzer ziekenhuis, Dordrecht, [3]HOVON Data Center, Rotterdam, [4]UZ Leuven, Leuven, Netherlands, [5]Herlev Hospital, Copenhagen, Denmark, [6]HagaZiekenhuis, van Den Haag, [7]Antonius Ziekenhuis, Sneek, [8]Universitair Medisch Centrum Utrecht, Utrecht, [9]AMC, Amsterdam, [10]Isala, Zwolle, Netherlands, [11]Roskilde Sygehus, Roskilde, Denmark, [12]Akershus, Oslo, Norway, [13]Turku University Hospital, Turku, Finland, [14]Uppsala University Hospital, Uppsala, Sweden

Background: Standard of care for patients with relapsed or refractory CLL (RR CLL) is rapidly changing. For patients with late relapse >2-3 years from 1st-line, repeated fixed duration therapy with chemoimmunotherapy may be used, while patients with early relapse or TP53 aberrations are treated with novel targeted agents until toxicity or progression. Thus, chemotherapy-free regimens with the option to safely stop treatment are warranted.
Aims: The aim of the VISION / HOVON 141 study is to evaluate whether combination treatment with venetoclax+ibrutinib in patients with RR CLL can lead to minimal residual disease (MRD) negativity, allowing MRD-guided treatment cessation. We here report on a preplanned safety interim analysis.
Methods: Patients were treated with ibrutinib monotherapy (420 mg daily) for the first two cycles of 28 days, in cycle 3 venetoclax was ramped up weekly from 20 mg to the final dosage of 400 mg daily from start of cycle 4. All patients are treated for 15 cycles; MRD negative patients (blood and bone marrow, by central flowcytometry, 10^{-4}level) are randomized 1:2 between maintenance ibrutinib or observation thereafter, MRD positive patients continue ibrutinib monotherapy. Patients in the observation arm are assessed for blood MRD every three months; venetoclax+ibrutinib is reinitiated upon MRD >10^{-2}. Enrollment is ongoing, 62 of 207 planned patients enrolled. This interim analysis includes data for the first 3 induction cycles of the first 15 eligible patients in the study with focus on safety including the incidence of adverse events (AEs) of special interest (atrial fibrillation, bleeding and tumor lysis) and initial response evaluation. Only grade 2 and above AEs were reported as per protocol. Registered at clinicaltrials.gov: NCT03226301.
Results: The median age for the first 15 patients was 59 years (range 40-79), eight (53%) were male, 11 (73%) showed WHO performance status 0, 14 (93%) were classified as Binet stage B/C, five (33%) as high and nine (60%) as medium risk of tumor lysis (one unknown), six (40%) had centrally assessed TP53 aberrations and nine (60%) were IGHV unmutated (one unknown). All patients completed the first two cycles of ibrutinib monotherapy and the third cycle of ibrutinib combined with venetoclax ramp up. Three and two patients had dose modifications of ibrutinib and venetoclax, respectively, no patients stopped treatment. Three (20%) patients experienced grade 2 AEs, three (20%) grade 3 and two (13%) grade 4 AEs, no grade 5 AEs were reported. Two (13%) patients experienced an SAE during ibrutinib monotherapy (febrile neutropenia, adenocarcinoma of the lung) while no SAEs were reported during cycle 3 venetoclax ramp up. No tumor lysis, atrial fibrillation or bleeding events reported. Eight (53%) patients achieved a clinical complete remission (CR) and six (40%) patients achieved a partial remission (PR) while one (7%) achieved PR with lymphocytosis as assessed by the local investigator at end of cycle 3 (the day before the ramp up to 400 mg venetoclax in the 4th cycle). Improvement in median hemoglobin and platelet count were seen while the median white blood cell count decreased from 260 to 8 x 10-E9 after an initial ibrutinib induced lymphocytosis. (Figure 1).



Preferred term for AEs reported for the first 15 patients within cycle 1-3:
(more than one type of AE per patient, each patient counts only once for -Any)

	grade 2	grade 3	grade 4
-Any	3 20%	3 20%	1 7%
General disorders and administration site conditions	3 20%	.	.
Respiratory, thoracic and mediastinal disorders	2 13%	1 7%	.
Infections and infestations	2 13%	.	.
Investigations	.	1 7%	1 7%
Musculoskeletal and connective tissue disorders	2 13%	.	.
Blood and lymphatic system disorders	.	1 7%	.
Hepatobiliary disorders	.	1 7%	.

Figure 1.

Summary and Conclusions: Treatment with ibrutinib and ramp up with venetoclax in the setting of RR CLL was manageable without any unexpected AEs; all patients responded, 53% had a clinical complete response within the first three cycles of treatment, before reaching the final dosage of venetoclax. The DSMB recommends continuing of the study.

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EFFECT OF ADDING IDELALISIB TO FRONTLINE OFATUMUMAB PLUS EITHER CHLORAMBUCIL OR BENDAMUSTINE IN LESS FIT PATIENTS WITH CLL: UPDATED RESULTS FROM THE NCRI RIALTO TRIAL

A. Pettitt[1,*], N. Kalakonda[1], F. Polydoros[2], M. Bickerstaff[2], G. Menon[3], S. Coupland [4], M. Oates[5], K. Lin[4], C. Pocock[6], S. Jenkins[7], A. Schuh [8], F. Wandroo[9], S. Rassam[10], A. Duncombe[11], P. Cervi[12], S. Paneesha[13], M. Aldouri[14], C. Fox[15], C. Knechtli[16], M. Hamblin[17], D. Turner[18], P. Hillmen[19]

[1] Department of Molecular and Clinical Cancer Medicine, [2]University of Liverpool /Liverpool Cancer Trials Unit, [3]Haemato-Oncology Diagnostic Service, [4]Department of Blood Sciences, Liverpool Clinical Laboratories, [5] Department of Molecular and Clinical Cancer Medicine, University of Liverpool, Liverpool, [6]Department of Haematology, Kent & Canterbury Hospital, Canterbury, [7]Haematology Unit, Russells Hall Hospital, Dudley, [8]Department of Oncology, University of Oxford, Oxford, [9]Department of Haematology, Sandwell Hospital, Birmingham, [10]Department of Haematology, Maidstone Hospital, Maidstone, [11]Department of Haematology, University Hospital Southampton, Southampton, [12]Department of Haematology & Blood Transfusion, Southend Hospital, Westcliff-on-Sea, [13]Department of Haematology, Heartlands Hospital, Birmingham, [14]Department of Haematology, Medway Maritime Hospital, Gillingham, [15]Department of Clinical Haematology, Nottingham University Hospitals NHS Trust, Nottingham, [16]Department of Clinical Haematology, Royal United Hospital, Bath, [17]Department of Haematology, Colchester General Hospital, Colchester, [18]Oncology Unit, Torbay Hospital, Torquay, [19]Faculty of Medicine and Health, University of Leeds, Leeds, United Kingdom

Background: The Phase 3 RIAltO trial opened in December 2011 to compare ofatumumab plus chlorambucil (O+C) with ofatumumab plus bendamustine (O+B) in patients with previously untreated chronic lymphocytic leukaemia (CLL) considered unfit for FCR (fludarabine, cyclophosphamide, rituximab). A protocol amendment was introduced in September 2014 to investigate the addition of idelalisib (first-in-class inhibitor of the p110δ isoform of phosphoinositol-3 kinase) or placebo. Review of safety data in January 2016 revealed excessive toxicity due to idelalisib, and recruitment was suspended pending the implementation of additional safety measures including mandatory antimicrobial prophylaxis and CMV surveillance. However, all idelalisib/placebo treatment was withdrawn from the trial in March 2016 following safety analysis of idelalisib registration studies and recommendations from Gilead Sciences Ltd and regulatory authorities.
Aims: Here, we present an updated ad-hoc analysis of the cohort of patients in RIAltO who received idelalisib or placebo.
Methods: Patients were eligible for inclusion if they had previously untreated CLL requiring treatment by NCI/IWCLL criteria, were considered unfit for FCR and did not have any contraindications to the study drugs. Consenting patients underwent an unblinded 1:1 randomisation to ofatumumab (300mg iv day 1 and 1000mg iv day 8 of cycle 1; 1000mg iv day 1 of cycle 2 onwards) plus either chlorambucil (10mg/m^2 day 1-7, repeated every 28 days for 3-12 cycles) or bendamustine (70mg/m^2 iv day 1-2 for 3-6 cycles) and a double-blinded 1:1 randomisation to concurrently administered placebo or idelalisib (150mg bd for up to 3 years). Co-trimoxazole prophylaxis was recommended. Study drugs were discontinued in the event of disease progression or unacceptable toxicity. The primary endpoint was progression-free survival (PFS). The mandatory post-treatment reporting period for serious adverse events (SAEs) was 6 months for grade ≥3 infections and 28 days for other events.
Results: 145 patients received idelalisib (73) or placebo (72), with a median idelalisib exposure time of 2.5 months (IQR 1.7-5.5 months). The two arms were well balanced for age, gender, stage, co-morbidity, performance status and chemotherapy allocation. As of January 2018, SAEs were reported in 80% of idelalisib-treated patients (84 grade 3-4 and 7 grade 5) compared to 47% of the placebo arm (35 grade 3-4 and 3 grade 5). The frequency of SAEs in the idelalisib arm was similar in both chemotherapy arms. Grade 5 events in this arm were sepsis (1), lung infection (2), febrile neutropenia (2), myocardial infarction (1) and sudden death NOS (1). After a median follow-up of 25 months (range 0.1-38.1 months), 15 PFS events have been reported in the idelalisib arm compared with 28 in the placebo arm (P=0.061, log-rank test). Regarding OS, 7 deaths have been observed in the idelalisib arm compared to 15 in the placebo arm (P=0.093, log-rank test). 0 and 11 deaths in the respective arms occurred beyond 6 months, while 0 and 10 deaths were associated with CLL progression (Figure 1).
Summary and Conclusions: In less fit patients with CLL, the addition of idelalisib to frontline O+C or O+B resulted in an increased rate of early grade 3-5 toxicity, much of it due to infection and febrile neutropenia. However, despite a median drug exposure time of only 2.5 months, there was a non-significant trend for superior PFS and OS in the idelalisib arm. Longer follow-up is required to establish whether the efficacy of frontline O+C/B is improved by brief co-administration of idelalisib.

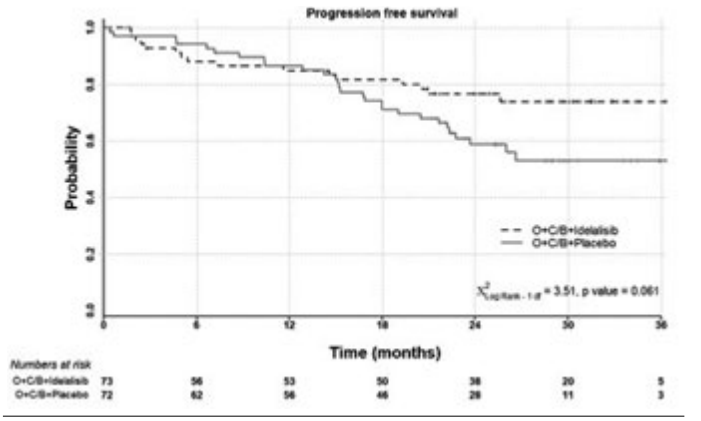

Figure 1.

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OBINUTUZUMAB PRE-INDUCTION ABROGATES HIGH TUMOR LYSIS RISK OF VENETOCLAX IN TREATMENT NAÏVE FCR-UNFIT PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA: A PLANNED INTERIM ANALYSIS

M.-D. Levin[1,*], S. Kersting[2], Y. van Norden[3], J. Dobber[4], C. Mellink[5], L. Evers[4], F. Croon - de Boer[6], J. Schreurs[7], E. van der Spek[8], H. Visser[9], C. Idink[10], S. Wittebol[11], M. Hoogendoorn[12], S. Tonino[13], A.P. Kater[13]

[1]Internal Medicine, Albert Schweitzer hospital, Dordrecht, [2]Internal Medicine, HAGA hospital, the Hague, [3]Statistics, Erasmus MC Institute HOVON data centre, Rotterdam, [4]Laboratory special hematology, [5]Laboratory of clinical genetics, Academic Medical Center, Amsterdam, [6]Internal Medicine, Ikazia hospital, Rotterdam, [7]Internal Medicine, Martini hospital, Groningen, [8]Internal Medicine, Rijnstate hospital, Arnhem, [9]Internal Medicine, Northwest Clinics, Alkmaar, [10]Internal Medicine, ZorgSaam hospital, Terneuzen, [11]Internal Medicine, Gelderland Valley hospital, Ede, [12]Internal Medicine, Medical Center Leeuwarden, Leeuwarden, [13]Hematology, Academic Medical Center, Amsterdam, Netherlands

Background: Combination of chlorambucil and obinutuzumab is currently the most effective treatment for CLL patients unfit for fludarabine or bendamustine containing regimens with 1/3 of patients reaching peripheral blood (PB) minimal residual disease negativity (MRD-). Early data on venetoclax containing regimens show promising results with deep remissions but are hampered by risk of tumor lysis syndrome (TLS). Whether optimal duration of venetoclax treatment can be guided by MRD- is currently unknown. We perform a randomized phase 2 study to address 1) whether TLS-risk can be mitigated in an unfit population by introducing pre-induction and 2) whether MRD-guided duration of venetoclax treatment is a feasible and efficacious approach.
Aims: Here we report on a planned interim safety analysis (especially TLS risk before and after obinutuzumab pre-induction) and MRD data of the first 30 patients in this HOVON 139 / GIVE trial.
Methods: Treatment regimen is as follows: Pre-induction (Figure 1): 2 cycles of obinutuzumab monotherapy (C1 d1:100 mg, d2: 900 mg, d8:1000 mg, d15: 1000mg and C2 d1: 1000 mg); Induction I: 6 cycles obinutuzumab (C3-8 d1: 1000 mg) in combination with venetoclax (C3: ramp-up weekly 20-50-100-200mg and 400mg daily thereafter); Induction II: 6 cycles venetoclax monotherapy (C9-14: 400 mg daily); MRD-guided maintenance: randomization of patients with at least partial remission (PR) after induction II: 12 additional cycles of venetoclax irrespective of MRD or venetoclax maintenance only in MRD+ patients. All cycles are 28 days. MRD–: <1 CLL cell/10^4 leukocytes (L) by flowcytometry. TLS risk groups according to Roberts.
Results: Until the 29th of January 2018, 46 patients were included in this trial. Thirty patients were followed for at least 3 cycles (median age 70, range 57-79 years) and are reported here, of whom 73% was male, 47% had RAI stage ≥3 and median CIRS-score was 3 (range 0-16). IGHV was mutated in 13 (43%) and unmutated in 13 (43%) patients (non-conclusive in 4 (13%). Deletion 17p was found in 3 (10%), 7 (24%) patients showed a TP53-mutation (≥10%) and 9 (30%) had complex karyotype. Baseline TLS risk scores are depicted in Table 1. Downgrading of TLS risk occurred in 25 patients (83%): 3 from high to medium, 3 from high to low and 19 from medium to low risk. Laboratory TLS grade 1 occurred in 4 times in 3

patients: 2 times during pre-induction with obinutuzumab, both with medium TLS risk and 2 times during venetoclax ramp-up with low TLS risk after pre-induction, of whom 1 with high and 1 with medium TLS risk at baseline. All TLS resolved with protocolled hydration, allopurinol +/- rasburicase. During pre-induction 9 patients (30%) experienced grade 3-4 toxicities, but none in the second pre-induction cycle. The intended dose of 3000 mg obinutuzumab in the first pre-induction cycle was reached in 28 patients (93%). During the first induction cycle 3 patients (10%) developed grade 3-4 toxicity. In addition, all patients received the total intended dose of venetoclax ramp-up. First PB MRD data showed 21 of 25 patients (84%) MRD-, 3 (12%) MRD intermediate (10^{-4}–$<10^{-2}$) and 1 (4%) MRD+ after 6 cycles of induction treatment and 4 of 4 (100%) MRD- after 12 cycles of induction treatment (see Table 2).

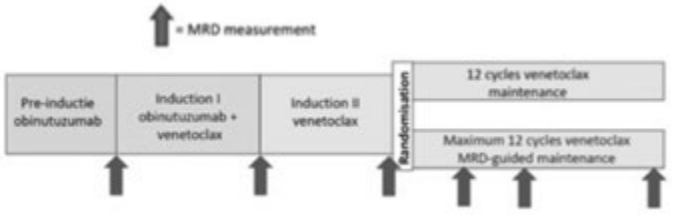

Figure 1.

Table 1.

TLS risk (%)	Baseline (n=30, %)	After pre-induction (n=28, %)*
Low	2 (7%)	24 (80%)
Medium	22 (73%)	4 (13%)
High	6 (20%)	0 (0%)

*Unknown in 2 patients

Table 2.

PB MRD	After 6 cycles induction (n=25, %)*	After 12 cycles induction (%) (n=4)**
$<10^{-4}$:	21 (84%)	4 (100%)
10^{-4}–$<10^{-2}$:	3 (12%)	0
$\geq 10^{-2}$:	1 (4%)	0

*in patients 5 MRD assessment after 6 cycles was not (yet) done
**only 4 patients complete 12 cycles of induction treatment

Summary and Conclusions: Obinutuzumab pre-induction is well tolerated in elderly patients and results in abrogating high TLS risk in all patients. MRD-negativity is seen in 84% of patients after 6 cycles and in all patients reaching 12 cycles of induction treatment with obinutuzumab and venetoclax.

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CONTINUING REMISSIONS AFTER VENETOCLAX AND OBINUTUZUMAB IN PATIENTS WITH PREVIOUSLY UNTREATED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AND COEXISTING MEDICAL CONDITIONS

K. Fischer[1,*], O. Al-Sawaf[1], A.-M. Fink[1], M. Dixon[2], S. Robrecht[1], J. Bahlo[1], M. Tandon[2], S. Warburton[2], T.J. Kipps[3], R. Weinkove[45], S. Robinson[6], M. Dreyling[7], S. Opat[8], C. Owen[9], J. López[10], R. Humerickhouse[11], C.-M. Wendtner[1,12], K.-A. Kreuzer[1], A.W. Langerak[13], J.J. van Dongen[13], M. Ritgen[14], S. Boettcher[14,15], V. Goede[1], B. Eichhorst[1], S. Stilgenbauer[16], M. Mobasher[17], M. Hallek[1]

[1]Department I of Internal Medicine, Center of Integrated Oncology Cologne-Bonn, University Hospital Cologne, Cologne, Germany, [2]F. Hoffmann-La Roche Ltd, Welwyn Garden City, United Kingdom, [3]Moores Cancer Center, University of California San Diego, San Diego, United States, [4]Wellington Blood and Cancer Centre, Capital and Coast District Health Board, [5]Malaghan Institute of Medical Research, Wellington, New Zealand, [6]Queen Elizabeth II Health Science Center, Halifax, Canada, [7]Department of Internal Medicine III, University Hospital Ludwig-Maximilians University Munich, Munich, Germany, [8]Monash Hematology, Monash University, Clayton, Australia, [9]Department of Hematology, Tom Baker Cancer Centre, Calgary, Canada, [10]Hospital Ramon y Cajal, Madrid, Spain, [11]AbbVie, Inc., North Chicago, United States, [12]Department of Hematology, Oncology, Immunology, Palliative Care, Infectious Diseases and Tropical Medicine, Klinikum Schwabing, Munich, Germany, [13]Department of Immunology, Laboratory for Medical Immunology, Erasmus Medical Center, Rotterdam, Netherlands, [14]Second Department of Medicine, University Hospital of Schleswig-Holstein, Campus Kiel, Kiel, [15]Department III of Internal Medicine, University Hospital Rostock, Rostock, [16]Department of Internal Medicine III, Ulm University, Ulm, Germany, [17]Genentech, Inc., South San Francisco, United States

Background: The combination of venetoclax and obinutuzumab was reported to achieve excellent responses regarding overall responses rates (ORR), complete responses (CR) and MRD-negativitiy with manageable toxicity in previously untreated patients with chronic lymphocytic leukemia (CLL).
Aims: The CLL14 trial is a prospective, open-label, multicenter, randomized phase-III trial to compare the efficacy and safety of obinutuzumab and venetoclax with obinutuzumab and chlorambucil in patients with previously untreated CLL and coexisting medical conditions. Prior to the randomized phase, a safety run-in phase was conducted. Here we present long-term follow-up results for safety and efficacy for patients treated within the run-in phase.
Methods: Thirteen previously untreated patients with confirmed CLL and with coexisting medical conditions assessed by cumulative illness rating scale (CIRS) total score >6 and/or estimated creatinine clearance (CrCl) <70 mL/min requiring treatment according to iwCLL criteria were enrolled. Treatment consisted of 6 cycles obinutuzumab and venetoclax followed by 6 cycles of venetoclax. Risk assessment for tumor lysis syndrome (TLS) was performed before treatment. Adverse events were graded per NCI CTCAE v.4. MRD in peripheral blood was assessed by ASO-PCR, flow cytometry and NGS. Progression free survival (PFS) was defined as the time between enrollment and first disease progression or death and overall survival (OS) as the time between enrollment and death.
Results: Baseline characteristics, acute toxicity and ORR of 100% three months after the end of treatment, were previously reported. As of this analysis, 12 patients had completed treatment and had at least 18 months of follow-up. One patient discontinued treatment early due to a grade 4 infusion-related reaction following the first infusion of obinutuzumab. Median observation time was 29.6 months (range 20.7-35.0). A total of 264 adverse events were reported for all 12 patients that had received obinutuzumab and venetoclax. Of these, 226 occurred during the treatment period, 27 during the first and 11 during the second year of follow-up period. No prolonged neutropenia was observed. A total of 35 infections were reported. One basal cell carcinoma, but no further secondary malignancies were reported. A 90 year old patient who was in CR died of cardiac failure nine months after the end of treatment, which was assessed as non-related by the investigator. Median PFS and OS have not been reached. At month 30, 80.2% of the patients were progression-free and 92.3% were alive (Figure 1A,B). At follow-up month 3, 11 of 12 treated patients had no detectable (<10-4) minimal residual disease by ASO-PCR in peripheral blood and one patient was assessed positive (≥10-4). At follow-up month 18, seven patients remained MRD negative and four patients were assessed positive. MRD results assessed by flow cytometry are shown in Figure 1C.

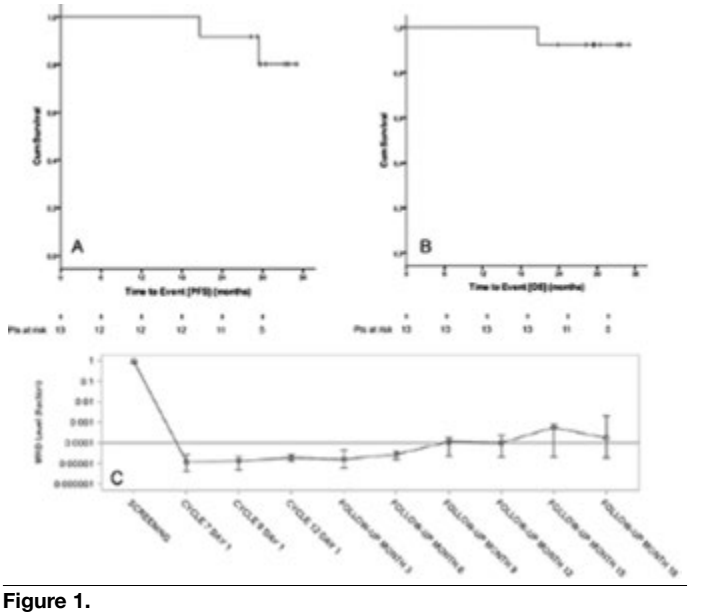

Figure 1.

Summary and Conclusions: The combination of venetoclax and obinutuzumab in previously untreated patients with CLL appears to induce long-lasting remissions. The trial population consists of 13 elderly patients (median age 75 years) with clinically meaningful comorbidities in addition to CLL including 2 patients with deletion 17p. After the fixed treatment duration of 12 cycles and with median follow-up of 30 months, 80.2% of patients are progression-free and 12 patients are still alive.

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TWO-COHORT, PHASE II STUDY IN R/R CLL (COSMOS): FIRST PRELIMINARY SAFETY AND EFFICACY RESULTS OF MOR208 TREATMENT IN COMBINATION WITH IDELALISIB IN PATIENTS WHO DISCONTINUED PRIOR IBRUTINIB THERAPY

W. Jurczak[1,*], R. Greil[2], A. Hellmann[3], M. Dirnberger-Hertweck[4], P. Kelemen[4], J. Weirather[4], J.M. Middeke[5], M. Montillo[6], M. Motta[7], P. Neumeister[8], S. Stilgenbauer[9], C.-M. Wendtner[10], J.A. Woyach[11], J. Schetelig[5]

[1]Department of Hematology, Jagiellonian University, Kraków, Poland, [2]Department of Internal Medicine III with Hematology, Medical Oncology, Hemostaseology, Infectious Diseases, Rheumatology, Oncologic Center, Paracelsus Medical University Salzburg, Salzburg Cancer Research Institute and Cancer Cluster, Salzburg, Austria, [3]Haematology and Transplantology, Gdanski Uniwersytet Medyczny, Gdansk, Poland, [4]Morphosys AG, Planegg, [5]Medizinische Klinik und Poliklinik I, TU Dresden, Dresden, Germany, [6]Niguarda Cancer Center, Niguarda Hospital Milan, Milan, [7]S.C. Ematologia, ASST Spedali Civili, Brescia, Italy, [8]Division of Hematology, Medical University of Graz, Graz, Austria, [9]Department of Internal Medicine III, Ulm University, Ulm, [10]Klinikum Schwabing, Department I of Medicine, Academic Teaching Hospital of University of Munich, Munich, Germany, [11]Division of Hematology, Department of Internal Medicine, The Ohio State University Comprehensive Cancer Center, Columbus, OH, United States

Background: Patients (pts) with relapsed or refractory (R/R) chronic lymphocytic leukaemia (CLL) who failed treatment with Bruton's Tyrosine Kinase inhibitor (BTKi) ibrutinib have a particularly dismal prognosis. A previous phase I study showed that the Fc-enhanced, humanized, anti-CD19 antibody MOR208 was well tolerated with encouraging single-agent activity in pts with R/R CLL. In preclinical studies, MOR208 showed synergistic potential in combination with idelalisib (IDE, PI3Kδ inhibitor) and venetoclax (VEN, BCL-2 inhibitor).
Aims: This ongoing phase II study assesses the safety and preliminary efficacy of MOR208+IDE (Cohort A) or MOR208+VEN (Cohort B) in pts with R/R CLL previously treated with a BTKi. Here we present the first results of Cohort A.
Methods: Adult pts with R/R CLL without transformation or Richter's syndrome, who progressed on BTKi therapy or were intolerant to a BTKi during last prior therapy, were eligible in case of having an ECOG status 0–2 and adequate organ function. In Cohort A pts were treated until progression or for up to 24 cycles (C; each C consists of 28 days) with MOR208 administered intravenously at a dose of 12 mg/kg body weight, weekly during C1-3 (with equivalent loading dose on day 4 of C1), every other week in C4-6, monthly in C7-24 and with IDE continuously administered orally at 150 mg BID. Primary endpoint is the incidence and severity of adverse events (AEs), secondary endpoints include overall response rate (ORR) as per IWCLL 2008.

Table 1.

Treatment Emergent Adverse Events in Cohort A

Preferred Term	n (%) , N=11			
	TEAEs (22 pts)		SAEs (Any)	
	All Grades	Grade ≥3	All Grades	Grade ≥3
Hematological				
Neutropenia	5 (45)	4 (36)	0	0
Anaemia	5 (45)	3 (27)	1 (9)	1 (9)
Thrombocytopenia	2 (18)	2 (18)	1 (9)	1 (9)
Pancytopenia	n.a	n.a	1 (9)	0
Non-Hematological				
Dyspnea	5 (45)	1 (9)	0	0
Pyrexia	5 (45)	0	0	0
Infusion related reaction	4 (36)	0	0	0
Cough	3 (27)	0	0	0
Amylase increased	2 (18)	2 (18)	0	0
ALT increased	2 (18)	1 (9)	0	0
Bronchitis	2 (18)	1 (9)	1 (9)	1 (9)
Hypertension	2 (18)	1 (9)	0	0
Cytomegalovirus infection	2 (18)	0	0	0
Urinary tract infection	2 (18)	0	0	0
CRP increased	2 (18)	0	1 (9)	0
Headache	2 (18)	0	0	0
General physical health deterioration	2 (18)	0	0	0
Pancreatitis acute*	n.a	n.a	1 (9)	1 (9)
Pulmonary sepsis	n.a	n.a	1 (9)	1 (9)
Upper respiratory tract infection	n.a	n.a	1 (9)	1 (9)
Overdose	n.a	n.a	1 (9)	0
Medication error	n.a	n.a	1 (9)	0

Abbreviation: n.a= not applicable. Data cut-off 29-Jan-2018.
*As pancreatitis may be an immune-mediated AE caused by IDE, the study's Independent Data Monitoring Committee recommended early use of steroids in pts suspicious of developing pancreatitis based on laboratory results.

Results: Eleven pts had completed at least 1 cycle of study treatment in Cohort A. Median age: 69 years (51-79); Female 45%; Rai stage ≥3: 36%; Binet stage B/C: 28%/36%; ECOG 0/1: 55%/45%. Nine pts (82%) discontinued prior BTKi treatment due to progressive disease and 2 pts (18%) due to toxicity. The median number of prior treatment lines including ibrutinib was 5 (2-9). Pts experienced prior FCR chemoimmunotherapy (73%), anti-CD20 mAb (100%) and allogeneic/autologous stem cell transplantation (SCT) (9%/18%). Table 1. summarizes treatment-emergent adverse events (TEAEs). Ten treatment-emergent serious adverse events (SAEs) were reported in 5 pts (45%), none was fatal. All 5 pts recovered, 1 with sequelae after acute pancreatitis. Two pts (18%) permanently discontinued MOR208+IDE due to TEAE (Pt#1: amylase increased Gr4, lipase increased Gr4 and pancreatitis acute Gr4; Pt#2: transaminase increased Gr4). With a median observation time of 4.2 months, investigator assessed ORR was 82%: 1 CR (9%) confirmed by bone marrow biopsy and 8 PR (73%). Of note, 1 patient with "a very good PR" was taken off the study and received allogeneic SCT. Two pts had progressive disease, 1 of them resulted in fatal cardiorespiratory failure. Two patients who discontinued prior BTKi treatment due to toxicity reached PR, 1 of them discontinued study treatment due to AE. Treatment of 6 pts is ongoing.
Summary and Conclusions: The novel combination treatment of MOR208 with idelalisib showed acceptable safety and tolerability as well as promising antitumor activity in heavily pre-treated pts with R/R CLL who failed prior treatment with ibrutinib.

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REAL-WORLD RESULTS ON IBRUTINIB IN RELAPSED/REFRACTORY CLL: 30-MONTH FOLLOW-UP OF 95 SWEDISH PATIENTS TREATED IN A COMPASSIONATE USE PROGRAM

M. Winqvist[1,*], P.-O. Andersson[2], A. Asklid[3], K. Karlsson[4], C. Karlsson[1], B. Lauri[5], J. Lundin[1], M. Mattsson[6], S. Norin[7], A. Sandstedt[8], R. Rosenquist[9], F. Späth[10], L. Hansson[1], A. Österborg[1]

[1]Department of Hematology, Karolinska University Hospital, Stockholm, [2]Department of Hematology, Borås Hospital, Borås, [3]Department of Oncology, Karolinska University Hospital, Stockholm, [4]Department of Hematology, Lund University Hospital, Lund, [5]Department of Hematology, Sunderbyn Hospital, Sunderbyn-Luleå, [6]Department of Hematology, Uppsala University Hospital, Uppsala, [7]Department of Internal Medicine, Karolinska Institutet, Stockholm, [8]Department of Hematology, Linköping University Hospital, Linköping, [9]Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, [10]Department of Radiation Sciences, Oncology, Norrland University Hospital, Umeå, Sweden

Background: Ibrutinib is standard of care for patients with chemoimmunotherapy-refractory CLL. There is often a discrepancy between data obtained in pivotal clinical trials with strict inclusion criteria and those later obtained in routine health care. We have previously reported the 10-month follow-up of real-world results on ibrutinib in consecutive Swedish CLL patients treated in a compassionate use program (CUP) (Winqvist *et al.*, Haematologica 2016).
Aims: This 30-month follow-up reports long-term PFS, OS and safety as well as the impact of inclusion/exclusion criteria (obtained from RESONATE trial, Byrd *et al.*, NEJM 2014) on the outcome of patients treated in the Swedish CUP.
Methods: All patients treated in the CUP (n=95) were identified for long-term follow-up and in-depth analyses of their files were performed. All patients started at 420 mg/d.
Results: Median age was 69 years, 63% had del(17p)/*TP53* mutation, 27% had ECOG PS grade 2-3 and median no. of prior therapies was 3. Median treatment duration was 27 months (range 0.6-38) and 51% remained on ibrutinib at follow-up. ORR was 84% (CR not evaluable). Median PFS and OS were not yet reached. At a median follow-up time of 30 months (range 1-38) the OS rate was 63% and the PFS rate was 52% (Figure 1A and B). We analyzed the impact of the inclusion/exclusion criteria obtained from the RESONATE pivotal study (Byrd 2014): 44% of CUP patients had at least one exclusion criteria, supporting real-world representativity of our cohort. OS was significantly ($p<0.05$) shorter for CUP patients not matching these criteria (Figure 1C), there was no difference in PFS. 51% of patients had a grade 3-4 infection; 22% pneumonia, 13% febrile neutropenia/septicemia and 31% other infections. 13% had grade 3-5 opportunistic infections. 41% and 20% had grade 3-4 neutropenia or thrombocytopenia. Richter transformation (RT) occurred in 12 patients (13%) after a median time of 14 months (range 4-36); no clear-cut RT plateau was yet observed. 37% of the patients had died due to RT (n=12), CLL progression (n=6), second malignancy (n=5), infection (n=7), miscellaneous toxicity (n=4) and sudden death (n=1). Ibrutinib was permanently stopped in 47 patients (49%). The most common reasons were toxicity (n=18), RT (n=11), second malignancy (n=3), allogeneic SCT (n=4) and CLL progression (n=6). The drop-out rate of 49% at 30 month follow-up in our study is in contrast to the 42% discontinuation rate at 17 months follow-up in the recent real-world report by Mato et al (Haematologica 2018).

Four of 6 patients who progressed while on ibrutinib were tested for BTK mutations at PD; all carried a C481S mutation in >50% of the cells (Sanger sequencing); baseline tests (n=2) were negative. 19 patients had totally 21 treatment breaks >14 d (median 22d). In contrast to others (Barr *et al.*, Blood 2017, UK CLL Forum, Haematologica 2016) we found no impact on PFS or OS (Figure 1D). In contrast to our 10-month report (Winqvist 2016), the negative impact of del(17p) was not significant, but del(17p) patients who had ≥3 previous therapies had a short OS on ibrutinib (median 20.7 months). In multivariate analyses OS was associated with number of prior therapies and PFS was associated with baseline comorbidities (CIRS).

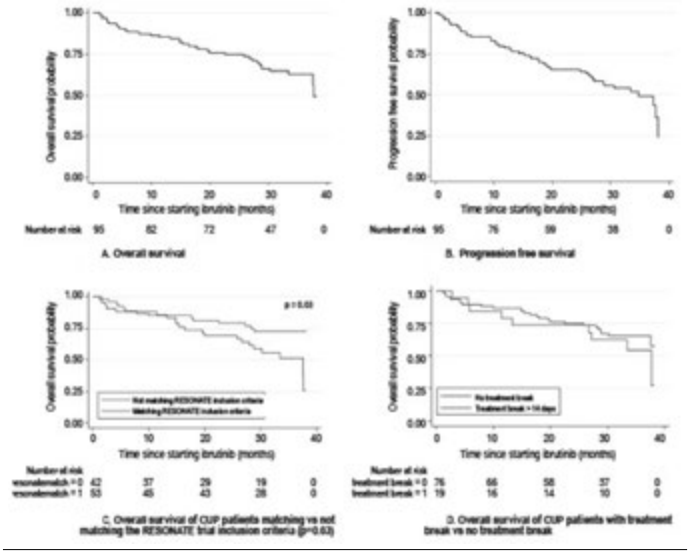

Figure 1.

Summary and Conclusions: The 30-month follow-up of the Swedish real-world cohort showed that 51% of CLL patients remained on ibrutinib. The impact of pivotal trial inclusion/exclusion criteria on the effects of new drugs in routine health care merits further investigations.

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ANALYSIS OF SUSTAINED HEMATOLOGIC IMPROVEMENT WITH IBRUTINIB TREATMENT IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LEUKEMIA

W. Wierda[1,*], J. Byrd[2], A. Chanan-Khan[3], J. Burger[1], P. Cramer[4], A. Tedeschi[5], J. Brown[6], P. Barr[7], S. O'Brien[8], F. Demirkan[9], J. Barrientos[10], G. Fraser[11], T. Robak[12], C. Phelps[13], A. Howes[13], C. Zhou[14], J. Dean[14], D. James[14], P. Hillmen[15], T. Kipps[16], M. Hallek[4]

[1]University of Texas MD Anderson Cancer Center, Houston, TX, United States, [2]The Ohio State University Comprehensive Cancer Center, Columbus, OH, United States, [3]Mayo Clinic Cancer Center, Jacksonville, FL, United States, [4]University of Cologne, German CLL Study Group, Cologne, Germany, [5]ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy, [6]Chronic Lymphocytic Leukemia Center, Dana-Farber Cancer Institute, Boston, MA, United States, [7]Wilmot Cancer Institute, University of Rochester Cancer Center, Rochester, NY, United States, [8]University of California Irvine, Chao Family Comprehensive Cancer Center, Orange, CA, United States, [9]Dokuz Eylul University, Izmir, Turkey, [10]Hofstra Northwell School of Medicine, Hempstead, NY, United States, [11]Juravinski Cancer Centre, McMaster University, Hamilton, ON, Canada, [12]Medical University of Lodz, Lodz, Poland, [13]Janssen Research & Development, LLC, Raritan, NJ, United States, [14]Pharmacyclics, LLC, an AbbVie Company, Sunnyvale, CA, United States, [15]The Leeds Teaching Hospitals, St. James Institute of Oncology, Leeds, United Kingdom, [16]University of California San Diego, Moores Cancer Center, La Jolla, CA, United States

Background: Ibrutinib (ibr), a first-in-class, once-daily oral inhibitor of Bruton's tyrosine kinase. Ibrutinib as a single agent is indicated for treatment of patients (pts) with chronic lymphocytic leukemia (CLL) by EMEA and for CLL/small lymphocytic leukemia (SLL) by US FDA and allows for treatment without chemotherapy. Superior efficacy of ibr over comparator drugs was demonstrated in clinical studies with ibr in CLL/SLL (Byrd, *NEJM*, 2014; Burger, *NEJM*, 2015; Chanan-Khan, *Lancet Oncol*, 2016).
Aims: To assess sustained hematologic improvement (SHI) with a side-by-side presentation of three phase 3 clinical studies with ibr *vs* comparator arm as first line treatment in pts with CLL/SLL or in pts with relapsed/refractory (R/R) CLL/SLL.

Methods: Ibr 420 mg daily was given until progressive disease or unacceptable toxicity in the RESONATE (PCYC-1112) and RESONATE-2 (PCYC-1115/1116) studies and in combination with bendamustine plus rituximab (BR) in the HELIOS study (CLL3001). Comparator/combination agents were of fixed duration. In the RESONATE study, pts with R/R CLL/SLL received up to 6 cycles of ofatumumab (ofa; Byrd, *NEJM*, 2014). In RESONATE-2, treatment naïve pts aged ≥65 y with CLL/SLL received up to 12 cycles of chlorambucil (clb; Burger, *NEJM*, 2015). In the HELIOS study, pts with R/R CLL/SLL received up to 6 cycles of combined placebo and BR (Chanan-Khan, *Lancet Oncol*, 2016). All patients provided written informed consent. SHI was compared using Fisher's exact test and was defined as ≥56 days with: 1) platelet count >100 x 109/L if baseline ≤100 x 109/L or increase ≥50% over baseline; or 2) hemoglobin >11 g/dL if baseline ≤11 g/dL or increase ≥2 g/dL over baseline (in pts without blood transfusion or growth factor support).
Results: The analysis included a total of 1238 pts (391 RESONATE, 269 RESONATE-2, 578 HELIOS). Compared to respective comparator arms, ibr treatment resulted in sustained increase in hemoglobin for pts with baseline anemia (RESONATE: 80% *vs* 44%, *P*<0.0001; RESONATE-2: 90% *vs* 45%, *P*<0.0001; HELIOS: 74% *vs* 65%, *P*=0.2528) or sustained increase in platelets for pts with baseline thrombocytopenia (RESONATE: 85% *vs* 20%, *P*<0.0001; RESONATE-2: 83% *vs* 46%, *P*=0.0032; HELIOS: 69% *vs* 59%; *P*=0.1935) (Figure 1). The rates of SHI were improved with ibr treatment *vs* comparators across specified high-risk genomic subgroups (del11q, complex karyotype, unmutated *IGHV* and trisomy 12) in all three studies and in del17p pts, which were enrolled in the RESONATE study only. Pts with SHI had improved progression-free survival (PFS) with ibr *vs* comparators at 36 months in all studies (Figure 1, RESONATE: 68% *vs* 7%; RESONATE-2: 80% *vs* 32%; HELIOS: 77% *vs* 21%) as well as overall response rates (ORR; RESONATE: 97% *vs* 29%; RESONATE-2: 95% *vs* 50%; HELIOS: 95% *vs* 76%). Improvement in fatigue was observed for all patients who achieved SHI on RESONATE and RESONATE-2. In long term follow-up, grade 3-4 hematologic adverse event (AE) rates overall and in pts with SHI continued to appear favorable for ibr (median treatment and AE collection periods up to 8-fold longer in ibr *vs* comparator arms).

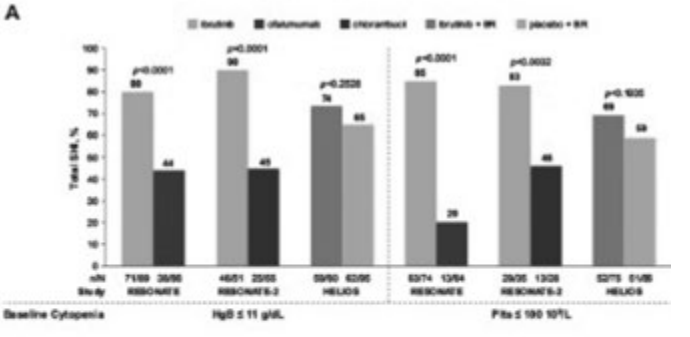

Figure 1.

Summary and Conclusions: Analyses showed that single-agent and combination treatment with ibr resulted in a high frequency of SHI in hemoglobin and platelet levels for both first-line and R/R pts *vs* comparators. ORR and PFS improvements with ibr, including in pts with SHI benefit, were maintained *vs* comparators. Over a median treatment duration of 34-41 months, the rates of severe hematologic AEs continue to remain low for patients treated with ibr.

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REAL WORLD TREATMENT PERSISTENCE OF AUSTRALIAN IBRUTINIB PATIENTS IN A NAMED PATIENT PROGRAM

C. Tam[1,2,*], B. Kuss[3], S. Opat[4], A. Puig[5], M. Acar[5], C. Zhou[6], S. Mulligan[7,8]

[1]Peter MacCallum Cancer Centre Parkville, [2]Haematology, St. Vincent's Hospital, Melbourne, [3]Molecular Medicine and Pathology, Flinders University/Flinders Medical Centre, Bedford Park, [4]Monash Health, Monash University, Melbourne, [5]Health Economics, Pricing and Outcomes Research, [6]Medical and Scientific Affairs, Janssen-Cilag Pty Ltd, [7]Royal North Sydney Hospital, [8]University of Sydney, Sydney, Australia

Background: NPPs (Named Patient Programs) can provide controlled access to treatment in response to unsolicited requests by physicians for specific patients before medicines become commercially available. In 2014, a global ibrutinib NPP was opened for patients with relapsed or refractory Chronic Lymphocytic Leukemia (CLL) in multiple countries, including Australia. The program closed in Australia in November 2017.
Aims: To analyse duration on ibrutinib treatment for patients with relapsed/refractory CLL enrolled in a NPP in Australia.
Methods: A retrospective cohort analysis was conducted using baseline patient characteristics such as age, sex, received 3 or more prior lines of therapy, etc. (collected on a binary scale, *e.g.*, age <65 years *versus* >=65 years) entered by treating physicians when enrolling patients into the NPP via the Janssen Managed Access portal (MacWeb). Patients were considered to be on treatment until they were reported as discontinued. Treatment duration was estimated from the date of first supply to date of last resupply. This real-world estimate of treatment duration was compared to time to treatment discontinuation in the ibrutinib arm of the RESONATE trial, as reported in Brown et al Leukemia, 32, 83-91 (2018). Time on treatment was evaluated descriptively using Kaplan-Meier (KM) curves, and statistical testing was conducted using log-rank test.
Results: Of the 1,015 patients treated with ibrutinib in the NPP in Australia from 2014 to 2017, 68% were male, 73% were 65 years or older, and 43% of patients had received 3 or more prior lines of therapy before commencing in the NPP. While 226 patients (22%) discontinued treatment by the end of the NPP, 85% (95% CI: 0.82-0.87) remained on treatment for at least 12 months; median duration on treatment was not reached over the NPP period. Duration on treatment in NPP for this analysis ranged from 0 to 39 months. There was no difference in the time on treatment between the RESONATE trial and the NPP (p= 0.11, HR= 0.81, 95% CI= 0.63-1.05) (Figure 1).

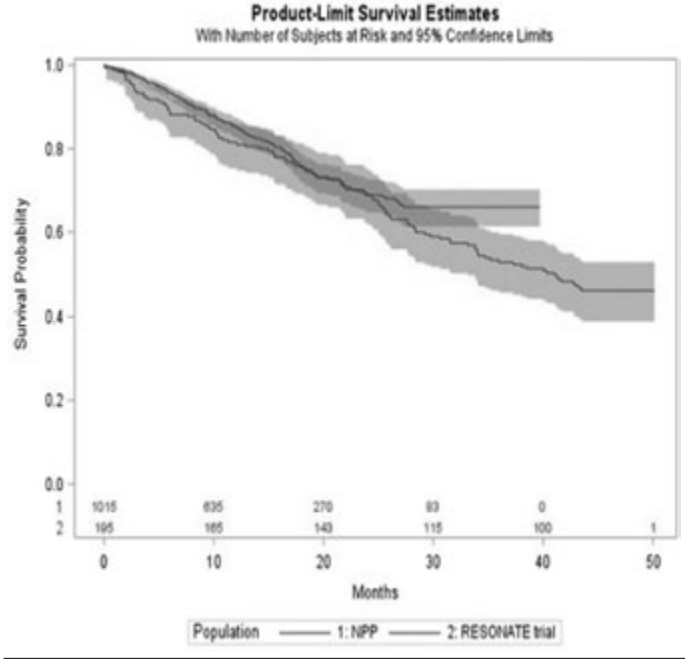

Figure 1.

Summary and Conclusions: The Australian NPP estimates of time on treatment closely tracks to the time on treatment observed in the pivotal Phase 3 trial for Ibrutinib in relapsed/refractory CLL, RESONATE, with caveats associated with the statistical comparability of these cohorts. While there are limitations to the NPP data given that it was based on unmonitored physician declarations, and the duration on treatment was estimated from resupply data, these findings provide a real-world estimate of time-on-treatment with ibrutinib that is similar to the time-on-treatment in the RESONATE trial.

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THE EFFICACY OF DUVELISIB MONOTHERAPY FOLLOWING DISEASE PROGRESSION ON OFATUMUMAB MONOTHERAPY IN PATIENTS WITH RELAPSED/REFRACTORY CLL OR SLL IN A PHASE 3 CROSSOVER EXTENSION STUDY

P. Hillman[1,*], B. Kuss[2], C. Moreno[3], M. Davids[4], N. Lammana[5], Z. Nagy[6], J. Essell[7], P. Ghia[8], C. Tam[9], U. Jaeger[10], S. Stilgenbauer[11], J. Delgado[12], N. Le[13], B. Jeglinski [13], M. Montillo[14]

[1]St. James's Institute of Oncology, The Leeds Teaching Hospitals, Leeds, United Kingdom, [2]Flinders Medical Centre (FMC), Bedford Park, Austria, [3]Department of Hematology, Hospital de Sant Pau i Santa Creu, Autonomous University of Barcelona, Barcelona, Spain, [4]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, [5]New York Presbyterian, Columbia University Medical Center, New York, United States, [6]1st Department of Internal Medicine, Semmelweis University, Budapest, Hungary, [7]OHC, Cincinnati, United States, [8]Università Vita-Salute San Raffaele and IRCCS Istituto Scientifico San Raffaele, Milan, Italy, [9]Peter MacCallum Cancer Centre, Melbourne,, Australia, [10]Medical University of Vienna, Vienna, Austria, [11]Department III of Internal Medicine, University Hospital Ulm, Ulm, Germany, [12]Hospital Clinic, Barcelona, Spain, [13]Verastem, Inc, Needham, United States, [14]Niguarda Cancer Center, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy

Background: Duvelisib, an oral dual inhibitor of PI3K-δ, γ, is being developed for the treatment of hematologic malignancies, including relapsed/refractory (RR) CLL/SLL. In the Phase 3 DUO study (NCT02004522) duvelisib monotherapy demonstrated significant improvement compared to ofatumumab monotherapy (PFS 13.3 *vs* 9.9 mo. p<0.0001; ORR 74% *vs* 45% p <0.0001) with a manageable safety profile (Flinn, ASH 2017). Study IPI-145-12 (NCT02049515) is an open-label, optional, crossover extension study where pts with confirmed progressive disease (PD) on DUO were given the option to receive the opposite treatment.
Aims: Herein we present data for the 89 pts who voluntarily rolled over following PD on ofatumumab on DUO and received duvelisib on Study IPI-145-12.
Methods: Eligible pts were enrolled within 3 months of PD on the DUO study (excluding Richter's transformation or prolymphocytic leukemia), and maintained adequate renal and hepatic function and an ECOG PS of 0-2. Duvelisib 25 mg BID was administered until PD, intolerance, death, or study withdrawal. Responses were determined by investigators per modified IWCLL/IWG criteria.
Results: Median age was 68 yrs (range: 39-89), 63% were male, and 90% Caucasian. Nearly half (49%) had Rai Stage III/IV or Binet Stage C, and 23% had del(17p) and/or *TP53* mutation. Median prior anticancer therapies was 3 (range: 2-8). Median exposure to duvelisib was 32 weeks on the extension study. The ORR for pts treated with duvelisib in the crossover was 73% (95% CI: 64, 82) (all PRs) compared to 28% (95% CI: 19, 37) (1% CR, 27% PR) when previously treated with ofatumumab in DUO. The median PFS for duvelisib was 15 mo. (95% CI: 10, 17) compared to 9 mo. (95% CI: 9, 11) for prior ofatumumab.
Summary and Conclusions: In an extension study, duvelisib monotherapy achieved robust and durable responses in 89 RR CLL/SLL pts with PD following ofatumumab treatment in the DUO study (ORR: duvelisib 73%; prior ofatumumab 28%), with a longer PFS with duvelisib than prior ofatumumab (PFS: duvelisib 15 mo.; prior ofatumumab 9 mo.). These data further support duvelisib monotherapy as an effective oral treatment option for pts with RR CLL/SLL.

PF355

A PHASE 1 DOSE ESCALATION STUDY OF ARQ 531 IN SELECTED PATIENS WITH RELAPSED OR REFRACTORY HEMATOLOGIC MALIGNANCIES

J. Woyach[1,*], I. Flinn[2], D. Stephens[3], F. Awan[1], R.E. Savage[4], F. Chai[4], S. Eathiraj[4], B. Schwartz[4], L. Szuszkiewicz[1], K. Tith[4], J.C. Byrd[1]

[1]Ohio State University, Nashville, [2]Sarah Cannon Research Institute, Columbus, [3]Huntsman Cancer Institute, Salt Lake City, [4]ArQule, Inc., Burlington, United States

Background: Bruton's tyrosine kinase (BTK) is a key regulator of the B cell receptor (BCR) signaling pathway that contributes to survival, proliferation and trafficking of malignant B cells. Despite impressive clinical response of ibrutinib in B-cell malignancies, cases of primary and secondary resistance have emerged with poor outcomes and no established treatment options. The underlying drug resistance mechanisms are complex and diverse, in ~80% of relapsing CLL patients develops resistance in association with BTK-C481S and PLCg mutations. ARQ 531 is a reversible ATP competitive inhibitor of BTK that does not require C481 residue for binding. With its distinct kinase selectivity profile, ARQ 531 targets multiple oncogenic signals of BCR signaling pathway and has demonstrated potent inhibitory activity in CLL and DLBCL mouse models.
Aims: The primary objectives of the study are to assess the safety and tolerability, and to determine the recommended Phase 2 dose and schedule of ARQ 531. The secondary objectives are to assess the pharmacokinetic (PK) profile, pharmacodynamics (PD) activity, and to generate preliminary evidence of anti-tumor activity of ARQ 531.
Methods: This is a first in human, phase 1 dose escalation study in patients

with relapsed or refractory CLL/SLL, WM, B-cell NHL who received at least 2 prior lines of systemic therapy including a BTK inhibitor approved for their diseae. Patients with DLBCL must have failed, refused, or be ineligible for autologous stem cell transplant. Patients with low grade lymphoma must be progressing and requiring treatment. Dose escalation was performed according to a 3+3 design. TEAEs were assessed per NCI CTCAE v.4.03. Tumor response were evaluated per disease specific guidelines. Blood samples were collected for PK and PD analysis.
Results: A total of 8 patients enrolled (2 DLBCL, 5 CLLs and 1 follicular lymphoma) and treated at dose levels of 5 mg QD, 10 mg QD, and 15 mg QD. All patients signed written informed consent prior to initiation of any study-specific procedures. No DLTs or drug-related AEs have been reported so far. Preliminary PK data show that increases in ARQ 531 exposure were close to dose proportional and that the estimated plasma half-life was ~24 hours. Sustained pBTK PD knockdown was observed. One patient treated at the dose of 5 mg QD has shown >30% tumor reduction after 8 weeks on treatment and is ongoing.
Summary and Conclusions: The study is actively enrolling patients. Updated data in safety, PK, PD and efficacy will be presented.

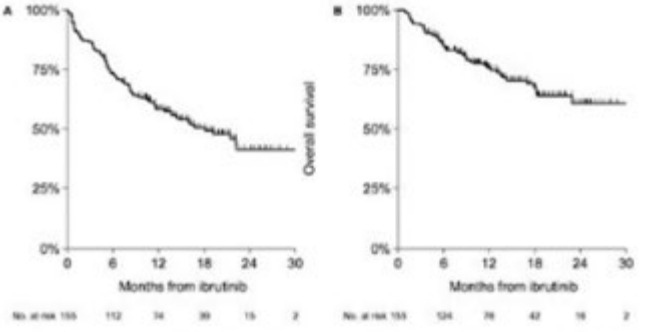
PF356

EFFECTIVENESS OF IBRUTINIB FOR THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA IN DAILY PRACTICE IN THE NETHERLANDS: A NATIONWIDE POPULATION-BASED COHORT STUDY

L. Van Der Straten[1,2,*], M.-D. Levin[2], O. Visser[3], N.M. Blijlevens[4], J.J. Cornelissen[5], J.K. Doorduijn[5], A.P. Kater[6], A.G. Dinmohamed[1,5,7]

[1]Research, Netherlands Comprehensive Cancer Organisation (IKNL), Utrecht, [2]Internal Medicine, Albert Schweitzer Hospital, Dordrecht, [3]Registration, Netherlands Comprehensive Cancer Organisation (IKNL), Utrecht, [4]Hematology, Radboud University Medical Center, Nijmegen, [5]Hematology, Erasmus MC Cancer Institute, Rotterdam, [6]Hematology, Academic Medical Center, Amsterdam, [7]Public Health, Erasmus University Medical Center, Rotterdam, Netherlands

Background: In the late 2014, ibrutinib was approved in the Netherlands for patients with relapsed/refractory chronic lymphocytic leukemia (CLL) and for CLL patients with p53 dysfunction in any line of therapy. At present, most of our knowledge on the effectiveness of ibrutinib arrive from compassionate-use programs (CUPs), which to a certain degree are associated with patient selection as in randomized clinical trials (RCTs).
Aims: The aim of this nationwide, population-based study was to assess the effectiveness of ibrutinib in CLL patients in a post-approval setting in the Netherlands.
Methods: We identified all adult (≥18 years) CLL patients who were treated with ibrutinib in the Netherlands between January 1, 2015 and December 31, 2016 from Dutch Hospital Data. These patients were subsequently linked to the nationwide Netherlands Cancer Registry to allow for additional data collection on patient, disease, and treatment characteristics. The primary endpoint was progression-free survival (PFS; see Figure 1 for definition). The secondary endpoints included prescription pattern of ibrutinib according to the HOVON-endorsed indications (see Table 1 for indications) discontinuation rate, and overall survival (OS; see Figure 1 for definition). Multivariable logistic regression (MLR) and multivariable Cox regression (MCR) was used to assess covariates (listed in Table 1) associated with permanent ibrutinib discontinuation and outcome (*i.e.* PFS and OS), respectively. A $P<0.05$ indicates statistical significance.
Results: A total of 155 ibrutinib-treated CLL patients (median age, 70 years; range, 48-91 years) were included in this analysis, of whom 94% received ibrutinib as subsequent treatment and 55% according to the HOVON-endorsed indications. At ibrutinib initiation, the majority of patients were male (74%), had anemia (51%), had RAI stage ≥3 (67%), received ≥3 prior lines of therapy (62%), received ibrutinib within a non-university hospital (68%), and had ≥1 comorbidity (71%). Del(17p) and *TP53* mutation was found in 27/86 (31%) and 10/16 (63%) tested patients, respectively. At a median follow-up of 12.0 months (range, 0.9-30.9), 47% of the patients were still on ibrutinib. The median PFS and OS was 18.2 months (95% confidence interval [CI], 11.6-not reached [NR]; Figure 1A) and NR (95% CI, 22.9-NR; Figure 1B). At 1-year, PFS and OS was 58% (95% CI, 50%>66%) and 76% (95% CI, 68%>82%), respectively. Of note, median PFS for patients with anemia was 14.3 months (95%CI, 7.3-21.5), as compared with NR (95%CI, 13.7-NR) for non-anemic patients (P=0.004). Furthermore, patients with anemia experienced more toxicity, as compared with non-anemic patients (57% *vs* 38%%; P=0.021). MLR analysis showed that anemia was the sole covariate that was associated with permanent ibrutinib discontinuation (OR 2.33; 95% CI, 1.20-4.52; P=0.012). In the MCR analysis, anemia was associated with lower PFS (P=0.014) and OS (P=0.001; Table 1). Further, hepatosplenomegaly (P=0.033) and number of previous therapies (P=0.002) were associated with inferior OS (Table 1).



- Progression-free survival was defined as the time from start of ibrutinib to progression/relapse, start of subsequent therapy, death resulting from any cause, or last follow-up (October 27, 2017), whichever occurred first. Seven patients who received an allogeneic hematopoietic stem cell transplantation (alloHSCT) after having responded to ibrutinib were censored at the date of alloHSCT.
- Overall survival was defined as the time from start of ibrutinib until death resulting from any cause or until the last date the patient was known to be alive (October 27, 2017), whichever occurred first.

Figure 1.

Table 1.

Results of the multivariable Cox regression

Covariate	Progression-free survival HR	95% CI	P	Overall survival HR	95% CI	P
Age, years (per one unit increase)	1.00	0.97 - 1.04	0.858	0.99	0.95 - 1.03	0.532
Female sex	0.73	0.41 - 1.31	0.293	0.66	0.30 - 1.43	0.288
No. of comorbidities (per one unit increase)	1.09	0.92 - 1.30	0.311	1.07	0.85 - 1.36	0.558
Lymphadenopathy (as per RAI stage)	1.21	0.42 - 3.45	0.720	1.00	0.22 - 4.47	0.999
Hepato- and/or splenomegaly (as per RAI stage)	1.45	0.76 - 2.76	0.254	**3.09**	**1.09 - 8.72**	**0.033**
Anemia (as per RAI stage)	**1.90**	**1.15 - 3.34**	**0.014**	**3.56**	**1.67 - 7.60**	**0.001**
Thrombocytopenia (as per RAI stage)	1.67	0.64 - 1.79	0.798	0.97	0.49 - 1.90	0.926
Treatment as per HOVON-endorsed indication[a]	1.48	0.91 - 2.40	0.112	1.85	0.96 - 3.57	0.065
No. of previous therapies (per one unit increase)	1.18	0.98 - 1.41	0.080	**1.46**	**1.16 - 1.85**	**0.002**
Prior therapy with purine analogue and/or bendamustine	0.83	0.47 - 1.47	0.529	0.87	0.39 - 1.94	0.731

[a]The current ibrutinib label in the Netherlands is defined by HOVON as follows: (i) salvage treatment for patients with CLL in need for treatment within 2 years after FCR or BR treatment, (ii) or within 6 months after chlorambucil in combination with an anti-CD20 agent, (iii) or as first-line treatment in the presence of del(17p) and/or a *TP53* mutation.

Summary and Conclusions: In this nationwide population-based study, we demonstrated that PFS among ibrutinib-treated CLL patients was substantially lower than reported in RCTs and CUPs. This might be explained by differences in patient characteristics and provision of care between patients treated in the real-world, as compared to those enrolled into RCTs and CUPs. The impact of anemia on ibrutinib discontinuation and outcome calls for confirmation in future RCTs and population-based studies.

PF357

RAPID DOSE ESCALATION OF VENETOCLAX IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA PREVIOUSLY TREATED WITH B-CELL RECEPTOR INHIBITOR THERAPY

K. Koenig[1,*], D. Konstantinou[2], A. Rogers[3], T. Wiczer[4], K. Rogers[3], J. Woyach[3], J. Byrd[3], F. Awan[3]

[1]Department of Internal Medicine, The Ohio State University Wexner Medical Center, [2]The Ohio State University College of Medicine, [3]Division of Hematology, [4]Department of Pharmacy, The James Cancer Hospital and Solove Research Institute at The Ohio State University, Columbus, United States

Background: Despite the remarkable disease control observed with B-cell receptor pathway inhibitors (BCRi), patients with chronic lymphocytic leukemia (CLL) who relapse after these agents frequently tend to have rapidly progressive, symptomatic disease that requires immediate intervention and disease control. Venetoclax selectively inhibits anti-apoptotic protein B-cell lymphoma 2 (BCL-2), and is the only agent that has demonstrated remarkable efficacy after BCRi therapy. However, its use requires a slow, weekly dosing ramp-up from 20mg to 400mg goal dose over 5 weeks, to reduce the risk of tumor lysis syndrome (TLS). Herein, we report our experience with a rapid dose escalation protocol for venetoclax that attains prompt disease control.
Aims: Given the need to promptly attain goal venetoclax dose, we aim to demonstrate the feasibility, safety/tolerability, and efficacy of a "rapid dose escalation" of venetoclax in relapsed/refractory CLL pts previously treated with BCRi in a properly equipped university institution.
Methods: With permission from an IRB approved protocol, data was retrospectively compiled from medical records of patients who received rapid venetoclax (dosing ramp up <5 weeks to goal dose). Patient demographics, treatment, disease, and survival outcomes were collected. Detailed safety and efficacy measures were also assessed.

Results: Fifteen patients received rapid venetoclax dose escalation as inpatients with close laboratory and clinical monitoring and aggressive supportive care for laboratory and clinical TLS. Median age was 65 years (range 48-77) and 80% were men with ECOG 0-1 in 93% of patients. Patients had received a median of 5 previous treatments (range 3-7). Most recent prior treatment was a BCRi alone in 10 patients, BCRi in combination with chemotherapy in 3 and high dose steroids in 2 patients. The BCRi treatments overlapped with venetoclax in 6 patients. High risk cytogenetics were present in the majority of patients including: complex karyotype in 60%, del17p in 67% and unmutated IGHV in 80%. Three patients had high tumor burden disease (per venetolax package insert and NCCN Guidelines) and 73% had confirmed BTK/PLCG2 mutations. The mean time to goal venetoclax dose was 12 days (range 5-21), and all patients reached goal dose. Seven (46.7%) pts developed tumor lysis syndrome (TLS) by lab criteria at doses ranging from 20-200 mg (1 patient at 20 mg, 2 patients each at the 50 mg, 100 mg and 200 mg dose); but only 2 patients developed TLS by clinical criteria, of grade 0 or 1 severity (per Cairo-Bishop definition). No patients required renal replacement; 53.3% had an elevated uric acid requiring rasburicase and 40% had hyperphosphatemia requiring phosphate binders. Twelve patients (80%) achieved a partial response, 2 had progressive disease and 1 patient died within 30 days from progressive disease. Mean time to best response was 71 days (range 13-428), and to subsequent treatment was 298 days (range 204-430).
Summary and Conclusions: Rapid dose escalation of venetoclax with close inpatient monitoring in experienced centers could be a feasible approach in patients with disease relapse after BCRi in patients who require urgent disease control. Further trials in these patients utilizing a consistent rapid dose escalation protocol are warranted.

PF358

INDIRECT TREATMENT COMPARISON OF VENETOCLAX PLUS RITUXIMAB WITH B-CELL RECEPTOR INHIBITORS IN PATIENTS WITH RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA

A. Mato[1,*], G. Follows[2], K. Sail[3], I. Diakite[4], D. Nicoloso[4], B. Dietz[5], J. Maher[3], D. Alexiou[3], V. Chirikov[4]

[1]CLL program, Leukemia Service, Division of Hematological Oncology, Memorial Sloan Kettering Cancer Center, New York, NY, United States, [2]Department of Hematology, Cambridge University Hospitals NHS Foundation Trust Addenbrooke's Hospital, Cambridge, United Kingdom, [3]AbbVie, Inc., North Chicago, IL, [4]Pharmerit International, Bethesda, MD, United States, [5]AbbVie Deutschland GmbH & Ko KG, Ludwigshafen, Germany

Background: The primary analysis of the MURANO (median follow up 23.8 months) Phase 3 trial (NCT02005471) in patients with relapsed/refractory chronic lymphocytic leukemia (CLL) demonstrated improvement in progression-free survival (PFS IRC: HR=0.19 [95% CI: 0.13-0.28], p<0.001) and strong pattern of clinically meaningful improvement in the secondary endpoint overall survival (OS: HR=0.48 [95% CI: 0.25-0.90], descriptive p=0.02) for venetoclax plus rituximab (V+R) compared to bendamustine plus rituximab (BR). Recent randomized trials HELIOS (NCT01611090) for ibrutinib+BR and Study 115 (NCT01569295) for idelalisib+BR have also showed improved efficacy *versus* BR. However, there is absence of head-to-head clinical evidence on the comparative efficacy of novel targeted therapies.
Aims: This study aimed to indirectly compare PFS and OS relative efficacy of V+R *versus* B-cell receptor inhibitor therapies.
Methods: Published survival data from HELIOS (median follow up 17 months) and Study 115 (median follow up 14 months) on PFS as assessed by independent review committee (IRC) and OS was used to conduct indirect treatment comparisons (ITC) *versus* V+R. Consistent with the exclusion criteria for HELIOS, MURANO patients with 17p deletion were excluded for V+R to ibrutinib+BR comparisons. Given the availability of a common comparator, the ITC for V+R *versus* ibrutinib+BR and V+R *versus* idelalisib+BR were anchored by accounting for cross-trial differences in Kaplan-Meier curves for the BR arms. Based on literature review and analysis of the patient-level MURANO data, it was determined that the efficacy of novel CLL selective inhibitors would not be significantly modified by differences in patient populations and the anchored ITC was conducted without further adjustment for baseline characteristics.
Results: PFS and OS relative efficacy estimates in MURANO patients without 17p deletion for V+R (N=123) compared with BR (N=119) were consistent with those reported for the general trial population. In the ITC analysis using BR as a common comparator, V+R was similar in efficacy to ibrutinib+BR with respect to PFS (HR=0.90 [95% CI: 0.50-1.64]) and OS (HR=0.70 [95% CI: 0.27-1.83]). Compared to idelalisib+BR, V+R had improved PFS (HR=0.57 [95% CI: 0.35-0.93]) but similar OS (HR=0.71 [95% CI: 0.33-1.51]) (Figure 1).

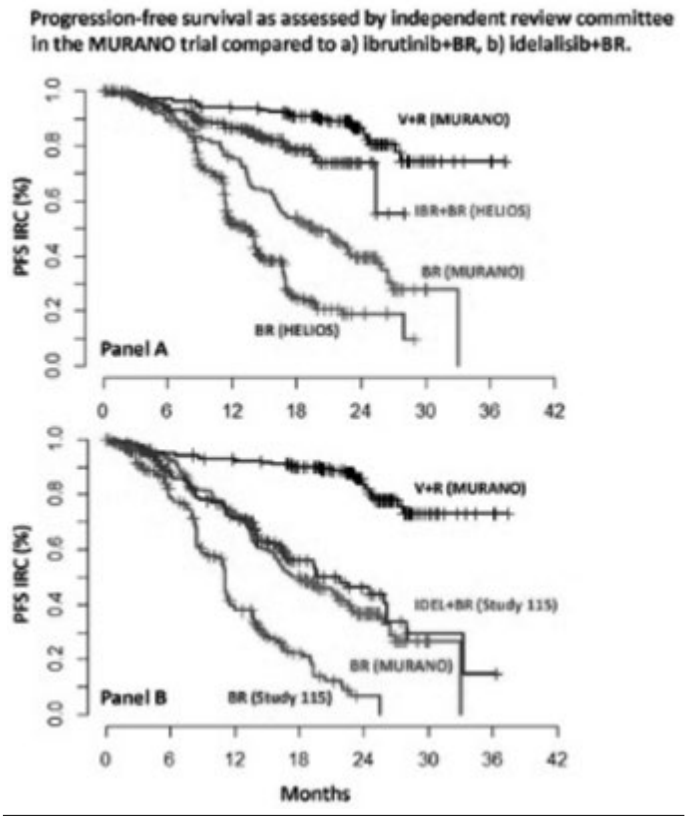


Figure 1.

Summary and Conclusions: V+R is an effective therapy for patients with relapsed/refractory CLL. Indirect comparisons suggest V+R to have improved PFS *versus* idelalisib+BR and similar PFS and OS compared to ibrutinib+BR. Future comparisons as data mature across trials will provide more robust estimates of OS comparisons.

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PF359

INHIBITION OF NUCLEOCYTOPLASMIC EXPORT ENHANCES SELECTIVE ELIMINATION OF LEUKEMIC STEM CELLS IN CHRONIC MYELOID LEUKEMIA

M. Deininger[1,2,*], H. Than[2,3], A. Pomicter[2], C. Mason[4], A. Eiring[2], W. Heaton[2], D. Yan[2], A. Senina[2], P. Clair[2], T. Holyoake[5], T. O'Hare[1,2]

[1]Division of Hematology and Hematologic Malignancies, University of Utah / Huntsman Cancer Institute, [2]Huntsman Cancer Institute, University Of Utah, Salt Lake City, United States, [3]Department of Hematology, Singapore General Hospital, Singapore, Singapore, [4]Division of Pediatric Hematology/Oncology, University of Utah, Salt Lake City, United States, [5]Paul O'Gorman Leukaemia Research Centre, Institute of Cancer Sciences, University of Glasgow, Glasgow, Ukraine

Background: Chronic myeloid leukemia (CML) is a hematopoietic stem cell malignancy driven by BCR-ABL1 tyrosine kinase and effectively managed with tyrosine kinase inhibitors (TKIs) such as imatinib. Reactivation of BCR-ABL1 through mutations in the kinase is a common mechanism of resistance, but fails to explain clinical resistance in a significant proportion of cases, a situation referred to as BCR-ABL1-independent resistance. Additionally, most patients require continuous TKI therapy to avoid recurrence of active leukemia, suggesting that primitive, fully leukemogenic CML stem cells (LSCs) are BCR-ABL1-independent. We have previously demonstrated that BCR-ABL1-independent CML cells from patients with overt resistance require functional nucleocytoplasmic export (NCE) and are sensitive to knockdown of the NCE regulator RAN and selective inhibitors of NCE (SINEs) such as KPT-330 (selinexor, Karyopharm) [Khorashad *et al.* Blood. 2015].
Aims: Test whether reliance on NCE extends to LSCs, the reservoir for persistent CML.
Methods: LSCs ($CD34^+CD38^-$) and leukemic progenitor cells (LPCs, $CD34^+CD38^+$) isolated from newly diagnosed chronic phase CML patients and their normal (cord blood, CB) equivalents were analyzed with colony forming assays +/- KPT-330 and/or imatinib. Cells were cultured for 72h in +/- KPT-330 and/or imatinib and analyzed for apoptosis, plated in LTC-IC assays or injected into NSG mice. LTC-IC colonies and human $CD45^+$ cells isolated from the NSG mice were genotyped for BCR-ABL1 by FISH. In a separate set of experiments, we assessed drug effects on cell division using CSFE.
Results: Treatment with KPT-330 reduced colony formation by LPCs and LSCs to 42% and 55% of controls (Table 1). When combined with imatinib, KPT-330 dramatically reduced colony formation by LPCs and LSCs to 8% and 2% of controls. Single agent KPT-330 had modest effects on apoptosis, but the combination increased apoptosis in LPCs and LSCs by 280% and 940%, compared to 180% and 270% respectively for CB. These data suggest that combining KPT-330 with imatinib has the most profound effects on LSCs, with a large differential compared to normal hematopoietic stem cells.

Table 1.

% Control		CML		CB		p(CML vs. CB)	
		LPC	LSC	HPC	HSC	PC	SC
CFU-GM Colony formation	Imatinib (2.5μM)	13%	8%	77%	63%	<0.01	<0.01
	KPT-330 (50nM)	42%	55%	78%	96%	<0.05	<0.01
	Combination treatment	8%	2%	66%	43%	<0.01	<0.01
Apoptosis	Imatinib (2.5μM)	200%	410%	130%	160%	ns	ns
	KPT-330 (50nM)	100%	200%	110%	80%	ns	ns
	Combination treatment	280%	940%	180%	270%	ns	<0.01

While no reduction in colonies was seen in LTC-IC experiments with either single agent, a 64% reduction in LTC-IC colonies was observed with combination. Genotyping of LTC-IC colonies by FISH revealed that *BCR-ABL1*$^+$ colonies were reduced by 11% and 22%, when treated with KPT-330 or imatinib respectively, while combination treatment reduced *BCR-ABL1*$^+$colonies by 38%, significantly lower than either single agent. Similarly, neither imatinib nor KPT-330 monotherapy reduced the proportion of *BCR-ABL1*$^+$cells, but combination treatment reduced *BCR-ABL*$^+$cells by 42%. CFSE staining showed that imatinib monotherapy promoted accumulation of undivided $CD34^+$CML cells, with little effect on CB, as previously reported. In contrast, KPT-330 promoted cell division and this effect overrode imatinib effects when the two drugs were combined. These data suggest that the efficacy of the KPT-330/imatinib combination may be due to KPT-330-enforced cell division, with subsequent killing of cycling cells by imatinib.
Summary and Conclusions: Our data illustrates that interrupting NCE by SINEs preferentially eliminates CML LSCs and enhances their sensitivity to TKIs, suggesting that combinations of TKI and SINEs represent a clinical strategy to target BCR-ABL1 independent resistance, as well as persistent residual CML. Mechanistic studies exploring the effects of nuclear entrapment of certain NCE cargo proteins of biological significance in CML LSCs are ongoing and will be reported.

PF360

IMMUNOMODULATORY EFFECTS OF IFN-ALPHA ON T AND NK CELLS IN CHRONIC MYELOID LEUKEMIA PATIENTS IN DEEP MOLECULAR RESPONSE IDENTIFY POTENTIAL CANDIDATES FOR TREATMENT DISCONTINUATION

M. Breccia[1,*], M.C. Puzzolo[1], P. Mariglia[1], M. Molica[1], G. Colafigli[1], S. Pepe[1], A. Guarini[2], R. Foà[1]

[1]Department of Cellular Biotechnologies and Hematology, [2]Department of Molecular Medicine, Hematology, Sapienza University, Rome, Italy, ROMA, Italy

Background: Recent studies indicate that about 40% of chronic myeloid leukemia (CML) patients are able to discontinue tyrosine kinase inhibitor (TKI) treatment without molecular recurrence, defined as the loss of major molecular response (MMR). Novel strategies aimed at increasing the cure rates are thus warranted. In this context, adoption of combined treatments capable of activating the immune system to control residual leukemic cells may represent a promising approach to improve the rates of treatment-free remission.
Aims: In order to better identify potential candidates of successful treatment discontinuation, we have examined the phenotypic and functional host immune compartment - in particular T cells, NK and NK cell subsets - by comparing patients who had received INFα prior to TKI treatment (IFNα+TKI) with patients treated only with TKIs (TKI-only).
Methods: CML patients in deep molecular response (DMR) eligible to TKI discontinuation according to recent NCCN and ESMO guidelines (stable MR4 for more than 2 years) were evaluated for the T/NK-cell subset distribution, NK and T-cell cytokine production, activation, maturation markers and cytotoxic NK activity. We compared 10 patients treated with IFNα+TKI with 19 samples from TKI-only treated patients. Patients included in the study had discontinued IFNα for 15 years on average. T and NK cells have been evaluated for the surface expression of CD3, CD4, CD8, CD16, CD56, NKp30, NKp44, NKp46, NKG2C, NKG2D, DNAM-1, CD25, CD69, CD62L, CD57 and, after activation and permeabilization, for intracytoplasmic IFNγ and TNFα production (BD Biosciences, San Jose, CA and R&D System, Minneapolis, MN).
Results: We observed an increased number of lymphocytes capable of producing IFNγ and TNFα in INFα+TKI patients compared to TKI-only. In particular, the cytokine production by $CD3^+CD4^+$cells was: IFNγ=36.9±16.8 *vs* 23.0±9.7% (p=0.01), TNFα=70.4±11.0 *vs* 62.3±21.1% (ns) and by $CD3^+CD8^+$: IFNγ=75.2 ±17.4 *vs* 63.5±18.9% (ns), TNFα=71.3±17.1 *vs* 63.6±22.4% (ns). We found no differences in the percentage of $CD4^+$ and $CD8^+$T cells, and of the $CD4^+/CD8^+$ ratio between the two groups of patients. No differences were also observed in the percentage of NK and NK-T cells in the two groups of patients, nor in the NK cell subpopulations ($CD56^{bright}$/CD16, $CD56^{bright}/CD16^{dim}$, $CD56^{dim}/CD16^+$), as well in the activation, maturation markers and activating receptors (NKp30, NKp44, NKp46 and NKG2D) for NK cell subsets. In INFα+TKI patients, the NKG2C mean fluorescence intensity (MFI) was significantly higher compared to the TKI-only group in the $CD56^{bright}/CD16^{dim}$ and $CD56^{dim}/CD16^+$ NK cell subsets (p=0.01 and p=0.005, respectively). Furthermore, we observed a significant increase of DNAM-1 MFI in the $CD56^{bright}/CD16^-$ NK cell subset (p=0.03).
Summary and Conclusions: Our data indicate that in CML patients treated with INFα+TKI, IFNα appears to induce substantial modifications in the immune system, in particular in memory T lymphocytes, differentiated $NKG2C^+$ "long-lived" NK cells and $DNAM\text{-}1^+$ "adaptive" NK cell responses, even after a long time period (12-17 years) from the last IFNα contact. Our results confirm that IFNα modulates and potentiates the host immunologic compartment and pave the way to design and carry out immunotherapeutic strategies aimed at maintaining a DMR after TKI discontinuation in CML patients.

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IDENTIFICATION OF GENES DIFFERENTLY EXPRESSED BETWEEN BONE MARROW CD34+/LIN- CELLS OF PATIENTS WITH CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA AT DIAGNOSIS *VS* 12 MONTHS OF FIRST-LINE NILOTINIB TREATMENT

A. Trojani[1,*], E. Pungolino[1], G. Rossi[2], M. D'Adda[2], M. Lodola[1], B. Di Camillo[3], A. Perego[4], M. Turrini[5], E. Orlandi[6], L. Borin[7], A. Iurlo[8], C. Bucelli[8], S. Malato[9], F. Spina[10], M.L. Latargia[11], F. Lanza[12], S. Artale[13], M. Anghilieri[14], M.C. Carraro[15], G. De Canal[16], E. Morra[17], R. Cairoli[1]

[1]Department of Hematology, ASST Grande Ospedale Metropolitano Niguarda, Milano, [2]Department of Hematology, ASST Spedali Civili, Brescia, [3]Department of Information Engineery, University of Padova, Padova, [4]Internal Medicine Hematology, Desio Hospital, Desio, [5]Department of Hematology, Valduce Hospital, Como, [6]Hematology Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, [7]Department of Hematology, San Gerardo Hospital, Monza, [8]OncoHematology Division, IRCCS Cà Granda-Maggiore Policlinico Hospital Foundation, Milano, [9]Hematology and Bone Marrow Transplantation Unit, San Raffaele Scientist Institute, Milano, [10]Department of Hematology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, [11]ASST Valle Olona Ospedale di Circolo, Busto Arsizio, [12]Department of Hematology, Hospital of Cremona, Cremona, [13]Department of Oncology, ASST Valle Olona Sant'Antonio, Gallarate, [14]ASST Lecco, Lecco, [15]Hematology and Transfusion Medicine, Sacco Hospital, Milano, [16]ASST Grande Ospedale Metropolitano Niguarda, Milano, [17]Rete Ematologica Lombarda, ASST Grande Ospedale Metropolitano Niguarda, Milano, Italy

Background: Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder with heterogeneous biological and clinical features. The biomolecular mechanisms of CML response to tyrosine-kinase inhibitors (TKI) are not fully defined. Despite the TKI nilotinib being a very effective drug for the treatment of CML, drug resistance can emerge.
Aims: In the contest of the REL-PhilosoPhi34 study (EudraCT: 2012-005062-34) on behalf of the Rete Ematologica Lombarda, we undertook gene expression profiling (GEP) of selected bone marrow (BM) CD34+/lin- cells of 30 patients with CP-CML at diagnosis *vs* the same patients after 12 months of nilotinib treatment to investigate molecular signatures characterizing both conditions.
Methods: MNCs and CD34+/lin- cells of 30 CML patients were counted at diagnosis and after 3, 6 and 12 months of nilotinib, respectively. We performed GEP of BM CD34+/lin- cells of 30 patients at diagnosis *vs* the same patients after 12 months of nilotinib.
Results: BM CD34+/lin- cells decreased between the diagnosis and after 3 as well as 6 months of nilotinib while the BM CD34+/lin- cells slightly increased between 6 and 12 months of nilotinib (Table 1 and Figure 1). FISH analysis detected CD34+/lin- Ph+ cells in 30 CML patients at diagnosis. No positive Ph+ nuclei were found in CD34+/lin- cells after 12 months of treatment. We identified 264 statistically significant differentially expressed genes at diagnosis *vs* 12 months of nilotinib. Functional enrichment analysis revealed groups of genes belonging to 14 pathways differentially active during nilotinib. CD34+/lin- cells after 12 months of nilotinib showed altered expression of genes involved in metabolic processes: *AGPAT4, LPCAT3, MBOAT2* (lipid metabolism), *HK1, PDK3, UGGT1* (glycaemia), *PRKAR2A, PRKAR2B, PTPN1* (insulin resistance), *CR1, HBB, HBBP1* (coagulation cascade), and *TLN1, FCGR2A, ITGA2B* (platelet activation). Several pathways involved in cell proliferation, RAS pathway, cell adhesion, B cell differentiation were up regulated whereas sphingolipid metabolism (*SGMS2* and *SGPP1*) and apoptosis (*CCNG1, GAS2, SPTA1, CDH*) were down regulated in CML CD34+/lin- cells at diagnosis, respectively. In addition, genes involved in the mechanisms of transport across extra- and intra-cellular membranes were overexpressed (*ABCC5, SLC25A33, SLC25A37, SLC25A38, SLC27A2, SLC43A3, SLC4A4, ABCC5)* in CD34+/lin- cells at diagnosis, providing a rationale for investigating this phenomenon in patients undergoing nilotinib treatment.
Summary and Conclusions: GEP analyses of CD34+/lin- cells of CP-CML patients at diagnosis *vs* 12 months of nilotinib demonstrated that 264 genes belonging to 14 pathways were significantly differently regulated. We can speculate that nilotinib might interfere with biologic mechanisms (lipid and glycemic metabolism, insulin resistance, coagulation cascade and platelet activation) that are relevant in CML patients as previously determined by several studies in literature. The alteration of pathways regarding apoptosis in CML CD34+/lin- cells at diagnosis may underlie the increased cell proliferation playing a significant role in recognizing resistance mechanisms of leukemic stem cells treated with TKI. Future GEP studies on a larger cohort of CML patients at diagnosis *vs* 12 months of nilotinib are ongoing. We believe that BM CD34+/lin- cells from CML patients at diagnosis and after nilotinib harbor differences in certain biologic and genetic properties, that may predict how well they will respond to nilotinib and we will test this hypothesis.

Table 1.

Range, average and standard deviation of the number of BM MNCs as well as the number of the BM CD34+/lin- cells among the 30 CML patients at diagnosis and after 3, 6 and 12 months of nilotinib.

	BM MNC at diagnosis ($x10^6$)	BM MNC at 3 months ($x10^6$)	BM MNC at 6 months ($x10^6$)	BM MNC at 12 months ($x10^6$)
Range (min-max)	36-3450	12-166	9-257	8-187
Average	974	61	57	51
Standard deviation	800	41	46	41
	CD34+ /lin- at diagnosis ($x10^6$)	CD34+ /lin- at 3 months ($x10^6$)	CD34+ /lin- at 6 months ($x10^6$)	CD34+ /lin- at 12 months ($x10^6$)
Range (min-max)	0.001-1.5	0.001-0.6	0.001-0.7	0.001-0.4
Average	0.371	0.111	0.062	0.073
Standard deviation	0.296	0.158	0.141	0.1

Boxplot of the number of BM CD34+/lin- cells at diagnosis and after 3, 6 and 12 months of nilotinib of the 30 CML patients.

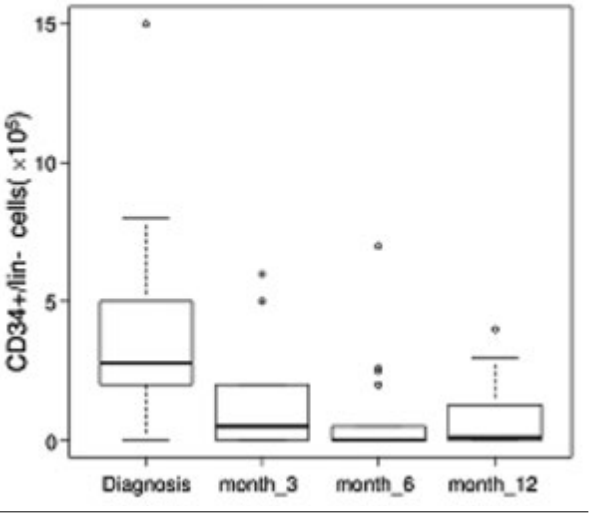

Figure 1.

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NK-CELLS AND LOSS OF MOLECULAR RESPONSE AFTER TKI DISCONTINUATION IN CML PATIENTS

N. Kapranov[1,*], Y. Davydova[1], I. Galtseva[1], A. Turkina[1], A. Petrova[1], E. Chelisheva[1], O. Shuhov[1], A. Bykova[1], I. Nemchenko[1], G. Gusarova[1], N. Tsyba[1], V. Savchenko[1]

[1]Research Center for Hematology of the Ministry of Healthcare of the Russian Federation, Moscow, Russian Federation

Background: Translocation t(9;22) in hematopoietic cells is the origin of chronic myeloid leukemia (CML). T(9;22) leads to BCR-ABL1 tyrosine kinase, which speeds up cells division and cause genomic instability. Tyrosine kinase inhibitors (TKI) such as imatinib and nilotinib specifically inhibit BCR-ABL1 activity and can induce complete remission in CML patients. Monitoring of residual disease in CML is carried out by quantitative real time PCR of BCR-ABL1. Over the last few years consequences of the cancellation of TKI have been actively studied. After prolonged use of the TKI and achievement of complete molecular remission, some patients develop an early molecular relapse (MR, *BCR-ABL1* level >0.1%) within six months after the cancellation of the TKI. Identification of factors predicting MR development could possibly allow optimization of TKI-discontinuation protocols.
Aims: The aim of the study is to estimate relations between different NK-cell subsets and *BCR-ABL* level in CML patients after TKI discontinuation.
Methods: The study included 66 CML patients with deep molecular response (DMR, *BCR-ABL1* <0.01%) for TKI therapy. 103 peripheral blood samples were studied: 28 on day of TKI discontinuation, 30, 19, 14 samples - after 3, 6, 12 months and 12 studies - on another time-points. First line therapy includ-

ed imatinib (46 patients) and nilotinib (7 patients) and 12 patients received nilotinib as the second line therapy. We used monoclonal antibodies anti-CD3, CD16, CD56 and CD45 to evaluate proportion of NK-cells and two distinct subsets (CD56bright/CD16- and CD56+/CD16+ NK cells) by flow cytometry. *BCR-ABL1* level was evaluated by RT-PCR by standard protocol. Frequencies of MR were compared by Fisher test, means were compared with Mann-Whitney test due to non-normal distribution of data.

Results: Analysis of MR frequency showed same MR rate for patients received imatinib (as 1st line therapy) and nilotinib as 2nd line (34% *vs* 30%, p=0.7). MR was not detected in patients received nilotib as 1st line therapy (n=7), but no significant differences compared to other therapy were found (p=0.1). All studies were divided into 3 groups: 1st group had no detectable level of *BCR-ABL1* (n=63), 2nd had *BCR-ABL1* level below 0.1% (n=33) and 3rd group had *BCR-ABL1* level above 0.1% (n=7). 3rd group showed significantly higher percentage of CD56bright+/CD16- NK-cells than other two groups (p<0.0094) (Figure 1). Differences in proportion of NK-Cell and CD16+/CD56+ subsets were not found.

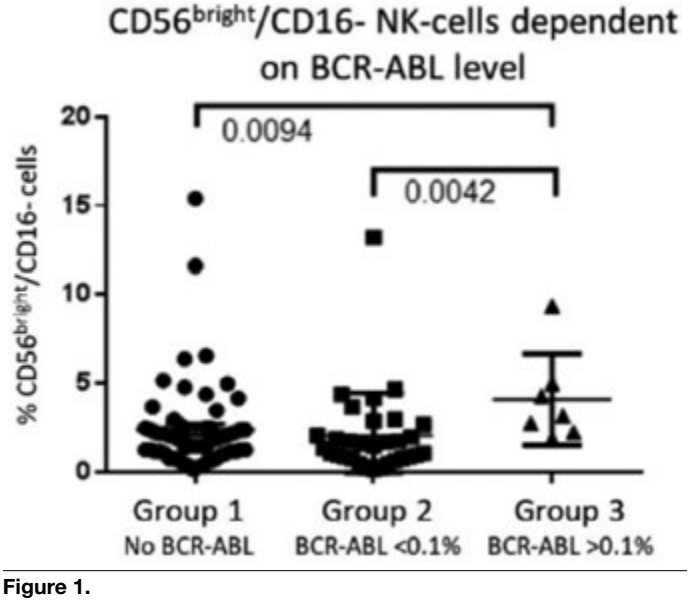

Figure 1.

Summary and Conclusions: These results show correlation between cytokine-producing NK cells and *BCR-ABL1* level, which potentially could be the evidence of participation of NK cells in the development of molecular relapse after TKI therapy discontinuation.

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LMC-TRIO, A REAL LIFE STUDY OF CHRONIC MYELOID LEUKEMIA CARE IN FRANCE BETWEEN 2006 AND 2013: FACTORS OF FAILURE FROM TREATMENT-FREE REMISSION

C. Jim[1,2,*], L.G.-P. Sandra[1,2], C.-M. Pascale[3], E. Gabriel[4], G.-B. Stéphanie[5], M. Marc[5], P. Jean-Marc[6], T. Xavier[6], O. Sébastien[1,2], M. Alain[1,2]

[1]Registre des hémopathies de Gironde, Institut Bergonié, [2]Team EPICENE, University of Bordeaux, INSERM, Bordeaux Population Health Research Center, UMR 1219, Bordeaux, [3]Service d'Hématologie, C.H. Annecy Genevois, Pringy, [4]Service d'hématologie, Institut Bergonié, Bordeaux, [5]Registre des hémopathies Malignes de Côte d'Or, Faculté des Sciences de Santé de Dijon, Dijon, [6]Registre régional des hémopathies malignes de Basse-Normandie (RRHMBN), CHU Caen, Caen, France

Background: With the occurrence of Tyrosine Kinase Inhibitor (TKI) in the beginning of 2000, Chronic Myeloid Leukemia (CML) survival has dramatically improved. To maintain CML under control, patients have to take daily dose of TKI. To reduce this burden, several clinical studies introduced treatment discontinuation exclusively proposed to patients with a deep molecular response. In case of loss of deep molecular response patients in Treatment-Free Remission (TFR) will have to start again daily treatment with TKI. Various clinical studies identified specific factors linked to TFR continuation such as TKI treatment duration before TFR or Sokal score. However, few data from real-life studies explored such factors.

Aims: In this study, we aim to investigate in a real life setting the factors influencing the failure of patients in TFR during its establishment. Population characteristics and care have been described according to their TFR status. In addition, we will describe the nature and the effect of factors leading to failure of TFR.

Methods: *Study design* retrospective cohort using data from three French haematological malignancies population based registries. *Population* patients with CML bcr-abl+ (ICD-O-3: 9875/3) diagnosed between 2006 and 2013. Atypical CML (ICD-O-3: 9876/3) and CML « without precision » (ICD-O-3: 9863/3) were excluded. *Analysis* End-point was set on 1st January 2017. Characteristics were described by TFR status: "failure" or "ongoing". Factors linked to failure were estimated with a logistic regression. Patients who have received an allograft before the establishment of TFR were excluded from the analysis.

Results: Between January 1st 2006 and December 31st 2013, 363 new patients with CML has been diagnosed in the regions covered by the registries. Median age at diagnostic was 61.6 years old (Q1 – Q3: 48.5 – 72.6 years old). Median of follow-up was 5.3 years (Q1 – Q3: 3.7 – 7.9 years). At end-point, 78 (21.5%) patients have been (at least once) in TFR, 41 (52.3%) patients failed after a median of 4.8 months (Q1 – Q3: 3.6 – 7.2 months) and treatment was restarted for all of them whereas six of them had a second TFR. At the end of follow-up, when counting the six patients who had already been in TFR once, ,43 patients were still in TFR after a median follow-up of 1.6 years (Q1 – Q3: 0.4 – 2.6 years). Eight of the 78 patients died during follow up (10%), two had stopped their TFR previously and were currently treated, four were still in TFR and two died after experiencing two TFR. Compared to patients still in TFR, patients who experienced a failure of the first TFR were mostly men (58.5% *versus* 39.5%), were less included in clinical trials (22.0% *versus* 30.2%), had poorer performance status at diagnosis (ECOG >0: 31.7% *versus* 20.9%) and were more frequently treated in non teaching hospital (general hospital or private clinic) (31.7% *versus* 16.3%). We estimated using a logistical regression that the following factors would statistically decrease the probability of leaving TFR: a longer TKI treatment duration before TFR, the absence of splenomegaly at diagnosis and to be treated in a teaching hospital (regional hospital or comprehensive cancer center). Table 1 presents the adjusted odds-ratios.

Table 1. Result for the logistical regression on the probabilities of failure of TFR (n=77).

Characteristics	$\widehat{OR}$	$CI_{95\%}$	*p-value*
TKI treatment duration to TFR (years)	0.78	[0.20 ; 0.82]	0.03
Care facility			0.02
Non-teaching hospitals	1		
Teaching hospitals	0.24	[0.06 ; 0.89]	
Splenomegaly at diagnosis			0.02
Yes	1		
No	0.23	[0.03 ; 0.47]	

Summary and Conclusions: Despite the low numbers, our study shows that factors influencing the failure of TFR in a real life setting are comparable to those identified in clinical studies. In addition, our study points out the eventual role of the care facility that need further investigations.

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POLYMORPHISMS IN MULTIDRUG RESISTANCE TRANSPORTER GENES AFFECT THE DURATION OF MOLECULAR RESPONSE TO NILOTINIB IN CHRONIC MYELOID LEUKEMIA PATIENTS

F. Loscocco[1,*], G. Visani[1], S. Galimberti[2], A. Ruzzo[3], I. Bagaloni[3], F. Stagno[4], P. Pregno[5], M. Annunziata[6], A. Gozzini[7], S. Barulli[1], M. Magnani[3], A. Isidori[1]

[1]AORMN, Hematology and Stem Cell Transplant Center, Pesaro, [2]Department of clinical and experimental medicine, University of Pisa, Pisa, [3]Department of biomolecular sciences, University of Urbino Carlo Bo, Urbino, [4]AOU Policlinico Vittorio Emanuele, Divisioni clinicizzata di ematologia con trapianto di midollo osseo, Catania, [5]AOU Città della scienza e della Salute di Torino, Hematology, Torino, [6]Hematology, Cardarelli Hospital, Napoli, [7]AOU Careggi, Unità funzionale di Ematologia, Firenze, Italy

Background: Despite the high efficacy of Nilotinib in chronic myeloid leukemia (CML), there is still a significant proportion of patients who fail to maintain a major molecular response (MMR, MR3). For those patients, treatment-free remission once in deep molecular response (DMR), which is the final goal of nilotinib treatment, will never be an achievable endpoint. Little is known about the impact of pharmacogenetic variability in influencing MMR/DMR. It has been reported that ABC multidrug transporters, responsible for multi-drug resistance, interact with tyrosine kinase inhibitors (TKIs). However, data are missing about how genetic variants in ABC genes may modify pharmacological proprieties of second generations TKIs.

Aims: We investigated the impact of 5 single nucleotide polymorphisms (SNP) in three ABC transporter genes, namely *ABCC1*, *ABCC2*, and *ABCB1*, on achieving and maintaining molecular response (MMR, DMR) in CML patients treated with nilotinib.
Methods: We prospectively genotyped 71 CML patients treated at 5 Italian sites. The following SNPs in ABC multi-drug transporter genes were studied: ABCC1rs212090, ABCC2rs3740066, ABCC2rs4148386, ABCC2rs1885301, and ABCB1rs13435. Genomic DNA was analyzed by High Resolution Melting assay and pyrosequencing. All patients gave informed consent before entering the study, that was approved by Institutional Ethical Committees. Hardy-Weinberg equilibrium was verified for all examined SNPs. Difference in genotype and allele's distributions among the patients and the associations of the various genotype with response, resistance o lost of response to Nilotinib were determined using the Fisher's exact test. The associations between SNPs status and progression free survival (PFS) were assessed using Kaplan-Meier method and Log-rank test.
Results: The characteristics of patients are listed in Table 1. MR3, MR4 and MR 4.5 were achieved by 90%, 59% and 48% of patients, respectively. Analysis of the difference in genotype distribution and alleles frequencies in responders and non-responders to Nilotinib showed that ABCC2 3972C>T SNPs (rs3740066) significantly impact on the loss of MR3, in dominant and codominant model (P=0.02 and P=0.01 respectively). Multivariate analysis in ongoing.

Table 1.

Clinical characteristic	CML patients
Gender	
Male	40
Female	31
Age at diagnosis	
median age (range)	50 (18-75)
18-50 years	37
=50-74 years	34
Nilotinib Treatment	
I line	34
II line	37
Sokal Score	
Low	37
Intermediate	28
High	6
Hasford score	
Low	37
Intermediate	28
High	6
Eutos score	
0	1
1	66
2	4
Best Molecular Response To Nilotinib	
MR3	90%
MR4	59%
MR4.5	48%

Summary and Conclusions: Our study hypothesizes, for the first time, that genetic variants in ABC genes may increase the number of patients not able to maintain a sustained MR3 with Nilotinib, thus limiting the number of patients able to achieve treatment-free remission. Further studies in lager series are warranted to confirm our preliminary experience.
Acknowledgements: The study was supported in part by AIL Pesaro Onlus.

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MYELOID CELL POPULATION DYNAMICS IN TKI-TREATED CML

S. Sopper[1,*], B. Hofer[1], S. Mustjoki[2], K. Porkka[2], J. Janssen[3], G. Ossenkoppele[3], B. Gjertsen[4], D. Wolf[5]

[1]Hematology and Oncology, Medical University Innsbruck, Innsbruck, Austria, [2]Hematology, Helsinki University Hospital, Helsinki, Finland, [3]Hematology, VU University Medical Center, Amsterdam, Netherlands, [4]Institute of Medicine, Hematology Section, University of Bergen, Bergen, Norway, [5]Hematology, Oncology and Rheumatology, University Hospital of Bonn, Bonn, Germany

Background: Immune mechanisms may impact treatment response of CML patients to TKI therapy and the success of therapy cessation. Myeloid populations such as classical and non-classical monocytes and different types of myeloid-derived suppressor cells (MDSCs) are important immune-mediators but their role in CML has not been systematically investigated. Therefore, we comprehensively characterized these population in 52 chronic phase CML (CML-CP) patients as a substudy to the ENEST1st study (NCT01061177).
Aims: Determination of myeloid cell populations in plasma of newly diagnosed CML-CP patients in the course of tyrosine kinase inhibitor therapy and definition of immunological surrogates for response prediction in CML-CP patients.
Methods: Whole blood was collected prior to and after 6 and 12 months of nilotinib therapy for immune-phenotyping by 9-color flow cytometry employing 6 antibody panels. Levels of circulating soluble factors were measured by immunoassays in plasma samples. Molecular response was quantified in central EUTOS reference laboratories. Changes in immune parameters were correlated to clinical and molecular endpoints.
Results: At diagnosis, the relative proportion of monocytes was lower ($p<0.0001$) whereas the absolute number was higher ($p<0.0001$) when compared to follow up samples at months 6 and 12 of therapy. Both, the CD16+CD14hi "intermediate" ($p<0.01$) and the CD56+ subset ($p<0.0001$) of monocytes were higher at baseline. $CD15^+$ polymorphnuclear and $CD15^-$ early stage MDSC, defined as Lin^-, $CD11b^+$ and $CD33^+$ cells according to recently published protocols (Bronte V *et al.*, Nat Comm 2016) were strongly increased at diagnosis ($p<0.0001$). However, a more detailed flow cytometric analysis revealed that these cells are identical to neutrophils and basophils, respectively. Monocytic MDSC are defined as CD14+ cells with low to negative HLA-DR expression. We therefore quantified HLA-DR expression levels on CD14+ monocytes, which increased ($p<0.05$) after initiation of therapy. Interestingly, HLA-DR expression at diagnosis negatively correlated with $BCR\text{-}ABL^{IS}$ levels throughout therapy ($p<0.05$). Cumulative response rates for MMR were higher in patients with high HLA-DR expression ($p<0.001$). HLA-DR expression negatively correlated with plasma levels of sCD62L, a predictive marker recently defined (Sopper *et al.*, J Clin Oncol 2017), but not with Arginase, a key enzyme for MDSC function.
Summary and Conclusions: Our comprehensive study demonstrates that several myeloid cell populations are substantially altered in CML patients. Specifically, a low HLA-DR expression on monocytes, associated with immunosuppressive functions, may have a negative impact on anti-leukemic immune response and may constitute a biomarker for response prediction to TKI therapy.

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THE EFFECT OF PREGNANE X RECEPTOR SNP AND TKI TROUGH CONCENTRATION ON CLINIC RESPONSE AND TOXICITY AMONG CHINESE CML PATIENTS

Z. Jiang[1,*], P. Xu[2], X. Zhao[1], Y. Zhu[2], Q. Wang[2], J. Zeng[2], Q. He[1]

[1]Department of Hematology, Central South University, Xiangya Hospital, [2]Department of Pharmacy, Central South Univeristy, the Second Xiangya Hospital, Changsha, China

Background: Imatinib mesylate (IM) has dramatically improved the outcomes of Chronic myeloid leukemia (CML) patients, with a significant effect on both quality of life and long-term survival rate. However, many patients still suffered from suboptimal response leading to treatment failure. Several mechanisms that lead to IM resistance have been investigated, including the suggestion that pharmacogenetic variability influences the pharmacokinetics of IM. On the other hands, trough concentration (C_0) of IM might play a role in the different outcomes of imatinib therapy and could serve as predictive markers but the results of different studies remain controversy. We had performed a early meta-analysis that revealed that the patients who achieved MMR have significantly higher C_0 of imatinib than those who failed to achieve MMR. We also found the patients achieved CCyR have significantly higher C_0 of imatinib than those not achieved CCyR (Figure 1). However, the reason behind the pharmacokinetic inter-individual variation is still unknown.
Aims: 1. To study the correlation between trough concentration and drug effect including toxicity and clinic response among Chinese CML patients. 2. To investigate the possible pharmacogenetic variation that could affect the IM metabolism then influence the clinic response or drug toxicity.
Methods: A total of 171 chinese chronic phase CML patients (97 males and 74 males) were enrolled in this study. All patients were initially orally administrated 400 mg per day and dose reduction was allowed for patients with severe adverse events. The steady-state trough concentrations of imatinib were determined by ultra performance liquid chromatography-mass spectrometry. Clinic response including CCyR, MMR and CMR was evaluated according to NCCN guidelines. Hematological toxicity was recorded and graded. Total genomic DNA was extraced and genotyping of CYP3A4, ABCB1, ABCG2, *SLCO1B3* and PXR was determined by using polymerase Chain Reaction (PCR) and then Sanger sequencing.
Results: The mean trough concentrations of the study population (109 patients available) was 1287.21±652.59 ng/mL, widely ranging from 318.83 to 3433.58 ng/mL. Association between imatinib trough concentrations and response was analyzed in patients orally administrated 400mg/day.

Patients with CCyR, MMR and CMR achieved significantly higher trough concentrations than those without these corresponding efficacy (1478.18±659.83 *vs* 984.89±454.06 ng/mL; 1486.40±703.38 *vs* 1121.17±527.14 ng/mL; 1528.00±709.98 *vs* 1112.67±518.35ng/mL, respectively Figure 1). This study also revealed patients with thrombocytopenia had significantly lower trough concentrations than those without thrombocytopenia (910.47±568.75 *vs* 1585.47±757.47 ng/mL, *p*=0.002 Figure 1).This result confirmed the relationship between metabolism of TKIs and effect of treatment as well as toxicity. We failed to find clinical significant results between CYP3A4, ABCB1, ABCG2, *SLCO1B3* genotype and trough concentrations and clinic response. However, we found rs3814055, a SNP of pregnane X receptor (**PXR**, **NR1I2**), showed significant higher trough concentration of imatinib (P=0.015) and dose-adjusted trough concentration of imatinib (P=0.039) than the wild-type. It was also showed the mutation group of PXR has significant more patients who achieved MMR(p=0.025) and CMR(P=0.022, Figure 1).

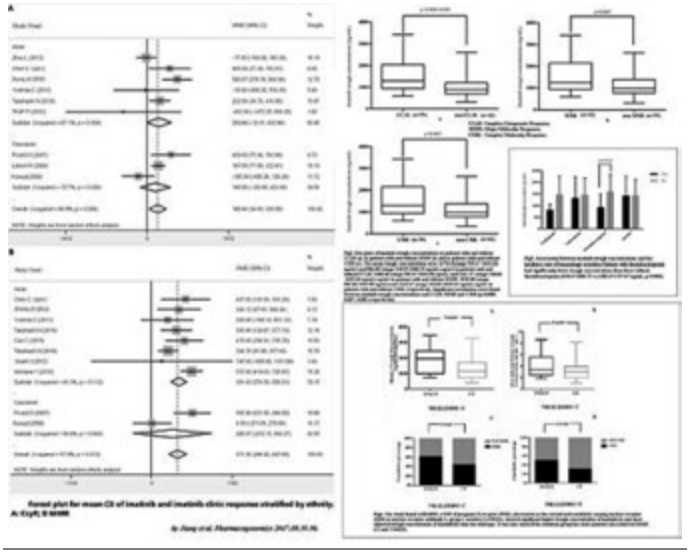

Figure 1.

Summary and Conclusions: This finding suggests nuclear receptors affect clinic response of imatinib by interfering drug metabolism and highlights the importance of investigation on the effect of nuclear receptors on TKI response among large cohort of CML patients.

PF367

WT1 EXPRESSION PREDICTS THE RESPONSIVENESS OF CHRONIC MYELOID LEUKEMIA TO FIRST LINE THERAPY OF TYROSINE KINASE INHIBITOR

E. Toffoletti[1,*], G. Maccari[1], M. Tiribelli[1], A. Chiarvesio[2], S. Di Giusto[2], L. Floreani[1], D. Damiani[1], R. Fanin[1]

[1]Department of Experimental and Clinical Medical Sciences, Division of Hematology and BMT, University of Udine, [2]Hematological Division of Udine, Azienda Sanitaria Universitaria Integrata di Udine, Udine, Italy

Background: The prognosis of Chronic Myelogenous Leukemia (CML) profoundly changed by the advent of Tyrosine Kinase Inhibitors (TKIs). Unfortunately, the biological factors affecting therapeutic response at the first line of TKIs remain under investigation and not all cases respond optimally to the therapy. BCR-ABL oncoprotein supports malignant hematopoiesis by multiple effects, mainly by promoting cell proliferation, abnormal cell adhesion and resistance to many anti-apoptotic stimuli. BCR-ABL induces WT1 expression and it is known that increasing levels of WT1 correlate with an advanced phase of disease. Recent evidence demonstrates that the anti-apoptotic effect of BCR-ABL is driven by a WT1-mediated pathway.
Aims: The aim of our study was to investigate the WT1 expression levels at onset and the response to first line TKI, in CML patients.
Methods: We studied WT1 expression levels retrospectively in 59 consecutive CML patients, followed-up from our Division from 2012 to 2016. For each patients, clinical scores, BCR-ABL type transcript, BCR-ABL IS levels and WT1 gene expression were collected on peripheral blood, at onset. Response to therapy was defined as European Leukemia Net reccomendations.
Results: We observed optimal response in 60% (35/58) of cases and warning/failure in 40% (23/58) of cases. The median age was 56 years (range 17-81 years). The majority of patients had a low EUTOS score (94% of patients) and 83% of cases had low/intermediate Sokal score. The optimal responding group showed a median expression level of WT1 equal to 94 copies/10000 ABL copies, significantly lower than the WT1 expression level of the warning/failure group (271 copies/10000 ABL copies, p=0,0024). B2a2 rearrangement was detected in 50% of cases in the warning/failure group, and in only 33% in optimal responder group. The BCR-ABL levels at onset were similar in optimal and warning/failure group (73,99%IS *versus* 63,86%IS) with no significant difference. In both groups the frequency of second generation administered TKI as first treatment was similar (respectively 40% *versus* 43% of cases). Moreover, all patients with WT1 expression level, at onset, lower than 100 copies/10000ABL copies turned out to be optimal responders. In low expressing WT1 samples, the b2a2 rearrangement was less frequent than in high expressing WT1 group (33% *versus* 44%). Finally, cases receiving a second generation TKI at onset were 23% in low expressing WT1 patients and 48% in high expressing WT1 patients.
Summary and Conclusions: In our cohort, WT1 expression levels, at onset, were shown to be correlated with the response to TKIs. Moreover, our data confirm the correlation between b2a2 rearrangement and the unfavorable response. Finally, the level of 100 copies of WT1 on 10000 ABL copies seems to be a crucial cut-off to guide the physician in therapy administration and CML monitoring.

Chronic myeloid leukemia – Clinical

PF368

LONG-TERM TREATMENT-FREE REMISSION (TFR) IN PATIENTS (PTS) WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) FOLLOWING FRONTLINE (1L) NILOTINIB (NIL): RESULTS FROM ENESTFREEDOM

G. Saglio[1,*], T. Masszi[2], M.T. Gómez Casares[3], A. Hellmann[4], J. Stentoft[5], E. Conneally[6], V. Garcia Gutierrez[7], N. Gattermann[8], B. Martino[9], S. Saussele[10], P. le Coutre[11], F.J. Giles[12], D.M. Ross[13], J.P. Radich[14], M. Sondhi[15], S. Acharyya[15], S. Chaturvedi[15], V. Bédoucha[16], A. Hochhaus[17]

[1]University of Turin, Orbassano, Italy, [2]Semmelweis University, Budapest, Hungary, [3]Hospital Universitario de Gran Canaria Dr Negrín, Las Palmas de Gran Canaria, Spain, [4]Medical University of Gdańsk, Gdańsk, Poland, [5]Aarhus University Hospital, Aarhus, Denmark, [6]St James's Hospital, Dublin, Ireland, [7]Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain, [8]Universitätsklinikum Düsseldorf, Düsseldorf, Germany, [9]Azienda Ospedaliera Bianchi Melacrino Morelli, Reggio Calabria, Italy, [10]III. Med. Klinik, Medizinische Fakultät Mannheim der Universität Heidelberg, Mannheim, [11]Charité - Universitätsmedizin Berlin, Berlin, Germany, [12]Developmental Therapeutics Consortium, Chicago, United States, [13]SA Pathology, Adelaide, Australia, [14]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, [15]Novartis Pharmaceuticals Corporation, East Hanover, United States, [16]Novartis Pharma AG, Basel, Switzerland, [17]Abteilung Hämatologie/Onkologie, Universitätsklinikum Jena, Jena, Germany

Background: ENESTfreedom (NCT01784068) is the first dedicated study evaluating pts with sustained deep molecular response (DMR) on 1L NIL for achievement of TFR, a new treatment goal in CML. Previous results from ENESTnd showed that more pts achieved sustained DMR (per ENESTfreedom criteria) by 6 y with 1L NIL (37.9% [300 mg BID]) *vs* 1L imatinib (IM; 21.6%), suggesting that more newly diagnosed pts may reach TFR eligibility with NIL.
Aims: In ENESTfreedom, TFR rates of 51.6% at 48 wk and 48.9% at 96 wk have been reported; here we present a long-term (144-wk) analysis of the durability and safety of TFR.
Methods: Pts with ≥2 y of 1L NIL who achieved $MR^{4.5}$ (*BCR-ABL1* ≤0.0032% on the International Scale [$BCR\text{-}ABL1^{IS}$]) entered a 1-y NIL consolidation phase; those with sustained DMR could enter the main TFR phase. Pts not eligible to enter the main TFR phase received another 1 y of NIL and, if sustained DMR was achieved, they could enter the TFR-2 phase. Pts in either TFR phase restarted NIL after loss of major molecular response (MMR; $BCR\text{-}ABL1^{IS}$ ≤0.1%). Data cutoff was Oct 11, 2017, by which all pts who entered the main TFR phase had completed ≥144 wk of TFR, restarted NIL, or discontinued the study. All pts gave informed consent.
Results: At the data cutoff, 89/190 pts in the main TFR phase remained in TFR (46.8%; 95% CI, 39.6%>54.2%); 94 pts (49.5%) lost MMR (91 restarted NIL) and 7 (3.7%) discontinued TFR for other reasons. Only 4/93 pts in TFR at 96 wk were no longer in TFR at 144 wk; 3 of these first lost $MR^{4.5}$ within the first 8 wk of TFR. Of 91 pts who restarted NIL, 90 (98.9%) regained MMR and 84 (92.3%) regained $MR^{4.5}$ (1 discontinued study without MMR, 5 discontinued with MMR but not $MR^{4.5}$, 1 remained in reinitiation phase with MMR but not $MR^{4.5}$). Of 84 pts who regained $MR^{4.5}$, 70 (83.3%) had stable $MR^{4.5}$ 48 wk later, 11 (13.1%) discontinued the study <48 wk after regaining $MR^{4.5}$, and 3 (3.6%) remained on NIL with <48 wk of follow-up after regaining $MR^{4.5}$. At the data cutoff, 5/10 pts (50%) who entered the TFR-2 phase remained in that phase; the other 5 lost MMR and restarted NIL (4 regained $MR^{4.5}$, 1 regained MR^{4}). TFR rate among these 10 pts was 40% at both 48 wk (1 pt had missing 48-wk data) and 96 wk (1 pt had <96 wk of follow-up). Including all pts who attempted TFR (after 1 or 2 y of consolidation), the overall TFR rate was 51% (102/200) at 48 wk and 48.5% (97/200) at 96 wk. No pt had disease progression. There were 10 deaths: 2 during consolidation (1 cardiac arrest, 1 suicide), 1 during TFR (unknown cause), 4 during reinitiation (1 acute myocardial infarction, 1 respiratory failure, 1 hepatobiliary cancer, 1 unknown cause), 3 >30 d after discontinuing study in the TFR phase (1 mesothelioma) or reinitiation phase (1 transitional cell cancer of renal pelvis and ureter, 1 unknown cause). 144-wk treatment-free survival (TFS) rate (based on main TFR phase) was 48.7% (95% CI, 41.4%>55.6%; Figure 1). Among 94 pts remaining in TFR for >96 wk, any-grade AE rates were 85.1%, 76.6%, 69.1%, and 45.7% in consolidation and the first, second, and third 48 wk of TFR, respectively; any-grade musculoskeletal pain–related AE rates were 16.0%, 40.4%, 9.6%, and 4.3%. Cardiovascular event rates were low across these periods.

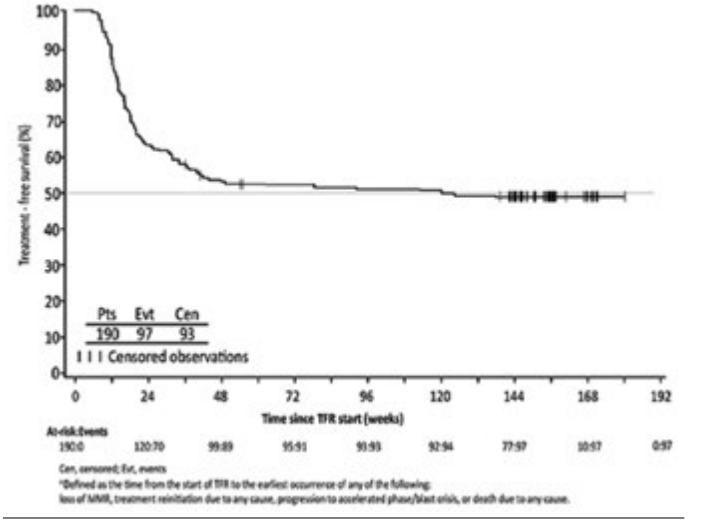

Figure 1. Treatment-free survival*.

Summary and Conclusions: These results support the long-term durability and safety of TFR following 1L NIL, with no disease progressions or deaths attributable to CML. Together with ENESTnd data showing higher sustained DMR rates with 1L NIL *vs* IM, these findings suggest more pts may be able to attempt and achieve TFR with 1L NIL.

PF369

BOSUTINIB *VERSUS* IMATINIB FOR NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN THE BFORE TRIAL: 24-MONTH FOLLOW-UP

C. Gambacorti-Passerini[1,*], M. Mauro[2], M. Deininger[3], C. Chuah[4], D.-W. Kim[5], V. Kota[6], J. Lipton[7], P. Rousselot[8], D. Milojkovic[9], P. le Coutre[10], V. Garcia-Gutierrez[11], R. Crescenzo[12], E. Leip[13], A. Viqueira[14], N. Bardy-Bouxin[15], A. Hochhaus[16], T. Brümmendorf[17], J. Cortes[18]

[1]University of Milano-Bicocca, Monza, Italy, [2]Memorial Sloan Kettering Cancer Center, New York, [3]University of Utah, Salt Lake City, United States, [4]Singapore General Hospital, Duke-NUS Medical School, Singapore, Singapore, [5]Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea, Republic Of, [6]Winship Cancer Institute, Emory University, Atlanta, United States, [7]Princess Margaret Cancer Centre, Toronto, Canada, [8]Hôpital Mignot, Université de Versailles Saint-Quentin-en-Yvelines, Versailles, France, [9]Hammersmith Hospital, Imperial College, London, United Kingdom, [10]Charité-Universitätsmedizin Berlin, Berlin, Germany, [11]Hospital Universitario Ramón y Cajal, Instituto Ramón y Cajal de Investigación Sanitaria, Madrid, Spain, [12]Pfizer Inc, Collegeville, [13]Pfizer Inc, Cambridge, United States, [14]Pfizer Spain, Madrid, Spain, [15]Pfizer International Operation-Oncology, Paris, France, [16]Klinik für Innere Medizin II, Universitätsklinikum Jena, Jena, [17]Universitätsklinikum RWTH Aachen, Aachen, Germany, [18]University of Texas MD Anderson Cancer Center, Houston, United States

Background: Bosutinib is a dual Src/Abl tyrosine kinase inhibitor approved for the treatment of newly diagnosed chronic phase (CP) chronic myeloid leukemia (CML) and CML resistant/intolerant to prior therapy.
Aims: Here we compare efficacy of first-line bosutinib and imatinib after ≥24 months (median: 27 months) of follow-up.
Methods: In the ongoing, open-label, phase 3 BFORE trial (NCT02130557), 536 patients were randomized 1:1 to bosutinib (n=268) or imatinib (n=268 [3 untreated]).
Results: Higher molecular and complete cytogenetic response (MR and CCyR) rates were observed for bosutinib *vs* imatinib at 12 months; these differences continued after ≥24 months (Table 1). The between-arm difference in major MR (MMR) rate was retained at 24 months; however, differences in rates of deeper MRs (MR^{4} and $MR^{4.5}$) were smaller. Times to MR and CCyR were shorter for bosutinib *vs* imatinib, consistent with 12-month data. In an analysis of event-free survival, 14 patients in the bosutinib arm and 17 in the imatinib arm had on-treatment events, with some patients experiencing multiple events. Events for bosutinib *vs* imatinib included death (1 *vs* 4), transformation to accelerated/blast phase (6 *vs* 7), loss of CCyR (3 *vs* 5), loss of complete hematologic response (CHR; 1 *vs* 3), and doubling of white blood cell count (confirmed by 2 values >20 × 10^9/L at least 1 month apart) for patients not achieving a CHR (4 *vs* 0). 71% *vs* 66% remained on bosutinib *vs* imatinib treatment.
Summary and Conclusions: At 24 months, a higher MMR rate was main-

tained with bosutinib *vs* imatinib, confirming the superior efficacy of bosutinib. The results continue to support the use of bosutinib as first-line therapy for CP CML.

Table 1.

	Intent-to-treat Population		
	Bosutinib n=268	Imatinib n=268	P*
Cumulative (any time on-treatment), %			
MMR	68.7	59.3	.024
MR[4]	39.9	31.3	.040
MR[4.5]	25.7	19.0	.063
CCyR†	82.5	76.8	.113
MMR by 24 months, %	67.2	57.5	.020
MMR, %			
At 12 months	46.6	36.2	.013
At 24 months	61.2	50.7	.015
MR[4], %			
At 12 months	20.5	11.6	.005
At 24 months	32.8	25.7	.073
MR[4.5], %			
At 12 months	7.5	3.0	.020
At 24 months	13.1	10.8	.428
Time to response (based on cumulative incidence), hazard ratio‡			
MMR	1.37		.004
CCyR†	1.34		.005
MR[4]	1.39		.025
MR[4.5]	1.42		.054
Overall survival,§ %			–
At 12 months	99.6	98.1	
At 24 months	99.2	97.0	

* 2-sided *P* values not adjusted for multiple comparisons; *P* value for overall survival not provided until 5-year analysis

† Modified intent-to-treat population (bosutinib n=246; imatinib n=241); includes Philadelphia chromosome-positive patients with e13a2/e14a2 transcripts

‡ Bosutinib vs imatinib; hazard ratio >1 indicates shorter time to response for bosutinib

§ 3 and 9 deaths in the bosutinib and imatinib arm, respectively, due to adverse event related (0 vs 1) or unrelated (2 vs 2) to study drug, disease progression (1 vs 3), and other causes (0 vs 3)

PF370

PREVALENCE AND OUTCOMES OF UNCOMMON BCR-ABL FUSION TRANSCRIPTS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA: DATA FROM A SINGLE CENTER

Y.-Z. Qin[1,*], Q. Jiang[1], H. Jiang[1], Y.-Y. Lai[1], H.-X. Shi[1], X.-J. Huang[1]

[1]Peking University People's Hospital, Peking University Institute of Hematology, Beijing, China

Background: Uncommon BCR-ABL junctions are observed in very few patients with chronic myeloid leukemia (CML). The data on the prevalence of uncommon BCR-ABL transcripts are limited. Moreover, response and outcomes have not been well-established in the era of tyrosine kinase inhibitors (TKIs).
Aims: To explore the types, prevalence and outcomes in CML patients with uncommon BCR-ABL transcripts receiving TKI therapy.
Methods: Data for 4750 CML patients were retrospectively reviewed. The patients were screened for uncommon BCR-ABL transcripts by multiplex polymerase chain reaction (PCR), fusion types were identified by Sanger sequencing, and type-specific real-time quantitative PCR was performed for molecular monitoring. Responses and outcomes were compared between newly diagnosed patients in chronic phase (CP) with uncommon BCR-ABL transcripts and patients with the common e13a2/e14a2 transcript receiving frontline imatinib therapy during the same period.
Results: A total of 19 uncommon BCR-ABL transcripts, including e1a2, e1a3, e6a2, e8a2, e12a2, unusual e13a2, e13a3, unusual e14a2, e14a3 and e19a2, were identified in 83 (1.7%) patients. The most frequent type was e19a2 (39.7%), followed by e13a3/e14a3 (20.5%) and e1a2 (16.9%). These transcripts accounted for three-quarters of cases with uncommon transcripts. Compared with the 571 newly diagnosed CP patients with common transcripts receiving frontline imatinib therapy, patients with the e19a2 (n=16) and e1a2 (n=11) transcripts had significantly reduced probabilities of 1-year complete cytogenetic response (CCyR, 44.4% *versus* 87.9%, p=0.0004 and 58.0% *versus* 87.9%, p=0.016, respectively, Figure 1A) and major molecular response (MMR, defined as a 3 log reduction of BCR-ABL from individual baseline level for patients with uncommon transcripts, 6.3% *versus* 48.6%, p=0.0018 and 0% *versus* 48.6%, p=0.0035, respectively, Figure 1B), and patients with the e13a3/e14a3 transcript (n=10) had significantly increased probabilities of 1-year CCyR (100% *versus* 87.9%, p=0.0072, Figure 1A) and MMR (83.3% *versus* 48.6%, p=0.0073, Figure 1B). With a median follow-up of 23 (range, 3-115) months, patients with the e19a2 transcript had low probabilities of 2-year event-free survival (EFS, 69.1% *versus* 94.4%, p=0.0004, Figure 1C) and progression-free survival (PFS, 76.6% *versus* 95.7%, p=0.0067, Figure 1D), and patients with the e1a2 transcript had low probability of 2-year EFS (30.3% *versus* 94.4%, p<0.0001, Figure 1C). However, patients with the e13a3/e14a3 transcript had similar outcomes as patients with common transcripts (Figure 1C and D).

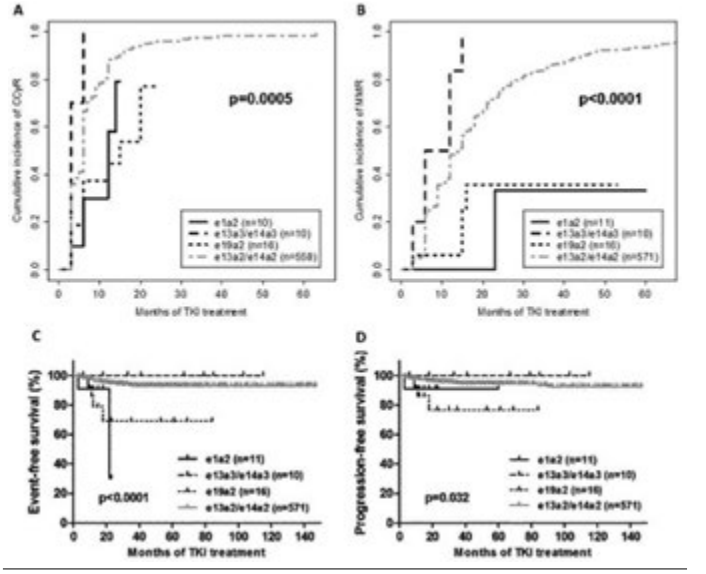

Figure 1.

Summary and Conclusions: Uncommon BCR-ABL fusion transcripts are rare and diverse in patients with CML and may be relevant for TKI therapy outcomes. Our data suggested that the identification uncommon BCR-ABL transcripts is essential at presentation to aid in diagnosing, appropriate monitoring and guiding treatment choices for patients with CML.

PF371

UPDATED RESULTS FROM THE ONGOING PHASE I STUDY OFPF-114 MESYLATE IN PATIENTS WITH CML WITH FAILURE OF PRIOR TKI THERAPY

A. Turkina[1,*], O. Shukhov[1], E. Chelysheva[1], I. Nemchenko[1], A. Petrova[1], A. Bykova[1], A. Zaritskey[2], E. Lomaia[2], N. Siordiya[2], O. Vinogradova[3], V. Shuvaev[4], E. Shatokhina[5], F. Novikov[6], V. Shulgina[6], G. Chilov[6]

[1]National Research Center for Hematology, Moscow, [2]Almazov Medical Research Centre, Saint-Petersburg, [3]Botkin Hospital, Moscow, [4]Russian Research Institute of Hematology and Transfusiology, Saint-Petersburg, [5]Federal State Budget Institution of Additional Postgraduate Education "Central State Medical Academy" Department for Presidential Affairs of the Russian Federation, [6]Fusion Pharma, Moscow, Russian Federation

Background: PF-114 is a third-generation oral tyrosine kinase inhibitor (TKI), which, according to pre-clinical studies, blocks native and mutated *Bcr-Abl* forms, including the T315I mutant. Currently PF-114 undergoes phase I clinical trial in patients with chronic or accelerated phase Ph+ CML who are resistant to at least one of the 2nd generation TKIs or intolerant to previous treatment with TKIs or who have T315I mutation (NCT02885766).
Aims: The primary objective of the current Phase I is to study the dose-limiting toxicities (DLTs) occurring in 1-st 28-day cycle of treatment and determine the maximum tolerated dose (MTD). Secondary objectives include safety, pharmacokinetics, anti-CML activity (based on hematologic, cytogenetic, and molecular assessments).
Methods: The trial design is a classical 3+3 dose escalation till the MTD followed by expanded cohorts planned for dose(s) below the MTD. The total expected enrollment is 65 patients. Treatment on a continuous QD regimen is being continued until disease progression, unacceptable toxicity, consent withdrawal, or death. Adverse events (AEs) are assessed and graded according to NCI-CTCAE v4.03.
Results: At data cut-off on January 24th, 2018, six dose-cohorts – 50mg, 100mg, 200mg, 400mg, 500mg and 600mg have completed cycle 1 and 750mg cohort had been opened for enrollment. Overall 27 patients (11 males) had been initially enrolled at the following doses: 50mg (n=3), 100mg (n=3), 200mg (n=3), 400mg (n=12), 500mg (n=3), 600mg (n=3), and the MTD has not yet been reached. A total of 13 patients with T315I mutation

were included into the trial thus far. Median age was 50 years (range, 30-66 years). Median time from diagnosis to treatment was 11 years (range, 20-1 years). All patients had baseline ECOG performance status 0-1. Patients were heavily pretreated: 14 (51,9%) had received ≥3 prior TKIs, 8 (29,6%) had received 2 prior TKIs and 5 (18,5%) patients with T315I had received 1 prior TKI. Intra-patient dose escalations occurred during the study in case the starting dose was well tolerated for at least 1 cycle and dose escalation seemed appropriate from the curative perspective. Median duration of treatment at each dose (including intra-patient dose escalations) comprised: 3 cycles for 50mg, 4 cycles for 100mg, 9 cycles for 200mg, 4 cycles for 400mg; 2 cycles for 500mg; 3 cycles for 600mg. In 10 patients the treatment is ongoing at doses 200-600mg, and 17 patients discontinued: lack of efficacy n=8, AEs n=4, patients' decision n=5. The pharmacokinetics of PF-114 was dose-proportional. So far, a single case of DLT was observed at the dose of 400mg – grade 3 erythematous rash. No non-hematologic AEs of grade≥3 occurred on doses below 400mg. Most common grade 3 AE on dose≥400mg was dermatologic toxicity (6/20). Other cases of grade 3 toxicities were presented by toxic hepatitis (n=1), neutropenia (n=2) and thrombocytopenia (n=2). No deterioration of the ankle-brachial index, which is being prospectively assessed during the trial, or vascular occlusive events were observed. The major cytogenetic response (MCyR) was obtained in 4 of 12 patients who completed 3 cycles and were not in MCyR at enrollment: 2 cases of complete CyR and 2 – partial CyR; 3 of responders had a T315I mutation

Summary and Conclusions: PF-114 exhibits antitumor activity in heavily pretreated patients with resistant forms of CML. The evaluation of the safety profile and efficacy continues. The dose escalation stage of the trial continues, while the enrollment of patients into expanded cohorts at doses below MTD has already started

PF372

VARIATION IN LIMIT OF BLANK FOR BCR-ABL1 DETECTION BETWEEN LABORATORIES IMPACTS ON SCORING OF DEEP MOLECULAR RESPONSE

H. White[1,2,*], M. Salmon[1], M.T. Bochicchio[3], J.-M. Cayuela[4], D. Colomer[5], F. Daraio[6], S. Dulucq[7], B. Izzo[8], T. Lion[9], K. Machova Polakova[10], J. Maier[11], K. Mokretar[12], R. Talmaci[13], M. Zawada[14], A. Hochhaus[15], N. Cross[12]

[1]Wessex Regional Genetics Laboratory, Salisbury NHS Foundation Trust, Salisbury, [2]Faculty of Medicine, University of Southampton, Southampton, United Kingdom, [3]Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy, [4]Laboratoire d'Hématologie, Hôpital Saint-Louis, Paris, France, [5]Hematopathology Unit, Hospital Clinic of Barcelona, Barcelona, Spain, [6]Department of Clinical and Biological Science, University of Turin, Turin, Italy, [7]Laboratoire d'hématologie-Biologie moléculaire, CHU Bordeaux, Bordeaux, France, [8]Department of Clinical Medicine and Surgery, University 'Federico II' of Naples, Naples, Italy, [9]Children's Cancer Research Institute, LabDia Labordiagnostik and Medical University, Vienna, Austria, [10]Institute of Hematology and Blood Transfusion, Prague, Czech Republic, [11]Abteilung für Hämatologie und internistische Onkologie, Universität Leipzig, Leipzig, Germany, [12]Department of Haematology, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom, [13]Hematology Department,Fundeni Clinical Institute, University of Medicine and Pharmacy 'Carol Davila', Bucharest, Romania, [14]Hematology Department, Jagiellonian University, Krakow, Poland, [15]Abteilung Hämatologie/Onkologie, Klinik für Innere Medizin II, University of Jena, Jena, Germany

Background: Accurate and reproducible scoring of deep molecular response (DMR) is critical for selection and monitoring of CML patients who may be eligible to stop tyrosine kinase inhibitor treatment. The current *BCR-ABL1* RT-qPCR molecular response guidelines assume that all laboratories are able to detect *BCR-ABL1* with maximal efficiency. However varying limits of blank (LoB) and limits of detection (LoD) between centers may result in variation in the way that DMR is scored. In particular, efforts by labs to increase the sensitivity of their tests could increase the false positive rate leading to detection of *BCR-ABL1* in samples that are negative (variation in LoB). Laboratory LoBs have not been examined systematically to date because of a lack of suitable control reagents and agreed methodology.
Aims: The aim of the project was to determine the LoB for *BCR-ABL1* RT-qPCR assays carried out in experienced EUTOS laboratories (n=12). The LoB is defined as the highest measurement result that is likely to be observed for a negative sample *i.e.* the likelihood of reporting a false positive *BCR-ABL1* result at a defined probability (α).
Methods: To determine the LoB the Clinical and Laboratory Standards Institute guidelines [EP17-A2; 2012] recommend the following minimum requirements: test 4 negative samples, using 2 reagent lots of RT-qPCR master mix, on 1 instrument, on 3 independent days, analysing 2 replicates per sample, generating 60 blank replicate results per reagent lot. Prior to the study, a questionnaire was sent to all labs to determine sample requirements (lysis type and volume). Fresh (<48hrs), 4ml non-leukaemic peripheral blood samples (n=360) were processed and pooled to generate 8x *BCR-ABL1* negative lysates with *ABL1* copy numbers comparable to clinical samples (Trizol n=4, RLT n=4). These samples (n=4) were provided to each lab and were used to generate RNA and cDNA to perform 18 RT-qPCR replicates (15x *BCR-ABL1*, 3x *ABL1*) per sample, per reagent lot using their standard protocols.
Results: *BCR-ABL1* copy number results of all samples from each lab were ranked in order from lowest to highest (n=60). The rank position corresponding to the chosen probability of α (0.05) was calculated as 57.5. The LoB was defined as the highest measurement value of the sample at the given rank position across both lots. The results are summarised in the Table 1. For 75% of labs (n=9) the likelihood of a true negative sample giving a result greater than zero was ≤5%, i.e acceptable. However, for 25% of labs (n=3: labs 5, 8 and 11) the likelihood of a true negative sample giving a result greater than zero ranged from 10-50%.

Table 1.

Lab Number	1	2	3	4	5	6
Final BCR-ABL1 LoB (95%)	**0**	**0**	**0**	**0**	**2.57**	**0**
Total BCR-ABL1 replicates tested	120	120	120	120	120	120
Number negative BCR-ABL1 replicates	120	120	120	119	3	120
% negative BCR-ABL1 replicates	100	100	100	99.2	2.5	100
Max BCR-ABL1 copy number	0	0	0	1.44	4.25	0
Min BCR-ABL1 copy number	0	0	0	0	0	0
Mean ABL1	2.94E+04	2.68E+04	4.08E+04	1.27E+04	1.63E+04	3.35E+04
Type of sample	RLT	Trizol	Trizol	RLT	RLT	Trizol

Lab Number	7	9	8	10	11	12
Final BCR-ABL1 LoB (95%)	**0**	**0**	**2.35**	**0**	**0.41**	**0**
Total BCR-ABL1 replicates tested	120	90	120	120	120	120
Number negative BCR-ABL1 replicates	120	90	110	120	117	119
% negative BCR-ABL1 replicates	100	100	91.7	100	97.5	99.2
Max BCR-ABL1 copy number	0	0	2.9	0	2.67	2.15
Min BCR-ABL1 copy number	0	0	0	0	0	0
Mean ABL1	1.41E+04	5.75E+03	2.90E+04	7.77E+04	1.66E+04	7.11E+04
Type of sample	RLT	Trizol	RLT	Trizol	RLT	RLT

Summary and Conclusions: Defining the LoB (and LoD) of quantitative assays is important for assay validation and is necessary for accreditation of a diagnostic test to ISO 15189 (2012). This study provides a practical recommended protocol for determining the LoB for *BCR-ABL1* RT-qPCR assays. A major challenge of the study was the production of *BCR-ABL1* negative samples. Initially material was prepared from several '*BCR-ABL1* negative' human cell lines from independent sources but these showed very low level but reproducible amplification of *BCR-ABL1*. Therefore the use of cell line derived material for LoB studies is not recommended. Preparation of pools of blood samples from non-leukaemic patients was time consuming but provided good quality material for the study. The study showed that 25% of labs had a LoB greater than zero which may compromise the scoring of DMR and demonstrates the importance of establishing a LoB.

PF373

DIGITAL PCR IN PH+ CHRONIC MYELOID LEUKEMIA PATIENTS FOR RECOGNITION OF "STABLE" DEEP MOLECULAR RESPONSE AND IDENTIFICATION OF BEST CANDIDATES TO TKI DISCONTINUATION

M. Malagola[1,*], S. Bernardi[2], C. Zanaglio[2], A. Iurlo[3], C. Bucelli[3], N. Polverelli[1], E. Toffoletti[4], M. Tiribelli[4], E. Abruzzese[5], C. Deambrogi[6], G. Gaidano[6], L. Franceschini[7], M.T. Voso[7], M. D'Adda[8], M. Farina[8], D. Gramegna[8], E. Cerqui[8], F. Stagno[9], P. Vigneri[10], F. Re[2], E. Dereli Eke[2], F. Cattina[1], V. Cancelli[1], B. Rambaldi[1], A. Turra[1], E. Morello[1], D. Russo[1]

[1]Clinical and Experimental Sciences, Unit of Blood Disease and Stem Cell Transplantation, University of Brescia, ASST Spedali Civili of Brescia, [2]Deep Sequencing and Molecular Biology Unit, Chair of Hematology, University of Brescia, CREA Laboratory, ASST-Spedali Civili of Brescia, Brescia, [3]Hematology Division, IRCCS Ca' Granda-Maggiore Policlinico Hospital Foundation, University of Milan, Milano, [4]Division of Hematology and SCT, University of Udine, Udine, [5]Hematology, S. Eugenio Hospital, Roma, [6]Division of Hematology, Department of Translational Medicine, University of Eastern Piedmont, Novara, [7]Department of Biomedicine and Prevention, University of Rome Tor Vergata, Roma, [8]UO Ematologia, ASST Spedali Civili di Brescia, Brescia, [9]Division of Hematology and Bone Marrow Transplant, University of Catania, [10]Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy

Background: Treatment of CML with TKIs aims to achieve a major molecular response and to possibly obtain a Deep Molecular Response (DMR), to gain the opportunity for treatment discontinuation. Due to its intrinsic limitations qPCR cannot be considered the optimal tool for monitoring the DMR. The digital PCR (dPCR) has emerged as a more sensitive and accurate method to detect and monitor MRD.
Aims: This study aims to comparatively monitor the DMR of CML patients treated with TKIs by qPCR and dPCR to evaluate the suitability and reliability of the dPCR for an improved recognition of "stable" DMR and for a better selection of the best candidates for TFR.
Methods: Using qPCR and dPCR (QuantStudio 3D Digital PCR System by Life Technologies), we comparatively analyzed 495 peripheral blood samples from 130 CML patients with DMR. qPCR analyses were performed according to the last International Guidelines and absolute quantifications of BCR-ABL1 transcript obtained by dPCR were expressed as number of BCR-ABL1 copies/ul of reaction. Out of 130 cases, 62 (48%) with a minimum of 12 months of observation were analyzed for evaluation of the"stable"DMR. 25 out of 62 (40%) discontinued the TKIs treatment and were also monitored by q/dPCR until the DMR was lost. 68 cases were analyzed for evaluating the positive predictive value (PPV) of q/d PCR for TFR. They included the above reported 25 patients who were monitored for the "stable" DMR and discontinued the TKI treatment and other 43 cases who had only one q/d PCR evaluation before the discontinuation of TKI and the q/d PCR at the time of DMR loss.
Results: At the time of enrollment 52/130 (40%) and 78/130 (60%) patients belonged to MR 4.0 and MR 4.5-5.0, respectively. According to the cut-off value determined by the previous dPCR curve ROC analysis, the patients were divided in 2 groups, based on the first dPCR evaluation: group 1 (dPCR BCR-ABL1 >0.468 copies/ul) and group 2 (dPCR BCR-ABL1 <0.468 copies/ul). 72% and 75% of the patients with MR 4.0 and MR 4.5-5.0 falled in group 2, respectively. Patients belonging to the same MR class, as determined by qPCR, at the time of enrollment showed variable transcript levels of dPCR BCR-ABL1 copies/ul. While qPCR did not allow the identification of the patients with "stable" DMR, dPCR BCR-ABL1 <0.468 copies/ul helped to discriminate the patients with deeper and more stable DMR. Furthermore, the dPCR evaluation made in the 68 patients before the TKIs discontinuation, resulted highly predictive for TFR. Indeed, 17 out of 68 patients had a dPCR BCR-ABL1 >0.468 copies/ul and 9/17 (53%) lost DMR. 51 out of 68 had a dPCR BCR-ABL1 <0.468 copies/ul and 9/51 (17%) lost the DMR. Conversely, the PPV, meaning the probability to maintain the TFR, was 47% in the cases with dPCR BCR-ABL1 >0.468 copies/ul and 83% in the cases with dPCR BCR-ABL1 <0.468 copies/ul, respectively.
Summary and Conclusions: The results suggest that dPCR would be more accurate than qPCR in order to recognize the CML patients with stable DMR and the best candidates for TKI discontinuation.
Acknowledgments: Reseach supported by DPT of Clinical and Experimental Sciences founds and by Lions Association Bassa Bresciana, and BTL Foundation.

PF374

FIRST INTERIM ANALYSIS OF THE RUSSIAN MULTICENTER PROSPECTIVE STUDY RU-SKI: DISCONTINUATION OF TYROSINE KINASE INHIBITORS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA AND DEEP MOLECULAR RESPONSE

E. Chelysheva[1,*], A. Petrova[1], O. Shukhov[1], A. Bykova[1], I. Nemchenko[1], G. Gusarova[1], L. Gavrilova[2], G. Miliutina[3], L. Vysotskaia[4], A. Abdullaev[1], A. Sudarikov[1], I. Martynkevich[5], A. Golenkov[4], N. Tsyba[1], S. Kulikov[1], A. Turkina[1]

[1]National Research Center for Hematology, Moscow, [2]Mordovian Republican clinical hospital № 4, Saransk, [3]Bryansk Regional Hospital №1, Bryansk, [4]Moscow Regional Research and Clinical Institute n.a. M.F.Vladimirsky, Moscow, [5]Russuan Research Institute of Hematology and Transfusiology, Saint-Petersburg, Russian Federation

Background: Treatment-free remission (TFR) is becoming a new goal for patents (pts) with chronic myeloid leukemia (CML). Discontinuation of tyrosine kinase inhibitors (TKI) in CML pts with stable deep molecular response (DMR) requires a careful molecular monitoring. A limited financial support of molecular tests and a limited number of the standardized molecular laboratories in Russia are the main obstacles to apply the TFR approach into routine practice. Russian Healthcare Ministry has approved the TFR approach in CML patients within the Clinical Approbation (ID 18-10) based on the protocol of the prospective multicenter study RU-SKI.
Aims: To describe the CML pts enrollment into prospective study of TKI discontinuation in Russia and to evaluate the first results of survival without loss of major molecular response (MMR).
Methods: CML pts with chronic phase (CP), TKI therapy for at least 3 years and a stable DMR (MR4 or BCR-ABL<0,01%) for at least 2 years were enrolled. Pts with previous imatinib failure were eligible for trial inclusion. The BCR-ABL level was evaluated by RQ-PCR within international scale (IS). The molecular tests were done monthly during first 6 months (mo) after TKI cessation, every 2 mo from 6 to 12 mo and every 3 mo thereafter. Treatment by the same TKI was resumed in case of MMR loss (BCR-ABL>0,1%). The survival without MMR loss after TKI cessation was the primary endpoint. The analysis was performed by Kaplan–Meier method.
Results: During a period from Aug.2015 till Dec.2017 151 eligible CML pts were evaluated as candidates for inclusion into the RU-SKI trial. Of those 151 candidates 51 (33%) pts were declined and 100 (67%) pts were enrolled which fulfilled the expected number of the trial participants. The reasons of inclusion failure were refusal from trial due to fear of relapse in 39 (26%) pts, lack of molecular monitoring or insufficient duration of DMR in 10 (6,6%) pts, non confirmed DMR at screening in 2 (1,3%) pts. The baseline characteristics of the enrolled 100 pts were as follows: male: 48%; age at TKI cessation 46 years (range 22-80); Me duration of TKI therapy 8,3 years (range 3-16,2); Me duration of DMR 3,2 years (range 2-10,7). Social characteristics: graduate degree n=72 (72%), non-smokers n=78 (78%). Therapy before treatment stop: imatinib in 69(69%) pts, second-generation (2G) TKI in 31(31%) pts including nilotinib in 29 pts and dasatinib in 2 pts. In 10 of 31 pts 2G TKI were used as 1st line; in 21 pts as 2nd line. Nine pts received 2G TKI after imatinib failure, 12 pts after imatinib intolerance. Pretreatment by interferon was in 16 (16%) pts. Me follow-up time after TKI stop was 14 mo (range 1-31). Loss of MMR after TKI cessation occurred in 46 (46%) pts. Me time to MMR loss was 3 mo (range 1-15). TKI therapy was resumed in all 46 patients. The survival without MMR loss at 6, 12 and 24 months after TKI discontinuation was 56%, 51% and 48% respectively (Figure 1).

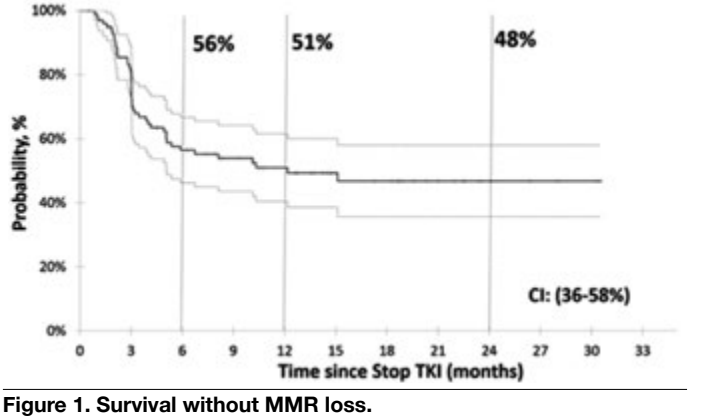

Figure 1. Survival without MMR loss.

Summary and Conclusions: The enrollment of CML pts with DMR into the first prospective trial evaluating the TFR approach in Russia was successful though a significant proportion of trial candidates was declined. The preliminary analysis of survival without MMR loss showed the results comparable to large international trials in a cohort of pts which was heterogeneous in terms of duration and lines of TKI therapy, icluding pts with previous resistance to imatinib. The results will be updated within a longer follow-up.

PF375

RESULTS OF TREATMENT OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA AND PREGNANCY IN ACCORDANCE WITH THE LEUKEMIC BURDEN AND TERM OF PREGNANCY (THE LET SCHEME)

E. Chelysheva[1,*], A. Turkina[1], E. Polushkina[2], R. Shmakov[2]

[1]National Research Center for Hematology, [2]FSBI National Research Center of Obstetrics, Gynecology and Perinatology of the Healthcare Ministry named after V.I. Kulakov, Moscow, Russian Federation

Background: Planning pregnancy in patients (pts) with chronic myeloid leukemia (CML) is safe when a stable deep molecular response (DMR) is achieved and a treatment free observation is possible in order to avoid the potential teratogenicity of tyrosine kinase inhibitors (TKI). However, unplanned pregnancies with high leukemic burden force to develop the treatment schemes with the possibility to use TKIs.
Aims: To evaluate the results of treatment scheme in CML pts with pregnancy considering the Leukemic burden and Term of pregnancy (LET).
Methods: The LET scheme has been developed in collaboration with obste-

tricians and used in years 2012-2017. The main principles were as follows: 1) to avoid TKI at early pregnancy till 15th week (organogenesis, no placental barrier) and to stop TKI immediately after pregnancy confirmation; to use interferon (IFN) before 15th week if there is no complete hematologic response (CHR); 2) after 15th week (at late pregnancy) to allow the use of imatinib (IM) or nilotinib (NIL) in case of no CHR or MR2 loss (BCR-ABL >1%) as these TKI have a moderate placental transfer; 3) if at least MR2 remains (BCR-ABL<1%) to observe without treatment or to use (IFN) at any pregnancy stage. The results were collected within CML Pregnancy Registry together with data of other treatment schemes.

Results: The data about 125 pregnancy cases in women with CML from Russia were gathered. The outcomes were as follows: labor n=83(66%), artificial abortion n= 34(27%), miscarriage n=8 (7%). The LET scheme was used in 45 pts with chronic phase CML including 49 subsequent pregnancy cases which ended in labor. Median (Me) age of pts at pregnancy onset was 29 years (range 20-43). A DMR (at least MR4 or BCR-ABL<0,01%) at pregnancy onset was observed only in 14 (29%) of pts, a spectrum of BCR-ABL levels was in other cases (Table 1a). No CHR in 1st trimester was in 13(27%) cases. At early pregnancy (till 15thweek) observation without treatment or IFN was used in 41(84%) and 7(14%) cases respectively; in 1(2%) case IM was used because a woman did not know about pregnancy. At late pregnancy (after 15th week) observation without treatment, IFN, IM at dose 400 mg and NIL at dose 400 mg QD were used in 16(38%), 3(6%), 26(53%) and 4(8%) of cases respectively (Table 1b). Me time of TKI cessation was 4thweek (range 3-17). Me time of TKI restart was 19th week (range 15-35). The newborns were healthy including 30 infants who underwent TKI exposure at late pregnancy. In 7 of these 30 infants a low birth weight (<2500 g) was observed. Hypospadia which is frequently seen in common population (1:150-180 cases) was found in 1(2%) infant. There was no association of this abnormality with TKI use as the mother had only IFN therapy throughout pregnancy. The follow-up of pts within 1 year after labor showed that CHR was in 47(96%) of cases; DMR, major molecular response (MMR or BCR-ABL≤0,1%) and no MMR were in 17(35%), 15(30%) and 17(35%) cases respectively. One woman with CML diagnosed during pregnancy unfortunately died after labor from blast crisis. She had no CHR on IFN therapy and was switched to IM at late pregnancy (3rd trimester); the subsequent dasatinib therapy after labor and allogenic bone marrow transplantation were insufficient. Three pts with DMR continued treatment free observation after labor for >24 months.

Table 1.

Molecular response	cases, n (%)
BCR-ABL >10%	10 (20%)
BCR-ABL 1% - 10%	7 (14%)
MR2 BCR-ABL 0,1 -1%	6 (12%)
MR3 BCR-ABL 0,01 -0,1%	5 (11%)
MR4 BCR-ABL <0,01%	14 (29%)
No molecular/cytogenetic data	7 (14%)
Total number	49 (100%)

a. Molecular response at pregnancy onset

Therapy	Period		
	conception	at 15th week	after 15th week
	cases, n (%)		
imatinib	28 (57%)	1 (2%)	26 (53%)
nilotinib	4 (8%)	0 (0%)	4 (8%)
dasatinib	1 (2%)	0 (0%)	0 (0%)
IFN	2 (4%)	7 (14%)	3 (6%)
no therapy	14 (29%)	41 (84%)	16 (33%)
Total number	49 (100%)	49 (100%)	49 (100%)

b. Therapy during pregnancy according to LET scheme

Summary and Conclusions: The LET scheme which considers a pregnancy stage and a leukemic burden may support a successful childbirth in different clinical situations for CML pts. Further analysis of treatment schemes at pregnancy may help to find a balance of risks both mother and baby.

PF376

ALLOGENEIC STEM CELL TRANSPLANTATION FOR CHRONIC MYELOID LEUKEMIA IN THE TKI ERA: POPULATION BASED DATA FROM THE SWEDISH CML REGISTRY

A. Lübking[1,*], A. Dreimane[2], F. Sandin[3], C. Isaksson[4], B. Märkevärn[4], M. Brune[5], P. Ljungman[6], S. Lenhoff[7], L. Stenke[8], M. Höglund[9], J. Richter[7], U. Olsson Strömberg[10]

[1]Dept of Hematology, Oncology and Radiation Physics, Skåne University Hospital, Lund, [2]Department of Hematology, Linköping University Hospital, Linköping, [3]Regional Cancer Center, Uppsala University Hospital, Uppsala, [4]Department of Hematology, Umeå University Hospital, Umeå, [5]Department of Hematology and Coagulation, Sahlgrenska University Hospital, Göteborg, [6]Department of Cellular Therapy and Allogeneic Stem Cell Transplantation, Karolinska University Hospital and Section of Hematology, Department of Medicine Huddinge, Karolinska Institutet, Stockholm, [7]Department of Hematology, Oncology and Radiation Physics, Skåne University Hospital, Lund, [8]Department of Medicine, Division of Hematology, Karolinska University Hospital Solna, Stockholm, [9]Division of Hematology, [10]Department of Medial Sciences, Uppsala University, Uppsala and Division of Hematology, Uppsala university hospital, Uppsala, Sweden

Background: The majority of patients with chronic myeloid leukemia (CML) in chronic phase (CP), will reach a near normal life expectancy under treatment with tyrosine kinase inhibitors (TKI). Nevertheless, about 400 patients with CML undergo allogeneic hematopoietic stem cell transplantation (allo-HSCT) in Europe each year, of which a sizeable number in first chronic phase.

Aims: We aimed to evaluate patients undergoing allo-HSCT regarding indication, phase of disease at transplantation and outcome in a population-based manner. Furthermore, data concerning relapse rate and management of relapse in different patient groups as well as post-transplant TKI treatment were analyzed.

Methods: From the in the Swedish CML registry, covering 98% of CML cases in the country, 119 patients diagnosed with CML between 2002 and 2016 that underwent allo-HSCT between 2002 and august 2017 were identified. Additional information was collected by systematic review of patient records.

Results: Patients diagnosed with CML at age <65 years had a cumulative probability to undergo allo-HSCT within 5 years of 12.4%. In patients transplanted in CP1 (N=57, 47.9%), indications for allo-HSCT were TKI resistance (57.9%); high risk disease/ T315I mutation (21.1%); TKI intolerance (8.8%); other causes (12.3%). Of patients, transplanted in >CP1 (n=47, 39.5%), 61.7% were initially diagnosed in accelerated phase (AP) or blast crisis (BC). Of 15 patients transplanted in AP/BC, 12 were diagnosed in CP initially. When transplanted in CP1, >CP1 or AP/BC, an unrelated donor was used in 68.4%, 70.2% and 93.3% of patients. Myeloablative conditioning was used in 45.6% of patients transplanted in CP1, 76.6% in >CP1 and 53.3% in AP/BC. Median follow-up for patients alive at last control was 7.2 years. 5-year overall survival (OS) for patients transplanted in CP1, >CP1 and AP/BC was 96.3% (95% CI 91.4-100%), 69.3% (95% CI 56.5-85.1%) and 34.3% (95% CI 16.2-72.4%). (Figure 1) In total, 37 of 119 patients had relapsed and 81% of relapses occurred within 2 years. Characteristics of relapse were linked to the disease phase at allo-HSCT with mainly molecular relapses in patients transplanted in CP1. In 10 of 12 relapsed patients in this group, MR3 was achieved by treatment with DLI and/or TKI. Hematological relapse occurred in 7 of 8 patients transplanted in AP/BC and 5 of these patients died due to relapse. The probability to develop GvHD of any grade was 63.9% for all patients, 50.9% when transplanted in CP1 and 80.9% in >CP1. Risk factors for death were AP/BC at time of allo-HSCT (p=<0,001) and EBMT score >2 (p=0.008). These factors were also associated with risk for relapse with p=0.007 and p=0.013. Sokal risk score, age at transplantation, time to transplantation, conditioning regimen, type of donor or time period of allo-HSCT were not significantly associated with death or relapse. At most recent follow up 29 of all 119 patients had died. Cumulative probability for non-relapse mortality (NRM)) for patients transplanted in CP1 or >CP1 compared to patients with AP/BC at time point of allo-HSCT was 11.6% and 21.4%.

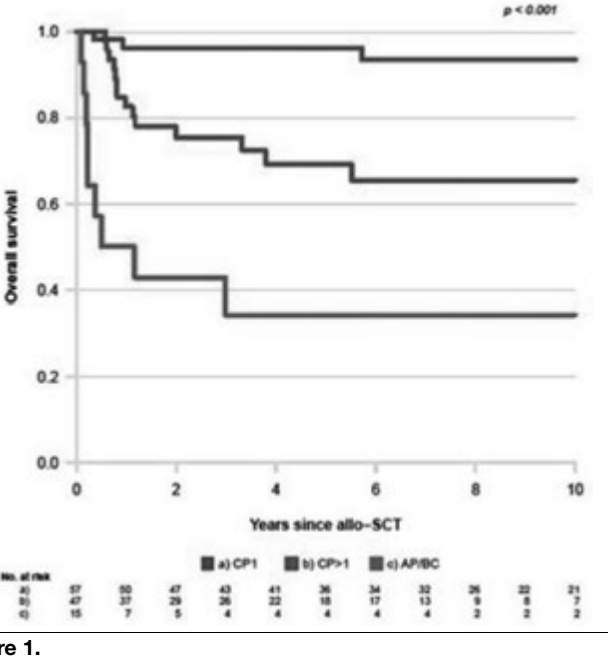

Figure 1.

Summary and Conclusions: Our population-based study demonstrates that there is still a considerable number of patients with CML in CP undergoing allo-HSCT each year. Patients transplanted in CP1 have an excellent OS, low NRM and respond to TKI and/or DLI treatment in case of relapse. We found a relatively low NRM even for patients transplanted in AP/BC but a high number of relapses, underlying the dismal OS in this group.

PF377

LONG-TERM TREATMENT-FREE REMISSION (TFR) FOLLOWING SECOND-LINE (2L) NILOTINIB (NIL) IN PATIENTS (PTS) WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP): ENESTOP 144-WK RESULTS

T.P. Hughes[1,*], C. Boquimpani[2], N. Takahashi[3], N. Benyamini[4], N.C.D. Clementino[5], V. Shuvaev[6], A. Merchant[7], J.H. Lipton[8], A.G. Turkina[9], R. De Paz Arias[10], B. Moiraghi[11], F.E. Nicolini[12], J. Dengler[13], T. Sacha[14], D.-W. Kim[15], R. Fellague-Chebra[16], S. Acharya[17], S. Chaturvedi[18], C. Bouard[19], F.-X. Mahon[20]

[1]SA Pathology and South Australian Health and Medical Research Institute, University of Adelaide, Adelaide, Australia, [2]Hemocentro do Rio de Janeiro, Hemorio, Rio de Janerio, Brazil, [3]Department of Hematology, Akita University Hospital, Akita, Japan, [4]Rambam Health Care Campus, Haifa, Israel, [5]Hospital Das Clinicas da Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [6]Russian Research Institute of Hematology and Transfusiology, Saint Petersburg, Russian Federation, [7]Keck School of Medicine, University of Southern California, Los Angeles, United States, [8]Princess Margaret Cancer Centre, University of Toronto, Toronto, Canada, [9]National Research Center for Hematology, Moscow, Russian Federation, [10]University Hospital La Paz, Madrid, Spain, [11]Hospital General De Agudos J. M. Ramos Mejia, Buenos Aires, Argentina, [12]Centre Léon Bérard, Lyon, France, [13]Onkologische Praxis Heilbronn, Heilbronn, Germany, [14]Department of Hematology, Jagiellonian University Hospital, Kraków, Poland, [15]Seoul St Mary's Hospital, Leukemia Research Institute, The Catholic University of Korea, Seoul, Korea, Republic Of, [16]Novartis Pharma SAS, Rueil-Malmaison, France, [17]Novartis Healthcare Pvt Ltd, Hyderabad, India, [18]Novartis Pharmaceuticals Corporation, East Hanover, United States, [19]Novartis Pharma AG, Basel, Switzerland, [20]Cancer Center of Bordeaux, Institut Bergonié, INSERM U1218, University of Bordeaux, Bordeaux, France

Background: The ENESTop study (NCT01698905) is assessing pts with CML-CP who achieved a sustained deep molecular response after switching from imatinib (IM) to NIL for achievement of TFR, a new treatment goal in CML. TFR rates of 57.9% at 48 wk (primary endpoint) and 53.2% at 96 wk were previously reported.
Aims: Assess long-term TFR durability and safety at 144 wk after stopping 2L NIL.
Methods: Pts treated with tyrosine kinase inhibitors for ≥3 y (IM for >4 wk followed by NIL for ≥2 y) and achieving $MR^{4.5}$ (*BCR-ABL1* ≤0.0032% on the International Scale [*BCR-ABL1*^{IS}]) on NIL were eligible to enroll and enter a 1-y consolidation (CS) phase; those with no confirmed loss of $MR^{4.5}$ could enter the main TFR phase. Pts who did not have stable $MR^{4.5}$ received another 1 y of treatment; those with stable $MR^{4.5}$ could then enter the TFR-2 phase. Pts with loss of major molecular response (MMR; *BCR-ABL1*^{IS} ≤0.1%) or confirmed loss of MR^4 (*BCR-ABL1*^{IS} ≤0.01%) during either TFR phase restarted NIL. Data cutoff for this analysis was Oct 18, 2017, when all pts in the main TFR phase had completed ≥144 wk of TFR, restarted NIL, or discontinued the study. All pts gave informed consent.
Results: By the data cutoff, 61/126 pts in the main TFR phase remained in TFR (48.4% [95% CI, 39.4%>57.5%]), 7 permanently discontinued the study while in this phase, and 58 reinitiated NIL (loss of MMR, 34; confirmed loss of MR^4, 24). 6/67 pts in TFR at 96 wk were no longer in TFR at 144 wk: 3 had confirmed loss of MR^4 (at 108, 120, and 144 wk of TFR), 2 died, and 1 discontinued the study due to pt decision. Of 34 pts who restarted NIL due to loss of MMR, 33 (97.1%) and 31 (91.2%) regained MMR and $MR^{4.5}$, respectively. The pt who did not regain MMR had been retreated with NIL for 19.7 wk. Of 24 pts who restarted NIL due to confirmed loss of MR^4, 23 (95.8%) regained $MR^{4.5}$; the remaining pt had only 1.1 wk of follow-up at the data cutoff. Of 54 total pts who regained $MR^{4.5}$ after restarting NIL, 42 (77.8%) had stable $MR^{4.5}$ 48 wk later. Of 26 pts who did not have stable $MR^{4.5}$ during the 1-y CS phase and received another 1 y of treatment, 7 achieved stable $MR^{4.5}$ and entered the TFR-2 phase. TFR rate among these pts was 71.4% (5/7 pts) at both 48 and 96 wk. The remaining 2 pts restarted NIL and discontinued the study due to AEs: arterial occlusive disease in 1 pt (last *BCR-ABL1*^{IS} 0.003%), cardiovascular disorder in 1 pt (last *BCR-ABL1*^{IS} 0.004%). Among all pts who attempted TFR (after 1 or 2 y of CS), TFR rate was 58.6% (78/133 pts) at 48 wk and 54.1% (72/133 pts) at 96 wk. No disease progressions were reported. Five pts died; 1 during CS (arterial hemorrhage), 2 during TFR (respiratory failure and arthritis bacterial, respectively; both after 96 wk of TFR), and 2 >30 d after discontinuing the reinitiation phase (1 cardiopulmonary failure, 1 adenocarcinoma). Based on pts who entered the main TFR phase, the 144-wk treatment-free survival (TFS) rate was 52.0% (95% CI, 42.9%>60.4%; Figure 1); median TFS was not reached. Of 68 pts remaining in TFR for >96 wk, any-grade AE rates were 77.9%, 83.8%, 67.6%, and 50.0% in the CS phase and first, second, and third 48 wk of TFR, respectively; any-grade musculoskeletal pain–related AE rates were 10.3%, 51.5%, 19.1%, and 11.8%. Any-grade cardiovascular-related AEs were low across these periods (CS, 1 pt; TFR, 2 pts during the second 48 wk).

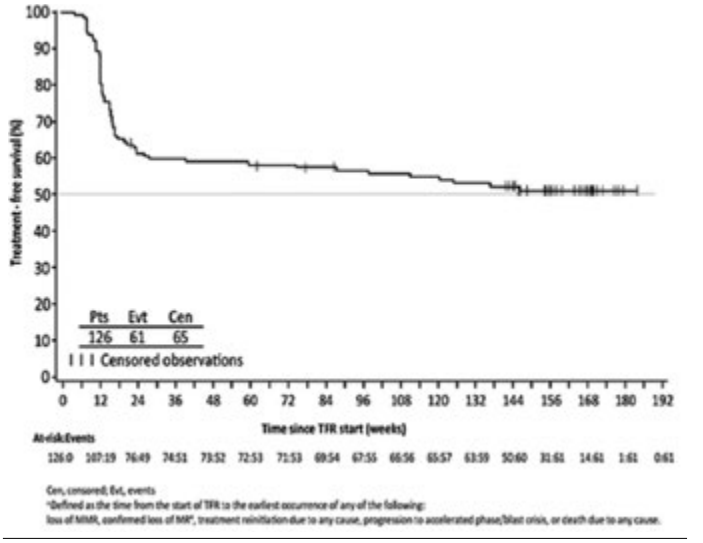

Figure 1. Treatment-free survival*.

Summary and Conclusions: These results show that following 2L nilotinib, long-term durable TFR is achievable in many pts, and pts should be monitored for potential late loss of response. Most pts restarting NIL regained stable $MR^{4.5}$.

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NILOTINIB *VERSUS* NILOTINIB COMBINED TO PEGYLATED-INTERFERON ALFA 2A IN FIRST-LINE CHRONIC PHASE CML PATIENTS. UPDATED INTERIM ANALYSIS OF A PHASE III TRIAL

F. Nicolini[1,*], G. Etienne[2], F. Huguet[3], A. Guerci-Bresler[4], A. Charbonnier[5], M. Escoffre-Barbe[6], V. Dubruille[7], H. Johnson-Ansah[8], L. Legros[9], V. Coiteux[10], P. Cony-Makhoul[11], P. Lenain[12], L. Roy[13], P. Rousselot[14], D. Guyotat[15], J.-C. Ianotto[16], M. Gardembas[17], F. Larosa[18], M.-L. Chretien[19], P. Turlure[20], S. Courby[21], P. Quittet[22], E. Hermet[23], S. Amé[24], S. Lapusan[25], V. Schwiertz[26], M. Etienne[27], S. Morisset[1], D. Rea[28], S. Dulucq[29], F.-X. Mahon[2]

[1]Hematology, Centre Léon Bérard, Lyon, [2]Hematology, Institut Bergonié, Bordeaux, [3]Hematology, Institut Universitaire du Cancer, Toulouse, [4]Hematology, CHRU Brabois, Vandoeuvre les Nancy, [5]Hematology, Institut Paoli Calmettes, Marseille, [6]Hematology, Hôpital de Pontchaillou, Rennes, [7]Hematology, Hôtel Dieu, Nantes, [8]Hematology, Hôpital Clémenceau, Caën, [9]Hematology, Hôpital de l'Archet, Nice, [10]Hematology, Hôpital Huriez, Lille, [11]Hematology, Hôpital Annecy-Genevois, Pringy, [12]Hematology, Centre Henri Becquerel, Rouen, [13]Hematology, Hôpital Henri Mondor, Créteil, [14]Hematology, Hôpital A. Mignot, Le Chesnay, [15]Hematology, Institut de Cancérologie Lucien Neuwirth, Saint Etienne, [16]Hematology, Hôpital Morvan, Brest, [17]Hematology, Hôpital universitaire, Angers, [18]Hematology, Hôpital J. Minjoz, Besançon, [19]Hematology, Hôpital universitaire, Dijon, [20]Hematology, Hôpital Dupuytren, Limoges, [21]Hematology, Hôpital A. Michallon, La Tronche, [22]Hematology, Hôpital Saint Eloi, Montpellier, [23]Hematology, CHU Estaing, Clermont-Ferrand, [24]Hematology, Hôpital Civil, Strasbourg, [25]Hematology, Hôpital Saint Antoine, Paris, [26]Pharmacy department, UPCO, [27]Hematology, Centre Hospitalier Lyon Sud, Pierre Bénite, [28]Hematology, Hôpital Saint Louis, Paris, [29]Laboratory of Hematology, Hôpital Haut-Lévêque, Pessac, France

Background: Nilotinib (NIL)+Pegylated IFN-a2a (Peg-IFN) are able to induce high deep molecular response (DMR) rates in chronic phase CML (CP CML) patients (pts), as first-line therapy (Nicolini FE., Lancet Haematol. 2015).
Aims: Comparison of DMR rates of NIL+Peg-IFN *vs* NIL alone, prospectively, in newly diagnosed CP-CML. (EudraCT 2013-004974-82).
Methods: Pts ≤65 yrs with no arterial history were randomized 1:1 to get

NIL 300 mg BID (Month (M) 0 to M72, arm A) *vs* Peg-IFN alone 30 days (M-1 to M0) 30 mg/wk as priming, prior to NIL 300 mg BID+Peg-IFN 30 mg/wk 2 wks, upgraded to 45 mg/wk thereafter, for up to 2 yrs (M0 to M24, arm B) followed by NIL alone for 4 more yrs. The primary endpoint is the rate of MR4.5 at M12.

Results: 201 pts were randomized (100 arm A, 101 arm B), 65 males in both arms, 35 females in arm A, 36 in arm B. The median follow-up was 25 (13.4–39) months. Results were analysed in ITT. Sokal was high in 25%, interm. in 35% and low in 39% pts; Eutos LTS was high in 2%, interm. in 17%, and 81% low; equally balanced. Median age was 46 (18-66) yr, equally balanced; 8 pts had a cryptic Ph, 12 a variant Ph, and 15 ACAs, all pts had a "Major" BCR transcript. CHR was obtained in 9.6% pts at M0 (arm B) and in 88% pts in arm A and 90.4% pts in arm B at M1. The rates of CCyR at M3 were 72.5% *vs* 76% in arm A *vs* B. At M12, the MR4.5 rates were 15.22% *vs* 24.21 (p=0.018) in arm A *vs* B respectively. By M12, the CI of MMR were 68.54% *vs* 90.79% (p=0.066), MR4 were 34.80% *vs* 48.89% (p=0.046), MR4.5 were 17.03% *vs* 24.11% (p=0.048), MR5 11.68% *vs* 36.13% (p=0.025), in arm A *vs* arm B respectively (Figure 1). Only 1 pt progressed toward accelerated phase in arm A with a Y253H mutation, to date. Fourty-two pts were withdrawn from study, 23 in arm A [toxicity 7, other cancer 2, resistance 14 (4 Y253H, 1 E255K, 1 F317L mutations), lost for follow-up 1] and 18 pts in arm B [toxicity 11, resistance 7 (T315I mutation 1)], no pt died. The median dose of Peg-IFN delivered in arm B during the first month was 30 (0-30)mg/wk, 45 (0-45)mg/wk at M2, 45 (0-45)mg/wk at M3, 30 (0-30)mg/wk M0-M12. The median daily doses of NIL were 600 mg in both arms M0-M12. The NIL doses delivered were not different between the 2 arms until M9 (p=0.053). Multivariate analysis demonstrated that female gender favourably impacted, but high Sokal and low cumulated doses of Peg-IFN (0-540 mg) negatively impacted on the M12 rate of MR4.5. The overall rate of grade 4 hematologic toxicities was 9.5%, with no anemia, 0.5% and 1.5% thrombocytopenia, 1.5% and 1% neutropenia, 0% and 0.5% leucopenia, and 0.5% and 0.5% pancytopenia in arm A *vs* B respectively. Grade ¾ non-hematologic toxicities consisted in 2% of cardiac disorders in arm A *vs* 2.5% in B, 0% vascular disorders in arm A *vs* 2% in B, 1.5% gastro-intestinal disorders in arm A *vs* 0.5% in B; 1% auto-immune disorders in arm B (1 recurrent pericarditis, 1 hemolytic anemia); 3 and 6 pregnancies (of the female partner except 2) were observed in arm A and B respectively, despite recommended contraceptive methods. We observed 8% lipase elevations in arm A *vs* 3% in B, 1% cholestatic episodes in arm A *vs* 0.5% in B. There were 1% depressive episodes in arm B; 2% infections in arm B and 1 before randomisation. Finally 3 intercurrent cancers were detected in arm A (cervix, breast, thyroid).

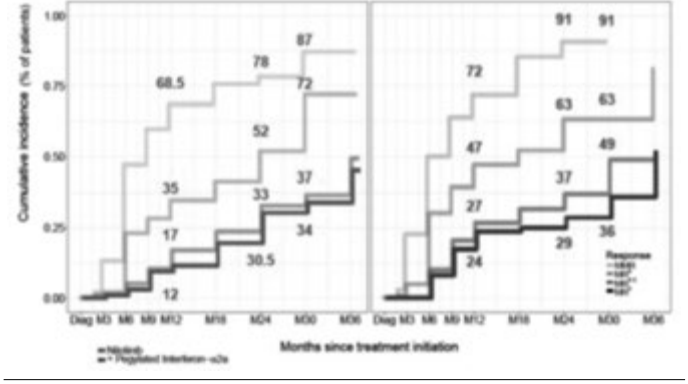

Figure 1. Cumulative incidence of molecular responses at definite time points.

Summary and Conclusions: The combination of NIL+Peg-IFN provides significantly higher rates of DMR rates by M12, in newly diagnosed CP CML pts without increasing the rate of early SAEs in such setting. M30 updated results will be presented during the meeting.

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THE VALUE OF BCR-ABL1 QPCR LEVEL AND DOUBLING TIME (DT) TO PREDICT SUCCESSFUL TREATMENT-FREE REMISSION AFTER IMATINIB DISCONTINUATION: TREATMENT-FREE REMISSION ACCOMPLISHED BY DASATINIB (TRAD) TRIAL

D. Kim[1,*], D. Forrest[2], L. Savoie[3], I. Bence-Bruckler[4], S. Couban[5], L. Busque[6], P. Laneuville[7], R. Delage[8], A. Xenocostas[9], E. Liew[10], K. Paulson[11], J. Lipton[1], S. Kamel-Reid[12], B. Leber[13]

[1]Princess Margaret Cancer Centre, Toronto, [2]British Columbia Cancer Agency, Vancouver, [3]Tom Baker Cancer Centre, Calgary, [4]Ottawa Hopsital, Ottawa, [5]Queen Elizabeth II Health Sciences Centre, Halifax, [6]Hopital Maisonneuve-Rosemont, [7]McGill University Medical Centre, Montreal, [8]Laval University, Quebec, [9]London Health Science Centre, London, [10]University of Alberta, Edmonton, [11]Cancer Care Manitoba, Winnipeg, [12]Toronto General Hospital, Toronto, [13]Juravinski Cancer Centre, Hamilton, Canada

Background: The ongoing Canadian tyrosine kinase inhibitor (TKI) discontinuation trial is designed to determine if a second generation TKI (dasatinib) can be used for successful attaining treatment-free remission (TFR) after an initially unsuccessful attempt of TFR with imatinib discontinuation (IM). Our preliminary result indicates that: 1) The 6-month relapse-free survival (RFS) rate after IM discontinuation is estimated as 58.0%; 2) Dasatinib re-treatment leads to rapid re-achievement of MMR and MR4.

Aims: One aim of our trial is to identify early surrogates that predict successful TFR by using serial measures of BCR-ABL1 qPCR and doubling time (DT).

Methods: This prospective clinical trial (BMS CA180-543, Clinicaltrial.gov NCT#02268370) has 3 phases: 1) IM discontinuation 2) dasatinib rechallenge 3) dasatinib discontinuation. Serial measures of BCR-ABL1 qPCR test and the DT of the transcript were evaluated monthly for the first 6 months. For the latter the baseline qPCR level prior to IM discontinuation or the qPCR level from the prior month was used and DT was calculated as x=ln(2)/K (x is the DT, and k is the fold change in BCR-ABL1 from the previous value divided by the number of days between measurements). To define cut-off levels for BCR-ABL1 qPCR and DT, a binary recursive partitioning method was applied for RFS and a decision tree analysis was performed incorporating the variables BCR-ABL1 monthly, and DTs.

Results: As of Feb 11 2018, 53/131 patients (40.4%) lost molecular response; the 12-month relapse-free survival (RFS) rate was estimated as 58.0% (42.1-71.0%). 51/53 patients who lost response received dasatinib. The incidence of MMR, MR4 and MR4.5 at 3 months was 97.7%, 89.9%, and 84.6%, respectively. 22/51 patients have currently attained MR4.5 for 12 months or longer and have discontinued dasatinib for a second TFR attempt. 11/22 patients (50.0%) have lost molecular response, with an estimated TFR2 rate of 39.3% at 6 months, and a median of 5.5 month to loss of molecular response. BCR-ABL1 qPCR kinetics after IM discontinuation showed rapid rise of BCR-ABL1 transcript level in first 3 months, followed by gradual rise after the 3 months. We have analyzed the monthly level of BCR-ABL1 qPCR at 0-6 months. Recursive partitioning suggested 2 months' BCR-ABL1 qPCR as strong predictive for successful TFR. The RFS rate at 12 months was 89.3%, 26.5% and 0% with BCR-ABL1 qPCR 5 log or deeper (n=71), 4-5 log (n=28), and less than 4 log level (n=24) at 2 months (p<0.001). The DT of BCR-ABL1 transcript was evaluated at each month for the first 6 months using qPCR level at screening or at the previous month as a baseline. The DT between 0 (baseline) and 2 months was very predictive of successful TFR after IM discontinuation. The RFS rate at 12 months was 0% with DT less than 14 days (n=26) *vs* 34.2% with DT 14 days or longer (n=23) *vs* 84.1% with DT equal to or below zero (n=80; p<0.001). The result of the DT at 2 months calculated based on the qPCR levels of the present and previous month (+/- 8 days) was also highly predictive (p<0.001). When combined qPCR level and DT at 2 months, three prognostic groups were identified as shown in the Figure 1.

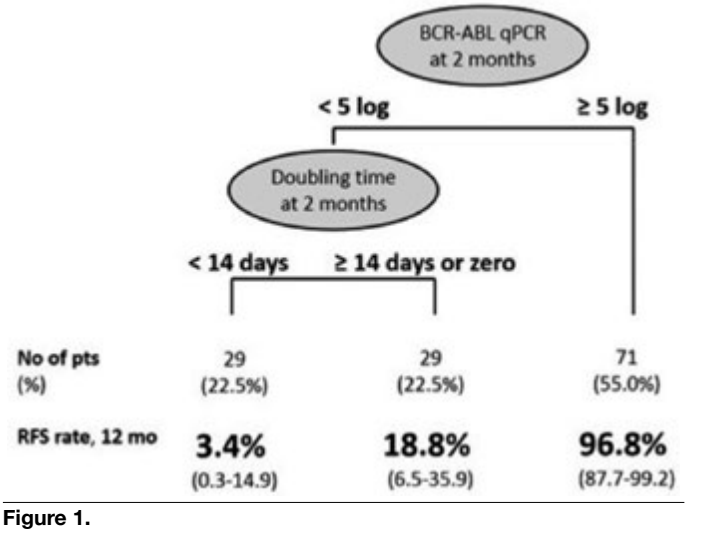

Figure 1.

Summary and Conclusions: BCR-ABL1 qPCR level at 2 months was very predictive of TFR success with a molecular cutoff of 5.0 logs.

Enzymopathies, membranopathies and other anemias

PF380

ADDRESSING THE DIAGNOSTIC GAPS IN PYRUVATE KINASE (PK) DEFICIENCY. CONSENSUS RECOMMENDATIONS ON THE DIAGNOSIS OF PK DEFICIENCY

P. Bianchi[1,*], E. Fermo [1], B. Glader[2], H. Kanno[3], A. Agarwal[4], W. Barcellini[1], S. Eber[5], J.D. Hoyer[6], D. Kuter[7], M.T. Magalhães[8], M.D.M. Mañu-Pereira[9], T.A. Kalfa[10], S. Pissard[11], J.C. Segovia[12], E. van Beers[13], P.G. Gallagher[14], D. Rees[15], R. van Wijk[16]

[1]Oncohematology Unit - Physiopathology of Anemias Unit, Foundation IRCCS Cà Granda Policlinico Milan, Milan, Italy, [2]Stanford University School of Medicine, Lucile Packard Children's Hospital, Palo Alto, CA, United States, [3]Department of Transfusion Medicine and Cell Processing, Faculty of Medicine, Tokyo Women's Medical University, Tokyo, Japan, [4]University of Utah/ARUP Laboratories, Salt Lake City -UT, United States, [5]Special Praxis for Pediatric Hematology and Childrens` Hospital, Technical Univ. Munich, Munich, Germany, [6]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, [7]Hematology Division, Massachusetts General Hospital, Boston - MA, United States, [8]Hematolgoy Department, University Hospital Center of Coimbra, Coimbra, Portugal, [9]Red blood cell and hematopoietic disorders research group, Josep Carreras Leukaemia Research Institute (IJC), Universitat Autònoma de Barcelona (UAB), Badalona, Spain, [10]Cancer and Blood Diseases Institute, Cincinnati Children's Hospital Medical Center and Department of Pediatrics, University of Cincinnati, College of Medicine, Cincinnati -OH, United States, [11]APHP-University Hospital Henri Mondor and Inserm IMRB U955eq2, Creteil, France, [12]Differentiation and Cytometry Unit. Hematopoietic Innovative Therapies Division, Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT) - Centro de Investigaciones Biomédicas en Red de Enfermedades Raras (CIBERER), Madrid, Spain, [13]Van Creveldkliniek, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands, [14]Departments of Pediatrics, Pathology and Genetics, Yale University School of Medicine, New Haven, CT, United States, [15]Department of Paediatric Haematology, King's College Hospital, London, United Kingdom, [16]Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands

Background: Pyruvate kinase deficiency (PKD) is the most common enzyme defect of glcolysis, causing hereditary non-spherocytic hemolytic anemia. PKD is an autosomal recessive disease, caused by homozygosity or compound heterozygosity for mutations in *PKLR* gene. The disease has a worldwide geographical distribution but there are no exact and verified data regarding its frequency. Difficulties in the diagnostic workflow and interpretation of PK enzyme assay likely play a role.
Aims: We studied the current gaps in the diagnosis of PKD in order to establish diagnostic guidelines that will improve diagnosis and increase awareness of the disease.
Methods: A global PKD Advisory Board Committee was established in 2016, involving 24 experts from 20 Centers of Expertise (CE). By means of a detailed survey and subsequent forum discussions, multiple aspects on the diagnosis of PKD were evaluated and discussed by 13 members of CEs from Europe (7), USA (5), and Asia (1). The outcomes were compiled and presented as recommendations.
Results: Broad consensus was reached on many clinical and technical aspects of the diagnosis of PKD. In particular: PKD should be suspected in: a) patients with a variable degree of chronic anemia and/or splenomegaly, and/or jaundice, with normal or near-normal red cell morphology; b) transfusion-dependent cases of unknown etiology; c) patients with severe neonatal indirect hyperbilirubinemia and hemolysis (mean degree of agreement: 95%). Clinical information, family history, and the time of last blood transfusion is essential and should be provided along with patient blood samples (98.5%). Similarly, laboratory parameters such as complete blood count, RBC morphology and markers of hemolysis (reticulocyte count, LDH, haptoglobin, unconjugated bilirubin) are considered mandatory upon sample acceptance (97%). Spectrophotometric PK activity assay (Beutler, A Manual of Biochemical Methods, 1984) is performed by all CEs and considered the reference assay (98.7%). >95% of agreement was reached on several technical aspects: sample is stable up to 21 days at 4°C or 15 days if the PK assay is performed together with hexokinase activity to evaluate the influence of high reticulocyte count or sample aging; anticoagulant (ACD/EDTA); sample purification by alpha-cellulose/microcrystalline cellulose column (buffy-coat removal may be considered as an alternative); interference of reticulocyte number and recent transfusion must to be taken into account when interpreting results of PK enzyme assay. Different positions were expressed on the need to confirm a PK enzyme deficiency by molecular testing (88%); two Centers consider this necessary only for atypical cases. Different approaches among Centers may be due to different levels of reimbursement for testing by national insurances. The use of Next Generation Sequencing panels is an alternative reliable method for PKD diagnosis, in particular in neonates (when parents are not available), in transfusion dependent, or recently transfused patients, or on samples with prolonged shipping times (91% degree of agreement).
Summary and Conclusions: A wide consensus on diagnosis of PKD was reached among 13 ECs worldwide. The results of this study will be presented as recommendations for the diagnosis of PK deficiency and will be useful in preparing a diagnostic algorithm. Since no external quality control program exists for PK testing, this information might be helpful for other centers to deliver timely and appropriate diagnosis and to increase awareness in PKD.

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IMPLEMENTATION OF THE DIAGNOSTIC WORKFLOW OF HEREDITARY ANEMIAS BY USING TARGETED-NGS PANEL ANALYSIS

R. Russo[1,*], I. Andolfo[1], F. Manna[1], A. Gambale[2], R. Marra[1], B.E. Rosato[1], P. Caforio[1], V. Pinto[3], P. Pignataro[1], K. Radhakrishnan[4], S. Unal[5], G. Tomaiuolo[6], G.L. Forni[3], A. Iolascon[1]

[1]Molecular Medicine and Medical Biotechnologies, University "Federico II", Naples, CEINGE - Biotecnologie Avanzate, [2]Molecular Medicine and Medical Biotechnologies, University "Federico II", Naples, CEINGE - Biotecnologie Avanzate, Naples, Naples, [3]Centro della Microcitemia e Anemie Congenite, Ospedale Galliera, Genova, Italy, [4]Department of Haematology, Monash Medical Centre, Paediatric Haematology/Oncology, Children's Cancer Centre, Monash Children's Hospital, Melbourne, Australia, [5]Division of Pediatric Hematology, Hacettepe University, Ankara, Turkey, [6]Dipartimento di Ingegneria Chimica, dei Materiali e della Prod. Indus., University Federico II, Napoli, Italy

Background: Mutations in more than 70 genes cause hereditary anemias (HA), a highly heterogeneous group of rare/low frequency disorders in which we included: hyporegenerative anemias, as congenital dyserythropoietic anemias (CDA) and Diamond-Blackfan anemia; erythrocyte membrane defects, as hereditary spherocytosis and stomatocytosis; hemolytic anemias due to enzymatic defects, as pyruvate kinase (PK) deficiency. The classification and the distinction among different types of HA is often difficult. The variety of unspecific and overlapping syndromic and non-syndromic phenotypes somewhat hampers a clear clinical diagnosis and prevents straightforward genetic testing. The diagnosis of these conditions may require several lines of lines of investigation.Due to the failure of the current diagnostic workflow to find a definitive and correct diagnosis of HA, next-generation sequencing (NGS) is making its way on this field. The major current application of NGS in diagnostics is through targeted (t)-NGS, in which a selected fraction of genes is analyzed (custom gene panels).
Aims: We propose a new diagnostic workflow for HA based on t-NGS.
Methods: We have developed two consecutive versions of a t-NGS panel, including 34 and 71 genes, respectively. Seventy-four probands from 62 unrelated families were investigated. The probe design was performed by web-based tool SureDesign (Agilent Technologies, USA). Sample preparation was performed following the instruction's manufacturer for HaloPlex Target Enrichment kit for Illumina Sequencing (Agilent Technologies). High-throughput sequencing was performed by Illumina NextSeq 500. Agilent SureCall software (v 3.0.3.1, Agilent Technologies) was used for bioinformatic and computational analyses. The pathogenicity of each variant was evaluated by gathering evidence from various sources, according to the guidelines of ACMG.
Results: We obtained an overall diagnostic yield of 64.9%. Despite 54.2% of cases showed conclusive diagnosis fitting well to the clinical suspicion, the multi-gene analysis modified the original clinical diagnosis in 45.8% of patients (non-matched phenotype-genotype). Of note, 81.8% of non-matched patients were clinically suspected to suffer from CDA. Particularly, 45.5% of the probands originally classified as CDA exhibited a conclusive diagnosis of chronic anemia due to enzymatic defects, mainly due to mutations in *PKLR* gene. Interestingly, we also identified a syndromic CDA patient with mild anemia and epilepsy, showing a homozygous mutation in *CAD* gene, recently associated to early infantile epileptic encephalopathy-50 and CDA-like anemia. Multi-target diagnosis also allowed us identifying polygenic conditions, in which the phenotypic variability could be explained by the co-inheritance of multiple disease mutations. This was the case of a *PIEZO1* patient that also exhibited the E109K-SEC23B genotype, causative of CDAII. The co-inheritance of *PIEZO1* and *SEC23B* mutations accounts for marked iron overload in this patient.
Summary and Conclusions: We herein described the diagnostic workflow that we established for molecular diagnosis of HA, based on the development of two consecutive versions of a t-NGS panel, including 34 and 71 genes, respectively. We showed the results obtained by the analysis of 74

probands. This is the largest cohort of patients and the most comprehensive gene set for this subset of anemias described so far. We also demonstrated that the multi-gene approach is valuable not only for achieving a correct and definitive diagnosis, but also for guiding treatment.

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GENETIC MARKERS AND NEW TARGET GENES IN THE DIAGNOSIS OF RARE ANAEMIAS

J.-L. Vives-Corrons[1,*], E. Llaudet Planas[1], V. Rizzuto[1], E. Krishnevskaya[1], J. Iraola[1], M.D.M. Mañú Pereira [1]

[1]*Erythropathology, Institute for Leukaemia Research Josep Carreras, Barcelona, Spain*

Background: In spite of the use of different phenotypic, clinical and laboratory markers, between 15 and 20% of rare anaemias (RA), rare diseases with a prevalence of less than 5 cases per 10,000 individuals, remain undiagnosed. In all these cases, the final diagnosis requires the use of genetic markers, where the approach will depend on the presence of suggestive phenotypic characteristics of congenital haemolysis or hereditary erythropoietic failure.
Aims: The main objective of our task is to provide a fast and efficient diagnosis of rare anaemias (RA) using New Generation Sequencing (NGS) and two panels of genes. One panel for analysing known gene mutations leading to hereditary haemolytic anaemia, and a second panel for analysing other genes related with erythropoiesis, or modulators of clinical expression
Methods: 166 patients from 117 unrelated families have been studied by Next Generation Sequencing (NGS) including a panel of 35 genes responsible of membranopathy (ANK1, EPB41, EPB42, SLC4A1, SPTA1 (HBA1, HBA2), haemoglobinopathy (HBA1, HBA2), enzymopathy (ADA, AK1, ALDOA, BPGM, CYB5A, G6PD, GCLC, GPI, GSR, GSS, HK1, NT5C3A, PFKM, PGK1, , HBB) and CDA (CDAN1, C15orf41, SEC23B, KLF1, GATA1, KIF23). In a second panel, additional 33 genes, potential modulators of clinical expression have been also analysed. The diagnosis of patients studied was: 1. Haemoglobinopathy (7 patients): alpha thalassemia (3), beta thalassemia (2), unstable haemoglobin (1) and high affinity haemoglobin (1), 2. Enzymopathy (33 patients): G6PD deficiency (6), Pyruvate kinase deficiency (17), Glucose phosphate isomerase (1),Phosphofructokinase (2) and cytochrome b5 reductase (7), 3. Membranopathy (75 patients): Hereditary spherocytosis (63) and elliptocytosis (9) Congenital Xerocytosis (3), 4. Haemolytic anaemia of unknown origin but suspected to be a hereditary membranopathy (24 patients), 5- CDA type I (1) and 6- Haemolytic anaemia of unknown origin but without clinical orientation (26 patients)
Results: The mutation has been identified in 1.Haemoglobinopathies: 5 of 6 patients, since NGS does not detect major deletions such as the 3.7KB HBA, 2. Enzymopathies: 24 of 33 patients (72.7%) 3-Membranopathies: 73 of 76 patients (96%). 49% of the variants identified (36/73) were missense, most in the SPTB gene (11 variants), and the remaining (37/73), nonsense or Frameshift, most in the ANK1 gene (12 variants) and SPTB (9 variants) 4. Haemolytic anaemia suspected to be a membranopathy: 17 of 22 patients (77%), 5- CDA type I (1 case), and 6. Haemolytic anaemia of unknown origin: 8 of the 26 patients (31%)
Summary and Conclusions: Using the NGS panel of genes causing anaemia, it was possible to identify the mutation in 142 of the 166 patients studied (86%), leaving 24 unidentified cases (14%); 5 with suspected membranopathy, and 19 without diagnostic orientation (unknown aetiology) . The next step is to carry out the NGS analysis with additional 33 genes candidates to be modulators of clinical expressivity. If after all the studies, some RA remain unidentified, we will participate in the H-2020 RD-Solve project with the cooperation of EuroBloodNet consortium

PF383

ACTIVATE: A PHASE 3, RANDOMIZED, MULTICENTER, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY OF AG-348 IN ADULTS WITH PYRUVATE KINASE DEFICIENCY WHO ARE NOT REGULARLY TRANSFUSED

M.-H. Jouvin[1,*], C. Bowden[1]

[1]*Agios Pharmaceuticals, Inc., Cambridge, United States*

Background: Pyruvate kinase (PK) deficiency is an under-recognized hereditary disease resulting in lifelong hemolytic anemia. PK deficiency is caused by mutations in the *PKLR* gene that lead to reduced red cell PK (PK-R) enzyme activity, resulting in defective glycolysis and decreased lifespan of red blood cells. AG-348 is a novel, first-in-class, oral, small molecule allosteric activator of PK-R under clinical testing as the first targeted disease-altering therapy for PK deficiency. In the DRIVE PK study (NCT02476916; a phase 2, open-label, dose-ranging trial in adults with PK deficiency who are not regularly transfused) twice-daily (BID) dosing with AG-348 for >6 months was well tolerated and induced rapid, durable responses (Grace RF *et al.* ASH 2017). As of July 14, 2017, 26 (50%) of 52 enrolled subjects had a maximum hemoglobin (Hb) increase of >1 g/dL. Among these 26 subjects, the mean maximum Hb increase was 3.4 g/dL and 25 (96%) had at least one missense *PKLR* mutation.
Aims: To report the design of the planned ACTIVATE study of AG-348.
Methods: ACTIVATE is a phase 3, multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of AG-348 in adult subjects with PK deficiency who are not regularly transfused. The study will consist of a screening period of up to 42 days, a 12-week dose optimization period, and a 12-week fixed-dose period (Figure 1). During the dose optimization period, AG-348 or matched placebo will be titrated up to each subject's individually optimized dose, with an initial dose of 5 mg BID for all subjects and two potential sequential dose increases (from 5 to 20 mg BID and from 20 to 50 mg BID), depending on safety and Hb change. Approximately 76 adults with PK deficiency who are not regularly transfused will be randomized in a 1:1 ratio to AG-348 (administered orally, BID) or matched placebo. Key inclusion criteria include: written informed consent; not regularly transfused (≤4 transfusion episodes in previous year and no transfusions in the 3 prior months); baseline Hb ≤10 g/dL; adequate organ function. Subjects who are homozygous for the R479H mutation or have two non-missense mutations (without the presence of another missense mutation) in *PKLR* will be excluded. The primary endpoint is the Hb response, defined as the proportion of subjects who achieve a ≥1.5 g/dL increase in Hb sustained over at least two of the last three visits in the fixed-dose period. Key secondary endpoints include change in Hb, markers of hemolysis, hematopoietic activity, health-related quality of life, and safety. An independent data monitoring committee will review the study data periodically and provide safety oversight. The ACTIVATE study will be initiated globally in the first half of 2018.
Results: Not yet available.

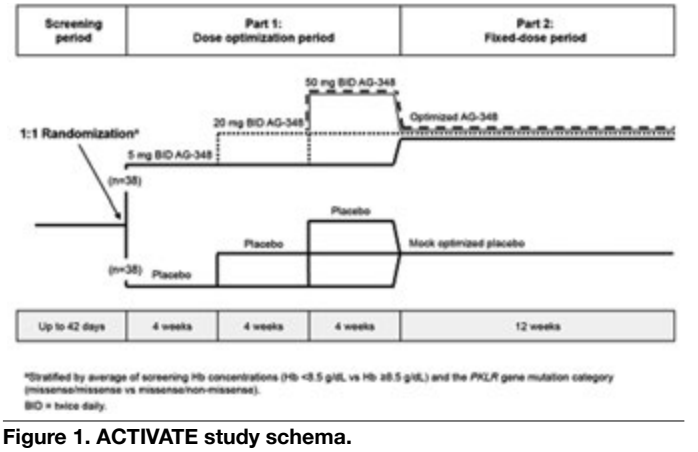

Figure 1. ACTIVATE study schema.

Summary and Conclusions: AG-348 is a novel, first-in-class PK-R activator undergoing clinical testing in subjects with PK deficiency. The ACTIVATE study of AG-348 in subjects with PK deficiency who are not regularly transfused will start enrollment in 2018.

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PYRUVATE KINASE DEFICIENCY IN JAPAN: A SUMMARY OF CLINICAL FEATURE, LABORATORY DATA AND ENZYMATIC DIAGNOSIS

T. Iwasaki[1,*], T. Utsugisawa[1], T. Yamamoto[2], H. Ogura[1], T. Aoki[1], Y. Okamoto[1], T. Kawakami[1], S. Ohga[3], A. Ohara[4], E. Ito[5], H. Kanno[1]

[1]*Transfusion Medicine and Cell Processing,* [2]*Institute of Medical Genetics, Tokyo Women's Medical University, Tokyo,* [3]*Pediatrics, Graduate School of Medical Sciences, Kyushu University, Fukuoka,* [4]*Pediatrics, Toho University, Tokyo,* [5]*Pediatrics, Hirosaki University, Aomori, Japan*

Background: Pyruvate kinase deficiency (PKD) is the most prevalent congenital hemolytic anemia due to a glycolytic enzyme defect, and inherited in an autosomal recessive manner. We have been working on the differential diagnosis of congenital hemolytic anemia and related disorders by the biochemical, physiological and genetic tests.

Aims: In this study, we aim to clarify the clinical entity of PKD in Japan: Clinical features, laboratory data, red cell enzyme analysis and genotypes of the Japanese PKD cases are summarized.
Methods: All PKD cases are enzymatically diagnosed by using the leukocyte-depleted erythrocytes. To exclude possible inactivation of the mutant enzymes, patients' blood samples are always transported to our laboratory with normal controls simultaneously sampled.
Results: We diagnosed 120 PKD cases since 1972 through 2017. The median age of diagnosis as PKD is 10 (min 0 - max 72), and gender of PKD showed 52 male and 68 female cases. The parents of 34.7% of PKD patients had consanguinity; 63.6% of patients experienced exchange transfusion at the neonatal period; 64.8% experienced RBC transfusion; 47.7% had acute hemolytic crisis; 53.1% were noticed as having splenomegaly and 58.6% were splenectomized. Mean values of Hb, reticulocytes, indirect bilirubin, serum LDH, transferrin saturation, and ferritin were 8.8g/dL, 13.0%, 3.7mg/dL, 587 IU/L, 57% and 497 ng/mL, respectively. We confirmed that 36 PKD cases harbored two *PKLR* mutations, and 42 family members were heterozygous by genetic testing. The relative erythrocyte PK activity of the PKD cases was 38.5±3.7% (mean±SE) of controls, whereas that of heterozygotes was 58.5±3.3%(mean±SE). By using a receiver operator characteristic (ROC) curve, we identified the cut-off limit as 79.8% (Figure 1 left) and 85.9% (Figure 1 right) to discriminate PKD patients from heterozygotes and heterozygotes from normal subjects, respectively.

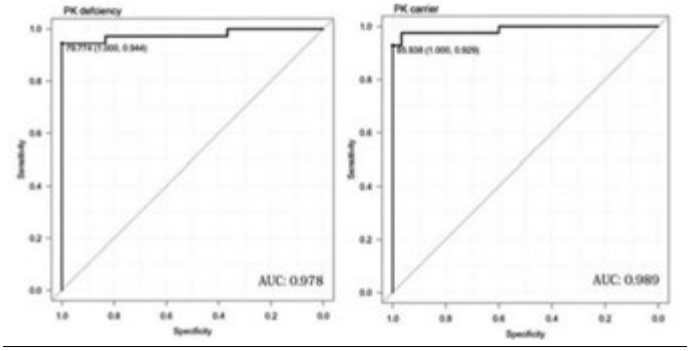

Figure 1.

Summary and Conclusions: According to the diagnostic guidelines of PKD currently being promoted as the international collaborative research, it is recommended that confirmation of mutation of both PK alleles by genetic testing be desirable for definite diagnosis of PKD. It is obvious that both next generation sequencing analysis or conventional PCR resequencing is more complicated, time consuming and more costly than enzyme assay. It is expected that a erythrocyte PK activity threshold that can be used for rapid and accurate clinical diagnosis can be determined by association analysis of the results of measurement of erythrocyte PK activity and genotype by a method standardized between PKD diagnostic facilities.

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ACTIVATE-T: A PHASE 3, OPEN-LABEL STUDY TO EVALUATE THE EFFICACY AND SAFETY OF AG-348 IN REGULARLY TRANSFUSED ADULTS WITH PYRUVATE KINASE DEFICIENCY

M.-H. Jouvin[1,*], C. Bowden[1]

[1]Agios Pharmaceuticals, Inc., Cambridge, United States

Background: Pyruvate kinase (PK) deficiency is an under-recognized hereditary disease resulting in lifelong hemolytic anemia. PK deficiency is caused by mutations in the *PKLR* gene that lead to reduced red cell PK (PK-R) enzyme activity, resulting in defective glycolysis and decreased lifespan of red blood cells. AG-348 is a novel, first-in-class, oral, small molecule allosteric activator of PK-R under clinical testing as the first targeted disease-altering therapy for PK deficiency. In the DRIVE PK study (NCT02476916; a phase 2, open-label, dose-ranging trial in adults with PK deficiency who are not regularly transfused) twice-daily (BID) dosing with AG-348 for >6 months was well tolerated and induced rapid, durable responses (Grace RF *et al.* ASH 2017). As of July 14, 2017, 26 (50%) of 52 enrolled subjects had a maximum hemoglobin (Hb) increase of >1 g/dL. Among these 26 subjects, the mean maximum Hb increase was 3.4 g/dL and 25 (96%) had at least one missense *PKLR* mutation. The ACTIVATE-T study is designed to assess the efficacy and safety of AG-348 for the first time in regularly transfused subjects with PK deficiency.
Aims: To report the design of the planned ACTIVATE-T study.
Methods: ACTIVATE-T is a global, multicenter, open-label study to evaluate the efficacy and safety of AG-348 in regularly transfused adults with PK deficiency. Approximately 15–20 adults with PK deficiency who are regularly transfused will be enrolled and treated with AG-348. The study will comprise an 8-week screening period, in which each subject's complete transfusion history from the prior 52 weeks will be documented, followed by a 16- to 24-week dose optimization period, and a 24-week fixed-dose period (Figure 1). During the dose optimization period, each subject will undergo individualized AG-348 dose optimization. All subjects will start on a dose of 5 mg BID, which may be increased twice over the course of 16–14 weeks (from 5 to 20 mg BID and from 20 to 50 mg BID). In the fixed-dose period, each subject will receive AG-348 at their optimized dose for 24 weeks. Key inclusion criteria include written informed consent, regular transfusion (≥6 transfusion episodes in the previous year), and adequate organ function. Subjects who are homozygous for the R479H mutation or have two nonmissense mutations (without the presence of another missense mutation) in *PKLR* will be excluded. An additional key exclusion criterion is an average transfusion frequency of more than once every 3 weeks in the previous year. During the study, subjects will be transfused when their Hb reaches their individual transfusion trigger calculated from their transfusion history. The primary endpoint is the proportion of subjects who achieve a reduction in transfusion burden, defined as a reduction of ≥33% in the number of red blood cell units transfused during the 24 weeks of the fixed-dose period compared with the historical transfusion burden standardized to 24 weeks. Secondary endpoints include safety. An independent data monitoring committee will meet at least once to review the study data. The ACTIVATE-T study will be initiated globally in the first half of 2018.
Results: Not yet available.

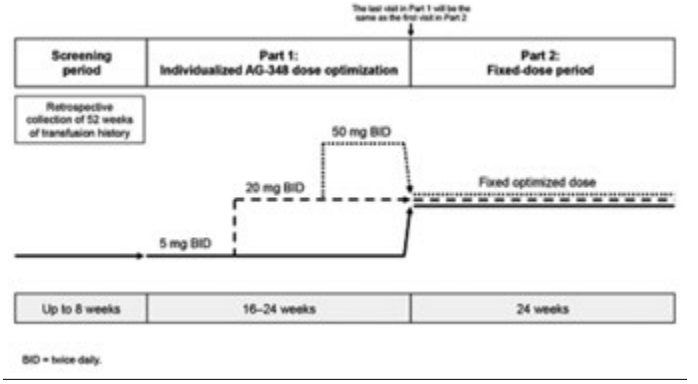

Figure 1. ACTIVATE-T study schema.

Summary and Conclusions: AG-348 is a novel, first-in-class PK-R activator undergoing clinical testing in subjects with PK deficiency. The ACTIVATE-T study of AG-348 in regularly transfused subjects with PK deficiency will start enrollment in 2018.

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CLASSIFYING, SHARING AND REVIEWING GENETIC VARIANTS ASSOCIATED WITH RED CELL DISORDERS

D. Philip[1], F. Smith[2], R. Mayhew[3], D. Brawand[4], S. Allouni[2], N. Parkin[3], B. Clark[4,5,*]

[1]SW Thames Regional Genetics, St Georges Hospital, [2]Molecular Diagnostics, [3]Molecular Pathology, Viapath LLP at Kings College Hospital, [4]Precision Medicine, Kings College Hospital, [5]Molecular Haematology, Kings College London, London, United Kingdom

Background: Having sequenced 212 genes in over a thousand individuals with a suspected red cell disorder we have amassed 1594 common genetic variants (allele frequency >10%) and classified 3408 unique variants, using a five class classification system. It is important to be able to share this genetic variation with other laboratories carrying out similar investigations to ensure patients receive an equitable service no matter which laboratory undertakes the testing. The Variant Scoring Assistant (VASA) that we have developed is a software tool that enables laboratories to upload variants in batch, as a VCF, or individually. This allows users to see how other laboratories have classified the same variant and gives them the opportunity to classify their own genetic variants and store any associated evidence. VASA scores variants according to the American College of Medical Genetics (ACMG) guidelines and automatically assigns a pathogenicity score. This score can be overridden by a user allowing scientists to exercise their scien-

tific judgment. By using VASA it is hoped that different laboratories will consistently score variants using the ACMG guidelines and will build their own institution specific dataset. Being able to see scores from other institutions should make pathogenicity classifications quicker and easier and reducing reporting times and improving patient care.
Aims: To develop a cloud based software tool that enables different laboratories to share genetic variant pathogenicity classifications.
Methods: Developed a SQL database that permits automated ACMG classification of genetic variants.
Results: Confirmed that the database scores variants using the ACMG guidlines by comparing manually scored variants to automatically scored variants. This indicated that the database rules and underlying code is functioning correctly. We have confirmed that variants can be uploaded in batch, as a VCF, or individually. When working from a VCF variants can be scored and the associated evidence stored using the VCF as a worklist. The VCF is not stored and therefore variants from the same individual are not linked. We have built an API allowing VASA to be linked to other databases.
Summary and Conclusions: By comparing scores submitted from different groups VASA can serve as a tool to identify genetic variants where there are differences in scores between groups. These variants could then be reviewed with the patient's haematology to understand the differences of opinion. If there is agreement on classification between multiple groups then laboratories can have greater confidence in these variant classifications. In this way VASA may work as a framework for collaboration on variant interpretation for red cell disorders. We aim to have the system hosted on a public cloud by June 2018.

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HIGH SUCCESS RATE OF TARGETED NEXT GENERATION SEQUENCING FOR THE DIAGNOSIS OF PATIENTS WITH RARE CONGENITAL ANEMIAS

O. Steinberg-Shemer[1,2,*], N. Shefer Averbuch[1], O. Dgany[3], T. Krasnov[3], S. Noy-Lotan[3], J. Yacobovich[1], A.A. Kuperman[4], A. Kattamis[5], A. Ben Barak[6], B. Roth[7], E. Chubar[8], E. Shabad[9], G. Dufort[10], M. Ellis[11], O. Wolach[12], I. Pazgal[13], A. Abu Quider[14], H. Miskin[15], H. Tamary[1]

[1]Hematology Oncology, Schneider Children's Medical Center of Israel, Petach Tikva, [2]Sackler School of Medicine, Tel Aviv University, Tel Aviv, [3]Pediatric Hematology Laboratory, Felsenstein Medical Research Center, Sackler School of Medicine, Tel Aviv University, Petach Tikva, [4]Blood Coagulation Service and Pediatric Hematology Clinic, Galilee Medical Center, Nahariya, Israel, [5]First Department of Pediatrics, National & Kapodistrian University of Athens, Athens, Greece, [6]Pediatric Hematology/Oncology Department, Rambam Medical Center, Haifa, [7]Department of Hematology, Hadassah Medical Center, Jerusalem, [8]Blood Bank, HaEmek Medical Center, Afula, [9]Blood Bank, Carmel Medical Center, Haifa, Israel, [10]Pediatric Hemato-Oncology Department, Centro Hospitalario Pereira Rossell, Montevideo, Uruguay, [11]Hematology Institute, Meir Medical Center, Kfar Saba, [12]Institute of Hematology, Davidoff Cancer Center, Rabin Medical Center, [13]Comprehensive Center of Thalassemia, Hemoglobinopathies & Rare Anemias, Institute of Hematology, Beilinson Hospital, Rabin Medical Center, Petach Tikva, [14]Pediatric Hematology, Soroka University Medical Center, Ben-Gurion University, Beer Sheva, [15]Pediatric Hematology Unit, Shaare Zedek Medical Center, Faculty of Medicine, Hebrew University, Jerusalem, Israel

Background: Most patients with anemia are diagnosed through clinical phenotype and basic laboratory testing, including a peripheral blood smear, iron body status, and hemoglobin electrophoresis. Nonetheless, in cases of rare congenital anemias, some patients remain undiagnosed despite undergoing an exhaustive workup. The diagnosis of those rare disorders often requires specialized analyses available in only a few dedicated laboratories. However, an accurate diagnosis in many of those disorders is crucial for optimal clinical management and for genetic counseling.
Genetic testing is complicated by the large number of genes involved in rare anemias and the similarities in the clinical presentation of the different syndromes. The advent of targeted gene capture technique followed by next generation sequencing (NGS) offers a rapid, accurate and cost-effective approach in these difficult cases.
Aims: We aimed to enhance the diagnosis of patients with congenital anemias by using targeted next generation sequencing.
Methods: Genetic diagnosis was performed by gene-capture followed by next generation sequencing of 78 genes known to cause anemia syndromes, including congenital dyserythropoietic anemia (CDA), red blood cell enzymopathies and membranopathies, sideroblastic anemia, and Diamond-Blackfan anemia (DBA).
Results: Genetic diagnosis was achieved in 13 out of 21 patients (62%). Six patients were diagnosed with pyruvate kinase deficiency, four with dehydrated hereditary stomatocytosis, two with sideroblastic anemia and one with CDA type IV. The mean lag-time from presentation to diagnosis was over 12 years. Overall, eight novel mutations were found. In seven patients the genetic diagnosis differed from the pre-test presumed diagnosis. One patient, who passed away due to recurrent pulmonary embolism post splenectomy, was eventually diagnosed with dehydrated hereditary stomatocytosis, a condition in which splenectomy is contra-indicated.
Summary and Conclusions: In our study, targeted next generation sequencing led to an accurate diagnosis in over 60% of patients with rare anemias. Earlier incorporation of this method into the workup of patients with congenital anemia may improve patients' care and enable genetic counseling.

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DIAGNOSIS OF RARE CONGENITAL HEMOLYTIC ANEMIAS ENABLED BY NEXT GENERATION SEQUENCING PANEL

E. Fermo[1,*], V. Cristina[1], A.P. Marcello[1], E.Y. Keskin[2], A. Feliu Torres[3], S. Perrotta[4], T. Aksu[5], A. Zaninoni[1], V. Brancaleoni[6], R. van Wijk[7], S.A. Koker[8], R. Colombatti[9], P. Corti[10], L. Farinasso[11], A. Zanella[1], A. Cortelezzi[12], W. Barcellini[1], P. Bianchi[1]

[1]Oncohematology Unit - Physiopathology of Anemias Unit, Foundation IRCCS Cà Granda Policlinico Milan, Milan, Italy, [2]Department of Pediatric Hematology and Oncology, Suleyman Demirel University, School of Medicine, Isparta, Turkey, [3]Servicio de Hematologia-Oncologia, Hospital de Pediatria "Dr Juan P. Garrahan" , Buenos Aires, Argentina, [4]Department of Woman, Child and of General and Specialist Surgery, I° Policlinico II° Università di Napoli, Naples, Italy, [5]Department of Pediatric Hematology and Oncology, University of Health Sciences, Ankara Child Health and Diseases Hematology Oncology Training and Research Hospital, Ankara, Turkey, [6]Medicina Generale, Foundation IRCCS Cà Granda Policlinico Milan, Milan, Italy, [7]Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht, Netherlands, [8]Pediatric Hematology and Oncology Department, Dr Behcet Uz Childrens Hospital, Izmir, Turkey, [9]Clinic of Pediatric Hematology-Oncology, Azienda Ospedaliera-Università di Padova, Padova, [10]Ematologia Pediatrica, Fondazione MBBM, Monza, [11]SCDU Pediatria 2, Struttura Semplice Ematologia, OIRM, Citta' della Salute e della Scienza, Torino, [12]Oncohematology Unit, Foundation IRCCS Cà Granda Policlinico Milan, University of Milan, Milan, Italy

Background: Hereditary chronic hemolytic anemias (CHAs) are heterogeneous rare disorders including defects of red cell membrane structure or cation permeability, defects of metabolism, hemoglobinopathies, and disorders of erythropoiesis. All these diseases are complex and frequently difficult to diagnose. The diagnostic laboratory tools include routine tests and more specialized analyses, available only in few dedicated laboratories. In our experience, a conclusive diagnosis is not reached in about 15-20% of cases despite detailed and exhaustive investigations. The advent of next generation sequencing (NGS) tools has made diagnosing complex genetic disorders feasible.
Aims: We developed and applied a NGS targeted panel to identify the underlying genetic cause in a selected series of patients with undiagnosed CHAs.
Methods: A total of 52 cases with CHAs from 40 families were studied. In 33 subjects no definitive diagnosis was previously done despite extensive investigation, in 12 the conventional diagnostic workup was unreliable due to transfusions or sample shipping. In addition three HS/HE families with intra-family clinical variability were studied to elucidate the molecular basis of the atypical phenotype. Using SureDesign software (Agilent) we created a NGS based panel containing 40 genes associated with RBC membrane disorders (13 genes), enzymopathies (20), CDAs (7), and 9 other candidate or modifier genes. Libraries were obtained by HaloPlexHS Target Enrichment System Kit and sequenced on a MiSeq platform (Illumina). The panel was validated on previously characterized patients. Mutations were confirmed by Sanger.
Results: We identified pathogenic variants that enabled a definitive diagnosis in 24 cases (17 families). Results are reported in Table 1. 15 mutations were novel and considered pathogenetic. Notably, we identified a PK deficient case with normal activity in absence of transfusions. Moreover, we found a TPI deficiency in a 7 month child with anemia but no neuromuscular manifestations at the time of the study, thus permitting an early diagnosis. A new pathogenic variant in *ALAS2* gene was detected in a female with macrocytic anemia, suggesting a diagnosis of congenital sideroblastic anemia; HUMARA analysis showed a skewed X-Chr inactivation pattern. Seven cases carried pathogenic variants in *PIEZO1* gene, associated with hereditary xerocytosis, five of them displaying p.R2456H mutation. We identified a new patient with a *de novo* R352H mutation in KCNN4 gene (Gardos Channellopathy) and one with a mutation in *ABCG8* gene associated with congenital sitosterolemia. Complex genotypes were identified in 3 families with HS/HE: case F11-817 (mixed HS/HE phenotype) carried two pathogenic variants in *SPTA1* and *SLC4A1*, whereas the brother with typical HE

had only the *SPTA1* variant; case F12-73 with combined 68% spectrin and 56% ankyrin deficiency, had two different *in trans* mutations in *SPTA1*, one of them associated with the alpha-LELY allele. Finally, 3 different pathogenetic *SLC4A1* variants were detected in case F13-723 with severe HS and 50% Band 3 deficiency, the father presenting only one missense mutation, thus justifying intra-family clinical variability.

Table 1.

Patient	Sex	Age** yrs	Gene	Position	HGVS (coding)	Effect	HOM/HET	Diagnosis	New variant
F1-888	M	55	PKLR	g.155261709G>A	NM_000298.5	R486W	HET	PK deficiency	n/a1610089***
			PKLR	g.155263047C>T	NM_000298.5	T384M	HET		n/a1613967***
F2-921	M	18	PKLR	g.155260203C>G	NM_000298.5	R139P	HOM	PK deficiency	No ExAC /10000
F3-475	M	12	GPI	g.34888643A>G	NM_001289790.2	G126D splicing	HET	GPI deficiency	n/a1801015
			GPI	g.34886477G>A	NM_001289790.2	R444H	HET		No ExAC /10000
F4-881	F	15	CYB5R3	g.43027421A>G	NM_001128913.2	F37S	HOM	NADH-cytob deficiency	No ExAC /10000
F5-897	M	7mo	TPI1	g.6979338G>C	NM_000365.5	E105D	HOM	TPI deficiency	n/a1984845***
F6-852	M	17	SEC23B	g.18505189delG	NM_001172745	V164WfsX3	HET	CDAII	No ExAC /10000
			SEC23B	g.18491520G>A	NM_001172745	R1HQ	HET		n/a1918022***
F7-804	F	26	ALAS2	g.55039948A>G	NM_001037967	H457R	HET	Sideroblastic anemia	No ExAC /10000
F7-920	F	49	ALAS2	g.55039948A>G	NM_001037967	H457R	HET		
F9-923	F	14	PIEZO1	g.88782120C>T	NM_001142864	R2456H	HET	DHS	rs587776988 ***
F9-396	F	41	PIEZO1	g.88782120C>T	NM_001142864	R2456H	HET	DHS	rs587776988 ***
F9-271	M	44	PIEZO1	g.88782120C>T	NM_001142864	R2456H	HET	DHS	rs587776988***
F17-916	F	3	PIEZO1	g.88782120C>T	NM_001142864	R2456H	HET	DHS	rs587776988***
F17-917	F	22	PIEZO1	g.88782120C>T	NM_001142864	R2456H	HET	DHS	rs587776988***
F15-914	M	18	PIEZO1	g.88786834G>A	NM_001142864	A2022T	HET	DHS	No ExAC /10000
F16-943	M	44	PIEZO1	g.88790120C>T	NM_001142864	R2113W	HET	DHS	No ExAC /10000
F14-920	F	1	KCNN4	g.44721790>A	NM_002250.2	R352H	HET	Gardos channelopathy	rs774455945***
F12-913	F	45	ABCG8	g.44729631G>A	NM_022437.2	R263Q	HOM	Sitosterolemia	rs137852890***
F11-917	F	44	SPTA1	g.158651385insTTG	NM_003126	L154FW	HET	HE/HS	No ExAC /10000
			SLC4A1	g.42704914C>G	NM_000342.3	S613R	HET		No ExAC /10000
F11-916	M	30	SPTA1	g.158651385insTTG	NM_003126	L154FW	HET	HE	
F12-73	M	37	SPTA1	g.158621158C>G -LelyA*	NM_003126.3	abnormal splicing	HET	severe/HPP	No ExAC /10000
			SPTA1	g.158623113G>A	NM_003126.3	R1047X	HET		No ExAC /10000
F12-74	M	65	SPTA1	g.158621158C>G -LelyA*	NM_003126.2	abnormal Splicing	HET	healthy father	
F12-75	F	65	SPTA1	g.158623113G>A	NM_003126.2	R1047X	HET	healthy mother	
F13-723	M	36	SLC4A1	g.42333213C>A	NM_000342.3	abnormal splicing	HET	HS	No ExAC /10000
			SLC4A1	g.42334875C>T	NM_000342.3	R490H	HET		No ExAC /10000
			SLC4A1	g.42337213 delTTGGGGAGCAGAGGC	NM_000342.3	QPLU*Q196Q	HET		No ExAC /10000
F13-776	M	72	SLC4A1	g.42334875C>T	NM_000342.3	R490H	HET	HS	No ExAC /10000

* presence of low expression α spectrin LELY allele in cis ** at the time of molecular diagnosis *** Known disease mutation (pathogenetic)

Summary and Conclusions: NGS platform is a powerful tool to elucidate the molecular causes in patients with CHAs where traditional hematologic testing failed. However, in this selected series we attributed pathogenetic variants in 40% of the examined families. Different approaches need to be considered to investigate the cause of hemolysis in the remaining cases.

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NEXT GENERATION SEQUENCING FOR DIAGNOSIS OF INHERITED HEMOLYTIC ANEMIAS

J. Nieto[1,*], C. Cuéllar PA[2], B. Prieto[1], F.A. Gonzalez[1], A. Villegas[1], A. Velasco[1], R. Trelles[1], A. Bobes[1], C. Vicente[1], A. Gonzalez[1], M. Ibarra[1], R. Martinez[1], P. Ropero[1]

[1]Hematology, Hospital Clínico San Carlos, [2]Hematology, Hospital Universitario La Princesa, Madrid, Spain

Background: Inherited hemolytic anemias (IHAs) are a group of congenital disorders with multiple etiologies, such as intrinsic erythrocyte enzyme deficiencies or membrane protein alterations. The typical clinical presentation is usually unspecific, with anemia, pallor, jaundice and often splenomegaly. Morphological and biochemical differential diagnosis between IHAs is challenging due to the limited availability of all the necessary techniques in one single centre. Mutations in many different genes can cause an IHA, thus, genetic analysis by Sanger sequencing become expensive and very time consuming. Of note, treatment options could be different based on the etiology of the IHA. In this scenario, we wonder if a Next Generation Sequencing (NGS) approach would be useful in the differential diagnosis between IHAs.
Aims: To define pros and cons of genetic analysis by NGS in patients with an IHA (with known or unknown cause).
Methods: Biological samples of 44 patients with IHA were sent to our hospital for genetic analysis to confirm or to find out a specific diagnosis. We have developed a custom sequencing panel (TruSeq Custom Amplicon, Illumina) for sequencing 40 genes (exons and intron-exon boundaries) previously associated to IHAs. Studied diseases encompass membranopathies (spherocytosis, stomatocytosis, etc.) and enzimopathies (deficiency in PK, G6PD, GPI, etc.). Genes causing congenital dyserythropoietic anemias were included in the panel. Hemoglobinopathies are studied in our hospital with other techniques. Only results concerning to variants classified as pathogenic, likely pathogenic and those with unknown significance are shown in this abstract. We have followed ACMG standards for variant classification.
Results: 34 out of 44 patients had an initial suspicion of a specific IHA. Eighty eight percent of them (n=30) have shown genetic variations that are likely causing the disease. In the other hand, 8 out of the remaining 10 patients with initial unknown cause for the IHA have genetic alterations. No significant alterations were found in 6 patients (14%).
Remarkably, NGS analysis helps us to identify 2 patients (#29 and #30) in which a spherocytosis was initially suspected but they actually have a PK deficiency. In this study we have found 17 new or novel genetic alterations. These results are summarized in Table 1.

Table 1. NGS results in 44 patients with suspected IHA.

Patient	Age (years)	Gender	Initial suspicion	Mutation 1	Mutation 2	Most probable diagnosis
#1	28	female	DHS (Xerocytosis)	PIEZO1 Lys2502Arg (LP)		DHS (Xerocytosis)
#2	25	female	DHS (Xerocytosis)	**PIEZO1 Ala1455Thr (US)**		DHS (Xerocytosis)
#3	25	female	DHS (Xerocytosis)	**PIEZO1 Leu597Phe (US)**		DHS (Xerocytosis)
#4	33	male	DHS (Xerocytosis)	PIEZO1 Met870Val (LP)		DHS (Xerocytosis)
#5	17	female	Elliptocytosis	**SPTA1 Gln2394Ter (LP)**	LELY (others)	Elliptocytosis
#6	4	female	Elliptocytosis	**EPB41 Glu248Ter (LP)**		Elliptocytosis
#7	43	male	Elliptocytosis	**EPB41 Val286ter (LP)**		Elliptocytosis
#8	37	male	G6PD deficiency	G6PD Haplotype A- (P)		G6PD deficiency
#9	42	female	G6PD deficiency	G6PD Ser218Phe (P)		G6PD deficiency
#10	11	male	G6PD deficiency	G6PD Arg488Cys (P)		G6PD deficiency
#11	21	female	GPI deficiency	GPI Lys561Glu (P)	GPI Lys561Glu (P)	GPI deficiency
#12	35	female	DHS	SLC4A1 Arg730Cys (P)		DHS (Cryohydrocytosis)
#13	30	male	PK deficiency	PKLR Arg486Trp (P)	PKLR His124Gln (LP)	PK deficiency
#14	45	male	PK deficiency	PKLR Arg510Gln (P)	PKLR Arg510Gln (P)	PK deficiency
#15	24	female	PK deficiency	PKLR Arg486Trp (P)	PKLR Glu241Stop (P)	PK deficiency
#16	43	female	PK deficiency	PKLR Arg486Trp (P)	PKLR His124Gln (LP)	PK deficiency
#17	59	female	PK deficiency	PKLR Arg486Trp (P)	PKLR Arg337Gln (LP)	PK deficiency
#18	16	male	Spherocytosis	**ANK1 Arg1293Ter (LP)**		Spherocytosis
#19	20	female	Spherocytosis	**ANK1 Arg1293Ter (LP)**		Spherocytosis
#20	60	female	Spherocytosis	SPTA1 Ala970Asp (US)	SPTA1 Ala970Asp (US)	Spherocytosis
#21	48	female	Spherocytosis	**SLC4A1 249Sdup0 (LP)**		Spherocytosis
#22	69	female	Spherocytosis	SLC4A1 Glu40Lys (US)		Spherocytosis
#23	5	male	Spherocytosis	SPTA1 c.798A>G (US)	LELY (others)	Spherocytosis
#24	76	male	Spherocytosis	SPTA1 Arg891Ter (LP)	SPTA1 Leu1485Phe (US)	Spherocytosis
#25	5	male	Spherocytosis	SPTA1 Leu1485Phe (US)	LELY (others)	Spherocytosis
#26	0	male	Spherocytosis	**ANK1 Asp68GlyfsTer6 (LP)**		Spherocytosis
#27	4	male	Spherocytosis	**ANK1 c.659_660dupGC (LP)**		Spherocytosis
#28	23	male	Spherocytosis in beta thal patient	KLF1 Phe182Leu (Others)	HBB Gln39Ter (P)#	Beta thalassemia intermedia
#29	52	female	Spherocytosis	PKLR Arg486Trp (P)	PKLR Arg486Trp (P)	PK deficiency
#30	5	male	Spherocytosis	PKLR Arg559Pro (LP)	**PKLR Gly411Cys (US)**	PK deficiency
#31	11	male	Not evident	**PIEZO1 Gly2365Arg (US)**		DHS (Xerocytosis)*
#32	7	male	Not evident	**PIEZO1 Gly2365Arg (US)**		DHS (Xerocytosis)*
#33	15	female	Not evident	**PIEZO1 Arg1527His (US)**		DHS (Xerocytosis)*
#34	0	male	Not evident	SPTA1 Ins666Ser (P)	LELY (others)	Pirospikelocytosis
#35	27	male	Not evident	SPTA1 Ala970Asp (US)	LELY (others)	Spherocytosis
#36	27	female	Not evident	**ANK1 Leu1524Arg (US)**		Spherocytosis
#37	40	male	Not evident	**CDAN1 Ala910Gly (US)**		CDA carrier
#38	33	female	Not evident	**CDAN1 Ser256Gly (US)**		CDA carrier
#39	0	female	Not evident	--		unknown
#40	30	female	Not evident	--		unknown
#41	78	male	Spherocytosis	--		unknown
#42	58	male	Spherocytosis	--		unknown
#43	1	female	Spherocytosis	--		unknown
#44	26	female	DHS (Xerocytosis)	--		DHS (Xerocytosis) (KCNN4 gene?)

* Cation permeability studies required
# Sanger sequencing
LELY: SPTA1 low expression allele; DHS: Dehydrated Stomatocytosis, DHS: Overhydrated Stomatocytosis, CDA: Congenital Dyserythropoietic Anemia; (P): Pathogenic, (LP): Likely Pathogenic; (US): Unknown significance. Novel mutations are indicated in bold.

Summary and Conclusions: Overall, there is a high percentage (86.4%) of patients with a recognizable genetic variant that is likely causing the IHA. In 6 cases we obtain no significant genetic results; 2 patients with no initial proposed cause for the IHA, 3 with low suspicion for spherocytosis and 1 with xerocytosis that could be caused by mutations in KCNN4 (not included in our panel). In some patients, biological significance of genetic variants is difficult to assess, especially when a gain of function variant is expected to cause the disease (*i.e.* PIEZO1 mutations in xerocytosis). Without complementing this information with functional analyses, many of the novel findings have to be classified as unknown significance variations. Therefore, it remains some degree of uncertainty in the diagnosis of a number of patients. In 10 cases (#29 to #38), NGS enabled us to better identify genetic variations that can cause IHAs. 8 of them had an unknown cause. Of special interest, there were 2 cases labeled as spherocytosis where NGS had a huge added value, since final diagnosis turned into PK deficiency (the former disease have new promising pharmacologic options in clinical trials).

PF390

REPORT OF THE FIRST CASE OF HEREDITARY SPHEROCYTOSIS DUE TO A LARGE DELETION OF THE SPTA1 GENE

J. Russello[1], I. Bertchansky[2], C. Guittard[1], O. Guillot[1], V. Cacheux[1], M. Giansily-Blaizot[1], P. Aguilar-Martinez[1,*]

[1]biological hematology, [2]addictology, CHU Montpellier, Montpellier, France

Background: Hereditary spherocytosis (HS), is caused by a defect in proteins forming the red cell membrane cytoskeleton, therefore leading to a progressive modification of the red cell shape and ultimately to red cell destruction by spleen macrophages. The typical clinical presentation is chronic haemolytic anaemia. Causative mutations are generally located in the *ANK1*,

SPTB, SLC4A1, EPB42 or *SPTA1* genes, which encode ankyrin, spectrin beta chain, the anion exchanger 1 (band 3) protein, protein 4.2 and spectrin alpha-chain, respectively.
Aims: Here, we report the first case of a large deletion of the *SPTA1* gene associated with HS.
Methods: The *proposita* is a18year-old girl who underwent a DAT negative haemolytic episode with anaemia (Hb 67 g/l) and hepatic cytolysis due to an acute cytomegalovirus infection. After recovery from the infection, haemoglobin levels spontaneously returned to normal (146 g/L) as well as LDH and bilirubin levels, but haptoglobin levels remained below 0.1g/L and the reticulocyte count ranged between normal and high values. A membrane defect was suspected because of increased osmotic fragility (positive pink test). The EMA test was in the normal range and osmotic gradient ektacytométrie displayed an atypical profile. Informed consent for genetic studies was obtained and analysis of a panel of eleven genes involved in HS was performed by next generation sequencing, using the Custom SureSelectQXT® Target Enrichment system (Agilent) on a MiSeq platform (Illumina). Each deleterious variation was independently checked using conventional Sanger sequencing. The large rearrangements were confirmed by Array-CGH using Sure Print G3 Human CGH Microarray 2x400K (Agilent).
Results: We did not identify any likely pathogenic (class 4) or pathogenic (class 5) variants among the eleven studied genes. The α^{LELY} allele (rs28525570; c.6531-12CNT) was present, apparently at the homozygous state. However, all *SPTA1* sequence variations were surprisinglyat the homozygous state, which may correspond to a loss of heterozygosity. Subsequent copy number variation (CNV) analysis revealed a large deletion of the *SPTA1* gene at the heterozygous state. The 392Kb deletion, confirmed by array-CGH, involved the following genes: *SPTA1, MNDA, OR6Y1, OR6P1, OR10X1,OR10Z1, OR6K2, OR6K3, OR6K6, OR6N1, OR6N2* on chromosome 1 (1q24.2) from 158496074 to 158888723. This is the first report of a complete deletion of the *SPTA1* gene, associated with hemizygozity of the α^{LELY} allele *in trans*. An interstitial deletion of the *SPTA1* gene leading to an expressed abnormal protein and associated with the α^{LELY} polymorphism *in trans* has been reported in a splenectomised patient with mild symptomatic elliptopoikilocytosis (Iolascon et al 2011).
Summary and Conclusions: SPTA1-related disorders have marked clinical, biochemical and genetic heterogeneity. In the reported case, despite the complete deletion of one *SPTA1* gene, the patients had a mild compensated haemolysis at the basic level and the disease was revealed by an acute episode triggered by viral infection. A possible explanation is that, when the α^{LELY} allele, which results in 50% reduction of alpha chains, is inherited *in trans* to a pathogenic *SPTA1* allele, a greater proportion of the defective α-spectrin is recruited by spectrin β-chains. This leads to a higher percentage of defective αβ dimers, resulting in a higher percentage of abnormal tetramers leading to a more severe clinical phenotype. In our case, the absence of abnormal alpha spectrin chains due the entire gene deletion may have impaired this phenomenon thus producing a milder phenotype.

PF391

CONGENITAL ERYTHROPOIETIC PORPHYRIA AS A MIMICKER OF CHRONIC HEMOLYTIC ANEMIAS IN INFANTS

Y. El Chazli[1,*], A. Elsharkawy[1], A. Salama[2], S. Hasan[3], B. Shanash[4], W. Shoman[5]

[1]Pediatric Hematology/Oncology, Faculty of Medicine, Alexandria University, [2]Pediatric hematology specialist, Alexandria University Hospitals, [3]Pediatric intensive care unit, [4]Pediatric clinical genetics, [5]Immunology/Rheumatology unit, Faculty of Medicine, Alexandria University, Alexandria, Egypt

Background: Congenital erythropoietic porphyria (CEP) is rare, with only approximately 200 cases reported in the literature. Cutaneous phototoxicity and hemolytic anemia are the predominant findings in CEP. The paucity of the reported cases makes an accurate description of the disease presentation and its diagnosis a real challenge, especially in resource-limited countries.
Aims: To report the experience of a single institution in the diagnosis of CEP and highlight the importance of considering this rare disease as a differential diagnosis of chronic hemolytic anemia in infants.
Methods: A descriptive study from the Hematology-Oncology clinic at Alexandria University Children's Hospital. After taking an informed consent, the patients have been subjected to history taking, clinical examination; Wood's light urine examination, urine and stool porphyrin quantification. Sanger sequencing to confirm Uroporphyrinogen III synthase (UROS) gene was done.
Results: Three patients (2 males and a female) have been diagnosed as CEP during 2016 spring season. All were born at term to consanguineous parents (first-degree cousins) and have presented to our clinic after several months of investigations. All three infants have been symptomatizing since birth, showing anemia requiring transfusions early in life. Two of them had received phototherapy for hyperbilirubinemia and have developed skin lesions that were thought to be incidental or due to neonatal sepsis. They had intermittently red urine and all have developed progressive hepatosplenomegaly during first months of life. They have undergone investigations for diagnosis of chronic hemolytic anemia and have been misdiagnosed as unclassified hemolytic anemia (patient 1) and as Evans syndrome (patient 2). It is only with exposure to the sun during the spring, that skin blisters appeared raising suspicion of CEP in the first two cases with an interval of several months between first presentation and final diagnosis. Patient '3' has been diagnosed earlier (at the age of 3.5 months) as awareness has been raised to CEP in our center. A positive Wood's light examination of urine strongly supported the diagnosis of CEP in the three cases. Confirmatory testing was done in collaboration with Triemli hospital in Zurich and revealed elevated urinary and fecal porphyrin and sequencing of exon 4 of UROS gene revealed a p.C73R mutation in the homozygote state in the three patients and in the heterozygote state in their parents (Figure 1).

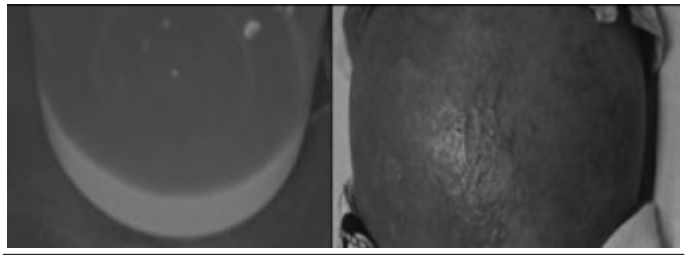

Figure 1.

Summary and Conclusions: Raising awareness about rare diseases like CEP is essential to prevent delayed diagnosis and increased complications. Manifestations of phototoxicity can be delayed and might not appear except during spring/summer months. The Wood's light examination is an easy and cheap way of supporting the diagnosis of CEP in resource-limited countries. Finally, although it is too early to determine the most common mutations causing CEP in Egyptian infants, this early report suggests testing for exon 4 mutations could be done as the first step of genetic testing in suspected cases.

PF392

CLINICAL CHARACTERISTICS OF PATIENTS WITH RED CELL PYRUVATE KINASE DEFICIENCY IN GERMANY

P. Klothaki[1,*], R. Grace[2], S. Eber[3], M. Wlodarski[4], J. Kunz[5], P. Bianchi[6], E. Fermo[7], S. Burdach[8], O. Andres[9], M. Nathrath[1], N. Kollmar[1]

[1]Pädiatrische Hämatologie und Onkologie, Klinikum Kassel, Kassel, Germany, [2]Dana-Farber Boston Children´s Cancer and Blood Disorder Center, Boston, United States, [3]Praxis für Kinder und Jugendmedizin, Munich, [4]Pädiatrische Hämatologie und Onkologie, Universitätsklinik Freiburg, Freiburg, [5]Pädiatrische Hämatologie und Onkologie, Universität Heidelberg, Heidelberg, Germany, [6]Fondazione IRCCS Ca´Granda, Ospedale Maggiore Policlinico, [7]Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Milan, Italy, [8]Medizinische Fakultät, Technische Universität München, Munich, [9]Erythrozytenlabor Universitäts-Kinderklinik Würzburg, Universitäts-Kinderklinik Würzburg, Würzburg, Germany

Background: Pyruvate kinase deficiency (PKD) is the most common enzymopathy of anaerobic glycolysis in erythrocytes. PKD is a rare autosomal recessive disease caused by mutations in the *PKLR* gene causing a hereditary non-spherocytic hemolytic anemia. There is a lack of research concerning clinical characteristics and molecular findings of patients with PKD in Germany.
Aims: This study describes clinical and molecular findings of patients with red cell PKD in Germany.
Methods: 28 patients (11 female, 17 male) with compound heterozygous or homozygous PKD who participated in Germany in the PKD Natural History Study (Bianchi et al 2017) were included in the analysis. Medical and laboratory data of the patients were retrospectively collected.
Results: 36% (n=10) of the patients were adult and 64% (n=18) children. The median age at diagnosis was 6.9 years (range: 0-48 years) and the median residual pyruvate kinase activity was 49% (range: 13- 100%). 57% of the patients (n=16) had severe anemia (Hb<8 g/dl), 11% (n=3) moderate anemia (Hb 8-10 g/dl) and 29% (n=8) mild anemia (Hb 10-12 g/dl). There was no correlation between the residual enzyme activity and the hemoglobin level. 39% (n=11) were homozygous and 61% (n=17) compound heterozygous for mutations in the *PKLR* gene. 23 different mutations were found.

The most common mutation was c. 1529 G>A (39% of the patients, n=11). 71% (n=20) of the patients had newborn hyperbilirubinemia and were treated with phototherapy (80%) and/or exchange transfusions (45%). 64% (n=18) needed at least 1 transfusion in the newborn time and 86% (n=24) of the patients needed at least 1 lifetime transfusion. 57% (n=16) received regular transfusions (≥6 transfusions over a 12 month period) after the newborn period. Chelation therapy for transfusion-associated iron overload was necessary in 36% (n=10) of the patients. Gallstones are common complication reported in 36% (n=10) of the patients. Liver cirrhosis was reported in 7% (n=2) of the patients. Splenectomy was reported in 29% (n=8) and cholecystectomy in 25% (n=7) of the patients with median age at splenectomy of 11.5 years (SD 11.9 years) and at cholecystectomy of 20.3 years (SD 12.7 years). The splenectomy led to a decrease in the transfusion burden and to an increase of median hemoglobin in 63% (n=5) of these patients but led to no change in hemoglobin or transfusion burden in 25% (n=2) of these patients. The median rise of the hemoglobin level was 1.7 g/dl (range: 0.9-3.3). In 12% (n=1) of these patients remained the effects of splenectomy unknown. 25% (n=2) of the patients developed a thrombosis after splenectomy.

Summary and Conclusions: This study suggests that patients with PKD living in Germany have wide clinical and molecular heterogeneity. Most patients have severe anemia and receive regular transfusions. Splenectomy leads to a decrease in the transfusion burden in the majority of patients. Careful monitoring for complications, such as iron overload, cholelithiasis and liver cirrhosis is essential.

Gene therapy, cellular immunotherapy and vaccination – Biology & Translational Research

PF393

ANTITUMOR ACTIVITY BY TEGS: ALPHA/BETA T CELLS ENGINEERED TO EXPRESS A DEFINED GAMMA/DELTA TCR IN A 3D BONE MARROW NICHE MODEL OF MULTIPLE MYELOMA

T. Straetemans[1], M. Braham[2], T. Aarts-Riemens[1], J. Alblas[2], M. Minnema[3], Z. Sebestyen[1], J. Kuball[3,*]

[1]Department of Hematology / Laboratory of Translational Immunology, [2]Department of Orthopaedics, [3]Department of Hematology, University Medical Center Utrecht, Utrecht, Netherlands

Background: γδT cells mediate cancer immune surveillance by sensing metabolic changes of malignant cells via their γδT cell receptor (TCR). Activation of the γδTCR is independent of MHC molecules, making them a valuable treatment strategie. Moreover, γδTCR are able to differentiate between healthy and leukemic stem cells (Sebestyen *et al.* Cell Reports 2016). This concept led to the development of next generation CAR T cells, so-called TEGs: aβT cells Engineered to express a defined γδTCR. A particular γ9δ2TCR, isolated from "clone 5", has been selected as the candidate for clinical testing (TEG001). TEG001 cells showed a strong and broad recognition of hematological malignancies (Straetemans *et al.* Clin Can Res 2015, Marcu-Malina *et al.* Blood 2011, Gruender *et al.* Blood 2012).

Aims: Important for the therapeutic success of TEGs is a better understanding of the cross-talk between malignant, stromal and immune cells present in the bone marrow, as well as investigation of the changes in the microenvironment during immune-therapy.

Methods: A 3D bone marrow niche model was established that allowed engraftment of multiple myeloma (MM) cell lines and primary MM cells within a humanized bone marrow niche consisting of mesenchymal stroma cells and endothelial progenitor cells (Braham *et al.*, Oncoimmunology 2018). TEG001 cells were engineered from both healthy donor and multiple myeloma patients' T cells and added when MM growth was established. Homing, efficacy and toxicity of TEG001 cell treatment was evaluated using a combination of confocal microscopy and luminex technology.

Results: TEG001 cells, but not mock engineered T cells, migrated into the 3D structure and exerted a killing response towards the tumor cells but not towards the supportive stroma cells. Importantly, this cognate recognition was associated with the differential production of chemokines, cytokines and inhibitory molecules. Amongst others, TEG001 cells induced CCL1 secretion, but also the secretion of IL-6 and GM-CSF, reported to be involved in cytokine release syndrome. Soluble Galectin-9, the proposed ligand for inhibitory receptor TIM-3, was reduced in the supernatant.

Summary and Conclusions: These findings demonstrate that TEGs are a promising addition to the currently available immune therapeutic strategies as they target cancer as a metabolic disorder. Moreover, we show that 3D-bioprinted bone marrow model allows the interplay between malignant, stromal cells and tumor-targeting TEG001 cells and enables studying of immune therapy in a complex tissue environment. In addition to tumor targeting, this system is able to model treatment-related immune escape mechanisms of bone marrow derived cancers and may be of great value to improve the chance to successfully translate novel immune therapy concepts, such as TEGs, into patients.

PF394

DUAL TARGETING STRATEGY OF ACUTE MYELOID LEUKEMIA BY ENGINEERED CYTOKINE-INDUCED KILLER CELLS COEXPRESSING AN INTERLEUKIN 3 CHIMERIC ANTIGEN RECEPTOR (CAR) AND AN ANTI-CD33 COSTIMULATORY RECEPTOR

V.M. Perriello[1,2,*], M.C. Rotiroti[2], M.P. Martelli[1], B. Falini[1], S. Tettamanti[2], A. Biondi[2]

[1]CREO, University of Perugia, Perugia, [2]Department of Pediatrics, University of Milano-Bicocca, Tettamanti Research Center, Monza, Italy

Background: Acute myeloid leukemia (AML) is the most common acute leukemia in adults. Chemotherapeutic regimens are initially effective in most patients but only a minority achieves long-term survival (about 25% 5-year survival). Adoptive cell therapy with chimeric antigen receptor (CAR) engineered T cells could represent a promising approach to prevent relapse by targeting chemoresistant quiescent leukemic stem cells (AML-LSCs). CD123 (known as IL-3 receptor alfa) and CD33 are myeloid surface markers co-

expressed in up to 70% AML, commonly upregulated in AML-LSCs and in both NPM1 and FLT-ITD mutant AML. Anti-CD123 CAR redirected Cytokine Induced Killer (CIK) cells demonstrated a better safety profile against normal hematopoietic stem/myeloid progenitor cells (HSPCs) compared to anti-CD33 CAR CIK cells in preclinical studies, probably related to the lower CD123 expression by normal HSPCs. Despite that, CD123 expression in healthy tissues such as endothelial cells represents a possible on-target off-tumor effect. Here, we probe a dual targeting model to improve selectivity for CD123+/CD33+ cells through a first generation anti-CD123 IL-3 cytokine CAR and an anti-CD33 without activation signalling domains.

Aims: The aim of the present study is to explore the anti-leukemic efficacy of genetically-modified CIK cells coexpressing anti-CD123 IL-3 cytokine CAR and anti-CD33 costimulatory receptor.

Methods: We evaluated the *in vitro* efficacy profile of dual targeting IL.3(z).CAR/CD33(CD28-41BB).CCR CIK cells together with single targeting first generation IL-3.CAR CIK cells and third generation (CD28-4.1 BB) anti-CD33.CAR CIK cells. IL-3.CAR and anti-CD33.CAR were generated in separate SFG-retroviral vectors while, for the dual targeting model, the IL-3.CAR and CD33.CCR constructs were cloned between a self-cleavage 2A peptide in a unique retroviral construct. Fresh and frozen peripheral blood mononuclear cells were transduced with retroviral vectors at day 4 and 5 of the CIK cell differentiation process. The anti-AML activity of all CAR-CIK cell conditions was assessed by means of cytotoxicity (4 hours at 5:1 E/T ratio), proliferation (72 hours at 1:1 E/T ratio) and cytokine production (5 hours at 1:3 E/T ratio) assays upon challenge with highly CD123/CD33 positive THP1 AML cell line, the CD123/CD33 negative MHH-CALL4 cell line was used as control.

Results: Dual targeting IL-3.CAR/CD33.CCR CIK cells display a potent and specific *in vitro* anti-leukemic efficacy against THP1 cells as compared to non-transduced CIK cells. The killing efficiency of the dual targeting model was up to 90%, analogous to third generation anti-CD33.CAR+CIK cells. In addition, IL-3.CAR/CD33.CCR CIK cells proliferate (up to 30%) and secrete IL-2 (up to 15% of IL-2 producing CAR-CIK cells) and IFNγ (up to 80% of IFNγ producing CAR-CIK cells), almost equally to anti-CD33.CAR, when stimulated with THP-1 cells. IL-3.CAR alone display also a similar activity, suggesting a high binding affinity for the CD123 target.

Summary and Conclusions: These data demonstrate a potent antitumor efficacy mediated by dual targeting IL-3.CAR/CD33.CCR CIK cells and single targeting IL-3.CAR- and anti-CD33.CAR CIK cells.The absence of a truly AML-restricted antigen supports the use of a double targeting approach in order to increase the selectivity of CAR-redirected T cells towards the leukemic target. Considering the power of the anti-CD123 IL-3.CAR, we next aim to find strategies that could retain efficacy and at the same time minimize toxicity by lowering the IL-3.CAR affinity.

PF395

SYNERGISTIC T CELL SIGNALING BY CD79A/CD40 COSTIMULATORY ENDODOMAIN ENHANCES CD19 CHIMERIC ANTIGEN RECEPTOR T CELL PROLIFERATION AND SURVIVAL

J. Julamanee[1,2,*], S. Terakura[1], K. Miyao[1], S. Okuno[1], S. Kamoshita[1], E. Takagi[1], T. Sakai[1], S. Okazaki[1], D. Koyama[1], T. Goto[1], T. Nishida[1], M. Murata[1], H. Kiyoi[1]

[1]Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan, [2]Department of Internal Medicine, Prince of Songkla University, Songkhla, Thailand

Background: Adoptive transfer of CD19 chimeric antigen receptor (CAR) T cell unveiled promising clinical outcomes, particularly in acute B-cell lymphoblastic leukemia (B-ALL). Among several studies, CAR-T cell persistence *in vivo* correlated with long-term clinical efficacy. Consequently, various costimulatory domains have been developed to improve T cell signaling, ultimately enhanced T-cell function and persistence. However, no novel signaling domains have been recently reported to be useful.

Aims: We adopted B-cell signal moiety, CD79A, and dendritic signal moiety, CD40, and generated the composite costimulatory domain of CD79A/CD40 to synergize T cell signaling in order to improve T cell proliferation and persistence.

Methods: The CD79A/CD40 costimulatory domain was developed and fused into anti-CD19scFv-CD28TM-CD3z-tEGFR construct. The CD19.79a.40z CAR gene was subsequently cloned into retroviral vector and transduced into human $CD3^+$ cells. The anti-EGFR mAb was used to assess CAR expression and purify, which resulted in achieving greater than 90% purity. We then expanded CD19 CAR-T cell by coculture with irradiated EBV-LCL. The expanded CD19.79a.40z CAR-T cells were further used for downstream experiments in order to compare with conventional CD19.28z CAR-T cells. In addition, CD19 CAR-transduced Jurkat-Dual luciferase reporter cells were also generated and used for NF- B signaling assay.

Results: The CD19 CAR genes were successfully transduced with similar transduction efficacy between the costimulatory domains. We next examined the NF- B activity and found significant higher NF- B signaling in CD19.79a.40z compared to CD19.28z upon $CD19^+$ target cells stimulation. Regarding cytokine production, the intracellular cytokine staining showed comparable proportions of IFN-γ^+ and IL-2^+ responders between CD19.79a.40z and CD19.28z CAR-T cells after stimulated with CD19-K562. The equal amounts of IFN-γ, IL-2, and TNF-α, produced by CD19.79a.40z and CD19.28z CAR-T cells, were also confirmed using ELISA. We next assessed T cell proliferation by coculturing CD19 CAR-T cells with CD19-K562 without exogenous IL-2. Surprisingly, the novel CD19.79a.40z exhibited robust T cell proliferation and persistence throughout 2 weeks of culture compared to CD19.28z (Figure 1). To analyze anti-tumor efficacy *in vitro*, we first performed a standard ^{51}Cr release assay and found similar cytotoxicity against CD19-K562 and two primary B-ALL samples between the costimulatory domains. We therefore did the coculture assay to examine the integrated activities of cytotoxicity as well as T cell expansion. Although CD19.28z CAR-T cells rapidly killed target cells in first few days, however, the target cell regrowth was observed after one week of culture. In contrast, CD19.79a.40z CAR-T cells could suppress target cell growth throughout the end of culture. To determine the plausible factors owing to T-cell survival advantage, we assessed T cell differentiation and exhaustion phenotypes upon antigen encounter and found no difference among the costimulatory domains. Lastly, we examined anti-tumor efficacy *in vivo* that a small survival advantage was observed in NALM-6 bearing mice treated with CD19.79a.40z CAR-T cells.

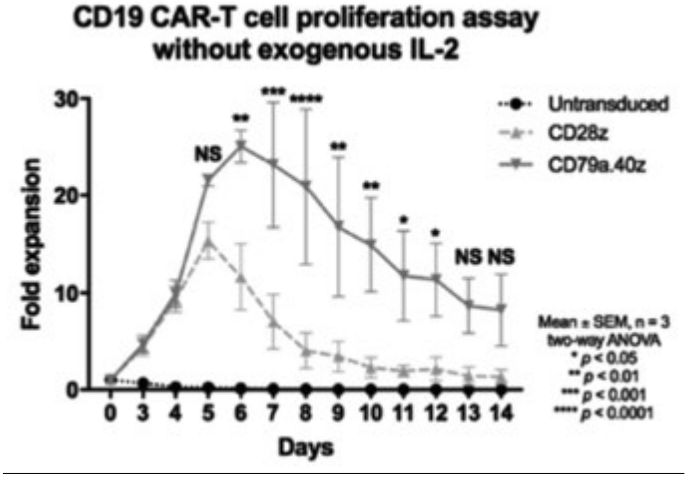

Figure 1.

Summary and Conclusions: The novel CD79A/CD40 costimulatory domain enhanced CD19 CAR-T cell proliferation and persistence, which improved anti-tumor efficacy. In addition, our construct exhibited higher NF- B activity upon antigen exposure, which possibly synergized to T-cell signaling and led to a proliferation advantage.

PF396

LENALIDOMIDE AND PROGRAMMED DEATH-1 BLOCKADE SYNERGISTICALLY ENHANCES THE EFFECTS OF DENDRITIC CELL VACCINATION IN A MODEL OF MURINE MYELOMA

S.-H. Jung[1], J.-J. Lee[1,2,*], M.-C. Vo[1], T.-H. Chu[1], H.-J. Lee[2], T.J. Lakshmi[1], H.-S. Park[1], H.-J. Kim[1], J.H. Rhee[2,3]

[1]Chonnam National University Hwasun Hospital, [2]VaxCell-Bio Therapeutics, [3]Chonnam National University Medical School, Hwasun-Eup, Korea, Republic Of

Background: The therapeutic efficacy of dendritic cell (DC)–based immunotherapy may be realized in combination with other anticancer therapies that enhance DC function by modulating immune responses and the tumor microenvironment.

Aims: In this study, we investigated the efficacy of DC vaccination in combination with lenalidomide and programmed death (PD)-1 blockade in a model of murine myeloma.

Methods: MOPC-315 cell lines were injected subcutaneously to establish myeloma-bearing mice and following five test groups were established: PBS

control, DCs, DCs+lenalidomide, DCs+PD-1 blockade, and DCs+lenalidomide+PD-1 blockade.
Results: The combination of DCs plus lenalidomide and PD-1 blockade strongly inhibited tumor growth compared to the other groups. This effect was associated with a reduction in immune suppressor cells (such as myeloid-derived suppressor cells, M2 macrophages, and regulatory T cells) and an increase in immune effector cells (such as $CD4^+$ and $CD8^+$ T cells, natural killer [NK] cells, and M1 macrophages) in the spleen. Functional activities of cytotoxic T lymphocytes and NK cells were also enhanced by the triple combination. Levels of immunosuppressive cytokines, such as TGF-β and IL-10, were significantly reduced in the tumor microenvironment.
Summary and Conclusions: These findings suggest that the combination of DCs plus lenalidomide and PD-1 blockade synergistically establishes a robust anti-myeloma immunity through a two-way mechanism, which inhibits immunosuppressive cells while activating effector cells with superior polarization of the Th1/Th2 balance in favor of the tumor immune response. This result would provide an experimental ground for incorporating check point inhibitors to existing immunotherapeutic modalities against multiple myeloma.

PF397

RNAI-MEDIATED SILENCING OF ENDOGENOUS TCR ENHANCES TUMOR KILLING ACTIVITY OF TCR-ENGINEERED WT1-SPECIFIC T CELLS

D. Campillo[1,*], F. Fujiki[2], J.M. Van den Bergh[1], H. De Reu[1], E.L. Smits[1,3,4], H. Goossens[1,5], H. Sugiyama[2], E. Lion[1,3], Z.N. Berneman[1,3,6], V.F. Van Tendeloo[1]

[1]Laboratory of Experimental Hematology, University of Antwerp, Edegem, Belgium, [2]Department of Cancer Immunology, Osaka University Graduate School of Medicine, Osaka, Japan, [3]Center for Cell Therapy & Regenerative Medicine, Antwerp University Hospital, [4]Center for Oncological Research (CORE), University of Antwerp, [5]Division of Clinical Biology, [6]Division of Hematology, Antwerp University Hospital, Edegem, Belgium

Background: The major bottleneck with standard cancer therapies is treatment failure leading to progressive disease or clinical relapse. The specificity of T cells for their cognate antigen turns them into an attractive and targeted cancer therapeutic. Unfortunately, the scarcity of tumor-reactive T cells and the difficulty of their isolation in sufficient numbers for adoptive cellular immunotherapy have hindered their clinical application. Gene transfer of a T cell receptor (TCR) specific for a tumor-associated antigen into T cells would confer redirected anti-tumor specificity to effector T cells for adoptive T-cell therapy. However, mispairing between transgenic and endogenous TCR alpha and beta chains results in reduction of transgenic TCR expression and potentially harmful reactivities.
Aims: Here, we sought to develop a novel non-viral, rapid and clinically safe strategy to promote transgenic expression of a Wilms' tumor 1 (WT1)-specific TCR by electroporation of Dicer-substrate small interfering RNA (DsiRNA) and codon optimized *TCR* mRNA electroporation.
Methods: First, we isolated and cloned an HLA-A*02:01-restricted WT1 peptide-specific TCR derived from a leukemia patient who demonstrated clinical benefit after receiving a WT1-targeted DC vaccine. Next, we produced a codon optimized *TCR* (WT1 *TCR*-co) sequence from the wild-type *TCR* construct (WT1 *TCR*-wt) and both *TCR* mRNAs were generated by *in vitro* transcription. In order to suppress the translation of endogenous *TCR* mRNA in $CD8^+$ T cells, DsiRNA duplexes were designed to specifically target the constant regions of endogenous TCR alpha (*TRAC*) and beta (*TRBC*) mRNAs, but not the codon optimized *TCR* mRNA. We further developed a sequential double electroporation protocol in which DsiRNA electroporation was performed 24 hours prior to transgenic *TCR* mRNA electroporation. To determine the epitope-specific activation and functionality of DsiRNA/TCR-engineered resting $CD8^+$ T cells, we analyzed the expression of CD69 and CD137, secretion of cytokines and cytotoxicity activity against epitope-bearing tumor cells.
Results: Our results show more than 2-fold increase in WT1 *TCR*-co TCR expression by HLA-A2/WT1 tetramer staining after DsiRNA treatment as compared to transgenic *TCR* mRNA electroporation only. Transgenic TCR expression was present at least 5 days after transgenic *TCR* mRNA electroporation in resting peripheral blood $CD8^+$ lymphocytes from healthy donors. The enhanced transgenic TCR expression in DsiRNA-transfected $CD8^+$T cells was also correlated with an increase of epitope recognition as shown by interferon (IFN)-γ ELISpot, higher levels of activation markers CD69 and CD137 and secretion of IFN-γ and granzyme B upon TCR triggering as compared to the non-DsiRNA treated T cells. Cytotoxicity, which was already present in WT1 *TCR*-wt and WT1 *TCR*-co mRNA-transfected cells, was significantly enhanced in WT1 *TCR*-co mRNA-electroporated T cells after suppression of the endogenous *TCR* by DsiRNA treatment.
Summary and Conclusions: In summary, we show a marked enhancement of transgenic codon optimized WT1-specific TCR expression upon silencing of the endogenous *TCR* using DsiRNA electroporation prior to *TCR* mRNA electroporation. Importantly, this enhancement in transgenic TCR expression was correlated with a significant increase in WT1-specific $CD8^+$ T-cell killing activity, expression of CD69 and CD137 and cytokine secretion after recognition of WT1 peptide-bearing target cells. These results pave the way for developing a clinically safer strategy for T-cell-based adoptive immunotherapy of patients with WT1-expressing malignancies.

Gene therapy, cellular immunotherapy and vaccination – Clinical

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ANTI-CD19 CAR T-CELL THERAPY IN ADULTS WITH RELAPSED/REFRACTORY AGGRESSIVE B-CELL MALIGNANCIES AS A BRIDGE TO ALLOGENEIC TRANSPLANTATION – THE EXPEIENCE OF A SINGLE CENTER

A. Avigdor[1,*], R. Shoval[1], A. Shimoni[1], O. Itzhaki[2], J. Schachter[2], A. Toren[3], E. Jacoby[3], M. Besser[4], A. Nagler[1]

[1]Division of Hematology and Bone Marrow Transplantation, Chaim Sheba Medical Center and Sackler School of Medicine, Tel Aviv University, [2]Ella Lemelbaum Institute for Immuno Oncology, Chaim Sheba Medical Center, Tel Hashomer and Sackler School of Medicine, Tel Aviv University, [3]Department of Pediatric Hematology-Oncology, Safra Children's Hospital, Chaim Sheba Medical Center and Sackler School of Medicine, [4]Ella Lemelbaum Institute for Immuno Oncology, Chaim Sheba Medical Center and Department of Clinical Microbiology and Immunology, Sackler School of Medicine, Tel Aviv University, Tel Hashomer, Israel

Background: Early encouraging results on treatment with T-cells expressing anti-CD19 chimeric antigen receptor (CAR) in relapsed/refractory (R/R) aggressive B non-Hodgkin lymphomas (B-NHL) have been recently published. The reported overall response rates (ORRs) compare favorably with historical data for patients treated with current standards of care. However, progression occurs in approximately 50% of responding patients in the first year. The impact of allogeneic stem cell transplantation (alloSCT) on outcome following CAR T-cell treatment is unknown.
Aims: We report the early results on the outcomes of adult patients (age 18-55) with R/R aggressive B-NHL, treated with locally produced CAR T-cells followed by consolidation with alloSCT.
Methods: This interim analysis is a part of a single center, phase 1b/2 study of CAR T-cell therapy in B-cell malignancies (NCT02772198) that is now underway in our center. Patients with R/R B-NHL and adequate organ function are eligible. The approach uses a CAR construct that is composed of an anti CD19 single-chain Fv, CD28 co-stimulatory and CD3-zeta intracellular domains. The CAR is transfected by retrovirus into bulk-stimulated autologous peripheral blood (PBL) cells. Treatment includes lymphodepletion with fludarabine and cyclophosphamide followed by infusion of fresh transduced T-cells. Responders proceed to an alloSCT. The primary end-points are safety and best ORRs 28 days after infusion.

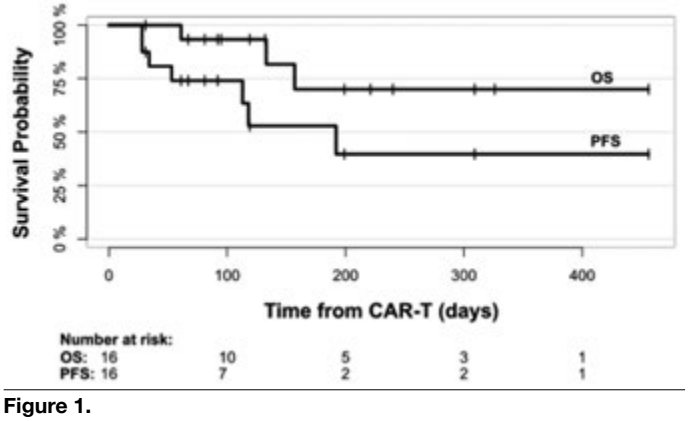

Figure 1.

Results: As of November 2016, 16 adult patients were enrolled. Thirteen patients were male. Median age was 36 years (range 23-52). Eleven patients were diagnosed with diffuse large B cell lymphoma (DLBCL, 8 – *de novo*, 2 - transformed and 1- primary mediastinal B cell lymphoma), 3 patients with B acute lymphoblastic leukemia and 2 with Burkitt lymphoma. Median number of prior therapies was 3 (range 2-5). Thirteen patients (81%) were refractory to their last therapy. Eight had a prior HSCT (4 - allogeneic and 4 - autologous). Ten days following apheresis patients received CAR T-cells at a median dose of 1 x 10^6/kg. Cells reached maximal expansion in PBL 7-14 days after infusion. Cytokine release syndrome occurred in 13 patients (83%), and was severe in one. Three patients (19%) experienced neurotoxicity (2 - grade 2, 1 - grade 3); one patient received tocilizumab and dexamethasone. No deaths were attributed to CAR T-cell therapy. alloSCT was performed in 11/16 patients (69%) at a median time of 59 days (range 48-126) after CAR T-cell infusion. Related toxicities were severe acute graft-*versus* host disease (2 patients) and sinusoidal obstruction syndrome (2 patients). At day 28 after infusion of CAR T-cells the ORs for the entire cohort and DLBCL group were 73% (47% CR) and 66% (33% CR), respectively. With a median follow-up of 7 months, 56% of patients continued to have a response. Median duration of response was 4 months (range 1-16). Three patients have died: 2 due to disease progression and one from transplant-related toxicity. Median progression free survival was 7 months and median overall survival was not reached (Figure 1).
Summary and Conclusions: Our interim results show that treatment patients with R/R B-cell malignancies with in-house produced CAR T-cells followed by consolidation with alloSCT is feasible. Our findings corroborate those from previous phase 2 studies, showing high ORRs. However, the durability of responses remains a major concern. Longer-term follow-up is needed to clarify the role of alloSCT in maintaining those responses.

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EARLY MRD NEGATIVITY PREDICTS DEEPENING MYELOMA RESPONSE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) PATIENTS TREATED WITH BB2121 ANTI-BCMA CAR T CELLS

N. Munshi[1,*], J. Berdeja[2], Y. Lin[3], J.M. Kochenderfer[4], N. Raje[5], M. Liedtke[6], S. Jagannath[7], D. Madduri[7], J. Rosenblatt[8], M. Maus[5], A. Turka[9], L.P. Lam[9], K. Hege[10], T. Campbell[10], M. Massaro[9], F. Petrocca[9], D. Siegel[11]

[1]Dana-Farber Cancer Institute, Boston, [2]Sarah Cannon Research Institute and Tennessee Oncology, Nashville, [3]Mayo Clinic, Rochester, [4]Experimental Transplantation and Immunology Branch, National Cancer Institute/Institutes of Health, Bethesda, [5]Massachusetts General Hospital Cancer Center, Boston, [6]Stanford University Medical Center, Palo Alto, [7]Mount Sinai Medical Center, New York, [8]Beth Israel Deaconess Medical Center, Boston, [9]Bluebird Bio Inc., Cambridge, [10]Celgene Corporation, San Francisco, [11]Hackensack University Medical Center, Hackensack, United States

Background: A high frequency of rapid minimal residual disease-negative (MRD-negative) responses has been seen in CRB-401, a phase I trial of bb2121 chimeric antigen receptor (CAR) T cell therapy for relapsed/refractory multiple myeloma (RRMM) (Kochenderfer, ASH 2017).
Aims: We report responses based on International Myeloma Working Group (IMWG) Uniform Response Criteria for Multiple Myeloma in MRD-negative patients and associated factors.
Methods: Patients treated with ≥150 × 10^6 anti-B-cell maturation antigen (BCMA) CAR+ T cells in the dose-escalation phase of CRB-401 and evaluable for MRD by Adaptive NGS-based MRD Assay (Adaptive Biotechnologies) were included in this analysis (n=10 as of 02 Oct 2017; median follow-up: 34 wks; min, max: 7, 67).
Results: Nine of 10 evaluable patients were MRD-negative with a sensitivity of 1 in 10^{-4} nucleated cells (1 patient), 1 in 10^{-5} (6 patients), and 1 in 10^{-6} (2 patients). Achievement of MRD negativity was independent of depth of response at first MRD-negative assessment; 2 patients had stable disease, 3 had partial response (PR), 2 had very good PR (VGPR), and 2 had complete response (CR)/stringent CR. Of 8 evaluable MRD-negative patients, all showed ≥85% and ≥97% decline in serum BCMA and involved free light-chain levels, respectively, at month (M) 1. Two of 9 MRD-evaluable patients were in CR at MRD-negative assessment and the remaining 7 achieved deeper response over time, 4 with CR or stringent CR, 2 with VGPR and 1 with PR between M1 and M15. One MRD-negative patient became MRD-positive at M12, and 1 MRD-negative patient had progressed as of data cut-off. Achievement of MRD negativity was independent of occurrence of cytokine release syndrome. MRD-negative status was observed across all active bb2121 doses (150 [n=3], 450 [n=5], and 800 x 10^6 [n=1] CAR+ T cells). Of 9 MRD-negative patients, 7 had at least 6 months follow-up and 1 had IMWG progression (at M6); 3 had at least 12 months follow-up, 1 had become MRD-positive, and none had IMWG progression. Attainment of MRD-negative status was independent of peak CAR T expansion (vector copy min, max: 93,744, 1,457,070 copies/µg gDNA; n=9) and was observed in high (>50%) bone marrow plasma cells (BMPC; n=6) and low BMPC (n=3) tumor burden patients. Eight of 9 MRD-negative patients had cytogenetic abnormalities including del(17), del(13), amp(1q21), or t(11:14).
Summary and Conclusions: bb2121 induced a high frequency of rapid MRD-negative responses, independent of IMWG MM responses. These early MRD-negative responses starting at M1 offer insights into bb2121 kinetics and may portend achievement of deeper responses.

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FIRST-LINE THERAPY WITH DONOR DERIVED HCMV-SPECIFIC T CELLS PROMOTES ANTIVIRAL IMMUNITY BY RESTORING THE QUANTITY AND FUNCTION OF RECIPIENT'S ENDOGENOUS HCMV-SPECIFIC T CELLS AFTER ALLO-HSCT

X. Zhao[1,*], X. Pei[1], Y. Chang[1], Y. Wang[1], L. Xu[1], X. Huang[1,2]

[1]Peking University People's Hospital, Peking University Institute of Hematology, [2]Peking-Tsinghua Center for Life Sciences, Beijing, China

Background: Our previous trial had demonstrated that adoptive transfer with donor HCMV-specific T cells is a promising therapeutic option for the treatment of persistent human cytomegalovirus (HCMV) infection after allogeneic stem cell transplantation (allo-SCT). However, the safety and antiviral activity of adoptive transfer with donor HCMV-specific T cells as for first-line therapy for HCMV have yet to be established. In addition, the mechanisms driving the sustained antiviral immunity induced by adoptive T cell transfer remain undetermined. Previous studies reported that adoptive therapy with donor HCMV-specific T cells can enhance antiviral immunity concomitantly with the reduction of HCMV viremia. However, whether the recovery of HCMV-specific T cells is delivered by adoptive infused T cells, recipient's endogenous immune recovery after stimulation, or both, remains unknown.
Aims: i) To evaluate the safety and efficacy of first-line therapy with HCMV-specific T cells for HCMV infection. ii) To explore where the adoptive transferred cells trafficked and persisted, and identify whether adoptive T cell therapy could promote HCMV-specific immunity. iii) To investigate the recovery of HCMV-specific immunity were from the *in vivo* expansion of adoptive T cells or the recovery of recipient's endogenous T cells.
Methods: i) We conducted a prospective study of 21 allo-SCT patients who had a high risk developing refractory HCMV infection, and treated with donor HCMV-specific T cells together with antiviral drugs as first-line therapy when HCMV infection was diagnosed. Another group of 21 matched patients only treated with antiviral drugs were selected as controls. The quantitative and functional recovery of HCMV-specific T cells were monitored in both cohorts. ii) We transferred HCMV-specific T cells labeled with both of BrdU and CFSE to HCMV infected humanized mice, and explored the recovery of HCMV-specific antiviral immunity in transferred mice and control mice.
Results: i) First-line therapy with HCMV-specific T cells promoted the quantitative and functional recovery of HCMV-specific T cells, and showed a trend to reduce the persistent HCMV reaction rate (4.8% *vs* 28.6%, *P*=0.038) and HCMV disease rate (0% *vs* 4.8%, *P*=0.311) compared to control group. There were no significant side effects attributable to adoptive T cell infusion. ii) Adoptive transferred HCMV-specific T cells had the ability homing to the bone marrow, spleen and liver, and diminished HCMV pathology in humanized mice. A preferential *in vivo* proliferation of HCMV-specific T cells were observed, and peak of HCMV-specific T cells were on day 14 after adoptive therapy. However, rate of the transferred HCMV-specific T cells was reduced over time, and on day 21 post infusion all of the proliferated HCMV-specific T cells were recipient's endogenous T cells. These observations indicated that adoptive T cell therapy most likely contributed to endogenous HCMV-specific T cell reconstitution.
Summary and Conclusions: First-line therapy for HCMV infection with donor HCMV-specific T cells showed prolonged benefit which promoted the quantitative and functional recovery of HCMV-specific T cells and decreased the rate of refractory CMV infection. However, the long-term recovery of the HCMV-specific immunity mainly developed from the restoring of recipient's endogenous T cells, not the expansion of transferred cells.

PF401

LONG TERM OUTCOMES OF TABELECLEUCEL (ALLOGENEIC THIRD-PARTY EBV-TARGETED CYTOTOXIC T LYMPHOCYTES) FOR RITUXIMAB-REFRACTORY POST-TRANSPLANT EBV+ LYMPHOMAS: A SINGLE CENTER EXPERIENCE

S. Prockop[1,*], E. Doubrovina[1], A. Feng[2], G. Koehne[3], P. Dahi[4], E. Papadopoulos[4], C. Sauter[4], S. Suser[1], W. Navarro[2], A. Sudhindra[2], R. O'Reilly[1]

[1]Pediatric BMT, Memorial Sloan Kettering Cancer Center, New York, [2]Clinical Development, Atara Biotherapeutics, Westlake Village, [3]BMT and Hematologic Oncology, Miami Cancer Institute, Miami, [4]Adult BMT, Memorial Sloan Kettering Cancer Center, New York, United States

Background: Monoclonal EBV+ post-transplant lymphoma disorders (PTLD) following solid organ transplant (SOT) or hematopoietic cell transplant (HCT) are life-threatening. Patients (pts) who fail rituximab(R)-based therapy have limited treatment options. R failure in EBV+ PTLD following HCT portends a dismal prognosis with a median overall survival (OS) in the range of 16-56 days (d). Pts with EBV+ PTLD following SOT tolerate anthracycline-based chemotherapy more poorly than non-immunocompromised lymphoma pts. Effective therapy with lower toxicity is needed. We have previously shown that treatment with tabelecleucel (tab-cel™) results in durable responses and a 1-year OS rate of 50-67% in R-refractory EBV+ PTLD pts after HCT (Prockop AACR 2015). Here we report longer-term study results of tabelecleucel for pts with EBV+PTLD following HCT or SOT.
Aims: Report updated long-term survival results of tabelecleucel in pts with EBV+ PTLD following HCT or SOT
Methods: Protocols 11-130 and 95-024 were single-center, open-label studies enrolling pts with EBV+ PTLD following HCT or SOT and other EBV-driven malignancies with measurable disease and adequate organ function and performance status. Pts providing signed informed consent were treated with tabelecleucel sharing ≥2/10 HLA alleles with the disease, including ≥1 HLA allele through which tabelecleucel exerts cytotoxicity (HLA restriction). Tabelecleucel was given at 2 x 10^6 cells/kg/dose (Protocol 11-130) or 1-2 x 10^6 cells/kg/dose (Protocol 95-024) on d1, 8, and 15 of every 4-6 week cycle with imaging at ~d35 of each cycle. Pts could receive multiple cycles of tabelecleucel.
Results: Across both studies from 1995-2017, 50 (35 HCT; 14 SOT) pts with PTLD after R-based failure received tabelecleucel (Table 1).

Table 1.

Median (range)	HCT-Associated PTLD (N=35)	SOT-Associated PTLD (N=14)
Age: yrs	28 (5-74)	18 (6-77)
Karnofsky Score—adults (Protocol 11-130 only)	70 (30-100, n=23)	60 (40-90, n=7)
Time from Diagnosis to Start of Tab-cel: months (mos)	1.2 (0.2-14.6)	13.3 (1.2-139)
Cell Dose: cells/kg/dose	2 x 10^6 (0.9-2.4)	1.9 x 10^6 (1.6-2.4)
Number of Cycles	2 (1-5)	2 (1-6)
Duration of Treatment: mos	1.4 (0.4-25.5)	1.4 (0.05-14.5)
Duration of Follow-Up: mos	23.3 (0.5-88.9)	21.3 (0.3-115)

The 1-yr Kaplan-Meier (KM) estimated OS for pts with PTLD following HCT and SOT is 68 and 64%, respectively. Median OS for HCT PTLD pts has not been reached after a median follow-up of 23.3 mos; median follow-up for SOT pts was 21.3 mos and median OS is 21.3 mos. Treatment-related serious adverse events (SAEs) were reported in 1 and 1 pts with PTLD following HCT and SOT, respectively.
Summary and Conclusions: Long term follow-up of pts with R-refractory PTLD following HCT or SOT treated with tabelecleucel shows durable responses and low incidence of related SAEs. Median OS for HCT pts with PTLD was not reached with a median follow-up time of 23.3 mos; recognizing limitations of cross-study comparisons, this represents a marked improvement compared to reports for this pt population prior to tabelecleucel. Phase 3 studies of tabelecleucel in pts with R relapsed/refractory PTLD after HCT or SOT are underway.

PF402

THE EFFICACY AND SAFETY OF HUMANIZED CD19 CAR-T CELLS FOR RELAPSED OR REFRACTORY B-CELL NON-HODGKIN LYMPHOMA (NHL): A PRELIMINARY REPORT

H. Huang[1,*], J. Cai[1], F. Xiao[1], X. Han[1], B. Ni[1], T. Yin[1], Z. Li[2], F. Chen[1], J. Hou[1]

[1]Department of Hematology, Ren Ji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, China, [2]State Key Laboratory of Oncogenes and Related Genes, Shanghai Cancer Institute, Ren Ji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, China

Background: Chemo-resistant, advanced B cell lymphomas are difficult to treat and often fatal. Recent studies have shown that chimeric antigen receptor (CAR) T cells have shown promising results in patients with B cell malignancy. However, CAR-T therapy-associated adverse systemic responses can be life-threatening. Therefore, how to optimizing CAR-T therapy regimen is key to the success of such innovative treatment.
Aims: The safety and efficacy of administering autologous humanized CD19 CAR-T cells to patients with relapsed or refractory (R/R) CD19(+) B-cell non-Hodgkin lymphoma (NHL) were assessed. Herein we report preliminary results from the single institute using CD19 CAR-T cells to treat R/R B-cell NHL. This study is registered at www.clinicaltrials.gov as #NCT02933775.
Methods: Patients with relapsed or refractory CD19+ B-cell NHL were eligible, provided there was no curative treatment available. Autologous CAR-T cells were manufactured by CARsgen Therapeutics. Patients received a conditioning chemotherapy regimen of cyclophosphamide and fludarabine

followed by infusion of CD19 CAR-T cells. Cyclophosphamide (400mg/m^2) was given from d-6 to d-5 and fludarabine (20mg/m^2) from d-6 to -4 or -3. The primary objective of the trial is to evaluate the safety of humanized CD19 CAR-T Cells as determined by the incidence of dose-limiting toxicities. Key secondary objectives include evaluating the overall response rate, duration of response, levels of CAR-T cells in the blood, and levels of serum cytokines. Cytokine release syndrome (CRS) was graded per revised criteria (Lee *et al.* Blood 2014).

Results: Three patients were enrolled into this prospective cohort until March 2018. Patient 1 was diagnosed as mantle cell lymphoma (MCL) in 2015, obtained complete remission (CR) after 6 cycles of R-CHOP and relapsed after 12-month follow-up. Patient 2 had diffuse large-B cell lymphoma (DLBCL) diagnosed in 2016 and she never achieved CR after several lines of immune-chemotherapy regimens. Patient 3 had DLBCL (double-expressor lymphoma) diagnosed in 2017, obtained partial remission (PR) after 3 cycles of R-CHOP and progressed after additional 3 cycles of same regimen. The patients received infusion of CAR-T cells (1x10^7 cells patient#1; 3x10^7 patient#2; 6x10^{7}patient#3). One month after CAR-T cells infusion, the PET-CT or CT scan showed all of the three patients responded to treatment (1/3 CR; 2/3 PR) without any symptoms of CRS. Laboratory test indicated the cytokines were dramatically increased in the peripheral blood 3 days after infusion and began to decrease gradually, especially serum IL-8 levels. The circulating CAR transgene can still be detected after 1 month and remained in high level. All patients had neutropenia (grade II adverse events) and lymphocytopenia (grade III to IV adverse events). No other adverse events were found during the treatment. Up to submission of this abstract, patient#1 and #3 remained in remission after 9 and 5 months post CAR-T infusion respectively. Patient#2 progressed after 3 months and was still alive after 8 months post CAR-T infusion.

Summary and Conclusions: This is the first report of successful treatment of R/R B-cell NHL with humanized CD19 CAR-T cells. The efficacy and safety demonstrated by these preliminary results provides strong support for further study of this approach.

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THE STUDY OF LYMPHOCYTES SUBPOPULATIONS IN PERIPHERAL BLOOD OF PATIENTS WITH AND WITHOUT GVHD PROPHYLAXIS BY MEANS OF MULTIPOTENT MESENCHYMAL STROMAL CELLS AFTER ALLOGENEIC BMT

Y. Davydova[1,*], N. Kapranov[1], I. Galtseva[1], N. Petinati[1], N. Drize[1], L. Kuzmina[1], E. Parovichnikova[1], V. Savchenko[1]

[1]*National Research Center for Hematology, Moscow, Russian Federation*

Background: Multipotent mesenchymal stromal cells (MSCs) are increasingly used to modulate immune responses in conditions related to auto-alloimmunity, including graft *versus* host disease (GVHD). The mechanisms that possibly underlie the capacity of MSCs to treat and prevent GVHD are still obscure. Most studies involve extensive characterization of the heterogeneity within and between different MSC preparations. Patient monitoring can help to identify the *in vivo* mechanisms of action in the treatment of GVHD.

Aims: The aim of the study is to analyze the alterations in lymphocytes subpopulations in patients soon after the injection of MSCs and a month later.

Methods: The study included 28 patients received allo-BMT from related donors after informed consent. The patients were randomized into 2 groups: first received standard prophylaxis of GVHD (n=13) and the second (n=15) were additionally infused with MSCs from the bone marrow of corresponding hematopoietic stem cells donor. MSCs were administered intravenously when the blood counts indicated recovery (WBC ≥1x10^9/l). Lymphocytes markers CD25, HLA-DR, PD-1 as well as distribution of naïve and effector cells were studied by flow cytometry on day 0 just before MSCs injection, day 3 and day 30 after MSCs injection. Differences between treatments groups were analyzed using Student's t-test for normally distributed data and the Mann–Whitney test for non-normal distributions.

Results: The Table 1 shows the composition of lymphocyte population. On day 0 before MSCs injection there were no significant differences between the groups. The absolute number of CD4+ transitional memory (TM) cells increased 3 times (p=0.029), CD8+ terminal effectors (TE) 8 times (p=0.05), CD4+PD-1+ 4 times (p=0.019) on 3rd day after MSCs injection in comparison with the control group. The subpopulations of CD4+ central memory (CM) increased 2.3 times (p=0.007); CD4+TM - 2.8 times (p=0.05); CD4+TE - 3.5 times (p=0.01); CD8+TE - 2.7 times (p=0.03); CD4+CD25+ - 2.8 times (p=0.02) and T reg 2.3 times (p=0.007) on 30th day after MSCs injection in comparison with the control group. The remaining subpopulations of lymphocytes did not differ significantly between the groups either 3 or 30 days after the MSCs administration. A significant increase in the number of central memory and effector memory cells, as well as CD4+CD25+ in the peripheral blood of patients after MSCs administration, in the absence of changes in the number of naive T cells, indicates the activation of cells participating in the adaptive immune response. The incidence of infections was lower in the group of patients with MSCs (27% *vs* 50%, non-significant). The number of CD8+TE was 4 times lower in patients with infections (p=0.004). The increase in the T-reg subset confirms the possibility of MSCs modulating immune response in the development of acute GVHD.

Table 1.

Cells number	Day 0		Day 3		Day 30	
	No MSCs	MSCs	No MSCs	MSCs	No MSCs	MSCs
WBC, ×10^9/L	1.57±0.4	1.36±0.08	2.18±0.56	2.22±0.25	2.7±0.53	3.6±0.72
% Lymphocytes	13.33±1.8	21.46±3.6	16.3±0.56	21.01±3.2	30.5±4.42	39.7±5.13
Lymph. ×10^3/uL	0.16±0.02	0.3±0.06	0.24±0.04	0.45±0.07	0.7±0.16	1.5±0.48
%CD4+	5.05±1.01	7.26±1.99	5.17±1.62	6.78±1.32	5.4±1.76	7.9±1.7
%CD8+	2.17±0.39	3.33±0.74	3.04±0.99	3.65±0.87	9.7±1.79	14.5±3.4
CD4+, /uL	0.06±0.01	0.10±0.03	0.06±0.03	0.15±0.04	0.1±0.02	0.2±0.05
CD8+, /uL	0.03±0.01	0.05±0.01	0.03±0.02	0.09±0.03	0.2±0.08	0.3±0.08
CD4+, /uL	2.06±0.48	2.16±0.61	4.58±2.62	4.33±1.18	0.9±0.42	2.3±0.65
CD4+NV, /uL	22.0±4.34	24.05±6.04	31.93±13.6	39.81±8.8	26.0±10.3	44.0±11.73
CD4+CM, /uL	15.12±3.76	20.66±6.2	18.27±4.85	32.99±7.52	17.7±6.91	40.6±8.78
CD4+TE, /uL	0.64±0.33	0.69±0. 4	0.62±0.22	1.82±0.73	1.5±0.98	5.3±1.82
CD4+TM, /uL	17.5±3.69	38.32±13.7	21.31±3.92	57.91±15.18	38.3±8.71	106.3±31.8
CD4+EM, /uL	0.96±0.29	1.19±0.49	1.01±0.46	1.85±0.58	9.9±5.38	34.6±20.5
CD8+SCM, /uL	6.99±2.55	8.33±3.86	3.82±0.82	30.81±11.52	14.0±6.12	14.0±4.07
CD8+NV, /uL	1.59±1.17	0.93±0.29	0.55±0.26	2.26±0.97	0.4±0.15	0.9±0.32
CD8+CM, /uL	2.61±1.67	1.67±0.69	1.59±0.89	5.18±2.13	2.5±0.71	3.9±0.88
CD8+TE, /uL	6.59±1.63	11.31±2.98	10.36±2.56	82.33±34.17	63.0±19.0	168.3±41.01
CD8+TM, /uL	9.03±1.78	11.57±2.56	22.06±9.27	76.14±32.52	39.6±12.2	91.4±46.03
CD8+EM, /uL	4.8±1.95	7.35±2.47	6.41±3.26	75.12±40.13	121.7±67.	346.7±250.1
Treg, /uL	7.1±1.99	9.84±2.9	9.2±3.5	16.68±4.01	4.8±2.20	11.2±2.44
CD4+DR+, /uL	5.0±1.93	8.25±2.29	5.26±2.11	18.86±10.18	21.7±9.19	88.2±37.83
CD4+PD1+, /uL	2.45±0.82	8.09±2.4	3.92±1.05	14.6±4.07	22.4±4.56	70.2±26.18
CD4+CD25+, /uL	13.92±3.16	28.23±7.85	25.78±12.27	38.94±9.14	10.01±4.4	28.0±6.63
CD8+DR+, /uL	7.12±1.63	8.2±2.63	25.62±11.76	30.08±12.44	151.7±59.	163.53±44.8
CD8+CD25+, /uL	1.50±0.67	2.67±0.94	2.71±1.08	4.24±1.91	2.6±0.98	4.0±1.35
CD8+PD1+, /uL	5.05±2.66	3.38±1.66	5.92±2.31	4.75±2.3	25.5±6.62	56.8±28.55

Summary and Conclusions: The alterations in lymphocytes subpopulations caused by MSCs may contribute to prevention of infectious complications and GVHD.

PF404

LIVE ATTENUATED VARICELLA ZOSTER VIRUS VACCINATION TO ADULT T-CELL LEUKEMIA/LYMPHOMA PATIENTS CAN INDUCE TUMOR SPECIFIC CELLULAR IMMUNITY

T. Jo[1,*], K. Horio[1], J. Taguchi[1]

[1]*Hematology, Japanese Red Cross Nagasaki Genbaku Hospital, Nagasaki, Japan*

Background: Adult T-cell leukemia/lymphoma (ATLL) is caused by human T lymphotropic virus type I (HTLV-I). ATLL is still quite difficult to be cured despite lots of researchers and clinicians have struggled for this entirely refractory hematologic malignancy for more than 40 years. Up-regulation of cellular immunity such as HTLV-I Tax-specific cytotoxic T cells (CTLs) has been proved to be important to win long time survival through knowledge of previous experiments and clinical experiences. However, no efficacious method to activate ATLL specific cellular immune response is available at present.

Aims: We planned this clinical study to investigate if Live Attenuated Varicella Zoster Virus (VZV) vaccination to ATLL patients can activate HTLV-I Tax specific cellular immune response. We also examined TCR V beta repertoire pre- and post-VZV vaccination. The primary objective is whether the percentage of Tax-specific CTLs after VZV vaccination increases.

Methods: It was planned that 3 indolent (smoldering or favorable chronic) type ATLL and 3 to 5 aggressive (acute, lymphoma, and unfavorable chronic) type ATLL patients to be enrolled in this study. VZV vaccination was conducted twice with at least 12 weeks interval between 2 doses. VZV vaccination method was changed for patients enrolled after May 2017 to 2 courses. Each course included up to 5 doses of vaccine with 1 to 4 weeks intervals.

Results: The enrollment of patients started from May 2016. Three indolent type (smoldering) patients and 2 aggressive type ATLL (1 acute, 1 lymphoma) patients were enrolled in this study. The aggressive type ATLL (lym-

phoma) patient was enrolled after May 2017 and subjected to the changed VZV vaccination protocol. The aggressive (1 acute and 1 lymphoma) type patients had VZV vaccination after the end of whole courses of anti-ATLL therapy including mogamulizumab (Moga, an anti-CCR4 monoclonal antibody) and combination chemotherapy including EPOCH therapy and VCAP-AMP-VECP therapy. Treatment effect after finishing Moga plus chemotherapy was complete remission in both patients. On the other hand 3 indolent type patients had VZV vaccination without any anti-tumor treatment. In all enrolled patients, no grade 3/4 adverse event due to VZV vaccination has been observed up to the present. Figure 1 shows the results of Tax-specific CTLs using tetramer assay in 1 smoldering and 2 aggressive type patients. There was no significant change in 2 indolent type patients (data not shown). However, moderate elevation from 0.15% (pre-vaccination) to 0.27% (post vaccination) was observed in 1 indolent type patient (Figure 1A). Obvious elevation of Tax-specific CTLs was observed in 2 aggressive type patients. The percentages changed from 0% to 0.07% in the acute type patient (Figure 1B) and from 0.02% to 0.75% in the lymphoma type patients (Figure 1C) after VZV vaccination. No disease progression has been observed in 3 indolent type patients, and complete remission has been maintained for more than 750 days in the acute type patient and 460 days in the lymphoma patient since the beginning of immunochemotherapy at present.

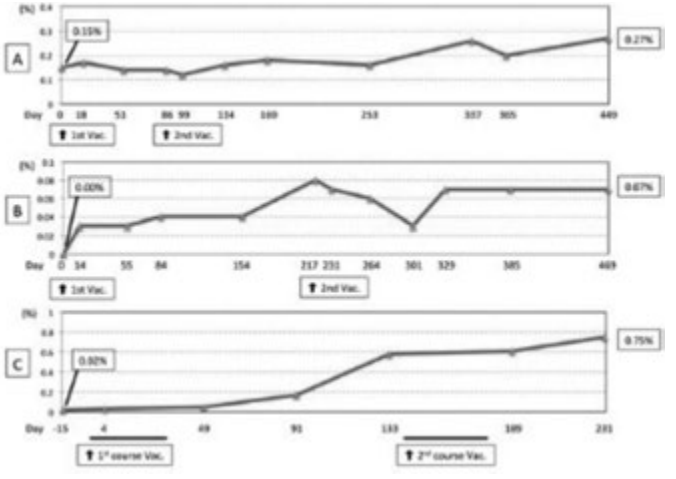

Figure 1. Tax-specific CTL/CD8+ T cell (tetramer assay).

Summary and Conclusions: These data suggest that VZV vaccination to aggressive type ATLL patients after Moga plus chemotherapy can up-regulate HTLV-I Tax-specific CTLs without any problematic adverse events. Enrollment for aggressive type ATLL patients is on going at present.

PF405

PRODUCTION OF ARI-0001 CELLS (A3B1:CD8:4-1BB:CD3Z CAR19 CELLS) IN PATIENTS WITH CD19+ RELAPSED/REFRACTORY B-CELL MALIGNANCIES USING THE CLINIMACS PRODIGY SYSTEM

M. Caballero-Baños[1], J. Delgado[2,*], S. Rives[3], M. Castella[1], V. Ortiz-Maldonado[2], A. Boronat[1], E. Garcia-Rey[4], J. Cid[5], M. Lozano[5], B. Marzal[1], J.M. Canals[6], A. Vilarrodona[4], J. Tabera[4], E. Trias[4], A. Urbano-Ispizua[2], J. Yagüe[1], M. Juan[1]

[1]Immunology, [2]Hematology, Hospital Clinic, [3]Hematology, Hospital Sant Joan de Deu, [4]Banc de Sang i Teixits, [5]Apheresis Unit, Hospital Clinic, [6]University of Barcelona, Barcelona, Spain

Background: The prognosis of relapsed or refractory (R/R) B-cell malignancies is very poor, particularly in patients relapsing after, or ineligible to, allogeneic hematopoietic cell transplantation (alloHCT). In the last decade, several chimeric antigen receptor targeting CD19 (CAR19) constructs have been developed. One of them (tisagenlecleucel) was approved by the FDA for pediatric or young adults with R/R ALL. The other construct (axicabtagene ciloleucel) was approved by the FDA for R/R diffuse large B-cell lymphoma after two or more lines of systemic therapy.
Aims: To produce our own CAR19 (ARI-0001) cells for clinical use in patients with R/R CD19-positive B-cell malignancies recruited into the CART19-BE-01 clinical trial
Methods: We selected the anti-CD19 A3B1 hybridoma licensed by our institution, identified the scFv sequence and incorporated the CD8, 4-1BB and CD3z modules next to it. We cloned it into a 3rd generation lentiviral vector and transduced PBMCs from buffy coats after activation with CD3 and CD28 TransACT polymeric nanomatrix (ARI-0001 cells). Once cytotoxicity and specificity were confirmed *in vitro* and *in vivo* (in NALM6-xenograft NSG murine models), we scaled-up both lentiviral and cell production, the latter using the CliniMACS Prodigy System (Miltenyi). After reaching all pre-specified acceptance criteria in lymphoaphereses from 3 healthy donors, the Spanish Agency of Medicines approved our IND and also our first pilot clinical trial (clinicaltrials.gov NCT03144583) on May/2017. Eligibility criteria included R/R ALL (adult and pediatric), non-Hodgkin's lymphoma and chronic lymphocytic leukemia (CLL) who had failed all standard available therapy.
Results: As of February 2018, we have recruited 14 patients with R/R CD19+ B-cell malignancies, 10 patients with ALL, three patients with DLBCL and one patient with CLL. Patients with ALL (with one exception) had relapsed after alloHCT. Median age was 22.5 years (range 3-54) and 42% were female. Six out of 10 patients with ALL had active disease at the time of study inclusion, while all 4 patients with DLBCL/CLL had progressive disease upon inclusion. We successfully prepared ARI-0001 cells in all but two patients, who required two procedures each (2/16 [12.5%] production failure rate). Causes of production failure were bacterial contamination (1 patient) and insufficient viral transduction (1 patient). The median total number of cells harvested after 8-10 days of culture was 1350 $x10^6$(range, 230-3400 $x10^6$). The median percentage of CD3+ cells was 97.5% (median of CD4+ cells 56%; median of CD8+ cells 42%). The median percentage of ARI-0001 cells in the cell product was 30.15% (range, 20.4-72.3). After fludarabine (90 mg/m^2) and cyclophosphamide (900 mg/m^2) chemotherapy, we have infused 0.5-5 $x10^6$ ARI-0001 cells/kg to 11 patients. In one patient, the infusion was delayed due to pneumonitis and the other two patients are awaiting quality control results. In all infused patients, ARI-0001 cells were detected in peripheral blood together with B-cell aplasia.
Summary and Conclusions: It is feasible to prepare CART19 (ARI-0001) cells in a purely academic setting using the automated CliniMACS Prodigy System and home-made lentiviral vectors. The production failure rate was 12.5% and yet enough cells were produced in all patients (two required two procedures).

Hematopoiesis, stem cells and microenvironment

PF406

BLOOD-CIRCULATING BILE ACIDS SUPPORT EXPANDING HEMATOPOIETIC STEM AND PROGENITOR CELLS AND IMPROVE RECOVERY FROM MYELOSUPPRESSIVE CHEMOTHERAPY

V. Sigurdsson[1,*], Y. Haga[2], V. Radulovic[1], H. Takei[3], M. Suzuki[1], C. Okamatsu-Haga[4], M. Van Der Garde[1], E. Mansell[1], S. Koide[1], H. Nittono[3], A. Ohara[2], K. Miharada[1]

[1]Dep. Molecular Medicine and Gene Therapy, Lund University, Lund, Sweden, [2]Department of Pediatrics, Toho University Omori Medical Center, [3]Junshin Clinic Bile Acid Institute, Tokyo, [4]Department of Pathology, Tokai University School of Medicine, Kanagawa, Japan

Background: Chemotherapeutic agents and irradiation can damage hematopoietic stem and progenitor cells (HSPCs) in bone marrow and cause prolonged myelosuppression or myeloablation in the worst cases. These side effects are among the most common life-threatening complications of cancer treatment. It is hard to predict in which patients this will be problematic but will limit the tolerability of the therapy, result in a delay and/or discontinuation of the treatment protocol and can impact cancer free survival of patients. Previously, we discovered a novel role for bile acids (BAs) as key components in the expansion of HSPCs in fetal development and that BAs suppress the activation of the unfolded protein response (*Sigurdsson et al., Cell Stem Cell, 2016*). Recently we have seen that BAs are upregulated in plasma of pediatric cancer patients undergoing chemotherapy.
Aims: The aim of the project was to explore if bile acids supported recovery of the hematopoietic system and HSPCs after chemotherapy.
Methods: To study the role of bile acids during hematopoietic recovery we used pediatric patient material, mouse models of altered BA levels and composition and BA supplementation to 5-Fluorouracil treated mice.
Results: We have discovered that BAs are highly increased in systemic circulation, specifically during recovery from myelosuppression and that this is correlated to improved clinical outcome in pediatric patients. Mice treated with 5-Fluorouracil (5-FU) showed a similar upregulation of BAs, during recovery. Using a mouse model of altered bile acid composition, *Cyp8b1* KO mice, we see that a subset of mice recover poorly after 5-FU chemotherapy. The poor recovery was connected to low expression levels of key BA production enzymes in liver and subsequent low levels and changed composition of total BAs in systemic circulation. In line with this, BA supplementation, using TUDCA, in 5-FU treated WT mice resulted in significantly improved recovery (Figure 1).

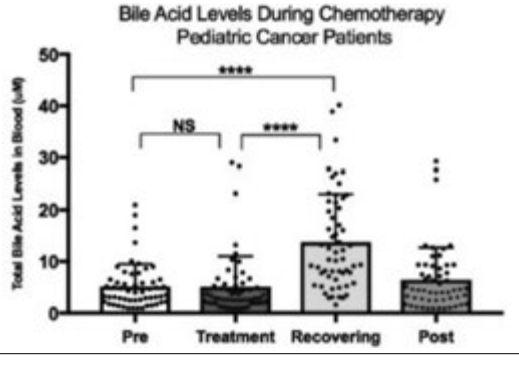

Figure 1.

Summary and Conclusions: Our results indicate that bile acids are a part of the recovery system in hematopoietic regeneration, and a potential supportive factor for rapid and efficient recovery from myelosuppression.

PF407

EARLY GROWTH RESPONSE (EGR)-1 IS A KEY REGULATOR OF HUMAN PRIMARY BONE MARROW STROMA CELLS WITH A DUAL ROLE IN PROLIFERATION AND HEMATOPOIETIC STROMA SUPPORT FUNCTION

H. Li[1], R. Ghazanfari[1], H. C. Lim[1], J. Hansson[1], D. Zacharaki[1], S. Scheding[2,*]

[1]Molecular Hematology, University of Lund, [2]Hematology, University Hospital Lund, Lund, Sweden

Background: Bone marrow mesenchymal stem cells (BMSCs) are essential constituents of the hematopoietic stem cell (HSC) niche with potent hematopoietic stroma function and multilineage differentiation capacity. Despite considerable progress in the identification of the bona fide MSC in human bone marrow, little is known about how BMSCs are regulated. Our previous gene expression profiling data demonstrated that the expression level of early growth response 1 (EGR1) in primary colony-initiating bone marrow MSCs was substantially higher compared to the non-colony-forming cells and cultured stromal cells, respectively.
Aims: The regulatory role of EGR1 in human BMSC has thus far not been investigated. In this study, we therefore studied the functional role of EGR1 for BMSC proliferation and hematopoietic supporting function.
Methods: EGR1 expression and function in human bone marrow stroma cells were evaluated by gene expression analysis, proteomics, gain-of-function and loss-of function approaches, and CD34 expansion and transplantation assays.
Results: EGR1 expression was 126.9±9.1-fold and 2.8±0.2-fold higher in highly fibroblast colony-forming cells (CFU-F) enriched human primary $lin^-/CD45^+/CD271^+/CD140a^-$ bone marrow cells compared to the non-colony forming $CD45^-/CD271^-$ cell population and $CD45^-/CD271^+/CD140a^+$ cells, which contains minimum CFU-F activity.
The hematopoietic supporting function of EGR1 was evaluated by co-culturing cord blood (CB) $CD34^+$ cells with EGR1 knockdown or EGR1 overexpressing stromal cells. HSC function of the expanded $CD34^+$ CB cells was evaluated by *in-vivo* transplantation into NSG mice. Both the percentage and absolute number of transplantable $CD34^+CD90^+$ hematopoietic stem cells (HSCs) were reduced in co-cultures with EGR1 knockdown cells as supporting stroma, while HSC production was significantly increased in co-cultures with EGR1 overexpressing cells. *Ex vivo* CB $CD34^+$ expansion experiments using transwell assays and stroma cell-conditional medium demonstrated that the EGR-1 mediated hematopoietic support was mediated by both soluble factors and direct cell-cell contact. Array-based gene expression profiling demonstrated upregulation of multiple hematopoietic supporting genes in EGR1 overexpressing stromal cells. Of these, a functional role was demonstrated for CCL28 (soluble factor) and for surface expressed VCAM1, which is mediating cell-cell contact.
EGR1 knockdown, on the other hand, resulted in enhanced colony-forming capacity and reduced population doubling times compared to controls. In contrast, CFU-F activity was virtually absent in EGR1 overexpressing cells. Proteomic profiling of EGR1 knockdown stromal cells identified a group of downregulated proteins related to oxidative-reduction processes, and reactive oxygen species (ROS) levels were indeed elevated in EGR1 knockdown cells, which explained the increased cell proliferation of these cells
Summary and Conclusions: In summary, EGR1 is highly expressed by primary BM-MSC compared with non-colony forming cells. EGR1 expression negatively regulates BMSC proliferation while positively regulating hematopoietic stroma support function. Increased proliferation of EGR1 knockdown cells is (at least in part) mediated by elevated ROS levels, while the enhanced hematopoietic support of EGR1 overexpressing cells is mediated by upregulation of a number of hematopoietic supporting genes. Our data thus indicate that EGR1 is an important MSC regulator coordinating the specific functions of BMSC in their different biological contexts.

PF408

A SINGLE-CELL RNA-SEQUENCING ATLAS OF THE MOUSE SPLEEN

R. Chawla[1,2,3,*], M. Wray-Dutra[4,5], D. Jackson[3], D. Rawlings[4,5,6], R. James[5,6,7], C. Trapnell[3,8]

[1]Department of Pediatric Hematology & Oncology, University of Basel Children's Hospital, [2]Department of Biomedicine, University of Basel, Basel, Switzerland, [3]Department of Genome Sciences, [4]Department of Immunology, University of Washington, [5]Center for Immunity and Immunotherapies, Seattle Children's Research Institute, [6]Department of Pediatrics, [7]Department of Pharmacology, [8]Molecular & Cellular Biology Program, University of Washington, Seattle, United States

Background: B cells constitute a principal component of humoral immunity and their dysregulation leads to numerous human diseases. Following activation, B cells undergo terminal differentiation within secondary lymphoid organs such as the spleen, giving rise to antibody-secreting plasma cells. Much effort has been devoted towards the classification and characterization of B-cell subsets. Yet, most studies to date are biased in that the subsets are classified on the basis of cell surface marker expression and characterized in bulk.
Aims: We set out to dissect the cellular components of the murine spleen in a completely unbiased manner using single-cell RNA-sequencing and to study their dynamics upon immunization with a T cell-dependent antigenic

stimulus. By comparing the cellular and molecular dynamics in wildtype mice *vs* different transgenic mouse models, we also aimed to shed light on mechanisms underlying lymphomagenesis.
Methods: We immunized wildtype and transgenic mice with degenerating sheep red blood cells and prepared single-cell suspensions from spleens harvested at different time points post-immunization. Following immunomagnetic B-cell enrichment, single-cell cDNA libraries were generated using a commercially available, droplet-based platform and sequenced using standard next-generation sequencing pipelines. Established computational methods were used for the processing of sequencing reads, as well as for the subsequent, in-depth analysis.
Results: We have thus far generated single-cell expression profiles of nearly 40,000 murine spleen cells across different conditions. Using unsupervised clustering, we were able to classify not only broad cell types but also many cellular subtypes and define their expression signatures in a completely unbiased manner. Besides validating the known splenic B-cell subsets, including transitional, follicular, marginal zone, B1a, germinal center (GC) B cells, and differentiated plasma cells, we found a previously uncharacterized follicular B-cell population with a strong type I interferon response signature. Differential gene expression analysis demonstrated that naïve B cells exhibit a much lower total mRNA abundance than activated B or plasma cells, revealed both known and new markers for the identified B-cell subsets, and uncovered novel insights into the patterns of immunoglobulin expression. Moreover, we were able to generate branched developmental trajectories which suggest that early B-cell maturation into plasma cells occurs primarily via an extrafollicular pathway rather than via the GC. Finally, we found that expression of a constitutively activate version of CARD11 in B cells promoted skewing of GC B cells towards a dark zone phenotype, along with enhanced class switching of both GC B and plasma cells.
Summary and Conclusions: In conclusion, we used single-cell RNA-sequencing to dissect the cellular components of the murine spleen, thereby defining the transcriptional signatures of multiple B-cell subtypes and maturational stages, and gain novel insights into the kinetics of B-cell maturation, both in wildtype and transgenic settings. Importantly, this dataset is the first to offer an unbiased classification of virtually all splenocytes, and we are hopeful that it will serve as a reference to immunologists and genomicists alike.

PF409

BONE MARROW ERYTHROPOIETIC DEFECT ACCOMPANYING EXTRAMEDULLARY HEMATOPOIESIS IS MIMICKED BY DOWNREGULATION OF NOTCH SIGNALING IN SPECIFIC MICROENVIRONMENT

T. Sakamoto[1,2,*], N. Obara[1], H. Nishikii[1], T. Kato[1], R. Fujimura[3], L. Cao Sy[4], M. Sakata-Yanagimoto[1], S. Takahashi[5], S. Chiba[1]

[1]Department of Hematology, Faculty of Medicine, University of Tsukuba, [2]Transfusion Medicine, University of Tsukuba Hospital, [3]School of Medical Sciences, [4]Department of Hematology, Graduate School of Comprehensive Human Sciences, [5]Department of Anatomy and Embryology, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan

Background: Nestin-expressing cells (NeC) have been characterized as one of many types of bone marrow (BM) stromal cells, including endothelial cells, osteoblasts, CXCL12-abundant reticular cells, etc. Recent studies have gradually provided information about anatomy and functions of each of these cells. Nevertheless, subcellular signaling and transcriptional regulations in individual stromal cells remains unclear. Several previous studies showed that Notch signaling in BM microenvironmental cells developed myeloproliferative phenotypes. Despite this, information is limited whether Notch signaling plays a role in NeC. Nestin was originally identified in neural stem cells (NSCs), in which Notch signaling is known to play a pivotal role in the NSCs. This linkage urged us to investigate whether and how downregulation of Notch signaling in BM NeC affects hematopoiesis.
Aims: To investigate whether and how downregulation of Notch signaling in BM NeC affects hematopoiesis.
Methods: Mice with an *Rbpj*-flox allele were crossed with mice with a *CreERT2/ Z/EG* transgene under the nestin promoter (*Nestin-CreERT2;Z/EG;Rbpj$^{f/f}$* mice). Tamoxifen was intraperitoneally injected 5 times/week for 4 weeks to delete the *Rbpj* gene only in NeC (*Rbpj* cKO mice), with once a week maintenance injection for up to 8 weeks until the analysis. In this experimental system, GFP is expectedly expressed as a surrogate marker for the *Rbpj* gene deletion. Then, transplantation assays were performed using *Rbpj* cKO BM cells as a donor to reconstitute hematopoiesis in the wild-type (WT) mice, or using *Rbpj* cKO mice as recipients to see reconstitution of hematopoiesis from WT BM cells. To investigate the mechanism of impairment of erythroid differentiation in the BM, the erythroid-island reconstitution assay was performed, using BM macrophages from *Rbpj* cKO and littermate control mice and BM Ter119$^+$ erythroid cells prepared from WT mice. The littermate *Rbpj*$^{null/wt}$or*Rbpj*$^{wt/wt}$ mice were used as controls.
Results: GFP was detected by flow cytometry in approximately 0.5% of CD45(-)Ter119(-)CD31(-) cells only after tamoxifen injection. Deletion of *Rbpj* was specifically confirmed in CD45(-)Ter119(-)CD31(-)GFP(+) BM cells. *Rbpj* and *Hes1* mRNA level was decreased in BM CD45(-)Ter119(-)CD31(-)GFP(+) cells of *Rbpj* cKO mice. BM of *Rbpj* cKO mice demonstrated marked decrease in the CD71(+)Ter119(+) mature erythroid cells without obvious anemia and without changes in the hematopoietic stem/progenitor cells. In contrast, mild splenomegaly was observed in *Rbpj* cKO mice. In transplantation analysis, the *Rbpj* cKO recipients transplanted with BM cells from WT mice also showed the maturation arrest in erythroid cells and mild splenomegaly. The WT recipients transplanted with BM cells from *Rbpj* cKO mice did not show any phenotypes. The erythroid island reconstitution capacity of macrophages from cKO BM showed the reduction compared with that of the macrophages from control mice. Additionally, mRNA level of *interleukin-6*, which is reported as the regulator of erythroid island formation, was decreased only in the macrophages forming erythroid islands.
Summary and Conclusions: *Rbpj* cKO in NeC induced impaired erythroid differentiation in BM together with mild splenomegaly, accompanied by the damaged erythroid island forming capacity of BM macrophages in *Rbpj* cKO mice, through the IL-6 hyperproduction of the macrophages. The *Rbpj* cKO mice may model a part of inflammation-induced disorder of erythropoiesis accompanying mild splenomegaly.

PF410

HIERARCHICALLY RELATED LINEAGE-RESTRICTED FATES BY MULTIPOTENT HEMATOPOIETIC STEM CELLS

P. Woll[1,*], J. Carrelha[2,3], Y. Meng[2,3], L.M. Kettyle[1], T.C. Luis[2,3], R. Norfo[2,3], V. Alcolea[2,3], H. Boukarabila[2,3], A. Gambardella[3], A. Grover[3], K. Högstrand[1], A. Lord[1], A. Sanjuan-Pla[3], C. Nerlov[3], S.E.W. Jacobsen[1,2,3]

[1]Dept. of Medicine, Huddinge and Dept. of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden, [2]HSC Laboratory, Weatherall Inst. of Molecular Medicine, [3]MRC Molecular Hematology Unit, Weatherall Inst. of Molecular Medicine, University of Oxford, Oxford, United Kingdom

Background: Rare multipotent hematopoietic stem cells (HSCs) in adult bone marrow (BM) with extensive self-renewal potential possess the ability to efficiently replenish all myeloid and lymphoid blood cells, securing long-term multilineage reconstitution following physiological and clinical challenges, including chemotherapy and hematopoietic transplantations. HSC-transplantation remains the only curative treatment for many hematological malignancies, but inefficient blood-lineage replenishment remains a major cause of morbidity and mortality. Single cell transplantation has uncovered considerable heterogeneity among reconstituting HSCs, supported by findings in unperturbed hematopoiesis and suggested to reflect different propensities for lineage-fate decisions by distinct myeloid-, lymphoid- and platelet-biased HSCs. However, other studies suggested that such lineage-bias might rather reflect generation within the phenotypic HSC compartment of unipotent or oligopotent self-renewing progenitors, and implicated uncoupling of the defining HSC properties of self-renewal and multipotency.
Aims: To monitor the long-term contribution to all mature blood lineages, by transplanted single long-term self-renewing HSCs
Methods: Kinetic monitoring of blood lineage replenishment from single HSCs transplanted into lethally irradiated mice, as well as genetic fate-mapping of the lineage contribution of platelet-biased HSCs.
Results: Highly sensitive tracking of progenitors and mature cells of the megakaryocyte/platelet, erythroid, myeloid, B and T cell lineages produced from singly transplanted HSCs revealed a highly organized, predictable and stable framework for lineage-restricted fates of long-term self-renewing HSCs. Most notably, a distinct class of HSCs adopts a fate towards effective and stable replenishment of a megakaryocyte/platelet-lineage tree but not other blood cell lineages, despite sustained multipotency, whereas no HSCs contribute exclusively to any other single blood-cell lineage. Single multipotent HSCs can also fully restrict towards simultaneous replenishment of megakaryocyte, erythroid and myeloid lineages without executing their sustained lymphoid lineage potential. Unpublished data will be presented with regard to the further characterization and significance of these distinct classes of multipotent HSC adopting lineage-restricted fates *in vivo* following transplantation and through genetic fate-mapping.
Summary and Conclusions: These findings uncover a limited repertoire of distinct HSC subsets, defined by a predictable and hierarchical propensity to stably adopt a fate towards replenishment of a restricted set of blood cell lineages, prior to loss of self-renewal and multipotency.

PF411

GATA2 DIRECTLY REPRESSES CARDIAC FATES TO PROMOTE HEMATOPOIETIC SPECIFICATION OF HUMAN MESODERM

A. Giorgetti[1,*], J. Castaño Cardoso[1], S. Aranda[2], C. Bueno[3], F.J. Calero-Nieto[4], E. Mejia-Ramirez[1], E. Blanco[2], X. Wang[4], C. Prieto[3], L. Zabaleta[5], M. Rovira[1], B. Göttgens[4], L. Di Croce[2], P. Menendez[3], A. Raya[1]

[1]Center of Regenerative Medicine, [2]Center for Genomic Regulation (CRG), [3]Josep Carreras Leukemia Institute and Department of Biomedicine, Barcelona, Spain, [4]Wellcome and MRC Cambridge Stem Cell Institute and Cambridge Institute for Medical Research, Cambridge, United Kingdom, [5] Fundación Inbiomed, Donostia, Spain

Background: In vertebrates, GATA2 transcription factor is a master regulator of hematopoiesis, repeatedly used throughout embryo development and the adult life. Studies in the mouse system have suggested an early key role of GATA2 promoting mesoderm specification toward hematopoietic lineages, at the expense of cardiac fates. However, investigating the function of GATA2 during early human hematopoietic development remains elusive.
Aims: We took advantage of the model of induced pluripotent stem cells (hiPSCs) differentiation to study the early GATA2 functions in this process.
Methods: To determine the developmental impact of GATA2 in early human hematopoiesis, we established transgenic hiPSCs in which the expression of transgenic GATA2 could be temporally controlled and specifically induced by doxycycline (Dox) administration.
Results: Here we show that transient induction of *GATA2* during mesoderm patterning robustly promotes hemogenic progenitor (HEP) cell specification and their further differentiation into hematopoietic progenitor cells (HPCs). Remarkably, *GATA2* knockout impaired hematopoietic development while simultaneously enhancing cardiac potential of mesodermal progenitors. Surprisingly, genome-wide transcriptional (RNA-Seq) and chromatin immunoprecipitation (ChIP-seq) analyses showed that GATA2 bound preferentially to regulatory regions, and repressed expression, of cardiac development-related genes. In contrast, gene important for hematopoietic differentiation were upregulated by GATA2 in human mesoderm precursors in a mostly indirect manner.
Summary and Conclusions: Collectively, our data reveal a previously unsuspected role of GATA2 as a direct repressor of cardiac fates, and highlight the importance of coordinating the specification and repression of alternative cell fates.

PF412

NORADRENALINE STIMULATES MITOCHONDRIAL REALLOCATION AND HAEMATOPOIETIC MIGRATION TO THE SPLEEN

A. Morfakis[1,*], C. Marlein[1], C. Hellmich[1], R. Horton[1], J. Mistry[1], K. Bowles[1], S. Rushworth[1]

[1]Norwich Medical School, UEA, Norwich, United Kingdom

Background: During a stressed state, catecholamines are released, leading to widespread changes, including altered haematopoiesis in the bone marrow (BM). Increased levels of catecholamines such as Noradrenaline (NA) have been previously shown to facilitate haematopoietic cell egress from the bone marrow, commonly seen in burn and trauma patients. These patients often have erythropoietin-resistant anaemia, independent of any blood loss, persisting after the initial injury has been treated. The length of anaemia is associated with the increased levels of circulating catecholamines and circulating primitive haematopoietic cells. This process requires energy resources that could not be made *de-novo* in such a short timeframe.
Aims: The aim of our study was to determine if mitochondrial content was reallocated in haematopoietic progenitor cells when stimulated with NA.
Methods: Animal experiments were conducted with approval from the UK Home Office and University of East Anglia Animal Welfare and Ethical Review Board. Mitochondrial content was quantified *in vitro* using C57/BL6 mouse BM stroma using MitoTracker Green FM and flow cytometry. MitoTracker content and cell population changes in C57/BL6 mice were quantified *ex vivo* using flow cytometry after NA intraperitoneal injections, or sham phosphate buffered solution (PBS) injection, and euthanised after 2 hours.
Results: We first quantified mitochondrial content of mature erythroid-lineage cells *in vitro* under different conditions. We found that culturing with noradrenaline (NA) at stressed levels *in vitro* led to decreased mitochondrial content compared to control. Moreover, culturing with atenolol (a beta-blocker which counters NA's actions) led to an increase in mitochondrial content of erythroid-lineage cells. Next, we found that intraperitoneal injection of NA led to a significant efflux of haematopoietic cells from the BM within 2 hours as well as a reduction in their mitochondrial content compared to control. This corresponded with an increased number of erythroid and myeloid lineage cells in the spleen, with an increased mitochondrial content. Finally, we investigated the effect of NA on haematopoietic progenitor cells (HPCs) in the BM. This revealed fewer progenitors (granulocyte-macrophage, common myeloid and megakaryocyte-erythroblast progenitors) in the BM with a decreased mitochondrial content in common myeloid progenitors. There was also an increase in the multipotent progenitor population.
Summary and Conclusions: NA is a major driver of mitochondrial reallocation and cell-lineage bias under stressed haematopoiesis. Migration of cells to the spleen, a major lymphatic organ, prepares the body to combat infection, but potentially at the expense of normal haematopoiesis in the bone marrow, slowing long-term recovery. This pathway has multiple therapeutic applications including stem cell transplant and post-trauma recovery.

PF413

CELLULAR AND MOLECULAR MAPPING OF HUMAN EARLY LYMPHOID PROGENITOR CELLS SUBMITTED TO LOW OXYGEN DOSES

S. Chabi[1,*], B. Uzan[1], I. Naguibneva[1], J. Rucci[1], L. Fahy[1], M.-L. Arcangeli[1], J. Calvo[1], S. Rahming[2], C. Waskow[2], F. Mazurier[3], F. Pflumio[1], R. Haddad[1]

[1]Cea/UMR967, Fontenay-aux-Roses, France, [2]T U Dresden, Dresden, Germany, [3]UMR7292, TOURS, France

Background: *In vitro* studies of human lymphoid development are routinely conducted in normoxic/hyperoxic conditions, however physiologic *in vivo* O_2 levels are lower. Indeed adult hematopoietic stem/progenitor cells (HSPC) are maintained in bone marrow (BM) within a microenvironment characterized by low oxygen (O_2) tension (pO_2: 1-6% O_2) commonly called hypoxia. Conventional *in vitro* cell culture thus appears not suitable for studying human lymphoid differentiation since it does not faithfully reproduce the *in vivo* tissue niches especially as it is related to *in vivo* oxygen tension.
Aims: In this study we aimed at measuring how low O_2 levels impact the biological characteristics of early lymphoid progenitors.
Methods: We addressed the functional, developmental and molecular consequences of direct exposure to hypoxia in distinct umbilical cord blood (UCB)-derived HPC more or less polarized towards the lymphoid lineage. HSPC, multipotent progenitors (MPP), lymphoid-primed multipotent progenitors (LMPP), Pro-T/NK and Pro-B cells were isolated and short-term co-cultured with BM derived stromal MS-5 cells in hypoxia (3.5% O_2) comparatively to normoxia (21% O_2) prior to *in vitro* analysis of cell cycle, metabolic profile, lymphoid cell differentiation in bulk and limiting dilution, *in vivo* hematopoietic reconstitution of NSGW41 immune-deficient mice, and to molecular profile of early lymphopoiesis related genes.
Results: Incubation of human hematopoietic progenitor cells in hypoxia enhanced cell quiescence, reduced autophagy and protected cells from mitochondrial activation. Functionally hypoxia empowered NK-cell development capability from MPP, LMPP and Pro-T/NK cells and B and T cell generation capacity from LMPP and Pro-T/NK respectively while it preserved LMPP and Pro-T/NK intrinsic lymphoid-cell generation capacity as compared to the original sorted cells. Furthermore, higher human hematopoietic chimerism was obtained in BM of NSGW41 immune-deficient mice injected with LMPP-derived cells from hypoxic cultures in comparison to normoxia. *In vivo* B cell production was enhanced from LMPP-derived cells maintained in hypoxia compared to normoxia. As expected, HIF-1α is highly expressed, is efficiently stabilized and robustly localizes in the nucleus under hypoxia in LMPP- and Pro-T/NK-derived cells. Accordingly, *VEGF*, *CXCR4* and *GLUT3* HIF-1α target genes are upregulated under hypoxia. Interestingly, hypoxia promoted lymphoid molecular identity of LMPP and Pro-T/NK cells in comparison to normoxia allowing the expression of early lymphoid genes such as *FLT3*, *SOX4*, *BCL11A*, *E2A*, *NOTCH1*, *IL15RA*, *IL7RA*, *RAG1*. Interplay of HIF-1/2α in hypoxia-related LMPP and Pro-T/NK lymphoid behavior is being investigated.
Summary and Conclusions: Our results indicate that hypoxia acts differentially in human hematopoietic progenitor cell populations and especially enhances lymphoid development from LMPP and Pro-T/NK with HIF-1/2α factors being important players. This study provides new insights into the contribution of O_2 levels in the lymphoid development of human hemato-lymphoid progenitors, thereby establishing valuable tools to explore the cellular and molecular events that direct the initiation of normal and pathological lymphoid cell fate. The work may enable to generate therapeutically useful cell types.

PF414

AGE-RELATED CLONAL HEMATOPOIESIS (ARCH) IN CENTENARIANS AND CARDIOVASCULAR DISEASE PATIENTS: A SURVIVAL BIAS HYPOTHESIS

L. Bertamini[1,2,*], C. Sala[3], N. Martinelli[1], C. Papayannidis[2], C. Giuliani[4], G. Malerba[5], M. Cavo[2], A. Curti[2], P. Garagnani[6], O. Olivieri[1], G. Martinelli[7], C. Franceschi[6], D. Girelli[1]

[1]Department of Medicine, Section of Internal Medicine, University of Verona, Verona, [2]DIMES, Institute of Hematology "L. e A. Seràgnoli", Univeristy of Bologna, [3]DIFA and Galvani center for Bioinformatics, University of Bologna, [4]BIGeA, Laboratory of Molecular Anthropology & Centre for Genome Biology, Univeristy of Bologna, Bologna, [5]Department of Neurosciences, Section of Medical Genetics, University of Verona, Verona, [6]DIMES, Univeristy of Bologna, Bologna, [7]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST), IRCSS, Meldola, Italy

Background: Accumulation of somatic DNA mutations is an unavoidable result of ageing, due to a lot of cell cycles and exposure to exogenous genotoxic agents (Tomasetti C, Science 2017). Hematopoietic tissue is particularly exposed to this somatic variation. Some mutations can give an advantage to a hematopoietic stem cell, leading to its clonal expansion. This condition, known as age-related clonal hematopoiesis (ARCH), has been linked to an increased risk of hematological malignancies, and of overall cardiovascular mortality (Jaiswal S, NEJM 2014). Noteworthy, in the elderly population suffering from cardiovascular diseases (CVD) there is a relatively low prevalence of classical cardiovascular risk factors (Baber U, JACC 2015), and ARCH could represent a novel entity explaining at least part of the still unknown "residual risk".
Aims: To estimate the prevalence and importance of ARCH as cardiovascular risk factor in patients with Coronary Artery Disease (CAD) and in a group of "super-controls" centenarians, free of atherosclerosis related diseases (Garagnani P, Aging 2013).
Methods: We performed whole-exome sequencing (WES) from peripheral-blood cells DNA in 99 patients with angiographically proven severe CAD from the Verona Heart Study (Girelli D, *NEJM* 2000), and 79 centenarians (of which 58 semi-supercentenarians, aged >105 years). The mean data coverage was between 30x and 100x (maximum in centenarians). After eliminating germline variants, we defined ARCH carriers as those subjects having at least 1 somatic mutations predicted to alter the protein function in a list of 6 key genes (*TET2*, *ASXL1*, *DNMT3A*, *JAK2*, *PPM1D*, *TP53)*, previously associated to ARCH (Jaiswal S, *NEJM* 2014).
Results: The prevalence of ARCH in CAD patients was 18.2%, a result that is comparable to the increased prevalence recently described in other CAD populations (Jaiswal S, NEJM 2017). Intriguingly, we found an extremely low prevalence (2.5%) of ARCH in centenarians, contradicting the expected exponential increase of this condition in subjects aged over 80. This counterintuitive result could be explained by a "survival bias" model, in which ARCH increases with ageing but, in the meantime, contributes to an higher risk of mortality. Of note, the majority (85%) of the driver mutations in CAD patients occurred in *TET2*, at variance with other studies where *DNMT3A* was the most recurring driver gene.
Summary and Conclusions: Our outcomes add further insights to the recent hypothesis that links ARCH to an increased cardiovascular risk and its possible role in elderly population with CVD. Our results raise the question over a possible multi-faceted clinical and biological significance of ARCH that might depend on the driver mutation. In particular, *TET2*-driven ARCH may be the most important driver of an higher cardiovascular risk, consistently with the pro-atherosclerotic and heart remodeling role of this gene in mouse models (Fuster JJ, *Science* 2017, Jaiswal S, *NEJM* 2017, Sano S, *JACC* 2018).

PF415

HUMAN ADULT HSCS CAN BE DISCRIMINATED FROM LINEAGE-COMMITTED HPCS BY THE EXPRESSION OF ENDOMUCIN

K. Reckzeh[1,*], H. Kizilkaya[1], A. Søgaard Helbo[1], M. Estruch Alrich[1], K. Theilgaard-Mönch[1]

[1]Novo Nordisk Foundation Center for Stem Cell Biology, DanStem, The Finsen Laboratory, Rigshospitalet & Biotech Research and Innovation Centre, University of Copenhagen, Denmark, Copenhagen, Denmark

Background: Hematopoietic stem cells (HSCs) promote the lifelong production of all mature blood cell lineages through their unique ability of durable self-renewal and multi-lineage differentiation. Given the extensive regenerative property of HSCs, various types of stem cell products including bone marrow (BM), cord blood (CB), and mobilized peripheral stem cells (PBSC) have been successfully utilized in the clinic for stem cell transplantation (SCT) of patients with hematological malignancies and monogenic diseases. Despite the clinical success of HSC therapies, much of our knowledge on HSC biology is based on either mouse studies, or studies on cord blood-derived HSCs of limited purity. Hence, identification of novel human HSC markers to refine the human HSC immunophenotype is instrumental to gain new insights into human HSC biology and advance HSC-based therapies in the clinic setting.
Aims: The goal of this study is to identify novel cell surface markers of human adult HSCs in order to refine their immunophenotype. In addition, this study aims at identifying HSC-exclusive markers allowing for tumor-free autologous stem cell grafts from patient-derived PBSC products.
Methods: To identify novel human HSC surface markers, we screened of microarray gene expression profiles of BM populations with the "classic" immunophenotypes of HSCs, MPPs, HPCs, and mature blood cells. Candidate markers were screened against AML gene expression data sets (HemaExplorer) and subsequently validated in sorted BM by qRT-PCR. To test enrichment for functional HSCs BM cells positive for the cell surface marker were transplanted into immune-deficient NOG mice. Assessment of long-term, multi-lineage engraftment was performed 20 weeks after transplantation.
Results: The screen identified Endomucin (EMCN) as a candidate HSC marker highly expressed by human HSCs and progressively down-regulated as HSCs differentiate via MPPs, CMPs, GMPs, MEPs, and mature monocytes and neutrophils. Flow cytometry analysis of adult BM samples from healthy subjects combining EMCN with current standard markers for the identification of lineage-committed HPCs, LMPPs, MPPs, and HSCs, demonstrated that EMCN is highly expressed on the surface of HSCs and to some extent on MPPs, but almost entirely absent on LMPPs and HPCs. Transplantation of 50,000 L-EMCN- or 2,000 L-EMCN+ highly purified BM cells into sublethally irradiated NOG mice demonstrated significant multi-lineage engraftment in BM and spleen including B-cells and myeloid cells only in L-EMCN+ transplanted mice.
Summary and Conclusions: These findings establish EMCN as a marker expressed by the majority, if not all, bona fide functional human adult HSCs. This study further highlights the use of EMCN in future studies in combination with "classic" HSC markers for refinement of the human HSC immunophenotype and purification of HSCs in order to gain new insights into human HSC biology. EMCN also represents a potential marker for simplified HSC purification strategies including two-color flow cytometry-based or two-step immunomagnetic cell sorting protocols that could be adapted in the clinical setting allowing for production of highly pure stem cell products for patients undergoing allogeneic or autologous SCT, respectively. In summary, our study identifies EMCN as novel HSC marker, which holds great potential to advance future studies on human HSC biology and improve HSC-based therapies.

PF416

ROS-DEPENDENT SELF-RENEWAL DEFECTS OF HUMAN HEMATOPOIETIC STEM CELLS AFTER EXPOSURE TO LOW DOSE OF IONIZING RADIATIONS

I. Souissi-Sahraoui[1], M. Deynoux[1], E. Henry[1], A. Lefèvre[1], V. Barroca[1], J. Calvo[1], F. Pflumio[1], M.-L. Arcangeli[1,*]

[1]iRCM-CEA, INSERM U967, Fontenay-aux-Roses, France

Background: Hematopoietic stem cells (HSC) are responsible for life-long blood cell regeneration. In the adult, HSC quiescence, self-renewal and differentiation capacities are tightly regulated. Nowadays, HSC endure more and more genotoxic stresses that can alter their self-renewal properties. Indeed, people are increasingly exposed to low doses of ionizing radiation (LDIR, <100mGy) due to the recurrent usage of medical imaging. Moreover, studies have shown that combination of several CT scans (thoracic or cranial) can have an incidence in cancer development risk. Up to 5 CT scans (corresponding to a cumulative dose of 30 mGy) can increase up to 3 times the risk to develop pediatric leukemia.
Aims: Therefore, it is crucial to study the consequences of LDIR exposure in human cells in particular in human HSC. We aim also to understand how LDIR can affect human HSC fundamental properties such as self-renewal potential and multipotency.
Methods: We studied the consequences of a single *ex vivo* or *in vivo* acute 20mGy LDIR on differentiation and self-renewal capacities of human cord blood-derived HSC using xenograft models, functional *in vitro* tests such as CFU-C and LTC-IC assays and flow cytometry.
Results: We showed that exposure of human HSC to a single 20mGy LDIR has an impact on long-term HSC self-renewal properties. No alteration in HSC differentiation potential can be detected after exposure to LDIR when tested in primary assays (transplantation, CFU-C assay or cultures). However, when HSPC were irradiated *in vivo* in the NSG mouse bone marrow, a defect

in human hematopoietic reconstitution was observed; deleterious effects observed in HSPC after *ex vivo* LDIR exposure were detected after the tertiary transplantation in NSG mice, strongly arguing for a HSC self-renewal defect. In addition, in serial CFU-C assay, HSPC exposure to 20mGy induced loss of secondary CFU-C potential as well as a loss in secondary LTC-IC frequency. Single 20mGy LDIR does not induce DNA double strand breaks in HSC, suggesting no major defect in DNA repair, but results in a fast and transient increase in ROS production resulting in the activation of p38MAPK pathway. Importantly *ex vivo* treatment of HSC with ROS or p38MAPK inhibitors prior to radiation allows rescuing the 20 mGy-induced-HSC defects.
Summary and Conclusions: Altogether our results indicate that exposure to a single 20 mGy LDRI induces HSC self-renewal defect through a ROS/p38MAPK pathway. P38MAPK activation as well as ROS increased levels are associated with decreased efficiency of HSC to transplant upon serial transplantation and aging. Thus, it is tempting to speculate that exposure to LDIR may induce early/accelerated aging of the young CB-derived HSC albeit not to the extent of biased myelopoiesis. Since radiation sensitivity as well as transplantation efficiency highly depends on HSPC origin, aged HSPC may have been more sensitive to LDIR. Moreover, another feature of HSC aging is higher risk of leukemic transformation, especially in presence of an oncogenic initiating event such as a mutation of the epigenetic modifier dnmt3a, as it is observed in blood cells from aged people. The next question will be thus to determine if aged HSC exposed to LDIR are more prone to (pre)leukemic transformation, especially in aged cells containing primary oncogenic mutations.

PF417

THE HISTONE CHAPERONE NAP1L3 IS REQUIRED FOR HEMATOPOIETIC STEM CELL MAINTENANCE AND DIFFERENTIATION

Y. Heshmati[1], S. Kharazi[2], G. Turköz[1,*], D. Chang[1], E. Kamali Dolatabadi[1], J. Boström[2], A. Krstic[3], T. Boukoura[1], E. Wagner[1], N. Kadri[1], R. Månsson[3], M. Altun[3], H. Qian[1], J. Walfridsson[1]

[1]Department of Medicine, Center for Haematology and Regenerative Medicine, Karolinska University Hospital, [2]Department of Medical Biochemistry and Biophysics, Research Division of Translational Medicine and Chemical Biology, [3]Department of Laboratory Medicine, Center for Haematology and Regenerative Medicine, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden

Background: Hematopoietic stem cells (HSCs) are rare cell populations, which possess multipotent activities and self-renewal capacities. Nucleosome assembly proteins (NAPs) are histone chaperones with an important role in chromatin structure and epigenetic regulation of gene expression. *NAP1L3* has previously been shown to be expressed predominantly in HSCs, indicative of a potential functional role in primitive hematopoietic cells.
Aims: The purpose of this study is to determine the *in vitro* and *in vivo* role of *NAP1L3* in HSC activities and hematopoietic differentiation.
Methods: The gene expression profile of *NAP1L3* in various hematopoietic cells was studied with quantitative real time PCR (qPCR) analysis. The functional importance of *NAP1L3* in HSCs, cell differentiation and hematopoiesis both *in vitro* and *in vivo* was investigated using a combination of shRNA, CRISPR-Cas9 and over expression approaches. The cellular and molecular mechanisms of NAP1L3 in hematopoiesis were delineated with a combination of cell cycle analysis, apoptosis studies and expression profiling.
Results: We find that high gene expression levels of murine *NAP1L3* are restricted to HSCs in mice. Importantly, with shRNA or CRISPR-Cas9 mediated loss of function of mouse *Nap1l3* and with overexpression of the gene, the number of colony-forming cells and myeloid progenitor cells *in vitro* are reduced. This manifests as a striking decrease in the number of HSCs, which reduces their reconstituting activities *in vivo*. Inhibition of human *NAP1L3* in umbilical cord blood (UCB) HSCs impairs the maintenance and proliferation of HSCs both *in vitro* and *in vivo*. *NAP1L3* inhibition in UCB HSCs causes an arrest in the G0 phase of cell cycle progression and induces gene expression signatures that significantly correlate with downregulation of gene sets involved in cell cycle regulation, including E2F and MYC target genes. Moreover, we demonstrate that *HOXA3*, *HOXA5*, *HOXA6*, and *HOXA9* genes are markedly upregulated when *NAP1L3* is suppressed in UCB HSCs.
Summary and Conclusions: Histone chaperones constitute a family of chromatin regulators that have an important function in epigenetic gene regulation. Our studies identified a novel role of the histone chaperone *NAP1L3* in hematopoiesis. Moreover, we found *NAP1L3* to be highly expressed in HSCs, and showed that loss of function of *NAP1L3* in primitive human UCB HSCs had a remarkable impact on the survival and proliferation of HSCs and cell differentiation *in vivo*. Taken together, our findings establish an important role for *NAP1L3* in HSC homeostasis and hematopoietic differentiation.

PF418

THE *IN VIVO* EFFECT OF CHEMOTHERAPY AND CXCR4 ANTAGONISM ON AML AND T-ALL CELL MIGRATION IN THE BONE MARROW

D. Duarte[1,2,3,*], S. Amarteifio[4], H. Ang[1], N. Ruivo[1], G. Pruessner[4], E. Hawkins[5], C. Lo Celso[1]

[1]Department of Life Sciences, Imperial College London, London, United Kingdom, [2]Department of Haematology, IPO-Porto / Porto Comprehensive Cancer Centre, [3]i3S - Instituto de Investigação e Inovação em Saúde / University of Porto, Porto, Portugal, [4]Department of Mathematics, Imperial College London, London, United Kingdom, [5]The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

Background: The majority of acute myeloid leukemia (AML) patients and a significant number of relapsed T-cell lymphoblastic leukemia (T-ALL) patients have a dismal prognosis with poor response to conventional chemotherapy. Leukemia-bone marrow (BM) microenvironment interactions are emerging as potential therapeutic targets, which could be shared across multiple diseases. Inhibition of CXCR4, a known regulator of cell migration, was shown to induce blast mobilization and improve disease outcomes of both AML and T-ALL in pre-clinical models. We recently showed that treatment-naïve and chemoresistant T-ALL cells are highly motile in the BM. In contrast, the features of AML cell migration *in situ* remain unexplored.
Aims: Characterize in detail the impact of (1) chemotherapy and (2) CXCR4 antagonism on the *in vivo* migratory behavior of T-ALL and AML within the BM.
Methods: In this study, we use the well-established Notch1-driven T-ALL and MLL-AF9-driven AML pre-clinical disease models combined with 2-photon and confocal intravital microscopy (IVM) of calvarium BM, which allows imaging at the single-cell level with high temporal resolution.
To model chemoresistance, drugs commonly administered in the treatment regimens of T-ALL and AML patients were used in our mouse models. For CXCR4 inhibition experiments, mice were i.v. injected with AMD3100.
We analyzed leukemic cells during disease establishment, when cells were either isolated or grouped in small clusters, and following treatment with chemotherapy, once chemoresistant populations had been selected. To understand the short-term effect of CXCR4 inhibition, we time-lapsed the same BM areas before and after administration of AMD3100. We used image and mathematical analysis to study the migratory behaviour of leukemic cells.
Results: AML cells migrated unexpectedly rapidly and faster than T-ALL cells in the BM at early stages of disease. These findings suggest that migration is an important trait of malignant cells of both lymphoid and myeloid origin. In contrast with chemoresistant T-ALL cells, AML cells surviving induction chemotherapy were less migratory than seeding AML cells.
We next investigated the effect of CXCR4 antagonism on non-mobilized, parenchymal T-ALL and AML cell migration. AMD3100 strikingly decreased T-ALL cell speed at both seeding and post-chemotherapy stages. However, we show that CXCR4 inhibition on AML cells elicits mobilization, but not significant changes in migration. Time-averaged mean-squared displacement (MSD) analysis revealed that in contrast with the typical leukemic diffusive/sub-diffusive cell movement, chemo-resistant T-ALL cells uniquely shifted to a super-diffusive and ballistic-like movement when exposed to AMD3100.
Furthermore, the size of T-ALL cell clusters diminished following AMD3100 treatment, as a result of combined intravasation and cell death. In contrast, AML cell clusters were maintained after AMD3100 injection.
Summary and Conclusions: We show that motility is a baseline feature of both AML and T-ALL cells. However, we demonstrate that increased cell migration following chemotherapy is specific to T-ALL cells. We also demonstrate that CXCR4 promotes residence of T-ALL and AML cells in the BM and regulates migration of T-ALL cells inside the BM. Our study suggests that migration is a feature of acute leukemias that can be targeted through CXCR4 inhibition in T-ALL but not in AML.

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PROSPECTIVELY ISOLATED BONE MARROW STROMAL CELLS FROM PATIENTS WITH HEMATOLOGICAL MALIGNANCIES DIFFER PHENOTYPICALLY AND RELATE TO DISEASE STAGE IN MGUS/MYELOMA

D. Zacharaki[1], R. Ghazanfari[1], H. Li[1], H. C. Lim[1], S. von Palffy[2], S. Soneji[1], T. Fioretos[2], S. Scheding[1,3,*]

[1]Molecular Hematology, [2]Clinical Genetics, University of Lund, [3]Hematology, University Hospital Lund, Lund, Sweden

Background: Bone marrow (BM) mesenchymal stromal cells (MSCs) are important elements of the hematopoietic stem cell (HSC) niche contributing to the tight regulation of hematopoiesis under homeostasis. MSCs have also been implicated in disease progression and even in disease initiation in hematologic malignancies. Studies of human bone marrow primary *bone-fide* MSCs – in contrast to cultured stroma cells which do not necessarily reflect the properties of their *in vivo* counterparts (Ghazanfari *et al.*, Stem Cells Development 2017) – have become possible by recent progress in the identification of the BM-MSC phenotype as reported by us and others (Li at al., Stem Cell Reports 2014).
Aims: Therefore, the current study aimed to characterize phenotypical and functional properties of primary MSC from patients with hematological malignancies.
Methods: BM samples were collected from patients and healthy age-matched controls. Surface marker expression of primary MSC was measured by flow-cytometry. RNAseq analysis was performed on FACS-sorted primary MSC using the Illumina NextSeq500 platform.
Results: We collected a total of 85 diagnostic BM aspirates from patients that were under investigation for suspected hematological diseases and 9 samples from healthy age-matched donors (HC). FACS analysis of primary MSC identified as lin$^-$CD45$^-$CD31$^-$CD71$^-$CD235a$^-$CD271$^+$cells was performed to investigate the co-expression of known and recently reported MSC markers (CD105, CD106, CD140, CD146, CD230, CD51, CD73, CD90, W5C5). These data formed the basis to generate a surface maker expression heat-map (% median frequency of each marker) that clearly showed disease-cluster-specific differences in several of the markers, *e.g.* lower CD146 expression in MSC from patients with monoclonal gammopathy of undetermined significance (MGUS) compared to multiple myeloma (MM) patients. CD105 expression was lower in MM-MSCs compared to MGUS-MSCs and age-matched controls. MSCs from myelodysplastic syndrome (MDS) patients expressed lower levels of CD105, CD106 and CD51 compared to age-matched controls. MSCs from patients with myeloproliferative diseases (MPDs) showed a low expression of most of the markers.
Intrigued by the differences between MGUS, MM and HC-MSCs we performed RNAseq on prospectively-sorted primary lin$^-$CD45$^-$CD71$^-$CD235a$^-$CD31$^-$CD271$^+$ stromal cells. Unsupervised clustering of the RNAseq data showed that MGUS-MSC clustered partly with HC-, but also with MM-MSC. When restricting the analysis to those MSC samples that clustered separately, a total of 3,758 genes was found to be differentially expressed between the three groups. Pre-ranked gene set enrichment analyses (GSEA) showed a strong enrichment of inflammatory response/oncogenic pathways in MM-MSC compared to HC-MSC. Venn diagram analysis identified commonly expressed genes among MM- and MGUS-MSC *versus* HC-MSC. Furthermore, a total of 17 genes was identified that were highly deregulated in MM- and intermediately deregulated in MGUS-MSC when compared to controls.
Summary and Conclusions: Taken together, phenotypical analysis of primary MSC from patients revealed disease-specific surface marker expression patterns. High throughput transcriptome analysis of primary MSC from HC, MGUS and MM identified genes that were specifically upregulated/suppressed in MM-MSC compared to MGUS-MSC and control-MSC. These genes thus represent potential candidates that might be related to the progression of MGUS to MM and studies investigating a possible functional role of these genes are ongoing.

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CD83 IS A NEW POTENTIAL BIOMARKER AND THERAPEUTIC TARGET FOR HODGKIN LYMPHOMA

Z. Li[1,2,*], X. Ju[1,2], K. Lee[2,3], C. Clarke[3], J. Hsu[1,2], E. Abadir[1,2], C. Bryant[1,4], S. Pears[5], N. Sunderland[5], S. Heffernan[5], A. Hennessy[5], T.-H. Lo[1], G. Pietersz[6], F. Kupresanin[1], P. Fromm[1,2], P. Silveira[1,7], C. Tsonis[1], W. Cooper[2,8], I. Cunngingham[9], C. Brown[4], G. Clark[1,2], D. Hart[1,2]

[1]Dendritic Cell Research, ANZAC Research Institute, [2]Sydney Medical School, University of Sydney, [3]Anatomical Pathology Department, Concord Repatriation General Hospital, [4]Institute of Haematology, [5]Animal Facility, Royal Prince Alfred Hopspital, Sydney, [6]Burnet Institute, Melbourne, [7]Anatomical Pathology Department, University of Sydney, [8]Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hopspital, [9]Haematology Department, Concord Repatriation General Hospital, Sydney, Australia

Background: Hodgkin lymphoma (HL) is a B cell neoplasm that is defined by the presence of Hodgkin and Reed-Sternberg (HRS) cells. New targeted therapies for HL are warranted, especially for refractory/relapsed patients and elderly patients where limiting treatment toxicity is essential. CD83 is a member of the Ig superfamily that is expressed as a membrane molecule (mCD83) and as a membrane cleaved soluble molecule (sCD83). Our group reported that Hodgkin lymphoma tumor cells expressed mCD83 and sCD83 can be detected in serum in lymphoma patients. CD83 was identified as one of the four classifiers to distinguish HL with ALK- anaplastic large cell lymphoma. Despite its potential as a relatively specific target and potential biomarker, CD83 has not been investigated as a therapeutic target on HL.
Aims: The aim of this study is to assess whether CD83 is a potential biomarker and therapeutic target in HL patients. We developed a therapeutic human anti-human CD83 mAb, 3C12C, and its toxin conjugate, 3C12C-MMAE. We will test the killing efficiency of 3C12C and 3C12C-MMAE on HL cell lines. To "de-risk" the antibodies before advancing 3C12C into a clinical trial, we performed dose-escalation studies of 3C12C in non-human primates (NHP).
Methods: Immunohistochemical (IHC) staining of CD83 was performed on formalin fixed paraffin embedded (FFPE) lymph node biopsies of 35 HL patients. Serum samples were collected from HL patients at diagnosis and during sequential standard chemotherapy. Human sCD83 was analyzed by human sCD83 ELISA. Antibody-dependent cell-mediated cytotoxicity (ADCC) was tested on HL lines using 3C12C mAb. 3C12C-toxin conjugate was prepared and tested for killing effect *in vitro*. For dose-escalation studies, five NHP received intravenous human-IgG (10mg/kg) or 3C12C mAb (1, 5, 10, 10 mg/kg) at days 0, 7, 14 and 21. PBMC were collected and analyzed for immune cell populations including DC, T and B cells on flow cytometer. Liver and kidney function were assessed by measuring alkaline phosphatase, aspartate transaminase & creatinine in serum samples.
Results: IHC staining of HL patient FFPE biopsy samples showed 8/35 expressed high levels (10-90% positive) of CD83 on the HRS cells (>90% positive), 21/35 expressed middle level and 6/35 expressed low levels (<10% positive) of CD83 (Figure 1A).

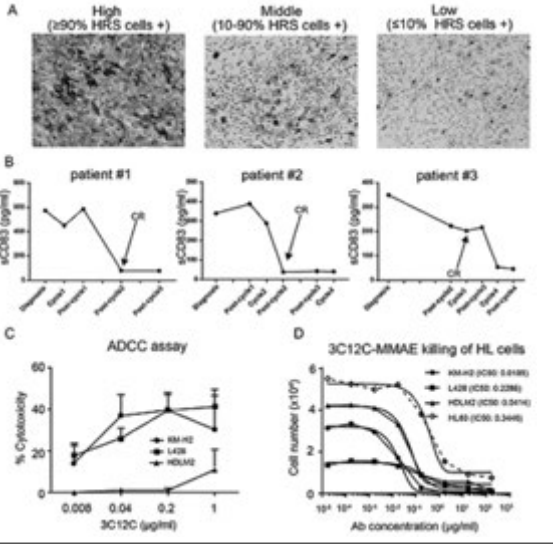


Figure 1.

HL patients had significantly higher serum sCD83 (360.5±54.82 pg/ml, n=10) at diagnosis than healthy donors (52.6±9.5 pg/ml). High levels of sCD83 returned to normal in patients who had good clinical responses to chemotherapy confirmed by positron emission tomography scans (Figure 1B). The ADCC activity of 3C12C was tested on the three HL lines: KM-H2, L428 and HDLM2. Whilst 3C12C killed KM-H2 and L428 efficiently, HDLM2 was relatively resistant to it (Figure 1C). To investigate further potential therapeutic applications, we generated a 3C12C toxin conjugate 3C12C-MMAE. 3C12C-MMAE killed CD83+ KM-H2 cells most efficiently, followed by HDLM2 and L428, while CD83- HL-60 cells were least sensitive to 3C12C-MMAE (Figure 1D). In NHP trial, no toxicity was observed but there was evidence of CD83 positive target cell depletion in lymph node in the 3C12C treated animals (10mg/kg) compared to the control animals. In addition, reductions in blood B cells were noted by flow cytometry.

Summary and Conclusions: Most HRS in HL LN biopsies were CD83+ and sCD83 may be a useful blood biomarker to monitor disease. Anti-CD83 mAb, 3C12C and its toxin conjugate, kill CD83+ HL cells *in vitro*. No toxicity was observed in 3C12C dose-escalation NHP study. These data establish CD83 as a potential biomarker and therapeutic target in HL.

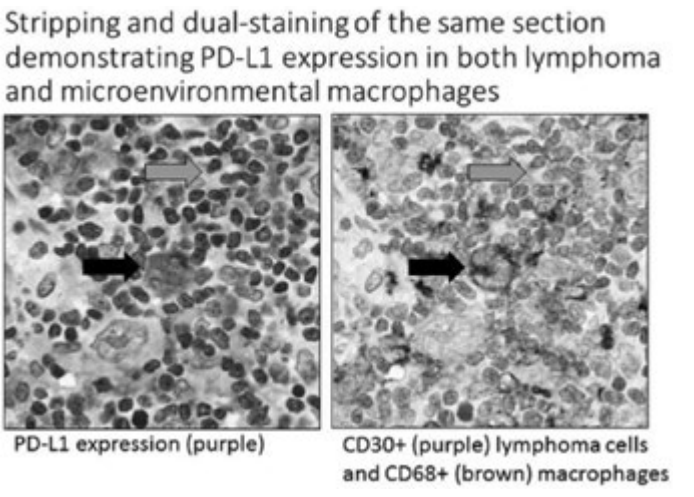


Figure 1.

PF421

PROGRAMMED DEATH LIGAND 1 EXPRESSION IN THE MICROENVIRONMENT OF CLASSICAL HODGKIN LYMPHOMA IS INDUCED BY LYMPHOMA CELLS VIA AN IL27-DEPENDENT MECHANISM

J. Taylor[1,2,*], A. Clear[1], M. Poynton[1], M. Caporale[1], M. Calaminici[1], J. Gribben[1]

[1]Centre for Haemato-Oncology, Barts Cancer Institute, [2]Haematology, St Barts Hospital, London, United Kingdom

Background: Tumor-associated macrophages (TAM) in Classical Hodgkin Lymphoma (CHL) are associated with relapse and inferior survival. TAM express immunosuppressive molecules including Programmed Death Ligand 1 (PDL1). The responses seen in CHL with PD1 inhibition highlight the importance of this axis. Mechanisms of lymphoma PDL1 upregulation are known in CHL but mechanisms of TAM PDL1 upregulation are unknown. IL27 is a heterodimeric cytokine made up of a and β subunits (IL27p28 and EBI3). It has immunosuppressive effects in the myeloid compartment including PDL1 upregulation. Isolated EBI3 expression by CHL lymphoma cells is known but its relevance was previously unclear.

Aims: To evaluate mechanisms contributing to TAM PDL1 upregulation in the CHL.

Methods: Immunohistochemistry (IHC) was used to assess lymphoma cells, TAM, PDL1 and IL27 subunit expression. Co-expression was assessed by IHC followed by antibody stripping and re-staining of the same section. *In vitro*, monocytes from healthy donors were differentiated into monocyte-derived macrophages (MdMφ). Conditioned media (CM) was harvested from the KMH2 CHL cell line. Western blotting was performed on supernatants and MdMφ lysates. PDL1 expression was determined by flow cytometry.

Results: IHC staining of PDL1 followed by stripping and re-staining with CD30 and CD68 (see Figure 1) demonstrated PDL1 expression on both lymphoma cells and TAM. Lymphoma-involved areas were enriched for PDL1+TAM. TAM were responsible for the majority of PDL1 expression. We assessed the role of IL27 in PDL1 upregulation. Lymphoma cells stained positive for EBI3 (IL27 subunit β) in 85% (47/55) of cases whilst TAM stained universally negative. TAM stained positive for IL27p28 (IL27 subunit a) in 76% of cases with positive staining in lymphoma cells in 14% of cases. EBI3 H score (incorporating intensity and area stained) correlated positively with TAM markers including CD68 (p=0.004) and CD163 (p=0.001) and with Il27p28 intensity (p=0.006). This suggeststhat higher EBI3 production coincides with increased TAM infiltration and higher IL27p28 production. Finally, EBI3 H Score correlated positively with PD-L1 expression (p=<0.001). This is consistent with EBI3 and IL27p28 formation of IL27 within the microenvironment promoting PDL1 upregulation. We modelled this process *in vitro*, treating healthy donor derived MdMφ with KMH2 CHL cell line conditioned media (CM). We demonstrated by western blotting that EBI3 but not IL27p28 was present in the KMH2 CM and that IL27p28 was upregulated in a dose-dependent manner in CM-treated MdMφ but was not expressed in lysates of RPMI control MdMφ. PDL1 was upregulated on CM-treated MdMφ compared to control (p=0.009). Finally, to assess the impact of IL27 on PDL1 expression we treated with neutralising doses of anti-IL27 antibody, partially abrogating PDL1 upregulation.

Summary and Conclusions: These data suggest that TAM PDL1 upregulation in CHL is promoted by IL27. EBI3 is secreted by the lymphoma and TAM are stimulated to express IL27p28 by lymphoma-secreted factors. These data support that IL27 is responsible, at least in part, for subsequent PDL1 upregulation. These findings are important as they provide a mechanism connecting TAM PDL1 expression with the lymphoma cell and identify an immunosuppressive role for monomeric EBI3 secretion. A similar mechanism is described in other lymphoma cell lines and monomeric EBI3 secretion is described in lung cancer demonstrating broader relevance. To date, monomeric EBI3 secretion is only described in the context of malignancy raising the possibility of its use as a therapeutic target.

PF422

TARGETING ROR1 RECEPTOR IN CLASSICAL HODGKIN LYMPHOMA: EFFECTS ON APOPTOSIS AND PROLIFERATION AND ITS EMERGING THERAPEUTIC IMPLICATIONS

L. Velentza[1,*], I. Zerdes[1], A. H. Daneshmanesh[1], N. Tsesmetzis[1], M. Hojjat-Farsangi[1], A. Ghaderi[1], E. Drakos[2], M. Palma[1], A. Österborg[1], H. Mellstedt[1], G. Rassidakis[1]

[1]Oncology-Pathology, Karolinska Institute, Stockholm, Sweden, [2]Pathology, University of Crete, Heraklion Crete, Greece

Background: Receptor tyrosine kinase-like orphan receptor 1 (ROR1) is a member of the ROR family consisting of ROR1 and ROR2. RORs have been involved in physiologic embryonic patterning and neurogenesis and, in addition, ROR1 is only detectable in embryonic tissue and generally absent in normal adult tissues. Accumulating evidence has established ROR1 as a marker for chronic lymphocytic leukemia and possibly other hematologic malignancies. ROR1 seems to be critically involved in the progression of these diseases. However, the potential role of ROR1 receptor signaling in the pathogenesis of classical Hodgkin lymphoma (cHL) is yet unknown. Wnt5a, a member of the Wnt family, binds as a ligand to ROR1 and activates ROR1 signaling in various *in vitro* models. Recent evidence suggests that non-canonical Wnt5a pathway activity is involved in the mutual interactions between cHL cells and microenvironment. Therefore, the Wnt5a/ROR1 signaling merits further investigation in cHL.

Aims: To investigate the potential role of ROR1 receptor in tumor cell survival and proliferation in cHL and assess the effects of a novel ROR1 inhibitor in an vitro model of cHL.

Methods: Five cHL cell lines including L428, L1236, HDML2, HDMyZ and MDA-V were used. Expression, and localization (surface *vs* cytoplasmic) of ROR1 protein in the cell lines were assessed by Western blot and flow cytometry, respectively. Standard cell viability, apoptosis, proliferation and cytotoxicity assays were performed to assess the biologic effects after pharmacologic inhibition of ROR1 using the novel inhibitor, KAN00440571-C alone or in combination with ibrutinib. Silencing of ROR1 gene was performed using specific si-RNA constructs and the Nucleofector transfection system (Amaxa). The levels of critical cell cycle and apoptosis regulators were assessed by Western blot analysis. In a cohort of 110 previously untreated patients with cHL (University of Crete), ROR1 protein expression was assessed by immunohistochemistry performed on a tissue microarray (TMA) that included duplicate tumor cores from each case.

Results: ROR1 was differentially expressed among cHL cell lines with L1236, HDML2 and MDA-V expressing the highest protein levels in the cytoplasm or the surface of the cells. Similarly, ROR1 receptor was detected in the Hodgkin and Reed-Sternberg cells (HRS) of cHL in the cohort of 110 patients at a variable level (number of positive HRS, staining intensity). Selective inhibition of ROR1 using the novel inhibitor KAN571-C in HDML2 and MDA-V cells resulted in significantly increased cytotoxicity (MTT assay), which was associated with increased apoptosis and cell death as well as decreased cell proliferation. The efficiency of KAN571-C was substantially higher as compared with a previous ROR1 inhibitor (KAN0493834). Combination of KAN0440571-C with ibrutinib resulted demonstrated additive effects in the same *in vitro* model of cHL. These effects were linked to decreased levels of critical regulators of both the intrinsic (*i.e.* BAX, MCL-1) and extrinsic (*i.e.* cFLIP) apoptotic pathways. Knocking -down of the ROR1 gene by specific siRNA constructs resulted in decreased cell viability and proliferation as well as induction of apoptosis (up to 61% of the cHL cells), which was associated with changes in the protein levels of apoptosis regulators including BAX and cFLIP.
Summary and Conclusions: Taken together, these findings show for the first time that ROR1 receptor-mediated signaling may contribute to cell survival and proliferation in cHL *in vitro* and thus, it represents a promising target for investigational therapy using ROR1 inhibitors in cHL.

Hodgkin lymphoma – Clinical

PF423

TREATMENT RELATED INCIDENCE OF DISEASES OF THE CIRCULATORY SYSTEM IN PATIENTS DIAGNOSED WITH HODGKIN LYMPHOMA

C. Weibull[1,*], M. Björkholm[2], I. Glimelius[3,4], P. Lambert[5], T. Andersson[1], K. Ekström Smedby[3], P. Dickman[1], S. Eloranta[3]

[1]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, [2]Division of Haematology, Department of Medicine, Karolinska University Hospital Solna, [3]Clinical Epidemiology Unit, Department of Medicine Solna, Karolinska Institutet, Stockholm, [4]Department of Immunology, Genetics and Pathology, Uppsala Academic Hospital, Uppsala, Sweden, [5]Biostatistics Research Group, Department of Health Sciences, University of Leicester, Leicester, United Kingdom

Background: With excellent cure rates for patients diagnosed with Hodgkin lymphoma (HL), focus has shifted towards reducing long-term treatment related morbidity and mortality. The two major sequelae, with risk of fatal outcome, are secondary cancers and diseases of the circulatory system (DCS). In Sweden, a reduction in excess mortality due to DCS (that is, the DCS mortality attributable to the HL and its treatment) has been observed among long-term survivors since the mid-1980s. This is likely explained by less cardiotoxic HL treatment, improved risk communication and prevention, and better prognosis for individuals diagnosed with DCS.
Aims: We present temporal trends in excess DCS incidence among HL patients diagnosed in Sweden between 1985 and 2013. By characterizing the incidence of treatment related DCS we aim to enhance the understanding of the overall disease burden of DCS, and to provide data that may serve as a basis for further improvements of existing DCS prevention and treatment strategies in HL survivors.
Methods: We identified 4,479 patients (aged 18-80 years) with an HL diagnosis recorded in the Swedish Cancer Register during the study period. Patients were followed from diagnosis until the first diagnosis of DCS (ICD-10 codes I00-I99, or equivalent in earlier versions). Excess incidence was defined as the difference between the observed DCS incidence in the patient population and the incidence in the general population (assumed free from HL) matched on age, sex and year. Excess incidence rate ratios (EIRRs) with 95% confidence intervals (CIs) were estimated using flexible parametric relative survival models. As a measure of absolute risk, cumulative excess DCS incidence in the presence of competing risks (*i.e.*, deaths due to a non-DCS cause) was further calculated based on these models.
Results: Among all HL patients, 945 (21%) had a diagnosis of DCS during follow-up and another 978 (22%) died (due to causes unrelated to DCS). The five- and ten-year excess incidence of DCS decreased slightly between 1985 and the late 1990s among young (25-year olds: $EIRR_{5\text{-year}}$=0.36, 95% CI: 0.15-0.83, and $EIRR_{10\text{-year}}$=0.28, 95% CI: 0.11-0.72 comparing 1999 to 1985) and middle-aged patients (60-year olds: $EIRR_{5\text{-year}}$=0.52, 95% CI: 0.29-0.91, and $EIRR_{10\text{-year}}$=0.40, 95% CI: 0.21-0.80 comparing 1999 to 1985). No improvements were observed among elderly patients. The cumulative probability of excess DCS within the first five years after diagnosis remained stable throughout the study period. For patients treated in 2009, the treatment related risk of DCS within five years of diagnosis was 3.5% (95% CI: 1.6-5.4) for 25-year olds, 15.0% (95% CI: 10.3-19.6) for 60-year olds, and 17.0% (95% CI: 9.0-24.9) for patient aged 75 years.
Summary and Conclusions: Although excess incidence of DCS has decreased since the mid-1980s, at least for young- and middle-aged HL patients, treatment related DCS morbidity still persists. Further efforts towards reducing cardiotoxicity from first- and second line HL therapy are needed, and this study can thus serve as a basis for future analysis on DCS incidence among contemporarily treated HL patients.

PF424

ACTIVATED CASPASE-3 EXPRESSION BY HODGKIN AND REED-STERNBERG CELLS (HRS) IS A POWERFUL PROGNOSTIC FACTOR IN CLASSICAL HODGKIN LYMPHOMA (CHL) TREATED WITH ABVD OR EQUIVALENT REGIMENS

T. Vassilakopoulos[1,*], G. Rassidakis[2], E. Drakos[3], L.J. Medeiros[2], S. Viviani[4], G. Nadali[5], B. Thakral[2], M. Herling[6], R. Giardini[4], M. Chilosi[5], A. Kanta[3], C. Kittas[7], A. Gianni[4], G. Pizzolo[5], J. Khoury[2], G. Pangalis[1], F. Cabanillas[6], A. Sarris[6]

[1]Department of Haematology and Bone Morrow Transplantation, Laikon General Hospital, National and Kapodistrian University of Athens, Athens, Greece, [2]Depart-

ment of Hematopathology, The University of Texas M.D. Anderson Cancer Center, Texas, United States, [3]Department of Haematology, EUROCLINIC, Athens, Greece, [4]Department of Haematology, Istituto Nationale Tumori, Milano, [5]Department of Haematology, University of Verona, Verona, Italy, [6]Lymphoma Service, The University of Texas M.D. Anderson Cancer Center, Texas, United States, [7]Department of Histology-Embryology, National and Kapodistrian University of Athens, Athens, Greece

Background: Most HRS cells are derived from B-cells that lack surface B-cell receptor due to transcriptional defects or rearranged immunoglobulin genes bearing crippling mutations. Although this should normally lead to apoptosis, HRS cells escape apoptosis via several mechanisms. Caspase-3 is the executioner pro-apoptotic protease and its activation by cleavage leads to irreversible cell death irrespective of the upstream activated apoptotic pathways.
Aims: To investigate the expression of active caspase-3 (aC3) in HRS cells of cHL and correlate them with aC3 levels with BCL2 expression, presenting features and clinical outcome.
Methods: Eligible patients had biopsy-proven cHL treated with ABVD or equivalent regimens, pretreatment archival tumor tissue available and no HIV infection. Expression of aC3 (clone C92-605, BD Pharmingen) and BCL2 (clone 124, DAKO) were assessed by immunohistochemistry. A cutoff of 1% was used to define high *vs* low aC3 expression ($aC3^{high}$ *vs* $aC3^{low}$). Any BCL2 expression was considered positive (Rassidakis, Blood. 2002;100:3935-41).
Results: This study included 260 patients from 4 Centers, with baseline features consistent with an unselected series of non-pediatric cHL population: Median age 30 years, males 50%, B-symptoms 47%, Ann Arbor Stage (AAS) I 14%, II 46%, III 20%, and IV 20%. Histology was nodular sclerosis (NS) in 76%, mixed cellularity (MC) in 17% and other cHL 7%. Low (<1% of HRS cells) aC3 expression was observed in 156/260 patients (60%) (range, 0-5.5%). aC3 expression did not differ significantly between NS and MC, with $aC3^{high}$ in 39% of NS *vs* 43% of MC cases. There was absolutely no correlation between aC3 expression and any baseline feature reflecting demographics, tumor burden or disease aggressiveness. However, $aC3^{high}$ was observed in 52% *vs* 34% of $BCL2^{neg}$ and $BCL2^{pos}$ cases [U1] respectively (p=0.008). After a median follow-up of 17.5 years, the 20-year freedom from progression (FFP) was 90% *vs* 65% for $aC3^{high}$ *vs* $aC3^{low}$ patients (p<0.0001). The corresponding 20-year overall survival was 76% *vs* 64% (p=0.056). $aC3^{low}$ was an adverse prognostic factor for PFS within AAS I/II (91% *vs* 74%, p=0.02) and III/IV as well (89% *vs* 50%, p<0.0001). As previously published, BCL2 expression also correlated with inferior FFP (p=0.004). In multivariate analysis, $aC3^{low}$ and $BCL2^{pos}$ were independent adverse prognostic factors for FFP [hazard ratio (HR) 3.6 (95% CI 1.7-7.3), p=0.001 and HR 2.2 (1.1-4.3), p=0.02 respectively]. The 20-year FFP for patients whose tumors were $aC3^{high}/BCL2^{neg}$, either $aC3^{low}$ or $BCL2^{pos}$, and $aC3^{low}/BCL2^{pos}$ was 91% *vs* 84% *vs* 60% (p<0.0001). Within stages I/II, the 20-year PFS for these groups was 89% *vs* 86% *vs* 74% (p=0.15). Within stages III/IV, it was 91% *vs* 80% *vs* 43% (p<0.0001), with 25/97 (26%) and 42/97 patients (43%) falling into the low- and the high-risk group. Other established prognostic factors, such as stage IV, low albumin, anemia and high LDH could add independent prognostic information to the aC3/BCL2 model (data to be presented at the Meeting).
Summary and Conclusions: In a large study with extremely long follow-up, $aC3^{high}$ HRS cell expression was associated with superior FFP after ABVD or equivalent regimens. The combination $aC3^{low}/BCL2^{pos}$ defined a sizeable (~43% of patients) subgroup of advanced cHL with very poor outcome reflected by PFS<50%. Similarly, a very low risk subgroup can be defined as $aC3^{high}/BCL2^{neg}$ for both early and advanced cHL, with 5- and 20-year failure rates of <5% and ~10%.
**TP Vassilakopoulos, GZ Rasidakis, E Drakos had equal contribution to this work.*

PF425

RISK OF SECOND MALIGNANCIES IN HODGKIN LYMPHOMA PATIENTS IN THE NEW MILLENNIUM: REAL WORLD EVIDENCE FROM THE SEER DATABASE

V. Shivarov[1,*], M. Ivanova[2]

[1]Laboratory of Clinical Immunology and Department of Clinical Hematology, Sofiamed University Hospital, [2]Laboratory of Clinical Immunology, Alexandrovska University Hospital, Sofia, Bulgaria

Background: The risk of long-term toxicity in patients treated for Hodgkin lymphoma (HL) including development of second solid cancer (SSC) and hematological malignancies (SHM) has been recognized early on during the development of curative approaches in this lymphoma subtype.
Aims: We decided to complement the existing data with an analysis of the risk of second cancers in adult patients diagnosed with HL after 2000 using the data from a population based cancer registry – The Surveillance, Epidemiology, and End Results (SEER) program of the NCI with its latest release from April 2017.
Methods: To numerically assess the incidence of second malignancies in HL patients we used the standardized incidence ratio (SIR). We estimated the 99% confidence intervals (CIs) for the calculated SIRs based on the exact method. The cumulative incidence (CumIn) of second hematological and solid neoplasms was calculated with a competing risk survival methodology. Fine and Gray model was used to model the effect of different variables on the CumIn.
Results: We identified a total of 28450 adult (≥18 years) patients with HL reported in the SEER registry between 2000 and 2014. A total of 1432 cases with second cancers were identified, conferring overall statistically increased risk – SIR: 1.75 (99% CI: 1.63-1.87). The risk was increased for both SSC (SIR: 1.32 (99% CI: 1.21-1.43)) and SHM (SIR: 6.34 (99% CI: 5.59-7.17)). The risk was increased irrespective of gender, race, periods of diagnosis, stages, histological subtypes, for use of chemotherapy and radiotherapy. All age groups also showed increased risk except for the elderly patients. The SIR for all solid cancers was 1.32 (99% CI: 1.21-1.43). The incidence of radiation exposure related cancers such as thyroid cancer (SIR: 3.07 (99% CI: 2.27-4.06)) and lung cancer (SIR: 1.71 (99% CI: 1.4-2.08)). However, the incidence of female breast cancer was not increased (SIR: 0.74 (99% CI: 0.54-0.98)). The SIR for all SHMs was 6.34 (99% CI: 5.59-7.17) with significant increase for NHLs (SIR: 9.17 (99% CI: 7.92-10.56)) and acute non-lymphocytic leukemia (SIR: 8.71 (99% CI: 6.2-11.87)). However, there was no significantly increased risk of ALL (SIR: 3.26 (99% CI: 0.7-9.23)) and multiple myeloma (SIR: 1.36 (99% CI: 0.62-2.55)). SSCs occurred later than SHMs with median latency of 53.03 and 41.18 months, respectively ($p<10^{-5}$). The 10-year CumIn for SSC and SHM was 4.69% and 1.96%, respectively. In univariate Fine and Grey models of death, SSCs and SHMs as competing risks, chemotherapy and radiotherapy alone were not significant risk factors for SSC for SHM (p=0.076 and p=0.106, respectively), whereas only radiotherapy was significant risk factor for any type of SHM (p<0.0001). Combined modality treatment (CMT), however, was significant risk factor for both SSC and SHM (p=0.002 and p<0.0001, respectively).
Summary and Conclusions: Our analysis based on the SEER database shows no significant decrease of the risk for second solid and hematological malignancies in HL survivors diagnosed in USA after 2000. Interestingly, the incidence of female breast cancer was not increased, which might be attributed to the shorter follow-up and the inclusion of only adult patients. Repeated analysis at a later time point would address this issue more adequately. In spite of the efforts for optimization of CMT for HL in the last decades it seems that consistent mitigation of risk for second cancer would be eventually achieved only with the bringing of immunotherapy to the forefront of HL management.

PF426

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION AFTER NIVOLUMAB TREATMENT IN RELAPSED/REFRACTORY HODGKIN LYMPHOMA

J.-B. Mear[1,*], E. Nicolas-Virelizier[2], J.-M. Schiano[3], B. Bareau[4], B. Deau[5], H. Lanic[6], A. Schmitt[7], L. Molina[8], K. Bouabdallah[9], A. Tempescul[10], W. Lescaut[11], T. Marchand[12], G. Manson[12], R. Houot[12]

[1]Hematology, CHU Rennes, Rennes, [2]Centre Léon Berard, Lyon, [3]Institut Paoli Calmette, Marseille, [4]Clinique Sévigné, Rennes, [5]Hematology, APHP (Cochin), Paris, [6]Hematology, Centre Henri Becquerel, Rouen, [7]Institut Bergonié, Bordeaux, [8]CHU Grenoble, Grenoble, [9]CHU Bordeaux, Bordeaux, [10]CHU Brest, Brest, France, [11]Centre Hospitalier Princesse Grace, Monaco, Monaco, [12]CHU Rennes, Rennes, France

Background: Anti-PD1 antibodies have been approved for the treatment of relapse/refractory (R/R) Hodgkin lymphoma (HL). Responders are likely to be candidates for allogeneic hematopoietic stem cell transplantation (alloHSCT). However, preliminary results (Merryman *et al.*, Blood 2017, N=39 HL) suggest that alloHSCT after PD-1 blockade may be associated with increased immune toxicity.
Aims: To evaluate the safety and efficacy of alloHSCT in patients with R/R Hodgkin lymphoma previously treated with nivolumab.
Methods: We retrospectively analyzed 14 patients with R/R HL who were treated with nivolumab in the French early access program (EAP) between March and August 2015 and underwent alloHSCT thereafter.
Results: The median age was 31 (19-44) and the median number of prior treatment lines was 6 (3-11). Ten (71.4%) patients had received prior autologous HSCT and all of them had received Brentuximab-Vedotin. Patients

had received a median of 7.5 (4-23) cycles of Nivolumab before transplantation. Median time between the last infusion of nivolumab and alloHSCT was 32 days (15-115). Graft source was haplo-identical donor in 7 (50%) patients. At the time of alloHSCT, 8 (57.1%) patients were in complete remission (CR), 4 (28.6%) were in partial remission, one patient had progressive disease (PD) and one patient was not evaluated. After a median follow up of 17.5 month (9-27), 12 (85.7%) patients were still alive, 1 patient died of hemoptysis and 1 patient died of acute Graft *versus* host disease (GvHD). Ten (71.4%) patients remained in CR (the two other patients were not evaluated). All evaluated patients converted to CR. Median PFS and OS have not been reached. Three patients (21,2%) developed grade 3-4 acute GVHD and 3 patients developed chronic GVHD (one mild, one moderate and one severe). The six patients not in CR before AlloHSCT were in CR at post-transplant evaluation (Figure 1).

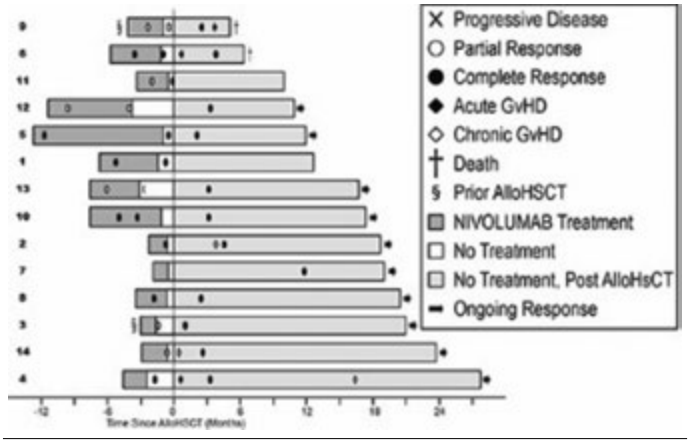


Figure 1.

Summary and Conclusions: AlloHSCT after Nivolumab treatment appears feasible and is associated with prolonged remissions in patients with R/R Hodgkin lymphoma. Updated results will be presented at the meeting.

PF427

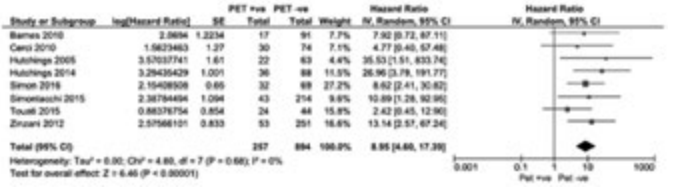
INTERIM- PET RESULTS FOR PROGNOSIS IN ADULTS WITH HODGKIN LYMPHOMA: A PROGNOSTIC FACTOR EXEMPLAR REVIEW (PRELIMINARY RESULTS)

A. Aldin[1,*], G. Collins[2], K. Moons[3], L.J. Estcourt[4], A. Engert[5], C. Kobe[6], B. von Tresckow[5], M. Trivella[7], N. Skoetz[1]

[1]Cochrane Haematological Malignancies, Department I of Internal Medicine, University Hospital of Cologne, Cologne, Germany, [2]Centre for Statistics in Medicine, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, United Kingdom, [3]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, Netherlands, [4]Haematology/Transfusion Medicine, NHS Blood and Transplant, Oxford, United Kingdom, [5]Department I of Internal Medicine, [6]Department for Nuclear Medicine, University Hospital of Cologne, Cologne, Germany, [7]Centre for Statistics in Medicine, University of Oxford, Oxford, United Kingdom

Background: [18F]-fluorodeoxy-D-glucose (FDG)-positron emission tomography (PET) has been increasingly used for staging, potential prognosis and treatment planning in patients with Hodgkin Lymphoma (HL). Particularly an interim FDG-PET scan identifies the state of disease after a few cycles of chemotherapy and it has been suggested to be a good predictor of prognosis, aiding the distinction between patients with poor prognosis from those with a better prognosis.
Aims: To identify all studies evaluating interim-PET scan results as a prognostic factor, and where treatment was not modified due to the interim-PET result. Further, to describe the characteristics and risk of bias of included studies and if possible, meta-analyse results on the association between PET scan results and overall survival (OS), progression-free survival (PFS) and adverse events (AE).
Methods: Based on a-priori Cochrane protocol, we developed sensitive search strategies for Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, databases of ongoing trials and conference proceedings (search date 07/2017). Two authors independently assessed studies for eligibility using pre-defined inclusion and exclusion criteria. Data were extracted using a self-developed data extraction form for prognostic factor studies. Methodological quality of studies was assessed using the Quality in Prognosis Studies (QUIPS) checklist. The primary outcome was OS and secondary outcomes were PFS and AE. We used hazard ratios (HR) as an effect measure. Where values were not available, we attempted to estimate it using available data according to the methods suggested by Tierney and colleagues. We included studies which evaluated interim-PET as a prognostic factor after a few cycles of first-line chemotherapy in adult patients with HL. We excluded studies which modified treatment based on interim-PET results. This project was funded by the German Federal Ministry of Education and Research, grant number 01KG1709.
Results: A total of 28 eligible studies were identified, including 6328 patients diagnosed with HL. However, during data extraction four studies were excluded as they did not present any results for our outcomes of interest. One additional study was excluded during data extraction because it included patients with additional disease. All studies reported values for OS and/or PFS, but no study reported values for AE. Here, we present the **preliminary results** of a meta-analysis of the primary outcome OS, including eight studies with 1151 patients. All patients received first-line chemotherapy with ABVD (doxorubicin, bleomycin, vinblastine and darcarbacine), with or without radiotherapy, and interim-PET was conducted after two cycles of chemotherapy. Meta-analysis shows a higher OS of PET-negative patients than PET-positive patients, with a HR of 8.95 [95% Confidence Interval (CI) 4.60, 17.39] (Table 1).

Table 1.



Summary and Conclusions: Despite the wide CI, we seem to have positive results that interim-PET can be considered a prognostic factor for OS in univariate analysis. As a next step, we need to examine other concurrent factors in order to identify the prognostic ability of interim-PET. However, the lack of standard reporting of prognostic studies, and the consequential poor quality and reliability of reported data in the primary studies makes it difficult to give final conclusions at this stage. We are currently in the process of contacting authors in order to receive more information and data in order to update our data set and analysis accordingly.

PF428

DOWNREGULATION OF STATS DETERMINE POOR PROGNOSIS IN HODGKIN'S LYMPHOMA

A. Zargari[1], K. D. Cummins[2], A. Pham[3], P. Hosking[4], E. Hawkes[5], S. Ting[1,2,*]

[1]Haematology, Eastern Health, Box Hill, [2]Haematology, [3]Anatomical Pathology, Alfred Health, Melbourne, [4]Anatomical Pathology, [5]Oncology, Eastern Health, Box Hill, Australia

Background: Patients with Hodgkin Lymphoma (HL) have a long term overall survival (OS) of approximately 85%. Despite prognostication tools, with the IPS-7 being the most commonly used, a small subset of patients deemed "favourable" unpredictably relapse and progress despite autologous stem cell transplant and novel salvage therapies. To investigate the mechanisms driving treatment-refractory HL, we hypothesised that upregulation of the JAK-STAT pathway within pathognomonic Hodgkin-Reed-Sternberg (HRS) cells may act as both biomarker of poor prognosis and a potential targeted pathway for therapy.
Aims: To stain diagnostic HL tissue with candidate biomarkers, pSTAT3, STAT1, p53, CD68 and PD1 by immunohistochemistry (IHC) and correlate these with clinical outcome.
Methods: An initial retrospective test cohort (n=29) from one institution with known OS was chosen for staining with pSTAT3 and PTP1B. Cohort A (n=18) had durable complete remission (100% 5-year OS) whilst cohort B (n=11) had relapsed/refractory (R/R) disease (54.5% 5-year OS). We sought to validate this with retrospective analysis of a separate cohort of HL patients at our institution whose clinical outcome was unknown; of the 98 patients assessed for diagnostic tissue availability, 53 patients were eligible for tissue staining with pSTAT3, STAT1, p53, CD68 and PD1.
In both the test and validation cohorts, anatomical pathologists were blinded to the patients' identity and scored HRS cells (based on a combination of morphology and CD15/30 staining; using previously published cut-offs) for:

(i) STAT intensity (low or high) and overall percentage of positive STAT stained HRS cells; (ii) p53 wild-type (weak positive) or dysregulated (absent or strongly positive) and surrounding (non-HRS) cells for CD68 and PD1. Kaplan-Meier OS and progression free survival (PFS) analyses were performed on this validation cohort based on each candidate biomarker assessment.

Results: The experimental design to assess the initial cohort was based on the hypothesis that upregulated JAK-STAT would correlate with worse prognosis; however, the reverse was found to be true with both low intensity (p=0.003) and reduced percentage (p=0.008) of pSTAT3 staining in HRS cells was seen in patients with poor outcomes. This loss of STAT signalling was further evaluated in the validation cohort.

The diagnostic samples from our validation cohort (n=53) showed different staining intensities of p53 in HRS cells and both CD68 and PD1 in surrounding "inflammatory" cells, but there were no differences in either 5-year OS or PFS between cohorts whose samples were stained for p53, CD68 or PD1. In contrast, as with the test cohort, low (n=23) *versus* high (n=30) intensity staining of pSTAT3 in HRS cells correlated with both inferior 5-year OS (73% *vs* 93%, p=0.0148) and PFS (47% *vs* 72%, p=0.113), shown in Figure 1. A similar pattern was demonstrated with STAT1 staining, with low (n=19) *versus* high (n=34) staining correlating with a 5-year OS of 69% *vs* 93% (p=0.0050) and PFS 54% *vs* 66% (p=0.4165). Demographics including: sex, age, stage at diagnosis and treatment received were similarly represented in the low and high staining groups for both pSTAT3 and STAT1.

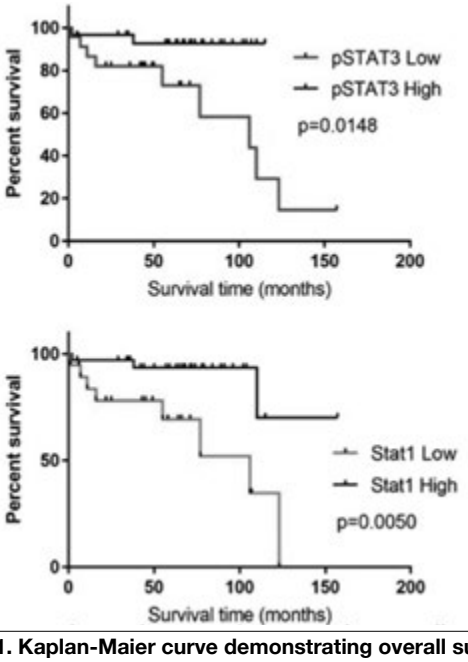


Figure 1. Kaplan-Maier curve demonstrating overall survival of low *vs* high intensity staining HRS cells for pSTAT3 and STAT1.

Summary and Conclusions: Contrary to our hypothesis of an upregulated JAK-STAT pathway portending poor prognosis in HL patients, these retrospective analyses in fact correlate down-regulation of pSTAT3/STAT1 (by IHC) with statistically significant inferior OS in both a targeted R/R and an independent validation cohort. These results suggest novel insights into HL biology where STAT transcription may function as a tumour suppressor.

PF429

A PHASE 1 STUDY INVESTIGATING THE COMBINATION OF AFM13 AND PEMBROLIZUMAB IN PATIENTS WITH RELAPSED/ REFRACTORY HODGKIN LYMPHOMA AFTER BRENTUXIMAB VEDOTIN FAILURE: UPDATED SAFETY AND EFFICACY DATA

N.L. Bartlett[1], R.W. Chen[2], A. Forero-Torres[3], P. Armand[4], I.S. Lossos[5], C.B. Reeder[6], A. Strassz[7,*], C. Choe-Juliak[8], S.M. Ansell[9]

[1]Washington University in St. Louis, St. Louis, MO, [2]City of Hope, Duarte, CA, [3]University of Alabama at Birmingham, Birmingham, AL, [4]Dana-Farber Cancer Institute, Boston, MA, [5]Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, FL, [6]Mayo Clinic, Scottsdale, AZ, United States, [7]Affimed GmbH, Heidelberg, Germany, [8]Affimed, Inc., New York, NY, [9]Mayo Clinic, Rochester, MN, United States

Background: AFM13 is a bispecific, tetravalent NK cell-engaging antibody construct binding to CD30 on Hodgkin Lymphoma (HL) cells and CD16A on NK cells. By engaging CD16A-positive NK cells, AFM13 leads to NK-cell mediated killing of CD30-positive lymphoma cells (Reusch *et al.*, 2014). Pembrolizumab is a PD-1 blocking antibody that prevents tumor immune evasion and has shown to induce high single-agent response rates in patients (pts) with relapsed or refractory (R/R) HL (Armand *et al.*, 2016). AFM13 has shown first signs of clinical activity in R/R HL as single agent in a preceding Phase 1 study (Rothe *et al.*, 2015). Preclinical *in vivo* data of the combination of AFM13 with PD-1 inhibition suggest potential synergistic activity and the potential for induction of cross-talk between innate and adaptive immunity (Zhao *et al.*, 2016). The combination of the two agents could potentially improve outcomes in pts with R/R HL.

Aims: A Phase 1b study is ongoing to evaluate the safety and tolerability of the combination of AFM13 with pembrolizumab (Keytruda) as salvage therapy after failure of standard therapies including brentuximab vedotin in HL (NCT02665650).

Methods: Patients (pts) receive escalating doses of AFM13 (Table 1) in combination with pembrolizumab at a dose of 200 mg flat administered every 3 weeks following the classical 3+3 design. Upon completion, recruitment continues into an extension cohort. Response assessment is performed every 12 weeks by PET/CT according to the Lugano Classification Revised Staging System for malignant lymphoma (Cheson *et al.*, 2014).

Results: As of January 31st 2017, 12 pts have been enrolled into the dose escalation part of the study and 3 pts have been enrolled into the extension part of the study. All pts have relapsed disease and have failed standard treatments including brentuximab vedotin. Preliminary data presented earlier showed that the combination of AFM13 and pembrolizumab is well-tolerated and that the 3-month ORR of the combination compares favorably to historical ORR of pembrolizumab alone. 3-month response data from the dose-escalation and extension cohorts will be reported at the 23rd Congress of EHA.

Table 1. Dose escalation and extension of AFM13.

	Weeks 2 & 3	Weeks 4, 5, 6, 7, 8, 9	Weeks 10, 13, 16, 19, 22 & 25
Cohort 1	0.1 mg/kg x 3	0.5 mg/kg	0.5 mg/kg
Cohort 2	0.5 mg/kg x 3	1.5 mg/kg	1.5 mg/kg
Cohort 3/ Extension	3 mg/kg x 3	7.0 mg/kg	7.0 mg/kg

Summary and Conclusions: Early data suggest that the combination of AFM13 and pembrolizumab is a well-tolerated salvage therapy in pts with R/R HL. While IRRs were observed frequently, most of these events were of mild or moderate severity. Moreover, the combination showed promising signs of antitumor activity which will be further analyzed in the extension cohort.

PF430

PAEDIATRIC HODGKIN LYMPHOMA IN VERY YOUNG PATIENTS: THE ITALIAN EXPERIENCE

P. Farruggia[1,*], G. Puccio[1], F. Locatelli[2], M.R. Vetro[3], M. Pillon[4], A. Trizzino[1], C. Mosa[1], F. Di Marco[1], A. Guarina[3], A. Sala[5], S. Buffardi[6], A. Garaventa[7], F. Rossi[8], M. Bianchi[9], M. Zecca[10], A. Pession[11], C. Favre[12], S. D'Amico[13], M. Provenzi[14], G.A. Zanazzo[15], A. Sau[16], N. Santoro[17], R. Mura[18], T. Casini[19], M. Mascarin[20], R. Burnelli[21]

[1]Paediatric Haematology and Oncology Unit, Oncology Department, A.R.N.A.S. Ospedali Civico, Di Cristina e Benfratelli, Palermo, [2]Oncoematologia Pediatrica, IRCCS Ospedale Bambino Gesù, Roma, University of Pavia, Pavia, [3]Department of Sciences for Health Promotion and Mother and Child Care, University of Palermo, Palermo, [4]Dipartimento di Oncoematologia Pediatrica, Università di Padova, Padova, [5]Clinica Pediatrica, Universita' Milano - Bicocca A.O. San Gerardo, Fondazione MBBM, Monza, Monza, [6]Dipartimento di Oncologia Pediatrica, A.O Santobono-Pausilipon, Napoli, [7]Dipartimento di Ematologia e Oncologia Pediatrica, Istituto G. Gaslini, Genova, [8]Servizio di Oncologia Pediatrica, Dipartimento di Pediatria II Ateneo di Napoli, Napoli, [9]Oncoematolgia Pediatrica e Centro Trapianti, Ospedale Infantile Regina Margherita, Torino, [10]Oncoematologia Pediatrica, Fondazione IRCCS Policlinico San Matteo, Pavia, [11]Oncoematologia Pediatrica "Lalla Seragnoli" Clinica

Pediatrica, Policlinico Sant'Orsola Malpighi, Bologna, [12]Oncologia Clinica Pediatrica e Trapianto Midollo Osseo, Azienda Ospedaliera Università di Pisa, Pisa, [13]Oncologia Pediatrica, Clinica Pediatrica, Catania, [14]14Sezione Oncoematologia Pediatrica, Dipartimento di Pediatria, Ospedali Riuniti di Bergamo, Bergamo, [15]U.O. Emato-Oncologia Pediatrica, Università degli Studi di Trieste Osp.le Infantile Burlo Garofolo, Trieste, [16]U.O. Oncoematologia Pediatrica, Ospedale Civile Spirito Santo, Pescara, [17]Dipartimento Biomedicina Eta' Evolutiva, U.O. Pediatrica I Policlinico, Bari, [18]Oncoematologia Pediatrica e Patologia della coagulazione, Ospedale Regionale per le Microcitemie, Cagliari, [19]Dipartimento di Oncoematologia Pediatrica, A.O.U Meyer, Firenze, [20]S.S. Radioterapia Pediatrica e Area Giovani, IRCCS, Centro di Riferimento Oncologico Aviano, Pordenone, [21]Oncoematologia Pediatrica, Azienda Ospedaliera Universitaria, Ospedale Sant'Anna, Ferrara, Italy

Background: Many studies described a more favorable outcome in younger patients affected by paediatric Hodgkin's Lymphoma (HL).
Aims: The first aim of this study was to find an appropriate age cut-off able to detect low risk children. After having identified the most reliable cut-off, the next step was to describe the natural history in a large group of very young HL patients.
Methods: By using the database of patients enrolled in in 3 following national A.I.E.O.P. (Associazione Italiana di Emato-Oncologia Pediatrica) trials MH89, MH96 and LH2004 for paediatric HL, we have evaluated data of 1584 patients, applied a ROC curve based procedure, identified a reliable age cut-off and described the natural history of 148 very young patients treated from 1.1.1989 to 1.4.2016. We considered the following data at diagnosis: age, sex, stage, symptoms A *vs* B, TG, date of relapse/progression, date of second malignant neoplasm (SMN), date of last follow-up (FUP) and FUP status.
Results: The ROC analysis to set an appropriate cut-off for age showed that, while age seemed to behave as a weak prognostic factor (AUC=0.532), using Youden's index as a parameter the best cut-offs were found in the range of 7 – 11 years ; we chose to use, among the values in that range, the 7 year age value, which is the one with highest sensitivity, because, in the perspective of using it as a cut-off to identify patients with a better prognosis, it is definitely preferable to have a low number of false negatives. Table 1 shows sex, stage, symptoms (A *vs* B) and Therapeutic Group (TG) distribution in the 2 age groups (≤7 years and >7 years of age): male sex, stage I, A symptoms and TG1 were much more frequent in the younger cohort. Significant difference between the two age groups are present also in terms of EFS (p=0.0267), PFS (p=0.0056) and OS (p=0.0378). Then we verified how sex, protocol, A - B symptoms, stage and TG influenced the EFS and PFS of the whole case study: sex was not relevant (p=0.16 for EFS and 0.3 for PFS), protocol showed borderline significance (p = 0.0424 and 0.0667), whereas symptoms A *vs* B (p=1.49e-09 and 5.31e-11), stage (I-II *vs* III-IV) (p=1.77e-05 and 0.0001) and TG (1-2 *vs* 3) (p=1.21e-10 and 9.89e-11) were extremely significant.. So, in order to verify if the better prognosis of younger patients could be attributed to the different risk factors, we carried out a multiple Cox model analysis of EFS and PFS over the entire case study, including as prognostic variables age (≤7 years), A - B symptoms, stage (I-II *vs* III-IV) and TG (1-2 *vs* 3). This analysis showed that age group was not independently associated to prognosis. More specifically for both EFS and PFS the only independently significant prognostic factors are TG (p=0.003 for EFS and 0.0012 for PFS) and A - B symptoms (p=0.0008 for EFS and 0.0002 for PFS).

Table 1.

Sex, stage, A - B symptoms and TG distribution in the 2 age groups

Sex distribution by age					
	F	%	M	%	p
≤ 7y	29	19.6	119	80.4	1.17e-09
> 7y	655	45.6	781	54.4	

Stage distribution by age									
	I	%	II	%	III	%	IV	%	p
≤ 7y	39	26.4	61	41.2	32	21.6	16	10.8	7.602e-13
> 7y	107	7.5	715	49.8	336	23.4	278	19.4	

A - B symptoms distribution by age					
	A	%	B	%	p
≤7y	122	82.4	26	17.6	2.518e-07
>7y	875	60.9	561	39.1	

TG distribution by age							
	1	%	2	%	3	%	p
≤7y	79	53.4	35	23.6	34	23.0	2.2e-16
>7y	279	19.5	328	22.8	829	57.7	

y: years; TG: therapeutic group

Summary and Conclusions: Children affected by HL older than 7 years of age present a higher rate of primary treatment failures and mortality, but the better prognosis in younger patients seems to be related to a different presentation of the disease.

PF431

NIVOLUMAB IN COMBINATION WITH BRENTUXIMAB VEDOTIN IN PATIENTS WITH RELAPSED OR REFRACTORY HODGKIN'S LYMPHOMA AFTER FAILURE OF NIVOLUMAB MONOTHERAPY

L. Tsvetkova[1,*], K. Lepik[1], A. Kozlov[1], E. Kondakova[1], Y. Zalyalov[1], E. Borzenkova[1], V. Baykov[1], N. Mikhailova[1], B. Afanasyev[1]

[1]Raisa Gorbacheva Memorial Institute of Children Oncology Hematology and Transplantation, Saint-Petersburg, Russian Federation

Background: Nowadays chemotherapy induces durable remission rates of 60% to 80% of newly diagnosed patients with Hodgkin's lymphoma. High-dose chemotherapy followed by autologous hematopoietic stem cell transplantation (auto-HSCT) induce responses in 50% of patients with relapsed or refractory Hodgkin's lymphoma (r/r HL), but some of the patients remain to be resistant. Brentuximab vedotin (BV) is a highly efficient agent for HL relapses after auto-HSCT. Immunotherapy with PD-1 blocking monoclonal antibody nivolumab demonstrated significant activity in patients with r/r HL after auto-HSCT and BV. However, the relapses still occur. There are data, that nivolumab and BV may have synergistic effects and improve outcomes of treatment r/r HL.
Aims: The goal of this analysis was to evaluate the safety and the efficacy of combination nivolumab+BV in patients with relapsed or refractory Hodgkin's lymphoma after failure of nivolumab in monotherapy.
Methods: The current analysis included 10 patients (6 male,4 female) with the median age of 28 (range, 23-35 years). All patients received nivolumab in monotherapy previously with the median number of infusions 18 (12-27). During previous therapy 40% of patients had undergone auto-HSCT, 70% had prior treatment with BV. The progression of disease was observed in 80% of patients at initiation of nivolumab+BV therapy and two patients had indeterminate response by LYRIC (LYmphoma Response to Immunomodulatory therapy Criteria). Patients were treated in 21-day cycles for up to 4 (range, 3-6) cycles. BV (1.8 mg/kg) and nivolumab (3 mg/kg) was given on day 1. Toxicity was graded according to the NCI CTC for Adverse Events (AEs) (version 5.0). After completion of treatment, the responses were evaluated by PET-CT scan and assessed by investigators according to the LYRIC.
Results: Median follow-up time was 10.8 months from first dose (range; 7,4–13). The objective response rate among all treated patients was 70% with the CR rate of 30%; 2 (20%) patients had indeterminate response and 1 patient - stable disease. A decrease of SPD was observed in 90% of patients. Relapse with an increase in tumor volume and metabolic activity occurred in one patient after achieving of CR. Six patients received subsequent salvage combination therapy nivloumab+bendamustine after nivolumab+BV treatment. One patient proceeded to allogeneic hematopoietic stem cell transplantation in the CR. Nine patients (90%) experienced treatment adverse events (AEs). Among the most common were nausea (40%) and peripheral neuropathy (30%). Grade 3 adverse events developed in one patient (10%) as a maculopapular rash. Combined treatment was discontinued in cases of severe adverse events. Rash was resolved using systemic glucocorticosteroids. At the time of analysis 9 (90%) were alive at the time of analysis. One patient died due to the secondary myelodysplastic syndrome, which preceded nivolumab therapy, with transformation to chemoresistant acute myeloid leukemia.
Summary and Conclusions: The conducted study suggests that nivolumab+BV combination may represent highly active salvage regimen for patients with r/r HL after failure of nivolumab monotherapy. The toxicity profile was favorable with manageable adverse events. Nivolumab and BV combination probably demonstrated synergism of action and it can be effective even in patients who had resistance to monotherapy of this agents.

PF432

THE COMBINATION OF ALISERTIB AND ROMIDEPSIN IS A SAFE AND EFFECTIVE REGIMEN FOR RELAPSED/REFRACTORY AGGRESSIVE HODGKIN AND T-CELL LYMPHOMAS: A PHASE 1 STUDY

P. Strati[1,*], Y. Oki[1], L. Fayad[1], L. Nastoupil[1], N. Fowler[1], F. Hagemeister[1], L. Kwak[2], H. Lee[1], M. Wang[1], J. Westin[1], C. Ruben[1], E. Wesson[1], M. Fanale[1]

[1]MD Anderson Cancer Center, HOUSTON, [2]City of Hope, Duarte, United States

Background: In preclinical studies, aurora A kinase inhibitors have shown to enhance lymphoma cell death to histone deacetylase inhibitor through repression of C-Myc and C-Myc-responsive micro RNAs, exhibiting highly synergistic effects in lymphoma cell lines through cytokine modulation.
Aims: Therefore, we initiated a phase I trial (NCT01897012) combining alisertib with romidepsin in patients with multiple lymphoma subtypes.
Methods: Patients with PTCL, Hodgkin lymphoma (HL) or aggressive BCL, relapsed or refractory to at least 1 line of therapy, were eligible for the study. Eight dose levels were defined and a standard 3+3 design used for dose escalation; cycles were repeated every 21 days for levels 1-4, amended to every 28 days for levels 5-8, for a maximum of 8 cycles. Dose limiting toxicity (DLT) was assessed during cycle 1 and defined as grade 4 neutropenia or thrombocytopenia lasting longer than 14 days and/or uncontrollable grade 3 or 4 non-hematological toxicity.
Results: Twenty-five patients have been enrolled in the study between 08/2013 and 10/2017. Median age was 56 (range, 23-77 years) and 21 (84%) were male; 12 (48%) patients had aggressive diffuse large B-cell lymphoma (DLBCL), 7 (28%) HL, 4 (16%) PTCL, and 2 (8%) Burkitt lymphoma (BL); median number or previous regimens was 4 (range, 1-10), 9 (36%) patients had received autologous stem cell transplant (SCT) and 7 (28%) allogeneic SCT; overall response rate (ORR) to latest regimen was 24% and 23 (92%) patients had relapsed within 6 months. One additional patient was enrolled at dose level 6, because of 1 withdrawal before DLT assessment. Median number of cycles delivered was 2 (range, 1-8) and 24 (96%) patients have discontinued treatment to date; reasons for study discontinuation were: progression in 19 (79%) patients, completion of treatment in 2 (9%), indication for SCT in 1 (5%), patient's choice in 1 (4%) and toxicity in 1 (4%). No DLTs have been observed to date. The most common (>10%) grade 3-4 toxicities were thrombocytopenia (40%), anemia (28%), neutropenia (24%), infections (24%) and fatigue (16%). Twenty-one patients were evaluable for response by PET/CT; among 12 patients who had a decrease in tumor burden, median reduction was 49% (range, 7-89%), and among 9 patients who had an increase in tumor burden, median increase was 49% (range, 4-300%). Three patients (12%) achieved complete remission (2 patients with HL, 1 with PTCL), 2 (8%) partial remission (1 with DLBCL and 1 with PTCL), and 7 (28%) stable disease, and ORR rate was 20%. Only 1 (5%) patient proceeded to SCT (allogeneic) after 3 months of treatment. After a median follow-up of 5 months (range, 1-46 months), median progression-free survival (PFS) was 2 months (range, 1-21 months), and the longest PFS (>6 months) was observed in 2 patients with heavily pretreated HL (lymphocyte depleted) and 3 patients with PTCL. At most recent follow-up, 24 (96%) patients have progressed, 1 after SCT. Median OS was 12 months (range, 1-46 months), and at most recent follow-up, 11 (44%) patients have died, all of disease progression.
Summary and Conclusions: The combination of romidepsin and alisertib can be safely used as salvage therapy in patients with relapsed or refractory aggressive Hodgkin lymphoma and T cell lymphoma. As the majority of patients developed disease progression during follow-up, further investigation is needed about the role of maintenance therapy, identification of predictive factors of response, and evaluation of other targeted therapy doublets or triplets.

PF433

ΔSUVMAX AND ΔSUVPEAK IMPROVE THE NEGATIVE PREDICTIVE VALUE OF INTERIM PET SCAN IN HODGKIN LYMPHOMA

T. Ribeiro[1,*], G. Ferreira[2], A.I. Marques[1], M. Tavares[1], C. Castro[3], J. Lobo[4], B. Loureiro[1], D. Pereira[1], S. Chacim[1], N. Domingues[1], A. Espírito Santo[1], Â. Martins[1], I. Moreira[1], I. Oliveira[1], L. Viterbo[1], C. Moreira[1], M. Mariz[1]

[1]Onco-hematology, [2]Nuclear Medicina, [3]Epidemiology, [4]Pathological Anatomy, Instituto Português de Oncologia - Porto, Porto, Portugal

Background: Current therapies for Hodgkin Lymphoma (HL) achieve high complete response rates and overall survival. Limiting therapy-associated toxicity is now one of the main goals. Early positron emission tomography (PET) scan, performed after 2-4 cycles of chemotherapy (interim PET, PETi), may represent an important tool for early recognition of good response to first-line therapy and to guide de-escalation of therapy. PET interpretation is usually performed according to the 5-point scale (5-PS), a qualitative visual scale. Although having shown a higher predictive value for patient outcome compared to the currently available prognostic scores, it has limitations related to the reproducibility between observers. Therefore, alternative semiquantitative strategies based on the standardized uptake value (SUV) have been developed. The maximum SUV (SUVmax) is widely used, although it is significantly influenced by some technical details and tumoral heterogeneity. On the other hand, peak SUV (SUVpeak) relates to greater intra-patient reproducibility, being the proposed measure in PET Response Criteria in Solid Tumors. It is still to be determined which strategy would be best in evaluating PET results in HL patients and how PETi results correlate with patient outcome.
Aims: To compare the interpretation of the PETi scan result according to the 5-PS with ΔSUVmax or ΔSUVpeak between staging PET scan and PETi scan, in predicting outcome in HL patients.
Methods: Retrospective single-center analysis of 99 HL patients diagnosed according to the WHO classification criteria between 2012 and 2016 in our Institution, with median follow-up of 46.5 months. Fifty-eight patients with available staging and interim PET scans were analysed. In order to define PETi result as positive or negative according to ΔSUVmax or ΔSUVpeak, we used receiver-operating-characteristics curves and Youden index to determine best cut-off values.
Results: A positive PETi result consisted of a score of 4 or 5 in the 5-PS, or ΔSUVmax below 93.5% or ΔSUVpeak below 88.4%, according to our results. Of the 58 patients in our analysis, 2 had a positive PETi[5-PS] result (only one had persistent disease) and 56 had a negative PETi[5-PS] result (9 of these had persistent or relapsed disease). Considering PETi[ΔSUVmax] and PETi[ΔSUVpeak], 18 and 17 patients respectively had a positive result (7 of these patients had persistent or relapsed disease). The interpretation of PETi result according to ΔSUVmax and ΔSUVpeak showed higher sensitivity (70% in both cases) compared to the 5-PS (sensitivity of 10%), but lower specificity (77% and 79% for ΔSUVmax and ΔSUVpeak respectively, with 98% for the 5-PS). Therefore, ΔSUVmax and ΔSUVpeak have a very high negative predictive value (93% and 98% respectively; *vs* 85% for the 5-PS), but a lower positive predictive value than the 5-PS (39% and 41% respectively; *vs* 50% for the 5-PS). There is statistically significant difference between the progression free survival (PFS) curves at 60 months of follow-up of patients with positive and negative PETi[ΔSUVmax] (56.9% and 88.0%, respectively, $p<0.05$) and PETi[ΔSUVpeak] results (55.9% and 88.1%, respectively, $p<0.05$). The PFS of patients with negative PETi[5-PS] at 60 months of follow-up was 73.8% (Table 1).

Table 1.

	VISUAL ANALYSIS (1,2,3, vs. 4,5)	ΔSUVmax (93≤ % VS. > 93%)	ΔSUVpeak (88≤ % VS. > 88%)
SENSITIVITY	10% IC 95%(0.25-44.5)	**70%** IC 95%(34.8-93.3)	**70%** IC 95%(34.8-93.3)
SPECIFICITY	**98%** IC 95%(89.5-99.9)	77% IC 95%(62.7-88.0)	79% IC 95%(65.0-89.5)
NEGATIVE PREDICTIVE VALUE	85% IC 95%(81.3-87.3)	93% IC 95%(82.5-97.0)	**98%** IC 95%(82.9-97.0)
POSITIVE PREDICTIVE VALUE	**50%** IC 95%(6.37-93.6)	39% IC 95%(24.8-55.2)	41% IC 95%(86.0-58.3)

Summary and Conclusions: ΔSUVmax and ΔSUVpeak appear to discriminate better between patients who respond well to therapy, so that a negative result could support de-escalating therapy intensity, and those with persistent or relapsed disease, which would otherwise go unnoticed. More studies are needed to address these questions and confirm these results.

PF434

OUTCOME OF ELDERLY PATIENTS WITH CLASSICAL HODGKIN LYMPHOMA IN CENTRAL SCOTLAND, UK

M. Rafferty[1,*], A. Ivanos[1], K. Leighton[2], F. Scott[2], P. McKay[1]

[1]Haematology, NHS Greater Glasgow and Clyde, Glasgow, [2]Haematology, NHS Lothian, Edinburgh, United Kingdom

Background: Survival of patients with Hodgkin lymphoma (HL) has improved in recent years due to more effective use of chemo-radiotherapy. HL in the young is generally associated with a good overall prognosis. Despite these advances in treatment, management of the older patient with HL remains challenging. 5-year overall survival in the UK is less than 50% in patients aged over 60 years. Reported outcome rates vary across Europe with location and regimen used. Observational Swedish data from Sjoberg *et al* in 2012 shows a 59% 5-year overall survival. The presence of co-morbidities and excess treatment related toxicities make delivery of combination chemotherapy in this age group difficult.
Aims: The aim of this study was to evaluate the outcome of older patients diagnosed with classical HL treated in Edinburgh and Glasgow, Scotland, UK.
Methods: Departmental records were used to retrospectively identify patients aged over 60 years with classical HL diagnosed between 2009 and 2014. The Cumulative Illness Rating Scale for Geriatrics was calculated in a subset of patients as a measure of elderly 'comorbid burden'. Statistical analysis was performed using GraphPad Prism statistical software ©.

Results: 71 consecutive patients were reviewed. Median age at diagnosis was 72 (range 60-85) years. 43 patients (61%) were male. 14 patients had early stage disease (EORTC: 100% unfavourable); 56 patients had advanced stage disease (median Hasenclever 4; 2-6); staging information was unavailable for 1 patient. CIRS-G score was available for 25 patients; median total CIR-G score was 7 (0-16); median CIRS-G severity index was 1.7 (0-2.3). 59 patients (83%) received intensive chemotherapy: 46 (65%) received ABVD (bleomycin omitted at outset in 10 patients); 7 (10%) received ChLVPP; 4 (6%) received VEPEMB; 2 (3%) received P-VACE-BOP. Consistency was seen within the 60-69 year old cohort where 85% of patients received ABVD chemotherapy. 2 patients (3%) received palliative radiotherapy and 10 patients (14%) were palliated supportively. Successful delivery of planned chemotherapy was achieved in 53% of patients receiving intensive treatment (59% ABVD, 29% ChLVPP, 25% VEPEMB, 50% P-VACE-BOP). Bleomycin was discontinued in 6 patients (17%) treated with full ABVD due to clinical or radiological concerns of pulmonary toxicity. 5-year overall survival within our patient cohort was 42%. Median overall survival (OS) and progression free survival (PFS) were 40 and 36 months for all patients, 56 and 52 months for patients receiving intensive therapy, and 1.5 and 1.5 months for those receiving non-intensive measures. OS and PFS in the 60-69 year cohort (47% of those treated intensively) were 75 and 75 months *versus* 20 and 14 months in the 70-85 year cohort (53% of those treated intensively. Of those patients treated with ABVD/AVD chemotherapy (n=46), median OS was 85 months in those achieving successful full delivery of planned chemotherapy. Total CIRS-G score was inversely associated with overall survival (p=0.0033).
Summary and Conclusions: The high overall survival rates seen in the young are not reproducible in older patients with current combination chemotherapy regimens. Older patients are more likely to require de-escalation of therapy due to toxicities although those elderly patients who receive the planned dose of chemotherapy have much improved outcome. Overall survival in our cohort was lower than that seen in large European cohorts which may reflect the inclusion of frail patients within our analysis. Alternative treatments must be explored, especially in frail patients >70 years, in the context of clinical trials.

Indolent Non-Hodgkin lymphoma – Clinical

PF435

LONG-TERM FOLLOW-UP OF VEMURAFENIB IN BRAF MUTANT HAIRY CELL LEUKEMIA SHOWS DURABLE REMISSIONS AND HIGH RESPONSE RATES WITH REPEATED TREATMENT WITH VEMURAFENIB AT RELAPSE

J. Park[1,*], S. Vemuri[1], S. Chung[1], O. Abdel-Wahab[1], M. Tallman[1]

[1]Memorial Sloan-Kettering Cancer Center, New York, United States

Background: We have previously reported high response rates of the BRAF inhibitor, vemurafenib, in 26 U.S. patients with BRAF mutant relapsed or refractory (R/R) hairy cell leukemia (HCL) (Tiacci and Park *et al.* N Engl J Med 2015). However, follow-up duration of the study was short (median 11.7 months), and outcomes of the patients who relapse after vemurafenib remain largely unknown.
Aims: We analyzed the rates and timing of relapse with a longer follow-up duration for a total of 36 patients in the completed trial with an emphasis on responses to retreatment with vemurafenib at relapse.
Methods: Adult patients with R/R HCL were enrolled to the phase II clinical trial (NCT01711632), and received vemurafenib 960mg bid for 3-6 months. The trial enrollment has now been completed. During subsequent follow-up, patients with relapsed disease were allowed to be re-treated with vemurafenib until disease progression or development of unacceptable toxicity. Vemurafenib dose could be adjusted to as low as 240mg bid.
Results: Among the 36 enrolled patients, 31 patients completed at least 1 month of therapy and were evaluable for response; 5 patients discontinued therapy before response assessment due to drug-related toxicities (n=3), patient desire (n=1) and infection (n=1). Of the 31 evaluable patients, 8 patients (26%) achieved complete response (CR), 22 patients (71%) achieved partial response (PR), and 1 patient achieved no response with an overall response rate of 97%. With a median follow-up of 24 months (range, 1-61 months), 17 of 31 patients (55%) experienced relapse. The median time to relapse from treatment initiation was 18 months (range, 6.7-47 months). All 17 patients with relapse had retained BRAF V600E mutation, and 11 patients (65%) received retreatment with vemurafenib, 4 patients (24%) received alternative treatment, and 2 patients (12%) were observed. With retreatment of vemurafenib at relapse, 9 of 11 patients (82%) achieved PR with complete hematologic recovery; 1 patient developed acquired resistance to vemurafenib with KRAS mutation; and 1 patient discontinued therapy after 1 week due to drug-related toxicity (photosensitivity). Vemurafenib retreatment was well-tolerated with a median duration of therapy of 7 months (range, 0.3-39 months) but all patients received a lower dose of 240-480mg bid due to prior toxicity experience from initial treatment.
Summary and Conclusions: With a longer follow-up duration of up to 61 months, we confirm the high anti-tumor efficacy and safety of vemurafenib monotherapy in patients with R/R HCL. While relapses were common, retreatment with vemurafenib, even at a lower dose of 240mg bid, was highly effective (82% response rate) and acquired resistance to vemurafenib was rare. However, optimal duration of vemurafenib at retreatment remains unknown, and an addition of anti-CD20 monoclonal antibody may improve the CR rate and remission duration.

PF436

METABOLIC (PET) AND MRD RESPONSE CONFER REDUCED RISK OF PROGRESSION OR DEATH IN PATIENTS TREATED WITHIN THE PHASE III GALLIUM STUDY

C. Pott[1,*], A. Davies[2], W. Hiddemann[3], E. Hoster[3], R. Marcus[4], C. Schmidt[3], C. Harbron[5], K. Mundt[6], T. Nielsen[6], J. Trotman[7]

[1]University Hospital Schleswig-Holstein, Kiel, Germany, [2]Cancer Research UK Centre, University of Southampton, Southampton, United Kingdom, [3]Department of Medicine III, University Hospital Munich, Munich, Germany, [4]Kings College Hospital, London, [5]Roche Products Ltd, Welwyn, United Kingdom, [6]F. Hoffmann-La Roche Ltd, Basel, Switzerland, [7]Concord Repatriation General Hospital, University of Sydney, Sydney, Australia

Background: In the randomised GALLIUM (NCT01332968) trial of 1202 previously untreated follicular lymphoma (FL) patients (pts), the primary endpoint of investigator (INV)-assessed progression-free survival (PFS) was significantly prolonged with obinutuzumab (GA101; G)-based *vs* rituximab (R)-based immunochemotherapy and maintenance (Marcus *et al.* NEJM

2017). Lymphoma metabolic activity by ^{18}F-FDG PET-CT (PET) (Lugano 2014 criteria) showed complete metabolic response (CMR) at end of induction (EOI) to be prognostic for prolonged PFS and overall survival (Trotman *et al.* Haematologica 2017); minimal residual disease (MRD)-negative status in peripheral blood (PB) and/or bone marrow (BM) was prognostic for prolonged PFS (Pott *et al.* Blood 2016). MRD is a sensitive molecular measure of disease in PB and/or BM, and may add to PET prognostic information.
Aims: We assessed the relationship between EOI MRD and PET responses in GALLIUM and prognostic implications for PFS in previously untreated pts with FL.
Methods: Pts in GALLIUM received induction with G or R plus bendamustine, CHOP or CVP chemotherapy followed by maintenance with the same antibody in responders. PET scans were performed at baseline and EOI (6–8 weeks after Day 1 of the last cycle of induction) and assessed by an Independent Review Committee (IRC). PET images were assessed according to the Lugano 2014 criteria, with CMR defined as a score of 1, 2 or 3 on the 5-Point Scale. For MRD assessment, diagnostic PB and BM samples were screened by consensus PCR to detect a clonal t(14;18) translocation and/or Ig variable domain rearrangement. In pts with a detectable clonal marker, standardised allele- or translocation-specific real-time quantitative (RQ)-PCR assays with a sensitivity of $\leq 10^{-4}$ were performed and evaluated by European Study Group criteria (van der Velden *et al.* Leukemia 2007). MRD at EOI was defined as positive if RQ-PCR or subsequent qualitative nested PCR were positive in PB or BM.
Results: At baseline, 595/609 (98%) pts with PET scans had detectable lesions and 815/1101 (74%) pts with MRD evaluable samples had an MRD marker meeting the predefined quality criteria; 298 pts were evaluable for both measures at EOI. These pts were slightly younger, and had more advanced disease and fewer Asian pts than the non-evaluable intent-to-treat population (n=904). CMR was achieved by 266/298 evaluable pts (89%); 250 (94%) were MRD-negative. Median follow-up in the evaluable group was 44 months. In 250 CMR pts with MRD-negativity, 2.5-year PFS from EOI was 85% (95% CI: 80–89; Figure 1). In Cox proportional hazards modelling, this group had better INV-assessed PFS than the other groups: hazard ratio (HR)=0.39 (95% CI: 0.17–0.93; p=0.03) *vs* pts with CMR and MRD-positive response (n=16); and HR=0.39 (95% CI: 0.19–0.81; p=0.01) *vs* pts without CMR who were MRD-negative (n=24). Too few pts without CMR but with MRD-positive response (n=6) were available for Cox analysis. Results for IRC-assessed PFS were similar to those for INV-assessed PFS.

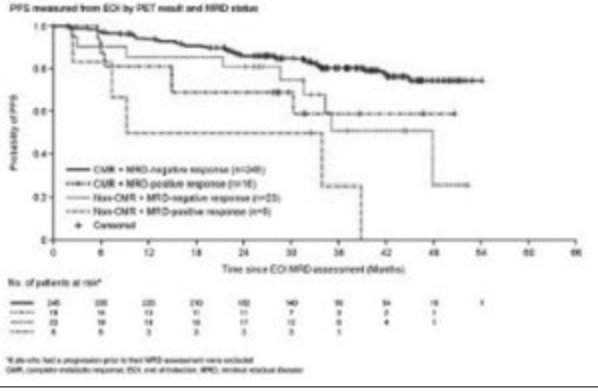


Figure 1.

Summary and Conclusions: Most evaluable pts achieved CMR and MRD-negativity, with a minority progressing despite a favorable prognosis. Risk of progression or death in pts achieving only CMR or MRD-negativity was 2.5-fold greater than that in pts who achieved both, suggesting that EOI PET and MRD responses could provide complementary information. Further evaluation of quantitative PET indices and molecular markers, and of early progressors, may help to identify additional prognostic markers.

PF437

THE DUAL SYK/JAK INHIBITOR CERDULATINIB DEMONSTRATES RAPID TUMOR RESPONSES IN A PHASE 2A STUDY IN PATIENTS WITH RELAPSED/REFRACTORY B- AND T-CELL NON-HODGKIN LYMPHOMA (NHL)

P.A. Hamlin[1,*], B.D. Cheson[2], C.M. Farber[3], T. Feldman[4], T.S. Fenske[5], B.T. Hess[6], J.L. Khatcheressian[7], C.B. Miller[8], J. Munoz[9], M.R. Patel[10], S.M. Smith[11], S.D. Smith[12], D.A. Stevens[13], J.C. Ye[14], A.J. Steele[15], A. Pandey[15], M.R. Birrell[15], J.M. Leeds[15], G.P. Coffey[15], J.T. Curnutte[15]

[1]Department of Medicine, Lymphoma Service, Memorial Sloan Kettering Cancer Center, Basking Ridge, NJ, [2]Lombardi Comprehensive Cancer Center, Georgetown University Hospital, Washington D.C, [3]Cancer Services, Summit Medical Group - MD Anderson Cancer Center, Morristown, NJ, [4]Hackensack University Medical Center, Hackensack, NJ, [5]Division of Hematology & Oncology, Medical College of Wisconsin, Milwaukee, WI, [6]Hollings Medical Cancer Center, Medical University of South Carolina, Charleston, SC, [7]Virginia Cancer Institute, Richmond, VA, [8]Cancer Institute, St. Agnes Hospital, Baltimore, MD, [9]Cancer Immunotherapy Program, Banner MD Anderson Cancer Center, Gilbert, AZ, [10]Drug Development Unit, Florida Cancer Specialists/Sarah Cannon Research Institute, Sarasota, FL, [11]Lymphoma Program, The University of Chicago Medicine, Chicago, IL,, [12]University of Washington/Fred Hutchinson Cancer Research Center, Seattle Cancer Care Alliance, Seattle, WA, [13]Norton Cancer Institute, Louisville, KY, [14]University of Michigan Cancer Center, Ann Arbor, MI, [15]R&D, Portola Pharmaceuticals, South San Francisco, CA, United States

Background: Subsets of B cell malignancies depend on tonic B cell antigen receptor (BCR) signaling for survival. SYK is a key regulator of BCR signaling (upstream of BTK and PI3K), and its inhibition using entospletinib has demonstrated clinical activity in B cell malignancies. SYK is also expressed in peripheral T cell lymphomas (PTCL), and pre-clinical evidence suggests it may be an oncogenic driver in this disease. Importantly, tumor growth may additionally be supported by autocrine or paracrine derived cytokines. IL2, IL4, and IFN alpha are all known to promote basal survival and protect against drug-induced cell death in chronic lymphocytic leukemia (CLL) *in vitro*. Furthermore compared to control tissues, lymph nodes from patients with follicular lymphoma (FL) have greater numbers of T follicular helper (T_{FH}) cells that express high levels of IL-4 which appears to drive tumor progression. Of particular interest, subsets of PTCL express either wild type SYK or overexpress SYK following an ITK-SYK translocation. Genetic or pharmacological inhibition of SYK expressing PTCL cell lines results in reduced growth and increased cell death. In contrast transgenic expression of ITK-SYK in mice CD4+ T cells generates a lethal T cell lymphoproliferative disease. Activating mutations in JAK/STAT pathways in PTCL are also frequently observed. These data suggest that combined SYK/JAK inhibition may have therapeutic activity in B and T cell malignancies. Cerdulatinib is a selective and potent inhibitor of SYK, JAK1, JAK3 and TYK2. A phase 1 dose escalation study of cerdulatinib in 43 patients with r/r CLL and B cell NHL was completed in 2016 (Hamlin *et al.*, EHA Congress 2016). Inhibition of SYK and JAK was well tolerated and consistent anti-tumor activity was seen in CLL and FL.
Aims: Here, we report the interim results on the efficacy and safety of cerdulatinib in an ongoing phase 2a study in patients with r/r B- and T-cell lymphoma.
Methods: An ongoing phase 2a study is to assess the antitumor activity of cerdulatinib in patients with specific subtypes of B cell or T cell NHL or CLL/SLL (ClinicalTrials.gov ID: NCT01994382). In this interim analysis, the safety and efficacy of cerdulatinib dosed 30 mg orally BID in patients with r/r B- and T-cell lymphoma was investigated. Response was assessed by Lugano Classification criteria.
Results: Ninety-nine patients were enrolled, 36 with FL, 28 with CLL/SLL, 18 with PTCL, 8 with MZL, 4 with WM, and 5 with aggressive NHL. Median age was 68 (42-93) and median number of prior therapies was 3 (1–13). 30 patients had prior BTK, PI3K or BCL-2 inhibitor therapy. The most common AEs of any grade were diarrhea (42%), fatigue (36%), and nausea (32%). Grade 3+ AEs occurring in ≥5% patients were neutropenia (18%), lipase increase (15%), pneumonia (12%), diarrhea (10%), and fatigue (7%). Five patients have had grade 5 infections considered potentially related to study drug, primarily in advanced stage CLL patients. The target PK range has been achieved with an average SSCmin of ~0.8 µM. Broad clinical activity has been observed, including: 61% ORR in CLL/SLL, 50% in FL, and 43% in PTCL (4 CRs, 2 PRs in 14 patients). The first PTCL patient enrolled achieved a CR and remains on drug at 12 months. Durable PRs have occurred in patients who relapsed on BTK inhibitor (CLL, 5+ months, WM, 7+ months, FL, 12 months), venetoclax (SLL, 18+ months).
Summary and Conclusions: The cerdulatinib phase 2a dose of 30 mg BID demonstrated good tolerability and efficacy in patients with heavily pretreated r/r B and T cell NHL.

PF438

ACALABRUTINIB ALONE OR IN COMBINATION WITH RITUXIMAB IN FOLLICULAR LYMPHOMA

N.H. Fowler[1,*], M. Coleman[2], D. Stevens[3], S. Smith[4], P. Venugopal[5], P. Martin[2], T. Phillips[6], R. Agajanian[7], D.M. Stephens[8], R. Izumi[9], J. Cheung[9], J.G. Slatter[9], M. Yin[9], M. Hiremath[9], N. Hunder[9], B. Christian[10]

[1]The University of Texas MD Anderson Cancer Center, Houston, [2]Weill Cornell Med-

icine, New York, [3]Norton Cancer Institute, Louisville, [4]University of Chicago, [5]Rush University Cancer Center, Chicago, [6]University of Michigan, Ann Arbor, [7]The Oncology Institute of Hope & Innovation, Downey, [8]University of Utah Huntsman Cancer Institute, Salt Lake City, [9]Acerta Pharma, Redwood City, [10]The Ohio State University Comprehensive Cancer Center, Columbus, United States

Background: Bruton tyrosine kinase (BTK) inhibition has shown clinical benefit in follicular lymphoma (FL). Acalabrutinib is a highly selective, potent, covalent inhibitor of BTK.
Aims: This Phase 1b study evaluated acalabrutinib±rituximab in patients with treatment-naive (TN) or relapsed/refractory (R/R) FL.
Methods: Patients with R/R FL (≥1 prior treatment) were randomized to acalabrutinib (mono) or acalabrutinib+rituximab (combo); TN patients received the combo. In 28-day cycles, rituximab (375 mg/m^2 IV) was given weekly in Cycle 1 and on Day 1 of Cycles 2-6; acalabrutinib (100 mg PO bid [2 patients received 200 mg qd]) was given until progressive disease (PD) or intolerance. The primary endpoint was safety. Secondary endpoints included overall response rate (ORR), duration of response (DOR), pharmacokinetics (PK) and pharmacodynamics.
Results: Thirteen TN and 27 R/R patients were treated. In all patients, the median age was 66 years (range 32-83), 98% of patients had ECOG PS ≤1, and 88% had stage III/IV disease. R/R patients received a median of 2 prior therapies (range 1-5). At a median follow-up of 22 and 7.6 months, 62% of TN patients and 26% of R/R patients, respectively, were still on treatment. Discontinuations were primarily due to PD (TN 15%; R/R 56%) and adverse events (AEs; TN 8%; R/R 11%). BTK occupancy and PK parameters were consistent with previous acalabrutinib studies. In all patients, the most common AEs of any grade were fatigue (48%), headache (43%), diarrhea (40%), nausea (30%) and sinusitis (25%). The most common Grade 3/4 AEs were hypertension (8%), increased alanine aminotransferase, increased aspartate aminotransferase, and cellulitis (all 5%), with no Grade 5 events. There were no cases of atrial fibrillation or Grade ≥3 hemorrhage. Efficacy outcomes are reported in the Table 1.

Table 1.

	TN combo (n=13)	R/R combo (n=13)	R/R mono (n=12)[a]
ORR[b] (≥ partial response), n (%)	12 (92)	5 (39)	4 (33)
95% CI	64, 100	14, 68	10, 65
Complete response	4 (31)	1 (8)	1 (8)
Median DOR, mo	NR	NR	NR
Range[c]	12.1 to 20.5+	10.8+ to 20.5+	0.03+ to 18.7+

NR: not reached.
[a]The 2 patients dosed at 200 mg qd are not included (1 stable disease, 1 PD).
[b]Investigator assessed using Cheson 2014 criteria.
[c]"+" indicates ongoing response.

Summary and Conclusions: Acalabrutinib, alone and combined with rituximab, was well-tolerated and yielded promising response rates in FL. These results support further evaluation of acalabrutinib in FL.

PF439

RESPONSE RATE TO LENALIDOMIDE PLUS RITUXIMAB (R2) IS INDEPENDENT OF RITUXIMAB-REFRACTORY STATUS: INITIAL INTERIM ANALYSIS OF MAGNIFY PHASE IIIB STUDY OF R2 FOLLOWED BY MAINTENANCE IN R/R INDOLENT NHL

M.J. Rummel[1,*], D.J. Andorsky[2,3], M. Coleman[4], A. Yacoub[5], J. Melear[3,6], H. Brooks[3,7], S. Fanning[3,8], K. Kolibaba[3,9], F. Lansigan[10], C. Reynolds[11], J. Li[12], D. Liu[12], M. Llorente[12], J.L. Ricker[12], J. Sharman[3,13]

[1]Justus-Liebig Universität, Giessen, Germany, [2]Rocky Mountain Cancer Centers, Boulder, [3]The US Oncology Network, The Woodlands, [4]Clinical Research Alliance Inc, Weill Cornell Medicine, New York, [5]The University of Kansas Cancer Center, Westwood, [6]Texas Oncology-Austin, Austin, [7]Oncology and Hematology Associates, Roanoke, [8]Greenville Health System, Greenville, [9]Compass Oncology, Vancouver, [10]Dartmouth-Hitchcock Medical Center, Lebanon, [11]IHA Hematology Oncology Consultants - Ann Arbor, Ypsilanti, [12]Celgene Corporation, Summit, [13]Willamette Valley Cancer Institute and Research Center, Eugene, United States

Background: Relapsed/refractory indolent non-Hodgkin lymphoma (iNHL) is generally considered incurable at advanced stages. Patients refractory to their last prior treatment, including rituximab, have poor prognosis with limited treatment options. Combination immunotherapy lenalidomide with rituximab (R^2) has shown promising efficacy and tolerability in multiple NHL studies.
Aims: This report explores the association between rituximab-refractory baseline status and response to R^2.
Methods: MAGNIFY (NCT01996865) is a phase IIIb, multicenter, open-label study of relapsed/refractory NHL patients, including follicular lymphoma (FL) grade 1-3a and marginal zone lymphoma (MZL). Upon informed consent, patients receive 12 cycles of R^2 (lenalidomide 20 mg/d, d1-21/28+standard rituximab). Patients with stable disease or better are randomized 1:1 to maintenance with R^2 or rituximab alone for a total of 18 cycles. This analysis focuses on the initial period prior to randomization (12 cycles R^2) comparing patients who were rituximab-refractory, defined as experiencing a best response of ≤SD or ≥PR lasting <6 months following patient's last prior rituximab dose, or rituximab-sensitive prior to enrollment.
Results: As of May 1, 2017, 232 patients have been enrolled in the initial treatment period, including 186 patients with FL grade 1-3a and 46 patients with MZL (n=26 nodal MZL, n=10 each MALT lymphoma and splenic MZL). Overall, median age was 66 years (range, 35-91), 54% were male, most had ECOG PS 0-1 (96%) and stage III/IV disease at study entry (89%). Analyzed subgroups included 138 (59%) rituximab-sensitive and 94 (41%) rituximab-refractory patients. Baseline characteristics were generally similar for these subgroups, including a median number of 2 prior systemic therapies for both groups. Rituximab-sensitive and rituximab-refractory patients have been on the study for a median follow-up time of 11.2 and 12.3 months, respectively. In efficacy-evaluable patients, the overall response rates (ORR) in the rituximab-sensitive and rituximab-refractory groups were 75% and 58%, respectively (Table 1). Median time to response (mTTR) was 2.8 months (range, 2-12) for both groups. For rituximab-sensitive and rituximab-refractory groups, 1-year rates for progression-free survival (PFS; 95% CI) were 76% (67% - 83%) and 60% (50% - 68%), and for DOR were 82% (68% - 91%) and 73% (51% - 87%), respectively. The most common grade ≥3 treatment-emergent adverse events for rituximab-sensitive and rituximab-refractory patients, respectively, were neutropenia (29%; 40%), thrombocytopenia (8%; 8%), anemia (6%; 2%), and leukopenia (5%; 7%).

Table 1. Efficacy of R^2 in efficacy-evaluable patients according to rituximab-refractory status.

	Rituximab-Sensitive (n=110)	Rituximab-Refractory (n=77)
ORR	75%	58%
CR/CRu	47%	35%
Median TTR, months (range)	2.8 (2-12)	2.8 (2-12)

Summary and Conclusions: In patients with relapsed/refractory FL grade 1-3a and MZL, initial treatment with R^2 showed clinically significant activity and tolerable safety profiles for patients, regardless of their prior response to a rituximab-containing regimen. Enrollment in MAGNIFY is ongoing in the USA and Europe.

PF440

AUTOLOGOUS STEM CELL TRANSPLANTATION MAY CURE FOLLICULAR LYMPHOMA PATIENTS WITH EARLY THERAPY FAILURE WHO REACH COMPLETE RESPONSE AFTER RESCUE TREATMENT IRRESPECTIVE OF PREVIOUS RITUXIAMB EXPOSURE.

A. Jiménez Ubieto[1,*], C. Grande [1], D. Caballero[2], L. Yañez[3], A. López-Guillermo[4], A. Salar[5], J.J. Lahuerta[1]

[1]Hematología, Hospital 12 de Octubre, Madrid, [2]Hematología, Hospital Universitario de Salamanca, Salamanca, [3]Hematología, Hospital Universitario Marqués de Valdecilla, Santander, [4]Hematología, Hospital Clinic de Barcelona, Barcelona, [5]Hematología, Hospital San Pau, Barcelona, Spain

Background: Patients with follicular lymphoma (FL) experiencing early therapy failure (ETF) within two years of frontline chemotherapy/immunochemotherapy have poor overall survival (OS) and lack of standard therapeutic options.
Aims: To analyse data from the GELTAMO registry to determine whether ASCT is an effective option in this high-risk subgroup of FL patients.
Methods: ETF was defined as failure to achieve at least PR after first line chemotherapy/immunochemotherapy (primary refractoriness) or relapse/progression within 2 years of starting first line chemotherapy/

immunochemotherapy. We identified two groups: the EFT cohort (n=155: 15 primary refractory patients, 95 transplanted in CR2 and 45 in PR2] and the non-EFT cohort [FL patients who received ASCT in either CR2 or PR2 but who did not experience ETF following first-line therapy (n=62: 50 transplanted in CR2 and 12 in PR2]. We additionally analyzed outcome of patients who need >1 therapy lines to reach CR1/PR1 and underwent ASCT in first response (n=152; CR1=85 and PR1=67).

Results: In the ETF cohort and non-ETF cohort, a total of 54 (35%) and of 16 (31%) patients were exposed to rituximab before ASCT. Median follow-up from ASCT was 12 years .In the ETF group a total of 74% of the patients received ASCT within the first year after treatment failure. Patient/disease characteristics were well balanced between the EFT and non–EFT except for: a) status of the disease at the moment of ASCT (CR2 60% *vs* 80%; *P*=.005, PR2 30% *vs* 22%; *P*=.005 and resistant/refractory 10% *vs* 0%; *P*=.01), b) median time from FL diagnosis to ASCT (28 months *vs* 67 months; *P*<.00001) and c) the number of therapy lines to reach first response (28% *vs* 8%; *P*=.01), respectively. In the ETF group a total of 74% of the patients received ASCT within the first year after treatment failure. There was a significant difference in PFS (*P*=0·016) between the ETF and non-ETF cohorts, with 5-year PFS from ASCT of 45% and 58%, respectively. Nevertheless, in patients experimenting EFT with an interval from first relapse after primary treatment to ASCT <1 year, the 5-year PFS was 55%; similar to that of the non-EFT cohort (*P*=0·5).There was no significant difference in OS (*P*=0·27) between the ETF and non-ETF cohorts, with 5-year OS of 70% and 77% respectively (Figure 1). In the EFT cohort, on multivariate analysis, the factors associated with inferior OS were age >46 years (HR=1.85; 95% CI=1.08-3.18; P=-02), male sex (HR=1.89; 95% CI=1.15-3.2; P=.01), a status of disease different to CR at ASCT (HR=2.24; 95% CI=1.44-3.56; P=.0005) and the use of bone marrow as stem cell source (HR=2.56; 95% CI=1.42-4.64; P=-.0002). In the ETF group, in patients transplanted in CR (n=95), there was a plateau in the PFS and OS curves beyond 12.7 years of follow-up at 40%. Finally; 5-year PFS and OS of patients needing >1 therapy line to reach CR1/PR1 (n=152; 33% of them exposed to rituximab before ASCT) and undergoing ASCT in first response presented a 5-year PFS and OS of 60% and 77%, respectively.

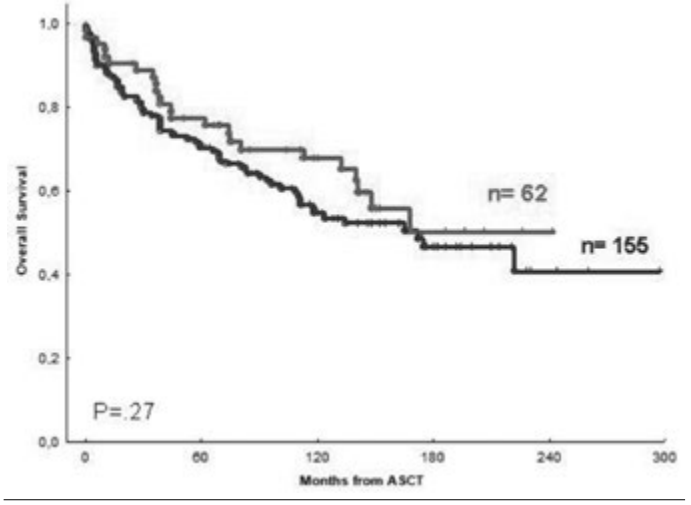


Figure 1.

Summary and Conclusions: ASCT can be a curative and cost-effectiveness option in ETF who respond to rescue treatments, irrespective of previous rituximab use. However, the value of ASCT in primary refractory patients remains to be elucidated. Additionally, patients requiring more the one line to reach CR1/PR1 are good candidates to consolation with ASCT. Nevertheless, large randomized trails focus on high risk patients and biological research are urgently needed.

PF441

RESULTS OF A DOSE ESCALATION STUDY OF ME-401, A SELECTIVE PI3KΔ INHIBITOR, IN RELAPSED/REFRACTORY (R/R) FOLLICULAR LYMPHOMA (FL) AND CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)/SMALL LYMPHOCYTIC LYMPHOMA (SLL)

J.M. Pagel[1,*], J.D. Soumerai[2], D. Jagadeesh[3], H.H. Salman[4], V.P. Kenkre[5], A.S. Asch[6], A. Stathis[7], N.M. Reddy[8], A. Iasonos[9], K. Patel[1], R. Ghalie[10], E. Zucca[7], A.D. Zelenetz[11]

[1]Center for Blood Disorders and Stem Cell Transplantation, Swedish Cancer Institute, Seattle, [2]Hematology/Oncology, Massachusetts General Hospital, Boston, [3]Hematology and Medical Oncology, Cleveland Clinic, Cleveland, [4]Stony Brook Cancer Center, Stony Brook University School of Medicine, Stony Brook, [5]Division of Hematology and Oncology, University of Wisconsin School of Medicine, Madison, [6]Hematology/Oncology, University of Oklahoma Stephenson Cancer Center, Oklahoma City, United States, [7]Lymphoma Unit, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland, [8]Hematology/Oncology, Vanderbilt University Ingram Cancer Center, Nashville, [9]Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York City, [10]Clinical Development, MEI Pharma, San Diego, [11]Lymphoma Service, Memorial Sloan Kettering Cancer Center, New York City, United States

Background: PI3Kδ mediates B-cell receptor signaling and microenvironmental support signals that promote the proliferation and survival of malignant B lymphocytes. ME-401 is a potent, structurally differentiated, second generation oral tyrosine kinase inhibitor highly selective for PI3Kδ. Pharmacokinetic (PK) and pharmacodynamic data from a prior study in healthy volunteers informed the starting dose for this first-in-patients trial.

Aims: This phase 1b study evaluated the safety, tolerability, dose limiting toxicities (DLT), maximum tolerated dose (MTD), efficacy, and PK of ME-401 in patients (pts) with R/R FL or CLL/SLL.

Methods: Eligible pts ≥18 years of age had FL or CLL/SLL, relapsed disease after ≥1 prior therapy, ECOG performance status ≤2, no prior PI3K therapy, and no prior progression of disease (POD) on BTK therapy. ME-401 was given once daily on days 1-28 of 28-day cycle until POD or unacceptable toxicity. All pts received PJP prophylaxis, and CMV monitoring was mandatory. Dose escalation was guided by a continuous reassessment model (CRM) design to identify the MTD. At least 6 evaluable pts were treated at each dose level, with option to expand to 12 pts for further efficacy assessment. DLT were assessed in the first 56 days window. Response was assessed after Cycles 2 and 6, and then every 6 months or as clinically indicated, using the IWCLL 2008 and Lugano 2014 criteria.

Results: 31 pts (21 FL, 10 CLL/SLL) received ME-401 and 30 were evaluable for DLT: 12 at 60 mg, 12 at 120 mg, and 6 at 180 mg. Median age was 65 years (range: 47-79), 12/30 (40%) pts received ≥2 prior therapies, and 9/21 (43%) pts with FL had POD <24 months after initial chemoimmunotherapy (POD24). Of 29 pts evaluable for response, the overall objective response rate (ORR) was 83% (24/29), including 21% (6/29) with a complete nodal response. An objective response was noted by Cycle 2 in 21/24 (88%) responders. In FL, the ORR was 75% (15/20), including 9/9 (100%) who had POD24. In CLL/SLL, the ORR was 100% (9/9). No DLTs were reported and dose escalation above 180 mg was closed given frequent responses across lower dose levels. With a median follow-up of 23 weeks (range: 5-55 weeks), 3 pts had POD at Weeks 8, 14 and 17. Five pts discontinued ME-401 due to adverse events (AEs): rash (n=3), colitis (n=1), and cardiomyopathy (n=1), and 2 pts discontinued for personal reasons at Weeks 4 and 21. Most common AEs (all grades/grade ≥3) were diarrhea (32%/16%), fatigue (29%/0%), cough (29%/0%), rash (29%/10%), and nasal congestion (26%/0%). All grade ≥3 AEs were reported in Cycle 3 or later. Dosing was interrupted in 9/31 pts (29%) to manage toxicities. PK parameters were dose proportional, and steady state pre-dose plasma concentrations exceeded the EC90 of the basophil activation test (BAT) at all 3 doses.

Summary and Conclusions: ME-401 resulted in frequent early responses among pts with R/R FL, including POD24, and CLL/SLL. No DLTs were reported in the first 56 days, and the MTD was not identified. Dose escalation was closed due to high ORR across lower dose levels. Toxicities were manageable, albeit still with limited follow-up. An alternative dosing (ME-401 given on days 1-7 of 28-day cycle starting with Cycle 3) is being evaluated, including dosing ME-401 at 45 mg and at 60 mg in combination with rituximab with various B-cell malignancies.

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SHORT DIAGNOSIS TO TREATMENT INTERVAL IS ASSOCIATED WITH POOR OUTCOMES IN PATIENTS WITH FOLLICULAR LYMPHOMA TREATED WITH IMMUNOCHEMOTHERAPY

M.R. Clausen[1,*], B.K. Link[2], C. Madsen[1], G. Nowakowski[3], T.E. Witzig[3], A. Feldman[4], S.L. Slager[5], T.M. Habermann[3], S.M. Ansel[3], F. d'Amore[1], J.R. Cerhan[5], M. Maurer[5]

[1]Department of Hematology, Aarhus University Hospital, Aarhus, Denmark, [2]Division of Hematology, Oncology and Blood & Marrow Transplantation, University of Iowa Hospitals and Clinics, Iowa City, [3]Division of Hematology, [4]Department of Laboratory Medicine and Pathology, [5]Department of Health Sciences Research, Mayo Clinic, Rochester, United States

Background: The time interval from diagnosis to treatment initiation (DTI) has prognostic implications in diffuse large B-cell lymphoma. Patients who

initiate therapy urgently (shorter DTI) have more adverse prognostic features and poor outcomes compared to patients with longer DTI.
Aims: To evaluate whether this also applies to patients with follicular lymphoma (FL) initially managed with immunochemotherapy (IC).
Methods: Discovery was performed in patients prospectively enrolled from Iowa/Mayo Lymphoma SPORE Molecular Epidemiology Resource (MER) with validation in the Danish Lymphoma Registry (LYFO). Patients had newly diagnosed FL grades 1 to 3a, were treated with rituximab(R)-CVP, R-CHOP or R-bendamustine based therapy and had treatment initiated within 90 days of biopsy leading to the diagnosis. We excluded patients who had CNS-lymphoma or had a discordant lymphoma including DLBCL at diagnosis. DTI was defined as days from the initial biopsy containing lymphoma to first day of treatment. We analyzed DTI both on a continuous scale and dichotomized in 0-14 days *vs* >14 days based on functional form assessment. Kaplan-Meier curves and Cox regression (adjusted for FLIPI and treatment) were used to assess the association of DTI with event free survival (EFS) and overall survival (OS) from initiation of therapy.
Results: 447 patients from the MER diagnosed from 2002-2015 and 918 from the LYFO diagnosed from 2001-2012 were included. In the MER, median age was 59 years (IQR: 49, 68), 56% were male, and FLIPI was 0-1 in 28%, 2 in 33% and 3-5 in 39%. At a median follow-up of 75 months (range: 1-168) 178 (40%) had events and 92 (21%) had died. In the LYFO, median age was 62 years (IQR: 54, 69), 52% were male, and FLIPI was 0-1 in 16%, 2 in 26%, and 3-5 in 58%. Median follow-up was 52 months (range: 2-173) with 331 (36%) events and 205 (22%) deaths. Median DTI was 22 days (IQR: 13, 35) in the MER with 29% initiating therapy within 14 days of diagnosis; median DTI in the LYFO was 24 days (IQR: 13, 41) with (30%) initiating therapy within 14 days. For both cohorts early DTI (0-14 days) was significantly associated with elevated LDH, anemia, and poor performance status; however, association between DTI and FLIPI did not reach significance (MER p=0.12, LYFO p=0.07). Early DTI was strongly associated with inferior EFS and OS (see Figure 1). Event-free survival at 24 months from treatment initiation was 63% (95%CI: 55-72%) (MER) and 70% (95%CI: 64-75%) (LYFO) for patients with early DTI *vs* 80% (95%CI: 76-85%) and 84% (95%CI: 81-86%) in MER and LYFO patients with DTI >14 days, respectively. Associations between early DTI and outcome remained significant in both the MER and LYFO cohorts after adjusting for FLIPI and type of therapy: EFS (MER: HR=1.98, 95%CI: 1.44-2.72; LYFO: HR=2.15, 95%CI: 1.59-2.90) and OS (MER: HR=2.23, 95%CI: 1.44-3.46; LYFO: HR=2.40, 95%CI: 1.56-3.69). Associations were consistent when analyzing DTI as a continuous variable.

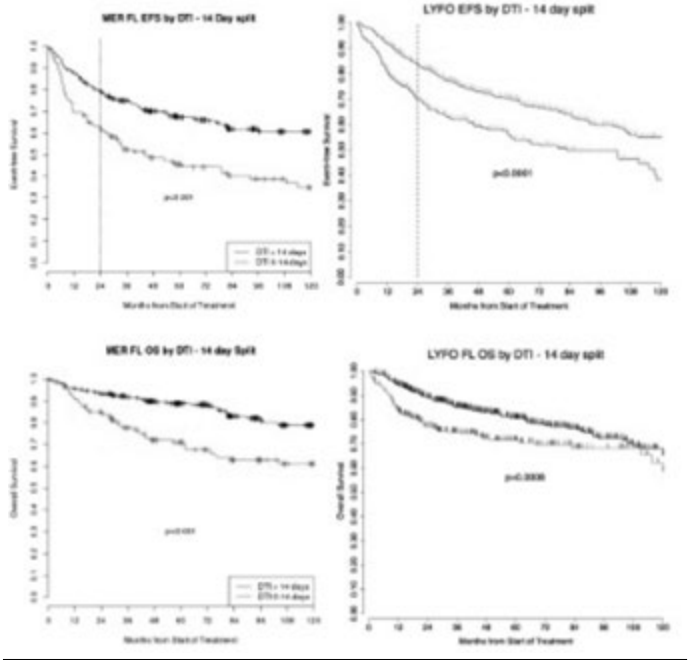

Figure 1.

Summary and Conclusions: We found that shorter DTI was strongly associated with inferior outcomes in IC treated FL in two independent cohorts. This association was independent of the FLIPI and type of therapy regimen. This finding has implications for clinical trials illustrating a potential selection bias since patients with urgent need of treatment are less likely to be enrolled in clinical trials.

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NEW ASSESSMENT INDEX OF CLINICAL TRANSFORMATION FROM FOLLICULAR LYMPHOMA (FL) TO DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) IN THE RITUXIMAB ERA

T. Shichijo[1,*], D. Maruyama[1], N. Yamauchi[2], A. Miyagi Maeshima[3], M. Sugano[4], K. Tajima[1], S. Yuda[1], T. Suzuki[1], K. Toyoda[1], S. Makita[1], S. Fukuhara[1], W. Munakata[1], T. Suzuki[1], Y. Kobayashi[1], H. Taniguchi[3], Y. Minami[2], K. Izutsu[1], K. Tobinai[1]

[1]Hematology, National Cancer Center Hospital, Tokyo, [2]Hematology, National Cancer Center Hospital East, Kashiwa, [3]Pathology and Clinical Laboratory, National Cancer Center Hospital, Tokyo, [4]Pathology and Clinical Laboratory, National Cancer Center Hospital East, Kashiwa, Japan

Background: Histological transformation (HT) is still a critical event because the prognosis of FL patients (pts) with HT is poorer than that of FL pts without HT even in the rituximab era. Although the histological diagnosis of HT by biopsy is the gold standard, it is not always possible to obtain a biopsy specimen. Indeed, more than half of the pts have been diagnosed with HT based on clinical criteria without histological confirmation according to the doctor's discretion in several previous reports. Hence, estimates of the true incidence of HT have wavered because the definition of clinical transformation has never been standardized. Thus, we proposed an assessment index for clinical transformation, which was retrospectively analyzed in a derivation cohort [Hematol Oncol. 2017; 35: 367]. Here, we further validated our assessment index in an independent cohort.
Aims: To propose a new assessment index for clinical transformation of FL that is easy to use in both daily practice and clinical trials in the rituximab era, we conducted a multicenter retrospective analysis.
Methods: In the derivation set, we retrospectively analyzed pts who were initially diagnosed with FL and underwent biopsy at the time of disease progression at the National Cancer Center Hospital (NCCH) from 2000 to 2016. We compared the clinical factors at disease progression and constructed an assessment index based on clinical covariates obtained with a multivariate logistic regression model. In the validation set, to confirm the accuracy of our assessment index, we retrospectively analyzed pts who were diagnosed at the NCCH East (NCCHE) from 2003 to 2014 based on the same criteria.
Results: In the derivation set, 459 pts were diagnosed with FL (grade 1-3a) at the NCCH with a median follow-up duration of 9 (range: 0.7-16) years. Disease progression was observed in 184 pts, 80 (43%) of whom had histological documentation (FL in 42, HT with DLBCL in 34, HT other than DLBCL in 4). Finally, we identified 76 pts with biopsy-proven FL or HT with DLBCL as subjects for the derivation analysis. HT occurred at a median of 5.5 (range: 0.2-16) years from the initial FL diagnosis and the 5-year overall survival rate from HT was 62%. In the multivariate analysis, rapid nodal growth, bulky disease ≥6 cm, elevated serum lactate dehydrogenase (LDH) level and hemoglobin level (Hgb) <12 g/dL at disease progression were associated with HT. The weights of variables were decided based on the regression coefficients, and we then constructed the final assessment index consisting of the above four factors (score 1 each) (Figure 1).

Risk factor	Regression coefficient	Odds ratio (95% CI)	P-value	Score
Rapid nodal growth	2.3419	10.4 (2.24–48.3)	0.003	1
Bulky disease (≥ 6 cm)	2.4996	12.2 (1.45–102)	0.021	1
Hgb < 12 g/dL	2.1521	8.6 (1.69–43.9)	0.010	1
LDH > normal upper limit	2.3567	10.6 (2.4–46.4)	0.002	1

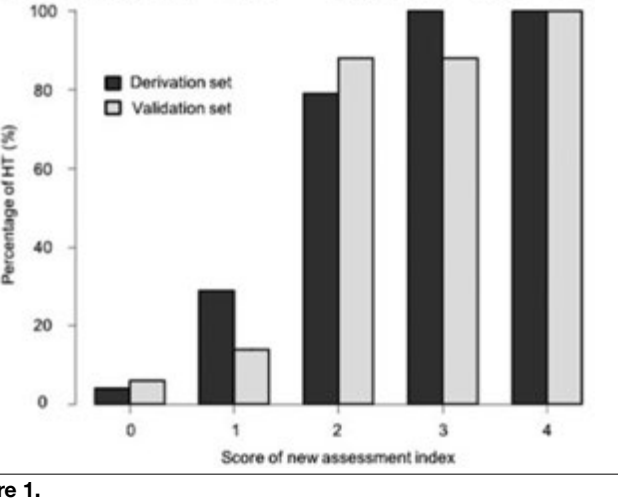

Figure 1.

The percentage of HT was score 0: 4%, score 1: 29%, score 2: 79%, score 3: 100% and score 4: 100% (Figure 1). In the validation set, 243 pts were diagnosed with FL (grade 1-3a) at NCCHE. Disease progression was observed in 95 pts, 50 (53%) of whom had histological documentation (FL in 30, HT with DLBCL in 20). Finally, our assessment index was applied to 50 pts with biopsy-proven FL or HT with DLBCL, and the percentage of HT was score 0: 6%, score 1: 14%, score 2: 88%, score 3: 88% and score 4: 100% (Figure 1). The probability of HT was high when the score was 2 or higher.

Summary and Conclusions: We developed a new assessment index for clinical transformation of FL that was confirmed by validation analysis. It is likely to be a simple and valuable tool for the diagnosis of HT, especially for pts from whom it is difficult to obtain a biopsy specimen. Further investigation is warranted in prospective studies on a large number of pts.

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LONG TERM INTEGRATED SAFETY ANALYSIS OF UMBRALISIB (TGR-1202), A PI3K-DELTA/CK1-EPSILON INHIBITOR WITH A DIFFERENTIATED SAFETY PROFILE, IN PATIENTS WITH RELAPSED/REFRACTORY LYMPHOID MALIGNANCIES

M.S. Davids[1,*], I.W. Flinn[23], A.R. Mato, [4], O.A. O'Connor[5], D.M. Brander,[6], M.A. Lunning[7], J.M. Vose[7], L. Nastoupil[8], N. Fowler[8], C.R. Flowers[9], J.R. Brown[1], M.T. Schreeder[10], N. Ghosh[11], F. Lansigan[12], B.D. Cheson[13], P.M. Barr[14], J.M. Pagel[15], A. Danilov[16], J. Pinilla-Ibarz[17], C. Deng[5], J.M. Burke,[18,19], T. Siddiqi,[20], M.R. Patel[2,21], C.M. Farber[22], P. Venugopal[23], J.G. Gribben[24], P.L. Zinzani[25], H.P. Miskin[26], P. Sportelli[26], M.S. Weiss[26], S.M. O'Brien,[27]

[1]Dana-Farber Cancer Institute, Boston, [2]Sarah Cannon Research Institute, [3]Tennessee Oncology, Nashville, [4]Memorial Sloan Kettering Cancer Center, [5]Center for Lymphoid Malignancies, Columbia University Medical Center, New York, [6]Duke University Medical Center, Durham, [7]University of Nebraska Medical Center, Omaha, [8]The University of Texas MD Anderson Cancer Center, Houston, [9]Winship Cancer Institute, Emory University, Atlanta, [10]Clearview Cancer Institute, Huntsville, [11]Levine Cancer Institute, Charlotte, [12]Dartmouth-Hitchcock Medical Center, Lebanon, [13]Georgetown University Medical Center, Washington DC, [14]Wilmot Cancer Institute, University of Rochester, Rochester, [15]Swedish Cancer Institute, Seattle, [16]Oregon Health & Science University, Portland, [17]H. Lee Moffitt Cancer Center & Research Institute, Tampa, [18]Rocky Mountain Cancer Centers, Aurora, [19]US Oncology, Woodlands, [20]City of Hope National Medical Center, Duarte, [21]Florida Cancer Specialists, Sarasota, [22]Summit Medical Group/MD Anderson Cancer Center, Summit, [23]Rush University Medical Center, Chicago, United States, [24]Barts Hospital Cancer Institute, Queen Mary University of London, London, United Kingdom, [25]Institute of Hematology "L. e A. Seràgnoli", University of Bologna, Bologna, Italy, [26]TG Therapeutics, Inc., New York, [27]University of California Irvine, Chao Family Comprehensive Cancer Center, Orange, United States

Background: First generation PI3Kδ inhibitors such as idelalisib and duvelisib are active in patients (pts) with lymphoid malignancies, but are often associated with significant immune-mediated adverse events (AEs), including transaminitis, diarrhea/colitis, and pneumonitis. These toxicities can be severe, and frequently lead to treatment discontinuation; moreover, these toxicities may arise only after pts have been on drug for several months. Umbralisib (TGR-1202) is a next generation, once-daily, oral PI3Kδ/CK1 inhibitor, that is active in pts with relapsed/refractory (R/R) lymphoid malignancies (Burris *et al.*, 2018). We previously have presented data suggesting that umbralisib has a favorable safety profile, though follow-up in the prior analysis was short, and the crucial information about the rate of late immune-mediated AEs with umbralisib was not available due to short follow-up.

Aims: We performed an integrated safety analysis focusing on key immune-mediated AEs with long term follow-up of pts on umbralisib.

Methods: Safety data were pooled from 5 completed or ongoing Phase 1 or 2 studies with umbralisib alone or in combination with other agents. All studies shared similar key eligibility criteria: pts had R/R lymphoid malignancies w/out limit to # of prior therapies. Umbralisib was dosed daily until PD or toxicity, while dosing of combination agents varied.

Results: A total of 347 pts were included in the analysis. Patients received the following regimens: umbralisib as monotherapy (146 pts) or umbralisib in combination with: the glycoengineered anti-CD20 mAb ublituximab ("U2", 98 pts), ibrutinib (32 pts), ublituximab+ibrutinib (38 pts), or ublituximab+bendamustine (33 pts). Among the 347 pts, 34% had CLL/SLL, 33% DLBCL, 21% indolent NHL, and 12% other lymphomas. Pts had a median 3 prior therapies. Median duration of exposure to umbralisib was 6.5 months, with the longest patient on daily umbralisib for 4+ years. The most common all-grade non-hematologic toxicities were: diarrhea (44% All – 29% GR1, 11% GR2, 4% GR3), nausea (39% All – 25% GR1, 13% GR2, 1% GR3), and fatigue (35% All – 19% GR1, 14% GR2, 2% GR3). The median time to onset for diarrhea events was early (32 days) and resolved in a median of 7 days. All-grade hematologic toxicities included neutropenia (22%), anemia (20%), and thrombocytopenia (18%). Grade 3/4 adverse events are described in Table 1. Key adverse events prevalent in prior generation PI3Kδ inhibitors were infrequent: transaminitis (8.6% All; G3/4 2.3%), colitis (1.4% All; G3/4 0.9%), and pneumonitis (1.4% All; G3/4 0.3%). Discontinuations due to treatment related adverse events were rare at under 10% for all studies. A total of 167 pts treated with umbralisib for a minimum duration of 6 months were included in a sub-analysis of long term safety. The median duration of exposure amongst these pts was 15.6 months (range 6.4 – 60.6 months). A summary of AEs occurring after 6 months on therapy is presented in Table 2.

Table 1.

Grade 3/4, All Causality, Adverse Events Occurring in >2% of Patients

	Study 101 Umbra Alone N=90	Study 201 Umbra Alone N=33	Study 105 Umbra + Ibrutinib N=32	Study 103 Umbra + Ubli (U2) N=75	Study 103 U2 + Ibrutinib N=38	Study 103 U2 + Benda N=33	Study 205 U2 or Umbra N=46	TOTAL N=347
Neutropenia	11%	18%	13%	20%	18%	24%	2%	16%
Anemia	8%	3%	9%	4%	3%	6%	4%	5%
Thrombocytopenia	6%	6%	9%	5%	8%	6%	0%	5%
Diarrhea	2%	9%	3%	8%	3%	9%	0%	4%
Pneumonia	4%	0%	0%	8%	11%	0%	2%	4%
Dyspnea	4%	0%	0%	3%	3%	3%	4%	3%
Hypokalemia	4%	3%	3%	3%	0%	9%	0%	3%
Febrile Neutropenia	3%	9%	0%	4%	3%	0%	2%	3%

Table 2.

All Grades, All Causality AEs Occurring After 6 Months on Umbralisib (N=167)

Adverse Event	All Grades		Grade ¾	
	N	%	N	%
Diarrhea	41	25%	12	7%
Nausea	25	15%	3	2%
Cough	21	13%	-	-
Neutropenia	20	12%	17	10%
Fatigue	20	12%	1	1%
Vomiting	18	11%	2	1%
Sinusitis	18	11%	-	-
Insomnia	15	9%	-	-
Abdominal pain	14	8%	3	2%
Pyrexia	14	8%	2	1%
Hypokalemia	13	8%	3	2%
Upper respiratory infection	13	8%	-	-
Thrombocytopenia	12	7%	5	3%
Anemia	12	7%	3	2%
Headache	12	7%	2	1%
Arthralgia	12	7%	-	-
Hypophosphatemia	10	6%	3	2%
Blood creatinine increased	10	6%	1	1%
Rash	10	6%	1	1%
Dizziness	10	6%	-	-

Summary and Conclusions: In this integrated analysis with long term follow-up, once-daily umbralisib exhibited a differentiated safety profile compared to prior generation PI3Kδ inhibitors. In particular, late onset diarrhea or colitis commonly associated with first-generation PI3Kδ inhibitors was infrequent. Umbralisib can be safely combined with a diverse array of other agents active in lymphoid malignancies, and is currently being studied in the global registration directed UNITY-CLL Phase 3 randomized trial (NCT02612311) and UNITY-NHL Phase 2b randomized trial (NCT02793583).

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POOLED ANALYSIS OF SAFETY DATA FROM ZANUBRUTINIB (BGB-3111) MONOTHERAPY STUDIES IN HEMATOLOGIC MALIGNANCIES

C.S. Tam[1,2,3,*], J. Trotman[4,5], D. Simpson[6], D. Ritchie[1,2], E. Verner[4], S. Ratnasingam [7], M.A. Anderson[2,5], P. Wood[8,9], J.F. Seymour[1,2], J. Zhu[10], J. Li[11], P. Marlton[8,9], D. Gottlieb[12], L. Lin[13,14], S. Ro[13,14], J. Hilger[13,14], A. Wang[13,14], X. Xu[13,14], M. Ji[13,14], A.W. Roberts[2,15], S. Opat[7,16], G. Cull[17,18]

[1]Peter MacCallum Cancer Center, East Melbourne, Victoria, [2]University of Melbourne, Parkville, Victoria, [3]St Vincent's Hospital, Fitzroy, Victoria, [4]Concord Repatriation General Hospital, [5]University of Sydney, Concord, Australia, [6]North Shore Hospital, Auckland, New Zealand, [7]Monash Health, Clayton, Victoria, [8]Princess Alexandra Hospital, [9]University of Queensland School of Medicine, Brisbane, Australia, [10]Beijing Cancer Hospital, Beijing, [11]Jiangsu Province Hospital, Nanjing, China, [12]University of Sydney, Westmead Hospital, Westmead, Australia, [13]Beigene (Beijing) Co. Ltd., Beijing, China, [14]Beigene (Beijing) Co. Ltd., Emeryville, CA, United States, [15]Royal Melbourne Hospital, Parkville, Victoria, [16]Monash University, Clayton, Victoria, [17]Sir Charles Gairdner Hospital, Perth, Western Australia, [18]University of Western Australia, Perth, Australia

Background: Bruton's tyrosine kinase (BTK) plays a critical role in B-cell receptor signaling, which mediates B-cell proliferation, migration and adhesion. Zanubrutinib is a potent, selective and irreversible BTK inhibitor. It has demonstrated profound BTK inhibition with minimal inhibition of off-target kinases such as EGFR, ITK, JAK3, HER2, and TEC, providing a good scientific rationale for a reduced toxicity profile.
Aims: To demonstrate the safety profile of zanubrutinib.
Methods: Safety data from patients (pts) in 6 ongoing zanubrutinib monotherapy studies were analyzed. All pts have been treated with ≥1 dose of oral (po) zanubrutinib at 40 mg once-daily to 160 mg twice-daily (bid). The analysis included frequency and severity of adverse events (AEs), AEs of special interest, and AEs leading to treatment discontinuation.
Results: A total of 424 pts were included in the pooled analysis, with a data cutoff date of September 15, 2017. The median age was 64 years (range 20-87) and 71.9% were males. The median follow-up duration was 4.8 months (range 0.03-36.0). The most common (occurring in ≥10% of pts) AEs were upper respiratory tract infection (23.8%), contusion (17.5%), diarrhea (14.2%), cough (13.0%), rash (12.7%), anemia (11.8%), and neutrophil count decreased (11.6%). Serious AEs (SAEs) were reported in 24.3%, including 7.5% that were assessed as related to zanubrutinib. The most common SAEs included pneumonia (3.5%), lung infection (1.7%) and febrile neutropenia (1.2%). AEs of special interest are shown in the Table 1. The most common bleeding events included contusion (17.5%) and hematuria (8.3%). Major hemorrhage, defined as serious or Grade ≥3 bleeding of any site, or central nervous system bleeding of any grade included gastrointestinal hemorrhage, purpura (0.5% each), melena, hemorrhagic cystitis, hematuria, renal hematoma, cerebral hemorrhage and hemothorax (0.2% each). The median time to first major hemorrhage was 23 days (range 3-262). The fatal event of cerebral hemorrhage was reported in a 70 year old male pt with mantle cell lymphoma who developed left occipital lobe hemorrhage after treatment with zanubrutinib 160 mg bid for 6 days. Amongst pts with atrial fibrillation/flutter (8 pts) a majority had known risk factors including hypertension (2 pts), pre-existing cardiovascular disease (2 pts) and concurrent infection (1 pt). The rates of Grade ≥3 infections were 10.1 events/100 pts in the first 3 months, 3.1 in months 3 to 6, and 5.5 after 6 months. The most common second primary malignancies included basal cell carcinoma (3.5%) and squamous cell carcinoma of skin (2.8%) with 2.1% of pts having a prior history of skin cancer. AEs led to treatment discontinuation in 5.9% of pts with 2.4% related to zanubrutinib.

Table 1.

AEs of Special Interest	All Patients (N=424)	
	All Grades, %	Grade ≥ 3, %
Hemorrhage	38.0	2.1
Major hemorrhage	2.1	2.1
Atrial fibrillation/flutter	1.9	0.2
Hypertension	4.2	1.4
Diarrhea	14.4	0.7
Infections	51.9	12.0
Second primary malignancies	7.1	2.4

Summary and Conclusions: Zanubrutinib has shown a favorable safety and tolerability profile in pts with various B-cell malignancies. In zanubrutinib's cumulative safety experience, events of interest with BTK inhibitors, such as atrial fibrillation (1.9%), major hemorrhage (2.1%), and severe diarrhea (0.7%) have been infrequent. Additionally, treatment discontinuation due to zanubrutinib-related adverse events was uncommon (2.4% of pts). These data suggest that exposure levels of zanubrutinib resulting in complete and sustained BTK inhibition can be safely achieved, resulting in low tolerability-related treatment failure rates.

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R-ESHAP IS NOT EFFECTIVE AS SALVAGE THERAPY IN REFRACTORY FOLLICULAR LYMPHOMA: RESULTS OF A RETROSPECTIVE MULTICENTRE STUDY ON BEHALF OF GELCAB GROUP

A. Muntañola[1,*], O. Garcia[2], T. Baumann[3], A.C. Caballero[4], B. Sánchez[5], A. Salar[5], S. Mercadal[6], L. Escoda[7], J.A. Soler[8], L. Iserte[9], M. Canet[1], M. Julià[1], M. Sorigué[2], A. López-Guillermo[3], J.M. Sancho[2]

[1]Hematology, Hospital Universitari Mutua Terrassa, [2]Hematology, IC-IJC-Hospital Germans Trias i Pujol, [3]Hematology, Hospital Clínic i Provincial, [4]Hematology, Hospital de la Santa Creu i Sant Pau, [5]Hematology, Hospital del Mar, [6]Hematology, Hospital Duran i Reynals. ICO Hospitalet, Barcelona, [7]Hematology, Hospital Joan XXIII, Tarragona, [8]Hematology, Hospital Parc Taulí, Sabadell, [9]Hematology, Hospital Arnau de Vilanova, Lleida, Spain

Background: Follicular lymphoma (FL) is an incurable disease with a clinical course of successive relapses. However, there is no standard treatment for relapsed and refractory patients. Even though platinum-based chemotherapy combinations are one of the most widely used treatment schemes, few data have been reported in this setting.
Aims: The aim of our study was to analyse the efficacy of R-ESHAP chemotherapy in relapsed or refractory FL patients who had previously received at least one containing-Rituximab regimen. These data have particular interest nowadays when new targeted drugs are available.
Methods: We retrospectively collected data from 80 FL patients diagnosed between January 2000 and December 2016 in 9 different centres belonging to GELCAB group. Patients must have been treated with R-ESHAP in the first or successive relapses. R-ESHAP cycles were administered every 4 weeks. The number of total cycles that each patient received was determined based on physician criteria. The use of G-CSF was allowed, and histologically transformed patients were also included in the study. Autologous or allogeneic stem cell transplantation performed following R-ESHAP salvage treatment was permitted.
Results: Median age of the whole series (n=80) was 50 years, 79% (n=63) were male. At the time of R-ESHAP initiation, 85% of the patients (54/65) were in advanced stage, 28% had bulky disease (18/65) and 40% had increased LDH (26/65). FLIPI was low, intermediate or high in 28%, 33% and 39% of the patients, respectively. R-ESHAP was administered as salvage treatment for the first relapse in 45 patients (56%), for the second relapse in 23 (29%) and for the third or successive relapses in 15% of the cases. At the moment of R-ESHAP initiation, 17 patients had histological transformation to DLBCL. These transformed cases were excluded from the former analysis of response. Among the 63 remaining patients, 47 were considered as early relapsed to first-line treatment (defined as refractoriness or progression within the 24 months after diagnosis, POD24). Excluding transformed patients, ORR was 77% (47/63), with 46% of CR (28/63). None of the clinical characteristics at the time of R-ESHAP was associated with the sort of response. There were no statistically significant differences between POD24 and non-POD24 patients in terms of response to R-ESHAP (ORR of 72% *vs* 93%, p=0.109 and CR rate of 43% *vs* 57%, p=0.336, respectively). Median OS was 3.8 years and median PFS was 1.5 years (calculated from the time of R-ESHAP). When analysing R-ESHAP efficacy according to the response to the immediately previous line of treatment, we found that patients achieving CR or PR to previous line chemotherapy (n=45) had better CR rates to R-ESHAP than those who did not respond (n=14) (CR rates of 57% *vs* 15%, respectively, p=0.009) and a trend to better ORR (84% *vs*, 54%, p=0.054), as well as differences in OS (median of 7.2 years *vs* 1.4 years, p<0.0001), and in PFS (median of 2.1 years *vs* 0.3,p<0.0001) (Figure 1). Finally, in the subgroup of transformed FL (n=17) R-ESHAP provided an ORR of 47% (CR in 24%), with a median (95% CI) of OS and PFS of 1.3 (0.5, 2.1) years and 0.3 (0.1, 0.5) years, respectively.

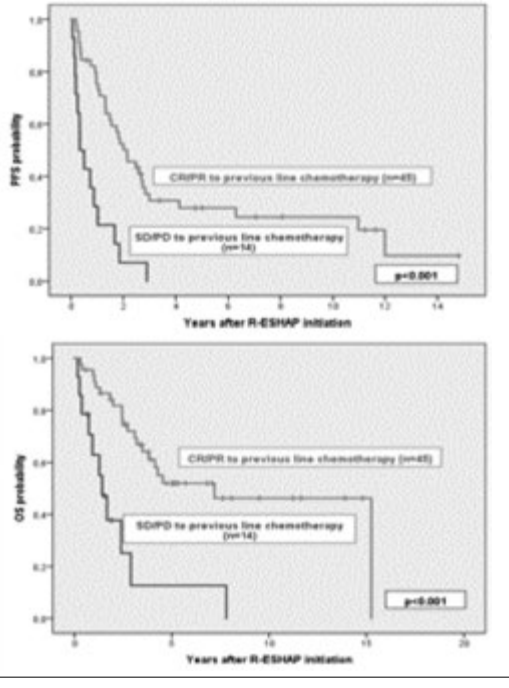


Figure 1.

Summary and Conclusions: In our series, R-ESHAP is not an effective salvage treatment in previously refractory FL patients, independently of response to first line treatment and clinical characteristics, nor in transformed FL. Our results support the use of new recently approved treatments (such as obinutuzumab or idelalisib) in these especially poor prognosis patients.

PF447

CHALLENGES IN DELIVERY OF MAINTENANCE ANTI-CD20 THERAPY IN ADVANCED FOLLICULAR LYMPHOMA: 5-YEAR EXPERIENCE AT THE ROYAL MARSDEN HOSPITAL

H. Kayhanian[1,*], A. Pender[1], M. Gleeson[1], N. Joharatnam[1], R. Taylor[1], J. Clark[1], T. Murray[1], M. Harris[1], L. Whiteman[1], C. Dearden[1], S. Iyengar[1], M. Ethell[1], M. Potter[1], D. Cunningham[1], I. Chau[1]

[1]Department of medicine, The Royal Marsden Hospital, London, United Kingdom

Background: The anti-CD20 monoclonal antibody rituximab (R) with chemotherapy followed by R-maintenance is standard of care for patients with symptomatic stage III/IV follicular lymphoma (FL). However, R is associated with potential complications such as hypogammaglobulinaemia (HG) and prolonged neutropenia. Furthermore, symptomatic HG may require intravenous immunoglobulin (IVIG).
Aims: 1. To benchmark the real-world occurence of infectious complications associated with anti-CD20 therapy 2. To measure the incidence of HG, infectious complications and need for IVIG in this population 3. To determine whether choice of induction regimen influences incidence of HG
Methods: All patients with stage III/IV histologically-confirmed FL (grade 1-3a) receiving at least 1 cycle of systemic therapy between January 2010 and December 2015 were identified from hospital and pharmacy records. Data were collated from electronic patient records.
Results: 68% (84/123) of patients received first-line chemo-immunotherapy: R-bendamustine (RB) in 40% (34/84), RCVP in 29% (24/84) and RCHOP in 24% (20/84).70% (59/84) of patients subsequently received MT. The reasons for MT not being administered are listed in Figure 1. Anti-CD20 therapy was with R in all patients except for one who received obinutuzumab within a clinical trial. Following induction the median time to starting MT was 9.1 weeks (range 3-19.7), with a delay of >8 weeks occurring in 31% (18/59); due to patient choice (n=8), prolonged neutropenia (n=3), recurrent infection (n=1) and other/unknown (n=6). The median number of MT cycles received was 12 (range 1-12). 42% (25/59) did not complete the planned 12 cycles and reasons for this are listed in Figure 1. Baseline Ig levels prior to commencing induction were available for 51% (43/84) and serum IgG, IgA and IgM were below normal range in 4% (2/43), 7% (3/43) and 21% (9/43) respectively. Post-induction 19% (16/84) had serum Igs tested and 81% (13/16) had a low level of at least one serum Ig. Following MT, 20% (12/59) had serum Igs tested and 92% (11/12) of those tested had a low level of at least one serum Ig. A total of 21% of patients (18/84) developed HG during follow-up and 94% (17/18) had received MT. For the two commonest induction regimens, RB and RCVP, there was no significant difference in rate of HG occurrence (71% *vs* 67%; Fisher's exact p=0.99). The proportion receiving MT was similar for these two groups (82% *vs* 72%; Fisher's exact p=0.36), however Ig testing was inconsistent with only 41%(14/34) of RB and 24%(6/25) of RCVP treated tested post-baseline. 12% (10/84) had recurrent infections and all had received MT, with respiratory infections seen most frequently (n=7). The majority (n=6/10) had received R-bendamustine induction. 6 (7%) cases of recurrent infection had concurrent HG diagnosed and 4 required IVIG (5%). The median duration of IVIG therapy was 8 months (range 4-30).

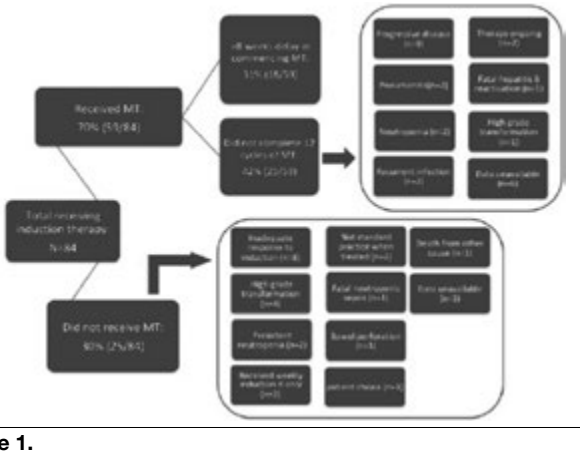


Figure 1.

Summary and Conclusions: The majority of delays to starting MT were due to patient choice, although delays due to recurrent infections or prolonged neutropenia were also observed. The incidence of recurrent infections was 12% overall and all occured in patients who had received MT; concurrent HG was diagnosed in 7%, consistent with published data on the incidence of symptomatic HG. Following systemic therapy Ig testing was only performed if clinically indicated so the frequency of asymptomatic HG may have been underestimated.

PF448

OUTCOMES FOR DIABETIC PATIENTS FROM THE CHRONOS-1 STUDY WITH RELAPSED OR REFRACTORY INDOLENT B-CELL LYMPHOMA TREATED WITH COPANLISIB

M. Dreyling[1,*], S. Leppä[2], T. Comeau[3], M. Kosinova[4], G. Lenz[5], D.S. Heo[6], S.Y. Oh[7], L. Huang[8], A. Miriyala[8], J. Garcia-Vargas[8], B.H. Childs[8], P.L. Zinzani[9]

[1]Medizinische Klinik und Poliklinik III, Klinikum der Universität München LMU, Munich, Germany, [2]Department of Oncology, Helsinki University Central Hospital Cancer Center, Helsinki, Finland, [3]Saint John Regional Hospital, New Brunswick, Canada, [4]Kemerovo Regional Hospital, Kemerovo, Russian Federation, [5]Translational Oncology, University Hospital Münster, Münster, Germany, [6]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, [7]Dong-A University Hospital, Busan, Korea, Republic Of, [8]Bayer HealthCare Pharmaceuticals Inc, Whippany, United States, [9]Department of Hematology, Institute of Hematology "L. e A. Seràgnoli"- University of Bologna, Bologna, Italy

Background: Copanlisib is a pan-Class I phosphatidylinositol 3-kinase (PI3K) inhibitor with predominant PI3K-α and PI3K-δ activity recently approved in the US for treatment of relapsed follicular lymphoma. In the CHRONOS-1 trial, treatment of patients with relapsed or refractory indolent lymphoma resulted in an objective response rate of 59% (*JCO* 35:2169-2178, 2017). The most common adverse event following intravenous administration of copanlisib is transient hyperglycemia, thought to be due to impaired glucose uptake associated with PI3K-α isoform inhibition.
Aims: We focus here on the efficacy and safety of copanlisib treatment in indolent lymphoma patients with a medical history of diabetes mellitus (DM).
Methods: Indolent B-cell lymphoma patients with well-controlled DM were eligible and required to have fasting glucose <160 mg/dL prior to each infusion. Patients received copanlisib 60 mg as a 1-hour infusion on days 1, 8, and 15 of a 28-day cycle. On C1D1, glucose was measured at pre-dose, and 3, 5, 6 and 8 hours after infusion. DM patients were instructed to check blood glucose at home 3x per day for 72 hrs after infusion until fasting glucose was <160 mg/dL or non-fasting glucose was <200 mg/dL.
Results: Twenty patients with DM out of a total of 142 patients were enrolled; 17 patients with a history of DM, 1 with history of impaired glucose tolerance, and 2 diagnosed at screening. Comparing non-DM patients (n=122) to DM patients, all-grade (G) hyperglycemia was 43% *vs* 85%, G3 31% *vs* 40%, and G4 2% *vs* 35%. In routine laboratory glucose assessments, G3 events were reported in 39% *vs* 70% and G4 in 2% *vs* 30%, non-DM *vs* DM respectively. Dose reductions due to any drug-related adverse event (AE) were higher in DM patients (40%) compared to non-DM patients (20%), as were permanent discontinuations due to AEs (30% *vs* 14%, respectively). Three patients overall discontinued treatment due to hyperglycemia, 2 of whom were DM patients. Objective responses were observed in 9 of 20 patients (45%; 2 non-evaluable), including one complete response and stable disease in 8 patients (40%). Of note, 6 responders were on treatment >300 days (or >30 infusions), with 5 of these patients remaining on treatment at data cut-off.
Summary and Conclusions: These results strongly suggest that the transient hyperglycemia seen with IV administration of copanlisib is manageable and should not preclude treatment of indolent lymphoma patients with DM. With appropriate management such patients can derive long-term clinical benefit.

Infectious diseases, supportive care

PF449

COMPARATIVE EFFICACY AND SAFETY OF DIFFERENT ANTIVIRAL AGENTS FOR CYTOMEGALOVIRUS PROPHYLAXIS AFTER HEMATOPOIETIC-CELL TRANSPLANTATION: A SYSTEMATIC REVIEW AND META-ANALYSES

N. Gagelmann[1,*], P. Ljungman[2], J. Styczynski[3], N. Kröger[4]

[1]Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [2]Karolinska Institutet, Stockholm, Sweden, [3]Nicolaus Copernicus University Torun, Bydgoszcz, Poland, [4]University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Background: No evidence synthesis available to date assessed the role of newly studied antiviral agents on cytomegalovirus (CMV) after transplantation.
Aims: We conducted a systematic review, conventional meta-analysis and network meta-analysis of all randomized controlled trials (RCTs) to examine the role of antiviral prophylaxis on CMV after allogeneic hematopoietic-cell transplantation, and to compare each agent's relative efficacy.
Methods: We performed a systematic literature review using MEDLINE®, MEDLINE® in-process, the Cochrane Library and the website www.clinical-trials.gov. In addition, meeting abstracts (ASH, ASCO, EBMT, EHA, BMT Tandem) were screened to include the most recent evidence. Primary objectives were CMV disease and infection. Survival and serious adverse events were considered as secondary end points. The analysis consisted of two steps, the first step being the computation of the conventional meta-analysis while the second step was the actual network meta-analysis. Pooled relative risks (RRs) with corresponding confidence intervals (CIs) were generated using a random-effects model in R (package meta). The GRADE approach was used to assess quality of evidence.
Results: Fifteen trials used seven different treatment options reporting on a total of 3924 patients: (1) acyclovir, (2) ganciclovir, (3) maribavir, (4) brincidofovir, (5) letermovir, (6) vaccine, and (7) valacyclovir. Twelve trials compared antiviral prophylaxis *versus* placebo while three trials compared different antiviral agents. Antiviral prophylaxis compared with control significantly prevented CMV disease (RR, 0.66; 95% CI, 0.48 to 0.90) in the conventional meta-analysis. Overall, antiviral prophylaxis reduced the risk for CMV disease with 34%. Ganciclovir was the only agent which significantly reduced the risk for CMV disease showing the most favorable RR of 0.35 (95% CI, 0.20 to 0.62). The use of ganciclovir provided the probability of 92% of being the best treatment option (high quality of evidence). Regarding CMV infection, antiviral prophylaxis compared with control significantly reduced the incidence of infection (RR, 0.63; 95% CI, 0.50 to 0.79). Ganciclovir and letermovir significantly prevented CMV infection showing the most favorable RRs of 0.34 (95% CI, 0.12 to 0.91) and 0.44 (95% CI, 0.21 to 0.93). The use of either ganciclovir or letermovir provided probabilities of 83% and 73% of being the best options (low quality of evidence). Regarding survival and safety, antiviral treatment appeared to reduce the risk for death (RR, 0.88; 95% CI, 0.73 to 1.06) while prophylaxis showed no impact on the risk for serious adverse events compared with any control condition with a RR of 1.09 (95% CI, 0.94 to 1.27). Across the network, the risk for death at 24 weeks was significantly reduced by letermovir (RR, 0.58; 95% CI, 0.34 to 0.98) as well as acyclovir (RR, 0.62; 95% CI, 0.40 to 0.95) leading to probabilities of 82% (letermovir) and 80% (acyclovir) of being the best treatment options. In terms of safety, letermovir showed the best relative efficacy compared with all other antiviral agents with a RR of 0.85 (95% CI, 0.54 to 1.32) and was thus at least similar in comparison with placebo with the probability being 76% that letermovir shows the best risk-benefit profile (moderate quality of evidence).
Summary and Conclusions: Cytomegalovirus disease and infection could be significantly reduced by antiviral prophylaxis in hematopoietic-cell transplantation. While ganciclovir and letermovir appeared to be the best options for CMV prophylaxis, only letermovir showed the best risk-benefit ratio.

PF450

CANDIDA-REACTIVE T CELLS FOR THE DIAGNOSIS OF INVASIVE CANDIDA INFECTION

F. Koehler[1,*], O. Cornely[1,2,3], H. Wisplinghoff[4,5,6], A. Schauss[1], J. Salmanton-Garcia[2], H. Ostermann[7], M. Ziegler[4], P. Bacher[8], A. Scheffold[8,9,10], R. Alex[11], A. Richter[11], P. Koehler[12]

[1]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), [2]Department I of Internal Medicine, ECMM Diamond Center of Excellence in Medical Mycology, German Centre for Infection Research (DZIF), [3]Clinical Trials Centre Cologne (ZKS Köln), University of Cologne, [4]Labor Dr. Wisplinghoff, [5]Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Cologne, [6]Institute for Clinical Microbiology, University Witten/Herdecke, Witten, [7]Department of Internal Medicine III, University of Munich, Munich, [8]Department of Cellular Immunology, Clinic for Rheumatology and Clinical Immunology, Charité – University Medicine Berlin, [9]German Rheumatism Research Centre (DRFZ), [10]Leibniz Association, Berlin, [11]Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Background: Invasive *Candida* infection (ICI) is the most common fungal bloodstream infection. Blood and tissue cultures are the current gold standard diagnostic methods, however false-negatives remain a clinical challenge.
Aims: *Candida*-reactive T cells were quantitated based on the upregulation of CD69/CD154 (CD40L) from peripheral blood as new diagnostic readout for ICI. In a pilot study, we examined healthy donors and three patient cohorts with either proven ICI, suspected ICI, or high risk of ICI
Methods: *Candida* cells were lysed mechanically by gentleMACS® dissociator (Miltenyi Biotec GmbH, Germany). Peripheral blood mononuclear cells (PBMC) of patients and healthy donors were isolated by density gravitation. Cultured cells were stimulated with CD28 and CD40 pure antibodies and co-incubated with lysate of either *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* or *C. krusei* for 5h in 5% CO_2. Missing challenge with fungal lysate served as negative control, Staphylococcal Enterotoxin B as positive control. PBMCs were stained with CD4FITC, CD8PerCP, CD14PerCP, CD20PerCP, CD69APC, CD154PE (all Miltenyi Biotec GmbH, Germany) and 7AAD (Miltenyi Biotec GmbH, Germany and eBioscience, USA) and measured on a MACSQuant® flow cytometer (Miltenyi Biotec GmbH, Germany). *Candida*-reactive $CD4^+$ T cells were detected based on the upregulation of CD69and CD154 (CD40L). Cut-off values discriminating between healthy donors, disease control and patients with proven ICI were calculated by receiver operating characteristic analysis using IBM SPSS Statistics software (Version 23, IBM Corporation, Armonk, NY, USA).
Results: In a prospective pilot study, we determined the performance of the *Candida*-reactive lymphocyte assay in 26 patients, including 16 proven ICI and one patient with probable hepatosplenic candidiasis. Nine hematological high-risk patients served as disease control and 14 healthy donors as negative control. To examine the mean frequency of *C. albicans*-reactive T cells in healthy individuals we included an additional cohort of 96 healthy blood donors. Thirteen of 16 patients with proven ICI and one patient with probable ICI had elevated levels of *Candida*-reactive $CD4^+$ T cells. Due to autofluorescence of cells we excluded 3 candidemia patients from analysis. In 10 of 12 proven ICI, T cell reaction matched the *Candida* spp. identified by conventional diagnostics. One histology proven ICI patient had no species identification by standard diagnostics. Disease and healthy control patients of the pilot study cohort had no elevated *Candida*-directed T cells counts. The sensitivity and specificity of the *Candida*-reactive lymphocyte assay identifying ICI and causing *Candida* spp. among evaluable ICI patients were 83.3% and 100%, respectively.
Summary and Conclusions: The *Candida*-reactive lymphocyte assay correctly identified the majority of ICI patients by species level. Autofluorescence of cells and insufficient cell count of T cells are limiting factors. The *Candida*-reactive lymphocyte assay has the potential to complement current diagnostic assays for invasive *Candida* infection.

PF451

TENOFOVIR *VERSUS* LAMIVUDINE FOR PREVENTION OF HEPATITIS B VIRUS REACTIVATION AMONG PATIENTS WITH AGGRESSIVE LYMPHOMAS UNDERGOING FRONT-LINE ANTHRACYCLINE-CONTAINING CHEMOTHERAPY

I. Zacheo[1,*], R. Della Pepa[1], C. Giordano[1], A. Giordano[1], C. Mortaruolo[1], M. Raimondo[1], M. Di Perna[1], F. Trastulli[1], M. Picardi[2], F. Pane[1]

[1]Hematology, [2]Advanced Biomedical Science, Federico II, University of Naples, Naples, Italy

Background: Primary antiviral prophylaxis (PAP) is essential for patients seropositive for hepatitis B surface antigen (HBsAg) who undergo intensive cytotoxic chemotherapy for lymphoma. Lamivudine (LAM) is the most commonly used drug, but alternative nucleos(t)ide analogs such as entecavir and tenofovir disoproxil fumarate (TDF) may provide a better efficacy.
Aims: We compared the efficacy and safety of LAM (systematically administered from July 2004 to June 2009; LAM-cohort) and TDF (systematically administered from February 2009 to June 2015; TDF-group) in preventing HBV reactivation in HBsAg-positive patients with advanced-stage diffuse large B cell (DLBC) non-Hodgkin lymphoma (NHL) or classic Hodgkin lymphoma (HL).
Methods: All patients were scheduled to receive full-course of anthracy-

cline-containing chemotherapy, according to R-CHOP or ABVD regimen.During the study period, of the 64 HBsAg-positive patients who received PAP with either LAM or TDF, 20 were excluded due to liver dysfunction and/or high serum HBV-DNA levels at baseline, chemotherapy reduction or delay for acute toxicity not due to HBV infection, or because lost to follow-up. Twenty-one patients received LAM and 23 received TDF.
Results: The rate was significantly lower in the TDF-group compared with the LAM-cohort for HBV reactivation (0% *vs* 52.3%, respectively; *p*=.0002), HBV-related hepatitis (0% *vs* 19%, respectively; *p*=.04) and chemotherapy disruptions (0% *vs* 23.8%, respectively; *p*=.02). No difference was observed between the two groups in terms of incidence of PAP-related adverse events (4.3% [TDF-group] *vs* 19% [LAM-cohort]; *p*=.17). Furthermore, at a median follow-up of 34 months there was no significant difference between the TDF-group and LAM-cohort in terms of lymphoma progression free survival (94.7% *vs* 84.8%; *p*= .37). In a multivariate analysis, TDF prophylaxis and serum HBV-DNA ≤73 IU/mL were associated with a lower risk for HBV reactivation (*p* values, <.0001 and =.03, respectively) (Table 1).

Table 1. Patients characteristics.

	No (%) of Patients[a]		P value
	Tenofovir (n=23)	Lamivudine (n=21)	
Age			
Median, y (range)	53.5 (25-77)	56 (21-83)	n.a.
Sex			0.323898
Male	14 (60.9)	10 (47.6)	
Female	9 (39.1)	11 (52.4)	
Hematological malignancy			0.217311
DLBC-NHL	15 (65.2)	16 (76.2)	
HL	8 (34.8)	5 (23.8)	
NS	5 (62.5)	4 (80)	
MC	1 (12.5)	1 (20)	
LD	1 (12.5)	0	
LR	1 (12.5)	0	
Ann Arbor stage[b]			0.987353
Stage III	11 (47.8)	10 (47.6)	
Stage IV	12 (52.2)	11 (52.4)	
International Prognostic Index [c]			0.675422
0-2	4 (17.4)	4 (25)	
3-5	11 (82.6)	12 (75)	
International Prognostic Score [d]			0.276278
0-1	1 (12.5)	0	
2-3	4 (50)	2 (40)	
4-7	3 (37.5)	3 (60)	
Liver involvement [e]			0.609425
Yes	1 (4.3)	2 (9.5)	
No	22 (95.7)	19 (90.5)	
Hepatitis B core antibody status			0.915946
Seropositive	19 (82.6)	19 (90.5)	
Seronegative	4 (17.4)	2 (9.5)	
Serum HBV-DNA level			n.a.
Median (range) IU/mL	200 (10-948)	192 (21-877)	
Cycles of R-CHOP			n.a.
median	6	6	
Cycles of ABVD			n.a.
median	6	6	

Abbreviation:
RCHOP, rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone.
ABVD, doxorubicin, bleomycin, vinblastine and dacarbazine.

Summary and Conclusions: Therefore, TDF should be suggested for frontline prophylaxis in HBsAg-positive patients receiving induction chemotherapy for aggressive lymphomas to prevent HBV reactivation and evolution in HBV-related hepatitis, which could lead to chemotherapy disruption. Further studies are warranted to confirm these findings.

PF452

SWITCHING FROM POSACONAZOLE ORAL SUSPENSION TO TABLETS INCREASES SERUM LEVELS, PRESERVES SAFETY AND IMPROVES TREATMENT SATISFACTION: A PROSPECTIVE CLINICAL TRIAL IN PATIENTS WITH ACUTE LEUKEMIA

R.F. Duarte[1,*], I. Sánchez-Ortega[2], I. Salcedo[1], C. de Miguel[1], A. Lerma[1], B. Pérez de Camino[1], M.E. Martínez-Muñoz[1], C. Fernández-Maqueda[1], A. de Laiglesia[1], G. Bautista[1], H. Pomares[2], A. Sureda[2], J.R. Cabrera[1], M. Cuenca-Estrella[3], A. Gómez-López[3]

[1]Hospital Universitario Puerta de Hierro Majadahonda, Madrid, [2]Catalan Institute of Oncology, Barcelona, [3]National Microbiology Centre - ISCiii, Madrid, Spain

Background: Posaconazole (POSA) is the standard anti-mold prophylaxis agent for high-risk hematology patients with acute myeloid leukemia and myelodysplastic syndromes (AML/MDS). Compared with the original oral suspension (OS), gastro-resistant POSA tablets (TAB) have shown improved pharmacokinetics (PK) in registration trials. However, there is only anecdotal retrospective data comparing both formulations in the same subjects [DS Jung, *et al.* 2014].
Aims: Here we present a prospective clinical trial on sequential administration of POSA OS and TAB in AML/MDS patients to assess the relative impact of the new formulation on PK and safety.
Methods: A phase IV, multicenter, open-label clinical trial in adult patients undergoing induction chemotherapy for AML/MDS, to receive primary antifungal prophylaxis with POSA sequentially, first as OS (200mg TDS) until steady state concentration, and then (day 10-14) switch to TAB (300mg OD) for two additional weeks until day 28. Endpoints included the comparative PO *vs* TAB PK profile (primary), clinical and laboratory safety, as well as patient satisfaction with both formulations. EudraCT# 2015-003940-38.
Results: Twenty-one patients were successfully screened and received at least one dose of POSA (May 2016 - July 2017). Of these, 5 patients stopped POSA prophylaxis (23.8%) for chemotherapy-induced mucositis and diarrhea (2; 9.5%) or to receive antifungal treatment (3; 14.3%). There were no cases of breakthrough invasive fungal infection. Sixteen cases (76.2%) completed sequential treatment with both POSA formulations and are the study population for PK endpoint analysis. As described in Table 1, POSA serum levels significantly increased in these patients with the shift from OS to TAB formulation. Although 9 subjects (56%) had steady-state average concentration (Cavg) <0.5 mg/L with the OS, all 16 subjects (100%) achieved the recommended steady-state Cavg >0.5 (and >0.7) mg/L after switching to POSA TAB (note: all differences P<0.001). Three patients experienced grade 3 adverse events (CTCAE version 4) including supraventricular tachycardia, increased GGT and acute cholecystitis. They were all considered unlikely to be related to the study drug and all resolved within the treatment period without discontinuation of POSA. No other patients experienced changes in the electrocardiogram or prolongation of the QT interval. All patients experienced grade 3 and 4 cytopenias as a result of intensive chemotherapy and not related to the study drug (data not shown). Other than in the two cases mentioned above, liver function tests including bilirubin, alanine aminotransferase and gamma-glutamyl transferase remained stable through the switch from OS to TAB POSA. All 21 patients survived the course of induction chemotherapy (measured at 1 week after neutrophil recovery and end of treatment). Finally, subjects completed a questionnaire of patient satisfaction at the end of treatment with both formulations. A large majority of >80% patients preferred the TAB formulation of POSA, with a significantly higher level of overall satisfaction (P=0.037) and better experience of interference with activities of daily life (P=0.012) with TAB POSA compared to OS.

Table 1.

Pharmacokinetics and Liver Function Test Results.

	OS day 1	OS day 8	TAB day 1	TAB day 8
AUC				
Mean +/- SD	2.674 +/- 1.818	11.041 +/- 9.323	23.811 +/- 12.793	55.072 +/- 37.412
Median	2.236	7.648	20.000	38.832
Range	0.552 - 6.810	1.660 - 33.238	4.277 - 49.105	19.838 - 126.768
Cavg				
Mean +/- SD	0.092 +/- 0.088	0.454 +/- 0.379	0.905 +/- 0.532	2.638 +/- 1.579
Median	0.059	0.330	0.788	2.352
Range	0.012 - 0.354	0.066 - 1.345	0.141 - 1.884	0.750 - 5.242
Bilirubin (mean +/- SD)	0.690 +/- 0.345	0.830 +/- 0.712	0.800 +/- 0.976	0.611 +/- 0.569
ALT (mean +/- SD)	23.6 +/- 7.3	35.7 +/- 37.3	40.2 +/- 20.9	48.2 +/- 28.7
GGT (mean +/- SD)	42.3 +/- 22.7	85.5 +/- 113.8	124.8 +/- 127.7	97.2 +/- 68.9

ALT: alanine aminotransferase (IU/mL); AUC: area under the curve (mcg/mL*h); bilirubin: mg/dL; Cavg: average concentration (mg/L); GGT: gamma-glutamyl transferase (IU/mL); OS: oral suspension; SD: standard deviation; TAB: gastro-resistant tablets.

Summary and Conclusions: This is the first clinical trial to evaluate prospectively the sequential use of POSA OS and TAB for primary antifungal prophylaxis. Our data show that in patients with AML/MDS receiving intensive chemotherapy, the switch from POSA OS to TAB increases drug serum levels, preserves its safety profile and improves patient tolerance and treatment experience.

PF453

MURCORMYCOSIS AND ASPERGILLOSIS IN ONCOHEMATOLOGICAL PATIENTS: WHAT'S THE DIFFERENCE?

O. Shadrivova[1], S. Khostelidi[1], Y. Borzova[2], E. Desyatik[2], A. Volkova[3], M. Popova[3], I. Markova[3], O. Uspenskaya[4], T. Bogomolova[2], S. Ignatyeva[2], L. Zubarovskaya[3], B. Afanasyev[3], N. Klimko[1,*]

[1]Department of Clinical Mycology, Allergy and Immunology, [2]I. Mechnikov North-Western State Medical University, [3]I.Pavlov First Saint Petersburg State Medical University, [4]Leningrad Regional Clinical Hospital, St. Petersburg, Russian Federation

Background: Invasive aspergillosis (IA) and mucormycosis (M) are the main mold invasive fungal diseases in oncohematological patients.
Aims: We compare M and IA in oncohematological patients from our registries.
Methods: In the study were included 59 M patients (males – 56%, age – 3-74 y, median – 27) and 541 IA patients (males – 57%, age – 1-78 y, median – 38). We used EORTS/MSG, 2008 criteria for M and IA diagnosis.
Results: The main underlying diseases in M and IA patients were leukemia (64% *vs* 51%), p= 0,03. M patients were more immunosuppressed: severe neutropenia was in 88% *vs* 82%, duration of neutropenia (median) - 30 d *vs* 14 d (p=0,0001), lymphocytopenia – 77% *vs* 65%, duration of lymphocytopenia – 25 d *vs* 14 d (p= 0,001), allogeneic hematopoietic stem cells transplantation – 44% *vs* 28% (p=0,01), and GVHD was observed in 42% *vs* 22% (0,0001). In 52% patients M was diagnosed in 1–225 days after IA. The main M etiological agents were *Rhizopus* spp. (47%), *Rhizomucor* spp. (28%) and *Lichtheimia cor mbifera* (17%), IA – *A. fumigatus* (43%), *A. niger* (33%) and *A. flavus* (17%). The main sites of infection were lungs (73% *vs* 96%), in M patients more frequently was identified disseminated process (42% *vs* 8%, p=0,001) and paranasal sinuses involvement (15% *vs* 6%, p=0,04). Typical clinical feature of M was hemoptysis (32% *vs* 6%), lung CT signs – hydrothorax (53% *vs* 7%), lesions with destruction (38% *vs* 8%, p=0,0001) and a "reverse halo" symptom (17% *vs* 3%). Antifungal therapy received 78% *vs* 99% (p=0,001) patients, surgery – 47% *vs* 3% (p=0,0001). Overall 12 weeks survival was significantly lower in M patients (49% *vs* 81%, p=0,0001). Unfavorable prognosis factors in M and IA patients were ≥2 organs involvement (p=0,0009), concomitant bacterial or viral infection (p=0,001; p=0,008, respectively). Unfavorable prognosis factor in M patients was unresolved hemoptysis (p=0,002), favorable – remission of underlying disease (p=0,006). Favorable prognosis factors in IA patients were early bronchoscopy (p=0,003), voriconazole use (p=0,0007) and secondary antifungal prophylaxis (p=0,0001).
Summary and Conclusions: In patients with mucormycosis more often observed agranulocytosis, lymphocytopenia, GVHD. The main sites of mucor and aspergillosis infection were lungs. Overall 12 weeks survival was significantly lower in M patients (49% *vs* 81%, p=0,0001). Unfavorable prognosis factors in M and IA patients were ≥2 organs involvement and concomitant bacterial or viral infection. Favorable prognosis factor in M patients – remission of underlying disease, in IA patients - early bronchoscopy, voriconazole use and secondary antifungal prophylaxis.

PF454

INVASIVE FUNGAL DISEASES CAUSED BY RARE PATHOGENS IN CHILDREN WITH HEMATOLOGICAL DISEASES AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION AND CHEMOTHERAPY

M.O. Popova[1,*], I. Markova[1], Y. Rogacheva[1], A. Volkova[1], I. Nikolaev[1], K. Ekushov[1], O. Paina[1], T. Bykova[1], O. Pinegina[1], S. Ignatieva[2], T. Bogomolova[2], O. Pirogova[1], E. Darskaya[1], M. Vladovskaya[1], I. Moiseev[1], S. Bondarenko[1], L. Zubarovskaya[1], N. Klimko[1], B. Afanasiev[1]

[1]Raisa Gorbacheva Memorial Institute of Children Oncology, Hematology and Transplantation, First Pavlov State Medical University, Saint-Petersburg, [2]Dept. of Clinical Mycology, I.I. Mechnikov North-Western State Medical University, Saint-Petersburg, Russian Federation

Background: Introducing a new antifungals and diagnostic procedures has improved prognosis of the invasive fungal diseases (IFD) in hematological patients. The number of publications on epidemiology of IFD caused by rare pathogens in children with hematological diseases after hematopoietic stem cell transplantation (HSCT) and chemotherapy (CT) is limited.
Aims: Our study focused on epidemiology of IFD caused by rare pathogens in children with hematological diseases.
Methods: Between 2009 and 2016 More than 3000 patients was observed and treated, 1337 allogeneic hematopoietic stem cell transplantation (allo-HSCT) and 617 autologous hematopoietic stem cell transplantation (auto-HSCT) were performed in our center. A retrospective study includes 30 cases of rare IFD in the period of time in patients with hematological malignances and non-malignant hematological diseases after CT and HSCT. The study group consist of children (n=11). Patients >18 y.o. with rare IFD (n=19) were used for the outcome analysis. EORTC/MSG 2008 criteria were used for the diagnosis of proven and probable IFD as well as to evaluate response to therapy.
Results: The incidence of rare IFD in children after allo-HSCT was 1,5% (n=7/461), auto-HSCT – 0,4% (n=1/232). Rare IFDs developed more often in patients with acute leukemia (45,4%). The etiological structure of confirmed rare IFD in children were *Mucorales* at six patients (54.5%), two cases of IFD caused by *Fusarium spp.* (18.2%), one – *Trichosporon asahii*(9.1%), one – *Scedosporium apiosperium* (9.1%), and combination of *Fusarium spp.* and*Paecilomyces spp.* were diagnosed in one patient (9.1%). In 36% of cases, IFD caused by rare pathogens develops after or in combination with invasive aspergillosis. The median day of onset of IFD was 92 days after allo-HSCT and 138 after auto-HSCT, 134 days after start of CT. Febrile fever was the main clinical symptom of IFD (100%). Main organ of defeat were lungs (87%). Antifungals were prescribed in 100% of cases of IFD caused by rare pathogens. All pediatric patients with mucormycosis had combination therapy. Overall survival at 12 weeks and 1 year from the diagnosis of IFD was 46,2% and 36,4% and was not differ in age groups. Combination antifungal therapy improves 1 year survival in children and adult with mucormycosis (n=30) (40% *vs* 10%, p=0,05).
Summary and Conclusions: The incidence of rare IFD in children after allo-HSCT was 1,5%, auto-HSCT – 0,4%. The main etiology agents of rare IFD were mucorales. Rare IFD were late complications after CT and HSCT. Rare IFD develops after or in combination with invasive aspergillosis. Combination antifungal therapy improves 1 year survival in patients with mucormycosis.

PF455

INVASIVE ASPERGILLOSIS IN HAEMATOLOGICAL PATIENTS: A REVIEW OF 625 CASES

C. Duval[1], M.-P. Ledoux[1], F. Danion[1], G. Morel[1], B. Guffroy[1], C. Simand[1], E. Toussaint[1], A. Cazes[1], M. Lamothe[2], J. Denis[3], V. Letscher-Bru[3], L.-M. Fornecker[1,4], R. Herbrecht[1,4,*]

[1]Oncology and Hematology, [2]Pharmacy, [3]Parasitology and Medical Mycology, Hôpitaux Universitaires de Strasbourg, [4]Université de Strasbourg, INSERM UMR-S1113/IRFAC, Strasbourg, France

Background: Invasive aspergillosis (IA) remains a frequent infection in haematological patients and diagnosis and treatment are still difficult despite significant progresses over the last two decades.
Aims: To review the cases of IA observed in our center in patients with an underlying haematological disease and to describe the characteristics of the disease and the evolution of outcome over time.
Methods: A retrospective review of consecutive cases of IA in a single haematology center over 20 years (1997 – 2016). IA episode were classified into possible, probable or proven according to EORTC/MSG criteria.
Results: We have identified 625 episodes of IA (possible: 137, 22%; probable: 429, 69%; proven: 59, 9%) in 585 different patients. Median age is 57 years (range: 5.5 – 90). Most frequent primary underlying condition is acute myeloblastic leukemia (AML) (227, 36%), allogeneic haematopoietic stem cell transplantation (HSCT) (119, 19%), aggressive lymphoma (85, 14%), low-grade lymphoma (42, 7%), acute lymphoblastic leukemia (ALL) (37, 6%), autologous HSCT (29, 5%), myeloma (22, 4%), and myelodysplastic syndrome (17, 3%). Neutropenia is present in 416 (67%) cases. Potential additional risk factors are recent chemotherapy in 484 (77%), T-cell suppressor including nucleoside analogues in 356 (57%) cases, active or prior use of tobacco in 280 (45%), prior respiratory disease in 157 (25%), systemic and/or aerosolized steroids in 273 (44%), diabetes in 90 (14%). Thirteen patients have also a history of solid organ transplantation prior to the onset of the haematological malignancy. Most frequent primary therapies are voriconazole (340, 54%), amphotericin B deoxycholate (AmBd) (104, 17%), liposomal amphotericin B (L-AmB) (40, 6%). Isavuconazole was given to 10 (2%), primary combination therapy to 52 (8%) and 16 (3%) patients did not receive any therapy. Overall 12-week survival is 58.0%. Long-term survival is 21.0% at 5 years and 16.2% at 10 years. Survival at week 12 is higher for possible IA (73.7%), than for probable (55.0%) and proven (42.4%) (p value: <0.001). Twelve-week survival is highest for isavuconazole (70.0%) and voriconazole (65.6%) treated patients compared to liposomal AmB (45.0%) and AmB deoxycholate (48.1%) treated patients (p value: 0.0003) (Figure 1A). Survival is similar for acute leukemia patients (64.8%) and alloHSCT recipients (58.8%), and is lower for patients with lymphoma, chronic lymphocytic leukemia and multiple myeloma (45.9%) (p value: 0.0019) (Figure 1B). Sur-

vival improved over time (p value: 0.034). This improvement over time correlates with availability of voriconazole in 2002.

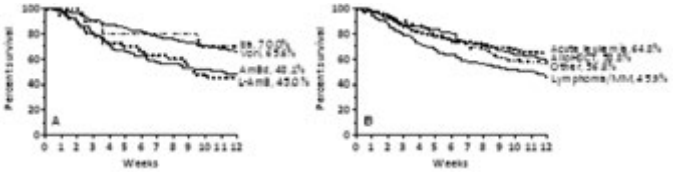


Figure 1.

Summary and Conclusions: This real-life study demonstrates that IA is still associated with a high mortality rate, greater than 30%, despite the significant improvement over time. Outcome is especially poor in patients with lymphoproliferations.

PF456

DIAGNOSIS OF INVASIVE ASPERGILLOSIS IN HIGH RISK HEMATOLOGICAL MALIGNANCY PATIENTS: PERFORMANCE OF BIOMARKERS AND CYTOKINES IN SAME DAY BLOOD AND BRONCHOALVEOLAR LAVAGE FLUID SAMPLES

S. Heldt[1], J. Prattes[1], S. Eigl[1], B. Spiess[2], J. Rabensteiner[1], F. Prueller[1], A. Wolfler[1], P. Neumeister[1], H. Strohmaier[1], R. Krause[1], D. Buchhheidt[2], H. Martin[1,*]

[1]Medizinische Universitaet Graz, Graz, Austria, [2]Mannheim University Hospital, Heidelberg University, Mannheim, Germany

Background: *Aspergillus* spp. and other molds have been shown to induce elevated levels of several cytokines. It remains unknown whether these cytokines hold value for clinical routine and enhance diagnostic performances of established and novel biomarkers/molecular tests for invasive aspergillosis (IA) and other invasive mold infection (IMI).
Aims: To determine the diagnostic potential of a number of cytokines, as well as established and emerging tests for IA and IMI in patients with underlying hematological malignancies in a setting that uses mold-active prophylaxis.
Methods: This cohort study included 106 prospectively enrolled (2014-2017) adult cases with underlying hematological malignancies and suspected pulmonary infection undergoing bronchoscopy. Serum samples were collected within 24 hours of bronchoalveolar lavage fluid (BALF) sampling. Both, serum and BALF samples were used to evaluate diagnostic performances of the *Aspergillus*-specific lateral-flow device test (LFD), *Aspergillus* PCR, galactomannan, β-D-glucan, and a bundle of cytokines for IA and IMI, classified according to the revised EORTC/MSG criteria.
Results: Among the 106 cases, 11 had probable IA, 32 possible IA (of which 7 had probable IMI), and 63 no evidence for IMI. Combinations of serum IL-8 with either BALF LFD (sensitivity 100%, specificity 94%) or BALF PCR (sensitivity 91%, specificity 97%) were highly sensitive and specific for differentiating probable IA from no IA. Serum IL-8 showed also potential for differentiating patients with possible/probable IMI from those without IMI, while serum and BALF GM and BDG did not.
Summary and Conclusions: Our study indicates that serum IL-8 testing may be a valuable addition to clinical routine for diagnosing IA and IMI in high risk patients who receive mold-active antifungals.

PF457

NEUTROPHIL EXTRACELLULAR TRAPS IMPAIR FUNGAL CLEARANCE IN A MOUSE MODEL OF INVASIVE PULMONARY ASPERGILLOSIS

A. Alflen[1,*], P. Aranda Lopez[1], A.-K. Hartmann[2], J. Maxeiner[3], M. Bosmann[4,5], H. Beckert[6], W. Ruf[4], M. Radsak[1]

[1]3rd medical department, [2]Institute for Immunology, [3]Asthma Core Facility, [4]Center for Thrombosis and Hemostasis, University Medical Center, Mainz, Germany, [5]Pulmonary Center, Department of Medicine, University Medical Center, Boston, United States, [6]Ruhrland Clinic, West German Lung Clinic of the University Medical Center, Essen, Germany

Background: Invasive pulmonary aspergillosis (IPA) is a major threat to patients with hematologic neoplasms or patients receiving immunosuppressive drugs after organ or hematopoietic stem cell transplantation. In this setting polymorphonuclear neutrophils (PMN) play a critical role in the immune response against fungal pathogens since they are rapidly recruited to sites of infection and kill microbes through a combination of cytotoxic mechanisms. Besides phagocytosis and the production of reactive oxygen species, they can expulse their nuclear contents to form neutrophil extracellular traps (NETs). Thereby they trap and kill microbes by forming highly decondensed chromatin structures that correlates with histone hypercitrullination catalyzed by peptidylarginine deiminase 4 (PAD4). Besides their ability to clear bacterial infections, the impact of NETs on fungal clearance is controversial. Furthermore, the toxicity of free DNA and histones is associated with organ damage.
Aims: Hence we asked whether NETs contribute to fungal clearance and outcome in a mouse model of IPA where PMN are essential for the survival.
Methods: IPA was induced by intratracheal application of *Aspergillus fumigatus* conidia in wildtype (C57BL/6J) or PAD4 deficient (*Pad4*$^{-/-}$) mice. After 24 h fungal load was assessed as colony forming units after plating and culturing lung homogenates on agar. Broncho alveolar lavage fluid (BALF) was analyzed for cell count, ELISA was performed for albumin amount and citrullinated Histone H3 (citH3), and cytokines were analyzed by a multiplex assay. One week after infection mice were anesthetized and invasive lung functions were performed. Afterwards, mice were sacrificed and blood, BALF and lungs were analyzed as described above. PMN functions (oxidative burst, phagocytosis, degranulation, CD62L shedding) from mice were analyzed by flow cytometry after lysis of whole blood and activation of cells. Human PMN were isolated from the blood of healthy donors by density gradient centrifugation. PMN were preincubated with a PAD4 inhibitor (GSK 484), activated and functions were analyzed by flow cytometry (see above) and viability by MTS assay.
Results: 24 hours after induction of IPA *Pad4*$^{-/-}$ mice revealed lower fungal burden in the lungs, which was accompanied by a significantly lower amount of albumin in the BALF, indicating lower acute lung injury. However, *Pad4*$^{-/-}$ and wildtype mice showed similar PMN recruitment in the blood and into the BALF. As expected, *Pad4*$^{-/-}$ mice showed significantly lower amounts of citH3 in the BALF of infected animals and also a lower amount of TNFa accompanied by a differential cytokine profile compared to wildtype mice. While GROa and IL-17A were also lower in BALF of *Pad4*$^{-/-}$ animals, MCP-1 was elevated in these mice and eotaxine, G-CSF and IL-18 showed only minor differences in the two mice strains. Invasive lung function tests one week after infection did not detect any significant differences in airway resistance in wildtype and *Pad4*$^{-/-}$ mice demonstrating no permanent lung damage. Furthermore, PMN functions from *Pad4*$^{-/-}$ and wildtype mice revealed no relevant differences when analysing them *ex vivo e.g.* for degranulation or phagocytosis and also pretreatment of human PMN with a PAD4 inhibitor did not results in differentially regulated PMN functions.
Summary and Conclusions: NETs impair fungal clearance in a mouse model of IPA and contribute to acute lunge injury. Inhibition of NET formation might therefore be a useful strategy to reduce fungal dissemination and to avoid lung damage in immunocompromised patients.

PF458

HEMOPHAGOCYTIC LIMPHOHISTIOCYTOSIS ASSOCIATED WITH LEISHMANIASIS: A HIDDEN PASSENGER IN ENDEMIC AREAS?

J. Badiola[1,*], J. M. Puerta-Puerta[1], P. González-Sierra[1], J. Sainz[2], L. Cabo-Zabala[3], F. Hernández-Mohedo[1], A. Romero-Aguilar[1], F. Ruiz-Cabello[3], R. Ríos-Tamayo[1], M. Jurado-Chacón[1]

[1]Hematology, Hospital Virgen de las Nieves, [2]GENYO. Centre for Genomics and Oncological Research, [3]Immunology, Hospital Virgen de las Nieves, Granada, Spain

Background: Hemophagocytic lymphohistiocytosis (HLH) is a rare and devastating heterogeneous disorder causing an uncontrolled immune inflammatory response and cytokine storm triggering tissue damage and leading to organ failure. The number of reported HLH cases in the adult population has increased dramatically in the last several years, probably due to the rise in recognition of the syndrome. In HLH the identification of the trigger is mandatory because it's elimination could remove the stimuli that prompts the abnormal immune system activation. Although the predominant causes differ in each country and age, infectious diseases and neoplasms are the most frequent triggers worldwide. An association between visceral leishmaniasis and HLH is well-described in the literature, especially in children. In adults, leishmaniasis is a rare cause of HLH, with less than 30 documented cases, being the trigger in less than 1% of adult HLH cases.
Aims: After identifying a few cases of HLH triggered by Leishmania, the objective of our study was to analyze the presentation, diagnosis and prognosis of this association in our area.
Methods: Retrospectively, we collected clinical and analytical data of adult patients diagnosed with HLH in the province of Granada (Spain), in the last 10 years (2008-2017).

Results: 21 patients were diagnosed of HLH in this period with the clinical and analytical characteristics that are shown in Table 1. Infectious diseases were the most common triggers, with 8 cases (5 leishmaniasis, 2 HIV and 1 CMV), followed by oncohematological triggers, 4 cases (1 T lymphoma, 1 B lymphoma, 1 acute lymphoblastic leukemia and 1 colorectal cancer), autoimmune triggers, 4 cases (2 systemic lupus erythematosus, 1 inflammatory bowel disease and 1 antiphospholipid syndrome), idiopathic, 4 cases, and HSCT, 1 case. It should be noted that no statistically significant differences were found in the characteristics or in the clinical-analytical presentation between HLH triggered by Leishmania and those that were not. In the cases of leishmaniasis, the PCR in the bone marrow was positive in 100% of the patients, the detection of Leishmania antigen in urine in 40% and the visualization of Leishmania in bone marrow analysis and the culture only in 20%. The mortality of the Leishmania group has been lower, (Figure 1) although it has not reached statistical significance (p=0.09).

Table 1. Clinical-analytical characteristics of the presentation (average).

Male sex, age, previous immunosuppression and temperature	52.4%, 44, 52.4% and 39.1ºC
Splenomegaly, hepatomegaly and adenopathies	100%, 70% and 25%
Hemoglobin, leukocytes and platelets	7.4 g/dL, 1978 x 10^3/L and 37100 x 10^3/L
Ferritin, triglycerides and fibrinogen	11199 ng/ml, 411 mg/dl and 202 mg/dl
ALT, AST, bilirubin, CRP and LDH	126 U/l, 159 U/l 5.4 mg/dl, 172 mg/L and 1357 U/l

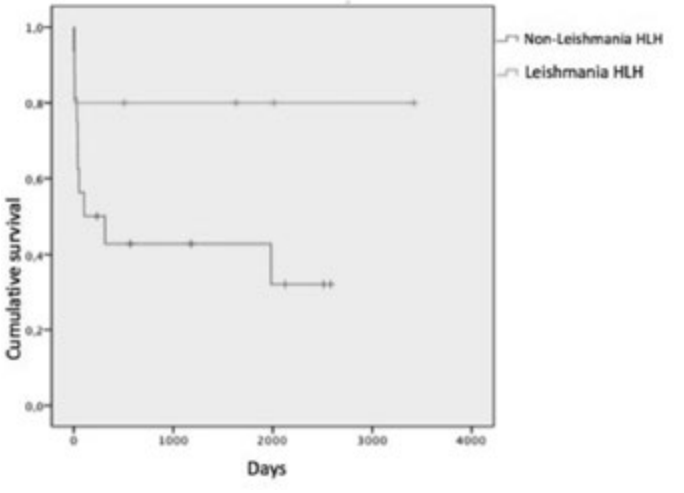


Figure 1.

Summary and Conclusions: Leishmania, far from being an infrequent cause of HLH, is the predominant trigger of adult HLH in our area. HLH triggered by Leishmania is indistinguishable from those not triggered by them, with an identical clinical and analytical presentation. Intense search of Leishmania is mandatory in every case of HLH using PCR. The rest of the techniques have low sensitivity in these cases, probably because of the paucimicrobial nature of the disorder. Diagnosis and adequate treatment of leishmaniasis could improve the prognosis of HLH in these patients.

PF459

ANALYSIS OF THE BURDEN OF CYTOMEGALOVIRUS REACTIVATION IN PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION

C. Tham[1,*], S. Monsalvo[1], S. Lozano[1], D. Avenoso[1], B. Sevillano[1], F. Fernando[1], N. Farah[1], C. Paliompeis[1], D. Milojkovic[1], R. Palanicawandar[1], E. Olavarria[1]

[1]Haematology, Imperial College London, London, United Kingdom

Background: CMV reactivation can be managed with pre-emptive antiviral therapy but this strategy is associated with significant morbidities and subsequent economic burden.
Aims: To analyse the incidence of CMV infection and the healthcare resource utilisation by estimating length of inpatient stay and recurrent hospitalisation rates.
Methods: We have analysed 385 consecutive patients undergoing allogeneic stem cell transplantation (AlloSCT) that were at risk of CMV infection (patient and/or donor CMV seropositive).
Results: Median age was 47 years (18-72). There were 229 (59%) males. CMV serology (Recipient/Donor) distinguished 3 groups: Pos/Pos 238 (62%); Pos/Neg 103 (27%); Neg/Pos 44 (11%). Transplant conditioning was myeloablative in 189 (49%) and reduced intensity in 196 (51%). Donor types were 178 (46%) HLA-identical sibling, 116 (30%) matched unrelated, 37 (10%) mismatched unrelated and 54 (14%) haploidentical donors. T-cell depletion with Alemtuzumab was performed in 194 (50%). All patients were managed with pre-emptive therapy after CMV reactivation was confirmed in 2 consecutive PCR samples. CMV reactivation occurred in 171 (44%) patients at a median of 35 (6-331) days post SCT. In 59 (34%) cases, CMV reactivated a median of 19 (2-314) days before the diagnosis of GVHD. Acute GVHD developed in 34 patients before CMV reactivation after a median of 25 (1-103) days post AlloSCT. Systemic steroids were started in 54(31%) patients a median of 9 (1-82) days before CMV reactivation. A total of 167 (98%) patients received antiviral therapy as per pre-emptive protocol. Intravenous Ganciclovir was given as first line antiviral therapy to 86 (50%) patients while Foscarnet was given to 39, oral Valganciclovir to 39 and Cidofovir to 2 patients. Four patients did not receive anti-CMV therapy. The median time between CMV reactivation and starting antiviral therapy was 1.5 days (0-26) with >96% of patients starting antivirals within 72 hours of reactivation. The median duration of antiviral therapy was 29 (3-130) days. The overall response to antiviral therapies was 83% complete remission (negative CMV PCR) and 10% partial response (reduction in CMV PCR copies). 13 (7%) patients died before their response could be evaluated (9 on Ganciclovir and 4 on Foscarnet). The respective response rates according with the different initial treatments was: Ganciclovir 75% CR and 11% PR, Foscarnet 75% CR and 10% PR and Valganciclovir 92% CR and 10% PR. A total of 92 (53%) patients required second-line antiviral therapy: 28 patients after a partial response and 64 patients (45% of CR patients) because of a second CMV reactivation (CMV relapse). In addition, 68 (41%) patients changed their initial antiviral for different reasons: renal toxicity (13%), intolerance (15%) and de-escalation to oral Valganciclovir (13%). A total of 63 patients required re-hospitalisation for their treatment (37% of CMV reactivations, 16% of all patients at risk of CMV reactivation). The median number of days in hospital was 21 (3-133).
Summary and Conclusions: CMV infection occurs in over 40% of patients at risk. Responses to first line antivirals are satisfactory, although there is a significant risk of relapse. Anti-CMV therapies impact on healthcare resources with a high rate of re-hospitalisation.

PF460

ANTIGFUNGAL DRUGS MODULATE HUMAN NEUTROPHIL ACTIVITIES *IN VITRO* AND *IN VIVO* IN PATIENTS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

F. Ries[1], A. Alflen[1], P. Aranda Lopez[1], E.-M. Wagner-Drouet[1], M. Theobald[1], H. Schild[2], M. Radsak[1], D. Teschner[1,*]

[1]Department of Hematology, Medical Oncology, & Pneumology, [2]Institute of Immunology, University Medical Center of the Johannes Gutenberg University, Mainz, Germany

Background: In high risk patients antifungal agents substantially contribute to reduced morbidity and improved survival. However, besides their well-known antifungal activity there is a growing body of evidence for their immunomodulatory side effects on different immune effector cells.
Aims: The aim of our study is to clarify the impact of antifungal drugs on the effector functions of human polymorphonuclear neutrophils (PMN) *in vitro* and *ex vivo* and on the clinical course of invasive pulmonary aspergillosis (IPA).
Methods: Isolated PMN from healthy donors were preincubated with different antifungals (fluconazole [FLU], voriconazole [VOR], posaconazole [POS], isavuconazole [ISA], caspofungin [CAS], micafungin [MIC], conventional [AmB], and liposomal amphotericin b [LAmB]) and stimulated with lipopolysaccharides (LPS) or zymosan *in vitro*. Afterwards, PMN were analyzed by flow cytometry regarding shedding of CD62L, degranulation of CD66b/CD11b, and phagocytosis or by dichlorofluorescein assay to detect reactive oxygen species (ROS). Furthermore, the influence of MIC and POS on IPA was investigated *in vivo*. Therefore, mice treated with antifungals were inoculated with *A. fumigatus* conidia and lungs were analyzed by fungal culture assays, histopathologic staining, and pulmonary damage (albumin ELISA). Afterwards, blood samples of patients after allogeneic hematopoietic stem cell transplantation (HSCT) under treatment with antifungal drugs were analyzed *ex vivo* as described above with PMN from untreated HSCT patients serving as controls.
Results: *In vitro*, POS led to enhanced PMN activation (CD62L: 44% +/- 8 *vs* 13% +/- 2, *; mean +/- SEM; p-value ≤0.05 [*]), increased degranulation, and intensified generation of ROS. In contrast, ISA pretreatment resulted in impaired activation, degranulation, and generation of ROS (6980 rfu +/- 1338 *vs* 28730 +/- 6893, LPS, *). MIC led to enhanced expression of acti-

vation marker CD62L but reduced expression of CD11b, and decreased degranulation. Phagocytosis (27% +/- 4 *vs* 44 +/- 1, LPS, *) as well as generation of ROS were substantially impaired. CAS showed an increased phagocytosis (75% +/- 6 *vs* 44 +/- 5, LPS, *), whereas degranulation and generation of ROS were reduced by trend. Pretreatment with AmB resulted in generally enhanced effector functions besides impaired phagocytosis (43% +/- 3 *vs* 59 +/- 3, LPS, *). In contrast, LAmB did not significantly alter any effector functions. Regarding IPA, treatment with POS resulted in generally reduced fungal burden but led to decreased pulmonary damage (111 ng/ml +/- 46 *vs* 380 +/- 31, *). Despite similar fungal culture assays, MIC resulted in reduced fungal load in histopathologic staining compared to neutropenic controls. As expected from our *in vitro* experiments, PMN isolated from a patient after HSCT treated with ISA displayed relevantly impaired generation of ROS especially after additional LPS stimulation (12365 rfu [ISA] *vs* 41605 [control]) compared to PMN taken from a HSCT patient without antifungal medication. Other PMN effector functions were affected by trend through ISA treatment *ex vivo* but not as pronounced as seen in the *in vitro* assays.

Summary and Conclusions: Independent from substance class, antifungals drugs show variable modification on PMN effector functions *in vitro*, *in vivo*, and *ex vivo*. These interactions are obviously multidimensional and potentially derive from involvement of different pathways. Further experiments regarding PMN taken from HSCT patients under treatment with various antifungal compounds are ongoing to clarify the impact of our findings in daily patients care.

PF461

(1-3)-β-D-GLUCAN ASSAY AND AIRWAY-INVASIVE RADIOLOGICAL FINDINGS AS EARLY SIGNS OF PULMONARY ASPERGILLOSIS IN HIGH-RISK HEMATOLOGIC PATIENTS IN THE POSACONAZOLE ERA: PRELIMINARY OBSERVATIONS

C. Giordano[1,*], R. Della Pepa[2], I. Zacheo[3], C. Mortaruolo[3], A. Giordano[3], F. Trastulli[3], M. Raimondo[1], M. Lucignano[1], M. Di Perna[1], M. Picardi[3], F. Pane[4]

[1]Hematology, Federico II University, [2]Hematology, Federico II, [3]Hematology, [4]Advance Biomedical Science, Federico II, University of Naples, Naples, Italy

Background: Invasive pulmonary aspergillosis (IPA) is a severe complication in immuno-compromised patients with neutropenia, due to chemotherapy for acute myeloid leukemia (AML) remission induction. The Serum Aspergillus galactomannan index (s-GMI) test is helpful in early diagnosing IPA, with excellent performance and the halo sign detected at the high resolution chest computed tomography (HRCT) may suggest early IPA. However, microbiological and radiological findings may vary according to several factors, including antifungal prophylaxis.

Aims: With the intention of optimizing the mycological and radiological diagnostic yield, we wondered whether the diagnostic criteria of EORTC 2008 for IPA diagnosis could be adapted based on the type of drug used as primary antifungal prophylaxis (PAFP).

Methods: In this prospective study, of the Hematology of the Federico II University of Naples (Italy), we analyzed the results of IPA diagnostic work-up of AML patients, neutropenic with persistent fever in prophylaxis with posaconazole or itraconazole. From 2009 to 2012 patients obtained itraconazole, from 2013 to 2016 patients received posaconazole as PAFP. In the event of febrile neutropenia, they underwent a baseline diagnostic work-up based on blood cultures and conventional radiological examinations. Patients with persisting fever after 96h unresponsive to broad-spectrum antibiotics (77 cases), underwent an intensive diagnostic work-up that included s-GMI and serum (1-3)-β-D-GLUCAN (s-BDG) assay twice a week, and HRCT. These results were compared in the two groups (Student *t* test and square test).

Results: Median value of s-BDG was 168 pg/ml (17-352) in the posa-group and 85 pg/ml (23-330) in the itra-group; s-GMI median result was 0.5 (0.1-0.8) in the posa-group and 0.8 (0.1-2) in the itra-group. Most frequent HRCT findings for the posa-group were aspecific whether, in the itra-group, frequent findings were included in the EORTC 2008 diagnostic criteria. During the study period, 11% of the patients in the posa-group had a proven or probable fungal infection, *vs* the 45% patients of the itra-group (*P*<.001). Patients with positive s-BDG value and a HRCT scan aspecific result, were classified in a new category of IPA called "probable invasive aspergillosis with positive s-BDG and aspecific radiological findings" (14 cases in the posa-group and 2 in the itra-group; *P*<.001). This new category of patients that are febrile, neutropenic and unresponsive to broad- spectrum antibiotics, but positive to indirect mycological tests with HRCT aspecific lesions during posaconazole prophylaxis can be considered as having a probable IPA in an airway bronchogenic phase (Figure 1).

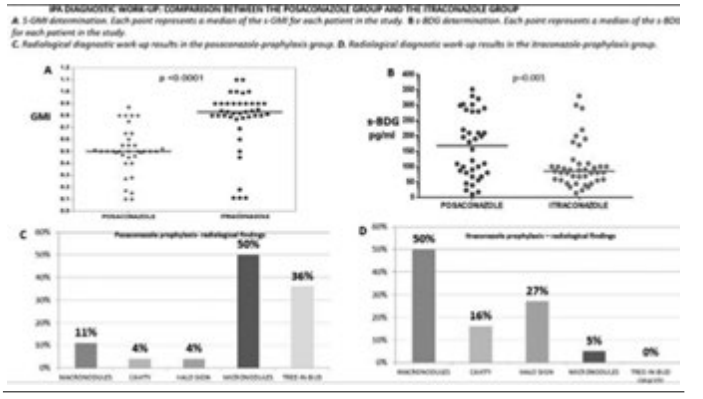


Figure 1.

Summary and Conclusions: Considering our data, the fungal dissemination and consequent radiologic and microbiologic signs are tightly related to the prophylaxis. Posaconazole seems to limit the angioinvasive disease while *Aspergillus* can disseminate through bronchi giving lesser expression in classical radiological findings and s-GMI results, thus supporting the hypothesis it's fungicide. More patients from the posa-group received an earlier systemic antifungal therapy (day 5 of work-up) in contrast with the itra-group (treated more frequently from day 7), thus affecting the 30-day OS (6 deaths *vs* 3). These results suggest the possibility of revising EORTC 2008 criteria by extending the suspicion of IPA to less specific HRCT features and a positive s-BDG value in patients with posaconazole prophylaxis, with regard to acting a pre-emptive therapeutic approach in an early stage of IPA.

PF462

COMPARATIVE ANALYSIS OF DETECTION OF EBV SMALL NON-CODING RNA (EBER) AND MARKERS OF ACUTE EBV INFECTION AT THE ONSET OF ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA

N. Chernova[1,*], D. Tikhomirov[2], M. Sinitcina[3], Y. Sidorova[4], A. Kovrigina[3], S. Kulikov[5], T. Tupoleva[2], E. Zvonkov[1]

[1]Department Chemotherapy of Lymphomas, [2]Laboratory of Virology, [3]Department of Pathology, [4]Department of Molecular Hematology, [5]Laboratory of Biostatistic, National Research Center for Hematology, Moscow, Russian Federation

Background: Angioimmunoblastic T-cell lymphoma (AITL) is associated with Epstein-Barr virus approximately in 70% of cases, which is proved by detection of EBV small non-coding RNA (EBER) in lymph node biopsies by *in situ* hybridization. At the same time, it is not known whether this is a sign of acute EBV infection or a tumor feature. The frequency of acute EBV infection markers at the onset of AITL is also unknown. A possible link between detection of EBER and the markers of acute EBV infection is uninvestigated.

Aims: The aim of the study was to perform a comparative analysis of detection EBER in lymph node biopsies and markers of acute EBV infection in primary patients with AITL.

Methods: 38 primary AITL patients were enrolled in the study. Male/female ratio was 20/18; a median age was 61 (29-81) years. Diagnosis was based on standard WHO criteria. EBV DNA concentration were screened by real-time PCR in peripheral blood (PB, n=38), bone marrow (BM, n=16), lymph node biopsies (LN, n=14) and bronco-alveolar aspirates (BAA, n=6). IgM against viral capsid antigen (IgM VCA EBV), IgG against early antigen (IgG EA EBV) and IgG against nuclear antigen type 1 (IgG ENBA-1 EBV) were tested by ELISA in all cases. Detection of DNA in low concentration (like less than 500 copies in 10^5 mononuclear cells of PB, BM and LN or in 1 ml of BAA) was regarded as latent infection. Detection of EBV DNA in concentration above 500 copies and/or of IgM VCA and/or IgG EA was regarded as acute infection. Discovery of markers of acute infection in absence of IgG ENBA-1 was referred to as primary infection. EBV small non-coding RNA (EBER) was tested by *in situ* hybridization in all lymph node samples.

Results: Laboratory markers of acute EBV infection were determined in 24 (63.2%) of 38 patients. EBV replication in PB were detected of 21 (55.3%) out of 38 patients, including 7 (18,4%) cases of primary infection. Serological markers of acute EBV infection (IgM VCA and/or IgG EA) were positive in 9 (23.7%) patients. EBV DNA in concentration relevant to active replication was detected in 14 (36,8%) cases. Comparison of viral DNA load in PB and/or BM and/or LN and/or BAA samples of a single patient showed that the highest level of viral load was determined in LN (up to

4.0x10^{6}copies/10^{5}cells) and BAA (up to 5.7x10^5/ml). EBV small non-coding RNA (EBER) was positive in 27 (71,1%) of 38 cases. Comparison of detection of EBER and acute markers of EBV infection showed good correlation (p<0,001). Patients with EBER-negative lymph node samples (n=11) didn't have any markers of acute EBV infection. Conversely, 24 of 27 (88,9%) EBER-positive cases accompanied by markers of acute EBV infection: 7 (25,9%) of them held markers of primary infection and 17 (63.0%) – reactivation. A pattern of markers of latent EBV was observed in the rest 3 (11.1%) EBER-positive cases. The results of detection of EBER and markers of acute EBV infection are summarized in the Table 1.

Table 1.

Comparison of detection of EBER in lymph nodes samples and patterns EBV markers in primary AITL patients

Pattern of EBV-markers		EBER-positive, n=27		EBER-negative, n=11	
		n	%	n	%
Acute EBV infection	Primary infection, n=7	7	25,9	0	0,0
	Reactivation, n=17	17	63,0	0	0,0
	TOTAL, n=24	24	88,9	0	0,0
No EBV replication	Latent infection, n=13	3	11,1	10	90,9
	Absence of infection, n=1	0	0,0	1	9,1
	TOTAL, n=14	3	11,1	11	100,0

Summary and Conclusions: The manifestation of AITL was accompanied by acute EBV infection (reactivation or primary infection) in almost half of enrolled patients. Markers of acute EBV infection were identified in most EBER-positive cases. Patients with EBER-negative lymph node samples didn't have any markers of acute EBV infection. Thus, screening for markers of acute EBV infection is strongly recommended at the onset of AITL.

PF463

THE ROLE OF SERUM GALACTOMANNAN ASSAY IN THE MANAGEMENT OF INVASIVE MOLD INFECTIONS IN AUTOLOGOUS HEMATOPOIETIC CELL TRANSPLANTATION.

M.E. Martinez-Muñoz[1,*], F. Portero[2], E. Ojeda[1], G. Bautista[1], R. Forés[1], I. Krsnik[1], A. de Laiglesia[1], B. Navarro[1], A. Ramos[3], J.R. Cabrera[1], R. Duarte[1]

[1]Hematology, [2]Microbiology, [3]Infectious Diseases, Hospital Universitario Puerta de Hierro Majadahonda (Madrid), Majadahonda, Spain

Background: Serum galactomannan (sGM)-based preemptive strategies are used for the management of invasive mold infections (IMI) in hematology patients. We have previously shown that effective anti-mold prophylaxis in high-risk patients makes sGM surveillance unreliable [RF Duarte, et al; Clin Infect Dis 2014]. Thus, these strategies would be particularly suited for patients not receiving anti-mold prophylaxis.
Aims: It remains to be tested wether they would be useful to manage patients for whom anti-mold prophylaxis is usually not recommended for an intermediate or low risk of IMI, such as autologous HCT recipients.
Methods: We present a single-center study on the use of sGM as part of a preemptive strategy to diagnose IMI in 86 consecutive episodes of autologous HCT (2009-2015) in 84 patients: 43 men; median age 52, range 16-69; 38 multiple myelomas, 28 non-Hodgkin lymphomas, 13 Hodgkin lymphomas, 2 systemic amyloidosis, 2 acute promyelocytic leukemias, 1 Crohn's disease. Patients received itraconazole prophylaxis up until 2011 (25; 30%) and fluconazole thereafter (61; 70%), and underwent twice weekly sGM surveillance throughout the risk episode.
Results: A total of 677 sGM tests were performed in this series, median 8 tests per risk episode (2-12). There were no cases of IMI according to EORTC/MSG criteria. One patient in the series (1.2%) died from septicemia with a single sGM positive test result (optical index [OI]: 0.9), no other features of IMI, and no necropsy (EORTC/MSG unclassified episode). A vast majority of 648 sGM tests (95.7%) and 72 episodes (83.7%) were all negative. Fourteen episodes (16.3%) had positive sGM test results (median 1, range 1-6) with a median OI of 0.9 (0.5-5.3). Five of these were false positive sGM episodes (5.8% of total, 35.7% of sGM positive episodes), as despite having positive sGM results (median OI 1.1, 0.7-1.6) patients remained afebrile, did not receive antifungal treatment and recovered from neutropenia with no other features of IMI (Table 1). The remaining nine episodes, with a median of 2 positive sGM results (range 1-6; OI 0.9, 0.5-5.3) were not evaluable to assess sGM performance, as test results led to the initiation of antifungal treatment (10.5% of total, 64.3.4% of positive episodes). All these non-evaluable episodes occurred in patients who survived and developed no other features of IMI, in five of them as antifungal surveillance in the absence of persistent fever. Despite the low incidence of IMI, positive sGM episodes were more likely to receive antifungal treatment (64.3% *vs* 23.6%; *p*<0.01). Of note, there were no differences in the occurrence of false positive or non-evaluable sGM episodes between patients on itraconazole or fluconazole prophylaxis (Table 1).

Table 1. Episode distribution according to serum galactomannan results.

Episode Type	No. (%)	Prophylaxis	
		Itra	Fluco
True positive	0	-	-
True negative	55 (64%)	15	40
False positive	5 (6%)	2	3
Non-evaluable	26 (30%)	8	18
• *sGM pos*	*9 (10%)*	*1*	*8*
• *sGM neg*	*17 (20%)*	*7*	*10*

Abbreviations: Fluco: fluconazole, Itra: itraconazole, sGM: serum galactomannan.

Summary and Conclusions: This study reproduces a very low incidence of IMI in recipients of autologous HCT. In keeping with our previous findings in high-risk patients on effective antifungal prophylaxis, our data show that sGM-based preemptive strategies are not reliable to manage IMI in this setting of low prevalence of infection, regardless of the use of mold-active prophylaxis. The vast majority of sGM tests are negative, and beyond confirmed false positive GM episodes, the remaining positive sGM test results can mislead diagnostic and therapeutic efforts, in particular when used as surveillance in patients without persistent fever.

PF464

CYTOMEGALOVIRUS (CMV) IMPACT ON CLINICAL OUTCOMES AND RESOURCE USE AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION: THE INFLUENCE OF RECURRENT EPISODES OF CMV INFECTION

C. de Miguel[1,*], A. Royuela[2], M.F. Portero[3], J.L. Cruz[4], M.E. Martínez-Muñoz[1], M.I. Sánchez-Romero[3], G. Bautista[1], R. Cabrera[1], R.F. Duarte[1]

[1]Hematology, Hospital Universitario Puerta de Hierro Majadahonda, [2]Clinical Biostatistics Unit, Biomedical Sciences Research Institute Puerta de Hierro-Segovia de Arana, [3]Microbiology, Hospital Universitario Puerta de Hierro Majadahonda, [4]IT Department, Hospital Universitario Puerta de Hierro Majadahonda - Universidad Politécnica de Madrid, Majadahonda, Spain

Background: Despite pre-emptive therapy (PET), Cytomegalovirus infection (CMVi) poses a negative impact on the outcome of allogeneic HCT recipients, and potentially on the use of resources for patient management.
Aims: The objective of this study is to analyse the impact of CMV infection on clinical outcomes and resource use in a tertiary hospital.
Methods: This retrospective study analyses the impact of CMVi on clinical outcomes and resource use including hospital length of stay (LOS) in consecutive allogeneic HCT recipients (2009-2016), with a particular focus on recurrent CMVi episodes.
Results: 183 allogeneic HCT in 172 recipients were included: 101 men (58.7%); median age 44 years (16-68); 51% AML/MDS, 16% ALL, 16% chronic lymphoproliferative disorders, 8% myeloma and 9% other; 45% matched related, 30% cord-blood, 20% unrelated and 5% haploidentical donors; 52% myeloablative conditioning; 161 (88%) at risk of CMVi (donor and/or recipient positive serology); 61 (33%) had acute GvHD grades II-IV. CMV disease occurred in only 4 cases (2%). CMVi occurred in 56% of patients (60% of patients at risk), at a median day +35 post-HCT (15-58), and had an impact on overall survival (44% *vs* 60% at 2 years; p=0.027). Patients above median age had higher CMV serology at risk (97% *vs* 80%; p<0.001) and CMVi rates (65% *vs* 48%; p=0.005). Compared to matched-related HCT, cord-blood and haploidentical HCT-recipients had higher CMVi rates (68% *vs* 49%; p=0.009, respectively) and earlier onset (70% *vs* 32% within day +35 post-HCT; p<0.001). Acute GVHD also increased CMVi rates (87% *vs* 43%; p<0.001). Among patients with a first CMVi, 57% had ≥2 and 20% had ≥4 recurrent CMVi episodes. CMVi recurrence

≥2 was also higher in cord-blood and haploidentical *versus* matched related HCT (HR 2.38; 95%CI 1.61-3.52; p<0.001), age at the time of HCT (+1.6% per year OR HR 1.02 (1.016); 95%CI 1-1.03; p=0.024) and acute GVHD (HR 2.3; 95%CI 1.62-3.26; p<0.001). In terms of clinical burden, hospital LOS throughout the first year post-HCT was overall >30 days longer in patients with CMVi (*vs* without, p<0.001; Table 1). This increase in LOS was >40 additional days in patients with ≥2 recurrent CMVi, significantly higher than in those with only one CMVi episode (19 days; p<0.001). In terms of treatment, 73.7% of CMV reactivations responded to 1st line of treatment, 60% to 2nd line and only 30% to 3rd line. The rate of adverse effects with PET was 31% after 1st line of treatment, and increased with usual drugs to 60% after 2nd line.

Table 1. Impact of CMV infection and recurrent infections on hospital admission length of stay.

Hospital LOS	No CMV infection	p	CMV infection (Any)	Number of CMV infection episodes		p
				Only 1	>=2	
1st HCT Hospital Admission	44.59 +/- 21.7	0.029	53.98 +/- 33.9			
1st Discharge to d +100	11.90 +/- 28.4	0.864	12.59 +/- 26.5	12.32 +/- 26.8	12.89 +/- 26.8	0.976
1st Discharge to d +365	24.75 +/- 39.1	0.002	47.30 +/- 56.5	35.93 +/- 46.2	54.59 +/- 62.1	0.004
d +100 to d +365	12.86 +/- 24.1	<0.001	34.65 +/- 52.3	23.59 +/- 37.9	41.69 +/- 59.7	0.001
HCT Admission to d +365	69.34 +/- 43.9	<0.001	101.28 +/- 63.2	88.49 +/- 52.5	109.7 +/- 69.2	<0.001

Abbreviations: CMV: Cytomegalovirus; HCT: Hematopoietic Cell Transplantation; LOS: Length of stay (expressed in days, d).

Summary and Conclusions: Despite preemptive therapy, CMVi remains a hurdle to the success of allogeneic HCT, with a significant impact on patients' outcome and use of resources. Recurrent CMVi episodes are very frequent, in particular in high-risk HCT-groups such as cord-blood, haploidentical and acute GVHD, pose a significant impact on outcomes and are a strong driver of hospital LOS and resource burden for the HCT program.

PF465

EPIDEMIOLOGY AND TREATMENT APPROACHES OF INVASIVE FUNGAL INFECTIONS MANAGEMENT IN HEMATOLOGICAL MALIGNANCIES: RESULTS FROM A SINGLE-CENTRE STUDY

N.S. Fracchiolla[1,*], M. Sciumè[1], N. Orofino[1], F. Guidotti[1], A. Grancini[2], F. Cavalca[1], A. Freyrie[1], M.C. Goldaniga[1], D. Consonni[3], A. Cortelezzi[1]

[1]Oncohematology Division, IRCCS Ca' Granda - Maggiore Policlinico Hospital Foundation and University of Milan, [2]UOS Microbiology, Central Laboratory, I.R.C.C.S. Foundation, Cà Granda Ospedale Maggiore Policlinico, [3]Unit of Epidemiology, Fondazione IRCCS Ca' Granda-Ospedale Maggiore Policlinico, Milan, Italy

Background: Invasive fungal infections (IFI) are a leading cause of morbidity and attributable mortality in oncohematologic patients (pts), particularly after intensive chemotherapy or hematopoietic stem cell transplant. Timely diagnosis is essential but challenging.
Aims: To describe the epidemiology and treatment of IFIs in a large, monocentric real-life cohort of hematological malignancies.
Methods: Oncohematologic pts treated with anti-fungal therapy (AFT) at our Hematology Department between January 2010 and July 2017 were identified from institutional databases. Clinical data, diagnostic work-up, treatment modalities, and outcomes were extracted. Diagnosis of IFI was carried out according to the revised definitions of European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG, 2008).
Results: Among 1719 consecutive hospitalizations, 221 AFT in 196 pts were recorded: the most represented disease was acute myeloid leukemia (103 pts) (p<0,001). Median age was 61 years (18-85) and male/female ratio was 58/42%. At fever onset 177 (80%) pts had a neutrophil count <0.5x10^9/L. Twenty-nine (13%) pts were receiving antifungal prophylaxis (26 posaconazole, 2 fluconazole, 1 itraconazole). The incidence of AFT was 13%. Serum galactomannan antigen (GM) was positive in 20% of the tested cases, while 85% of the pts had a CT scans suggestive for IFI, but only 23% of these cases had a GM positivity (p=.02). Only one case with a negative CT scan had a positive GM. Yeasts and moulds were identified in 42 (19%) and 22 (10%) pts, respectively. Four (2%) pts presented multiple positivity. Moulds were isolated from the respiratory tract, except for a case of hepatic mycoses at liver biopsy and a fungemia (Fusarium spp). Yeasts were isolated from peripheral blood (15 cases) and respiratory tract in the remaining cases. According to EORTC/MSG criteria, 22 (10%) cases were proven IFIs, 61 (28%) probable and 81 (36%) possible, but 57 (26%) cases could not be classified. Fifty-nine percent of the patients were treated with single agent AFT, 37% with sequential AFT, 8% with a combination regimen. Caspofungin (Caspo) was used in 100 cases, liposomal amphotericin B (L-AmB) in 123 and voriconazole (VCZ) in 93. The most used sequences were L-AmB and VCZ (20 cases), L-AmB and Caspo (17 cases), VCZ and Caspo (13 cases), L-AmB and Caspo (4 cases). Most frequent combination therapies were L-AmB plus VCZ (6 cases), Caspo plus L-AmB (6 cases), and Caspo plus VCZ (4 cases). IFI attributable mortality was 20%. At multivariate analysis, age, Caspo and L-AmB use were associated with increased mortality (p 0.007, p 0.002, p 0.008).
Summary and Conclusions: We described IFI epidemiology and treatment in a large monocentric cohort of consecutive oncohematologic pts, and correlated it with EORTC/MSG criteria, evidencing their potential weakness (high incidence of unclassifiable cases). This epidemiologic survey evidenced a persistent significant necessity of AFT, with high frequency of sequential and combination therapy. Interestingly, we failed to demonstrate an impact on outcome of diagnosis, GM or CT scan positivity, suggesting that the timely and aggressive therapeutic strategy applied seems to be efficacious in hematological diseases with different degree of immunosuppression and for IFI with different aggressiveness.

PF466

HERPES VIRUSES AT THE ONSET OF ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA. IS THERE AN ASSOCIATION?

D. Tikhomirov[1,*], N. Chernova[2], N. Yaroslavtseva[1], T. Romanova[1], F. Filatov[3], E. Zvonkov[2], T. Tupoleva[1]

[1]Laboratory of Virology, [2]Department Chemotherapy of Lymphomas, National Research Center for Hematology, [3]Laboratory of Molecular Biotechnology, Research Institute of Vaccines and Sera. named after I.I. Mechnikov, Moscow, Russian Federation

Background: Angioimmunoblastic T-cell lymphoma (AITL) is traditionally thought to be associated with Epstein-Barr virus (EBV) or rarer with Human Herpes Virus type 6 (HHV 6). It has been speculated that these viruses could directly or indirectly trigger AITL. However, data on association with other human herpes viruses and on frequency of detection of herpes viruses' markers in primary patients with AITL are scarce.
Aims: The aim of the study was to analyze the patterns of herpes viruses' markers at the onset of AITL.
Methods: 40 primary AITL patients were enrolled in the study. Male/female ratio was 22/18; a median age was 61.5 (29-81) years. Diagnosis was established based on standard WHO criteria. Markers of EBV, HHV 6, Cytomegalovirus (HCMV) and Herpes simplex virus types 1 and 2 (HSV 1, 2) were investigated. Antiviral IgM and IgG were tested in serum (40 samples) via ELISA. IgM detection was considered a marker of acute infection. Viral DNAs concentration were screened by real-time PCR in peripheral blood (PB, 40 samples), bone marrow (BM, 16 samples), lymph node biopsies (LN, 14 samples) and bronco-alveolar aspirates (BAA, 6 samples). Because of tropism to lymphoid cells and pathogenesis features of EBV and HHV 6, detection of EBV DNA or HHV 6 DNA in low concentration (like less than 500 copies in 10^5 mononuclear cells of PB, BM, LN or in 1 ml of BAA) was considered as marker of latent infection. HSV 1,2 DNA was tested only in LN and BAA due to absence of permissive cells for viral replication in PB and BM. Detection of HCMV DNA or HSV 1,2 DNA in any sample was regarded as markers of acute infection. Discovery of markers of acute infection in absence of IgG was considered as primary infection otherwise – reactivation
Results: Laboratory markers of acute herpes virus infection were detected in PB of 29 (72.0%) out of 40 patients, including 10 cases of primary infection (7 – EBV, 2 – HCMV, 1 – EBV and HCMV simultaneously). The data are presented in Table 1. The most frequent acute infection as expected was EBV. It's markers were detected in PB of 21 (52.5%) out of 40 patients. Markers of acute HSV 1,2 (IgM) and HCMV (IgM and DNA) infection in PB were rarer (15 (37.5%) and 14 (35.0%) respectively). Surprisingly high frequency was observed for anti-HSV 1,2 IgM detection (15 (37.5%) of 40 patients). Comparison of viral DNAs load in PB and/or BM and/or LN and/or BAA samples of single patient showed that the highest level of viral load was determined in LN (up to 4.0x10^{6}copies/10^{5}cells) and BAA (up to 5.7x10^5/ml). Marker of acute HHV 6 (HHV 6 DNA in BAA in concentration 700 copies/ml) was detected only in 1 patient
Summary and Conclusions: AITL during the onset appears to be associated

with acute herpetic infections (reactivation of endogenous virus or primary infection). Their markers, mainly markers of acute EBV, were observed in 29 (72.0%) out of 40 patients. High frequency of HSV 1, 2 IgM in primary AITL patients' needs further investigation. The highest level of viral load was determined in LN and BAA.

Table 1.

Virus	Viral DNA in concentration, relevant to acute infection	Antiviral IgM detection	Total	Primary infection among them
EBV	14 (35,0%)	9 (22.5%)	21 (52.5%)	8 (20.0%)
HCMV	9 (23,1%)	4 (10.0%)	14 (35.0%)	3 (7.5%)
HHV 6	0 (0%)	n/a*	0 (0.0%)	n/a*
HSV 1, 2	n/a*	15 (37.5%)	15 (37.5%)	0 (0.0 %)
Markers of any acute infection	19 (47,5%)	21 (52.5%)	29 (72.5%)	10 (25.0%)

n/a* - data not available

PF467

POST-TRANSPLANT EBSTEIN-BARR VIRAL LOAD MONITORING EXPERIENCE IN A HEMATOPOIETIC STEM CELL TRANSPLANTATION CENTER

M.S. Aydın[1,*], A.C. Inkaya[2], E. Karakulak[1], R. Çiftçiler[1], M. Turgut[3], H. Goker[1], E. Kelkitli[3], H. Demiroglu[1], Y. Buyukasik[1], N. Sayinalp[1]

[1]Hematology, [2]Infectious Diseases, Hacettepe University Faculty of Medicine, Ankara, [3]Hematology, 19 Mayıs University Faculty of Medicine, Samsun, Turkey

Background: Ebstein-Barr virus (EBV) viremia is a cause of post-transplant lymphoproliferative disorder (PTLD) and a significant cause of morbidity and mortality in hematopoietic stem cell transplant (HSCT) recipients. Risk of EBV viremia differs between different donor sources and T- cell depletion status. Prolonged immunosuppression increases EBV viremia risk. Most of the EBV viremias are asymptomatic and do not require treatment. Higher viral loads may require decreasing the grade of immunosuppression and pre-emptive use of rituximab.
Aims: To evaluate EBV viral load monitoring attitudes and clinical correlations of EBV viremia in HSCT patients
Methods: Retrospective data from a total of 565 hematopoietic stem cell transplant patients were analyzed who were transplanted between 01.01.2001 and 01.02.2018. EBV viral loads, dates, types of transplantation procedure and donor sources were recorded. Univariate analyses were done by Chi-square test for categorical and by t-test for numerical variables, respectively. Multivariate analyses were done by Cox regression analysis.
Results: There were 96 patients with post-transplantation EBV viral load test at least once. Median age was 43 (range, 16-75). There were 8 patients with EBV viremia. Four patients had acute leukemia, 1 had chronic myeloid leukemia (CML), 2 had non-Hodgkin lymphoma and 1 had multiple myeloma. Three of them were autologous and there was no difference between autologous and allogeneic HSCTs (p=1.000) and also there was no difference between myeloablative, reduced-intensity-conditioning and haploidentical HSCTs (p=0.898) in viral load positive and negative groups. EBV viral loads range between 73-14234 copies/mL. Only 1 patient who was transplanted (matched-related) for CML had EBV viral load >1000 copies/mL and had chronic graft *versus* host disease (GVHD) with severe thrombocytopenia. Rituximab 375 mg/m^2 was given for 2 times (3 week intervals) and after re-checking it was negative and thrombocytopenia was resolved. Three more patients with EBV viremia also had GVHD and 2 of them were negative when re-checked. All EBV viral loads were ordered incidentally with no suspicion of PTLD also neither patient had PTLD or second other malignancy with a median follow-up of 31 months. Also overall survival was not different in EBV viral load positive patients with respect to viral load negative patients (p=0.881).
Summary and Conclusions: As EBV viremia is mainly a problem of mismatched- or matched-unrelated donor and umbilical cord source for transplants. Our center mainly uses matched-related peripheral blood stem cell source and also T-cell depletion is not a generally issue. Our data shows, in low risk patients there may be no need for EBV viral load monitoring; it may be ordered in whom PTLD was suspected.

PF468

ISAVUCONAZOLE SHORTENS THE QTC INTERVAL

S. Mellinghoff[1,2,*], M. Bassetti[3], D. Dörfel[4], S. Hagel[5], N. Lehners[6], A. Plis[7], E. Schalk[8], A. Vena[3], O. Cornely[1,2,9]

[1]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, [2]Department I for Internal Medicine, Excellence Center for Medical Mycology (ECMM), University Hospital of Cologne, Cologne, Germany, [3]Santa Maria Misericordia University Hospital, Udine, Italy, [4]Medizinische Klinik II, Universitätsklinikum Tübingen, Tübingen, [5]Centre for Infectious Diseases and Infection Control, Jena University Hospital, Jena, [6]Department V of Internal Medicine, University Hospital Heidelberg, Heidelberg, [7]Department of Haematology, Oncology, Palliative Medicine and Transplantation Centre, University Hospital Greifswald, Greifswald, [8]Department of Haematology and Oncology, Medical Centre, Otto-von-Guericke University Magdeburg, Magedburg, [9]11. Clinical Trials Centre Cologne (ZKS Köln), University of Cologne, Cologne, Germany

Background: Isavuconazole is a novel antifungal drug, approved by both, the US as well as the European regulatory agencies in 2016 for treatment of adults with invasive aspergillosis and mucormycosis. While azoles as a class effect are known to prolong QTc interval, clinical trials have shown that isavuconazole administration may shorten QTc interval in a dose-related manner. Here, we assessed the effects of isavuconazole on the length of QTc interval.
Aims: To evaluate the effect of isavuconazole on QTc.
Methods: A total of 26 adult patients from seven hospitals were included. Patients received isavuconazole for treatment of invasive fungal disease and, in one case, for prophylaxis due to QTc prolongation under fluconazole. 12-channel electrocardiograms (ECG) were performed before and during treatment.
Results: 24 out of 26 patients showed shortening of QTc interval, while no changes were found in two patients. In those patients with QTc shortening, QTc during isavuconazole treatment showed a mean decrease of 7.4±5.8% (36.5±38.8 ms, range 7 to 202; p=0.004), compared to pre-isavuconazole ECG. One patient, with available long-term follow-up showed further decrease in QTc on day 55 and day 110.
Summary and Conclusions: Apart from one case report, these are the first data outside controlled clinical trials showing QTc shortening. Knowledge about cardiac effects of isavuconazole will serve to better manage the use of concomitant medications.

Iron metabolism, deficiency and overload

PF469

ALPHA LIPOIC ACID REDUCES THE TOXIC EFFECTS INDUCED BY IRON OVERLOAD TREATMENT: *IN VITRO* AND *IN VIVO* MODELS

G. Camiolo[1,2,*], D. Tibullo[1,2], C. Giallongo[1], N.L. Parrinello[1], P. La Cava[1], A. Romano[1], F. Puglisi[1], G. Li Volti[2], R. Avola[2], G.A. Palumbo[1], F. Di Raimondo[1]

[1]Division of Hematology - Policlinico Hospital "Vittorio Emanuele" (CT), [2]Biometec, University of Catania, Catania, Italy

Background: Secondary iron overload syndromes are due to hematological diseases such as thalassemias, transfusion-dependent anemias and myelodysplastic disorders. Several organs are affected by iron overload including liver, heart and endocrine glands. A comprehensive approach including tailored transfusion protocols, monitoring and assessment of total body iron levels and iron chelation is currently the mainstay in hindering iron overload.
Aims: Numerous studies suggest that iron chelation improves survival of trasfusion-dependent patients. The aim of the present study was to investigate whether alpha- Lipoic Acid (ALA), a naturally occurring substance, reduces cellular damage induced by iron overload focusing on its antioxidant and chelating properties *in vitro*, on a stromal cell line and *in vivo*, using an adult zebrafish model.
Methods: HS-5 cell line (stromal cells) was treated with Ferric Citrate Ammonium (FAC) 25µg/mL alone and in combination with ALA 20µg/ml. Oxidative stress was evaluated by flow-cytometry, western blot, immunofluorescence, autophagy induction by AVO-test and Fe3+ stores using the Perls staining. Concerning *in vivo* model, adults zebrafish were treated with FAC 120µg/ml alone and in combination with ALA 20µg/ml up to 48h. Specimens were fixed in formaldehyde 4% and Hematoxilin-Eosin and Perls staining were performed. Gene expression analysis of oxidative stress markers (HMOX1b, mtSOD and FPN1) was carried out by real time quantitative RT-PCR.
Results: The co-treatment of HS5 cells with FAC plus ALA was able to reduce the oxidative stress, measured by all the different methods, induced by FAC alone ($p<0.001$). After 24h of FAC treatment, iron overload induced the upregulation of oxidative stress marker genes and proteins HO-1 (heme oxygenase1) and SOD (superoxide dismutase). Interestingly, ALA co-treatment was able to induce glutathione synthesis ($p<0.0001$) and to restore the mitochondrial membrane potential ($p<0.001$) after mitochondrial damage induced by iron accumulation ($p<0.001$). In addition, co-treatment improves both mitochondrial integrity, increasing EF-Tu protein levels, and cellular homeostasis, decreasing the autophagolysosomes formation ($p<0.001$) compared to iron alone treatment. Looking at *in vivo* results, ALA protects zebrafish intestine, liver, heart and gills from iron overload showing its ability to prevent histological alterations and to reduce both the oxidative stress expression markers (HMOX1b, mtSOD; $p<0.001$) and Ferroportin1 (FPN1; $p<0.001$).
Summary and Conclusions: Both *in vitro* and *in vivo* model data suggest that ALA protects against iron overload mediated damages through suppression of oxidative stress induced by iron overload, reduction of cell autophagy, restoring mitochondrial integrity and preventing iron overolad-induced organ damages. Our findings back up the novel idea that ALA supplementation could be of help in countering secondary iron overload related diseases.

PF470

A PHASE 1, PLACEBO-CONTROLLED STUDY TO DETERMINE THE SAFETY, TOLERABILITY, AND PHARMACOKINETICS OF ESCALATING SUBCUTANEOUS DOSES OF LJPC-401 (SYNTHETIC HUMAN HEPCIDIN) IN HEALTHY ADULTS

D. Yaeger[1], A. Piga[2], A. Lal[3], A. Kattamis[4,*], S. Salman [5], B. Byrnes[1], G. Tidmarsh[1], L. Chawla[1]

[1]La Jolla Pharmaceutical Company, San Diego, United States, [2]Università degli Studi di Torino, Turin, Italy, [3]University of California San Francisco Benioff Children's Hospital, Oakland, United States, [4]National and Kapodistrian University of Athens, Athens, Greece, [5]Linear Clinical Research, Nedlands, Australia

Background: LJPC-401, synthetic human hepcidin, is being developed as a therapeutic treatment for various conditions of iron overload, including transfusion-dependent anemia and hemolytic anemia.
Aims: To determine the safety, tolerability, and pharmacokinetics (PK) and pharmacodynamics (PD) of escalating doses of LJPC-401 in healthy adults.
Methods: This was a phase 1, placebo-controlled, double-blind, randomized, single-center study. Healthy adult subjects (aged 18-65 years) were assigned to 1 of 4 dose cohorts (5, 10, 20, and 30 mg) with 6 subjects per cohort receiving LJPC-401 and 2 receiving volume-matched placebo. A single-dose subcutaneous injection was administered on day 1, and dose cohort escalations occurred only after the final subject at each dose level had been observed for ≥3 days with no evidence of study drug–related toxicity. Safety assessments included treatment-emergent adverse events (TEAEs), physical and laboratory evaluations, and immunogenicity. PK parameters of baseline-corrected serum LJPC-401 were obtained by noncompartmental analysis, with blood samples collected at predose, and at 0.5, 2, 4,8, 24, 48, and 168 hours postdose. PD endpoints included effects on serum iron, ferritin, transferrin, and iron-binding capacity.
Results: Overall, 32 subjects (mean age 29.1 years; 53.1% female; 93.8% white) were enrolled, completed the study, and were included in all analyses. All subjects (100%) in the LJPC-401 dose groups (n=24) and 2 subjects (25%) in the placebo group (n=8) experienced at least 1 TEAE. All TEAEs were mild in severity, and there were no serious AEs, TEAEs leading to early discontinuation, or deaths reported during the study. The most frequently occurring TEAEs were injection site reactions (ISRs; LJPC-401 100% *vs* placebo 12.5%), catheter site phlebitis (12.5% *vs* 0%), and headache (8.3% *vs* 0%). ISRs did not generally require treatment, and all but one resolved before study end. No serum samples were confirmed positive for anti-LJPC-401 antibodies, and there were no trends or changes in physical or laboratory test results with LJPC-401. PK results indicated that LJPC-401 maximum serum concentration (C_{max}) and area under the serum concentration-time curve from time 0 to 24 hours postdose (AUC_{0-24}) increased with dose over the tested dose levels; increases were generally linear between 5 and 20 mg. Peak concentrations of LJPC-401 occurred ~2 hours postdose for all doses with a return to baseline hepcidin levels by ~24-48 hours postdose. The mean (standard deviation [SD]) baseline serum iron value was 88.46 (22.70) g/dL, and all 4 doses of LJPC-401 significantly decreased serum iron levels compared with baseline ($P<0.0001$ overall); the mean maximum reduction was 33% to 65% at 8 hours postdose. A larger reduction in serum iron was generally associated with an increase in dose up to 20 mg. There was no apparent difference in the maximum reduction of serum iron between the 20- and 30-mg dose levels.
Summary and Conclusions: LJPC-401 was well tolerated at doses between 5 and 30 mg in healthy adults and showed decreased serum iron levels that returned to baseline levels within 48 hours. In patients at risk for iron overload (abstract submission by Lal et al) serum iron reductions were sustained up to day 8 in most patients.

PF471

REPEATABILITY OF LIVER IRON CONCENTRATION MEASUREMENTS WITH MRI AND AN ARTIFICIAL NEURAL NETWORK DATA ANALYSIS SYSTEM

T. St Pierre[1,*], Y. Aydinok[2], A. El-Beshlawy[3], S. Bayraktaroglu[2], A. Ibrahim[4], M. Hamdy[3], W. Pang[5], S. Khorshid[6]

[1]The University of Western Australia, Crawley, Australia, [2]Ege University Hospital, Izmir, Turkey, [3]Cairo University, [4]Ain Shams University, Cairo, Egypt, [5]Resonance Health Ltd, Burswood, [6]Three Springs Technology, Perth, Australia

Background: Recent studies have shown that artificial neural networks (ANNs) can be trained to estimate liver iron concentration (LIC) from raw magnetic resonance image (MRI) data. Such ANNs open up the possibility of MRI data analysis without the need for special expertise or training in image data analysis.
Aims: The aim of this study was to determine the repeatability of measurements of LIC using an ANN for data analysis and compare with the repeatability of a well established R2-MRI method of data analysis.
Methods: Patients at risk of transfusional iron overload (N=50) and healthy control subjects (N=10) were recruited (age range 12 to 67 years, 44 with thalassemia major, 4 with sickle cell disease, 1 with non-transfusion-dependent thalassemia, 1 with myelodysplastic syndrome). Each subject was scanned twice using a standard R2-MRI data acquisition protocol. The median period between the two scans was 1.9 hours with the minimum time between scans being 1.0 hours. Subjects were scanned on either a 1.5T Siemens Symphony Vision (N=45) or a 1.5T Philips Intera (N=15). Image data were analysed by an analyst (with no formal training in MRI, physiology, or MRI data analysis) using an ANN (mse184ssw2). The image data were also analysed by highly trained and experienced MR data analysts using spin-density projection assisted (SDPA) R2-MRI analysis. Bland-Altman statistics were used to assess the repeatability of the measurements by both methods.

Results: Image data upload, analysis, and report production using the ANN took about 1 minute. Image data upload, analysis, and report production by SDPA R2-MRI took about 30 minutes per dataset. The 120 LIC results obtained from the ANN were compared with the corresponding 120 LIC results obtained from the SDPA R2-MRI method, each pair of values resulting from image datasets from visit 1 and visit 2 with one dataset analysed by the ANN and the other by SDPA R2-MRI. The results are plotted in Figure 1 together with the line of equivalence (solid line). Bland-Altman analysis of the logarithmically transformed LIC results indicated non-uniform variance of the differences between the logarithmic values of LIC across the range of LIC encountered, with the variance being significantly larger below 3 mg Fe/g dw. Bland Altman analysis indicated a mean ratio of the ANN LIC to R2-MRI LIC of 1.04 and 0.87 below and above 3 mg Fe/g dw respectively. The upper and lower 95% limits of agreement for ratios of ANN LIC to R2-MRI LIC were 1.80 and 0.60 (<3 mg Fe/g dw) and 1.20 and 0.63 (>3 mg Fe/g dw). The repeatability tests for the ANN LIC yielded 95% of pairs of repeat measurements having ratios between 0.65 and 1.54 (<3 mg Fe/g dw) and between 0.80 and 1.26 (>3 mg Fe/g dw). The repeatability tests for the R2-MRI LIC yielded 95% of pairs of repeat measurements having ratios between 0.63 and 1.59 (<3 mg Fe/g dw) and between 0.86 and 1.16 (>3 mg Fe/g dw).

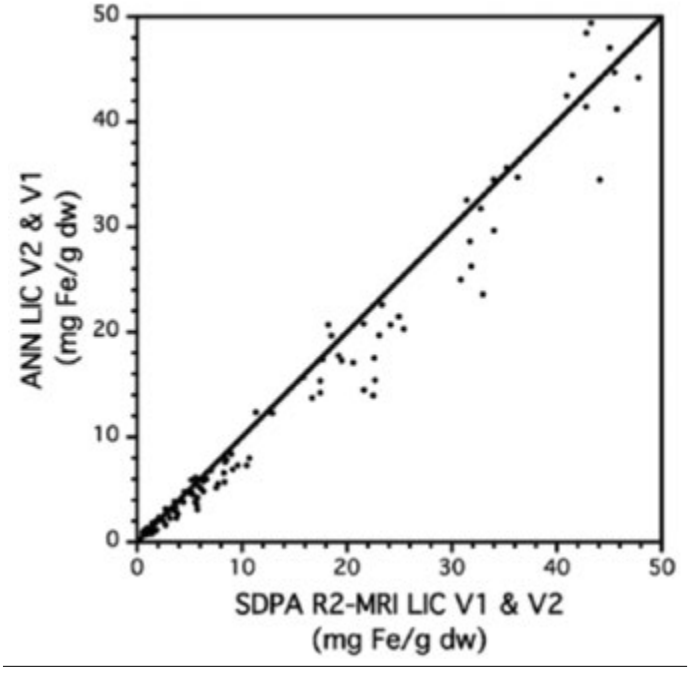


Figure 1.

Summary and Conclusions: Use of the ANN for data analysis eliminates the need for image analysis expertise and speeds up data analysis to an extent that point-of-care analysis could be performed and costs associated with analysis greatly reduced. The limits of agreement between the ANN analysis method and the SDPA R2-MRI method, together with the repeatability coefficients of each method, suggest that the ANN analysis method could be used in place of the SDPA R2-MRI method with good agreement and repeatability below 3 mg Fe/g dw but with a small but measurable bias and slightly decreased repeatability above 3 mg Fe/g dw.

PF472

IRON OVERLOAD FOLLOWING ALLOGENEIC HEMATOPOIETIC TRANSPLANTS: LONG-TERM OUTCOMES

G. Obi[1,*], A. Mukherjee[2], A. Scholoff[1], N. Vu[1], G. Carrum[1], R. Kamble[1]

[1]Bone Marrow Transplant, Center for Cell and Gene therapy, Baylor College of Medicine and Houston Methodist Hospital, [2]Hematology Oncology, Houston Methodist Hospital, Houston, United States

Background: Iron overload (IOL) is associated with increased transplant related morbidity and mortality related to oxidative damages from the free iron. Lack of prospective studies and variable definition of IOL in retrospective analyses precludes our understanding of IOL incidence and its long-term outcomes in patients receiving allogeneic hematopoietic transplantation (AHT).
Aims: To determine, incidence, IOL related morbidity and mortality in AHT.
Methods: Patient, disease and transplant related variables were retrospectively analyzed in 238 patients receiving AHT from January 2005- February 2017. Clinically significant IOL was defined as excess iron leading to organ dysfunction with SF of at least 1000 ug/dl along with abnormal transferrin saturation. Therapeutic intervention for IOL comprised of phlebotomy in all 25 patients with 2 receiving concurrent oral chelation. Phlebotomy program comprised of removing 250-500 ml of blood every 1-4 weeks when hemoglobin >11.5 gm/dl.
Results: Of the 238 patients receiving AHT, 37 (15.54%) were found to have post-transplant serum ferritin (SF) of >1000 ug/dl. Of these, a total of 25 (10.5%) developed clinically significant IOL. The median age of 19 males and 6 females was 55 years (24-70). Primary diagnosis for AHT included, AML/MDS (n=16), ALL (n=3), SAA (n=4) and others (n=2). Patients received median of 21 (7-66) life-time cumulative PRBCs transfusions.
The median pre-transplant SF and that at IOL was 1848 ug/dl (590-4658) and 3625 ug/l (1263-10696) respectively. IOL developed at median of 8.5 months (2-26) post AHT. All but 2 patients had cGvHD (limited skin=17, extensive=6). Liver dysfunctions (median AST=149 u/l (32-775), ALT=230 u/l (33-802) and alkaline phosphatase=249 u/l (48-841) was thought to be potentially IOL related. With a median of 9 (1-115) phlebotomies, SF <1000 ensued in 9 (2-30) months with corresponding improvement in transferrin saturation. Liver functions normalized at median of 3 months (1- 29) months. HFE analyses of both donor and recipients available in 11 patients had no impact on IOL occurrence of its response to treatment.
Interestingly, we noted improvement in hematopoiesis with iron chelation. Hemoglobin of 13 gm/dl and platelets count of 147 ug/L at initiation of phlebotomy rose to 13.9 and 178; a rise of 7% and 21% respectively. At the median follow-up of 45 (11-97) months, 18/25 (72%) patients are alive with median survival of 68 months (60- not reached). Cause of death included fungal infections in 2 patients, septic shock in 2 patients and relapse in 4 patients.
Summary and Conclusions: Phlebotomy for IOL in AHT produces predictable and safe iron reduction in patients with post AHT IOL with modest improvement in hematopoiesis.

PF473

IRON OVERLOAD IN RARE HEREDITARY HEMOLYTIC ANEMIA

S. van Straaten[1,2,*], J.-L. Kerkhoffs[3], J. Gitz-Francois[1], B.J. Biemond[4], R. van Wijk[1], E.J. van Beers[2]

[1]Laboratory of Clinical Chemistry & Haematology, [2]Van Creveldkliniek, University Medical Center Utrecht, Utrecht, [3]Haga Hospital, The Hague, [4]Department of Hematology, Academic Medical Hospital, Amsterdam, Netherlands

Background: Iron overload is an important, yet often overlooked complication in rare hereditary hemolytic anemia. Untreated, it may impair quality of life and shorten life expectancy. Currently, guidelines are mainly based on experience in the field of β-thalassemia. There are significant gaps in our knowledge with regard to prevalence and monitoring of iron overload in other forms of rare hereditary hemolytic anemia.
Aims: The aim of this study is to evaluate the prevalence of iron overload in non-transfused and transfused patients with different types of hereditary hemolytic anemia and to evaluate the predictive value of ferritin levels for liver iron overload.
Methods: This is a cross sectional analysis of iron overload in all patients with rare hereditary hemolytic anemia, from three expert centers for rare anemias in the Netherlands, who had MRI data on iron overload available.
Results: A total of 130 patients were included. Main disease categories were disorders of hemoglobin (*e.g.* sickle cell disease, β-thalassemia, HbH disease, unstable hemoglobins), disorders of red cell metabolism (*e.g.* pyruvate kinase deficiency, G6PD deficiency) and red cell membrane and hydration disorders (*e.g.* hereditary spherocytosis and hereditary xerocytosis). Mild liver iron overload (LIC≥3 mg Fe/g dry weight liver (DW) on MRI) was present in 82/130 of patients with MRI data available. Moderate/severe liver iron overload (LIC≥7mg/gDW) was present in 52/130 of patients and occurred in all forms of hemolytic anemia included. Importantly, 14/28 of patients who never received red cell transfusions did have mild or moderate/severe liver iron overload. Two of these 14 patients were diagnosed with sickle cell disease. Within the group of patients that had a plasma ferritin level below 1000 ng/mL at time of MRI (n=81), 20 (25%) patients had moderate/severe iron overload. Two of these patients had never received red cell transfusions. 7/100 patients had cardiac iron overload on MRI. Cardiac iron overload did not occur in patients with LIC<7mg FE/g DW and did not occur in patients without transfusion history (Figure 1).
Summary and Conclusions: We show that iron overload occurs in all forms of hereditary hemolytic anemia, even in patients without transfusion history. The currently used threshold of plasma ferritin ≥1000ng/ml is therefore a

poor predictor for iron overload. Diagnostic algorithms in current guidelines for patients with hereditary hemolytic anemia do not identify all patients with iron overload and we propose that these guidelines should be adapted.

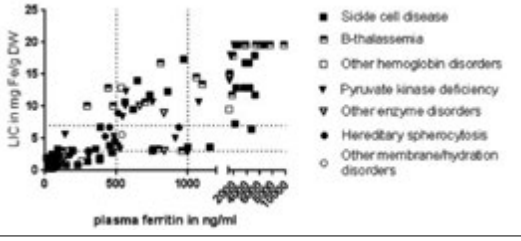


Figure 1. Ferritin *versus* LIC per disease category.

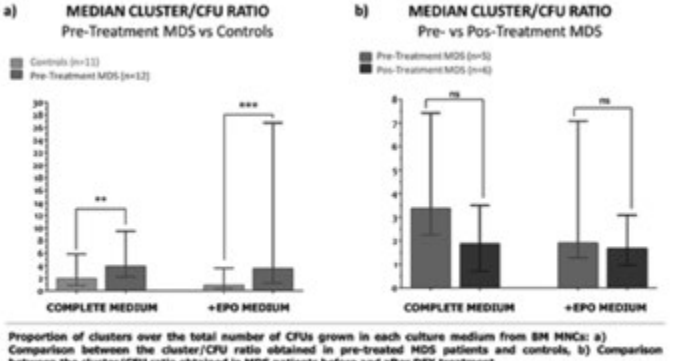
PF474

IRON OVERLOAD INCREASES OXIDATIVE STRESS AND DECREASES CLONOGENIC CAPACITY OF CELLS IN MDS PATIENTS: CAN BE RESTORED AFTER DEFERASIROX TREATMENT?

T. Jiménez Solas[1,2,*], F. López Cadenas[1,2], I. Aires Mejía[1,2], J.C. Caballero Berrocal[1], R. Ortega[1,2], A. Redondo Guijo[1,2], F. Sánchez-Guijo Martín[1,2], S. Muntión Olave[1,2], L. García Martín[1], B. Albarrán Severo[3], J.M. Alonso Alonso[3], C. Del Cañizo Férnandez-Roldán[1], Á. Hernández Hernández[2,4], M. Díez Campelo[1,2]

[1]Servicio de Hematología, Hospital Universitario de Salamanca, [2]Instituto de Investigación Biomédica de Salamanca (IBSAL), Salamanca, [3]Servicio de Hematología, Complejo Asistencial Universitario de Palencia, Palencia, [4]Departamento de Bioquímica y Biología Molecular, Universidad de Salamanca, Salamanca, Spain

Background: Low-risk myelodysplastic syndromes (MDS) patients usually presents iron overload due to the transfusion therapy they receive to ameliorate the effects of severe anemia. This overload may lead to a high level of oxidative stress in bone marrow (BM) cells, as free non-transferrin bound iron (LPI) catalyzes the production of reactive oxygen species (ROS).
We hypothesized that iron overload involved in ROS level rise in BM, may increase the hematopoietic cells (HPCs) and mesenchymal stromal cells (MSCs) dysfunction.
Aims: Our main objective is to analyze whether chelation therapy with deferasirox (DFX) could have a positive influence on the oxidative state of BM cells, on DNA damage and on clonogenic capacity of HPCs.
Methods: We analyzed samples from 13 iron-overloaded MDS patients before and 5-10 months after they were treated with DFX, and compared them with healthy donors (controls, n=20). We measured 3 oxidative stress parameters (intracellular ROS levels, DNA oxidation and DNA double strand breaks) in different BM populations by multiparametric flow cytometry analysis, and *in vitro* differentiation capacity of BM mononucleated cells (MNCs) by colony forming unit (CFU) assays.
Results: Comparing samples from pre-treated MDS patients with controls, our results showed a higher oxidative stress level in most patients HPCs, as well as greater DNA oxidative damage. After DFX treatment, the oxidative damage in DNA trends to decrease in HPCs populations: both for oxidation and for double strand breakage. Besides, the clonogenic assays carried out before treatment point to impairment in patients MNCs-derived CFU growth compared with controls (Figure 1a). This capacity trends to improve after DFX treatment, when the ratio cluster/CFU decreases and even approximates to control values (Figure 1b).



Proportion of clusters over the total number of CFUs grown in each culture medium from BM MNCs: a) Comparison between the cluster/CFU ratio obtained in pre-treated MDS patients and controls, b) Comparison between the cluster/CFU ratio obtained in MDS patients before and after DFX treatment.
Data expressed as the median ± range of the n indicated in each case. U from Mann-Whitney test: * = p < 0,05; ** = p < 0,01; *** = p < 0,001; ns: not statistically significant.

Figure 1.

Summary and Conclusions: We can conclude that BM cells in pre-treated MDS patients are subjected to higher oxidative stress conditions and present poor hematopoietic differentiation *in vitro*. These adverse features seem to be partially restored after DFX treatment *in vitro*.

PF475

RISK OF OVERTREATMENT OF HAEMOCHROMATOSIS DUE TO CO-INHERITED HYPERFERRITINAEMIA-CATARACT SYNDROME

H. Cutts[1,*], R. Farnell[1], H. Dreau[1], W. Atoyebi[1], J. Ryan[2], R. Aitchison[3], J. Porter[4], P. Bignell[1], E. Wilson[1]

[1]Haematology, [2]Gastroenterology, Oxford University Hospitals NHS Foundation Trust, Oxford, [3]Haematology, Buckinghamshire Healthcare NHS Trust, High Wycombe, [4]Haematology, University College London Hospitals NHS Foundation Trust, London, United Kingdom

Background: Haemochromatosis is a common genetic disorder most frequently caused by mutations in the HFE gene which can lead to progressive iron overload and eventually organ damage. These mutations however have variable penetrance and not all patients will progress to clinical iron overload or require therapy. Body iron stores in these patients are most commonly assessed by measuring serum ferritin and this is used to help determine the need to initiate therapy with phlebotomy and then to monitor the response to treatment and guide the frequency of venesections.
Aims: We present a series of three patients (ages 29, 50 and 70) who were found to have an elevated transferrin saturation and serum ferritin during investigation for lethargy, cardiac failure and abnormal liver function tests respectively.
Methods: All three patients were diagnosed with type one genetic haemochromatosis (one patient was homozygous for the C282Y HFE mutation and the other two were compound heterozygous for the C282Y and H63D mutations) and subsequently started on therapy with venesections for clinically significant iron overload, as assessed by elevated serum ferritin. They all however developed a microcytic anaemia during their venesection therapy (Hb 83-106g/L) despite the serum ferritin remaining elevated (Ferritin 160-1370mcg/L) above the treatment target of 50mcg/L. This is not consistent with the response of a haemochromatosis patient to venesection and specialist investigation was undertaken with an in-house NGS panel (TSCA v1.5, run on the MiSeq platform) comprising of sixteen genes involved in iron metabolism.
Results: In all three cases the HFE variants were confirmed but an additional variant (c.-160A>G, c.-160A>G and c.-162A>G) was detected in the iron regulatory element of FTL consistent with a diagnosis of the Hyperferritinaemia-cataract syndrome. This is a rare autosomal dominant genetic disorder resulting in an elevation of serum ferritin disproportionate to body iron stores but does not lead to pathological iron overload and early cataract is the only clinical consequence.
Summary and Conclusions: Hyperferritinaemia-cataract is well known as a cause of potential diagnostic confusion with haemochromatosis in patients being investigated for an elevated serum ferritin however these two conditions can usually be distinguished by the transferrin saturation which is elevated in haemochromatosis but not in the hyperferritinaemia-cataract syndrome. The co-inheritance of both conditions however leads to loss of the usefulness of ferritin as a marker of response to therapy in haemochromatosis and the potential for over treatment as occurred in our patients described above. Although there have been a couple of previous case reports of this association it is not widely recognised and our case series may imply that it is a more common occurrence than previously thought.

PF476

TISSUE IRON OVERLOAD ASSESSMENT IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

K. Lukina[1,*], N. Tsvetaeva[1], O. Nikulina[1], E. Sysoeva[1], E. Mershina[2], E. Lukina[1]

[1]Department of Orphan Diseases, National Research Center for Hematology, [2]Radiology Department, Federal Center of Treatment and Rehabilitation, Moscow, Russian Federation

Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired clonal non-malignant hematological disorder characterized by complement-mediated intravascular hemolysis and a prothrombotic state. PNH is characterized by diverse changes in iron metabolism. Chronic hemolysis leads to massive iron loss due to hemoglobinuria and hemosiderinuria, whereas chronic transfusions in severely anemic, transfusion-depen-

dent patients (pts) might cause the development of iron overload (IO).
Aims: To assess iron metabolism in PNH pts based on both laboratory parameters and MRI data.
Methods: The study group included 21 PNH patient followed up in our Center between 2015 and 2017. Laboratory parameters including hemoglobin (Hb), reticulocytes (RET), PNH clone size and LDH levels were analyzed. Chronic kidney disease (CKD) stage was assessed based on the estimated GFR. Iron metabolism was characterized by measurement of serum ferritin (SF), transferrin, iron concentration, total iron binding capacity (TIBC), transferrin saturation (TS). Multiecho gradient-echo T2* magnetic resonance imaging (T2* MRI) was performed in all pts to assess both liver and kidney IO. All pts were divided into 2 cohorts: 1 – eculizumab-naïve pts and 2 – pts treated with eculizumab.
Results: Cohort 1 included 14 eculizumab-naïve pts (12 men and 2 women aged from 29 to 58 yrs, median age 35 yrs). The median Hb level was 8,6 g/dl, median RET count – 9,1%. LDH levels ranged from 1322 to 8322 U/l (median 4721 U/l). The median erythrocyte PNH clone size was 52%. Median granulocyte and monocyte PNH clone sizes were 94% each. CKD Stages 1-3 were diagnosed in 8 (57%) pts. *Iron metabolism indices:* the median SF was 34 ng/ml, median TIBC – 60 umol/l, median TS – 28%. Laboratory signs of IO were detected in 1 (7%) patient. T2* MRI revealed signs of hepatic IO in 2 (14%) pts. One of these pts had laboratory signs of IO. Kidney IO was revealed in 100% pts. Cohort 2 consisted of 7 pts (2 men and 5 women aged from 31 to 64 yrs, median age 38 yrs). The median duration of eculizumab treatment in these pts was 1,5 yrs. The data of 6 pts was obtained in a period of 0,5 – 2 yrs after treatment termination due to administrative reasons. One patient continued to receive eculizumab at the time of analysis. The median Hb level was 7,7 g/dl, median RET count – 8,3%. LDH levels ranged from 567 to 7444 U/l (median 2623 U/l). The median erythrocyte PNH clone size was 35%. Median granulocyte and monocyte PNH clone sizes were 98% each. CKD Stages 1-2 were diagnosed in 3 (43%) pts. *Iron metabolism indices:* the median SF was 1118 ng/ml, median TIBC – 47 umol/l, median TS – 53%. T2* MRI revealed signs of hepatic IO in 6 (86%) pts which also had laboratory signs of IO. Signs of kidney IO were detected in 6 (86%) pts. The only patient with no signs of kidney IO was treated with eculizumab at the time of analysis (1,2 yrs).
Summary and Conclusions: Our findings prove that laboratory parameters of iron metabolism are not sufficient to identify tissue iron overload in PNH pts. T2* MRI is a noninvasive and reproducible technique that plays an impotrant role in the assessment of tissue iron overload. Our study revealed significant differences in iron metabolism parameters in the cohorts of eculizumab-treated and untreated pts. Hyperferritinemia was associated with the MR signs of liver iron overload in 86% of treated pts. On the other hand, 93% of untreated pts had either normal iron indices or signs of iron deficiency, MRI revealed signs of liver iron overload in 14% and signs of kidney overload – in 100% of untreated pts.

PF477

AN OPEN-LABEL, MULTICENTER, SINGLE-ARM, PHASE II STUDY ASSESSING PATIENT PREFERENCE FOR THE DEFERASIROX FILM-COATED TABLET COMPARED TO THE REFERENCE DISPERSIBLE TABLET FORMULATION: THE JUPITER STUDY

V. Viprakasit[1,*], R. Merad-Boudia[2], A. Das Gupta[3], A. T. Taher[4]

[1]Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand, [2]Novartis Pharma AG, Basel, Switzerland, [3]Novartis Healthcare Pvt Ltd, Hyderabad, India, [4]American University of Beirut Medical Center, Beirut, Lebanon

Background: Compliance with iron chelation therapy (ICT) influences the frequency and severity of iron overload-related complications.[1] Once-daily deferasirox (DFX) dispersible tablets (DT) have a well-defined safety and efficacy profile and, compared with parenteral deferoxamine, provide greater adherence, patient (pt) satisfaction, and quality of life.[2] However, barriers to optimal adherence remain, including gastrointestinal (GI) tolerability and palatability, leading to development of film-coated tablets (FCT) that contain the same active substance (strength adjusted to maintain comparable exposure to DT), can be taken with a light meal, and offer ease of administration. Results of the 24-week, randomized, open-label Phase II ECLIPSE trial (NCT02125877) indicated that the DFX FCT had a comparable safety profile to the DT with fewer severe GI-related adverse events (AEs) and more favorable pt-reported outcomes, including preference in favor of FCT.[3] However, preference was compared between two pt groups. A Phase II, open-label, multicenter, single-arm study has been initiated (currently enrolling pts) to evaluate preference in a broader pt population, including pediatrics, over a longer time period using one pt population that will cross over from DFX DT to FCT.
Aims: To evaluate pt preference for DFX FCT over DT in transfusion-dependent thalassemia (TDT) or non-transfusion-dependent thalassemia (NTDT) pts.
Methods: ICT-naïve or pre-treated (≥6 mths with ICT other than DFX) pts aged ≥2 yrs with TDT or NTDT, and serum ferritin (SF) >1000 ng/mL [≥800 ng/mL for NTDT] and, if available, LIC >3 mg Fe/g dw [≥5 mg Fe/g dw for NTDT] until 6 mths prior to screening will be included from 11 countries: Thailand, Lebanon, Malaysia, Iran, Saudi Arabia, Turkey, Oman, Vietnam, Algeria, Morocco and Egypt. Key exclusion criteria: creatinine clearance <60 mL/min or <40 mL/min per local label, serum creatinine >1.5x upper limit of normal (ULN), alanine aminotransferase >5xULN (unless LIC <10 mg Fe/g dw), urine protein/urine creatinine ratio >0.5 mg/mg, or impaired GI function. All pts/representatives will provide written consent for participation. 170 pts are planned to be enrolled in the study, consisting of two phases (Figure 1): Phase 1 (day 1 to week 24), pts to receive DFX DT starting at 20 mg/kg/day (10 mg/kg/day for NTDT) max 40 mg/kg/day (20 mg/kg/day for NTDT); Phase 2 (week 25–48), pts to receive DFX FCT starting at 14 mg/kg/day (7 mg/kg/day for NTDT) max 28 mg/kg/day (14 mg/kg/day for NTDT). An extension phase is planned for a max of 48 weeks with DFX FCT. Pts with difficulty swallowing the FCT could crush tablets. At the investigators discretion, pts can switch DFX formulation at any time. The primary objective is to evaluate preference at week 48, by Patient Preference questionnaire. Preference will also be evaluated at weeks 4, 24, and 28, alongside questionnaires to evaluate palatability, GI symptoms, and satisfaction conducted at screening, weeks 4, 24, 28, and 48. SF, pill counts, and the frequency/severity of AEs and changes in laboratory values will be monitored.
Results: -

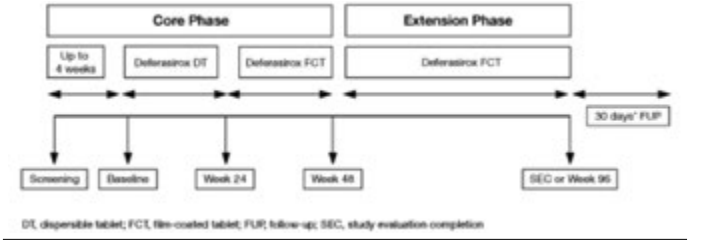


Figure 1. Study design.

Summary and Conclusions: This multicenter study will evaluate pt preference for DFX FCT compared to DT in pts with TDT or NTDT, providing valuable insight into the utility of DFX FCT to improve pt experience with ICT, as well as further evaluate the overall safety, tolerability, and efficacy profile.
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PF478

A RARE CAUSE OF HEREDITARY HEMOCHROMATOSIS: COMPOUND HETEROZYGOSITY ASSOCIATING THE C282Y VARIATION WITH A PRIVATE HFE MUTATION - TWO CASES COMBINING NEW NONSENSE MUTATIONS

S. Cunat[1,*], C. Serrier[2], F. Danton[1], O. Guillot[1], M. Giansily-Blaizot[1], P. Aguilar-Martinez[1]

[1]Département d'Hématologie biologique, CHU de Montpellier Hôpital Saint Eloi, Montpellier, [2]Service d'Hématologie clinique, CH de Perpignan, Perpignan, France

Background: Homozygosity for the *HFE* p.C282Y mutation (C282Y) is the most common genotype associated with adult forms of genetic hemochromatosis (GH). The association of the C282Y mutation with the H63D variant in *trans* is also involved in milder forms of GH. Much rarer forms, combining another *HFE* private variant with the C282Y mutation, have been reported in GH patients (ref 1.2).
Aims: Here we describe two novel nonsense mutations in the *HFE* gene co-inherited with C282Y in 2 patients with iron overload.
Methods: After exclusion of acquired causes, genetic analysis of 40 patients with unexplained iron overload was undertaken by using next generation sequencing (Agilent SureSelectQXT®, Miseq® Illumina). A diagnostic panel, including the six most common genes involved in rare forms of iron overload, namely *HAMP, HFE, HJV, SLC40A1, TFR2* and *BMP6* was analyzed. Each identified pathogenic variant was subsequently checked by Sanger sequencing.
Results: Two unrelated C282Y carriers, a 39-year-old man and a 22-year-old woman, with a liver iron concentration of 250 and 100 mol/g respec-

tively, were found to be compound heterozygotes with a rare *HFE* variant. Both had hyperferritinemia >800 g/L and transferrin saturation >80%. The two nonsense mutations associated with C282Y were HFE:c.308G>A (p.W103*) and HFE:c.1004C>G (p.S335*), respectively. The *HFE* W103* and S335* mutations are not referenced in literature or in databases. Both generate a stop codon, truncating the synthesized protein at exon 2 and exon 5, respectively. Their combination in *trans* with the C282Y mutation could be responsible for the severe (man) or early (woman) iron overload presented by the two patients. These two mutations extend the small number of private *HFE* variants co-inherited with C282Y, described so far in literature. The majority of these rare *HFE* variants are missense mutations, seven are frameshifts and only four generate a stop codon.
Summary and Conclusions: This report confirms the importance of searching for a private *HFE* mutation in *HFE* C282Y heterozygotes with mild to severe iron overload (ref. 2). As exemplified here, the association of the C282Y mutation to a nonsense *HFE* mutation result in clinical forms of iron overload as severe as C282Y homozygosity.

References:
(1) Porto G *et al.* Eur J Hum Genet 2016;24:479. EMQN best practice guidelines for the molecular genetic diagnosis of hereditary hemochromatosis.
(2) Aguilar-Martinez P *et al.* Haematologica 2011;96:507. Iron overload in HFE C282Y heterozygotes at first genetic testing: a strategy for identifying rare HFE variants.

PF479

THE RETICULOCYTE PARAMETERS: A NEW TOOL FOR ASSESSING AND MONITORING IRON HOMEOSTASIS

M.J. Teles[1,*], M. Coutinho[2], G. Melo[2], G. Porto[2,3,4,5]

[1]Clinical Pathology, Sao Joao Hospital Centre, [2]Clinical Hemathology Department, Porto Hospital Center, [3]Instituto de Ciências Biomédicas de Abel Salazar, University of Porto, [4]Instituto de Investigação e Inovação em Saúde, i3S, [5]Institute for Molecular and Cell Biology, University of Porto, Porto, Portugal

Background: Complete blood counts(CBC), serum ferritin(Ft) and transferrin saturation(TfSat) are useful markers to assess systemic iron homeostasis but their specificity is hampered by confounding effects of infection, chronic diseases or inflammatory states. Modern haematology equipments give additional RBC and reticulocyte(RET) parameters better reflecting iron acquisition and utilization for erythropoiesis. The hemoglobin content of RET (RET-He) is recognized as a marker of functional iron deficiency, used in the anemia of inflammation. The immature RET fraction(IRF) and RET counts(RC) offer a good picture of RET maturation and production used to evaluate erythroid response. To our knowledge, these parameters have never been described in the context of iron overload(IO) nor during iron depletion treatment. Phlebotomy is the standard therapy for hereditary hemochromatosis(HH), but its use in other acquired IO conditions, such as alcoholic or dysmetabolic liver diseases, is still debated.
Aims: 1.To test the discriminatory values of RET-He, IRF and RC in IO disorders, with or without inflammation; 2.To test the value of RET-He to monitor iron depletion therapy in patients with IO.
Methods: CBC, RET-He, IRF and RC were determined in Sysmex XE-5000 equipment in 127 adult patients who presented some form of IO, classified in 5 categories: 77 genetically confirmed HH; 9 secondary IO due to compensated chronic hemolysis(CCH), 25 hyperferritinemias associated with alchool abuse (AA), and 16 dysmetabolic hyperferritinemias (DHFt). For comparisons, 26 additional cases were included: 13 with absolute iron deficiency (ID) (TfSat <20%,Ft <20 ng/ml), 8 with non-ID inflammation (normal TfSat,Ft>400,CRP>3,0 ng/ml) and 8 controls (normal TfSat, Ft and CBC). Iron parameters, GGT and CRP were determined by routine standard methods. HH patients were analyzed at different stages: 9 at diagnosis (8 also followed during weakly intensive phlebotomy treatment) and 68 during maintenance treatment (every 3-4 months). Severity of IO in HH was estimated retrospectively by the total amount of iron removed by intensive phlebotomies (TBIS): 10 had<2g, 19 had 2-4g, 17 had 4-8g, 20 had>8g. Differences among groups were tested by ANOVA. A multiple regression analysis was performed to test the relative impact of sex, age, TfSat, AA and HH on RET-He values.
Results: The average RET-He values (controls 32,4+/-1,7) was significantly decreased in ID (23,4+/-3,6) and significantly increased in CCH (35,3+/-2,2), AA (35,3+/-2,0) and HH (34,4+/-1,6) but not in DHFt (33,7+/-1,4). RET-He in HH was significantly lower in patients who presented at diagnosis with TBIS<2g (33,8+/-0,6) than those who presented with TBIS>8g (35,0+/-1,1). Both in HH and AA (but not in CCH) neither RC nor IRF were increased, suggesting a limited/blunted erythroid response in the presence of excessive iron content. The major component influencing RET-He is TfSat (P<0,00001), but this relationship is affected by the presence of AA (P<0,00001) or HH (P=0,0280), evidenced by higher values under ID conditions. But while HH patients consistently maintain (or even increase) hemoglobin levels during intensive phlebotomy treatment, this was not observed in acquired IO (illustrative case reports will be shown).
Summary and Conclusions: The innovative RET parameters offer a new valuable and inexpensive tool to assess and monitor IO disorders. We speculate that HH patients have more effective iron avalilability/incorporation resulting in resistance to ID while patients with AA associated IO do not respond effectively, thus questioning the indication for phlebotomy treatment.

Myelodysplastic syndromes – Biology & Translational Research

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SOMATIC MUTATIONS AS MARKERS OF OUTCOME AFTER AZACITIDINE TREATMENT FOLLOWED BY ALLOGENEIC STEM CELL TRANSPLANTION (HSCT) IN HIGHER-RISK (HR) MDS AND LOW-BLAST COUNT ACUTE MYELOID LEUKEMIA (LBC-AML)

G. Falconi[1,*], E. Fabiani[1], A. Piciocchi[2], M. Criscuolo[3], L. Fianchi[3], C. Finelli[4], E. Cerqui[5], E.L. Lindfors Rossi[1], L. Iaccarino[1], T. Ottone[1], A. Molteni[6], M. Parma[7], S. Santarone[8], A. Candoni[9], S. Sica[3], F. Lo-Coco[1,10], M.T. Voso[1]

[1]Department of Biomedicine and Prevention, Università Tor Vergata, [2]Fondazione GIMEMA, [3]Department of Hematology, Università Cattolica del Sacro Cuore, Rome, [4]Department of Hematology, S. Orsola-Malpighi Hospital, Bologna, [5]Department of Hematology, A.O. Spedali Civili, Brescia, [6]Department of Hematology, Ospedale Niguarda, Milan, [7]Department of Hematology, San Gerardo Hospital, Monza, [8]Department of Hematology, Centro Trapianti Midollo Osseo, Pescara, [9]Department of Experimental and Clinical Medical Sciences, Azienda Ospedaliero-Universitaria di Udine, Udine, [10]Laboratorio di Neuro-Oncoematologia, Fondazione Santa Lucia, Rome, Italy

Background: The outcome of patients with HR-MDS has improved after the introduction of hypomethylating treatment, in particular azacitidine (AZA), which is considered the standard treatment. However, HSCT remains the only curative option in eligible patients. Somatic mutations play a pathogenic role in MDS/AML, and may predict clinical outcome.
Aims: Our aim was to evaluate the association between specific somatic mutations, response to AZA treatment and HSCT outcome in HR-MDS and LBC-AML.
Methods: Sixty-five patients (57 *de novo* HR-MDS and 8 LBC AML) with median age 59 years (range 21-66 years) enrolled in BMT-AZA multicenter trial, were studied. All patients were homogeneously treated with a standard AZA regimen (75 mg/sqm/day sc for 7 days every 28 days), for at least 4 cycles, followed by HSCT in 44 patients. Patients were considered responders to azacitidine, if they achieved complete remission (CR), partial remission (PR) or haematological improvement (HI). Patients exhibiting stable disease (SD) or progressive disease (PD) were considered resistant. Ultra-deep NGS (Myeloid Solution panel by Sophia Genetics on an Illumina platform) was performed on DNA extracted from BM mononuclear cell samples obtained before starting AZA treatment. Thirty genes known to be involved in MDS and AML pathogenesis were studied. Analysis of variant allele frequency (VAF) was performed using a standardized approach and the SOPHiA DDM® software. Changes in mutation burden were studied in selected cases after 4 cycles of AZA, using specific pyrosequencing assays.
Results: At diagnosis, we identified at least 1 mutation, at VAF greater than 1%, in 62 out of 65 patients (95.4%). The median number of mutations per sample was 3 (range, 0-6) and 57 patients (88%) carried more than 3 mutations. The most commonly mutated genes were: *ASXL1* (37%), *RUNX1* (29%), *SETBP1* (25%), *DNMT3A* (21%), and *TET2* (21%). Thirty-one of 62 patients (50%) had more than 1 mutation in the same gene (Figure 1). Response to AZA was documented in 30 patients (12 CR, 12 PR and 6 HI), while SD and PD were observed in 23 and 12 patients, respectively. Univariate analyses revealed that mutations in *DNMT3A* (VAF greater than 10%) were associated with lower response rate as compared to wild type cases (p=0.051). In particular, *DNMT3A* mutations localized in the methyltransferase domain were found in 11 of 65 patients (16.9%): only 1 patient in this subgroup achieved HI, whereas the remaining 10 patients were unresponsive (5 SD and 5 PD). The R882 mutation, present in 50% of DNMT3A-mutated patients (7 of 14), was identified only in unresponsive patients (4 SD and 3 PD). Allelic frequency of most mutations did not change upon AZA treatment, while in four AZA-responsive patients (2 CR and 2 PR) clearance of *TP53*-mutations was observed after 4 cycles. However, at the multivariable analysis, *TP53* mutations retained its unfavourable prognostic significance in terms of either PFS or OS (p=0.0013 and p=0.0008, respectively), together with AZA response (p=0.0003 and 0.0068, for PFS and OS). Moreover, mutations in *SETBP1* were associated to decreased OS (p=0.0241), whereas *TET2* mutations were a favourable prognostic factor (p=0.0237). When restricting the analysis to patients who underwent HSCT, *TP53, ZRSF2* or *PTPN11* mutations were negative prognostic factors for survival.
Summary and Conclusions: Our data show that mutational screening of HR-MDS and LBC-AML using a standardized NGS approach may predict response to AZA treatment and survival after HSCT.

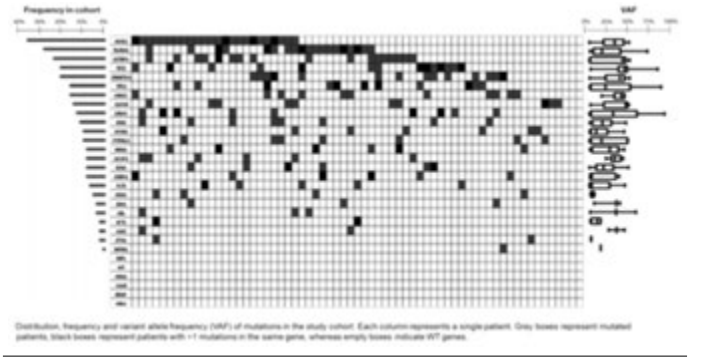

Figure 1.

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SYNERGISTIC EFFECTS OF PRIMA-1MET (APR-246) AND AZACITIDINE (AZA) IN TP53-MUTATED MYELODYSPLASTIC SYNDROMES

N. Maslah[1,*], E. Verger[1], N. Partouche[2], P. Ly[3], P. Aubin[3], N. Naoui[3], M.-H. Schlageter[3], C. Bally[3], A. Wisniewska[3], R. Rahmé[3], J. Lehmann-Che[3], L. Adès[3], P. Fenaux[3], B. Cassinat[1], S. Giraudier[1]

[1]Hôpital Saint-Louis, INSERM, [2]Hôpital Henri Mondor, [3]Hôpital Saint-Louis, APHP, Paris, France

Background: Myelodysplastic syndromes (MDS) mutated for the *TP53* gene form a subgroup characterized by frequent relapses, poor survival and poor response to current therapies such as 5-azacitidine (AZA). PRIMA-1^{Met} (APR-246, APR) is a methylated derivative of the compound PRIMA-1 that induces apoptosis in human tumor cells through restoration of the transcriptional transactivation function of mutant *TP53*. We show here that low doses of APR reactivates P53 pathway and induces an apoptosis program alone and in combination with AZA.
Aims: - Testing the effect of APR-246 alone and in combination with AZA in TP53-mutated MDS cells *in vitro* and *in vivo*. - Look for a synergy between APR and AZA in MDS cells. - Decipher the mechanism of the synergy between the two drugs.
Methods: Myeloid cell lines SKM1, UT-7 / JAK2V617F and K562 were selected because each exhibiting different *TP53* mutations. Proliferation and viability of cells in the presence of APR-246, 5-Azacytidine (AZA) or simultaneous or sequential combination of the two drugs were evaluated. In parallel, bone marrow samples from MDS patients with a high frequency of TP53 mutations (complex karyotypes, 5q deletion, or known to be mutated for TP53) were evaluated for their clonogenicity with or without AZA. APR-246. The efficacy of the treatments was also evaluated *in vivo* in nude immunocompromised mice (n=30) after subcutaneous xenograft of SKM1 cells. Finally, a transcriptomic analysis was performed on SKM1 cells treated or not with APR, AZA or both.
Results: Functionally, we demonstrated that APRshows efficacy alone and synergize with AZA in *TP53* mutated MDS/AML cell lines and in *TP53* mutated primary cells from MDS/AML patients. *In vivo*, low doses of APR alone or in combination with AZA also induced major efficacy. Lastly, using transcriptomic analysis, the AZA+APR synergy was demonstrated as mediated by downregulation of the FLT3 pathway in treated cells. The overstimulation of the FLT3 pathway by FLT3 ligand (FLT3-L) rescues APR+AZA treated cells from apoptosis, thus demonstrating the involvement of this pathway in the survival of myelodysplastic cells.
Summary and Conclusions: These data therefore may open a whole new avenue to TP53-mutated MDS/AML treatment using combination therapies in this subgroup of poor prognosis.

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GENE PANEL ANALYSIS BY NEXT GENERATION SEQUENCING FOR THE DETECTION OF CLONAL HAEMATOPOIESIS IN IDIOPATHIC CYTOPENIA OF UNDETERMINED SIGNIFICANCE (ICUS) AND MYELODYSPLASTIC SYNDROME (MDS)

Y. Wouters[1,*], F. Nollet[1], B. Cauwelier[1], J. Emmerechts[1], H. Devos[1]

[1]Laboratory medicine, AZ Sint-Jan Hospital, Brugge, Belgium

Background: According to the WHO 2016 definition, patients showing different degrees of cytopenia can only be classified as myelodysplastic syn-

drome (MDS) when concurrent bone marrow dysplasia, excess blasts or typical cytogenetic abnormalities are present. Cases lacking these major criteria but having persistent cytopenia are now classified as idiopathic cytopenia of undetermined significance (ICUS). It was reported that a fraction of these patients carry somatic mutations and are potentially at risk of progression to MDS or leukemia.
Aims: Aim of this study was to determine the frequency of gene mutations in patients with ICUS and overt MDS in our hospital using a custom next generation sequencing (NGS) based 19-gene panel analysis.
Methods: Bone marrow aspirates were collected from patients investigated for cytopenia. Samples were found eligible for testing when cytopenia was below the thresholds established by the original international prognostic scoring system (Haemoglobin <10 g/dL, Platelet count <100 $*10^9$/L, Absolute neutrophil count <1.8 $*10^9$/L). A total of 95 bone marrow aspirates were included: 61 patients were diagnosed with MDS because of morphological evidence of dysplasia, excess blasts or presence of a specific cytogenetic abnormality, whereas 34 patients were categorised as ICUS. A Qiagen GeneRead DNAseq custom panel was used to detect mutations in 19 genes associated with myeloid malignancies (ASXL1, CALR, CSF3R, DNMT3A, FLT3, IDH1, IDH2, JAK2, KIT, MPL, NPM1, RUNX1, SETBP1, SF3B1, SRSF2, TET2, TP53, U2AF1, WT1) and analysed on a MiSeq NGS sequencer (Illumina Inc., San Diego, CA, USA). A minimal read coverage of 300 reads was required, resulting in a sensitivity of the assay of 5% variant allele frequency (VAF). Statistical differences were calculated with the Mann-Whitney U test using Medcalc® version 18.2 (Medcalc Software, Mariakerke, Belgium).
Results: Out of 34 ICUS samples 6 (18%) bone marrow aspirates showed evidence of clonality by detection of at least one somatic mutations in either DNMT3A, SRSF2, TET2 or TP53. A higher mutation frequency was observed in MDS patients where 37 (61%) out of 61 samples carried at least one somatic mutation in a spectrum of 15 genes. No mutations were found in FLT3, JAK2, KIT or NPM1. Both groups showed an average of 2 mutated genes per sample in case of clonality. The overall average mutated gene per subject was however significantly lower in the total ICUS group compared to the total MDS group (p=0.0007). A lower mean VAF of 24% was also observed in ICUS subjects compared to a mean VAF of 36% in MDS patients (p=0.0029). An overview of the 4 most frequent mutated genes is shown in the following Table 1.

Table 1.

ICUS			MDS		
Gene	# samples	Mean VAF	Gene	# samples	Mean VAF
TET2	8	24%	TET2	31	39%
TP53	3	20%	ASXL1	13	35%
SRSF2	2	32%	TP53	11	36%
DNMT3A	1	16%	SF3B1	8	35%

Summary and Conclusions: Gene panel analysis by next generation sequencing revealed the presence of somatic mutations in bone marrow aspirates of 18% patients with ICUS compared to 61% patients with MDS. Both a significant higher number of mutated genes per subject and a higher mean VAF was observed in the MDS group.

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IMPACT OF SOMATIC MUTATIONAL TESTING BY NEXT-GENERATION SEQUENCING (NGS) FOR SUSPECTED MYELODYSPLASIA OR MYELODYSPLASTIC/MYELOPROLIFERATIVE SYNDROMES IN "REAL-LIFE"

S. Vanthyghem [1], A. Ménard[1], C. Debord[1], T. Guillaume[2], O. Theisen[1], A. Garnier[2], M. Eveillard[1], S. Thepot[3], S. Wuilleme[1], A. Lebourgeois[2], C. Godon[1], M. Loirat[4], E. Lestang[4], M. Voldoire[5], M. Tiab[5], R. Boutault[1], F. Subiger[6], H. Maisonneuve[5], J. Delaunay[7], P. Chevallier[2], V. Ugo[8], M.C. Béné[1], P. Peterlin[2], Y. Le Bris[1,*]

[1]Hematology Biology, [2]Hematology, CHU Nantes, Nantes, [3]Hematology, CHU Angers, Angers, [4]Hematology, CH Saint Nazaire, Saint Nazaire, [5]Hematology, [6]Hematology Biology, CHD Vendee, La Roche Sur Yon, [7]Hematology, Le Confluent, Nantes, [8]Hematology Biology, CHU Angers, Angers, France

Background: Recurrent genomic alterations that could contribute to an integrated diagnosis have been identified in such myeloid disorders as myelodysplasia (MDS). Such somatic mutations (SM) can be screened by next-generation sequencing (NGS) but their impact on clinical decision is still ill-known.
Aims: The aim of this study was to evaluate the impact of NGS on diagnosis (group A) and prognosis assessment (group B) of myeloid disorders in 90 patients. Group A (n=48) included patients with idiopathic cytopenia of undetermined significance (ICUS), with (ICUSwtd, n=13) or without dysplasia (ICUSwod, n=18) (Bejar *et al.* 2015), suspected chronic myelomonocytic leukemia without evidence of dysplasia (sCMML, n=4) or ambiguous diagnosis between aplastic anemia (AA) and hypoplasic MDS (n=13). Group B included patients with MDS [n=26, no del(5q)], CMML (n=10) or mixed MDS/myeloproliferative syndrome (MMS, n=6).
Methods: A custom targeted panel of 35 genes (145kbp) was applied on DNA extracted from peripheral blood or bone marrow. The DNA libraries, built with a Haloplex® target enrichment protocol were sequenced by an Illumina MiSeq®. Data analysis used an in-house pipeline, public databases and *in silico* predictors. Variants of Undetermined Significance (VUS) had no clear association to disease. Final diagnoses consolidated by NGS were evaluated in group A. Group B allowed to assess patients whose prognosis and/or therapy were modified by the identification of high risk SM for MDS(Bejar *et al.* 2012) or CMML (Itzykson *et al.* 2013) (*TP53, EZH2, ETV6, RUNX1, ASXL1*).
Results: The cohort included 52 men and 39 women (median 59 yo [10-86]) with a median follow up after NGS of 10 months (0-36). Among the 48 group A patients, none of the 46 informative karyotypes was conclusive for MDS. SM were detected for 40% of group A patients (average 1 [0-5]). These mutated patients were significantly older (63 yo *vs* 46 yo p<0.001). The most frequent mutated genes were *DNMT3A* (17%), *ASXL1* (12%) and *TET2* (10%). Identification of at least 1 SM changed the final diagnosis from ICUSwtd to MDS (3/13), from ICUSwod to CCUS (7/18), and from sCMML to cCMML (2/4). *DNMT3A* and *ASXL1* mutations were found in 7/13 AA. *PIGA* and *BCOR* were both mutated in 11% of this subgroup. SM-negative NGS allowed to definitely exclude MDS in 21/48 cases with no dysplasia nor cytogenetic abnormalities. VUS were found in 21% of group A patients and 8% displayed non-contributive SM. Among the 42 group B patients, R-IPSS identified the 5 categories of risk from very low to very high as follows (%): 23-47-23-3-3. CMML patients were 70% low risk and 30% intermediate risk[2]. NGS results modified therapy for 5 group B patients (12%). SM of *ASXL1* (n=4), *EZH2* (n=2) and *RUNX1* (n=2) were found in 7 patients, who thus switched from low (6) or intermediate (1) standard risk to high molecular risk. This prompted allogeneic-stem cell transplantation (Allo-SCT) for 3 of them (MDS). Conversely 8 patients had a favourable *SF3B1* SM, which led to cancel Allo-SCT for one intermediate standard risk MDS. VUS were found in 24% of group B patients and 7% of VUS+ were detected in the high risk gene *RUNX1* but with no treatment impact.
Summary and Conclusions: NGS was confirmed here to be contributive in an integrated diagnosis of CCUS, MDS, CMML or AA for 40/48 patients. The benefit in terms of prognosis and therapeutic impact is interesting and could have even more weight if international recommendations emerge in the future through more comprehensive biological/clinical analyses of the type reported here.

PF484

PROGENITOR HYPERSENSITIVITY TO FLT3L GENERATES CLONAL PLASMACYTOID DENDRITIC CELLS IN CHRONIC MYELOMONOCYTIC LEUKEMIA

N. Lucas[1,*], M. Duchmann[1], F. Noël[2], P. Michea[2], O. Kosmider[3], G. Pierron[4], P. Rameau[4], M. Fernandez-Zapico[5], W. Lunt[5], J. Davila[5], S. Niyongere[6], M. Diop[1], P. Fenaux[7], R. Itzykson[8], C. Willekens[1], M. Fontenay[3], E. Padron[6], V. Soumelis[2], N. Droin[1], M. Patnaik[9], E. Solary[1]

[1]U1170, Gustave Roussy Cancer Campus Grand Paris, Villejuif Cedex, [2]U932, Institut Curie, [3]U1016, Institut Cochin, Paris, [4]INSERM US23, CNRS ULS3655, Gustave Roussy Cancer Campus Grand Paris, Villejuif Cedex, France, [5]Schulze center for novel therapeutics, Mayo Clinic, Rochester, Minnesota, [6]Malignant Hematology Department, H Lee Moffitt Cancer Center, Tampa, United States, [7]Senior Hematology Department, [8]department of hematology, Hopital Saint-Louis, Paris, France, [9]division of hematology, Mayo Clinic, Rochester, Minnesota, United States

Background: In the 1980s, pathologists identified the presence of irregular islands of CD123-positive cells in the bone marrow of a fraction of patients with a chronic or acute myeloid malignancy, with a strong predominance in chronic myelomonocytic leukemia (CMML). These cells were initially described as plasmacytoid T cells because of their plasma cell-like morphology and the expression of CD4, then as plasmacytoid monocytes because of the expression of myelomonocytic markers. They share somatic chromosomal

abnormalities with leukemic cells, indicating their common clonal origins. However, the mechanisms promoting the generation of these tumor nodules and their impact on disease evolution have not been explored thus far.
Aims: The present study was initiated to characterize CD123-positive cells infiltrating the bone marrow of CMML patients and their impact on disease outcome.
Methods: We set up a multiparametric flow cytometry assay to detect lineage-negative mononucleated cells expressing CD45, CD123, HLA-DR, BDCA-2, BDCA-4 and CD4 in the bone marrow of 161 CMML patients compared to 24 samples collected from age-matched healthy donors. Conventional and electron microscopy, additional cell surface markers, gene expression analyses in sorted cells (RNA sequencing) and cytokine production in response to Toll-like receptor (TLR) agonists were used to further characterize these cells. Whole exome sequencing of sorted cell populations, *ex vivo* differentiation of patient and cord blood CD34$^+$cells in the absence and presence of FMS-like tyrosine kinase 3-ligand (FLT-3L) and co-culture experiments with CD34$^+$ cells explored the origin and impact of these cells. Finally, we explored the relationship between these cells and CMML patient outcome.
Results: An increased fraction of CD45, CD123, HLA-DR, BDCA-2, BDCA-4 and CD4-positive cells was detected in mononucleated cells collected from the bone marrow of 39/161 (24%) CMML patients. All analyses converged to demonstrate that these cells were *bona fide*, interferon type I producing, plasmacytoid dendritic cells (pDCs) whose accumulation was associated with an expansion of bone marrow Tregs. A signature of 74 differentially expressed genes distinguished pDCs collected from pDC-poor and pDC-rich CMMLs. Whole exome sequencing of sorted monocytes and pDCs suggested that pDC generation may occur through the emergence of a Ras pathway activating mutation-containing sub-clone. CD34$^+$ cells from pDC-rich CMML patients produced a greater number of pDCs in culture than cord blood CD34$^+$ cells or CD34$^+$ cells from pDC-poor CMML patients, even in the absence of FLT-3L. Proliferation of CD34$^+$cells from pDC-rich CMML patients and cord blood CD34$^+$ cells were both decreased when CD34$^+$ cells were cultured with pDCs sorted from pDC-rich CMML patients, and this decrease was more important in the presence of TLR 9 agonists. Finally, retrospective immunohistopathological analysis of 216 CMML bone marrow biopsies associated pDC islands with a higher risk of leukemic transformation.
Summary and Conclusions: About one fourth of CMML patients demonstrate bone marrow enrichment in clonal pDCs through Ras-mediated hypersensivity of myeloid progenitors to FLT-3L. Therapeutic targeting of these cells, whose presence correlates with an increased risk of acute transformation, may deserve to be explored in these patients.

PF485

ASXL1 IS A MOLECULAR PREDICTOR IN IDIOPATHIC CYTOPENIA OF UNDETERMINED SIGNIFICANCE

D.-Y. Shin[1,2,*], J.-K. Park[1], S.-M. Kim[3], K. Im[3], J.-A. Kim[4], S.Y. Kim[5], S.M. Hwang[6], S.-S. Yoon[13], D.-S. Lee[34]

[1]Internal Medicine, Seoul National University Hospital, [2]Biomedical Research Institute, Seoul National University Hospital, [3]Cancer Research Institute, Seoul National University College of Medicine, [4]Laboratory Medicine, Seoul National University Hospital, Seoul, [5]Laboratory Medicine, Chungnam National University School of Medicine, Daejeon, [6]Laboratory Medicine, Seoul National University Bundang Hospital, Seongnam, Korea, Republic Of

Background: Idiopathic cytopenia of undetermined significance (ICUS) is a challenging disease for clinical hematologist.
Aims: We studied to investigate the prognostic factors for survival of patients with ICUS in the context of genetic mutations.
Methods: Patients who were diagnosed as ICUS in a single institution were enrolled in our study. Patients with myelodysplastic syndrome (MDS) were also included for the comparison of mutational profiles. Targeted sequencing of 88 genes which have been reported to be associated with myeloid malignancies were performed from bone marrow mononuclear cells of each patient. The presence of mutation as well as clinical variables were analyzed for event-free survival (EFS) and overall survival (OS).
Results: A total of 40 patients with ICUS and 128 patients with MDS were included in this study. Median age of ICUS patients was 67 yrs (range, 31 – 83), and 23 pts were female (57.5%). A total of 28 mutations in these 16 genes were detected in patients with ICUS. The median mutational burden was 0.7 mutation/person in ICUS and 2.2 mutation/person in MDS, respectively. The most frequently mutated gene in ICUS was ASXL1 (7 pts). Every patient with ICUS and mutated ASXL1 showed cytopenia of bilineage or trilineage, while only half of patients (57.6%) without ASXL1 mutation harbored cytopenia of more than one lineage (p=0.033). ASXL1 mutation was enriched in elderly population (>60 years in all 7 ASXL mutants). ASXL1 was a significant prognostic factor for EFS in univariate and multivariate analysis (HR=12.8 and 10.07, 95% CI 3.08-53.17 and 2.13-47.72, p <0.001 and 0.004, respectively). In the multivariate analysis for OS, the presence of ASXL1 mutation was an independent significant prognostic factor for OS (HR=30.63, 95% CI 3.15 – 297.71, p=0.003) (Figure 1).

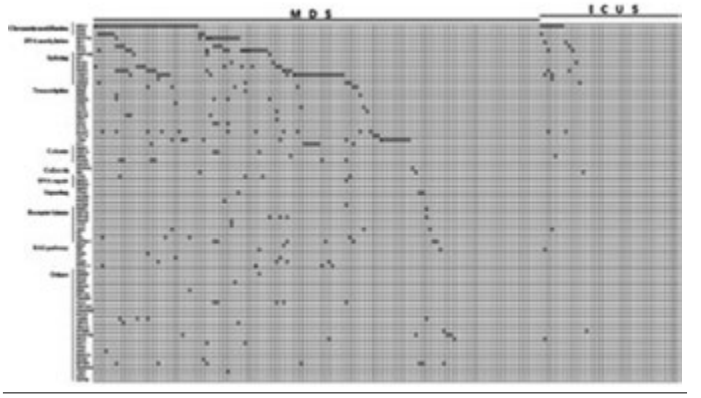

Figure 1.

Summary and Conclusions: ASXL1 mutation which is frequently detected in elderly patients is a molecular predictor for pancytopenia and survival in ICUS. A larger prospective study is warranted to validate the role of genetic mutation on the prognosis of ICUS.

PF486

MUTATIONAL PROFILE IN LOW RISK MDS WITHOUT RING SIDEROBLASTS

M. Abaigar[1,*], F. López Cadenas[2], J.M. Hernández Sánchez[1], F. Ramos[3], M. Martín Izquierdo[1], T. Bernal[4], R. de Paz[5], C. Pedro[6], M. Tormo[7], A. Insunsza[8], G. Sanz[9], B. Xicoy[10], J.C. Caballero Berrocal[11], F. Prosper[12], A. Alfonso[12], T. Jimenez Sola[1], D. Valcarcel[13], J.M. Hernández Rivas[2], M. Díez Campelo[2]

[1]Cancer Research Center-University of Salamanca (IBMCC, USAL-CIC), [2]Hematology department, Hospital Universitario de Salamanca, Salamanca, [3]Hematology department, Complejo Asistencial Universitario de León, León, [4]Hematology department, Hospital Universitario Central de Asturias (HUCA), Oviedo, [5]Hematology department, Hospital Universitario de la Paz, Madrid, [6]Hematology department, Hospital del Mar Medical Research Institute (IMIM), Barcelona, [7]Hematology department, Hospital Clínico de Valencia, Valencia, [8]Hematology department, Hospital Universitario Marqués de Valdecilla, Santander, [9]Hematology department, Hospital Universitario La Fe, Valencia, [10]Hematology department, Hospital Universitari Germans Trias I Pujol, Catalan Institute of Oncology (ICO), Badalona, [11]Hematology department, Hospital Clínico de Valladolid, Valladolid, [12]Hematology department, Clínica Universidad de Navarra, Pamplona, [13]Hematology department, Hospital Universitario Vall d'Hebron, Barcelona, Spain

Background: The molecular pattern of low risk MDS patients without ring sideroblasts (RS) have not been addressed as occurs in RS MDS patients.
Aims: The study of somatic mutations in low-risk MDS patients without ring sideroblasts (RS) could help to better define the clinical and prognostic characteristics of the disease and identify potential mutational targets to personalized medicine.
Methods: The study cohort comprised a selected group of 135 **low-risk MDS** patients (IPSS: low or int-1; IPSS-R: very low, low or int) **without ring sideroblasts** (RS<5%) recovered in our institution. Sequencing was carried out in all patients by using a targeted custom capture enrichment panel (*Nextera Rapid Capture Custom Enrichment*) consisting of 117 myeloid-related genes on an Illumina MiSeq/NextSeq instrument. Data analysis was performed with an *in-house* pipeline for quality assessment, alignment, PCR duplicates removal, and variants detection and annotation. Funding PI17/01741, GRS1349/A/16, GRS1179/A/15, FEHH 2015-16 and CM17/00171.
Results: Median age was 77.3 years (p10-p90 62.2 – 85.1) and 58.5% of the patients were males. According to the WHO 2008 classification diagnoses were as follows: RCUD (11.1%), RCMD (58.5%) y MDS with del(5q) (17%), MDS-U (5.2%) and RAEB-1 (8.2%). Ring sideroblasts (RS) count was below 5% in all of patients (p10-p90 0.0%>3.4%). Conventional cytogenetic studies (according to IPSS-R, revealed that the majority of the patients (89.6%) had normal cytogenetics or very good/good clonal abnormalities, while a 6.7% and 2.2% carried intermediate and poor/very poor clonal

lesions, respectively. Regarding prognostic classification, all patients were considered as low risk MDS, most of the patients belonged to IPSS-R categories very low (32.6%) and low (48.1%), and only 17% had an intermediate IPSS-R. During disease evolution, 49.6% of patients died and 17% progressed to a higher risk entity (3 to RAEB and 20 to sAML). A total of 287 mutations were identified in the exonic regions of 70 genes. Among them, 41 genes were recurrently mutated (≥2 patients), with *TET2* (30.4%), *SRSF2* (14%), *DNMT3A* (11%), *RUNX1* (10.4%) and *ZRSR2* (8%) the most frequently mutated ones. Of note, 55 variants were found in *TET2*, affecting 41 patients, and interestingly, 13 of these had double mutations (32%), highlighting that one of them had 3 different mutations. The remaining genes were mutated with a lower incidence, below 6% of patients. In this selected cohort a median of 2 mutations per patient was found (p10-p90 0-4), with an 80% of cases having at least one mutation in one of the studied genes. Of these, 23.7% had one mutation, 16.3% had two mutations, while 15.6%, 15.6%, 6.6%, 1.5% and 0.7% of patients had 3, 4, 5, 6 and 7 mutations, respectively and 20% did not carry any mutation. Additionally, in relation to the pathways affected by these mutations, 65% of the patients presented mutations in epigenetic regulators (*i.e.* DNA methylation, 46%; chromatin modification, 19%), 25% in transcriptional regulators and 37% in components of the RNA splicing machinery. After a median follor up of 4.66 years, median OS was 5.13 years (95CI 3.636-6.709). Mutated genes with significnatly adverse influence on OS were *EZH2* (0.012), *IDH2* (p=0.04) or *U2AF1* (p=0.011). In this sense, the number of mutations detected, >2 *vs* ≤2 also decreased survival (p=0.049, Figure 1).

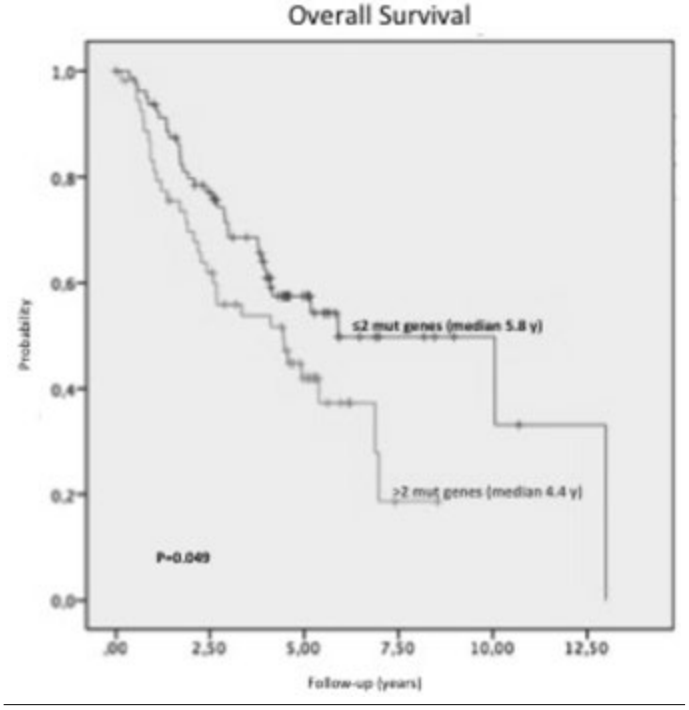

Figure 1.

Summary and Conclusions: Mutational profile in this subset of patients could help us to identify poor prognostic patients candidates to novel drugs that directly target the mutated gene and could improve prognosis in this subset of patients.

PF487

COMPARISON OF MUTATION DYNAMICS IN HIGHER RISK MYELODYSPLASTIC SYNDROME PATIENTS TREATED WITH AZACITIDINE AND DECITABINE

J. H. Moon[1,*], T. Kim[2], J.-S. Ahn[3], S.-S. Lee[3], S.-Y. Ahn[4], S.-H. Jung[5], D.-H. Yang[4], J.-J. Lee[4], S.H. Choi[6], D.W. Baek[1], Y.J. Lee[1], S.K. Sohn[1], H.-J. Kim[5], Z. Zhang[7], D.D.H. Kim[8]

[1]Hematology/Oncology, Kyungpook National University Hospital, Daegu, Korea, Republic Of, [2]Computer Science, University of Toronto, Toronto, Canada, [3]Chonnam National University Hwasun Hospital, Hwasun, Korea, Republic Of, [4]Hematology/Oncology, Chonnam National University Hwasun Hospital, [5]Hematology/Oncology, Chonnam National University Hospital, [6]Genomic Research Center for Hematopoietic Diseases, Chonnam National University Hwasun Hospital, Hwasun, Korea, Republic Of, [7]Molecular Genetics, University of Toronto, [8]Medical Oncology and Hematology, Princess Margaret Cancer Centre, Toronto, Canada

Background: Hypomethylating agents (HMAs) such as azacitidine (AZA) and decitabine (DEC) are used to treat higher risk myelodysplastic syndrome (HR-MDS). However, underlying genetics and mutational dynamics upon treatments with AZA and DEC have not been compared longitudinally.
Aims: To assess mutational dynamics on treatments between AZA and DEC, and to investigate the clinical relevance of these mutational allelic burdens using serial samples.
Methods: This study included bone marrow samples from 64 patients (34 AZA and 30 DEC) taken at diagnosis and follow-up after median 4 cycles. Targeted deep sequencing on a custom myeloid gene panel of 84 genes (Agilent SureSelect) was performed on trios of T-cell, pre-HMA, and post-HMA samples on 64 HR-MDS patients.
Results: At diagnosis, we detected 135 mutations from 54 patients. Most frequently mutated genes were *TP53* (n=9), *DNMT3A* (n=7), *ASXL1* (n=7), DDX41 (n=7) and *U2AF1* (n=6). HMAs were administered median 4 cycles (range 1-14). At follow-up, allelic burdens were decreased from mean 19.4% to 5.8%, where from 14.6% to 2.3% in patients treated with AZA (p<0.001) and from 23.4% to 10.6% in those treated with DEC (p=0.005). Nevertheless, 2-year overall survival (OS) rate did not differ between AZA and DEC groups (66.6±12.1% *vs* 60.6±13.4%, p=0.875) with median follow-up duration of 583 days (range 4-3012 days). TP53 mutations were detected in 9 patients including 4 with AZA and 5 with DEC group. CR/mCR was not achieved in the patients with TP53 mutation treated with AZA (n=0/4), while 2 patients (40%) achieved CR/mCR when treated with DEC (p=0.132). The presence of TP53 mutations did not affect the achievement of CR/mCR (p=0.379). In both groups, 2-year OS rates were lower in patients with TP53 mutations than those without: 25.0 ± 16.7% *vs* 59.7±9.3% in AZA group (p=0.057) and 20.0±17.9% *vs* 52.1±11.1% in DEC group (p=0.004). Cumulative incidence of leukemia transformation was associated with the presence of mutations in activated signaling pathway in AZA group but not in DEC group.
Summary and Conclusions: This study showed that allelic burden is decreased after treatment with both azacitidine and decitabine in HR-MDS patients. However, the mutation dynamics are independent to long-term survival. Presence of TP53 mutation remained independent prognostic factor in HR-MDS patients treated with HMAs, even with decreased allelic burden.

PF488

USING PU.1 AND JUN DIMERIZATION PROTEIN 2 TRANSCRIPTION FACTOR EXPRESSION IN MYELODYSPLASTIC SYNDROMES TO PREDICT TREATMENT RESPONSE AND LEUKEMIA TRANSFORMATION

K. Boasman[1], M. Simmonds [1], Y. Sauntharajah[2], C. Rinaldi[1,*]

[1]Life Science, University of Lincoln, Lincoln, United Kingdom, [2]Hematologic Oncology and Blood Disorders, Cleveland Clinic, Cleveland, United States

Background: Myelodysplastic syndromes (MDS) are malignant disorders of the myeloid progenitor leading to acute leukaemia (AML). Altered DNA methylation, and associated abnormal gene expression, results in aberrant cell growth and cytopenia seen in MDS. Demethylating agents are used to treat intermediate and high risk MDS with variable success. Recent breakthroughs identified significant down-regulation of transcription factor PU.1 in high risk MDS and AML patients, with work within NPM1 mutated AML cells showing PU.1 relocates in the cytoplasm causing cell differentiation arrest. In PU.1 overexpressing cell line model, microarray analysis revealed that Jun Dimerization Protein 2 (JDP2), a downstream of PU.1 which represses acetylation of core histones *in vitro* and *in vivo*, was significantly suppressed.
Aims: In this study, we show consistent and progressive reduction of PU.1 and JDP2 during MDS progression, with upregulated PU.1 and JDP2 levels only seen in patients achieving a clinically significant response to Azacitidine.
Methods: We investigated the gene expression of PU.1 and JDP2, in total bone marrow and selected CD34+ cells, from 12 newly diagnosed MDS patients stratified according to IPSS-R score (6-low, 3-intermediate, 3-high risk), 2 AML patient and 10 normal controls. Samples were enriched for the mononuclear fraction by Ficoll separation. Total RNA was extracted and analysed by Real Time PCR for PU.1 and JDP2 expression using the 2-ΔΔCT method. Protein expression was analysed by western blot. Results obtained were also compared with data from Bloodspot. PU.1-knockdown was performed in K562 using PU.1 short interfering RNAs.
Results: We revealed both PU.1 and JDP2 are down regulated in MDS patients. In addition, our data suggests that PU.1 and JDP2 expression

inversely correlates with disease, with expression of these genes consistently reducing according to IPSS-R groups. Furthermore, a positive correlation of PU.1 and JDP2 expression <R=O.9333, s=0.0004 >, provides additional evidence that suppression of JDP2 by PU.1 could contribute to the pathogenesis of AML. Investigating PU.1, and JDP2 expression data in MDS *vs* normal samples from the Bloodspot expression data showed they were significantly downregulated in MDS patients compared with normal controls, further confirming their potential role in predicting MDS progression. To confirm that JDP2 suppression is a direct result of reduced PU.1 we performed PU.1-knockdown in K562 cells stably expressing PU.1 short interfering RNAs *versus* control cells. Interestingly, these analyses reveal only a partial reduction in JDP2 expression when analysed by RT-PCR and Western blot, suggesting a more complex regulatory mechanism. During AML evolution, the expression level of both PU.1 and JDP2 in CD34+ cells, significantly and progressively reduces, suggesting their role in AML transformation but also highlight the possibility to early detection of AML transformation in MDS patient. This can allow a better stratification of MDS patients and help us in identifying the ones who might benefit sooner from a therapy change. Furthermore, in patients achieving a clinically significant response to Azacitidine, we demonstrated a significant upregulation in PU.1 and JDP2 expression compared with non-responders. This suggests that PU.1/JDP2 could be targets of the drug and their expression during treatment.

Summary and Conclusions: PU.1 and JDP2 expression correlates with patient's prognosis and leukaemia transformation in MDS, highlighting a potential role as new diagnostic and prognostic markers in MDS.

PF489

ASSOCIATION OF EMERGENT PTPN11 MUTATIONS WITH CLINICAL RESISTANCE TO THE COMBINATION OF AZACTIDINE AND RIGOSERTIB IN PATIENTS WITH HIGHER-RISK MYELODYSPLASTIC SYNDROME

L. Silverman[1,*], S. Navada[1], S. Melana[1], G. Garcia-Manero[2], R. Odchimar-Reissig[1], E.P. Demakos[1], M.E. Petrone[3], P. Zbyszewski[3], N. Azarnia[3], S.M. Fruchtman[3], E.P. Reddy[1]

[1]ICAHN School of Medicine, Mount Sinai, New York, [2]MD Anderson, MD Anderson, Houston, [3]Onconova Therapeutics, Onconova Therapeutics, Newton, United States

Background: The treatment of patients with MDS with Azacitidine (AZA) is associated with hematologic responses, and a significant increase in overall survival. AZA has become the standard of care for patients with higher-risk disease. However, all pts ultimately fail treatment due to either primary or secondary resistance. MDS, a hematopoietic stem cell disorder, is characterized by intricate complexities at the molecular, genetic and epigenetic levels contributing to the therapeutic challenge. Rigosertib (RIG) is a small molecule Ras mimetic that interferes with cellular signaling. This is believed to be mediated by the binding of RIG to the Ras-binding domain (RBD) and may inhibit multiple Ras-driven signaling pathways including RAS-RAF-MEK and the PI3K. *In vitro*, we demonstrated that the combination of RIG with AZA synergistically inhibits growth and induces apoptosis of leukemic cells in a sequence-dependent manner (Skidan *et al.*, AACR 2006). RIG combined with AZA in a phase I/II study in MDS pts demonstrated an overall response rate of 77%; 84% in pts that were HMA naïve and 64% in pts following HMA failure (Navada EHA 2017). Reversal of the clinical resistance phenotype represents a novel observation. Recently, we observed that RIG appears to act as a chromatin modifying agent and is associated with histone post-translational modifications (Silverman EHA 2017). Mutations that activate Ras signaling such as mutations in Ras genes or Ras regulators (NF1, PTPN11, or CBL) have been found in 30% of AML patients (Papaemmnanuil, 2016). Exploring the Ras pathway in patients treated with these agents can inform on potential mechanisms associated with clinical resistance.

Aims: Identify potential mechanisms of resistance

Methods: Patients participating in a clinical trial of the combination of RIG in combination with AZA had gene mutation analyses conducted serially at one of the participating institutions (Mount Sinai) while on the study. RIG was administered on day 1 -21 orally and AZA was administered parenterally on day 8 – 15. The cycle was repeated every 28 days. Serial samples were obtained from bone marrow and/or peripheral blood for NGS mutation analysis in conjunction with scheduled bone marrow samples. Data from the patients on this study were compared to a broad data set of patients in the MDS program at Mount Sinai.

Results: Comparison of changes in mutational analysis of pts treated with RIG/AZA revealed an emergence of PTPN11 mutations in a higher percentage of patients compared to the larger MDS population not treated with the combination. PTPN11 mutations were present at baseline in a total of 9 (2.7%) of 333 MDS patients studied; 4/300 (1.3%) comparator group (CG) *vs* 5/33 (15%) RIG/AZA group (P=0.0002). Of note the mutation emerged in 3 of the responding patients to RIG/AZA who had received treatment for >6 months, and the appearance of the mutation correlated with the onset of clinical resistance to the combination. The emergence of the mutation in 3 (10%) patients compares to 1 (0.03%) in the CG, P=0.0036. The appearance of the mutation was associated with progressive marrow failure and transformation to AML.

Summary and Conclusions: PTPN11 mutations occur much less frequently than other mutations in MDS. The appearance of these mutations in patients treated with the RIG/AZA combination in a disproportionately higher rate than the CG suggests that the dysregulation of the Ras pathway may lead to a clinical resistance phenotype to this combination. Further correlative studies of the Ras pathway are underway.

PF490

A NOVEL CULTURE MODEL FOR STUDYING TERMINAL ERYTHROID DIFFERENTIATION IN PATIENTS WITH SF3B1 MUTATED MYELODYSPLASTIC SYNDROMES

E.M. Elvarsdóttir[1,*], T. Mortera-Blanco[1], M. Dimitrou[1], T. Bouderlique[2], M. Jansson[1], S. Conte[1], I. Hofman[1], M. Creignou[1], M. Karimi Arzenani[1], B. Sander[2], I. Douagi[1], P. Woll[1], E. Hellström-Lindberg[1]

[1]Medicine, [2]Laboratory Medicine, Karolinska, Stockholm, Sweden

Background: Myelodysplastic syndrome with ring sideroblasts (MDS-RS) is characterized by recurrent mutations in the splicing factor gene *SF3B1* and terminal erythroid failure associated with formation of ring sideroblasts (RS). Previously established *in vitro* models used to study MDS-RS are based on CD34-enriched (CD34+) primary cells cultured in suspension, which facilitate production of erythroid progenitors and accumulation of aberrant mitochondrial ferritin. However, these models fail to support terminal erythropoiesis, including efficient production of erythroid islands and RS. Recently we demonstrated that RS can be generated in mouse bone marrow by transplanting HSCs from *SF3B1* mutated MDS-RS patients into immune-deficient mice (Mortera-Blanco, Blood 2017). We hypothesized that the bone marrow microenvironment and/or 3D structure plays a significant role in generating the MDS-RS phenotype.

Aims: The aim was to establish an erythroid culture model that simulates the human bone marrow and could be used to study terminal erythropoiesis in healthy individuals and MDS-RS patients.

Methods: Mononuclear cells (MNCs) and CD34+ cells isolated from 10 healthy individuals (NBM) and 9 MDS-RS patients were cultured for 4 weeks in scaffolds made out of collagen-coated polyurethane (3D culture), or in previously reported suspension cultures. Cells extracted before seeding and during week 2, 3 and 4 of culture were evaluated for proliferation via MTS assay, erythroid maturation by flow cytometry and self-renewal potential was assessed with long-term colony forming cell (LTC-CFC) assays. Cells were stained with Perl s Prussian blue for morphological assessment and quantification of RS and the allelic burden of *SF3B1* mutations were measured using pyrosequencing. Erythroid islands were counted after May-Grünwald Giemsa staining and visualized *in situ* with fluorescent staining of cryosectioned scaffolds.

Results: The proliferative capacity of MNCs and CD34+ cells isolated from NBM and MDS-RS patients was maintained for 4 weeks of 3D culture. In suspension however, only the MNCs grew beyond 2 weeks of culture. Cells extracted during the fourth week of NBM cultures, other than CD34+ cells in suspension, gave rise to colonies following LTC-CFC assays, indicating retained self-renewal potential. CD34+ cells cultured in 3D showed superior proliferation and generated the highest percentage of erythroid cells including enucleated erythrocytes. Importantly, *SF3B1* mutation allele frequency was maintained in all MDS-RS cultures. The RS seeded from MNCs disappeared after 2 weeks of culture followed by re-generation from the third week, coinciding with their appearance when CD34+ cells were cultured in 3D. Erythroid islands were identified in all cultures except for the CD34+ suspension.

Summary and Conclusions: We report a novel culture model able to mimic the terminal erythropoiesis of NBM and MDS-RS with ample *in vitro* generation of RS. Although we could induce terminal erythropoiesis, erythroid island formation and RS generation by seeding either MNCs or CD34+ cells, the latter produced a higher number of cells with the highest percentage of enucleated erythrocytes. Interestingly when seeding the same CD34+ cells in suspension the cells could only survive for two weeks without inducing erythroid maturation or RS generation, suggesting that the 3D simulation of the human bone marrow is important to model the disease. This culture system could be used for studying new treatment options for MDS-RS and to study other malignancies with erythroid maturation defects *in vitro*.

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PROGNOSTIC SIGNIFICANCE OF THE ASXL1 GENE ALTERATION IN PATIENTS WITH ISOLATED 20Q DELETION

J. Brezinova[1,*], I. Sarova[1], K. Svobodova[2], H. Lhotska[2], S. Ransdorfova[1], S. Izakova[2], L. Pavlistova[2], L. Lizcova[2], K. Skipalova[2], L. Hodanova[2], J. Markova[3], Z. Zemanova[2], J. Cermak[3], A. Jonasova[4], K. Michalova[2]

[1]Cytogenetic Department, Institute of Hematology and Blood Transfusion, [2]Center of Oncocytogenetics, Institute of Medical Biochemistry and Laboratory Diagnostics, General University Hospital and First Faculty of Medicine, Charles University, [3]Clinical Department, Institute of Hematology and Blood Transfusion, [4]1st Medical Department, General University Hospital and First Faculty of Medicine, Charles University, Prague, Czech Republic

Background: In bone marrow cells of patients with myelodysplastic syndromes (MDSs), acute myeloid leukemia (AML) or myeloproliferative neoplasms (MPNs) the isolated deletion of 20q [del(20q)] is the common chromosomal abnormality associated with a favourable outcome. On contrary, alterations of the *ASXL1* gene, which maps to 20q11.21 region, generally represent a poor prognosis across the spectrum of hematologic malignancies.
Aims: Our objective was to determine the frequency of *ASXL1* alterations in deletion 20q cases, to characterize breakpoints in the *ASXL1* gene using microarray techniques [array comparative genomic hybridization (aCGH)] and to evaluate the difference in survival between patients with and those without *ASXL1* alterations.
Methods: FISH with locus specific probes for 20q11, 20q12 and 20q13.12 regions (Abbott, Kreatech Diagnostics, MetaSystems) confirmed the cytogenetically observed deletions of 20q in a cohort of 39 patients (29 male, 10 female, median age at diagnosis 68 years) with hematologic disorders: MDSs (n=24), MPNs (n=10), non-Hodgkin lymphomas (n=2), AML (n=1), thrombocytopenia (n=1), anemia (n=1). In all patients, deletion of 20q was the sole aberration; however, in 3 patients, a variant of del(20q), an isochromosome of deleted 20q, was detected. Metaphase FISH mapping using a set of 5 bacterial artificial chromosome (BAC) probes (RP11-600A9, RP1-316I5, RP11-358N2, RP4-669H2 and RP5-823G15, BlueGnome, Empire Genomics) that target sequences in 20q11.21 and 20q13.2, along with a chromosome-20-specific centromeric probe (Kreatech Diagnostics) as a control, were used for determination of the breakpoints. aCGH [CytoChip Cancer 4x180K, CytoChip Cancer SNP 4x180K (Illumina); SurePrint G3 Cancer CGH+SNP Microarray 4x180K (Agilent)] was done on DNA samples of bone marrow cells of 9 patients with suspected partial deletion in *ASXL1* to characterize the breakpoints.
Results: According to the FISH results, three groups of patients were established: (1) 9 patients (23%) had a proximal breakpoint in *ASXL1* with partial deletion of the gene; (2) 11 patients (28%) had complete deletion of *ASXL1*; and (3) 19 patients (49%) had no alterations in either copy of *ASXL1*, with the proximal breakpoint of the deletion located downstream of this gene. In summary, *ASXL1* gene was altered (partially or completely deleted) in 20 out of 39 patients (51%). aCGH confirmed the high heterogeneity of the breakpoints in the *ASXL1* with the most common frequency in/downstream of the exon 4 and with partial deletion of the 3 end of the gene. Kaplan-Meier survival curves for the patients groups with and without *ASXL1* gene alterations showed significant difference (overall survival 48.6 months and 119.0 months, respectively; p=0.05).
Summary and Conclusions: Alterations of the *ASXL1* gene have a prognostic impact in patients with hematologic malignancies. In our cohort of patients with isolated del(20q), FISH with specific BAC probes revealed the *ASXL1* partial/total deletions in 51% of cases with significantly shorter overall survival. aCGH confirmed all findings implied by FISH. We consider FISH using specific BAC probes to be a reliable and robust technique, that can readily detect *ASXL1* alterations in routine analyses of del (20q) patients and that may identify those patients who need to be monitored more carefully and more frequently than those without this alteration.
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PARP1 EXPRESSION PROFILE AND DIVERGENT CLINICAL IMPACT IN MYELODYSPLASTIC SYNDROMES AND CHRONIC MYELOMONOCYTIC LEUKEMIA

A.M. Hurtado López[1,*], T.H. Chen-Liang[1], L. Palomo[2], E. Lumbreras[3], M. Díez-Campelo[4], L. Zamora[2], F.J. Ortuño[5], V. Vicente[1], C. del Cañizo[4], F. Solé[2], A. Jerez[1]

[1]Hematology and Medical Oncology Department, Hospital Morales Meseguer, IMIB, Murcia, [2]Josep Carreras Leukaemia Research Institute, ICO-Hospital Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, [3]Hematology, Hospital Universitario de Salamanca, [4]Hematology, Hospital Universitario de Salamanca, Salamanca, [5]Hematology and Medical Oncology Department, Hospital Morales Meseguer, IMIB, Murcia, Spain

Background: Clinical, pathological, and genomics disparities with MDS suggest that specific underlying biological mechanisms exist within CMML that might confer differential sensitivity to treatment. Independent studies have shown that neoplastic cells with homologous recombination-defective lesions are notably sensitive to *PARP1* inhibition.Recently, it has been reported in MDS patients, that mRNA PARP1 levels distinguish a subgroup with significant survival disadvantage, but there is a lack of information about *PARP1* in CMML.
Aims: To characterize mRNA *PARP1* expression in bone marrow at diagnoses in a large series of patient with MDS and CMML, assessing its potential clinical impact.
Methods: We measured by RT-qPCR, using TaqMan gene expression assays, mRNA *PARP1* in 82 CMML and 101 MDS bone marrow samples collected at diagnosis and 15 bone marrow samples from healthy donors. To validate our findings in the common cell of origin of myeloid neoplasms, CD34+ cells were enriched from thawed BM of 15 CMML patients, 15 MDS cases, and 15 controls and studied by RT-qPCR.We used Receiver Operating Curves (ROC) to determine the best *PARP1* expression cut-off point to discriminate prognoses, both in MDS and CMML. We performed univariate COX for overall survival and time to AML transformation. In case significant results were obtained, a multivariate analysis confronting IPSS-R or CPSS was planned.
Results: The MDS cohort included one hundred and one cases: 53% were male and median age was 69 y.o. (30-90). At a median follow-up of 20 months, 12% of patients experienced leukemic transformation. Compared with BM samples from 15 controls, we found a significant downregulation of *PARP1* in CMML (0.6 FC, p=0.04), and a statistical trend for downregulation in MDS cases (0.8 FC, p=0.09). We obtained similar results when assessing *PARP1* expression in bone marrow CD34+ cells from CMML (0.45 FC, p=0.08), MDS (0.25 FC, p=0.06), when comparing whit healthy donors. Those MDS patients with higher *PARP1* mRNA level (best sensitivity and specificity ROC cut-off point >0.00175) had a median time of survival of 22 months compared with 84 months in those cases with lower levels of *PARP1* mRNA (*P*=0.009; RR=2.3;95%CI[1.2-4.5]). When we performed a multivariate analysis including mRNA *PARP1* levels and IPSS-R (dichotomized as high risk-very high and high- *versus* low risk-intermediate, low and very low) both of them reached a statistical significance (*P*=0.016; RR=2.2;95%CI[1.1-4.3]) and (*P*=0.009; RR=7.5;95%CI[2.82-19]) respectively.The CMML cohort included eighty-two patients: 63% were male and median age was 74 y.o. (44-91). Conversely, no significant differences in survival were observed between those CMML patients with higher PARP1 mRNA levels, with a median survival of 36 months, compared to 39 months in patients with lower levels (cut-off point >0.00175; P=0.9;RR=0.9;95%CI[0.5-1.8]). Even when recalculating the best sensitivity and specificity ROC cut-off point for the CMML cohort (>0.00143), no significant differences in survival were found (P=0.66;RR=0.8;95%CI[0.4-1.6]).
Summary and Conclusions: In conclusion, we have identified important disease-specific differences for *PARP1* mRNA expression and its prognostic relevance comparing MDS and CMML cases, with potential translational ramifications for the positioning of novel therapeutic strategies. Our findings suggest that the promise of *PARP1* inhibition appears less pertinent to CMML than for other myeloid diseases. These differences might also shed insights into their distinctive biology.

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CHROMATIN REGULATORS AS RESPONSE-PREDICTING BIOMARKERS OF AZACITIDINE THERAPY IN MDS PATIENTS

J. Diesch[1,*], K. Götze[2], J. Zuber[3], L. Zamora[4], M. Buschbeck[1]

[1]Josep Carreras Leukaemia Research Institute, Badalona, Spain, [2]Department of Medicine III, Klinikum rechts der Isar, Technische Universität München, München, Germany, [3]Research Institute of Molecular Pathology (IMP), Vienna, Austria, [4]Hematology Laboratory, ICO, Josep Carreras Leukaemia Research Institute, Badalona, Spain

Background: Myelodysplastic syndrome (MDS) is one of the most common bone marrow disorders in the elderly characterized by an ineffective hematopoiesis and strong predisposition to acute myeloid leukemia (AML). MDS is associated with a variety of genetic modification, many of which affect epigenetic regulators, however their biological and prognostic significance is not well understood. Currently the demethylating agent azacitidine (AZA) and its analogs are the major treatments given to high-risk MDS

patients. Nevertheless, only 40% to 50% of treated patients show hematological improvements and 10% to 15% can achieve complete response. However, even in responding patients, the drug is not curative and response is lost over time leading to disease relapse. Therefore, response-predicting biomarkers and new treatment options are urgently needed.
Aims: The goal of this project is to identify response-predicting biomarkers for AZA therpy and new treatment options to improve the clinical management of MDS patients.
Methods: To identify genes affecting AZA sensitivity and resistance we performed two loss-of-function short-hairpin high-throughput screens targeting 912 chromatin regulators in the AML/MDS cell line SKK1. The first shRNA screen was set out as resistance screen using a high AZA concentration only allowing the survival of resistant cell clones. Additionally, we performed a sensitivity shRNA screen using lower AZA concentrations and thus focusing on genes affecting drug sensitivity rather than resistance. In parallel, we are performing a longitudinal study of AZA treatment in MDS patients. Therefore, we are collecting samples at diagnosis, during treatment and, if occurring, at relapse and will determine gene expression changes as well as the mutational status in disease relevant genes.
Results: Since AZA is a chromatin-modifying agent, this project is focused on chromatin and transcriptional regulators. Performing loss-of-function shRNA screens targeting 912 chromatin regulators led to the identification of several genes conferring resistance and differential responses to AZA treatment. While some of these genes have already been implicated in hematological diseases, others have never been mentioned in this context and thus are currently being examined in greater detail. Furthermore, the expression of selected top hits is currently being assessed in a cohort of patient samples and correlated to treatment response, thus allowing to identify potential markers of treatment success or failure. In addition, we identified genes whose knockdown causes greater sensitivity towards AZA treatment. Hence, targeting these genes could potentially be employed to improve AZA response, and thus treatment success. This is currently being assessed in drug combination studies.
Summary and Conclusions: The combination and cross-validation of the results of our clinical and experimental approaches will help to identify markers of AZA response as well as dissect the molecular mechanisms underlying AZA action. This will not only improve patient stratification but also enable us to establish novel strategies for therapeutic intervention.

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MECHANISTIC CHARACTERIZATION OF HDACI MEDIATED KIT DOWNREGULATION IN SYSTEMIC MASTOCYTOSIS

K. Lyberg[1,*], H. Abdulkadir Ali[1], J. Ungerstedt[2]

[1]Medicine Huddinge, Karolinska Institutet, Huddinge, [2]Institutionen för medicin, Huddinge (MedH), H7, Enheten för hematologi, M 54, Karolinska Universitetssjukhuset Huddinge, Karolinska Institutet, Stockholm, Sweden

Background: In systemic mastocytosis, there is an accumulation of mast cells in various tissues due to a point mutation in the tyrosine kinase receptor KIT rendering it continuously active. We have previously shown that histone deacetylase inhibitors (HDACi) induce apoptosis selectively in KIT mutated SM mast cells by downregulation of KIT at both mRNA and protein level.
Aims: The aim of this project is to elucidate the mechanism of action of HDACi induced mast cell apoptosis.
Methods: To further understand the mechanism of action of HDACi in SM, we have used the KIT mutated SM cell line (HMC-1.2). Following treatment with HDACi we have performed RNA seq to investigate alterations of the transcriptome, ATAC seq for whole genome nucleosome positioning and ChIP seq to assess repressive (H3K27me3) and active (H3K27ac, H3K4me3) chromatin marks.
Results: Already at 24h of HDACi treatment, there was a profound decrease in H3K4me3 in the KIT promoter region, and gene ontology analysis shows that the most silenced pathways (decreased H3K4me3) all are downstream pathways of KIT (*e.g.* MAPK cascade, phosphorylation). This is also evident when studying the RNA seq data, already at 6 hours but more profoundly at 24 hours of treatment with HDACi several regulators in KIT downstream pathways are significantly downregulated, *e.g.* STAT5, AKT, MAPK and FYN. Gene ontology analysis of RNA seq data shows upregulation of genes involved in negative regulation of phosphorylation and kinase activity, and downregulation of genes involved in heterochromatin formation processes.
Summary and Conclusions: Combining these results demonstrate that HDACi treatment silences not only KIT but also directly targets KIT downstream signaling pathways. We are now further analyzing the ChIP seq data from the additional active, repressive chromatin marks, and the ATAC seq data to understand the HDACi mechanism of action and selectivity for KIT mutated mast cells, to understand how we can use HDACi in the clinic for systemic mastocytosis, a disease that currently has limited treatment options.

Myelodysplastic syndromes – Clinical

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MYELODYSPLASTIC SYNDROMES WITH HYPOCELLULAR MARROW: CLINICAL CHARACTERISTICS AND EVALUATION OF OUTCOME

V. Santini[1,2,*], D. Gioia[2], E. Masiera[2], A. poloni[3], D. Ferrero[4], A. Fragasso[5], C. Finelli[6], F. Salvi[7], G. Gaidano[8], E.N. Oliva[9], M. Catarini[10], G. Cametti[11], M. Bonferroni[12], P. Danise[13], R. Tassara[14], B. Allione[15], D. Cilloni[16], A. Castelli[17], R. Centurioni[18], G. Ciravegna[19], G. Visani[20], P. Musto[21], E. Balleari[22]

[1]Experimental and Clinical Medicine, MDS UNIT- Hematology AOU Careggi-university of Florence, Florence, [2]FISMonlus, Alessandria, [3] Clinica di Ematologia, Dipartimento Scienze Mediche e Chirurgiche Ospedali Riuniti di Ancona, Ancona, [4]Ematologia U, AOU Città della Salute e della Scienza, Torino, Torino, [5]Ematologia Azienda Sanitaria Locale di Matera, Matera, [6]Istituto di Ematologia e Oncologia Medica – Policlinico S. Orsola Malpighi di Bologna, Bologna, [7]S.C. Ematologia, AO SS. Antonio e Biagio e C. Arrigo di Alessandria, Alessandria, [8]Ematologia, AOU Maggiore della Carità, Novara, Novara, [9]A.O. "Bianchi-Melacrino-Morelli" di Reggio Calabria, Reggio Calabria, [10]U.O. di Medicina, ASUR, Zona Territoriale 9 – Distretto di Macerat, Macerata, [11]U.O.A. di Medicina- Ospedale Maggiore di Chieri, Chieri, [12]Divisione di Ematologia ASO S. Croce e Carle di Cuneo, Cuneo, [13]Medicina Interna e Onco-Ematologica- Presidio Ospedaliero Umberto I – ASL SA 1 di Nocera Inferiore, Nocera inf, [14]SC Medicina Interna – Ospedale San Paolo- ASL 2 Savonese di Savona, Savona, [15]Ematologia, AOU Città della Salute e della Scienza, Torino, Torino, [16]daniela.cilloni@unito.it, SCDU Medicina interna 2 ad indirizzo Ematologica AOU San Luigi Gonzaga di Orbassano, Orbassano, [17] Divisione di Medicina B Ospedale degli Infermi di Biella, Biella, [18]Divisione di Ematologia Ospedale Civile di Civitanova Marche, Civitanova Marche, [19]Medicina Interna, Ospedale Cardinal Massaia di Asti, Asti, [20]Divisione di Ematologia Ospedale San Salvatore di Pesaro, Pesaro, [21] Dipartimento Onco-Ematologico IRCCS CROB di Rionero in Vulture, Rionero in Vulture, [22]Dipartimento di Emato Oncologia Clinica di Medicina Interna 3 – AOU San Martino, Genova, Italy

Background: Myelodysplastic syndromes (MDS) may be characterized by hypocellular marrow, irrespective of their WHO classification or molecular characteristics. The prognostic weight of this specific feature must still be completely evaluated. MDS with hypocellular marrow tend to be considered an aplastic anemia "overlap syndrome" or a pre-aplastic anemia stage. There are no strong specific therapy recommendations, and large studies analyzing the outcome of hypocellular MDS are lacking. While selective sensitivity to immunosuppressive therapy is suggested, evidence in this sense is controversial.
Aims: We wanted to evaluate the clinical characteristics,outcome and choice of therapy of patients with hypocellular MDS, and compare them with normocellular MDS.
Methods: We analyzed 2559 consecutive MDS cases with complete clinical annotations and with evaluable bone trephine biopsy, enrolled in our Italian National Registry FISMonlus. In this cohort of patients, 438 had a bone marrow cellularity <= 30%,and 2121 cellularity above 30%. We proceeded by comparing these two groups in terms of age, gender, WHO classification, IPSS-R categories,overall survival and choice of first line therapies.
Results: Median age was 72.5 yrs in the hypocellular group and 72,3 yrs in the normocellulare group; M/F were 53.2%/46.8% for hypocellular MDS *vs* 62.6%/37.4% in normocellular MDS. IPSS-R risk categories were distributed as follows: Hypocellular MDS Very Low risk 15.5%,Low 35.1%, Intermediate 30.1%, High 11.3%, Very high 8%; Normocellular MDS Very Low risk 12.8%, Low 37.2%, intermediate 23.7%, High 15.5%, Very High 11.4%. Global median overall survival (OS) was 77 months for hypocellular MDS and 56 months for normocellular MDS. When OS was evaluated in the different IPSS-R risk groups, Lower risk MDS cases with hypocellular BM had a median OS of 125 mos while normocellular had a median OS of 74 mos (p<.001). Higher risk MDS with hypocellular BM had 19 mos median OS *vs* 20 mos OS in normocellular MDS. Regarding the choice of firts line therapy, the comparison of hypocellular MDS with normocellular ones yielded the following: watch and wait 33.8% *vs* 31.6% for IPSS-R lower risk, 12.1% *vs* 16% for higher risk cases; AML like chemotherapy and HSCT were chosen for<1% of lower risk cases overall, and in 1.7% of higher risk hypocellular MDS, while higher risk MDS with normocellular marrow received it in 6.2% of the cases. Azacitidine was first line treatment of choice for 36.2% of the higher risk MDS patients with hypocellular BM and 25% of normocellular BM. Immunosuppressive treatments were emploied for <1% and 1.5% respectively in lower risk cases only. Erythroid stimulating agents were administered in 42.6% and 41.2% MDS IPSS-R lower risk, hypo- and normocellular, respectively.
Summary and Conclusions: Our results are based on an unbiased analysis

of "real life" MDS patients with hypocellular BM compared to normocellular ones.Clinical characteristics between the two groups were not significantly different in terms of age, gender, and distribution in the various IPSS-R risk categories.The outcome of the hypocellular marrow-MDS cases was better in comparison with that of normocellular MDS, with significantly longer OS in IPSS-R lower risk cases. Such advantage in OS for hypoplastic MDS was not present for IPSS-R higher risk cases. Finally, the choice of first line therapy dose not seem to be influenced at all by the BM cellularity, with a surprising very low proportion of patients receiving immunosuppressive agents, despite several guideline recommend of this treatment for hypoplastic MDS.

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DESCRIPTION OF MUTATIONAL PROFILE IN PATIENTS WITH LOWER-RISK MYELODYSPLASTIC SYNDROMES AT THE TIME OF LEUKEMIC PROGRESSION

B. Pedrote[1,*], J.F. Falantes[1], E. Carrillo[1], C. Calderon-Cabrera[1], F.J. Marquez-Malaver[1], I. Espigado[1], J.A. Pérez-Simón[1]

[1]Hospital Universitario Virgen del Rocio, Seville, Spain

Background: Despite refinement on prognostic stratification in myelodysplastic syndromes (MDS), clinical heterogeneity, particularly in the group of lower risk disease (LR-MDS), characterizes these disorders. Recent progress in mutational analysis supports the prognostic value of certain somatic mutations not only with regard to overall survival but also with response to azacitidine (AZA) [1-3]. By contrast, mutational profile at the time of leukemic progression remains poorly characterized.
Aims: A recent analysis from Chronic Myeloid working group of the International Cancer Genome Consortium detected *ASXL1* and *RUNX1* as most frequent mutations in 3 out 7 LR-MDS patients with mutational data available at progression [4].
Methods: Retrospective analysis of a large cohort (N=409) of patients with primary LR-MDS [defined as either an IPSS score of low/intermediate-1 and/or favorable cytogenetic categories (good/intermediate by IPSS or very good/good/intermediate by IPSS-R)] followed until progression to acute myeloid leukemia (AML) with mutational analysis available at the time of leukemic progression. The Ion AmpliSeq™ AML Cancer Research Panel targeting 19 genes implicated in AML (entire coding regions: *CEPBA, DNMT3A, GATA2, TET2, TP53; hotspot regions: ASXL1, BRAF, CBL, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, NPM1, NRAS, PTPN11, RUNX1, WT1*) was used to detect driver mutations in an "Ion S5™ Sequencer". Nonsense, frameshift and splicing mutations with frequency >3% were considered for analysis. Somatic confirmed mutations in COSMIC database were considered pathogenic and mutations not described in COSMIC but with a pathogenic prediction, were considered probably pathogenic.
Results: After 103 months median follow-up for the entire cohort (95% CI: 81-123), 66 patients (16%) progressed to AML. Data on somatic mutations at the time of leukemic progression was available in 17 patients (25%). Median time from diagnosis of MDS to leukemic progression was 10.2 months (range: 2-98 months). Mean number of somatic mutations detected per patient was 2 (range: 1-5), being *RUNX1* (5 pts; 29,4%) and *TET2* (5 pts; 29,4%) the most frequently mutated, followed by *CBL* and *GATA2* (found in 4 pts each; 23,5%) and *TP53, DNMT3A,CEBPA, ASXL1, BRAF, IDH2, JAK2, KRAS, NPM1 and WT1* (detected in 3 pts, each; 17.6%). According to WHO classification, refractory cytopenia with multilineage dysplasia was the most frequent MDS subtype. Karyotype was diploid in 12/17 cases at MDS diagnosis. Four pts acquired additional cytogenetic abnormalities at AML progression (pts#2, 10, 14 and 15 in Table 1). Interestingly, 3 out of those 4 had *TP53* mutation at progression, all of them with complex karyotype at AML evolution, with 1 remaining pt presenting *TET2, ASXL1* and *CBL* mutation. Ten pts received AZA at AML progression. Among AZA treated pts, 3/10 experienced long lasting and complete responses (2 pts with *TET2* and *CBL* and 1 pt with *TP53* mutations) while pts with remaining mutations identified at leukemic progression did not or lost response promptly.
Summary and Conclusions: This study shows a heterogeneous mutational landscape in MDS at progression to AML, some of them displaying a higher frequency than expected at MDS diagnosis (>29% pts had *RUNX* and 23,5% pts had *CBL* mutation at progression compared to 10-20% and 1% reported in MDS, respectively). Whether these represents ancestral mutations or were present as small subclones and expanded over time needs to be clarified in larger studies. Risk models including mutation data should evaluate this information, not only predicting survival, but also, the probability of leukemic progression.

Table 1.

Main baseline characteristic of patients and mutation information

Patient	MDS subtype	IPSS-R	Karyotype[1]	Karyotype[2]	Mutated genes	AZA Response
#1	RCMD	0.5-3.0	46,XY[20]	[illegible]	RUNX1	NR
#2	CMML	0	46,XY[20]	[illegible]	TET2, JAK2, CBL, RUNX1	CR
#3	RCMD	0-2	46,XX[20]	46,XX[20]	CBL	NA
#4	RA	0.5-2	46,XY[20]	46,XY[20]	TET2, TP53,CBL,NRAS	-
#5	RCMD		47,XX,+8[20]	47,XX,+8[20]	GATA2,PTPN11, RUNX1	NR
#6	CMML	0	46,XX[20]	46,XX[20]	TET2, NRAS	CR
#7	CMML	1.0	46,XY,del(7)(q11.2q34)[8]/46,XY[12]	46,XY,der(7)(q11.2)[20]	GATA2,CBL	CR
#8	RCMD	0.5-2.0	46,XY[20]	46,XY[20]	RUNX1, KIT	-
#9	RCMD	0.5-2.5	46,XX[20]	46,XX[20]	[illegible]	NR
#10	RCMD	1.0-4.5	46,XX[20]	Complex (>5 abn)	TP53, RUNX1	-
#11	5q	0-2.0	46,XX,del(5)(q13)[20]	46,XX,del(5)(q13)[20]	[illegible]	PR
#12	RCMD	0.5-6.0	46,XY[20]	Dil	TET2	-
#13	RCMD	0.5-3.0	46,XY[20]	46,XY[20]	PTPN11, RUNX1	-
#14	5q	0-2.0	46,XY,del(5)(q22q33)[8]/46,XY[12]	Complex (>5 abn)	TP53	-
#15	RCMD	-	Dil	Complex (>5 abn)	TP53	CR
#16	CMML	0.5-2.0	46,XY[20]	46,XY[20]	KIT	-
#17	CMML		46,XY[20]	46,XY[20]	[illegible]	NR

[1]Karyotype at MDS diagnosis
[2]Karyotype at AML progression
RCMD: Refractory cytopenia with multilineage dysplasia; CMML: Chronic myelomonocytic leukemia; RA: Refractory anemia; Dil: Inefficient metaphases; NR: No response; CR: Complete remission, NA: Not evaluable (less than 4 courses); PR: Partial remission

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CLONAL EVOLUTION IS MUCH MORE FREQUENT IN MDS AS KNOWN AS YET

C. Ganster[1,*], K. Shirneshan[1], S. Dierks[1], R. Martin[1], B. Glass[2], H.K. Al-Ali[3], M. Metz[4], U. Germing[5], U. Platzbecker[6], C. Müller-Thomas[7], K. Götze[7], N. Kröger[8], F. Braulke[1], J. Schanz[1], L. Trümper[1], D. Haase[1]

[1]Clinics of Hematology and Medical Oncology, University Medicine Göttingen, Göttingen, [2]Clinic for Hematology, Oncology, Tumor Immunology and Palliative Care, Helios Clinic Berlin Buch, Berlin, [3]Universitätsklinikum Halle, Halle (Saale), [4]Joint Practice Göttingen, Göttingen, [5]Department of Hematology, Oncology and Clinical Immunology, Heinrich Heine University Düsseldorf, Düsseldorf, [6]Department of Internal Medicine I, University Hospital Carl Gustav Carus Dresden, Dresden, [7]III. Department of Medicine, Technical University of Munich, Munich, [8]Department of Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Background: The acquisition of genetic aberrations during the course of the disease (clonal evolution, CE), is known to be associated with poor outcome in MDS. Considering only cytogenetic aberrations (CA), up to almost 30% of MDS patients are affected by CE. Little is known about the additional impact of cryptic CA, that cannot be detected by chromosomal banding analysis (CBA), and molecular mutations (MM, *e.g.* point mutations, indels) on the incidence of CE.
Aims: We aimed to identify the true incidence of CE in MDS by including obvious CA, cryptic CA, and MM in our study.
Methods: To accomplish this goal we retrospectively reviewed 108 pts with proven MDS (35x MDS MLD, 19x MDS EB2, 12x MDS SLD, 13x sAML, 12x MDS EB1, 6x CMML, 3x MDS del(5q), 3x MDS U, 2x hypoplastic MDS, 2x ICUS, 1x MDS/MPN-RS-T). The karyotype from CBA, and the molecular karyotype from SNP-array analysis (SNP-A) were available for all pts. Cryptic CA were detected by SNP-A and fluorescence *in situ* hybridization (FISH) analyses. Results from mutational analysis were included for 73 pts. Genetic follow-up by bone marrow and/or frequent sequential genetic monitoring of CD34+ peripheral blood cells was available for 61 pts.
Results: By CBA CA were detected in 59/108 pts (55%, at any time during the course of the disease). In 36 pts we detected cryptic CA which increased the number of informative cases to 65/108 (60%). Included were cryptic copy number (CN) variations in 12 pts and CN neutral losses of heterozygosity (CN-LOH) in 24 pts. We identified a homozygous MM or a cryptic deletion in the region of the CN-LOH in 17 pts. Affected were *EZH2* (7q36.1, 5 pts), *DNMT3A* (2p23.3, 3 pts), *RUNX1* (21q22.12, 3 pts), *CBL* (11q23.3, 2 pts), *TET2* (4q24), *IKZF1* (7p12.2), *TP53* (17p13.1),

and *SRSF2* (17q25.1). CE by the stepwise formation of a "double hit" aberration from a heterozygous MM to a homozygous MM due to a cryptic CN-LOH was observed in consecutive samples of 3 pts. Regarding the pts with genetic follow-up data and taking obvious CA, cryptic CA and MM into account, CE was detected in 35/61 (57%) pts. The median observation time was 42 months. Although 31/61 pts were genetically analyzed under treatment with disease modifying therapies, the first CE event was observed before treatment in 26/35 pts. Furthermore, 19/35 pts had <5% bone marrow blasts at first CE. Median time from first diagnosis to first CE was 13 months (range 0-176 months). Manifestation of antecedent CE at first diagnosis was observed in 8 pts (2x sub-clones; 6x homozygous MM in CN-LOHs). In 27 pts first observation of CE was after first diagnosis. There was no significant difference in median survival from time of detection of CE between these two groups (25 *vs* 18 months, P=0.92, n.s.). However, for pts without CE median survival from first diagnosis was significantly longer (78 months, P=0.04 and 0.01). While just 7/27 (26%) pts with first detection of CE after first diagnosis showed further CE events, subsequent CE events were observed in 7/8 (88%) of pts with patterns of CE at diagnosis.

Summary and Conclusions: There is a clear tendency that cytogenetic LOH preferentially affects regions with MM, resulting in homozygous MM. The development of these patterns seems to be an important step towards progression. Since we considered obvious CA, cryptic CA, and MM, the incidence of CE was much higher in our cohort of MDS pts than observed ever before in MDS. The occurrence of CE is important for therapy management of MDS patients, especially if targeted therapies are used in future and the target is moving over time.

PF498

MUTATIONAL AND SUBGROUP ANALYSES OF LOWER-RISK MYELODYSPLASTIC SYNDROMES (MDS) PATIENTS TREATED WITH LUSPATERCEPT: PHASE 2 PACE-MDS STUDY

U. Platzbecker[1,*], A. Giagounidis[2], P. Kiewe[3], U. Germing[4], K. Götze[5], K. Mayer[6], M. Radsak[7], T. Wolff[8], J. Chromik[9], U. Oelschlägel[1], J. Reynolds[10], C. Barron[10], C. Rovaldi[10], R. N. Suragani[10], X. Zhang[10], A. Laadem[11], P.G. Linde[10], M.L. Sherman[10]

[1]Universitätsklinikum Carl Gustav Carus, Dresden, [2]Marien Hospital Düsseldorf, Düsseldorf, [3]Onkologischer Schwerpunkt am Oskar-Helene-Heim, Berlin, [4]Universitätsklinikum Düsseldorf, Düsseldorf, [5]III. Department of Medicine, Hematology and Medical Oncology, Technical University of Munich, Munich, [6]University Hospital Bonn, Bonn, [7]Johannes Gutenberg-Universität, Mainz, [8]OncoResearch Lerchenfeld UG, Hamburg, [9]Universitätsklinikum Frankfurt, Goethe Universität, Frankfurt/Main, Germany, [10]Acceleron Pharma Inc, Cambridge, [11]Celgene, Summit, United States

Background: Serious hematologic conditions such as MDS are associated with an erythroid maturation defect leading to anemia and other clinical sequelae. Luspatercept (ACE-536) is being developed as an erythroid-maturation agent (EMA) for treatment of anemia in lower-risk MDS. Luspatercept binds to select TGF-β superfamily ligands reducing aberrant Smad2/3 signaling and promoting late-stage erythroid maturation and increased hemoglobin (Hgb) levels (Suragani R, Nat Med, 2014; Platzbecker U, Lancet Oncol, 2017).

Aims: This ongoing, phase 2, multicenter, open-label study followed by a long-term extension (ext) study evaluates the effects of luspatercept in pts with lower-risk MDS. Endpoints include long-term safety and tolerability, erythroid response (IWG HI-E), RBC transfusion independence (RBC-TI, ≥8 weeks), duration of HI-E, pharmacodynamic and iron metabolism biomarkers, and pt-reported quality of life (QoL). New analyses focus on mutational and bone marrow characteristics of luspatercept responders.

Methods: Inclusion criteria: MDS IPSS low or int-1, age ≥18 yr, Hgb <10 g/dL (if <4U RBC/8 weeks), no prior HMA, and no current lenalidomide or erythropoiesis-stimulating agent (ESA). Pts are treated every 3 weeks subcutaneously for up to 5 doses (titration up to 1.75 mg/kg) in the base study (NCT01749514) and are then eligible for long-term treatment up to 5 additional years (NCT02268383). Baseline bone marrow and blood samples were analyzed by central morphology and flow cytometry according to ELN guidelines for evaluation of erythroid precursors and soluble transferrin receptor.

Results: Data (as of 08Sept2017) were available for 88 pts treated at dose levels ≥0.75 mg/kg with evaluable mutational data at baseline. All 88 pts were evaluable for IWG HI-E. Increased response rates were seen in pts with EPO <500 IU/L. Response rates were also increased in the SF3B1 mutated group *vs* the non-SF3B1 mutated group. Responders in both the SF3B1 mutated and non-SF3B1 mutated groups had lower M/E ratios at baseline than non-responders with a parallel increase in erythroid precursors (Table 1).

Table 1.

	SF3B1 mutated N=46		non-SF3B1 mutated N=42*	
HI-E Response Rate, n/N (%)	33/46 (72%)		15/42 (36%)	
EPO < 500 IU/L	29/40 (73%)		14/29 (48%)	
EPO ≥ 500 IU/L	4/6 (67%)		1/13 (8%)	
	R	NR	R	NR
n	33	13	15	27
Erythroid cells and precursors (%, bone marrow morphology)	50	38	34	17
M/E ratio (bone marrow morphology)	1.00	1.63	1.98	4.00
Erythroid precursors (%, flow cytometry)	13.07	8.43	8.39	4.05
Soluble transferrin receptor (nmol/L)	63.6	57.8	42.4	34.9

Data presented as median
NR=non-responder; R=responder
*Non-SF3B1 group includes RS+ patients without an SF3B1 mutation

Summary and Conclusions: Increased response rates in patients with lower M/E ratios suggest that an expanded erythroid population at baseline may be associated with response to luspatercept, supporting the concept that luspatercept is acting as an erythroid-maturation agent (EMA). Patients with and without the SF3B1 mutation with EPO <500 IU/L experienced substantial response rates, consistent with earlier results. Additional analyses are ongoing to understand the role of other biomarkers such as mutation status and how they may impact the biology and influence response. As there is not one distinct profile of responder characteristics, this may suggest the possibility that luspatercept may work in a broad range of genotypes and phenotypes.

PF499

ROLE OF SOMATIC MUTATIONS IN PATIENTS WITH LOW-RISK MYELODYSPLASTIC SYNDROMES TREATED WITH ERYTHROPOIESIS STIMULATING AGENTS

J.C. Caballero[1,*], J.M. Hernández Sánchez[2], M. Abáigar[2], F. Ramos[3], J. Cervera[4], E. Such[4], E. Luño[5], B. Navarro[6], M. Tormo[6], C. Chillón[7], M. Martín Izquierdo[2], K. Janusz[2], C. Robledo[2], E. Lumbreras[2], M. Cabrero[1], F. López Cadenas[1], A. Redondo Guijo[1], J.M. Hernández Rivas[2,8], C. Del Cañizo[1], M. Díez Campelo[1]

[1]Hematology Department, Hospital Universitario de Salamanca, [2]Centro de Investigación del Cáncer (CIC), IBSAL, Salamanca, [3]Hematology Department, Hospital Universitario de León, León, [4]Hematology Department, Hospital Universitario La Fe, Valencia, [5]Hematology Department, Hospital Universitario Central de Asturias, Oviedo, [6]Hematology Department, Hospital Clínico Universitario de Valencia, Valencia, [7]Molecular Biology Department, [8]Hematology, Hospital Universitario de Salamanca, Salamanca, Spain

Background: Up to 90% of patients with Low-Risk Myelodysplastic Syndromes (LR-MDS) have somatic mutations in driver genes by Next Generation Sequencing (NGS). Erythropoiesis Stimulating Agents (ESAs) are the most frequently used therapy for anemia in these patients.

Aims: To evaluate the impact of somatic mutations in patients with LR-MDS receiving ESA.

Methods: The results of HSCT in 65 MDS patients from five centres in Spain were retrospectively analyzed. DNA from bone marrow samples from diagnosis was screened for somatic mutations by NGS, using a MiSeq platform (Illumina) and a 117 myeloid genes panel. This project has received the following funds: PI17/01741; GRS 1349/A/16; GRS 1179/A/15; CM17/00171.

Results: We selected 24 patients (36.9%) with a prolonged erythroid response (>1 year) and 41 (63.1%) without response (no statistical differences between both groups). Median age was 76 years (p25-p75 78-80) and 50.8% were male. According to WHO 2008 classification 4 (6.5%) were RCUD, 18 (29%) RARS, 25 (40.3%) RCMD, 1 (1.6%) RAEB-1, 1 (1.6%) RAEB-2, 5 (8.1%) 5q- syndrome, 3 (4.8%) Unclassifiable MDS, 4 (6.5%) CMML and 1 (1.6%) was other MDS/MPL. Among patients with calculated IPSS (IPSS) (63 of 65 patients) 45 (71.4%) had low risk and 18 (28.6%) were intermediate-1 risk. Regarding R-IPSS (62 of 65) 17 (27.4%) were very low risk, 38 (61.3%) low risk and 7 (11.3%) were intermediate risk. Forty five patients (69.2%) were RBC transfusion dependent, 25 (67.9%) had EPO levels <100 U/L and 36 (67.9%) had ferritin levels <500 mg/dL. Regarding mutational status, median number of mutated genes per patient was 2, with a mean VAF of 32.5%. Only 8 patients (12.3%) did not presented mutations; 20 patients (30.8%) had 1 mutated gene, 19 (29.2%) had 2, 6 (9.2%) had 3, 7 (10.8%) had 4, 5 (7.7%) and 5 (7.7%) had 5 mutated genes. The most frequently mutated genes were: *SF3B1* in 34 patients

(52.3%), *DNMT3A* in 11 (16.9%), *TET2* in 9 (13.9%) and *SRSF2* in 7 (10.8%); the other mutated genes were presented with a frequency <10%. Most of the patients had mutations in the splicing gene family (47, 72.3%). We observed a trend to a better erythroid response among patients with ≤2 or less mutated genes (42.6% *vs* 22.2% in those with >2; p=0.159). No significant association was found between erythroid response and specific mutated genes, but a trend toward to a best response was observed in patients with *TET2* mutations (66.7% responses; p=0.066). The only significant variable related with erythroid response was transfusion dependency (p=0.001). After a median of follow up for survivors of 3.30 years, median Overall Survival (OS) was 5.04 years. Univariate analysis determined that age as a continuous variable, sex, WHO 2008 diagnosis, IPSS, R-IPSS, erythroid response, number of mutated genes (≤2 *vs* >2) and somatic mutations in *SRSF2*, *GNAS* and *STAG2* genes significantly influenced on OS. We did not found differences in OS among patients with mutations in *SF3B1* alone or in association with mutations in other genes. In the multivariate analysis variables that significantly influenced on OS were age, sex, IPSS, erythroid response and number of mutated genes (≤2 *vs* >2) (Table 1).

Table 1. Multivariate analysis.

	Sig.	HR	95,0% IC Low	95,0% IC Upper
Sex (ref. women)	,007	**3,520**	1,412	8,770
Age (continous)	,000	**1,137**	1,073	1,206
IPSS (ref. low)	,000	**4,885**	2,161	11,040
Erythroid response (ref. No R)	,009	**,286**	,113	,728
Number of mutated genes (≤2 vs. >2) (ref. ≤2 mut.)	,011	**3,146**	1,303	7,594

Summary and Conclusions: We conclude that the number of mutated genes in patients with LR-MDS receiving ESAs have a significant impact on OS and could be related with the erythroid responses.

PF500

MOLECULAR SCORING SYSTEM INTEGRATED WITH IPSS-R IN KOREAN MYELODYSPLASTIC SYNDROME

H.S. Park[1,*], J. Park[2], K. Im[3], S.-M. Kim[3], J. Yun[1], D. Jeong[1], S.M. Hwang[4], D.S. Lee[1]

[1]Department of Laboratory Medicine, Seoul National University, Seoul, Korea, Republic Of, [2]The Webb schools, Claremont, United States, [3]Seoul National University, Seoul, [4]Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seongnam, Korea, Republic Of

Background: Revised International Prognostic Scoring System (IPSS-R) is global prognostic system in myelodysplastic syndrome (MDS). Recently, multi-gene target sequencing is widely performed for the purpose of prognostic prediction and application of targeted therapy. In spite of many reports on the prognostic implication of somatic mutation, combined implication with conventional IPSS-R have not been reported yet.
Aims: Here we proposed a new scoring system that encompass gene variations and IPSS-R together.
Methods: In 153 patients diagnosed with MDS in Seoul National University Hospital, G-banding, fluorescence *in situ* hybridization (FISH), targeted capture sequencing for 88 hematopoiesis-related genes, and measurement of telomere length (TL) were performed. Kaplan-Meier survival analysis with the log-rank test and Cox proportional hazards regression analysis were used to develop a new prognostic system using Mathematica.
Results: We developed new model including targeted capture sequencing and telomere length addition to IPSS-R scoring. Overall, 128 of the 153 patients (83.7%) harbored at least one mutation. We calculated prognostic implication of genes with frequency over 5% or more (*ASL1, U2AF1, TP53, RUNX1, TET2, DNMT3A, SRSF2, BCOR, EZH2, SF3B1, STAG2*, and *WT1*) and prognostic implication of TL. Patients with telomere length <5.58 showed an adverse survival. In univariate analysis, age, IPSS-R score, mutation in *ASXL1, EZH2, TP53* and telomere length were significantly associated with OS. We developed a new scoring model incorporating the weighted coefficients of these variables: age 0.017+IPSS-R score 0.220+*ASXL1*mutation 0.375+ *EZH2* 0.706+*TP53* 0.897+Telomere length 3.376. The age and IPSS-R score were used as a continuous variable. The presence of gene mutations and TL below 5.58 was scored as 1. According to this new scoring system, patients were divided into *four* groups: low score cutoff (≤5.44), intermediate-1 (5.44-5.98), intermediate-2 (5.98-7.30), high (>7.30). The median OS was 100.0, 49.7, 33.5, 10.6 months for low, intermediate-1, intermediate-2, and high, retrospectively (p<0.001). Meanwhile, according to conventional IPSS-R scoring system, the median OS was 97.2, 78.2, 79.1, 62.8, 33.7 months for very low, low, intermediate, high and very high, retrospectively (p <0.001).
Summary and Conclusions: The newly developed model incorporating molecular variations and TL yielded more clear separations of the survival curves. By adding the presence of gene mutation and telomere length to the existing IPSS-R, its predictive ability can be further improved in MDS.

PF501

PROGNOSTIC IMPACT OF ASXL1 MUTATIONS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND MULTILINEAGE DYSPLASIA WITH OR WITHOUT RING SIDEROBLASTS

A. Mangaonkar[1,*], T. Lasho[1], C. Finke[1], N. Gangat[1], A. Al-Kali[1], M. Elliott[1], K. Begna[1], C. Hanson[2], R. Ketterling[2], A. Wolanskyj[1], W. Hogan[1], M. Litzow[1], A. Pardanani[1], A. Tefferi[1], M. Patnaik[1]

[1]Hematology, [2]Pathology, Mayo Clinic, Rochester, United States

Background: The 2016 iteration of World Health Organization (WHO) classification of myeloid neoplasms removed the sub-category of 'refractory cytopenia with multilineage dysplasia' and reclassified patients with myelodysplastic syndromes (MDS) and multilineage dysplasia (MLD) into either myelodysplastic syndromes, ring sideroblasts with multilineage dysplasia (MDS-RS-MLD) or myelodysplastic syndromes with multilineage dysplasia (MDS-MLD); primarily based on the presence or absence of RS &/or *SF3B1* mutations (Arber *et al. Blood* 2016).
Aims: We sought to validate this morphology-based distinction in classification, in the context of myeloid relevant genomic alterations.
Methods: Consecutive cases of WHO-defined MDS-RS-MLD & MDS-MLD, meeting the 2016 WHO criteria were identified from our institutional database. Bone marrow (BM) biopsy reports & cytogenetics were re-reviewed. A targeted exome sequencing for myeloid-relevant genes was performed on BM specimens obtained at diagnosis.
Results: Ninety-eight patients were included in the study (Table 1); 59 with MDS-MLD & 39 with MDS-RS-MLD. Median age of the entire cohort was 73 years (range: 34-96) with 71 (72%) males. Fifty-five (56%) patients had an abnormal karyotype; while 9 patients (9%) had a monosomal karyotype. Targeted exome sequencing in 95 patients demonstrated the following mutational frequencies; *ASXL1* 32%, *SF3B1* 25%, *TET2* 21%, SRSF2 16%, *U2AF1* 12%, *TP53* 8%, *IDH2* 4%, *DNMT3A* 3%, 2% each for *IDH1*, *RUNX1* & *EZH2* and 1% each for *CALR, JAK2 & CBL*.
Among the significantly different variables; median platelet counts (p=0.03), percentage of patients with abnormal karyotypes (p=0.03) and as expected, BM ring sideroblast% (p<0.001) & frequency of *SF3B1* mutations (p<0.0001) were higher in the MDS-RS-MLD group; while *TET2* mutations were more frequent in the MDS-MLD group (31% *vs* 6%, p=0.002) (Table 1). At last follow up (median 95 months), 85 (87%) deaths & 11 (11%) leukemic transformations were documented. Median overall survival (OS) for the entire cohort was 25 months (Range: 1-102); with no significant difference between MDS-MLD 25 (0.5-100) and MDS-RS-MLD 25 (1-102) groups respectively (p=0.6, Figure 1A). In univariate survival analysis that included the aforementioned clinical, cytogenetic & molecular parameters; only older age (age>75 years, p=0.02), lower (<10 g/dL) Hb (p=0.009), lower (<100 x 10^9/L) platelet count (p=0.01), higher risk IPSS (p=0.007) and R-IPSS (p=0.017) cytogenetic categories (p=0.007), lack of *SF3B1* mutations (p=0.024), presence of *ASXL1* (p=0.007) & *TP53* (p=0.03) mutations adversely impacted OS. Presence of ring sideroblasts did not affect OS (p=0.98). In a multivariate model that included the variables significant on univariate analysis, only lower (<100 x 10^9/L) platelet count (HR 2.1, 95% CI 1.25-3.44, p=0.004) & presence of *ASXL1* mutation (HR 2.4, 95% CI 1.35-4.1, p=0.003) retained independent prognostic significance (Figure 1B). Further, in the context of IPSS & R-IPSS stratification, presence of *ASXL1* mutations retained prognostic relevance (p=0.003). Given the small number of events, leukemia free survival analysis was not performed.
Summary and Conclusions: While the 2016 iteration of the WHO classification of myeloid neoplasms has favored the re-introduction of sub-classifying MDS-MLD into cases with and without RS, in the context of genomic data this segregation is prognostically irrelevant. With this study, we once again, demonstrate the adverse prognostic impact of *ASXL1* mutations in MDS patients with multilineage dysplasia.

Table 1.

Variable; Median value (range or %)	*Cohort (n=98)*	*MDS-MLD (n=59)*	*MDS-RS-MLD (n=39)*	*P value*	
Age at diagnosis (years)	73 (34-96)	75 (34-96)	72 (44-91)	0.2	
No. of males;	71 (72)	39 (66)	32 (82)	0.08	
Hb; gm/dl	10.3 (7-14.8)	10.4 (7-14.8)	10 (7.4-14.4)	0.15	
WBC count x 10^9 per liter	4.15 (0.9-20.3)	4.1 (0.9-20.3)	4.5 (1.2-11.1)	0.83	
ANC x 10^9 per liter	2 (0-16)	1.8 (0-16)	2.4 (0.3-8.6)	0.7	
Platelet count x 10^9 per liter	92 (4-515)	85 (4-515)	167 (9-438)	0.03*	Survival
BM ring sideroblasts	5 (0-80)	0 (0-10)	25 (7.5-80)	<0.001*	
BM blasts	0 (0-3)	0 (0-1)	0 (0-3)	0.07	
Cytogenetics					
Abnormal karyotype	55 (56)	28 (47)	27 (69)	0.03*	
Monosomal karyotype	9 (9)	3 (5)	6 (15)	0.09	
R-IPSS cytogenetics Very good	7 (7)	4 (7)	3 (8)	0.09	
Good	59 (60)	35 (60)	24 (62)		
Intermediate	18 (19)	15 (25)	3 (8)		
Poor	6 (6)	2 (3)	4 (10)		
Very poor	8 (8)	3 (5)	5 (13)		
Genomic abnormalities					
ASXL1	30 (32)	22 (37)	8 (22)	0.13	
SF3B1	24 (25)	4 (7)	20 (56)	<0.0001*	
TET2	20 (21)	18 (31)	2 (6)	0.007*	
SRSF2	15 (16)	12 (20)	3 (8)	0.11	
U2AF1	11 (12)	7 (12)	4 (11)	0.9	
TP53	8 (8)	3 (5)	5 (14)	0.2	Survival
IDH1/2	7 (7)	4 (7)	3 (8)	0.8	
DNMT3A	3 (3)	1 (2)	2 (6)	0.2	
RUNX1	2 (2)	1 (2)	1 (3)	0.24	
EZH2	2 (2)	2 (3)	0 (0)	0.4	
Others (CALR,JAK2,CBL)	4 (4)	4 (7)	0 (0)	0.04*	
Treatment					
HMA treatment	24 (24)	13 (22)	11 (28)	0.5	
Lenalidomide	9 (9)	7 (12)	2 (5)	0.24	
Allogeneic HSCT	4 (4)	1 (2)	3 (8)	0.14	
Outcomes					
Leukemic transformation	11 (11)	7 (12)	4 (10)	0.8	
Overall survival (OS)	25 (1-102)	25 (0.5-100)	25 (1-102)	0.6	

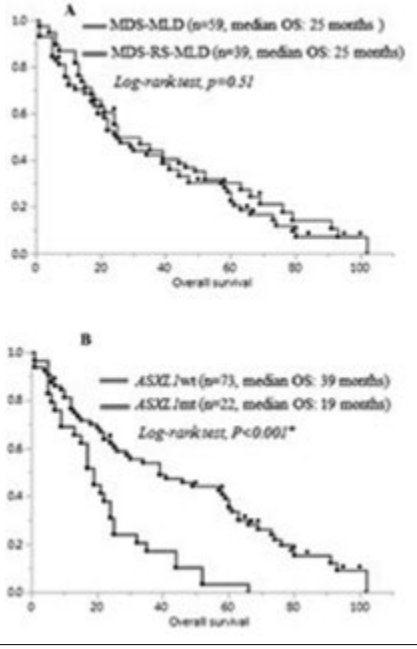

Figure 1.

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CLINICAL OUTCOMES OF PATIENTS WITH MDS/MPN OVERLAP SYNDROMES

S. Kavanagh[1,*], C. McNamara[1], S. Chan[1], D. Maze[1], M. Minden[1], A. Schimmer[1], A. Schuh[1], H. Sibai[1], V. Gupta[1], K. Yee[1]

[1]Department of Medical Oncology and Haematology, Princess Margaret Cancer Centre, Toronto, Canada

Background: The myelodysplastic/myeloproliferative overlap syndromes (MDS/MPN) are a group of rare myeloid malignancies. They are a clinically diverse group with highly variable prognosis, often limited by transformation to acute myeloid leukaemia (AML), and have limited treatment options.
Aims: To examine the clinical outcomes, particularly following disease progression, in patients with MDS/MPN.

Methods: Patients with newly diagnosed MDS/MPN, treated at Princess Margaret Cancer Centre between January 2008 and December 2016, were identified through PMCC databases. Charts were reviewed. Diagnoses were classified as per the WHO (2016) schema. MDS/MPN treatment responses were classified under the 2015 Working Group proposal; AML responses were classified by 2017 ELN guidelines. DNA sequencing, using a 54-gene myeloid panel, was performed at diagnosis since February 2015.
Results: We identified 149 patients with a diagnosis of MDS/MPN. The median age was 67 years (28-99). Ten (6.7%) patients had atypical CML, 96 (64.4%) CMML, 10 (6.7%) MDS-MPN-RS-T and 33 (22.1%) MDS-MPN-unclassifiable. Twenty-three (15.4%) had previously received cytotoxic chemo- and/or radiation therapy and were diagnosed with therapy-related disease. Patients with CMML had low (37), intermediate-1 (20), intermediate-2 (29) or high risk (4) disease as per CPSS classification. Patients with other MDS-MPN syndromes had low (27), intermediate-1 (15), intermediate-2 (4) and high-risk (1) disease according to IPSS classification. Six patients with CMML and six with other MDS/MPN could not be classified in the absence of cytogenetic results. Molecular data was available in 44 patients and will be presented at EHA. Sixty-one patients (40.9%) experienced disease progression, 43 (70.5%) to AML, 5 (8.2%) isolated myeloid sarcoma and 13 (21.3%) accelerated phase, defined as 10-19% blasts in peripheral blood or bone marrow. The median time to progression from diagnosis was 226 days (range 21 – 1636). Twenty-six received induction chemotherapy, 12 of whom achieved CR. The remaining patients received low-intensity therapy or supportive care; 2 achieved CR on clinical trial and 1 achieved CR with azacitidine. Nine patients received allogeneic bone marrow transplant following progression (6 in CR; 2 after reversion to chronic phase; 1 unknown). Overall survival of the cohort is shown in Figure 1A. Median survival was not reached in patients with MDS-MPN-RS-T and was significantly longer than survival in aCML (938 days), CMML (779 days) and MDS-MPN-u (491 days) ($p<0.05$ for all). There was no difference in survival between other MDS-MPN subgroups. OS was significantly shorter in patients who had disease progression compared to those patients who did not (538 *vs* 1005 days; p=0.012). No difference in survival, calculated from time of progression, was seen between those receiving intensive chemotherapy or less-intense therapy/supportive care for disease progression. In those achieving CR following treatment for disease progression, bone marrow transplantation was associated with superior OS (Figure 1B; 1866 *vs* 486 days, p=0.01).

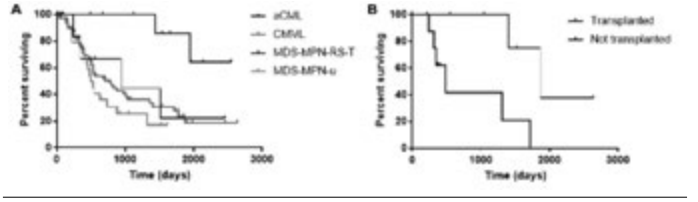


Figure 1.

Summary and Conclusions: In conclusion, survival is poor in MDS-MPN patients who progress. Allogeneic bone marrow transplantation remains the only option for long-term disease control in this situation. There is an unmet need to better characterise, prognosticate and develop appropriate treatment strategies for patients with these neoplasms.

PF503

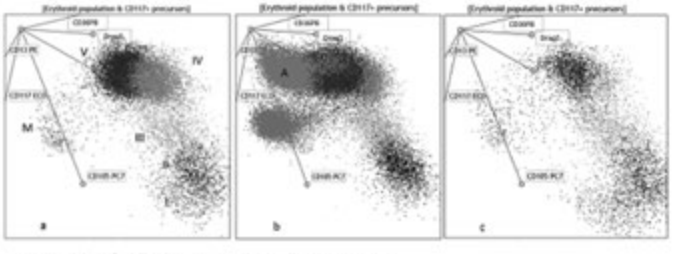
FLOW CYTOMETRY ANALYSIS OF ERYTHROPOIETIC MATURATION PATTERNS HELP TO DIAGNOSE AND FOLLOW-UP ERYTHROPOIETIC DYSPLASIA IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

A. Porwit[1], D. Violidaki[1,*], O. Axler[1], M. Ehinger[1], K. Jafari[2], K. Navred[3], L. Nilsson[4]

[1]Clinical Sciences, Division Oncology and Pathology, Lund University, Lund, Sweden, [2]Pathology, Cross Cancer Institute, Edmonton, Canada, [3]Laboratory Medicine, Blekinge Hospital, Karlskrona, [4]Hematology, Lund University, Lund, Sweden

Background: Anemia, erythropoietic dysplasia and morphological signs of disturbance in erythropoietic maturation in the bone marrow (BM) are seen in most patients with myelodysplastic syndromes (MDS). By flow cytometry (FC), the reported changes in the erythropoietic compartment in MDS are increased CD36 CV, CD71 CV, MFI of CD71 and increased fraction of CD117+ erythroid cells.
Aims: To evaluate the recently developed single tube FC panel (ERY) and radar plot analysis protocol that facilitate adequate estimation of various BM cell compartments and assessment of erythropoietic differentiation pattern.
Methods: 250x10^3 BM cells were incubated with ERY panel: CD71FITC, CD13PE, CD117ECD, CD105PC7, CD36PB and CD45KO and 10 ml of 0.5

mM DRAQ5 (10 resp. 15 min in the dark). $10x10^5$ $DRAQ5^+$ events were acquired with a Navios flow cytometer and analyzed using Kaluza software. Myeloid compartment was evaluated by Ogata score using 10-color, 14 antibody screening tube (SC). The results were compared with cytological evaluation of BM smears by 500 cell differential count. Dysplasia was evaluated as high (>30% of erythropoietic cells), low (10-30%) and absent (<10%).
Results: BM Samples included 19 normal BM, 35 BM samples from patients with anemia but no dysplasia (hospital controls, HC) and 66 BM samples from patients with MDS (30% MDS-MLD, 17% MDS-RS-MLD, 21% MDS-EB1, 23% MDS-EB2 and 9% MDS 5q, according to WHO 2016). The frequency of erythropoietic cells derived from ERY tube showed better correlation with morphological differential count (R^2=0.7759) than counts obtained by the SC tube. The frequency of erythropoietic cells in SC was significantly lower, which could lead to overestimation of CD34+ cell counts. In normal BM, CD117+ myeloid cells were 0.4% (range 0.1-1.1%). Erythroid cluster was gated as CD71+/CD45- (mean 19%, range 3.4-29.5%) and we could confirm that CD36 and CD71 CV in erythroid compartment were higher in MDS patients than in control groups (p<0.05). The following subpopulations could be recognized using Radar plots analysis: the earliest precursors (CD117+/CD105+, mean 1.8% of erythropoiesis) followed by CD117-/CD105++ (mean 4.5%), CD117-/CD105dim (mean 6.6%), CD105-/CD36++ (mean 25.8%), and CD105-/CD36dim (mean 60.9%). These subpopulations created a reproducible pattern of maturation in all normal BM (Figure1a). Deviations from this normal pattern could be seen in 68% of MDS patients. In 45% of MDS patients, an aberrant cluster of CD105- erythroids with low expression of CD71 and CD36 (2-48% of erythroids) was noted (Figure 1b). This cluster was seen in 16% HC patients (0.5%>12% of erythroids). In patients showing this cluster, the fractions of early and proliferating erythroids were decreased. In 23% of MDS patients and 12% of HC, fractions of immature CD105+ erythroids were high and later stages were decreased (Figure 1c). 58% of MDS patients showed an increased cluster of CD117+ myeloid blasts (mean 3.6%, range 0.2-16%). In 8 MDS patients, follow-up samples were available. Changes in erythropoietic maturation patterns could be observed after VIDAZA treatment and normalization was seen after BM transplantation.



Radar analysis of erythroid maturation pattern and CD117+ precursors.
1a.Normal pattern, 1b.Aberrant pattern 1 with aberrant CD71dim/CD36dim population (A, light brown).
1c. Aberrant pattern 2 with high fraction of CD105+ immature erythroids.

Figure 1.

Summary and Conclusions: The developed panel allows adequate assessment of erythroid cell compartment, which could be of use in diagnosis of erythroid dysplasia and help in evaluation of treatment response.

PF504

MUTATIONS AND KARYOTYPE PREDICT TREATMENT RESPONSE IN MYELODYSPLASTIC SYNDROMES

D. Idossa[1,*], T. Lasho[2], M. Mudireddy[2], C. Finke[2], K. Rhett[3], M. Patnaik[2], A. Pardanani[2], N. Gangat[2], A. Tefferi[2]

[1]Internal Medicine, [2]Division of Hematology, [3]Laboratory Medicine, Mayo Clinic Rochester, Rochester, United States

Background: Myelodysplastic syndromes (MDS) comprise a heterogeneous group of malignant hematopoietic stem cell disorders that result in dysplastic and ineffective hematopoiesis, with variable risk of death and transformation to acute myeloid leukemia. Thus, treatment options also vary from watchful waiting and supportive care to disease modifying therapy and allogeneic stem cell transplant. With the advent of next generation sequencing (NGS), a number of prognostically-relevant mutations and karyotype have now been described in MDS.
Aims: To examined the influence of mutations and karyotype on conventional treatment response in myelodysplastic syndromes (MDS).
Methods: Cytogenetic and NGS-derived mutation information and treatment details were available for 159 patients evaluated and followed at our institution for MDS. A retrospective chart review was conducted for these patients to obtain information regarding treatments received and their response.
Results: 159 patients (median age 72 years; 68% males) were included in the analysis. IPSS-R risk distribution was very high in 11%, high 18%, intermediate 17%, low 38% and very low 16%. At least one mutation was detected in 83% of the patients; most frequent were *ASXL1* (27%), *TET2* (25%), *SF3B1* (21%), *SRSF2* (16%), *U2AF1* (14%), *TP53* (13%), *RUNX1* (11%) and *DNMT3A* (10%). At median follow-up of 30 months, treatment with hypomethylating agents (HMAs) was documented in 65 (41%) patients, immunomodulatory drugs (IMiDs) in 31 (19%) and erythropoiesis stimulating agents (ESAs) in 68 (43%). Genetic markers predicted treatment response to HMAs and IMiDs, but not to ESAs. HMA treatment response was positively affected by *IDH1* mutations (100% *vs* 47%; p=0.04) and negatively by *TP53* mutations (11% *vs* 55%; p=0.03) and monosomal karyotype (MK) or trisomy 8 (13%, 13% *vs* 71% for normal karyotype; p=0.007). IMiD response was unlikely in patients with *U2AF1* mutations (0% *vs* 46%; p=0.02) and in those with MK (0%). Multivariate logistic regression confirmed HMA treatment response effect from *TP53* and *IDH1* and IMiD response effect from *U2AF1* mutations. Absence of red cell transfusion need was the only other clinical variable with additional value in predicting response to HMAs or ESAs.
Summary and Conclusions: We conclude that MDS patients with *TP53* mutations are unlikely to respond to HMAs and those with *U2AF1* to IMiDs.

PF505

GENDER AND THE PATTERN OF RECURRENT MYELOID MUTATIONS ARE STRONGLY AND INDEPENDENTLY ASSOCIATED TO BLOOD TRANSFUSION INTENSITY IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

J. Rydén[1,2,*], G. Edgren[3,4], M. Karimi[1], G. Walldin[1,2], M. Tobiasson[1,2], A. Wikman[5,6], E. Hellström-Lindberg[1,2], P. Höglund[1,5]

[1]Department of Medicine, Karolinska Institutet, Huddinge, Stockholm, [2]Department of Hematology, Karolinska University Hospital, [3]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, [4]Department of Cardiology, Stockholm South Hospital, [5]Department of Clinical Immunology and Transfusion Medicine, [6]Department of Laboratory Medicine, Karolinska University Hospital, Stockholm, Sweden

Background: Patients with myelodysplastic syndromes (MDS) frequently receive large numbers of blood transfusions over the course of the disease, but little is known about the clinical and disease-specific factors that drive the transfusion need and intensity in these patients.
Aims: This study aimed to identify clinical and disease-specific factors associated to transfusion need and to study the association between transfusion intensity and patient survival.
Methods: We established a retrospective cohort of MDS patients from the Karolinska University Hospital, Stockholm, Sweden, with detailed clinical information, complete transfusion data, and the pattern of recurrent myeloid mutations. Variables associated to transfusion intensity were estimated using a time-dependent Poisson regression and the association between transfusion intensity and risk of death was estimated using a time-dependent Cox regression.
Results: In addition to very high-risk disease according to the revised International Prognostic Scoring System (IPSS-R), male sex and mutational patterns were independently and significantly associated to high red cell and platelet transfusion requirements. Specifically, mutations involved in histone modulation, signaling and transcriptional regulation were independently associated with a higher transfusion need. Transfusion intensity during the past year was strongly associated with poor survival, when accounting for this variable, IPSS-R lost significance and only mutations in histone modulators were independently linked to survival.
Summary and Conclusions: The mutational profile in MDS is strongly associated to transfusion intensity, which indicates the involvement of specific gene pathways in ineffective hematopoiesis. Moreover, transfusion intensity has a stronger association to poor survival than previously recognized, which warrants further investigation.

PF506

DIFFERENCES IN MUTATIONAL PROFILE AND CLONAL ARCHITECTURE OF MYELODYSPLASTIC SYNDROMES WITH ISOLATED DELETION OF 5Q, HYPERPLASTIC AND HYPOPLASTIC MYELODYSPLASTIC SYNDROMES

F. Hernández Mohedo[1,*], J. Badiola[1], E. Cornejo[1], M. Bernal[2], P. Montes[2], F. Ruiz Cabello[2], A.R. Gonzalez[3], M. Jurado Chacón[1]

[1]Hematology, [2]Inmunology, [3]Investigation FIBAO, Complejo Hospitalario Universitario de Granada, Granada, Spain

Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of hematologic malignancies. Although most MDS patients have normal or increased cellularity (NH- MDS), approximately 10-20% of them have hypocellular bone marrow (h-MDS). The reports concerning the differences in genetic alterations between them are limited, but h-MDS seems to be a different clinical-biological entity with a different molecular profile, and a better overall survival.
Aims: Our aim has been to investigate the molecular profile in MDS, in the sense of number of driver mutations, the dominant molecular pathway altered and the incidence of high risk mutation (HRM) between three categories of MDS: h-MDS, NH-MDS and MDS with islolated del(5q).
Methods: We performed a prospective analysis from January 2016 to December 2017, in 69 patients with MDS diagnostic divided in 3 groups: (A) NH-MDS, (B) h-MDS (≤25% bone marrow cellularity) and (C) MDS with isolated del(5q). The molecular profile was analyzed by next-generation gene sequencing technology (NGS Sophia Genetics-Illumina Sequencing analysis v.1.8.37). We analyze the number of driver mutations by molecular pathway (Splicing, Cohesin, DNA Metylation, Transcription, Chromatin modification, tumor suppressor,etc) and we categorize as High Risk molecular score if at least one of these conditions is present: (1) ≥1 High risk mutations (HRM) TP53, RUNX1, ASXL1, EZH2, and ETV6 (Bejar, *et al.* N Engl J Med 2011), (2) ≥2 driver HRM mutations or ≥1 HRM plus TP53 and (3) High variant allele frequency in HRM mutations (VAF ≥40%). The statistical analysis was performed with software IBM SPSS.21. Categorical variables were compared using a Chi-squared test, while continuous variables were compared using a Wilcoxon rank-sum test. P-values are two-sided and considered significant at the <0.01 level.
Results: In the 69 MDS, diagnostic classification was: 47 NH-MDS, 11 h-MDS and 11 MDS with isolated del(5q). The analysis by molecular pathway don't show differences between the 3 subtypes of MDS patients. By contrast, MDS with isolated del(5q) had lower mutational score than NH-MDS(HRM 0,5 *vs* 2;p=0.01), lower variant allele frequency HMR than NH-MDS(VAF ≥40%:1,7% *vs* 39,7%;p=0.019) and NH-MDS had higher bone marrow blast than h-MDS(4% *vs* 2%;p=0,006). The IPSS-R risk category don't show differences between the 3 subtypes of MDS patients.
Summary and Conclusions: In patients with MDS with isolated del(5q), we observe a lower incidence HMR driver mutations, lower score of HRM and VAF than NH-MDS, and don't seam to be depended on IPSS-R score. The analysis of driver mutations by molecular pathway, don't show diferences by disease subtypee. Our results suggest that MDS with isolated del(5q) have a different clonal architecture with lower risk molecular score, and this can explain the better overall survival previously described in this disease. In the majority of patients with MDS-h we olso observe a lower incidence of HMR driver mutations compared with MDS-NH, but the differences were not statistically significant. In the near future, integration of the somatic mutation with IPSS-R score could better stratifie the risk group and the therapeutic plan on Hypopplastic MDS and MDS with isolated del(5q).

PF507

CLINICAL SIGNIFICANCE OF REDUCED EXPRESSION OF THE GENES LOCATED WITHIN COMMON DELETED REGION OF DEL(20Q) IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

M. Shiseki[1,*], M. Ishii[1], M. Ohwashi[1], Y.-H. Wang[1], K. Yoshinaga[1], N. Mori[1], J. Tanaka[1]

[1]Department of Hematology, Tokyo Women's Medical University, Tokyo, Japan

Background: Deletion of long arm of chromosome 20 (del(20q)) is observed in 5 to 10% of patients with myelodysplastic syndromes (MDS). Although biological significance of del(20q) in MDS pathogenesis has not been fully elucidated, it has been considered that common deleted regions (CDRs) of del(20q) contain target genes involved in molecular pathogenesis of MDS. Previously, we determined a CDR of del(20q) in MDS patients by an array comparative genomic hybridization analysis. The CDR contains more than 150 genes.
Aims: We examined expression of selected 28 genes within the CDR, including candidate tumor suppressor genes, and/or the genes involved in cell growth, death, and differentiation,in order to investigate clinical significance of each gene expression level.
Methods: Bone marrow samples of the patients with MDS at the time of diagnosis and those of control subjects were used for analysis. RQ-PCR was carried out to examine expression of 28 genes by the TaqMan probe method. To compare expression level of each gene between MDS patients and control subjects, a non-parametric test was used. Kaplan-Meier plots were used for estimation of survival. The COX proportional hazards model was employed to analyze impact of expression of each gene examined on overall survival (OS). The study was conducted according to the Declaration of Helsinki, and the protocol was reviewed by institutional ethics committee.
Results: We examined expression of the 28 genes in 48 MDS patients with (n=28) or without (n=20) chromosome 20 abnormalities, and control subjects (n=15). A total of 15 genes examined showed significant reduced expression in MDS patients with chromosome 20 abnormalities, compared to control subjects. In addition, expression of *BCAS4, ADA, and YWHAB* genes was significantly reduced in MDS patients without chromosome 20 abnormalities. We analyzed impact of expression level of each gene on OS to evaluate clinical significance of reduced expression, by employing the Cox proportional hazards model. Univariable analysis indicated association between inferior survival and reduced expression of five genes, *MMP9 BCAS4, BGALT5, SULF2,* and *ZNF335.* Multivariable analysis indicated that reduced *BCAS4* expression showed trend toward for association with inferior OS (P=0.0509). According to *BCAS4* expression level, we divided 48 MDS patients into two groups, low expression (less than median value) and high expression group (median or higher). Patients with high BCAS4 expression (median or higher) (H-group) showed superior survival to those with low BCAS4 expression (less than median) (L-group) (log-rank test, P=0.0022). Estimated median OS times were 67 months (H-group) and 32 months (L-group).
Summary and Conclusions: In conclusion, we analyzed expression of 28 genes located within CDR of del(20q), and expression of 15 genes was significantly reduced in MDS patients with chromosome 20 abnormalities, compared to control subjects. In addition, expression of *BCAS4, ADA,* and *YWHAB* genes was significantly reduced in MDS patients without chromosome 20 abnormalities. Five genes (*MMP9, BCAS4, B4GALT5, SULF2,* and *ZNF335*) showed clinical significance.

PF508

HIGH WT1 EXPRESSION LEVELS AT DIAGNOSIS PREDICTED POOR OUTCOMES OF PATIENTS PRESENTING ISOLATED THROMBOCYTOPENIA AT ONSET OF MYELODYSPLASTIC SYNDROME

Q.-S. Huang[1,*], X. Liu[1,2,3], R.-Y. Gui[1], J. Wu[1], X. Cai[1], Q. Jiang[1,2,3], H. Jiang[1,2,3], J. Lu[1,2,3], H.-X. Liu[4], X.-Y. Zhao[1,2,3], Y.-J. Chang[1,2,3], Y.-Z. Qin[1], Y.-R. Liu[1], L.-P. Xu[1,2,3], K.-Y. Liu[1,2,3], X.-J. Huang[1,2,3], X.-H. Zhang[1,2,3]

[1]Peking University Institute of Hematology, Peking University People's Hospital, [2]Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, [3]Collaborative Innovation Center of Hematology, Peking University, [4]Department of Clinical Epidemiology, Peking University People's Hospital, Beijing, China

Background: Myelodysplastic syndromes (MDS) comprise a heterogeneous group of clonal disorders of hematopoietic stem cell origin. Isolated thrombocytopenia is rare as the first presentation of patients with MDS. There is no information available on clinical characteristics, appropriate treatment, and prognosis of isolated thrombocytopenia MDS.Our findings demonstrate that haploidentical stem cell transplantation (haplo-SCT) is a reliable therapy for patients with MDS. WT1 is a transcription factor overexpressed in diverse neoplasms, including MDS. Accumulating evidence has suggested that WT1 expression levels increased in accordance with the aggressiveness of the disease in MDS. Therefore, whether WT1 expression could predict responses to specific interventions, including chemotherapy alone and haplo-SCT in patients with MDS presenting with isolated thrombocytopenia is unclear.
Aims: The aim of this study was to determine the WT1 expression level, morphologic, cytogenetic and the prognostic features of MDS presenting with isolated thrombocytopenia, to investigate the prognostic effect of WT1 expression level and to explore proper treatment methods.
Methods: We performed a retrospective nested case-control study in patients presenting with isolated thrombocytopenia at the onset of MDS. Patients were reviewed retrospectively from all the patients who were diagnosed with MDS between July 2007 and February 2018 at Peking University People's Hospital. WT1>1.0% was defined high-level expression according to our previous study(Zhao XS, 2012). In total, 280 patients with isolated thrombocytopenia as the first presentation (group A), 300 patients with two cytopenias (group B), and 300 patients with three cytopenias (group C) were enrolled in this study.
Results: During the 11-year period, 8100 patients were diagnosed with MDS. A total of 280 patients had isolated thrombocytopenia as the first presentation of MDS. Median cell counts at the time of first presentation were Hb 129.5g/L, ANC 3.6X10^9/L, and PLT 46.5X10^9/L. The most common cytogenetic profile, IPSS-R risk score, and WHO classification were normal karyotype, low, and MDS-SLD, respectively. Leukemia transformation occurred in 42 patients (15%). At the time of diagnosis with MDS, WT1 expression >1.0% was identified in 70.6%, 74.7%, and 78.5% of group A, group B, and group C, respectively. Median duration of isolated thrombocytopenia before diagnosis of MDS was 14.6 months (range 0-105

months). Median follow-up of the whole 280 patient cohort was 120 months, and the overall survival(OS) of group A was significantly longer than that of group B and group C, reflecting the favorable prognosis of isolated thrombocytopenia. In group A, WT1 high-expressing patients had a shorter OS than that of the WT1 low-expressing patients. Among the 280 patients, 69 patients underwent haplo-SCT, and 152 patients underwent chemotherapy. Patients undergoing haplo-SCT had a longer OS and progression-free survival(PFS) than that of chemotherapy patients. For patients undergoing haplo-SCT, in uni-variable and multi-variable analysis, WT1>1.0% was associated with shorter OS and PFS. Three-year OS in patients with WT1≤1.0% was longer than patients with WT1>1.0%.
Summary and Conclusions: Isolated thrombocytopenia as the first presentation of MDS had a low WT1 expression level and favorable outcome, compared to two cytopenias and three cytopenias. Haplo-SCT is superior to chemotherapy for these patients, after which the WT1 expression level could predict clinical outcomes and provide a potential way for suitable interventions.

PF509

ROLE OF DNA METHYLATION GENE AND TP53 MUTATIONS IN PREDICTING THE CLINICAL EFFICACY OF EPIGENETIC THERAPY IN MYELOID NEOPLASM PATIENTS - META ANALYSIS

L. Cai[1,*], H. Wang[1], Q. Chen[1]

[1]Hematology, Huazhong University of Science and Technology, Wuhan, China, Wuhan, China

Background: Myelodysplastic syndromes and acute myeloid leukemia (MDS and AML) are both heterogeneous myeloid disorders. Numerous genetic and epigenetic gene mutations are now identified in these patients such as mutation of TET2, DNMT3A, IDH1/2 and TP53. Hypomethylating agents (HMAs, azacitidine and decitabine), which results in overall response rates (ORR) of up to 50% and overall survival(OS) improvement, are now routinely used in MDS/AML patients. However, patients who do not response to HMAs get very poor outcome with a median OS of less than 6 months. Therefore, reliable markers predicting the clinical efficacy of HMAs to assist better selection of patients who really benefit from the HMAs are urgently needed in clinic.
Aims: This study is aim to assess the predictive role of mutations($TET2^{MUT}$, $DNMT3A^{MUT}$, $IDH1/2^{MUT}$ and $TP53^{MUT}$) on clinical efficacy of HMAs in MDS/AML patients.
Methods: We performed a Meta-analysis to summarize the existing evidences. We systematically searched PubMed, Embase, Cochrane and the Wan Fang database until 01 October 2017. Studies including MDS/AML patients treated with HMAs and providing available response and survival data were eligible. All data were analyzed within Revman 5.3 software.
Results: 317 papers were initially searched. Finally, 20 studies(participants, N=1710 patients) focusing on one or more genes mutations were included. Especially, 10 studies focus on $DNMT3A^{MUT}$,11 on $TET2^{MUT}$, 8 on $IDH1/2^{MUT}$, and 11 on $TP53^{MUT}$. For ORR, the pooled ORs (odds ratio, MUT *vs* WT) were calculated, only $TET2^{MUT}$ and $IDH1/2^{MUT}$ are significant factors ($TET2^{MUT}$OR=1.44, P=0.03; $IDH1/2^{MUT}$ OR=0.50, P=0.001; $DNMT3A^{MUT}$ OR=1.14, P= 0.53; $TP53^{MUT}$ OR=1.25, P=0.44). $TET2^{MUT}$ which acquires a slightly improvement of ORR(MUT *vs* WT:52.7% *vs* 46.7%) are further identified as having no significant impact on ORR. The combined mutations of $TET2^{MUT}$, $ASXL1^{MUT}$, $SF3B1^{MUT}$ and $RUNX1^{MUT}$, have no additional influence on treatment effect ($TET2^{MUT}$ $ASXL1^{WT}$*vs* others, OR=1.13, P= 0.78; $ASXL1^{MUT}$, OR=1.09, P= 0.59; $RUNX1^{MUT}$ OR=0.92, P= 0.73; $SF3B1^{MUT}$ OR=0.91, P= 0.77). For OS, the pooled HRs(hazard ratio) were calculated, $TP53^{MUT}$ and $DNMT3A^{MUT}$ turn to be poor factors ($DNMT3A^{MUT}$ HR=1.60, P=0.007; $TP53^{MUT}$ HR=2.53, P<0.00001; $TET2^{MUT}$ HR=1.12, P=0.34), the pooled HR was not assessed for $IDH1/2^{MUT}$ owing to limited studies.
Summary and Conclusions: Our results show that, in MDS/AML patients, $TP53^{MUT}$ patients who are resistant to conventional chemotherapy would benefit form HMAs, while $IDH1/2^{MUT}$ patients who proved to be resistant to HMAs should explore other new agents. $TET2^{MUT}$ and $DNMT3A^{MUT}$ patients do not get additional benefits from HMAs. In a word, these mutations can be used together to better select patients who really benefit form the epigenetic treatment.

PF510

THE EVALUATION OF OXIDATIVE STRESS AND IRON METABOLISM MARKERS IN MYELODYSPLASTIC SYNDROMES

M.-A. Gaman[1,*], C. Horescu[2], A.M. Gaman[2,3]

[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Bucharest, [2]Pathophysiology, University of Medicine and Pharmacy of Craiova, [3]Hematology, Filantropia City Hospital of Craiova, Craiova, Romania

Background: Myelodysplastic syndromes are clonal hematopoietic disorders characterized by hypercellular bone marrow with dysmyelopoiesis and peripheral blood cytopenias. Oxidative stress is involved in the aging process and in a manifold of hematological disorders (essential thrombocythemia, chronic lymphocytic leukemia or immune thrombocytopenia). Studies hypothesize that oxidative stress may be involved in the clonal expansion and transformation to AML of MDS, with prior studies reporting elevated levels of reactive oxygen species and a reduced total antioxidant capacity in plasma of MDS patients[1-8].
Aims: To evaluate the level of reactive oxygen species and the antioxidant capacity in MDS patients and healthy volunteers in relation to the iron metabolism.
Methods: We evaluated 30 patients with MDS hospitalized in the Clinic of Hematology, Filantropia City Hospital Craiova, Romania. Informed consent was obtained from all patients and healthy controls enrolled in the study. Oxidative stress was evaluated using a CR3000 analyzer from a single drop of capillary blood. Reactive oxygen species were evaluated by FORT (Free Oxygen Radicals Testing) and the total antioxidant capacity by the FORD (Free Oxygen Radicals Defense) assays. The normal range for the assays is: FORT <2.3 mmol/L H_2O_2 and FORD=1.07 – 1.53 mmol/L. Hemoglobin and parameters of iron metabolism (ferritin and serum iron) were also evaluated. The statistical analysis was performed using the student T-test. A p value ≤0.05 was considered significant.
Results: The male/female ratio was 1.5/1 (mean age=68 years). The distribution on MDS type was: refractory anemia (RA) – 14 patients, refractory anemia with ringed sideroblasts – 2 patients, refractory cytopenia with multi-lineage dysplasia – 4 patients, refractory anemia with excess blasts type 1 (RAEB1) – 3 patients, refractory anemia with excess blasts type 2 (RAEB2) – 5 patients, 5q deletion syndrome – 1 patient, and unclassified MDS – 1 patient. We registered medium high FORT levels in all MDS patients compared to controls (p≤0.05), but higher in patients with RAEB1 and RAEB2, and especially in patients with AML transformation (p≤0.05). FORD levels were lower in patients with MDS compared to controls (p≤0.05), and even lower in patients with RAEB2 transformed in AML (p≤0.05). MDS patients had high levels of ferritin compared to controls (p≤0.05), with significant differences in patients with RAEB2 and blastic transformation. We found a positive correlation between FORT and ferritin levels (Pearson correlation coefficient=0.774) and a weak negative correlation between FORT and hemoglobin values (Pearson correlation coefficient=-0.498) (Table 1).

Table 1.

	RA	RAEB1	RAEB2	RAEB2 → AML
Hemoglobin [g/dL]	8.57	8.3	7	6.87
Serum iron [μg/dL]	152.5	194.33	247.5	279.33
Ferritin [ng/mL]	370.3	1074.67	1923	3090
FORT [mmol/L H_2O_2]	2.51	3.03	3.45	3.95
FORD [mmol/L]	0.88	0.42	0.37	0.23

Summary and Conclusions: In our study, increased markers of oxidative stress and of iron metabolism were found in MDS patients compared to healthy controls. We may hypothesize that the level of oxidative stress and iron markers may be linked to the clonal expansion in MDS and possibly in the transformation to AML.
References: 1. Gaman AM, *et al.* Aging Dis. 2016 May 27;7(3):307-17. doi: 10.14336/AD.2015.1022. 2. Gaman AM, *et al.* Oxid Med Cell Longev. 2014;2014:158135. doi: 10.1155/2014/158135. 3. Gaman AM, *et al.* Haematologica. 2014; 99(s1):773. abstract n. PB2030. 4. Gaman MA, *et al.* Haematologica. 2017; 102(s2):840. abstract n. PB2119. 5. Gaman MA, *et al.* Haematologica. 2017; 102(s2):835. abstract n. PB2105. 6. Gaman AM, *et al.* Haematologica. 2015; 100(s1):772. abstract n. PB1967. 7. Tatic A, *et al.* Rom J Morphol Embryol. 2013;54(4):1177-82. 8. Udensi UK, *et al.* J Exp Clin Cancer Res. 2014 Dec 18;33:106.

Myeloma and other monoclonal gammopathies – Biology & Translational Research

PF511

MUTATIONS IN 19S PROTEASOME SUBUNITS REPRESENT A NOVEL MECHANISM OF ACQUIRED RESISTANCE TO PROTEASOME INHIBITORS IN MULTIPLE MYELOMA PATIENTS

S. Barrio[1,*], M. Simicek[2,3], M. DaVia[1], K. Growkova[2,3], M. Bittrich[1], H. Einsele[1], R. Hajek[2,3], K. M. Kortüm[1]

[1]Medizin II, Universitätsklinikum Würzburg, Würzburg, Germany, [2]Haematology, Ostrava University Hospital, [3]Faculty of Medizine, Ostrava University, Ostrava, Czech Republic

Background: The 26S proteasome is a large multiprotein complex responsible for protein degradation. It is composed of two parts: the regulatory complex (19S) and the catalytical core (20S). Inhibition of this molecular machine is a backbone in the treatment of multiple myeloma (MM). Mutations in *PSMB5* (20S subunit, a direct target of proteasomal inhibitors (PIs)) have been described *in vitro and in vivo*. Recently, down-regulation of genes encoding 19S subunits was related to PIs resistance (Tsvetkov, *et al* eLife 2017).
Aims: Through a meta-analysis of published MM genomic data, we aimed to explore the impact of proteasome mutations in MM patients before and after the PI therapy, and define their potential role in the PI resistance.
Methods: We have analyzed publically available genomic data from 1.714 MM patient samples. From these, 1.219 come from Whole Exome Sequencing (WES) dataset and includes 910 patient samples at diagnosis and 309 from patients previously exposed to the PI therapy. We have combined this data with 495 patient samples analyzed by targeted sequencing (M3P sequencing panel containing *PSMA1*, *PSMD1*, *PSMB5*, *PSMB8* and *PSMB9* subunits). Additionally, we included our WES data from 7 MM patients with minimal residual disease.
Results: We have found mutations in 27 proteasome subunits. The mutational rate at the time of diagnosis was low (<0.5%) for all the 26S subunits, suggesting on any or little importance in the development of MM. However, we observed a significant increase in the mutational rate in the proteasomal subunits after the therapy. In total, 50 out of 910 (5,5%) newly diagnosed patients and 30 out of 316 (9,5%) of pretreated patients have mutations in at least one proteasome subunit. Besides *PSMB5*, most of these alterations occurred in the 19S subunits. Mutations were detected mainly in the subunits responsible for binding of ubiquitinated proteins (PSMD1 and PSMD2) and in the enzymes forming the hexameric AAA ATPase (PSMC1-6). For the ubiquitin receptor *PSMD1* the mutational rate increased from 0,24% at diagnosis to 2,3% after therapy (Z score 4,3. p<0.001), and 5 out of the 11 mutations detected in *PSMD1* clustered in the PSMD2 binding region. From the ATPase subunits, the two mutations detected in PSMC2 were not present at diagnosis (p.R284C 0% *vs* 28% and p.Y429S 0% *vs* 44%) and were located in close proximity to the ATP binding site. Indeed most of the ATPase subunits mutations (9 out of 10) were located directly or in close proximity of the ATP binding suggesting that mechanisms of the PI resistance could be related to the compromised functions of the 19S proteasome regulatory complex (Figure 1).

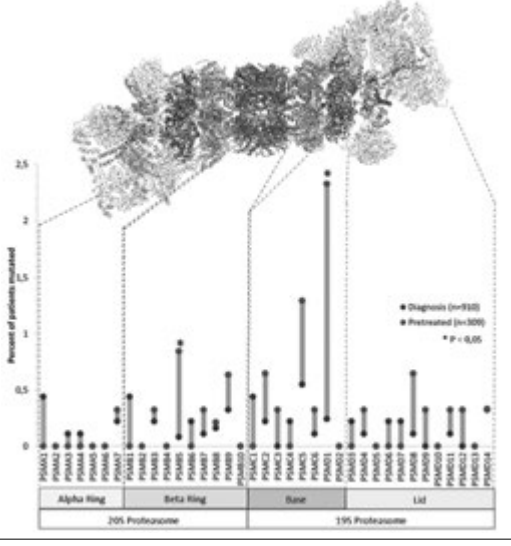


Figure 1.

Summary and Conclusions: The mechanisms of resistance to PIs are not fully understood. We and others have identified mutations in the PI binding subunit *PSMB5* after PI treatment. However, this proteasomal subunit is affected only very rarely in MM patients. Recently, downregulation of the 19S proteasome subunits was proposed as an alternative mechanism of PIs resistance due to the increase in the 20S/26S ratio and changes in transcription. Here, we present an analysis of a large cohort of MM patients with mutations in the 19S subunits, which were significantly enriched after treatment. Identified mutations in 19S will likely affect recognition of the ubiquitinated proteins by proteasome as well as protein unfolding and 20S gate opening. The patient-derived mutations indicate on novel mechanisms in the development of PI resistance in MM. Functional analysis of the mutations is ongoing and results will be presented at the meeting.

PF512

EXPRESSION OF P53 ISOFORMS AT MRNA LEVEL IN MULTIPLE MYELOMA: IMPLICATIONS FOR CLINICAL OUTCOME

E.D.L.A. Rojas[1,*], L.A. Corchete[2], C. De Ramón[3], A.B. Herrero[1], D. Qwaider[4], P. Krzeminski[4], J. Martínez-López[5], R. García-Sanz[3], J. Bladé[6], J.-J. Lahuerta[7], J. San Miguel[8], M.V. Mateos[3], I. Misiewicz-Krzeminska[1], N.C. Gutiérrez [3]

[1]Hematology, Institute of Biomedical Research of Salamanca (IBSAL); Cancer Research Center-IBMCC (USAL-CSIC),, [2]Hematology, Cancer Research Center-IBMCC (USAL-CSIC), [3]Hematology, University Hospital of Salamanca, [4]Hematology, Cancer Research Center-IBMCC (USAL-CSIC), [5]Hematology, Hospital 12 de Octubre; CNIO; Complutense University; CIBERONC, Salamanca, [6]Hematology, Hospital Clinic, Barcelona, [7]Hematology, Hospital 12 de Octubre, Madrid, [8]Hematology, Center for Applied Medical Research (CIMA); IDISNA; CIBERONC, Pamplona, Spain

Background: Loss and/or mutation of the *TP53* gene are associated with short survival in multiple myeloma (MM). Although both genetic abnormalities are routinely explored in the clinical practice, the p53 landscape goes far beyond the *TP53* deletion or mutation. At least 9 p53 mRNA isoforms have been found to be expressed by the *TP53* gene, due the combination of alternative splicing, usage of alternative promoter and/or alternative transcription site start: p53α, p53β, p53γ, Δ40p53α, Δ40p53β, Δ40p53γ, Δ133p53α, Δ133p53β and Δ133p53γ. They can be grouped depending on the molecular mechanisms that lead to their formation as α, β, γ, TA (full length), Δ40 and Δ133 subclasses. Although the deregulation of p53 isoform expression has been described in several cancers, to date, there are no studies evaluating their expression in MM.
Aims: To analyze the relative expression of p53 mRNA isoforms in MM and to evaluate the impact on MM treatment response and survival.
Methods: A total of 89 CD138-selected cell samples from newly diagnosed MM patients enrolled in the clinical trial of the Spanish Myeloma Group, VRD-GEM followed by ASCT conditioned with Mel-200 *versus* BuMel, were included in the study. The quantification of *TP53* gene and the subclasses of p53 mRNA isoforms were carried out by Taqman Real-Time PCR assays. Specific primers were used to quantify TA/∅40, ∅133, α, β, and γ subclasses. The gene/isoforms that had a Ct value ≥35 were considered as not expressed in that sample. The relative expression of the p53 gene and isoforms was normalized to 18S rRNA (ΔCt) and expressed as the fold change (FC) calculated using the $2^{\Delta\Delta Ct}$ method. The statistical analysis was carried out with IBM SPSS Statistics 22.0 and the Simfit package. Progression free survival (PFS) and overall survival (OS) were calculated for each isoform subclass. Survival curves were plotted by means of the Kaplan-Meier method and statistical significance was tested using the log-rank test. The Cutoff Finder software was used to obtain the optimal cutoff.
Results: The α and TA/∅40 p53 subclasses were expressed in all MM samples analyzed, while β, ∅133 and γ subclasses were expressed in 87% (77/89), 71% (63/89), and 48% (43/89) of MM samples, respectively. *TP53* gene was also expressed in all MM samples analyzed. At the time of study, the median follow-up for survivors was 25.3 months (range, 16-41), 76 patients remained free from disease progression and 80 remained alive. After induction treatment, 45% of patients achieved CR or sCR. There was no association between the p53 isoform subclasses expression and response to treatment. Kaplan-Meier log-rank analyses revealed that patients with higher expression of TA/∅40, ∅133, α, β and γ subclasses showed a significantly shorter PFS compared with the patients displaying lower expression of those isoforms (2-years PFS rate was 78% *vs* 93% for TA/∅40, 82% *vs* 88% for ∅133, 78% *vs* 96% for α, 78% *vs* 94% for β, and 77% *vs* 94% for γ; *p*-values <0.05). In addition, high expression levels of TA/∅40 and α subclasses were associated with inferior OS (TA/∅40, *p*-value=0.03 and α, *p*-value=0.04). The expression of β, γ and ∅133 subclasses did not influence OS (Figure 1).

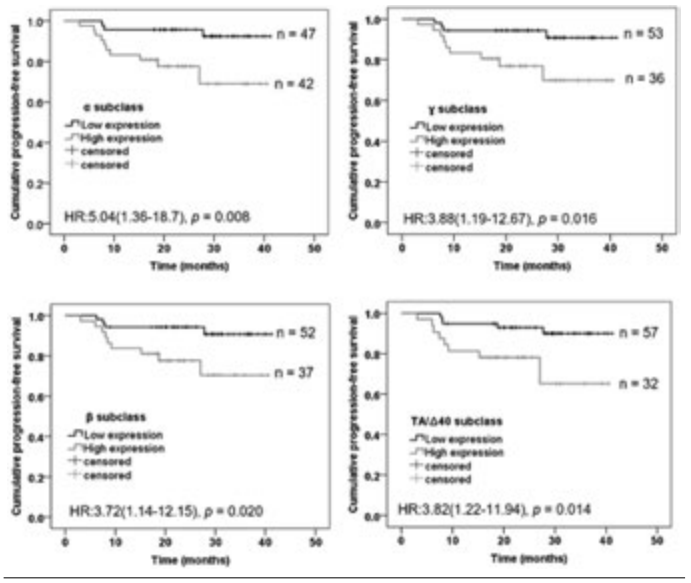


Figure 1.

Summary and Conclusions: The different expression of p53 isoforms at mRNA level is associated with prognosis of MM. The quantification of p53 isoforms in a larger number of patients as well as the analysis of mutation status of *TP53* is ongoing.
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PF513

KRAS BUT NOT NRAS MUTATIONS ARE ASSOCIATED WITH A FAVORABLE OVERALL SURVIVAL IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS TREATED WITH INTENSIVE THERAPY

C. Langer[1], L. Röhner[1], A. Dolnik[1], S. Held[2], M. Kull[1], V. Teleanu[1], D. Bunjes[1], D. Miller[1], S. Köpff[1], M. Schreder[3], L.-O. Mügge[4], M. Engelhardt[5], F. Bassermann[6], H. Döhner[1], R. Bargou[3], H. Einsele[3], L. Bullinger[7], S. Knop[3], J. Krönke[1,*]

[1]Internal Medicine III, Ulm University, Ulm, [2]ClinAssess, Leverkusen, [3]Internal Medicine II, Würzburg University Medical Center, Würzburg, [4]Internal Medicine II, Jena University Hospital, Jena, [5]Internal Medicine I, Freiburg University Hospital, Freiburg, [6]Internal Medicine III, Technical University of Munich, Munich, [7]Hematology, Oncology and Tumorimmunology, Charité, Berlin, Germany

Background: Intensive chemotherapy combined with immunomodulatory drugs or proteasome inhibitors is the standard treatment for patients with newly diagnosed multiple myeloma. The clinical outcome varies substantially among patients with a given treatment what may be due to genetic alterations in the multiple myeloma cells.
Aims: We performed targeted sequencing in order to determine the impact of gene mutations on outcome in newly diagnosed multiple myeloma patients treated with an intensive treatment protocol comprising lenalidomide.
Methods: Targeted sequencing using the HaloPlex™ Target Enrichment System (Agilent) for 28 genes frequently mutated in multiple myeloma was performed in CD138+ enriched baseline bone marrow samples from 76 patients treated in the DSMM XII clinical trial. Patients received four cycles lenalidomide/ adriamycin/ dexamethasone followed by two cycles of high-dose melphalan with autologous stem cell transplantation or sequential autologous/ allogeneic stem cell transplantation for cytogenetically defined high-risk patients. All patients received lenalidomide maintenance for 12 months.
Results: In total, we found 68 mutations in 76 patients. The median number of mutations per patient was 1 (range, 0-3) and 62% of the patients had at least one mutation. The most frequently mutated genes were *NRAS* (N=17/ 22%), *KRAS* (N=13/ 17%), *TP53* (N=5/ 7%), *FAM46C* (N=4/ 5%), *DIS3* (N=3/ 4%), *CCND1* (N=3/ 4%), and *CYLD* (N=3/ 4%). With regard to genes involved in lenalidomide activity, we found one mutation in *IRF4*, but no mutations in *IKZF1* or *IKZF3*. Four of the 5 patients with *TP53* mutations had a concurrent del17p13. *TP53* mutations had an adverse impact on progression-free survival (PFS) and overall survival (OS) but had no additional negative impact in patients with del17p13. *RAS* mutations were more prevalent in patients without t(4;14) and/or t(14;16) and/or del17p13 than in those with these abnormalities (53% *vs* 9%, P=0.0003) and in patients with +9q31 as a marker for hyperdiploidy (83% *vs* 46%, P=0.002). Consistent with previous studies, *NRAS* mutations affected mainly codon Q61 when compared to codon G12, while both codons were equally affected by mutations in *KRAS*. None of the patients had a *BRAF* mutation. The majority of *KRAS* (median VAF 0.21, range 0.07-0.46) and *NRAS* (median VAF 0.25, range 0.07-0.45) mutations were subclonal. Response rates were similar between patients with *NRAS* mutations, *KRAS* mutations or no *RAS* mutations. The type of *RAS* mutation had also no impact on PFS. In contrast, patients with a *KRAS* mutation had an OS of 100% after 4 years *versus* 70% in *NRAS*-mutated patients and 63% in patients without *RAS* mutations (P=0.04 for *KRAS vs NRAS* mutation; P=0.02 for *KRAS vs* no *RAS* mutation).
Summary and Conclusions: The type of (mostly subclonal) *RAS* mutations has no impact on response or PFS in patients treated with intensive therapy and lenalidomide. The superior OS of *KRAS versus NRAS*-mutated patients suggests that subsequent treatment lines were less effective in *NRAS*-mutated patients. *NRAS*-mutated patients were shown to be less sensitive to bortezomib comprising therapies (Mulligan *et al.*, Blood 2014; Bolli *et al.*, Leukemia 2017). Data on 2nd line treatment (*i.e.*, bortezomib) in our DSMM XII patients will be presented.

PF514

ADAR1-MEDIATED-A-TO-I-EDITING IS BIOLOGICALLY AND CLINICALLY RELEVANT IN MULTIPLE MYELOMA

P.J. Teoh[1,*], O. An [1], T.-H. Chung[1], J.Y. Chooi[1], S. Toh[1], S. Fan[2,3], L. Chen[1,4], S.B. Ng[2,3], H. Yang[1], W. J. Chng[1,5,6,7]

[1]Cancer Science Institute of Singapore, [2]Pathology, National University Hospital, [3]Pathology, [4]Anatomy, National University of Singapore, [5]National University Cancer Institute, [6]Medicine, National University of Singapore, [7]Haematology-Oncology, National University Cancer Institute of Singapore, Singapore, Singapore

Background: While alterations at the DNA level have been extensively studied in multiple myeloma (MM), the genomic changes including mutations, indels and translocations cannot yet fully explain all its biological and molecular abnormalities, which remains till this day an incurable disease with eventual emergence of refractory disease. In recent years, it has been shown that abnormalities at the RNA levels may have biological relevance in cancers. The role of microRNAs has been implicated in MM but the significance of other phenomenon such as RNA editing has been relatively underexplored. ADAR1-mediated-A-to-I-editing is a physiologically important post-transcriptional mechanism, its abnormality of which has emerged as a driver for carcinogenesis. Chromosome 1q21 consisting of the ADAR1 locus is amplified in more than 35% of MM, and this abnormality is a poor prognostic marker. Considering co-occupancy of other genes in 1q21, it is unknown if ADAR1 is an important driver gene.
Aims: We aimed to elucidate the RNA editome landscape of MM and to characterize the critical function of a disrupted editome in driving myelomagenesis. We would also like to delineate if ADAR1 is a critical gene in 1q21.
Methods: We analysed several publicly available MM datasets, including CoMMpass RNA-seq dataset from the MMRF and performed a systematic whole transcriptome sequencing on normal CD138+ cells and some primary patient samples (n=17) to identify the ADAR1 expression trend. For the elucidation of ADAR1 protein expression, immunofluorosence was conducted on patient's tissue microarray (TMA, n=200). The functional importance of ADAR1 in MM was investigated by modulating its expression level through the lentivirus system and the phenotypes were examined through cell viability (CTG), cell cycle (PI staining) and colony formation (Methocult semi-solid medium) assays.
Results: Our analyses on the gene expression datasets, RNA seq datasets and TMA identified that ADAR1 was overexpressed in MM. Its expression was gradually increasing along the disease progression route (Normal to MGUS to SMM to MM to Relapsed) and was higher in the high-risk TC classes, namely the 4p16 and MAF- translocation groups, signifying the role of ADAR1 in disease progression and in conferring disease aggressiveness. RNA sequencing revealed that A-to-G editing was the most common form of RNA modification. Critically, relative to the normal CD138+ cells, MM transcriptome was found to be aberrantly hyper-edited, with the highest frequency detected at the intronic and intergenic regions. Level of global editing events was closely correlated with ADAR1 expression. Additionally, artificial manipulation of ADAR1 expression could confer differential level of A-to-G editing, indicating that ADAR1 is a critical player of editing in MM. Importantly, high ADAR1 expression with the

consequent hyperediteme were closely associated with patients' survival. Although there was a close correlation between 1q21 copy number and ADAR1 expression, we found that ADAR1 was actually a poor prognostic factor independent of 1q21 amplification. ADAR1 level could also influence patients' responsiveness to different treatment regimes, including proteasome inhibitors. Physiologically, our functional assays established ADAR1 to be potentially oncogenic, driving growth and proliferation of MM cells.

Summary and Conclusions: Collectively, our data demonstrated that ADAR1- mediated-A-to-I editing is both clinically and biologically relevant in MM. These data unraveled novel insights into MM molecular pathogenesis, the current knowledge of which is deemed unsatisfactory.

PF515

MOLECULAR MECHANISMS OF THE ACTIVITY OF THE NOVEL C-MYC TARGETED-COMPOUND IDP-501 IN MM

P. Krzeminski[1,*], L. González Méndez[1], L. San Segundo[1], P. Mogollón[1], A. Díaz[1], S. Hernández-García[1], M. Algarín[1], M. Martín-Sánchez[1], L. Corchete[1], T. Paíno[1], N. C. Gutiérrez[1], M. V. Mateos[1], L. Nevola[2], S. Esteban[2], M. Garayoa[1], E M. Ocio[1]

[1]Hematology, Hospital Universitario de Salamanca (IBSAL). Centro de Investigación del Cáncer (IBMCC-CSIC-USAL), Salamanca, [2]IDP Discovery Pharma, Barcelona, Spain

Background: c-Myc is a well know player in the pathogenesis of cancer, and particularly in MM. The reduction of c-Myc protein level or inhibition of its binding to Max protein can lead to cell-cycle arrest, apoptosis and tumor regression, representing an appealing anticancer strategy. However, despite the existence of a large number of compounds targeting c-Myc, none of them is currently being effectively used in the clinics. IDP-501 is a new chemical entity specifically designed to target c-Myc protein.

Aims: To evaluate the antimyeloma activity and the underlying mechanisms of the novel cMyc inhibitor IDP-501

Methods: The experiments were performed in several MM cell lines (MM1S, MM1R, U266, NCI-H929, RPMI-8226 and OPM-2). Cell viability of myeloma cells was evaluated by the MTT method and potential synergy was quantitated using the Chou-Talalay Method with the Calcusyn Software. For mechanistic experiments, Western blot, flow cytometry and immunoprecipitation were employed. Förster resonance energy transfer (FRET) experiments were performed with MM1S and RPMI cell lines transiently transfected with plasmids coding GFP-cMYC and dsRED Express2-Max. FRET efficiency was measured by photobleaching method. For *in vivo* studies, MM1S cells were injected subcutaneously into 6-week-old female CB17-SCID mice and tumor development after treatment with IDP-501 ip at 6 mg/Kg was monitored.

Results: IDP-501 reduced the viability of all MM cell lines in a concentration and time dependent manner, with IC50s in the micromolar range. No correlation was found between the sensitivity and the levels of cMyc or p^{Ser62}-cMyc in the different cell lines tested. Interestingly, the effect of the compound was really quick as 3 hours of exposure were sufficient to exert its anti-myeloma effect. Similar reduction of cell viability was observed in primary samples *ex vivo* incubated with the compound. These results were further confirmed *in vivo* as IDP-501 inhibited tumor growth in mice bearing a subcutaneous plasmacytoma with a 75% reduction in tumor volume at day +38 of treatment. These results are particularly compelling since the previously investigated cMyc inhibitors failed to have any effect in animal models of MM. Regarding the mechanism of this anti-myeloma action, the cytotoxic effect revealed by MTT was confirmed by Annexin-V/PI assay what indicated an apoptotic mechanism of action; furthermore, experiments with caspase inhibitors suggested the presence of caspase-independent apoptosis. IDP-501 treatment resulted in an increase of DNA damage with marked by H2Ax phosphorylation and reduction of the mitochondrial potential. Unlike other cMyc inhibitors, IDP-501 did not induce phosphorylation of AMPK in MM, but, interestingly, nuclear fraction of cMyc and Max was reduced, and immunoprecipitation and FRET experiments demonstrated a dissociation of Myc-Max complexes caused by the inhibitor, pointing out at this mechanism as the main potential target of IDP-501. Finally, and importantly, IDP-501 potentiated the activity of bortezomib and dexamethasone and cyclophosphamide and dexamethasone, with combination indexes below 0.1.

Summary and Conclusions: The results here presented suggest that IDP-501 could be the first effective strategy able to inhibit the activity of cMyc in MM, and its preclinical activity (both *in vitro* and *in vivo*) prompts its clinical evaluation for the treatment of relapsed MM patients.

This project was supported by IDP-Discovery Pharma and by the Spanish National Projects RTC-2016-5056-1 and FIS PI15/00067.

PF516

SNPS ARRAY ANALYSIS OF COPY NUMBER ALTERATIONS (CNAS) IN NEWLY DIAGNOSED TRANSPLANT-ELIGIBLE MULTIPLE MYELOMA (MM) PATIENTS: DEFINITION OF A PROGNOSTIC RISK-CLASSIFIER

C. Terragna[1,*], V. Solli[2], A. Poletti[2], M. Martello[2], B. Santacroce[2], A. Baraldi[3], L. Dozza[2], A. Sementa[4], A. Larocca[5], L. Pantani[2], N. Pescosta[6], P. Tacchetti[2], G. Leonardi[7], E. Zamagni[2], R. Termini[2], C. Musolino[8], E. Borsi[2], G. Marzocchi[2], L. Canepa[9], G. Ameli[2], B.A. Zannetti[2], A. Pascarella[10], L. De Rosa[11], N. Testoni[2], G. Martinelli[12], P. Sonneveld[13], M. Cavo[2]

[1]DIMES, University of Bologna, [2]"Seràgnoli" Institute of Hematology, Bologna, [3]Dipartimento di Ematologia e Medicina Trasfusionale, Az.Osp. SS. Antonio e Biagio, Alessandria, [4]A.O.R.N. San G.Moscat, Avellino, [5]Myeloma Unit, Division of Hematology, University of Torino, Torino, [6]Ospedale Generale Regionale-Divisione di Ematologia e Centro Trapianto Midollo Osseo, Bolzano, [7]Department of Medical and Surgical Sciences, University of Modena and Reggio Emilia, Modena, [8]Section of Hematology, A.O.U. Policlinico, Messina, [9]Clinic of Hematology, Department of Internal Medicine (DiMI), Policlinico San Martino, IRCCS per l'Oncologia, University of Genoa, Genova, [10]Ospedalle Dell Angelo, Venezia, [11]Ospedale San Camillo Forlanini, Roma, [12]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) Srl - IRCCS, Meldola, Italy, [13]Erasmus Medical Center, Rotterdam, Netherlands

Background: The genomic heterogeneity that characterizes all MM patients (pts) is the consequence of an evolutionary process operating during the pre-neoplastic phase of the disease. Among the MM genomic heterogeneity, several lesions are recurrent and their presence significantly impacts the prognosis.

Aims: To deeply characterize the MM clone of newly diagnosed MM pts by means of a genomic approach, aimed to identify CNAs affecting clinical outcomes.

Methods: 471 pts were included in the present study: 329 and 74 were treated in the context of the EMN02/HO95 or the BO2005 study, respectively. Overall, 286 pts received an autologous stem cell transplantation (ASCT). Genomic data were obtained by SNPs array on BM-CD138+ cells. At a median follow-up of 43 months (m), the estimated PFS and OS was 55% and 71%, respectively.

Results: We first dissected the genomic complexity observed in the overall population: as expected, most pts had a complex genomic background, while occasionally few of them (27/397, 7%) carried only one of the following alterations: CN losses (L) on chr. 13q, CN gains (G) on chr.1q, hyperdiploidy and any IgH translocations. Remarkably, these 4 alterations were also the most frequently observed across the overall pts population, and their combination identified 16 subgroups harboring a progressively more complex genomic profile. This suggests that these 4 alterations – either combined or alone - are both necessary and sufficient to describe the genomic background of the whole population. Analysis of clinical outcomes showed that inferior outcomes correlated with the presence of both CN-L on chr.13q and CN-G on chr.1q, regardless of others genomic lesions. Based on this, we re-stratified pts according to the absence or presence of 1 or both of these lesions, to define the following 3 groups: group 1 (125 pts), carrying both CN-L on chr.13q and CN-G on chr.1q; group 2 (160 pts), carrying either CN-L on chr.13q or CN-G on chr.1q; group 3 (186 pts), lacking both these lesions. We observed that the 43-m estimates of PFS was significantly shorter in group 1 (48%, 61% and 72% respectively; p=0.0021). OS rates were 72% in group 1 *vs* 88% in group 2 and 91% in group 3 (p=0.0001). PFS hazard ratio (HR) of pts in group 1 (HR=2,51, 95%CI: 1.48-4.25, p=0.0006) was comparable to that of pts carrying either del(17p) (HR=2.09, 95%CI: 1.20-3.65) or t(4;14) (HR=1.84, 95%CI: 1.14-2.99), resulting as an independent factor in predicting PFS in a Cox multivariate analysis (both from del(17p) and t(4;14), p=0.01). PFS benefit correlated with ASCT was statistically significant in groups 2 and 3, while only a trend was observed in group 1. The genomic background of pts belonging to group 1 was characterized by the presence of several genomic aberrations, which overall severely compromise genes involved in the control of cell cycle progression (Rb1, CKS1B, MDM4, MCL1, genes coding for the DREAM-complex components, YAP1, MYC, FANCA).

Summary and Conclusions: Based on an evolutionary rationale, we proposed a simply, CNAs-based risk-classifier, which was able to segregate our cohort of pts with newly diagnosed MM into 3 well-populated groups, thus recapitulating most of the genomic aberrations commonly described in MM. The 3 groups have different clinical outcomes and PFS benefit imparted from ASCT. According to the observed genomic background, a strong de-regulation of cell cycle control might be responsible of the dismal outcome of pts carrying both CN-L on chr.13 and CN-G on chr. 1q.

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PF517

PEPTIDE STIMULATION OF PD-L1 SPECIFIC T CELLS NATURALLY OCCURING IN MYELOMA PATIENTS AUGMENT THE ADCC ACTIVITY OF DARATUMUMAB

N.G. Jørgensen[1,*], *S.M.* Ahmad[1], *S.K.* Bendtsen[1], *E.* Martinenaite[1], *M.O.* Holmström[1], *P.* Andersen[2], *Ö.* Met[134], *M.H.* Andersen[13]

[1]Center for Cancer Immune Therapy (CCIT), Department of Hematology, [2]Stem Cell Center, University Hospital Herlev, Herlev, [3]Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, [4]Department of Oncology, University Hospital Herlev, Herlev, Denmark

Background: Daratumumab, an anti-CD38 IgG1k monoclonal antibody, represents a resent major advance in the treatment of patients with multiple myeloma (MM). One of the mechanisms of action of daratumumab is antibody-dependent cell-mediated cytotoxicity (ADCC) via interaction with Fcγ-receptors on natural killer (NK) cells. We have identified naturally occurring T cells specific to programmed death ligand 1 (PD-L1). These PD-L1 specific T cells are naturally present in healthy donors as well as cancer patients. We have previously shown that stimulation of PD-L1 specific T cells has a boosting effect on anti-viral immunity (Ahmad *et al., Leukemia* 2013) and enhances the immunogenicity of an antineoplastic dendritic cell vaccine (Munir *et al., Oncoimmunology*, 2016). Thus, PD-L1 specific T cells seem to have a broad immune-enhancing effect which might also boost the effect of antineoplastic antibodies.
Aims: The purpose of this study was to assess the presence of PD-L1 specific T cells in MM. In case of their presence in MM patients, we hypothesized that stimulation with PD-L1 peptide can boost the ADCC effect of daratumumab against MM cells.
Methods: To test the presence of PD-L1 specific T cells, leukapheresis products were assayed by interferon-γ (IFN-γ) enzyme-linked immunospot (ELISPOT) against a 19-amino acid peptide from the signal peptide of PD-L1. ^{51}Cr-release cytotoxic assays were used to assess the cytotoxic capacity of a PD-L1 specific T cell culture, leukapheresis products and healthy donor peripheral blood mononuclear cells (HD PBMCs). Target cells in the cytotoxic assays were the myeloma cell lines U266 and RPMI-8226. ADCC was assessed in ^{51}Cr-release cytotoxic assays +/- daratumumab, when HLA-types of effector and target cells were not matching.
Results: PD-L1 specific T-cells were present and responded to stimulation with PD-L1 peptide in INF-γ ELISPOT assays in 13 of 19 leukapheresis products. Furthermore, an HLA-A2 restricted PD-L1 specific T cell culture showed dose-dependent lysis of the HLA-matched PD-L1 positive myeloma cell line U266. This lysis was enhanced when PD-L1 expression on the myeloma cell line was increased by treatment with INF-γ. Finally, both HD PBMCs and leukapheresis products from myeloma patients lysed the CD38-positive myeloma cell line RPMI-8226, when the cell line was treated with daratumumab. Importantly, this lysis was frequently increased when the leukapheresis products or HD PBMCs were stimulated with PD-L1 peptide. Since the leukapheresis products and myeloma cell line did not have matching HLA-types, the boosting effect was not directly T-cell mediated but more likely due to an increased cytotoxicity by NK-cells, *i.e.* ADCC. Interestingly, the PD-L1 peptide mediated enhancement of the activity of daratumumab was only seen in patient samples showing presence and activity of PD-L1 specific T cells, as evidenced by IFN-γ ELISPOT responses against the PD-L1 peptide.
Summary and Conclusions: PD-L1 specific T cells are present in patients with MM, and myeloma cells can be lysed by PD-L1 specific T cells. Stimulation with a PD-L1 peptide augments the daratumumab mediated ADCC against myeloma cells. We have an ongoing phase I trial of PD-L1 peptide vaccination as monotherapy as consolidation after high dose chemotherapy and stem cell support for patients with MM. These findings are to our knowledge the first evidence that a peptide vaccine can boost the activity of a monoclonal antibody. The data support a clinical trial combining daratumumab with PD-L1 peptide vaccination.

PF518

NOVEL KINASE INHIBITORS AFURESERTIB AND PIM447 ARE ACTIVE IN A PREDICTIVE MYELOMA *IN VIVO* MODEL, AND A CRISPR GENOME-WIDE SCREENING APPROACH IDENTIFIES BIOMARKERS DETERMINING SUSCEPTIBILITY

N. Keane[1,*], M. Sharik[2], A. Natoni[1], M. Chesi[2], L. Bergsagel[2], O. Michael[1]

[1]Haematology, National University of Ireland Galway, Galway, Ireland, [2]Research, Mayo Clinic, Scottsdale, United States

Background: Novel therapies for advanced Multiple Myeloma (MM) constitute an unmet clinical need and increasing knowledge of the molecular abnormalities driving MM places an onus on clinicians to tailor treatment to the individual. The overlapping Pim and PI3K/AKT/mTOR pathways play a key role in MM pathogenesis. Our group reported potent synergy with Pim inhibitor pim447 and Akt inhibitor afuresertib in combination in MM however investigation of this combination in a murine MM model failed to identify a therapeutic window.
Aims: We hypothesise that these inhibitors will complement current standard therapies proteasome inhibitors (PIs) and Immunomodulatory drugs(Imids) without significant toxicity given their non-overlapping mechanisms of action, and that use of a CRISPR screen will identify molecular signature of patients likely to benefit from these novel combination strategies.
Methods: The CoMMpass dataset was analysed for patterns of expression of Akt and Pim kinases in MM. Cell viability was determined using MTS assay. pAKT was measured by immunoblotting and Pim-2 levels by RNA sequencing. *In vivo* studies were performed in the VK*MYC *de novo* (for single drug treatments) or transplantable (for combination treatment) models. Cas9-expressing KMS11 cells were transfected with TKO CRISPR knockout library. Cells were treated with concentrations that induce >90% cell death. gDNA was extracted from surviving cells, gRNAs amplified and sequencing performed on Illumina HiSeq2500. Differential gene expression was determined between combinations and controls, and pathways identified using Ingenuity™ pathway analysis.
Results: Pim-2 expression is highest in MM featuring Ig translocations, while Akt expression increases with advancing disease across all subtypes. Afuresertib and pim447 activity increases with greater pAkt and Pim-2 expression, respectively. Combination of each inhibitor with PIs/Imids is synergistic in MM cell lines. *In vivo* investigation of combinations with bortezomib in VK*MYC12598 model reveals synergy with bortezomib/pim447, with significant reduction in M-Spike in the combination arm. CRISPR screening identifies Ras pathway activation as the key determinant of sensitivity to this combination. By contrast we observed no synergy *in vivo* with the bortezomib/afuresertib combination, although we did observe improved survival in the afuresertib-only arm in this highly resistant model unexpectedly. Furthermore, afuresertib was inactive in the *de novo* VK*MYC model, indicating that afuresertib will likely find a niche in treatment of advanced, highly proliferative MM. Though Imid combinations cannot be tested in this model we establish that synergy in combining Imids with afuresertib/pim447 results from additive degradation of Ikaros and Aiolos followed by downstream synergistic depletion of MYC and IRF4. CRISPR screening of these promising combinations suggest that the Ras signaling pathway is also important in determining sensitivity to combined Imid/AKT inhibiton and that upregulation of NF B signaling may predict resistance to combined Imid/Pim inhibition.
Summary and Conclusions: We identify novel therapeutic strategies to exploit kinase signaling in MM. Combination of bortezomib with pim447 is synergistic in a clinically-predictive *in vivo* model. Afuresertib is identified as a therapeutic option for advanced MM. MM subtypes with Ras pathway activation and suppression of NF B pathway may be more likely to benefit from combinations incorporating Pim- or Akt-inhibition with standard therapies.

PF519

CD26 IS A POTENTIAL THERAPEUTIC TARGET BY HUMANIZED MONOCLONAL ANTIBODY IN MULTIPLE MYELOMA

H. Nishida[1,*], M. Hayashi[1], H. Suzuki[1], M. Sakamoto[1], T. Yamada[1]

[1]Department of Pathology, Keio University, School of Medicine, Tokyo, Japan

Background: CD26, a 110-kDa transmembrane glycoprotein which is expressed on several tumor cells including malignant lymphoma, has been implicated in tumorigenesis, whereas little is known about its role in plasma cell malignancies. More recently, we have identified CD26 expression on human osteoclasts (OCs) in MM. *In vitro* studies showed that although CD26 expression was low or absent on MM cell lines cultured alone, it was intensely and uniformly expressed on MM cell lines co-cultured with OCs.
Aims: In the present study, we examine the therapeutic impact of huCD26mAb, a humanized monoclonal IgG_1 antibody specifically targeting CD26 on MM cell growth and clarify CD26 could be a target for the treatment of MM.
Methods: Antibody-dependent cellular cytotoxicity (ADCC) assay and complement-dependent cytotoxicity (CDC) assay by huCD26mAb were conducted against MM cell lines, cultured alone or co-cultured with OCs. *In vivo* effect of huCD26mAb was also analyzed using xenograft murine model.
Results: Immunostaining on bone marrow biopsy specimens showed that CD26 is expressed on plasma cells in several MM patients. First, we inves-

tigated the impact of huCD26mAb on growth of MM cell lines (KMS18, KMS26, KMS27, KMS28, KMS34, U266). huCD26mAb had no direct effect on viability of tested CD26- MM cell lines cultured alone, but it inhibited growth of CD26+ MM cell lines co-cultured with OCs, chiefly at higher concentrations (>10 g/ml) (p<.05). Next, we conducted calcein-AM release assay to analyze the ability of huCD26mAb to lyse MM cell lines via ADCC. huCD26mAb triggered ADCC against CD26+ MM cell lines co-cultured with OCs in the presence of natural killer (NK) cells in a dose-dependent manner with lytic activity by huCD26mAb starting at 0.0001 g/ml and maximum lysis at 10 g/ml (p<.05) and in an E/T ratio-dependent manner. In contrast, huCD26mAb did not exhibit dose-dependent ADCC against CD26-MM cell lines cultured alone. Furthermore, CD26+ KMS18, KMS26 and KMS28 after co-culture with OCs were pretreated with dexamethasone (Dexa, 25nM) or bortezomib (BTZ, 3nM). Then, subsequent MM cell lysis triggered by huCD26mAb (10 g/ml) was significantly increased by NK cell-dependent ADCC at the E/T ratio of 20 (p<.05). huCD26mAb revealed synergistic ADCC against CD26+ MM.1R, pretreated with lenalidomide (Lena, 0.05, 0.5 M) (p<.05, <.01). Pretreatment of NK cells with Lena also potentiated subsequent huCD26mAb-induced lysis of CD26+ MM.1R cells in a dose-dependent fashion (p<.05, <.01). Next, the effect of CDC by huCD26mAb against CD26+ MM cells was tested. However, in the presence of human serum as a source of complement, huCD26mAb exerted low or absent potential to confer CDC against CD26+ MM cell lines. Moreover, we examined ADCC activity by huCD26mAb against SP fractions in MM cells. Interestingly, although Lena alone did not decrease SP ratio in CD26+ RPMI8226 or KMS11, huCD26mAb substantially reduced its ratio and its further reduction was observed with both in combination (p<.01). Lastly, *in vivo* effect of huCD26mAb using NOD/SCID-hu mice by direct intrabone injection of MM cells into subcutaneously implanted human bone grafts showed that huCD26mAb significantly reduced CD26+ MM tumor burden and TRAP+ OC formation (p<.05).

Summary and Conclusions: huCD26mAb elicited significant anti-MM efficacy by impairing both CD26+ MM cell growth and OC formation. Our results suggest that CD26 could be a potential therapeutic target of antibody-based therapy in MM.

PF520

DNA REPAIR PROCESSES TARGET TRANSCRIBED REGION IN MULTIPLE MYELOMA

M. Samur[1], R. Szalat[2], M. Lawlor[3], F. Maura[4], C. Ott[3], N. Bolli[5], K. Anderson[2], S. Minvielle[6], P. Campbell[4], H. Avet-Loiseau[7], N. Munshi[2,*]

[1]Biostatistics and Computational Biology, [2]Dana Farber Cancer Institute, [3]Massachusetts General Hospital, Boston, United States, [4]Wellcome Trust Sanger Institute, Cambridge, United Kingdom, [5]University of Milan, Milan, Italy, [6]CRCINA, INSERM, CNRS, Université de Nantes, Nantes, [7]L'Institut Universitaire du Cancer Oncopole, Toulouse, France

Background: Multiple myeloma (MM) is a plasma cells malignancy characterized by a complex and heterogeneous genomic landscape. Using whole-exome and targeted sequencing, two main active mutational processes have been identified in MM provide initial insight into both initial pathogenic mechanisms and the processes affecting disease progression. However, limited numbers of mutations identified using WES does not allow interrogation of question whether different parts of the genome are targeted by different mutational processes.

Aims: Here we interrogate various genomic regions and mutation types such as non-coding regions and protein coding regions with missense mutations, to understand activated processes that cause DNA alteration in MM.

Methods: We have processed 39 purified CD138+ MM cells samples with Whole Genome Sequencing; ten samples also have RNA and ATAC sequencing. Public WES data from 999 samples were collected from dbGaP and CoMMpass study. Mutational processes were analyzed using non-negative matrix factorization and multiple linear regression model.

Results: We identified an average >5000 SNVs per patient sample with a total of over 200K SNVs from WGS and additional >170K SNVs from public WES data. Overall C>T mutations constituted 30% of all detected mutations across the genome, including a small fraction of C>T mutations within CpG islands. Majority of the mutations were observed in the intergenic (IGR), introns and non-coding RNAs regions (lincRNAs and ncRNA). Transcribed strand of the genome showed enrichment in C>A and C>T mutations. With non-negative matrix factorization we were able to identify 8 mutational processes in MM genome six of which were not described before. Although APOBEC/AID processes were the majority for the non coding genome, DNA repair related processes were highly active in the coding genome. We have integrated WGS, ATACseq and RNAseq data from same patients and identified that DNA damage activity increased in the expressed genes and sub clonal populations. We have confirmed our results using publicly available WES data from 999 samples.

Summary and Conclusions: C>A and C>T enrichment on the transcribed strand has been connected to high transcriptional activities and different mechanisms such as single *versus* double-strand DNA repair functions. Targeting of non-coding regions by APOBEC/AID could be part of ongoing somatic hypermutation in MM. However, missense mutations may be driven by DNA double-strand break-repair by homologous recombination and DNA mismatch repair processes. Increased intensity for DNA repair signatures in subclonal missense mutations may indicate that these processes may occur late during the MM progression. Future studies that can compare paired samples from early stage to relapsed and refractory myeloma can help us understand if DNA repair processes become dominant during the MM development.

PF521

EFFECT OF DARATUMUMAB ON NORMAL PLASMA CELLS, POLYCLONAL IMMUNOGLOBULINS AND VACCINATION RESPONSES IN EXTENSIVELY PRETREATED MYELOMA PATIENTS

K. Frerichs[1,*], P. Bosman[1], J. van Velzen[2], I. Nijhof[1], A. Bloem[2], T. Mutis[1], S. Zweegman[1], N. van de Donk[1]

[1]Hematology, VU University Medical Center, Amsterdam, [2]Laboratory of Translational Immunology, University Medical Center, Utrecht, Netherlands

Background: Daratumumab (DARA) is a CD38-targeting monoclonal antibody, which induces multiple myeloma (MM) cell death via several immune-mediated mechanisms. DARA is approved as monotherapy and in combination with standards of care in relapsed/refractory MM, but the effect of DARA on normal plasma cells (PCs) is still unknown.

Aims: We studied the effect of DARA on frequency of normal PCs, polyclonal immunoglobulin levels and antibody responses upon vaccination in MM patients.

Methods: We analyzed baseline CD38 expression levels (median fluorescence intensity, MFI) on PCs from relapsed/refractory (RR) MM patients treated with DARA monotherapy (GEN501 or DARA-ATRA trial), and from healthy controls (HC). The frequency of normal PCs in bone marrow (BM) samples from these MM patients was analyzed before and during DARA treatment. We also measured serial levels of polyclonal immunoglobulins (IgG, IgA, IgM and IgE). Additionally, in accordance with (inter)national guidelines, these patients received *Haemophilus influenzae type B (HiB)* and *Streptococcus pneumoniae* vaccination. Specific serum antibody titers (IgG) against Hib and pneumococcal serotypes were measured before, and 4, 8, 12 and 16 weeks after the first vaccination.

Results: CD38 expression on normal PCs from HCs (n=5) was significantly higher (median MFI: 141288; range: 112426-164626) compared to MM PCs (n=25; median MFI: 36174; range: 6997-160005; *P<0.0001*). As a consequence, DARA monotherapy significantly reduced the frequency of normal PCs in serial BM samples after the initiation of DARA treatment (baseline: n=17, 0.03% normal PC; 3 months: n=10, 0.0007%, *P=0.0078*; disease progression: n=15, 0.001%, *P=0.002*). Surviving normal PCs had reduced CD38 expression levels, as well as a decrease in CD19 levels. Such phenotypic changes are similar to what we previously observed on MM cells. This reduction in normal PCs resulted in a decrease in polyclonal IgA, IgM and IgE in 30 patients treated with DARA monotherapy, while polyclonal IgG levels remained unchanged. Furthermore, antibody responses upon vaccination were assessed in these heavily pretreated RR MM patients (median 4 prior lines of therapy [range 2-9]). Seventeen patients received HiB (Act-HiB) and pneumococcal vaccination (PCV-13, followed 8 weeks later by PPV-23). At this moment, antibody responses for HiB are available in 14 of these patients. Twelve patients (86%) showed a good response, either by obtaining a protective titer ≥1.0µg/mL (if baseline levels <0.15µg/mL) or by a 4-fold increase in antibody titer (if baseline titers were ≥0.15µg/mL, Figure 1A). Pneumococcal antibody responses are currently available in 9 of these vaccinated patients. 7 patients (78%) achieved a ≥2 fold increase in antibody titer for at least 6 out of 9 analyzed subtypes (6B, 8, 9, 14, 15B, 19F, 20, 23F and 33F, Figure 1B). None of the vaccinated patients suffered from pneumococcal or HiB infections thusfar.

Summary and Conclusions: Normal PCs have higher CD38 expression compared to MM cells. We found that the frequency of normal PCs in BM is reduced upon DARA treatment in MM patients. The surviving normal PCs showed reduced CD38 cell surface expression. We also observed reduced levels of polyclonal IgA, IgM, and IgE in these patients, while polyclonal IgG levels remained stable. Furthermore, a majority of heavily pretreated MM patients was able to produce protective antibodies in response to vaccination, even during DARA treatment. Given the high incidence of respi-

ratory infections, we recommend to vaccinate patients also during CD38-targeted therapy.

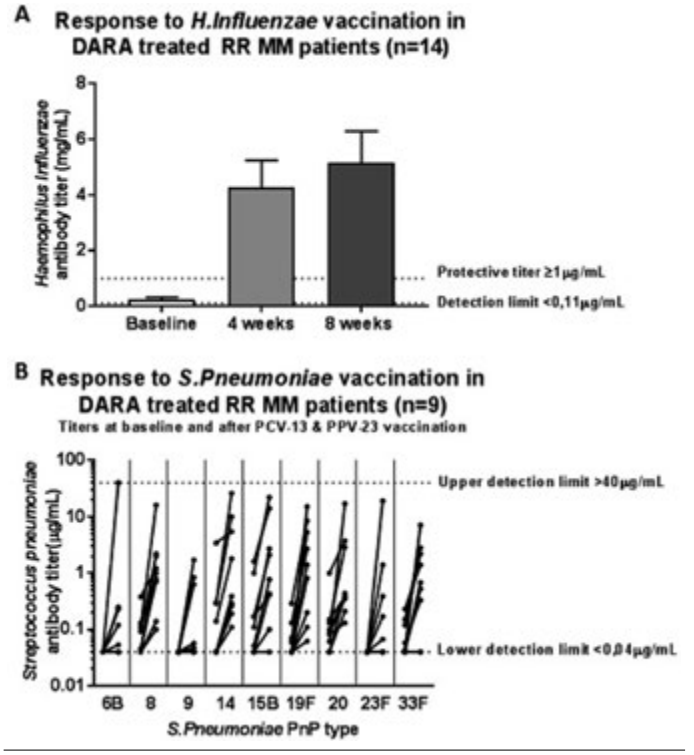


Figure 1.

PF522

STUDY OF PLASMA CELL TRASCRIPTOME IN SMOLDERING MYELOMA PATIENTS IN RELATIONSHIP TO PROGRESSION TO ACTIVE MULTIPLE MYELOMA

P. Storti[1,*], B. Dalla Palma[1,2], K. Todoerti[3,4], L. Agnelli[3,4], V. Marchica[1], M. Bolzoni[1], F. Costa[1], D. Toscani[1], E. Vicario[1,5], G. Sammarelli[2], F. Accardi[1,2], G. Todaro[1], L. Notarfranchi[2], I. Manfra[2], F. De Luca[1], L. Craviotto[1,2], F. Aversa[1,2], A. Neri[3,4], N. Giuliani[1,2]

[1]Medicine and Surgery, University of Parma, [2]Hematology and BMT Center, Azienda Ospedaliera Universitaria di Parma, PARMA, [3]Onocology and Hemato-oncology, University of Milan, [4]Hematology Unit, Fondazione IRCCS Ospedale Maggiore Policlinico, Milan, [5]Biopathology and Medical Biotechnologies, Biology and Genetic Section, University of Palermo, Palermo, Italy

Background: The molecular mechanisms involved in the progression of smoldering (SMM) to active multiple myeloma (MM) are not completely understood. Several genomic data indicate that genetic alterations that characterized MM patients are already present in SMM ones. However, few data are available on the transcriptional profiles of SMM patients in relationship to the progression to active MM.
Aims: In order to address this aim, in this study, we firstly compared plasma cells (PCs) from paired samples (SMM evolved in MM), then we investigated transcriptional differences between progressed and not progressed SMM patients.
Methods: All the SMM patients included in this study were defined according to the IMWG revised diagnostic criteria and stratified by known risk factors of progression. Median age at diagnosis was 72 years (range 58-84) in progressed patients and 74 years (range 38-86) in non-progressed patients. The median percentage of bone marrow plasma cells (BMPCs) at diagnosis in the 8 subsequently progressed SMM was 30% (range 13-40). High-risk FISH features (either del(17p), t(4;14) or t(14;16)) was detected in 3 out of 8 patients. The median time to progression was 14 months and all patients progressed with onset of CRAB features. The 12 non-progressed SMM patients had a median percentage of BMPCs of 14% (range 10-25%); high-risk FISH features was detected in 3 out of the 5 patients with enough BMPCs to allow examination. 55% of the patients were classified as intermediate-risk by Mayo score, whereas the remaining were low-risk. Median follow up in this cohort of patients was 49 months. Primary CD138$^+$ PCs were purified by magnetic beads from bone marrow (BM) aspirate of 8 paired SMM and MM samples and from 12 non-progressed SMM. Global expression profiles of 19.012 protein-coding and 13.972 non-coding genes were obtained on GeneChip® ClariomD arrays using annotations from Gencode v26. Hierarchical clustering was applied on the most variable coding/non-coding genes across the entire dataset. Rank Product and Gene Set Enrichment Analysis were used for differential and functional analyses.
Results: Hierarchical clustering analysis evidenced paired groupings of the 8 progressed SMM with the corresponding MM samples, whereas no markedly agglomerative similarities have been found with/between the non-progressed SMM cases. No significant differentially expressed coding/non-coding genes were observed between paired SMM and MM cases. Conversely, progressed SMM compared to non-progressed SMM down-regulated antigen processing and presentation and natural killer mediated cytotoxicity gene sets, whereas genes associated with proliferation and hyperdiploidy were positively modulated. Specifically, among the 28 most significantly deregulated genes, the Wnt inhibitors *FRZB* and *DKK1*, the TGF-beta targeting gene *SMAD1* and the pro-angiogenic one *CTGF* were found up-regulated in relation to progression to MM. This agrees with the recent finding that BM DKK-1 protein level is a new independent factor for progression in SMM (Dalla Palma B et al Br J Haematol 2017). Finally, 45 non-coding genes, mostly down-regulated (69%), were found modulated in the comparison between progressed and non-progressed SMM cases.
Summary and Conclusions: Our data indicate that the transcriptome of the PCs of SMM patients who progressed to MM did not significantly change throughout the progression; conversely, the upregulation of inhibitors of canonical Wnt signaling in PCs differentiated progressed from non progressed SMM patients.

PF523

GENOMIC CHARACTERIZATION OF HEMATOPOIETIC STEM AND PROGENITOR CELLS IN MULTIPLE MYELOMA REVEALS DISTINCT PATTERNS OF DISEASE EVOLUTION

A.-M. Leppä[1,*], S. Eldfors[1], P. Anttila[2], C. Heckman[1]

[1]Institute for Molecular Medicine Finland, [2]Helsinki University Hospital Comprehensive Cancer Center, Helsinki, Finland

Background: The expected overall survival of multiple myeloma (MM) patients has remarkably improved over the recent decades, but as a result, cases of secondary myelodysplastic syndrome and secondary acute myeloid leukemia (sMDS/sAML) have increasingly been recognized. Even though pathogenic alterations in MM have been well characterized in the malignant plasma cell fraction, little is known about the mechanisms leading to secondary myeloid disease. Also, it is unknown if molecular-level alterations can be detected in the hematopoietic stem and progenitor cells (HSPCs) of MM patients, that may be used to identify those patients predisposed to develop sMDS/sAML, as well as to better understand the clonal evolution of MM.
Aims: The aim of this study was to investigate if the HSPC fraction of MM patients harbor mutations associated with MDS/AML, potentially predisposing the patients to development of secondary disease, and to study the clonal evolution of MM.
Methods: Bone marrow (BM) aspirates were collected from 45 MM patients. For 6 patients, more than one sample was collected, thus, 51 samples were used in total. 30 samples were collected at the time of diagnosis and 21 at relapse. BM mononuclear cells (BM-MNCs) were isolated by gradient centrifugation, and processed by immunomagnetic bead selection to enrich for CD138+ cells. The CD138- fraction was further processed to isolate CD34+ HSPCs. DNA was prepared from the isolated cell fractions as well as matching skin biopsies. Whole exome sequence analysis was performed using the CD138+ and skin DNA, while targeted gene capture was performed using the TruSight Myeloid Sequencing Panel (Illumina, San Diego, CA, USA) using the CD34+ cell and skin DNA. High confidence somatic mutations were called for each CD34 and CD138-enriched sample.
Results: In 20 of the 51 HSPC-enriched MM samples, we detected a total of 42 mutations in 23 genes (39.2%) (Figure 1). The most commonly mutated gene was DNMT3A with 7 mutations observed. We compared the measured variant allele frequency (VAF) in the mutated samples and noticed substantial variance between the patients. Most of the mutations observed had a low VAF, more specifically 29/42 (69%) mutations had a VAF below 10%. The highest VAF was detected in TET2 (33.8% VAF, R1282H), SRSF2 (33.1% VAF, P95H) and IDH1 (28.5% VAF, R132H). Moreover, the first two were detected in the only patient in this cohort who proceeded to develop MDS, while the IDH1 hotspot mutation is considered an early driver event in leukemogenesis. Notably, exposure to alkylating agents and relapse-status were significantly associated with a higher VAF (both, P<0.01), which is an indication of clonal expansion.

The majority of the mutations we detected in the HSPC enriched samples were not observed in the CD138+ plasma cells. Intriguingly, four patients did, however, share mutations specific for MDS/AML in both cell fractions. Thus, MM patients with detectable MDS/AML associated mutations in HSPCs seem to present two different clonal evolution patterns during MM development: the malignant plasma cell population may arise from a genetically distinct subpopulation as well as from the genetically normal cell population.

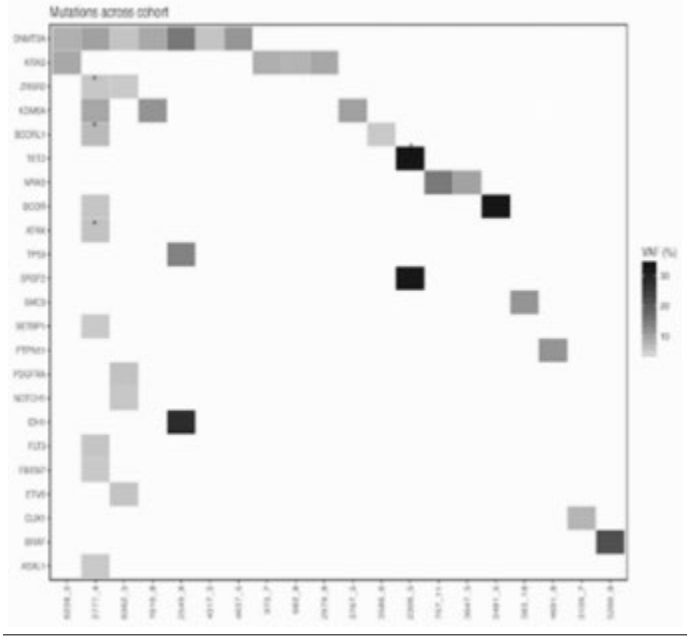


Figure 1.

Summary and Conclusions: Mutations in genes associated with MDS/AML are fairly frequent in HSPCs of MM patients, and exposure to alkylating agents is significantly associated with clonal expansion. For some patients, the mutations detected in the HSPCs were also observed in the malignant plasma cells, suggesting that MM can arise from a mutation-bearing, pre-malignant stem cell progenitor.

PF524

LONGITUDINAL CHROMOSOMAL ABNORMALITIES ANALYSIS IN MULTIPLE MYELOMA DISEASE PROGRESSION REVEALS CLONAL EVOLUTION AFTER THERAPY

S. Oliva[1,*], C. Simona[1], M. Ruggeri[1], G. Cetani[1], S. Spada[1], D. Oddolo[1], R. Troia[1], E. Marzanati[1], G. Gazzera[1], M. Gilestro[1], E. Saraci[1], C. Pautasso[1], V. Muccio[1], S. Bringhen[1], M. Boccadoro[1], P. Omedé[1]

[1]Myeloma Unit, Division of Hematology, University of Torino, A.O.U. Città della Salute e della Scienza di Torino, Torino, Italy

Background: Multiple myeloma (MM) is a very heterogeneous disease and prognosis is influenced by the presence of cytogenetic abnormalities (CA), such as del(17p), t(4;14) and/or t(14;16). In some cases, these mutations might change after receiving chemotherapy. However, it is unclear whether CA changes at disease progression have an impact on the prognosis of MM patients, as at diagnosis.
Aims: We investigated CA changes in bone marrow plasma cells (BMPC) from Monoclonal Gammopathy of Undetermined Significance (MGUS)/Smouldering Myeloma (SM) to Multiple Myeloma (MM)/Plasma Cell Leukemia (PCL) and from diagnostic samples to progressive disease.
Methods: Samples were collected from 2002 to 2018 at several timepoints for FISH analyses: MGUS/SMM phase, MM/PCL diagnosis and MM relapses (first and/or subsequent relapses). FISH analysis was performed on purified BMPC using anti-CD138-coated magnetic beads. Nuclei from fixed BMPC were prepared for interphase FISH using standard methods. DNA probes were used to detect Rb1 and TP53 deletions, t(11;14)(q13;q32), t(4;14)(p16;q32) and t(14;16)(q32;q23). 1p/1q detection was subsequently added to the standard FISH panel in 2012, thus it was not available for all patients. High risk (HR) was defined by the presence of at least one CA among del(17p), t(4;14) and t(14;16). In the absence of the aforementioned cytogenetic anomalies, patients were considered at standard risk.
Results: A total of 103 patients were analysed with a median follow up of 60 months (IQR 37-88 months). Median age was 64 years (IQR 56-72), and male patients were 54 (53%). According to the International Staging System (ISS), 36% had ISS-I,, 41% ISS-II, and 19% ISS-III (4% had no ISS data available). Twenty-two patients had both MGUS/SMM and MM/PCL diagnostic samples, 55 had MM diagnostic and first relapse samples, all the other patients had at least one subsequent relapse sample. Forty-three (42%) patients received front-line proteasome inhibitor (PI)-based therapy, 56 (54%) lenalidomide-based therapy, 3 (3%) melphalan-prednisone-thalidomide (MPT)/cyclophosphamide, whereas 1 patient did not start any therapy. Finally, 32 (31%) patients received autologous stem cell transplantation (ASCT). Twenty-eight/103 (27%) patients presented high risk CA at diagnosis: 17 had del(17p), 11 had t(4;14) (4 patients had both CA), no one had t(14;16). Almost one third of patients (33/103, 32%) showed changes in terms of FISH results during disease progression. Three different patient populations were identified: 25/33 patients (76%) with gain of new CA among Rb1, TP53 deletions, t(4;14), t(14;16) and 1q CA (group 1 – CA acquisition); 7/33 patients (21%) with loss of previously detected CA (group 2 – CA loss); the other 70 patients with no changes (group 3 – CA unchanged); only 1 patient had gain of t(11;14). The worst survival was observed in patients with gain of new CA during disease evolution (group 1): median OS was 66 months (95% CI, 58-89 months) in this group, 82 months (95% CI 68-NR months) in group 2 and 87 months (95% CI 78-NR) in group 3 (group 1 *vs* 3, P=0.002; group 2 *vs* 3, p=0.79) (Figure 1).

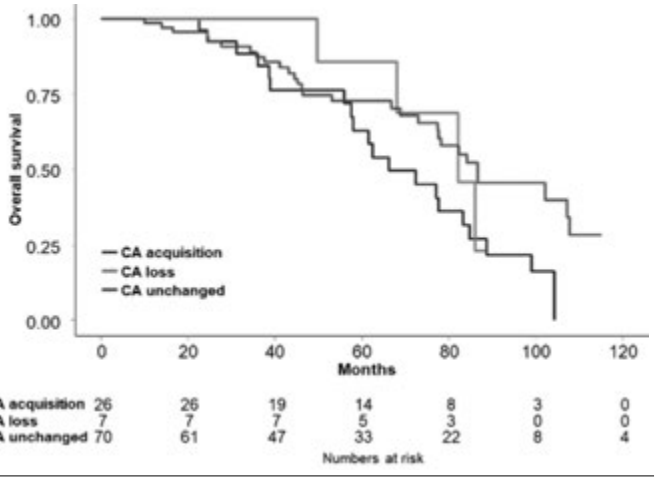


Figure 1.

Summary and Conclusions: Our results confirm that clonal evolution occurs as disease progresses after different chemotherapy lines, and patients who acquired high risk CA had the poorest prognosis. This could be explained by the fact that some clones might have existed at the diagnosis as a minor population, and became predominant during treatment, turning into resistant clones. Our findings also confirm the importance of performing FISH analysis both at diagnosis and relapse.

PF525

MYTYPE TARGETED NEXT GENERATION SEQUENCING ASSAY FOR DETECTION OF IGH TRANSLOCATIONS AND COPY NUMBER ALTERATIONS IN MULTIPLE MYELOMA

M. Hultcrantz[1,2,*], V. Yellapantula[3], E. Holth Rustad[1], H. Landau[4], C. Iacobuzio-Donahue[5], T. Akhlaghi[1], I. Amit[6], G. Ledergor[6,7], N. Korde[1], S. Mainlankody[1], D. Kazandjian[8], A. Patel[9], N. Bolli[10,11], E. Papaemmanuil[3], O. Landgren[1]

[1]Department of Medicine, Myeloma Service, Memorial Sloan Kettering Cancer Center, New York, United States, [2]Department of Medicine, Division of Hematology, Karolinska University Hospital and Karolinska Institute, Stockholm, Sweden, [3]Department of Medicine, Center for Epidemiology & Biostatistics, [4]Department of Medicine, Bone Marrow Transplant Service, [5]Human Oncology & Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, United States, [6]Immunology Department, Weizmann Institute of Science, Rehovot, [7]Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel, [8]National Cancer Institute, Center for Cancer Research, National Institutes of Health, Bethesda, [9]Center for Hematologic Malignancies, Memorial Sloan Kettering Cancer Center, New York, United States, [10]Department of Oncology and Onco-Hematology, University of Milan, [11]Department of Medical Oncology and Hematology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

Background: Multiple myeloma is a genetically complex disease where early

hits include chromosome 14 (IGH) translocations or hyperdiploidy. Later events are additional copy number alterations (CNAs), *e.g.* gain 1q, deletion 13q or 17p as well as somatic mutations. Currently, translocations and CNAs are commonly assessed via fluorescent *in situ* hybridization (FISH) in the clinical setting. Recently, targeted DNA assays have been evaluated in myeloma cell lines and showed correlation with conventional methods.
Aims: To interrogate genomic aberrations in primary samples from patients with multiple myeloma and we developed a targeted next generation sequencing panel, called myTYPE, and compared detection rates to those from FISH.
Methods: In the myTYPE assay, baits were designed to capture the entire IGH locus where the vast majority of the chromosome 14 breakpoints occur, genome wide single nucleotide polymorphisms (SNPs) for hyperdiploidy and other CNAs, as well as exons of 120 frequently mutated somatic genes in multiple myeloma.
To validate the capture of IGH translocations and CNAs using myTYPE, 46 samples from 22 patients with multiple myeloma as well as bone marrow samples from 16 healthy individuals were analyzed. All samples were sequenced using 126 bp paired end reads using Illumina HiSeq with a mean target depth of ~600x. All patient samples contained a high percentage of plasma cells. After sequencing, CNAs and translocations were identified using validated bioinformatic algorithms such as CNVkit, Brass and Delly.
Results: We found that the targeted sequencing assay myTYPE was equally good or better for detecting IGH translocations and CNAs compared to FISH. Using myTYPE, a higher number of t(4;14) and t(11;14) and equally many t(14;16) were captured using myTYPE in relation to FISH. (Table 1) Hyperdiploidy was detected in 14% and 22% with FISH and myTYPE, respectively. Greater numbers of 1q gains and 13/13q deletions were found using myTYPE while the number of 17p deletions were similar. Since myTYPE covers genome-wide SNPs, this assay was able to detect additional CNAs such as 6q deletion, 8p deletion, 16q gain, and trisomy 8 that were not targeted by FISH. In 4 multiple myeloma patients enrolled on a study protocol allowing for several parallel bone marrow/extramedullary disease biopsies in the same patient, the same IGH translocations and CNAs were detected across all sites of extramedullary disease except in two samples where a 6q deletion and 8p deletion were not detected in one sample each. This is likely explained by clonal heterogeneity, *i.e.* that the CNA was not present since we have no reason to suspect sequencing failure in these samples.

Table 1.

IGH Translocations and Copy Number Alterations	FISH	myTYPE
t(4;14)	9%	14%
t(11;14)	23%	36%
t(14;16)	9%	9%
Hyperdiploidy	14%	23%
Gain 1q	32%	32%
Deletion 13/13q	32%	59%
Deletion 17p	14%	14%

FISH=fluorescent in situ hybridization. myTYPE=targeted next generation sequencing panel designed to capture IGH translocations, copy number alterations, as well as exons of 120 frequently mutated genes in multiple myeloma

Summary and Conclusions: In this analysis, based on the targeted DNA capture assay myTYPE used head-to-head with FISH on primary samples from patients with plasma cell myeloma, we found that the sensitivity for detecting IGH translocations and CNAs was higher using the targeted sequencing approach as compared to FISH. Furthermore, using myTYPE, we found additional CNAs not covered by FISH, as FISH detects only the probe-specific alterations. Translocations and CNAs can be detected with high sensitivity also using whole genome sequencing, however, targeted sequencing is less complex and more cost-effective. In summary, targeted sequencing using myTYPE is an effective and sensitive approach to assess IGH translocations and chromosomal gains and losses in multiple myeloma and there are ongoing efforts to implement sequencing-based assays to replace FISH in the clinical setting.

PF526

RAPID AND SCALABLE GENOME-WIDE PROFILING OF CLINICALLY RELEVANT GENETIC ABERRATIONS IN MULTIPLE MYELOMA

D. Alpar[1,*], L. Atanesyan[2], S. Kosztolanyi[3], R. Kiss[1], A. Gango[1], K. de Groot[2], M. Steenkamer[2], P. Jakso[4], A. Matolcsy[1], B. Kajtar[4], L. Pajor[4], K. Szuhai[5], S. Savola[2], C. Bodor[1]

[1]1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary, [2]MRC-Holland, Amsterdam, Netherlands, [3]1st Department of Internal Medicine, [4]Department of Pathology, University of Pecs, Clinical Center, Pecs, Hungary, [5]Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, Netherlands

Background: Multiple myeloma (MM) has a heterogeneous genomic landscape coupled with diverse clinical outcome. Copy number aberrations (CNAs) including whole chromosome and subchromosomal gains and losses are common contributors of the pathogenesis and progression of MM. Genome-wide analysis of recurrently altered regions with short turn-around time is therefore highly warranted in order to stratify patients into risk groups and predict their responses to various treatment strategies.
Aims: Comprehensive, high-resolution and high-throughput profiling of all disease-relevant CNAs in MM.
Methods: A novel digital multiplex ligation-dependent probe amplification (digitalMLPA) assay has been developed for the molecular characterization of MM by combining conventional MLPA and next-generation sequencing (NGS). The probemix contains 500 probes and allows for screening for all chromosomal and focal CNAs recurrently occurring in MM as well as for specific detection of BRAF V600E mutation. Diagnostic bone marrow samples from 56 patients were analyzed, copy number status of each target locus was assessed by relative sequencing read count quantification. Plasma cell purity as measured by flow cytometry was considered at the interpretation of copy number changes. Results were compared to conventional MLPA, fluorescence *in situ* hybridization (FISH), pyrosequencing and droplet digital PCR data (ddPCR).
Results: CNAs indicating focal or whole chromosome aberrations were detected in all but one patient. Aneusomies were identified in 47 patients (84%), with predominant occurrence of monosomy 13 and trisomies of odd-number chromosomes, characteristic features of MM. The number of detected recurrent subchromosomal aberrations specifically affecting putative driver genes/regions varied between 0 and 13 (mean: 4.4) per patient. Gain(1q) was the most common lesion among the 53 patients harboring focal CNAs, followed by loss(1p), loss(8p), loss(16q), loss(12p), loss(14q), gain(8q), gain(Xq), loss(13q), loss(6q), gain(14q), loss(17p), loss(20p), loss(22q), loss(5q), gain(6p) and gain(9q). Biallelic deletions reported to be associated with disease progression were observed in *CDKN2C*, *FAF1*, *BIRC3*, *TRAF3*, *CYLD* and *TP53* genes. Investigating the location, allelic burden and extension of CNAs along chromosome 1, a high variability with 24 distinct patterns was observed across 38 patients. FISH, MLPA and digitalMLPA results at genomic loci investigated by multiple methods showed a congruency of 95%. The vast majority of whole chromosome changes and 63% of focal CNAs revealed by digitalMLPA were not detected by MLPA or FISH. BRAF V600E mutation was observed in two patients and the results were successfully validated by pyrosequencing and ddPCR
Summary and Conclusions: digitalMLPA has successfully been tested and validated for comprehensive, high-resolution profiling of disease-related unbalanced genetic aberrations and for screening a specific point mutation in MM. The whole protocol can be completed within 48 hours, thus, the method could represent a valuable addition to the diagnostic pipeline of MM by replacing more tedious procedures. Due to its targeted approach, data processing and evaluation are computationally less demanding as compared to most NGS methods. Owing to its specific probe composition, digitalMLPA allows both genome-wide aneusomy and large CNA detection as well as targeted interrogation of genomic driver regions in a single reaction, thus facilitating an efficient patient stratification to improve personalized treatment strategies.
Grants: NKFIH K119950 and NVKP_16-1-2016-0004; UNKP-17-4-III-SE-9; HAS LP95021.

PF527

INDUCTION OF STRUCTURAL AND FUNCTIONAL EFFECTS ON MYELOMA CELLS AFTER DARATUMUMAB TREATMENT

A.C. Faini[1,*], B. Castella[2], Y. Yakymiv[1], A. L. Horenstein[2], C. Bracci[1], F. Morandi[3], A. Larocca[4], S. Oliva[4], M. Massaia[5], M. Boccadoro[4], F. Malavasi[6]

[1]Department of Medical Sciences, Torino, Laboratory of Immunogenetics, University of Turin, [2]Department of Medical Sciences, Torino, Laboratory of Immunogenetics and CeRMS, University of Torino, Turin, [3]Dipartimento Ricerca Traslazionale, Medicina di Laboratorio, Diagnostica e Servizi, Stem Cell Laboratory and Cell Therapy Center, Istituto Giannina Gaslini, Genova, [4]Division of Hematology, Myeloma Unit, AOU Città della Salute e della Scienza, Turin, [5]Hematology Division, AO S. Croce Carle, Cuneo, Italy, Cuneo, [6]Department of Medical Sciences, Laboratory of Immunogenetics and CeRMS, University of Torino, Turin, Italy

Background: CD38 is a pleiotropic cell surface glycoprotein with receptorial

and enzymatic functions. The molecule is highly expressed in plasma cells which score the highest surface levels of CD38 among mature lymphoid cells, therefore CD38 has become the target of therapeutic antibodies in multiple myeloma (MM). Daratumumab (Dara) has been approved as monotherapy or in combination. The results obtained are good in patients refractory to standard myeloma therapies. Dara mechanisms of action comprehend: complement- and antibody-dependent cell cytotoxicity, antibody-dependent phagocytosis, programmed cell death, modulation of enzymatic activities and immunomodulation (in addition to its direct effects on cytotoxicity).
Aims: The aim of this study is to investigate the nature of the structural and functional modifications occurring in MM cells and in immune effectors after Dara treatment. Such modifications and effects could partially clarify the ways through which Dara acts on MM cells and even possible mechanisms of resistance.
Methods: Cell lines were cultured in IMDM supplemented with 10% FCS and MV were isolated from the culture supernatant through differential centrifugation steps. The phenotype of MV was analyzed by flow cytometry, using appropriate conjugated mAbs. MV internalization was evaluated by confocal microscopy with Alexa-conjugated mAbs and Vybrant Did-labelled MV. NK proliferation, viability and cytotoxicity after MV exposure was assessed by flow cytometry using conventional assays (CFSE, Annexin V/PI). Analyses of gene modulation were performed with NGS.
Results: CD38 engagement by Dara on MM cells is followed by a selective polar aggregation of the target molecule in myeloma membranes, with subsequent release into the extracellular space of microvesicles (MV) of 100–1,000 nm in diameter. We validated the hypothesis that MV released by MM in the bone marrow (BM) niche express functional ectoenzymes (CD38, CD39, CD73, and CD203a), potentially capable of metabolizing both ATP and NAD+ and to produce adenosine (ADO), a potent immunosuppressive molecule. Results indicate that MV obtained after Dara treatment tend to be internalized in NK cells, monocytes, dendritic cells and MDSC, cells all expressing Fc Receptors (FcR). Since NK cells apparently disappear in patients during Dara treatment, they were selected for testing MV-mediated effects. Comparative analysis of mRNA and small RNA modulated after exposing NK cells to the MV/Dara complex were followed by functional *in vitro* experiments. Both sets of results confirmed reduced proliferative ability and enhanced NK cell-mediated killing of MM cells. A further support to the immune modulatory roles exerted by Dara comes from the observation that MV surface represents not only a clustering of the expected CD38/Dara complex (flanked by a set of ectoenzymes involved in the generation of ADO), but also of inhibitory complement receptors such as CD55 and CD59, which impair the ability of the complement to exert *in situ* a cytolytic activity. Another set of observations indicate that PD-L1 tends to accumulate on the surface of MV after Dara treatment, anticipating a role in the modulation of immune checkpoint pathways (PD-1/PD-L1).
Summary and Conclusions: Results of the observations collected during the present work indicate that MV obtained from MM cells treated with Dara may be a particulate system to influence both the BM niche (local modulation) and at distance the immune response against the malignancy. Therefore, MV could also be partially responsible for Dara resistance.

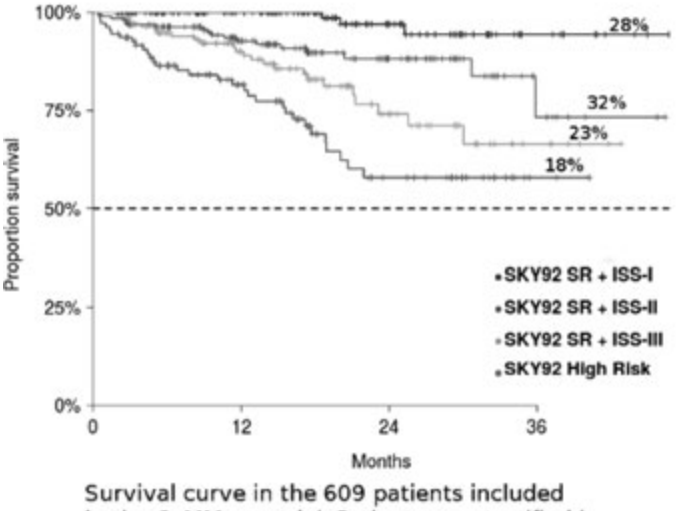
PF528

RNA-SEQ BASED RISK STRATIFICATION IN MULTIPLE MYELOMA PATIENTS VALIDATES SKY92 AS A HIGH RISK MARKER IN THE COMMPASS TRIAL

R. Kuiper[1,2,*], M.H. van Vliet[2], M. van Duin[1], E.H. van Beers[2], P. Sonneveld[1]

[1]Department of Hematology, Erasmus MC Cancer Institute, [2]SkylineDx, Rotterdam, Netherlands

Background: Multiple myeloma (MM) is a plasma cell cancer characterized by large variation in survival between patients; in this disease prognostic markers include gene expression (GEP) classifiers and FISH. Currently, GEP is mostly measured by microarray approaches (predominantly Affymetrix U133 Plus 2.0). Alternative technologies for GEP exist, such as RNA-Seq. Although measuring the same – *i.e.* relative mRNA abundance per gene specific sequence – the approach of both methods is entirely different. This raises the question how well RNA-Seq can be applied to microarray based markers.
Aims: Here we describe the conversion of the SKY92 classifier, from microarray to RNA-seq, and its validation in an independent cohort of MM patients.
Methods: The CoMMpass trial (NCT145429) is a longitudinal observational study in MM patients, performed by the Multiple Myeloma Research Foundation (MMRF). Each patient received a treatment regimen containing a proteasome inhibitor, IMiD or both. Preprocessed paired-end RNA-Seq and corresponding patient annotations were downloaded from the MMRF Researcher Gateway. In short, RNA-Seq (Illumina TruSeqRNA library kit; >60M 86 bp read pairs) was performed on purified plasma cells (>80% CD138+) obtained at diagnosis. The sequenced reads were mapped against the GRCh37 reference genome and resulting counts were converted to TPM (transcripts per million) values using SALMON v0.5.1.
The 92 Affymetrix probeset identifiers that define the SKY92 classifier were matched with Ensembl genes. In case of multiple matches, the corresponding TPM values were averaged. Five of 92 probesets were excluded: four probesets could not be matched and one had no expression in the RNA-Seq data in any of the patients. The weights of the linear SKY92 model as reported previously (Kuiper *et al.* Leukemia 2012) were adjusted using independent microarray data to correct for the missing probesets. This RNA-Seq classifier will be indicated as SKY92r and is applied to the $\log_2(1+TPM)$ values.
Results: For 632 patients from the CoMMpass trial, overall survival and corresponding RNA-Seq data was available. These patients were risk classified by the SKY92r gene classifier. In accordance with previous results, the patients with the 18% highest SKY92r scores were classified as high-risk (HR). In a Cox proportional hazards survival analysis, SKY92r HR classified patients had a hazard ratio of 3.3 (95%CI:[2.1 – 5.2]; p=1.4x10^{-7}) relative to the standard-risk (SR) patients. As shown previously, the SKY92 classification is further refined by addition of the international staging system (ISS), resulting in a low risk group, two intermediate and a high-risk group (Figure 1). The 3-yr overall survival rates are 94% (SKY92r SR with ISS-I), 73% (SKY92r SR with ISS-II), 66% (SKY92r SR with ISS-III) and 58% (SKY92r HR).



Survival curve in the 609 patients included in the CoMMpass trial. Patients are stratified by SKY92r and ISS.

Figure 1.

Summary and Conclusions: These results are in line with previous results published in Kuiper *et al.* (Blood 2015) using microarray data. This is a strong indication that the SKY92 classifier can effectively be converted from a microarray to an RNA-Seq platform. Further validation of the SKY92r in independent RNA-Seq datasets is required for future use.

PF529

MDSCS CONTRIBUTE TO CANCER STEM CELLS ACCUMULATION VIA PIRNA 823 UPREGULATION AND *DE NOVO* DNA METHYLATION IN MULTIPLE MYELOMA

L. Ai[1,*], S. Mu[1,1], Y. Hu[1]

[1]Hematology, Wuhan Union Hospital, Wuhan, China

Background: MDSCs (myeloid derived suppressor cells) promote tumor growth and invasion, exert immunosuppression and host immune evasion by suppressing lymphocyte activation and antigen recognition. A small population of cells, cancer stem cells (CSCs), are reported to persist in myeloma bone marrow, with unlimited self-renewal capacity to initiate tumor formation and disease relapse. Piwi-interacting RNAs (piRNAs) are non-coding RNAs (ncRNAs) of 26–31 nucleotides in length, which plays important role of epigenetic regulation in multiple myeloma (MM) via *de novo* DNA methylation.
Aims: Here, we sought to demonstrate the promoting effects of MDSCs on MM-CSCs accumulation, and investigate the involvement of piRNA-823 in the crosstalk between MDSCs and myeloma cells, thus providing theoretic basis of therapeutic strategy for MM patients.

Methods: CD11b^{+}HLA-DR$^{low/-}$MDSCs were isolated from BM via magnetic activated cell sorting (MACS). And Co-cultures of MDSCs with MM cell lines (RPMI8226 and NIH929) were performed in a non-contacted transwell system. Side population (SP) cells detection, cell colony formation, and stemness related genes expression were measured to validate the MDSCs' promoting effects on MM-CSCs accumulation. Besides, Expression of piRNA 823 and its target DNMT 3B were measured by qRT-PCR and Western blot, and total genome DNA methylation level was measured by 5-mC ELISA test according to the manufacturer's instructions. Specific antagomir for piRNA 823 was added to the co-culture system to investigate the regulating mechanisms in the crosstalk between MDSCs and MM cells.
Results: SP cells percentage in MM cells co-cultured with MDSCs was much higher than that in MM cells clone, and the experiment group showed greater sphere formation, with elevated expression of stemness related genes, such as Oct-4, Sox2, and Nanog. In addition, MDSCs co-culture induced the expression of piRNA 823 and its target DNMT 3B in MM cells, and the results of 5-mC ELISA test showed elevated DNA methylation level in MM cells co-cultured with MDSCs. Moreover, the promoting effects were almost abolished by the specific antagomir of piRNA 823. Adding antagomir-823 to the MDSCs/MM co-culture system not only decreased SP percentages in MM cells, hampered sphere formation, but also inhibited the CSC genes expression. That is to say, piRNA 823 is involved in the mechanisms about MDSCs' promoting effects on MM-CSCs accumulation (Figure 1).

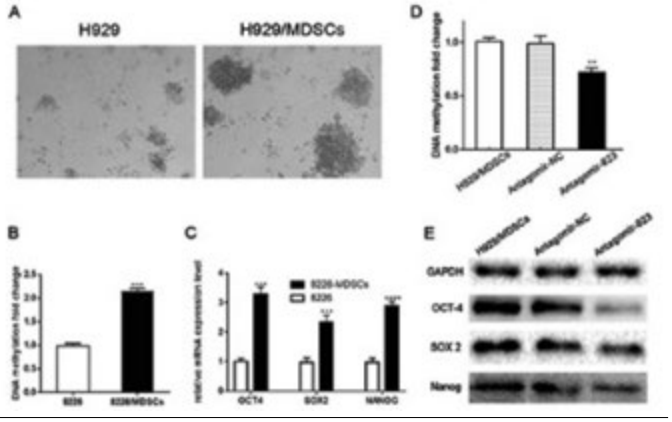


Figure 1.

Summary and Conclusions: Our results demonstrated that MDSCs worked as an important contributor to MM-CSCs accumulation *in vitro*. piRNA 823 and relevant *de novo* DNA methylation were involved in the regulatory mechanisms on MDSCs-MM interactions. These findings suggested that targeting MDSCs or other demethylating agents may become a new therapeutic strategy to improve the prognosis of MM patients.

PF530

MESENCHYMAL STROMAL CELL SIALYATION ENHANCES IMMUNE SUPPRESSION: IMPLICATIONS IN MULTIPLE MYELOMA

K. Lynch[1,2,*], K. De Veirman[3], G. O'Malley[1,2], K. Vanderkerken[3], A. Ryan[1,2,4], M. O'Dwyer[1,5], T. Ritter[1,2]

[1]College of Medicine, National University of Galway, [2]Regenerative Medicine Institute (REMEDI), Galway, Ireland, [3]Labo Hematology and Immunology, Vrije Universiteit Brussel, Brussels, Netherlands, [4]Discipline of Pharmacology and Therapeutics National University of Ireland, [5]Haematology Services, Galway University Hospital, Saolta University Health Group, Galway, Ireland

Background: Multiple myeloma (MM) is a plasma cell malignancy involving the bone marrow (BM) where the tumour microenvironment plays an important role in mediating survival, proliferation, resistance to therapy as well as disease evolution. Signals in the MM microenvironment are known to influence bone marrow mesenchymal stromal cells (BM-MSCs) facilitating MM progression and drug resistance. Little is known about the mechanisms of immune modulation mediated by BM-MSCs in MM. Aberrant glycosylation is a hallmark of cancer cells, playing an important role in tumour progression. In this study we investigate if regulation of BM-MSC sialylation alters their ability to inhibit effector T-cell function in an inflammatory microenvironment and if BM-MSCs isolated from MM mice have elevated levels of sialic acid and if this correlates with T-cell modulation.
Aims: 1. Characterise inflammatory mesenchymal stromal cells. 2. Profile sialic acid changes on inflammatory mesenchymal stromal cells and MM-MSCs. 3. Investigate if regulation of MSC sialylation alters their ability to inhibit effector T-cell function in an inflammatory microenvironment.
Methods: MM-MSCs and BM-MSC were isolated from mice and extensively characterized *in vitro*. BM-MSC were treated with both tumour necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) (i-MSC) for 72 hours and the sialic acid levels were analysed by flow cytometry. MM-MSCs, BM-MSC and i-MSC were co-cultured in mixed lymphocyte reactions (MLRs) for 96 hours. T-cell proliferation and activation were determined by flow cytometry. To assess the role of increased sialylation in lymphocyte suppression, both BM-MSC and i-MSC were pre-treated with the sialyltransferase inhibitor (SI) 3Fax-Neu5Ac for 72 hours prior to TNF-α and IL-1β stimulation.
Results: i-MSC displayed significant phenotypical changes with CD73 and CD44 increasing significantly. MM-MSCs and i-MSCs have significantly increased levels of both α2-3 and α2-6 linked sialic acid when compared to BM-MSC. *In vitro*, both MM-MSCs and i-MSCs displayed an enhanced ability to inhibit the proliferation of lymphocytes when compared to BM-MSC alone. Both MM-MSCs and i-MSC inhibited the proliferation of stimulated CD3^{+}CD4^{+} and CD3^{+}CD8^{+} lymphocytes significantly. SI BM-MSC and i-MSC displayed no significant changes in phenotype, viability, proliferation and cell size. However, following SI treatment, i-MSC lost the ability to suppress both CD3^{+}CD4^{+} and CD3^{+}CD8^{+}lymphocytes resulting in significant restoration of lymphocyte proliferation. Sialic acid expression on the cell surface correlated with both CD3^{+}CD4^{+} and CD3^{+}CD8^{+} lymphocyte suppression.

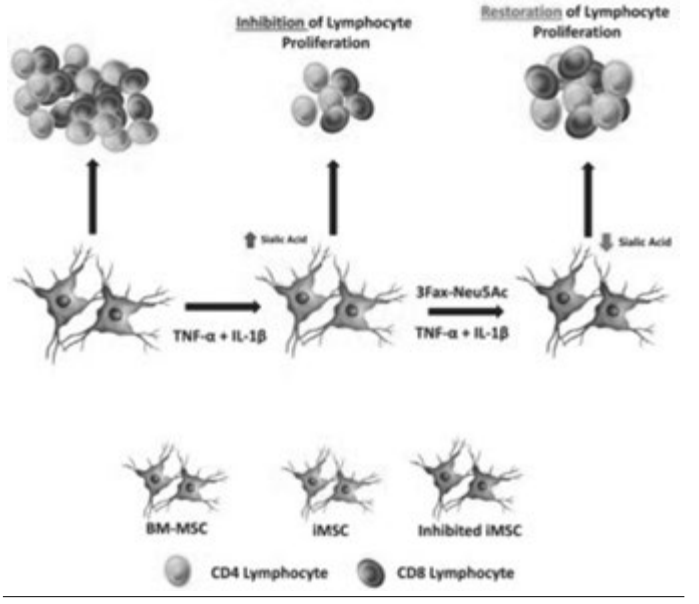


Figure 1.

Summary and Conclusions: Our findings confirm that inflammation, characteristic of the MM TME, induces BM-MSC sialylation and enhances their ability to suppress activated adaptive and innate immune effectors. Understanding the potential utility of targeting BM-MSC sialylation and consequently their immunomodulatory potential, may enhance immune cell activation in the MM microenvironment, providing further rationale to targeting BM-MSC sialylation in the context of MM. We suggest that understanding the functional importance of the BM stroma and its interaction with MM and immune cells is likely to lead to the identification of sialylation, as a new molecular target.

PF531

LOW-PASS SEQUENCING OF PLASMA CELL DNA AND OF CIRCULATING CELL-FREE DNA FOR THE DETECTION OF COPY NUMBER ABERRATIONS AND EARLY RESPONSE MONITORING IN MULTIPLE MYELOMA

B. Dewaele[1,*], S. Smits[1], L. Michaux[1], M. Delforge[2], G. Ameye[1], L. Dehaspe[1], N. Brison[1], J. Ding[1], S. Lehnert[1], N. Boeckx[3], J. Van Houdt[1], J. Vermeesch[1], P. Vandenberghe[2]

[1]Center for Human Genetics, [2]Hematology, [3]Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium

Background: Cytogenetic abnormalities are powerful prognosticators in multiple myeloma (MM). Presently, FISH on bone marrow (BM) plasma cells (PLCs) is the gold standard in diagnostics. Recently, array-CGH has been introduced as an alternative method for the detection of somatic copy number aberrations (CNAs) in PLCs, which allows to reduce the number of FISH tests needed per case, and yields genome-wide information. Next generation sequencing (NGS) technologies offer new perspectives for the diagnostic work-up of malignant disorders.
Aims: In this study, we compared low-pass sequencing with array-CGH for the detection of somatic CNAs in PLCs from BM aspirates in patients with MM at diagnosis and/or relapse. In addition, we explored whether low-pass sequencing of circulating cell-free DNA (ccfDNA) in plasma can reveal CNAs at diagnosis and can be used to monitor early response, as a non-invasive method.
Methods: BM aspirates were obtained from 31 cases with MM. All 31 cases were analyzed by FISH either on CD138+ cell-sorted samples or combined with cytoplasmic immunoglobulin (cIg) staining. When sufficient material was available, DNA was extracted from purified PLCs or from whole BM and subjected to array-CGH and/or low-pass sequencing. Array and genome-wide sequencing data were analyzed by the Cytosure Interpret Software (OGT) and our in-house pipeline, respectively. Paired samples of ccfDNA obtained at diagnosis and at early time points after therapy initiation were analyzed by low-pass sequencing.
Results: Low-pass sequencing of purified PLC (n=16) or whole BM cells (n=1) revealed CNAs in all cases. The CNAs identified by low-pass sequencing were confirmed by array CGH for each of these 17 cases. For 14 of the latter cases, paired ccfDNA samples were available. In 7 of these, sequencing of ccfDNA yielded a genomic representation profile compatible with the DNA profile on BM PLC. The 7 remaining cases had normal ccfDNA profiles despite abnormal profiles from BM aspirates. This could be due to a lower myeloma burden in these cases (mean 20,3% PLC in BM aspirates, range 10-30%) in comparison to those with abnormal ccfDNA profiles (mean 37,7% PLC, range 10-97%). Twelve patients with an aberrant ccfDNA profile at diagnosis were treated during the course of this study. The ccfDNA profiles of 11 of these patients returned to normal within 3 to 10 weeks after treatment initiation. The 12th patient is clinically not responding well, consistent with persistence of the abnormal ccfDNA profile under treatment.
Summary and Conclusions: Low-pass sequencing of PLCs DNA yields identical CNAs profiles in comparison with array-CGH: this validates low-pass sequencing as a novel and cost-efficient tool for the detection of CNAs in MM at diagnosis and relapse. In addition, we provide proof-of-principle that ccfDNA contains ctDNA from MM PLCs in a substantial fraction of patients. Therefore, analysis of ccfDNA in MM could be developed as a novel and non-invasive approach for monitoring of early disease response in a dynamic fashion.

PF532

RNA-SEQ ANALYSIS UNRAVELS SPECIFIC LONG NON-CODING RNA TRANSCRIPTIONAL FINGERPRINTS IN MULTIPLE MYELOMA SUBTYPES

E. Taina[1,2,*], C. Vinci[1,2], V. Ferla[1], D. Ronchetti[1,2], A. Pompa[1], K. Todoerti[1,2], M. Manzoni [1,2], L. Nobili[1,2], V. Favasuli[1,2], A. Cortelezzi[1,2], L. Baldini[1,2], A. Neri[1,2], L. Agnelli[1,2]

[1]Hematology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, [2]Department of Oncology and Hemato-oncology, University of Milan, Milano, Italy

Background: Long non-coding RNA (lncRNAs) represents the largest class of non-protein coding genes in the human genome. The increasing studies on lncRNAs have been changing the landscape of cancer biology. In multiple myeloma (MM), they are promising candidate to provide further insights into the molecular mechanism of the pathology.
Aims: The present study was aimed at characterizing lncRNAs expression in MM by next-generation sequencing, and unraveling their putative transcriptional relationships with the different molecular subtypes and biochemical-clinical parameters.
Methods: The study was performed in a cohort of 30 MM patients admitted to our hematology unit from 2000 to 2014. Cytogenetic and mutational data of TP53, KRAS, NRAS, BRAF, FAM46C, DIS3 were available for all patients. RNA-sequencing (RNA-seq) of lncRNA was performed using TruSeq® RNA Sample kit (Illumina) and HiSeq2000 (Illumina) platform. Fragments were aligned to the human genome using *STAR* and Gencode v25 annotations based on Ensembl database version 87. Transcript abundance was estimated using featureCounts. FPKM (Fragments Per Kilobase Million) quantification was performed using *cufflinks*. Differentially expressed lncRNAs were identified using DeSeq. Array analysis was run under manufacturer's standard procedure on GeneChip® Human Gene 2.0ST platform.
Results: The natural grouping of the transcriptional profiles of the most variable lncRNA across the dataset generated by RNA-seq could be associated with the main MM molecular subgroups, characterized by the presence of t(11;14), t(4;14), MAF-related translocations or hyperdiploid status (P<0.0001). Then, a differential analysis revealed 391 unique lncRNAs differentially expressed between the four cytogenetics subgroups of MM with prognostic impact. As a validation, we assessed the transcriptional signature of 391 lncRNAs differentiating MM subgroups in the same cohort of patients evaluated on microarrays (that contain specific probes for 262/391 transcripts), gaining full reproducibility. In addition, this analysis demonstrated that a robust transcriptional signature distinguish the tumor samples from 4 available healthy donors. Finally, to select lncRNA with a possible role in MM, we considered the levels of lncRNAs expression together with their proximity to genes that, based on literature, are relevant in MM, according to the reported evidence of cis-regulatory relationships between the transcription of proximal mRNA and lncRNA transcripts. For this purpose, a list of 707 genes described as associated to MM was downloaded from the NCBI database and studied in the genomic context. For 409 of them we found at least one of the 9540 lncRNAs detected in our dataset in at least one sample that mapped at upstream or downstream distance lower than 4 Mbps. Next, for each pair of lncRNA / MM genes, the correlation of the expression level was evaluated and unraveled 43 significantly correlated pairs (r>|0.4| and p-value <0.01) constituted by the combination of 39 genes with 35 lncRNA with significant co-expression.
Summary and Conclusions: In the present study, we have investigated the lncRNA expression profiling in MM patients by RNA-seq, with the aim of providing an exhaustive catalogue of lncRNAs specifically associated with the main molecular subgroups and genetic alterations in MM. Furthermore, we defined a *repertoire* of lncRNAs and genes linked by putative cis-regulatory relationship with a potential involvement in MM.

PF533

COMBINATION TARGETING OF SIALYLATION AND THE PROTEASOME INHIBITS TUMOR GROWTH AND INCREASES SURVIVAL IN A HUMANIZED MOUSE MULTIPLE MYELOMA MODEL

M. Reagan[1,*], M. Farrell[1], M. Macauley[2], A. Natoni[3], H. Fairfield[1], M. O'Dwyer[3]

[1]Center for Molecular Medicine, Maine Medical Center Research Institute, Scarborough, United States, [2]Faculty of Science, University of Alberta, Alberta, Canada, [3]Dept. of Medicine/Haematology, National University of Ireland Galway, Galway, Ireland

Background: Multiple myeloma (MM) is a cancer of clonal plasma cells that hijack the bone marrow (BM) to create a drug resistant, incurable malignancy. Aberrant sialic acid glycosylation, sialylation, has been linked to immune cell evasion, drug resistance, and metastasis in cancer. In several cancers, sialyltransferases, including ST3GAL1, ST3GAL4 and ST3GAL6 (1), are aberrantly expressed.
Aims: We have previously shown that inhibiting sialylation of BM-homing cells by targeting ST3GAL6 inhibits the ability of MM cells to extravasate and colonize the BM in mouse models (1,2). Based off these findings, we herein investigated changes in BM homing, growth, and bortezomib response of MM cells treated with 3Fax-Neu5Ac, a small molecule sialyltransferase inhibitor. We hypothesized that inhibiting homing of MM cells to the BM will improve survival and that co-treatment with bortezomib and 3Fax-Neu5Ac will have a synergistic effect.
Methods: We first enriched human GFP$^+$/Luciferase$^+$ MM1S MM cells for a sialofucosylated cell population using the HECA-452 antibody to select for cells expressing sialofucosylated E-selectin ligands, using FACS. We then determined the 3Fax-Neu5Ac dose and exposure times needed to decrease sialylation on these MM cells without causing toxicity. HECA-452 -enriched MM1S cells were pretreated with 3Fax-Neu5Ac or vehicle for 7 days before being injected into SCID-beige mice and then treated with vehicle or bortezomib (0.3 mg/kg twice a week). Mice were analyzed via bioluminescence imaging to monitor tumor progression and euthanized when they began to show paralysis under our IACUC protocol.
Results: We successfully identified and enriched for a sialofucosylated subclone of MM1S using HECA-452 sorting. Treatment with 3Fax-Neu5Ac, at 300 M for 7 days significantly reduced sialylation on these cells. Importantly, reducing sialylation with 3Fax-Neu5AC reduced tumor burden and increased survival, although this did not reach significance for survival (Figure 1). Both vehicle- and 3Fax-Neu5Ac-treated cells significantly responded to bortezomib

in the first 5 weeks of the *in vivo* study (Figure 1). However, unlike 3Fax-Neu5Ac-treated cells, HECA-452-enriched cells did now show increased survival when treated with bortezomib, suggesting that their aggressive nature overrode the inhibitory effects of bortezomib that were seen in the earlier on with BLI and indicating a form of drug resistance (Figure 1). Overall, the lowest tumor burden and best survival outcomes were observed in the combination group of bortezomib plus 3Fax-Neu5Ac.

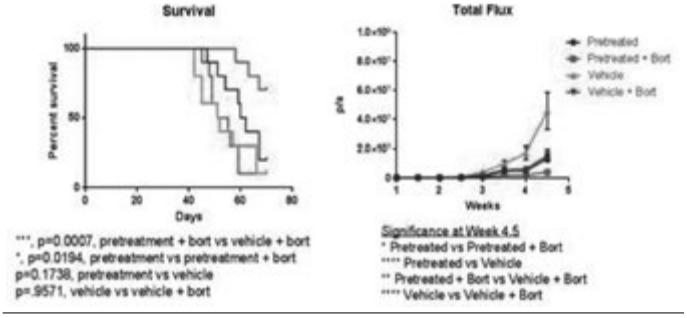


Figure 1.

Summary and Conclusions: In conclusion, sialylation plays an instrumental role in bone homing, BM colonization, and drug resistance of MM cells. Our data indicates that drug resistance can be overcome in HECA-452-enriched MM cells through the combination of 2 drugs: bortezomib and 3Fax-Neu5Ac which decreases sialylation. This is likely because 3Fax-Neu5Ac pretreatment increased MM circulation and reduced BM homing and bortezomib resistance, but it is possible that decreased sialylation also affected immune cell activity. This study supports prior work showing the importance of targeting sialylation in MM and demonstrates the feasibility and efficacy of targeting sialylation therapeutically, providing a strong rationale for further clinical translation of this novel approach.

References:
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PF534

ACTIVATION OF NFAT (NUCLEAR FACTOR OF ACTIVATED T CELLS) SIGNALING AND INDUCTION OF AUTOPHAGY CONFER BORTEZOMIB RESITSTANCE IN MULTIPLE MYELOMA

M. Ng[1,*], K.-M. Lau[1], C.-K. Cheng[1], N. Chan[1], J. Xia[1]

[1]Anatomical & Cellular Pathology, The Chinese University of Hong Kong, HKSAR, China

Background: Bortezomib (BTZ) is a proteasome inhibitor. It suppresses multiple myeloma (MM) cell growth by inducing apoptosis through endoplasmic reticulum (ER) stress. However, drug resistance remains an issue. Of note is a recent study demonstrating that BTZ conflictingly could promote MM cell proliferation and aggressiveness. Besides apoptosis, ER stress also stimulates pro-survival signals, particularly nuclear factor of activated T cells (NFAT) signaling. Whether BTZ could activate NFAT signaling, thereby counteracting its own growth inhibitory effects in MM deserves further studies.
Aims: We investigated the effects of BTZ on NFAT signaling in MM cells and determined how the signaling activation could enhance MM cell survivals. The potential therapeutic values of the chemical inhibitors blocking NFAT signaling in potentiation of BTZ induced MM cell growth inhibition were also evaluated.
Methods: Cellular calcineurin phosphatase (the upstream regulator of NFAT) activity was measured and NFAT luciferase reporter assay was used. Nuclear localization (activation) of NFAT proteins were studied by immunofluorescence staining and Western blot. RT-qPCR was performed to study the effects of BTZ on gene expressions and LC3 conversion assay for studying autophagy. MM cell growth and survival were studied by AccuCheck bead counting and Annexin V apoptotic assays respectively.
Results: We found that BTZ increased cellular calcineurin activity and NFAT transcriptional activity together with nuclear translocation of NFATc1 in MM1s myeloma cells. The BTZ-induced NFAT transcriptional activity was also confirmed in another MM cell line, ARH77. Mechanistically, BTZ up-regulated expressions of NFAT-related anti-apoptotic *BAG3* and *DDIT4* in both MM cell lines. Because these genes are also involved in autophagy as an adaptation strategy to deal with ER stress, concordantly we observed BTZ-induced autophagy in these MM cells. Further, we demonstrated that co-treatment with cyclosporin A or FK506, which inhibit calcineurin activity thereby blocking NFAT signaling, dramatically enhanced BTZ-mediated MM growth inhibition through intensification of BTZ-triggered ER stress-induced apoptosis as reflected by the concomitant up-regulation of fatal ER stress markers and down-regulation of anti-apoptotic *BCL2* expression in MM1s and ARH77 cells. The intensified BTZ-triggered apoptosis was also observed in CD138+ immunosorted MM cells from clinical bone marrow samples of the patients with MM (n=15, p=0.017).
Summary and Conclusions: These findings indicated that BTZ activates NFAT signaling, which induces pro-survival and adaptation signals to confer BTZ-resistance in MM. For clinical application, inhibition of NFAT signaling potentiates the therapeutic efficacy of BTZ treatment by circumventing the chemo-resistance in MM.

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THE CONICAL AGAROSE MICROWELL ARRAY (CAMA) ENABLES 3D LONG-TERM CULTIVATION OF PRIMARY SAMPLES AND ENHANCED HIGH THROUGHPUT SCREENING FOR RATIONAL EPIGENTIC/ANTIBODY COMBINATION IN MULTIPLE MYELOMA

S.J. Mueller[1,*], D. Wider[1], M. Follo[1], S.M. Dold[1], C. Miething[1], G. Ihorst[2], J.M. Waldschmidt[1], J. Felthaus[1], J. Schueler[3], A.R. Thomsen[4], A.K. Azab[5], J. Duyster[1], M.J. Hug[6], M. Jung[7], R. Waesch[1], M. Engelhardt[1]

[1]Hematology, Oncology and Stem Cell Transplantation, [2]Clinical Trials Unit, Medical Center, [3]Oncotest GmbH, [4]Radiation Oncology, Medical Center, Freiburg, Germany, [5]Radiation Oncology, Cancer Biology Division, Washington University School of Medicine, St. Louis, United States, [6]Pharmacy, Medical Center, [7]Pharmaceutical Sciences, University of Freiburg, Freiburg, Germany

Background: Over the past decade, numerous small molecules and monoclonal antibodies have substantially improved the median survival of multiple myeloma (MM) patients (pts). However, the variety of anti-MM agents also constitutes a challenge to establish rational and best possible drug combinations to furthermore enhance long-term efficacy and tolerability.
Aims: For this purpose we here screened 33 primarily subclass selective epigenetic modifying agents for their anti-MM activity, to subsequently assess their influence on antigen expression levels (CD138, CD38, SLAMF7, PD-L1, and CXCR4) and to finally derive best antibody drug combinations using our 3D *ex vivo* high throughput screening platform.
Methods: Initially, our screening approach included 4 inhibitors for epigenetic reader proteins as well as 8 compounds impeding writer enzymes and 21 drugs inhibiting epigenetic erasers. Their anti-MM cytotoxicity was assessed after 48h, 120h and/or 168h using ATP-dependent viability assay. Multiple myeloma cell lines (MMCLs) as well as primary bone marrow samples were seeded into 2 mm deep conical agarose-based microcavities of the CAMA platform (Figure 1) and cluster growth or treatment response were analyzed via confocal imaging and non-destructive transmitted light scanner analysis. For antigen quantification the aggregates were harvested on days 2 and 4 with or without epigenetic treatment and measured via FACS.

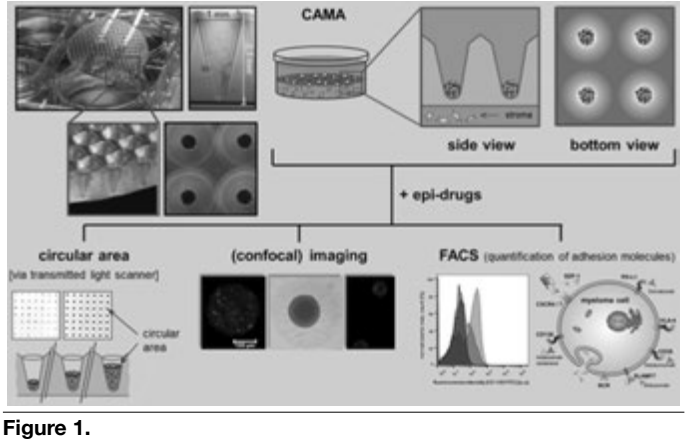

Figure 1.

Results: The well-defined, steep microwells of our CAMA enabled semi-adherent plasma cells (PCs) to form 3D-aggregates and furthermore mediate drug resistance to proteasome-inhibitors and epi-drug treatment. In this context qualitative confocal analysis of mCherry-transduced RPMI 8226 cells and fluorescent dye staining excluded uneven drug distribution within the CAMA. Moreover, PCs seeded into the microcavities showed prolonged

sigmoidal cluster growth in accordance to intratibial MM engraftment in our *in vivo* NOD/SCID mouse model. Using our established transmitted light scanner technique, stable *in vivo*-like proliferation of primary MM cells could be monitored for at least 2 weeks in a non-destructive manner and correlated to ATP concentrations and microscopic imaging. The evaluation of the epigenetic library identified 12 out of 33 compounds with potent anti-MM activity, including G9a, EZH2, panKDM and KDM5-selective inhibitors, but also 1 highly selective HDAC6 (JS28) and 2 HDAC10 selective inhibitors (HDACi: TH95 and TB76). For JS28, we could demonstrate a more than 200-fold selectivity for HDAC6 over HDAC1 or 8 via enzymatic *in vitro* assay. Consistently, we also assessed the selectivity on target via western blot as well as the functional synergism with bortezomib in 2 MMCLs. Our subsequent antigen analysis showed a dose-dependent increase of CD38, up to 140% in the presence of the class I selective HDACi entinostat as compared to persisting or decreased CD38 expression levels with vorinostat or panobinostat and JS28, respectively.
Summary and Conclusions: Our CAMA provides various advantages in MM, namely 1.) solid cluster formation of semi-adherent PCs including more *in vivo*-like proliferation and drug resistance, 2.) simultaneous, non-destructive observation of approx. 880 spheroids per matrix over time and 3.) cultivation of proliferating and viable primary MM-pt samples for at least 2 weeks. By using our investigated epigentic toolbox for subclass selective functional analysis, we found class I HDACis to upregulate CD38 expresion levels, which indicates that specific HDACi are promising and rationally-tested combination partner for daratumumab.

PF536

ENHANCED CYTOTOXICITY OF MULTIPLE MYELOMA CELLS USING DARATUMUMAB IN COMBINATION WITH NK CELLS ENGINEERED TO EXPRESS HIGH-AFFINITY CD16 (F158V)

S. Chauhan[1,2], S. Sarkar[1,2], T. Duggan[1], J. Daly[1], M. O'Dwyer[1,2,*]

[1]Apoptosis Research Centre, National University of Ireland, [2]Onkimmune Ltd., Galway, Ireland

Background: Multiple Myeloma (MM) is a clonal plasma B-cell malignancy, associated with strong expression of the CD38 antigen. Recent clinical trials with the anti-CD38 monoclonal Antibody (moAb) Daratumumab (Dara) have shown promising results. NK cells are important immune effectors of moAb therapy, mediating ADCC via FcγRIII (CD16). 15% of the population expresses a higher affinity (HA) form of CD16, due to a single point polymorphism (F158V) and this has been linked to higher responsiveness to therapeutic moAbs in the clinic. Therefore, we hypothesize that the combination of Dara with "off-the-shelf" NK cells, engineered to express high affinity (HA) CD16, could offer a new therapeutic strategy for treating MM patients.
Aims: In the current study, we investigated a "safer" m-RNA-based approach to develop high-affinity CD16 expressing NK cells for application as an off the shelf therapeutic.
Methods: CD38 expression was determined on a panel of *n=5* MM cell lines RPMI-8226, H929, MM.1S, U266 and JJN3. m-RNA transcripts coding for high-affinity CD16 protein was synthesized using *in-vitro* transcription (IVT), and KHYG1 NK cells were subsequently nucleofected using AMAXA Nucleofector II. 24 hours post-nucleofection cells were analyzed for surface expression of CD16, and further co-cultured with MM cell lines either alone or in combination with Dara. NK cell-induced cytotoxicity was measured by FACS-based methods. Experimental assays were performed with *n=5* MM cell lines at E:T ratios of 0.25:1, 0.5:1, 1:1 and 2:1. Cytotoxicity assays with each cell line were performed in *n=4* independent experiments.
Results: Immunophenotyping revealed that MM cell lines have a broad-spectrum cell surface expression of CD38, and therefore we classified them as $CD38^{hi}$ (RPMI 8226, H929), $CD38^{mod}$ (MM.1S), and $CD38^{lo}$ (U266, JJN3). Thereafter, ADCC assay was set-up against a panel of *n=5* MM cell lines (JJN3, H929, RPMI 8226, U266 and MM.1S) with HA-CD16 nucleofected KHYG1 either alone or in combination with Dara. HA-CD16 nucleofected KHYG1 in combination with Dara was significantly more cytotoxic towards NK resistant MM cell line H929 at E:T 0.5:1, 1:1, and 2:1, as compared to HA-CD16 KHYG1 alone. Furthermore, the combination was also significantly cytotoxic against $CD38^{lo}$ JJN3. Although RPMI 8226 cells and MM.1S cells are sensitive towards NK mediated killing, we found significant increase in cytotoxicity at multiple E:T ratios. Correlation plot analyzing sensitivity towards NK cells and change in cytotoxicity revealed than MM cells which are intrinsically resistant towards NK cells may benefit significantly more from this combination therapy (r^2=0.79).
Summary and Conclusions: This study provides the proof-of-concept for combination therapy of Dara and an "off-the-shelf" m-RNA approach based HA-CD16 expressing NK cells for treating MM patients.

PF537

IMMUNOLOGICAL PROFILING BY MASS CYTOMETRY REVEALS THAT TIM3 EXPRESSION IS REDUCED ON NK CELL SUBSETS IN NEWLY DIAGNOSED MYELOMA AND IS ASSOCIATED WITH LOSS OF NK FUNCTION AND DECREASED SURVIVAL

F. Seymour[1,*], J. Cavenagh[1], J. Gribben[1]

[1]Haematology, Barts Cancer Insititute, London, United Kingdom

Background: Natural killer (NK) cells have the potential to target malignant plasma cells and contribute to immunological control of myeloma. NK cells lose CD56 expression as they mature allowing the identification of bright and dim subsets with distinct functional roles.
Malignant plasma cells are known to have a range of immune modifying effects including the expression of PDL1 and the induction of a pro-tumour bone marrow environment. It is hypothesised that this downregulates the NK cell response, however the consequences for the function of NK subsets and its clinical significance is poorly understood.
Aims: To assess the phenotype and function of NK subsets in newly diagnosed myeloma (NDMM)
Methods: Bone marrow samples were interrogated by mass cytometry using a 36 parameter antibody panel to assess expression of 9 immune checkpoint regulators in addition to markers of cytotoxicity, cytokine production and proliferation.
Results: Total NK cell numbers were well preserved in NDMM with no numerical differences identified between control and disease. NK cell proliferation, as assessed by Ki67 expression, was reduced in myeloma (p=0.034). There was a marked reduction in proliferation within the NK CD56 dim subset while proliferation within the NK CD56 bright subset was well preserved. A reduction in TIM3 expression was seen in both NK cell subsets in myeloma compared to control (p=0.0018) suggesting loss of NK cell activation. Furthermore the NK CD56 bright subset had reduced expression of the NK cell activating receptor DNAM1 (p=0.0168), reduced production of IL10 (p=0.0077) and increased levels of the pro-tumour cytokine TGFb (p=<0.0001). The NK CD56 dim subset demonstrated reduced expression of IFNy (p=0.0103) and TNFa (p=0.0426) with increased levels of TGFb (p=0.0048). Perforin and granzyme production was preserved. PD1 expression was increased in the CD56 dim subset (p=0.0417). In addition to TIM3 and PD1, NK cells in myeloma also express the receptors DNAM1 (24%), NKG2D (19%) and 2B4 (69%) which may offer therapeutic targets to improve NK cell function. Expression of PD1 was of moderate, not high, intensity and was lower than that seen on CD8 cells in myeloma. Individuals with PD1 expression above the mean also had higher expression of Granzyme (p=0.0196), Perforin (p=0.0427) and DNAM1 (p=0.0152) suggesting that moderate NK cell PD1 expression may be a marker of activation rather than exhaustion. Total NK cell expression of TIM3 (p=0.0234) but not PD1 (p=0.0667) positively correlated with survival with individuals surviving more than 36 months having a 2.5 fold increase in NK cell TIM3 at diagnosis. NK cell TIM3 expression may therefore have prognostic value.
Summary and Conclusions: This data demonstrates that NK cell activation is reduced in NDMM with an associated reduction in proliferation and anti-tumour cytokine production. The functional deficits are most marked in the more mature NK CD56 dim subset and are likely to contribute to a pro-tumour microenvironment. Given that NK cells express a range of activatory receptors is may be possible to therapeutically target these in order to reverse the functional deficits. PD1 or TIM3 blockade may also produce beneficial functional responses. Interestingly those individuals with higher levels of NK TIM3 expression at myeloma diagnosis had a longer duration of survival, suggesting that the presence of activated NK cells is playing a key role in disease control. NK cell TIM3 levels at diagnosis may be a useful prognostic biomarker.

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AUTOLOGOUS STEM CELL TRANSPLANTATION AS A MODEL FOR ACCELERATED T-CELL AGING

F. Lucas[1,*], J. Woyach[1], S. Zhang[2], X. Guan[2], C. Burd[3], D. Benson[1], Y. Efebera[1], A. Rosko[1]

[1]Department of Medicine, [2]Comprehensive Cancer Center, [3]Cancer Biology & Genetics, The Ohio State University, Columbus, United States

Background: Physiological aging is characterized by T-cell cellular senescence, immunosenescence and exhaustion. Cellular senescence results in permanent cell cycle arrest generally characterized by increased p16INK4a (p16) expression, which has been shown to be a robust marker of physiological age. Hall-

marks of T-cell immunosenescence include subset changes, declining effector function, reduced receptor diversity, and changes in transcriptome and epigenome. T-cell exhaustion occurs in the context of chronic antigen stimulation and is association with the upregulation of inhibitory molecules. In patients with multiple myeloma (MM), high-dose chemotherapy (HD-CHT) followed by autologous stem cell transplantation (ASCT) increases T-cell p16 to levels equivalent of 30 years of aging (Rosko *et al*., BMT 2015), indicating that ASCT is a useful model to study accelerated aging.
Aims: We hypothesized that ASCT in MM is a suitable model of accelerated T-cell cellular- and immunosenescence. Our aims were to: (1) confirm acceleration of T-cell cellular senescence in an expanded cohort of MM patients; (2) characterize T-cell immunosenescence before and after ASCT.
Methods: 100 patients with plasma cell dyscrasias were enrolled on OSU13135 to receive standard-of-care induction treatment, followed by high-dose melphalan (HD-M) with ASCT rescue, and standard-of-care maintenance therapy (proteasome inhibitor or IMiD). T-cell p16 was measured by Nanostring before treatment, before ASCT, 90 days after ASCT, and 1 year following transplant. T-cell subsets were characterized by flow cytometry before and after ASCT in 20 representative trial patients and 10 age matched controls.
Results: Induction and maintenance therapy did not significantly alter T-cell p16 levels. However HD-M followed by ASCT increased these levels at least 2-fold ($p<0.01$), confirming this regimen as a model of accelerated T-cell senescence. Similarly, an inversion in the CD4:CD8 ratio, reduced CD28 expression, and increased levels of CD4+ T-regulatory cells confirmed immunosenescent phenotypes in transplanted patients. ASCT also altered T cell subset compositions: central memory (CM) and effector memory (EM) cell populations were increased in CD4 T cells, whereas naïve T cells were lost. In CD8 T cells, EM and terminally differentiated EM (TEMRA) cells were expanded at the expense of CM and naïve T cells. After ASCT, immune senescent CD57+PD1- cells were significantly enriched in CD8 EM and EMRA cells. By contrast, both anergic and exhausted CD4 T cells were preferentially expanded after ASCT.
Summary and Conclusions: Phenotypic hallmarks of accelerated T cell cellular- and immunosenescence are replicated in MM patients undergoing ASCT. This suggests that transplant serves as a model to study mechanisms and biomarkers of age-related T-cell impairment. Additional studies assessing T-cell function and transcriptional and epigenetic signatures, and a direct correlation between the immune senescent phenotype and T-cell p16, are needed. A comprehensive immune assessment might serve as a modifiable clinical predictor of outcome of treatment and frailty in older cancer patients.

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GENE EXPRESSION PROFILING OF PROSPECTIVE SERIES IN PRIMARY PLASMA CELL LEUKEMIA REVEALED GENE SIGNATURE ASSOCIATED WITH SURVIVAL

M. Naudot[1,*], M. Diouf[2], S. Minvielle[3,4], A. Caulier[1,5], H. Avet-Loiseau[6], J.-P. Marolleau[1,5], B. Royer[7]

[1]LNPC - EA4666, Université de Picardie Jules Verne, [2]Direction de la Recherche Clinique et Innovation, CHU Amiens, Amiens, [3]Service d'hématologie, CHU de Nantes, [4]CRCINA, INSERM, CNRS, Université de Nantes, Nantes, [5]Service d'Hématologie, CHU Amiens, Amiens, [6]Unité Génomique du Myélome, IUCT, IUCT, Toulouse, [7]Service Immuno-Hématologie, Hôpital Saint Louis, Paris, France

Background: Primary plasma cell leukemia (pPCL) is a rare, yet aggressive form of *de novo* plasma cell tumor, distinct from secondary PCL which represents a leukemic transformation of pre-existing multiple myeloma (MM). The prognosis of pPCL is poor, with median survival less than 1 year. The recent therapeutic progress in MM treatment offers novel agents (lenalidomide and bortezomib) to treat pPCL. A prospective phase II clinical trial of forty patients newly diagnosed pPCL combined novel agents and transplantation procedures (Royer *et al*., 2016). Patients received induction therapy composed of bortezomib, doxorubicine, cyclophosphamide and dexamethasone, followed by either a double autograft and long consolidation or an auto-allo-graft tandem. Overall response rate was 69% and median overall survival of 36 months.
Aims: pPCL shows complex and heterogeneous molecular patterns: the aim is to investigated the transcriptome of pPCLs and characterized gene signature correlated with PFS and/or OS.
Methods: Samples were available for 29 patients from the prospective phase II clinical trial and were analized with nCounter PanCancer pathways panel (nanostring). 770 genes from 13 cancer-associated canonical pathways were screened. Dataset was analyzed with nCounter advanced analysis software. To define statistically significant genes, p-value were adjusted with Benjamini-Yekutieli (BY) method.
Results: We found some upregulated genes CCND1, WHSC1 and FGFR3 in patients corresponding to genomic alterations, respectively t(11-14) and t(4-14), already characterized by FISH. nCounter advanced analysis software allocated patients into 4 groups according to the similarity of gene expression. This distribution is proved to be independent of the major cytogenetic alterations. These groups have strong expression characteristics and are correlated with PFS and OS. Group 3 overexpresses a large number of genes compared to all patients and has the more favorable outcome (PFS and OS). We investigated the dataset for differentially expressed genes in patients who failed to respond to induction therapy. No significant genes signatures were identified. Next, we assessed the relationship between each of the 770 genes across the pPCL dataset and OS. 2 genes: WNT5B and CD40, reached a highly significant correlation (BY. P value <0,05) with OS (Figure 1).

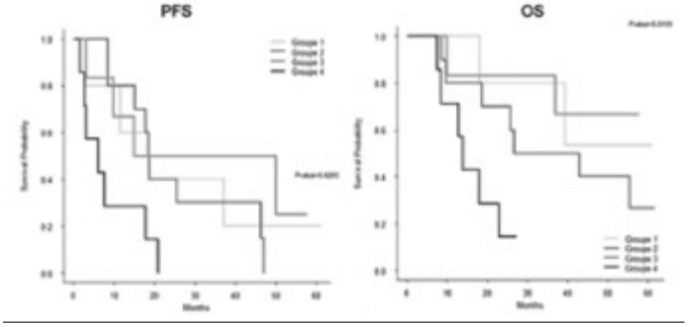

Figure 1.

Summary and Conclusions: This analysis, performed in a homogeneously treated population for a pPCL, allowed us to find 2 genes predictive of survival, whereas not predictive of the response to induction therapy. However the low number of patients in the non-responder group makes identification of gene signature less powerfull. These first hopeful results need to be confirmed with more patients.

PF540

MDSCS PROMOTED TUMORIGENESIS OF MULTIPLE MYELOMA BOTH *IN VITRO* AND *IN VIVO*

L. Ai[1,*], S. Mu[1], Y. Hu[1]

[1]Hematology, Wuhan Union Hospital, Wuhan, China

Background: Multiple myeloma (MM) is a clone malignancy of plasma cells, whose progression largely relies on support from the bone marrow (BM) microenvironment. MDSCs (myeloid derived suppressor cells) are heterogeneous population of myeloid lineage cells, which are highly increased in cancer patients as well as tumor bearing animal models.
Aims: Here, we sought to evaluate the clinical significance of MDSCs in MM development and progression, and validate the promoting effect of MDSCs on MM both *in vitro* and *in vivo*.
Methods: MDSCs frequency in the BM of MM patients or health donors (HDs) was detected by the multicolor flow cytometry (FCM), and other related clinicopathological parameters, such as age, gender, ISS staging, etc. were collected to analyze the clinicopathological significance of MDSCs in MM. Primary MDSCs were isolated from BM cells after removing red blood cells, using CD11b and HLA-DR microbeads selection according to the manufacturer's instructions. And Co-cultures of MDSCs with MM cell lines (RPMI8226 and NIH929) were performed in a non-contacted transwell system, and cell proliferation, apoptosis, cell cycle distribution were measured to validate the MDSCs' promoting effects on MM cell growth. BALB/c nude mice xenograft model was established to test the MDSCs' promoting capacity of tumorigenesis and progression *in vivo*.
Results: MDSCs frequency in the BM from MM patients was much higher than that in the HDs, and there existed positive association between MDSCs levels and the ISS staging for MM patients. Besides, MDSCs co-culture increased MM cells viability in a time-dependent manner, and the promoting effect was the strongest when MM cells were co-cultured with MDSCs for 48h *in vitro*. In addition, MM cells' apoptosis was inhibited by co-culturing with MDSCs, and much more MM cells co-cultured with MDSCs were at S phase in the cell cycle. thus contributing to MM growth *in vitro*. Accordingly, related proteins such as Bcl-2 and Cyclin D1 were up-regulated in MM cells co-cultured with MDSCs. Moreover, the study *in vivo* showed that pre-treatment with MDSCs to MM cells led to modest increase in tumor incidence and volume growth rate compared to MM cells-xenograft-

ed mice, with an average size of tumors of 530.03±69.91 $mm^{3 vs. 301.41 \pm 51.25 mm^3 after 4 weeks (}p<0.05$). Besides, immunohistochemistry and Western blot results showed that pre-treatment with MDSCs increased Ki-67, Bcl-2 and Cyclin D1 expression in MM cells (Figure 1).

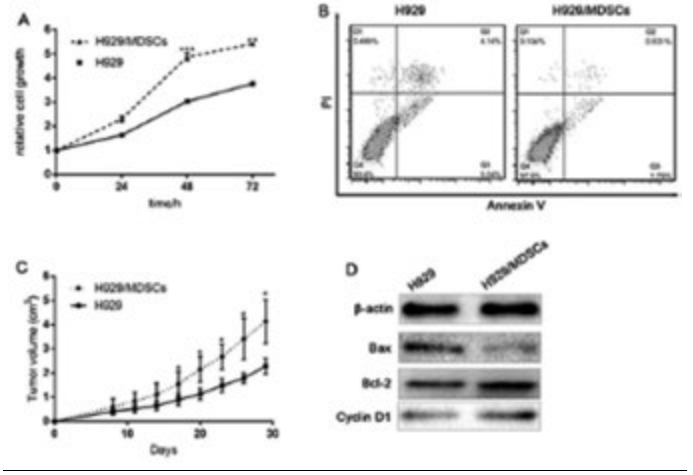

Figure 1. A. Promoting effect of MDSCs on NIH929 cells proliferation after 24h co-culture. B. Apoptotic effects of MDSCs on NIH929 cells as determined by flow cytometry. C. MDSCs co-culture accumulated tumor growth in the myeloma xenografted BALB/c nude mice model. D. Effects of mogrol on the expression of Bax, Bcl-2, and Cyclin D1.

Summary and Conclusions: Our results showed elevated MDSCs levels in MM patients and revealed the positive association between MDSCs and ISS staging in MM. Thus, we concluded that MDSCs might grow to be an important biomarker for MM diagnosis and prognosis. Furthermore, the study *in vitro* and *in vivo* both validate the promoting effects of MDSCs on MM proliferation and progression. These findings suggested that targeting MDSCs may become a new therapeutic strategy to improve MM patients' survival.

PF541

TARGETING THE INTERACTION OF MULTIPLE MYELOMA AND THE BONE MARROW MICROENVIRONMENT THROUGH NOVEL SMALL MOLECULES DIRECTED TO NOTCH PATHWAY

N. Platonova[1,*], C. Parravicini[2], L. Palazzolo[2], A. Paoli[1], S. Saporiti [2], M. Colombo[1], V. Vallelonga[1], D. Giannandrea[1], A. Neri[3,4], I. Eberini[2], R. Chiaramonte[3]

[1]Department of Health Sciences, [2]Department of Pharmacological and Biomolecular Sciences, [3]Department of Oncology and Hemato-oncology, Università degli Studi di Milano, [4]Hematology Unit, Fondazione Cà Granda, IRCCS Policlinico, Milan, Italy

Background: Multiple myeloma (MM) is an incurable hematological cancer characterized by plasma cells accumulation in the bone marrow (BM), where they shape the nearby BM milieu, inducing it to support tumor progression and acquisition of drug resistance. Despite recent advances, poor clinical response and relapse remain major problems. The oncogenic Notch pathway consists of 4 receptors (Notch1-4) activated upon binding to two families of ligands, Jag (Jag1 and 2) and Dll (Dll1, 3 and 4) and plays a crucial role in the pathological interaction between MM and BM cells. In MM the aberrant expression of Notch receptors and ligands on tumor cell results in homotypical interactions, which affect myeloma cell biology and heterotypical interactions with the BM cells, that favor tumor progression, osteoclastogenesis and drug resistance. In particular, aberrant Notch2 activation and overexpression of Jag2 ligand in MM cells play an important role in MM progression by stimulating osteoclast differentiation, release of pro-tumor cytokines by BM cells and MM cell self-renewal. Therefore uncoupling the interaction between Notch2 and Jag2 is critical to affect MM cell growth along with their pathological interaction with the BM niche. Currently, indirect approaches to inhibit Notch signaling are mainly based on inhibition of γ-Secretase, an enzyme that catalyzes Notch activation and the cleavage of several other γ-Secretase substrates. Moreover, γ-Secretase-mediated inhibition of all four Notch receptors is associated with gut toxicity, that might be avoided by selectively blocking of Notch signaling triggered by only one of the two family of ligands, Jag or Dll.
Aims: These lines of evidence prompted us to develop a therapeutic tool to selectively inhibit Notch2 signaling triggered by Jag2 using an unprecedented approach based on drug-like small molecules.
Methods: To select the small molecules, we performed *in silico* a protein-protein docking and virtual high-throughput screening (HTS) of an Asinex chemoteque of small molecules. The biological efficacy was validated through Notch responsive gene reporter and viability assays.
Results: We have set-up a strategy to exclusively uncouple Notch2::Jag2, leaving unaltered the interaction with Dll. The lack of crystallographic structures for Notch2::Jag2 was overcome by exploiting the differences in the surfaces of the Notch2::Jag2 and Notch2::Dll4 complexes, modeled by protein::protein docking on the bases of the crystallographic structures of Notch1::Jag1 and Notch1::Dll4, respectively. It allowed us to select *in silico* 100 top-scoring compounds supposed to be exclusively directed to Notch2::Jag2 surface by HTS of the small molecules chemoteque . Initially, 2 of 100 compounds were validated *in vitro*. A Notch responsive reporter assay on HEK293T cells showed that the compounds were able to significantly reduce Notch transcriptional activity. A viability assay of MM cell lines showed a dose-dependent cell growth inhibition in the presence of the compounds. Finally a Notch responsive reporter assay on co-culture systems allowed us to measure Notch2 activation triggered either by Dll4 or Jag2 ligands and to demonstrate that one of the two tested compounds specifically inhibited Notch2::Jag2 but not Notch2::Dll4 interactions.
Summary and Conclusions: Our integrated pipeline represents a successful strategy to identify compounds that directly and selectively antagonize Notch activation and lays a basis for the development of an entirely novel class of drugs to inhibit Notch signaling in cancer.

PF542

EXPRESSION LEVELS OF THE THREE GENES CRBN, IKZF1, AND IKZF3 IN PRIMARY MULTIPLE MYELOMA CELLS AT PRE- AND POST- LENALIDOMIDE TREATMENT

S. Kinoshita[1,*], M. Ri[1], T. Kanamori[1], S. Aoki[1], T. Yoshida[1], T. Narita[1], H. Totani[1], A. Ito[1], S. Kusumoto[1], H. Komatsu[1], S. Iida[1]

[1]Department of Hematology and Oncology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

Background: Lenalidomide (Len) binds to cereblon and alters its substrate specificity, which results in immunomodulatory and anti-tumor effects. Among the substrates ubiquitinated by cereblon by Len exposure, the degradation of the two transcription factors IKZF1 and IKZF3 is considered critical in anti-tumor effect of Len. Although expression levels of *CRBN* and its related genes have been analyzed in association with treatment outcome of Len, their results are still controversial. In addition, the expression levels of these genes after Len treatment have not been fully investigated.
Aims: This study was conducted to explore if expression levels of *CRBN*, *IKZF1* and *IKZF3* mRNAs before treatment with Len plus dexamethasone (Ld) in primary multiple myeloma (MM) cells were associated with its treatment outcome and to investigate their alteration at post-Ld treatment.
Methods: A total of 83 patients with relapsed MM were treated with Ld therapy in our hospital between July 2010 and May 2017 and their samples and data were retrospectively analyzed. Forty-eight bone marrow (BM) specimens were collected just prior to Ld therapy. Among these, 25 paired BM samples were obtained at pre- and post- Ld therapy. After purification of CD138 positive cells from mononuclear cell fraction, mRNA levels of *CRBN*, *IKZF1* and *IKZF3* were quantified using real-time RT-PCR. Next, their expression levels were analyzed in association with the following outcomes such as response levels, progression-free survival (PFS) and overall survival (OS). Alteration of the expression levels of these genes in primary MM cells were compared between pre- and post-Ld treatment.
Results: Out of 48 patients who provided BM specimens with their informed consents, 47 patients were evaluated for the efficacy of Ld therapy. Expression levels of any of the three genes, *CRBN*, *IKZF1* and *IKZF3*, were not significantly associated with the PFS or OS. When tested using the ratio of *IKZF1* divided by *CRBN* expression, poor responders (SD+PD, n=15) to Ld therapy showed a significantly lower ratio than good responders (CR+VGPR+PR, n=32) (P=0.01). In addition, when the median value of the ratio of *IKZF1/CRBN* was set as a cut-off value, the patients with lower ratio of *IKZF1/CRBN* showed a significantly shorter OS than those with higher ratio (Figure 1a, median OS: 17 *vs* 38 months, P=0.034). There was no association of the ratio of *IKZF3* to *CRBN* with the efficacy of the Ld therapy. Of the 25 paired BM samples collected at both pre- and post-Ld therapy, 22 post-Ld samples were obtained when the patients became refractory to Ld. The expression levels of *CRBN* were reduced in 17 patients (17/25, 68%) and increased in 8 patients (8/25, 32%) at post-Ld compared to pre-Ld (Figure 1b). In the analysis of 22 refractory MM

cases to Ld, most of them (16/22, 73%), showed reduced expression of *CRBN* and two of them lacked *CRBN* expression after Ld therapy. In terms of *IKZF1* and *IKZF3* genes, 19 patients (19/25, 76%) showed increased expression of *IKZF1* and 16 patients (16/25, 64%) showed reduction of *IKZF3 mRNAs*.

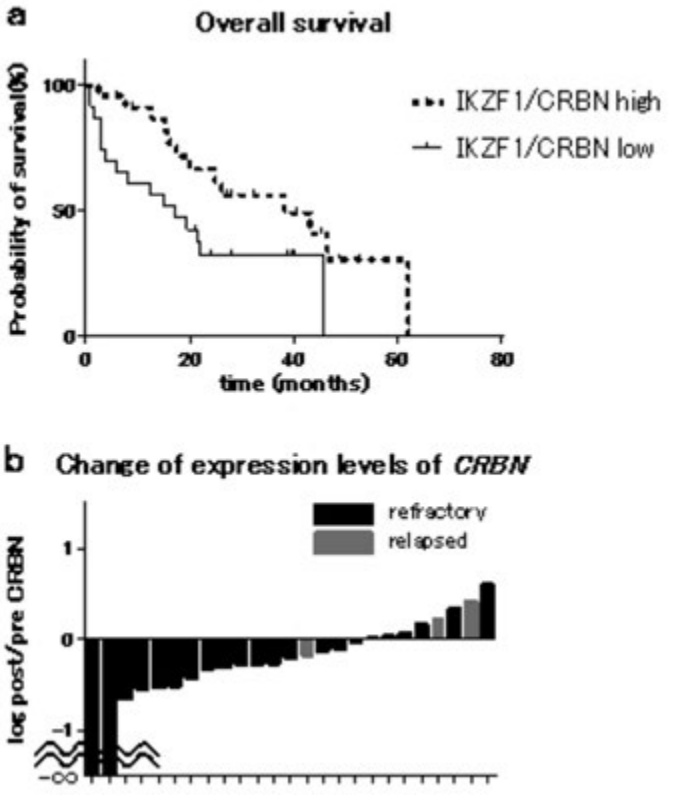

Figure 1.

Summary and Conclusions: A low ratio of *IKZF1/CRBN* may predict for the poor efficacy to Ld therapy and may serve as a biomarker in Len treatment. Reduced or lack of *CRBN* expression was observed in most refractory patients to Ld, suggesting an existence of lower dependence on cereblon and related pathways during development of Len resistance in primary MM cells.

PF543

MYC INVOLVES MULTIPLE MYELOMA PROGRESSION BY ATTENUATING TUMOR SUPPRESSIVE FUNCTION OF TP53-MICRO RNA 34 AXIS

H. Handa[1], Y. Murakami[2,*], K. Masuda[2], T. Oda[3], Y. Masuda[2], H. Hattori[4], R. Ishihara[2], K. Honma[2], S. Watanabe[2], Y. Kuroda[2], N. Kobayashi[1], T. Ishizaki[1], H. Shimizu[1], H. Koiso[1], Y. Osaki[1], A. Yokohama[5], N. Tsukamoto[6], T. Kasamatsu[2], T. Saitoh[2], H. Murakami[2]

[1]Department of Hematology, [2]Graduate School of Health Science, [3]Institute for Molecular and Cellular Regulation, Gunma University, Maebashi, [4]Graduate School of Medicine, Nagoya University, Nagoya, [5]Blood Transfusion Service, [6]Oncology Center, Gunma University, Maebashi, Japan

Background: Micro RNAs (miRs) are small non-coding RNAs of 19-25 bases in length having ability to modulate gene expression. Many miRs act as tumor suppressor genes (TSG) via degrading their target mRNA and/or inhibiting translation. MiR-34 is a transcriptional downstream and mediator of tumor suppressive function of TP53 by targeting MYC and CDK6. MiR29 acts as a tumor suppressor targeting DNA methyltransferase (DNMT). Those miRs cooperatively play a role in MM progression, however precise mechanism underlying miR dysregulation has not been elucidated yet. MYC simultaneously activates and silences many genes transcription including miR29.
Aims: In this study, we try to clarify mechanism of miR dysregulation in MM in the context of interaction between MYC and TP53-miR34 axis.
Methods: Purified bone marrow plasma cells by using anti-CD138 antibody and magnetic beads obtained from 123 MM patients, 57 MGUS patients 20 control subjects and 9 myeloma cell lines are subjected to the study after informed consent. This study was approved by IRB of Gunma University under the declaration of Helsinki. MiRs and their target gene mRNA values were determined by RQ-PCR. DNA methylation status was determined by methylation specific PCR. DNA demethylating agent decitabine, MDM2 inhibitor nutlin-3, MYC inhibitor 10058-F4, and miRNA-mimic™ and siRNA *in vitro* were used. U2OS/MYC-ER and KMS27/MYC-ER cell lines whose MYC are activated by incubation with 4-OHT were used.
Results: We found significantly reduced expression of miR 29a, 29b, 29c, 34a, 34b, and 34c in MM patients than in MGUS patients and control subjects (all: p<0.001). DNMT1 and 3A, 3B were elevated in MM patients than in MGUS patients and control subjects (p<0.001, p<0.001, p=0.01). The promoter of miR-34a and miR-34b/c were methylated with higher rate in MM (45.4%, 70.2%) than in MGUS patients (15.8%, 26.3%) (p<0.001). Significant positive correlations among miRs expressions were found: 29a-34a r=0.448, p<0.001; 29a-34b r=0.309, p=0.001; 29b-34a r=0.500, p<0.001; 29b-34b r=0.297, p=0.002). Unexpectedly, TP53 and its downstream p21 was rather higher in MM patients than MGUS and control subject (p=0.004, p<0.001), however TP53 level was not correlated with miR-34a, b, c. TP53 accumulation by nutlin-3 increased miR34a, b in U2OS, KMS28PE and KMS27 with wild type (wt) TP53, but did not increase miR34s in TP53 null KMS11 and TP53 mutated KMS18, KMS12PE, KMS28BM with wt TP53 and methylated miR34. Upregulation of miR34a by nutlin-3 was abrogated by MYC activation in U2OS/MYC-ER and KMS27-ER. MYC inhibitor increased pri-miR-29a/b-1 and miR-29a,b, pri-miR-34a and miR34a, b, c, however, knockdown by siRNAs did not show increase of miR29s and miR34s, probably because of insufficient suppression of MYC. Si-RNA-MAX showed slight increase of miR29b in KMS12PE. Decitabine increased miR34a, b and pri-miR-34a in, suggesting that miR-34s were suppressed by methylation. Transfection of miR-29a or 29b reduced DNMT3A and 3B and increased pri-miR-34s, and miR-34 transfection reduced CDK6 expression.
Summary and Conclusions: We found significantly reduced miR29 and 34 expression in MM discordant with higher TP53 expression. Escape from normal regulation of TP53-miR34 is promoted by MYC and promoter methylation. Correlations in between miRs in patients and *in vitro* study suggest that MYC involves multiple myeloma progression by attenuating tumor suppressive function of TP53-micro RNA 34 axis via abrogating micro RNA-29-DNA methyltransferase axis.

PF544

PREVENTING AND REPAIRING MYELOMA BONE DISEASE BY COMBINING CONVENTIONAL ANTI-RESORPTIVE TREATMENT WITH A NOVEL BONE ANABOLIC TREATMENT

A. Chantry[1,*], J. Paton-Hough[1], S. Tazzyman[1], H. Evans[1], L. Darren[1], J. Down[1], G. Alanna[1], S. John[1], M. Lawson[1]

[1]Oncology and Metabolism, University of Sheffield, Sheffield, United Kingdom

Background: Multiple myeloma is a plasma cell malignancy, which develops in the bone marrow and frequently leads to severe bone destruction. Current anti-resorptive therapies to treat the bone disease do little to repair damaged bone; therefore, new treatment strategies incorporating bone anabolic therapies are urgently required.
Aims: We hypothesised that combination therapy using the standard of care anti-resorptive zoledronic acid (Zol) with a bone anabolic (anti-TGFβ/1D11) would be more effective at treating myeloma-induced bone disease than Zol therapy alone.
Methods: JJN3-bearing mice (n=8/group) were treated with vehicle, Zol or 1D11 alone or Zol and 1D11 combined. Bone loss was analysed at the end stage of disease by *ex vivo* micro-CT and histomorphometry. U266-bearing mice (n=8/group) with established lytic bone lesions were treated with vehicle, Zol or Zol and 1D11 combined. Bone changes were monitored overtime by *in vivo* micro-CT, serum bone markers and dynamic histomorphometry. JJN3 myeloma-bearing mice treated with combined Zol and 1D11 resulted in a 48% increase (P≤0.001) in trabecular bone volume compared to Zol alone and a 65% (P≤0.0001) increase compared to 1D11 alone.
Results: The most significant finding was the substantial repair of U266-induced lytic bone lesions with combination therapy, which resulted in a significant reduction in lesion area compared to vehicle (P≤0.01) or Zol alone (P≤0.01). These results reveal a novel finding and demonstrate that combined anti-resorptive and bone anabolic therapy are significantly more effective at treating established myeloma-induced bone disease than Zol alone.
Summary and Conclusions: This is a highly translational strategy which could significantly improve bone outcomes and quality of life in myeloma patients.

PF545

TRYPTOPHAN SHORTAGE DUE TO IDO-1 EXPRESSED BY HIGH-DENSITY NEUTROPHILS INDUCE IMMUNE-SUPPRESSION AND PLASMA CELL FITNESS IN MULTIPLE MYELOMA

A. Romano[1,*], P. La Cava[1], N.L. Parrinello[1], C. Giallongo[1], D. Tibullo[2], G. Camiolo[2], F. Puglisi[1], V. Calafiore[1], M. Parisi[1], V. Del Fabro[1], C. Bellofiore[1], G. Sapienza[1], F. Cremasco[3], C. Conticello[1], G.A. Palumbo[2], S. Cenci[3], F. Di Raimondo[1]

[1]CHIRMED- Dipartimento Chirurgia Generale e Specialità Medico-Chirurgiche-Hematology Section, [2]Università degli Studi di Catania, Catania, [3]Age and Disease Related Unit, Fondazione Centro San Raffaele, Milano, Italy

Background: There is an increasing interest about the role of amino acid degrading enzymes in cancer immunotherapy. In multiple myeloma (MM), several groups including ours showed that immune-suppression due to arginine starvation is clinically relevant. However, Arginase inhibition or arginine supplementation cannot recover completely T-cell immune dysfunction.
Aims: In the attempt to investigate the role of amino acid deprivation in MM progression, we tested if availability of tryptophan could hamper immune function in the progression from MGUS through MM.
Methods: We first measured the amount of tryptophan and its degrading enzyme 2,3-indoleamine deoxygenase (IDO-1) in sera obtained from bone marrow and peripheral blood of 15 MGUS, 10 smoldering MM (sMM), 15 newly diagnosed and 10 relapsed MM. Second, we evaluated the main cellular source of circulating IDO by western blot and immune fluorescence of high- and low-density neutrophils (LDN), monocytes and neoplastic plasma cells. Third, we explored if the immune-suppressive activity of MM-LDN could be recovered by treatment *in-vitro* with 200 nM epacadostat, an IDO-1 inhibitor currently under investigation in phase I-II trials of immunotherapy in solid cancers. Fourth, we explored if tryptophan shortage could induce an adaptive response to MM cells MM1.s, OPM2 and U266 *in vitro* and mediate refractoriness to bortezomib, melphalan and lenalidomide.
Results: IDO-1 was increased in both bone marrow and peripheral blood of MM patients compared to MGUS and healthy subjects (p=0.002). Conversely, tryptophan was reduced (more in peripheral blood than in bone marrow) in MM *versus* MGUS patients and kynurenine (a product of tryptophan degradation) increased (p=0.001). T- cell function, evaluated as expression of HLA-DR and CFSE expression upon stimulation with 5ng/mL phytohaemagglutinin (PHA) for 72 hours, was hampered by co-colture at ratio 1:4 with MM-derived HDN, and only partially reverted by treatment with epacadostat.
MM cells expressed IDO-1 but their viability was not affected by exposure to epacadostat up to 72 hours. Tryptophan shortage (1000-10nM) did not affect cell proliferation and cell cycle of MM cell lines tested either, while induced T-cell apoptosis within 48 hours. In two human myeloma cell lines MM1.s and U266, progressive tryptophan shortage induced an adaptive response through increased expression, time and dose-dependent, of ATF4-ASNS-CHOP-GADD34, part of GCN2 signaling, followed by autophagy induction and fitness marker IRF4 and Blimp1 upon 96 hours of starvation. *In-vitro*, sub-toxic treatment with 5nM bortezomib, 10uM lenalidomide or 10uM melphalan for 24 hours showed synergic effect only between melphalan and epacadostat.
Summary and Conclusions: IDO-1 and tryptophan shortage are associated to MM progression. HDN are IDO-1 positive and mediate immune-suppression that can be reverted only partially by treatment with 200nM epacadostat. Epacadostat has synergic effect with melphalan *in vitro*. Experiments *in vivo* are ongoing to understand the contribute of IDO-1 in MM establishment in immune competent models of disease.

PF546

CHARACTERIZATION OF ST3GAL6-AS1, A NOVEL LNCRNA DEREGULATED IN MULTIPLE MYELOMA

C. Vinci[1,2,*], E. Taiana[1,2], V. Favasuli[1,2], D. Ronchetti[1,2], L. Agnelli[1,2], S. Galletti[1], M. Manzoni[2], F. Pelizzoni[2], M. Lionetti[1,2], K. Todoerti[1], A. Neri[1,2]

[1]Oncology and Hemato-Oncology, University of Milano, [2]Hematology Unit, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy

Background: During the last two decades, long non-coding RNAs (lncRNAs) relevance has been highlighted by a large number of studies. LncRNAs are non-protein-coding transcripts, longer than 200 nucleotides, which can act to regulate gene expression and be involved in cancer. More recently, some groups evidenced the impact of deregulated lncRNAs in Multiple Myeloma (MM). In previous report, we demonstrated that deregulated patterns of lncRNAs expression are associated with distinct MM molecular subtypes (Ronchetti *et al.*, Oncotarget, 2016).
Aims: (i) Confirming and extending data on lncRNA expression in MM (ii) Characterizing the RNA structure and explore the functional role in MM human cell lines (HMCLs) of ST3GAL6-AS1, the unique up-modulated lncRNA in MM patients compared with healthy donors.
Methods: A custom annotation pipeline was used to investigate lncRNA profiles on GeneChip® Human Gene 2.0 ST microarray in highly purified bone marrow PCs from 50 MM primary tumors and 4 normal donors. Real-time PCR (QT-PCR) was performed to confirm array data. Predicted splicing of ST3GAL6-AS1 transcripts were examined by qualitative PCR and Sanger sequencing in HMCLs and primary tumors. lncRNAs subcellular localization was evaluated by fractionated HMCLs nuclear and cytoplasmic RNA. HMCLs were treated with actinomycin D to determine the half-life of the lncRNAs. siRNA silencing of ST3GAL6 on HMCLs was performed by Neon Transfection system.
Results: We analyzed the long non-coding RNA fraction of the transcriptome of 50 MM patients using arrays that investigate more than unique 10000 sequences. First, we confirmed that the expression of the 5% most variable lncRNAs across the dataset grouped MM samples based on the main cytogenetics prognostic alterations (p<0.0001). One-hundred sixty-four lncRNAs showed significant differential expression in hyperdiploid, 11q14, 4p16 and MAF translocations groups. Importantly, we found ST3GAL6-AS1 as the unique significant overexpress lncRNA in MM samples compared to healthy donors. ST3GAL6-AS1 maps to 3q12.1 and is antisense to ST3GAL6, a protein involved in homing and *in vivo* engraftment of HMCLs and correlated with shorter overall survival in MM patients (Glavey *et al.*, Blood, 2014). The validation of array data by QT-PCR confirmed the overexpression of ST3GAL6-AS1 in MM sample. Analysis on HMCLs revealed that ST3GAL6-AS1 was overexpressed and equally localised in nuclear and cytoplasmic fractions. Furthermore, molecular analysis of the lncRNA in HMCLs and primary tumors showed the presence of a polymorphic splicing nucleotide variant (rs13065271) with the retention of a 128bp intron in the transcript. In homozygous mutated HMCLs we observed a prevalent nuclear localization of ST3GAL6-AS1, as well as a lower expression and reduced half-life of its transcripts. Moreover, ST3GAL6-AS1 and ST3GAL6 displayed a significant correlation in their expression levels. siRNA silencing of ST3GAL6 in HMCLs caused down-regulation of ST3GAL6-AS1, suggesting a possible co-regulation mechanism.
Summary and Conclusions: Our data indicate that ST3GAL6-AS1 is significantly deregulated in MM patients. Furthermore, the occurrence of a polymorphic variant leading to an alternative splicing in ST3GAL6-AS1 may have potential relevance in the transcript stability and its functional role.

PF547

MULTIPLE MYELOMA RELATED ANGIOGENESIS: ROLE OF NOTCH-JAG AXIS IN MODULATING ENDOTHELIAL CELLS BEHAVIOR

M.T. Palano[1,*], M. Colombo[1], N. Platonova[1], A. Neri[2], R. Chiaramonte[1]

[1]Department of Health Sciences, [2]Department of Oncology and Hemato-oncology, Università degli Studi di Milano, Milan, Italy

Background: Multiple myeloma (MM) is an incurable malignancy characterized by plasma cells accumulation in the bone marrow (BM). MM cells are able to shape the BM niche, inducing BM resident cells to support malignant cells survival and proliferation. In this process, a key role is played by alterations of Notch pathway. Indeed, the overexpression of Notch1/2 receptors and Jag1/2 ligands by MM cells promotes Notch hyperactivation not only in tumor cells, but also in the surrounding normal cells, stimulating different processes aimed to sustain MM cell progression. One of the mechanisms that promotes MM growth and contribute to its fatal outcome is the increased level of angiogenesis within BM. Interestingly, even if the link between MM progression and the angiogenic switch is well known, the contribution of Notch activity in this process still needs to be clarified.
Aims: This study aims to investigate the role of Notch-Jag axis in MM-associated angiogenesis evaluating the contribution of MM cells and of BMSCs.
Methods: We used 3 different cell lines, RPMI8226, U266 and OPM2 in which the Notch ligands, Jag1/2, were silenced ($MM^{KDJAG1/2}$) using specific siRNAs to study the role of Notch-Jag axis in promoting angiogenesis. The human pulmonary artery endothelial cells (HPAECs) were used to mimic the endothelial compartment while BMSCs were represented by the GFP^{+}HS5 cell line. The outcome of Jag1/2 silencing on MM-ECs interaction or on MM cells ability to secrete pro-angiogenic factors was analyzed using three assays that allowed us to evaluate different biological processes involved in tumor angiogenesis: motility (wound healing assays), adhesion on ECM-like substrate (adhesion assay) and EC tube formation assay. Finally, we analyzed MM capability to activate Notch signaling in BMSCs and induce the secretion of VEGF by qRT-PCR and flow cytometry. To investi-

gate the biological outcome of these variations, the above-mentioned assays were performed using CM collected from MM-BMSCs co-culture system.
Results: Our results indicate that MM cells promote tube formation both through direct contact and by releasing soluble factors. This effect is significantly reduced in the absence of Jag1/2 ligands, sustaining the hypothesis of a key role of Notch signaling in ECs stimulation. Comparable results were obtained in wound healing and in adhesion assays. Experiments performed on MM-BMSCs co-culture system showed that MM cells are able to boost BMSCs ability to produce the angiogenic factor VEGF. Interestingly, this effect is reverted by Jag1/2 silencing. As expected, MM-driven modulation of stromal-derived VEGF impacts on EC motility, adhesion and tube formation.
Summary and Conclusions: These findings indicate a novel role for the Notch-Jag axis in MM ability to promote angiogenesis in the BM microenvironment. Tumor-associated angiogenesis is supported by MM-ECs direct interaction and by the action of Notch-dependent MM-derived soluble factors. Moreover, Notch-Jag axis is involved in promoting stromal-derived VEGF production that further stimulates angiogenesis.

PF548

ADIPORON, THE FIRST ORAL ADIPONECTIN RECEPTOR AGONIST, INHIBITS THE PROLIFERATION OF MYELOMA CELLS VIA THE AMPK / MTOR /AUTOPHAGY SIGNALING PATHWAY

S. Wang[1,*], Y. Liu[1]

[1]The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

Background: Multiple myeloma (MM) is the second most common hematological malignancies. New agent therapy and hematopoietic stem cell transplantation have significantly improved the survival of MM, but patients will eventually progress to death. To develop new drugs with different mechanisms of action is the fundamental ways to overcome this problem. Adiponectin has been reported to play an important role in the pathogenesis of MM and is a potential therapeutic target. However, it's difficult to directly use adiponectin in clinical practice. AdipoRon, the first oral adiponectin receptor agonist, plays a similar role as adiponectin in improving glycolipid metabolism while the role of AdipoRon in hematological malignancies remains blank.
Aims: The aim of this study was to investigate the inhibitory effects of AdipoRon on proliferation of myeloma cells and to explore the underlying mechanisms.
Methods: RT-PCR was used to quantify the AdipoR1 and AdipoR2 mRNA copy number in the bone marrow cells from 21 patients with MM. 23 normal marrow samples were served as control. Informed consent was obtained for every marrow sample. The cell proliferation was detected by CCK-8 and cell apoptosis was analyzed by flow cytometry. Western blot was used to determine the protein level of the signaling pathway.
Results: We found that the expression of AdipoR1 in MM was significantly higher than that in normal controls, while the expression of AdipoR2 in MM was significantly lower than that in normal controls, suggesting that adiponectin receptors are differentially expressed between MM patients and normal individuals. AdipoRon significantly inhibited the proliferation of MM cell lines Sp2 and MPC-11 in a concentration-dependent and time-dependent manner without affecting the proliferation of normal mesenchymal stem cell. Flow cytometry showed that AdipoRon induced apoptosis of MPC-11 cells in a concentration-dependent manner; Western blot showed that AdipoRon increased the expression of apoptosis-related proteins cleaved caspase-3 and cleaved PARP. AdipoRon upregulated p-AMPK and its downstream p-ACC and down-regulated p-mTOR in MPC-11. In addition, AdipoRon upregulated LC3- II / LC3-I level and down-regulated the protein level of p62, suggesting the activation of autophagy.
Summary and Conclusions: AdipoRon, the first adiponectin receptor agonist, could inhibit the proliferation and induce the apoptosis of myeloma cells, activate AMPK, inhibit the mTOR signaling pathway and activate autophagy in myeloma cells, suggesting that AdipoRon might play an important role in anti-proliferation of myeloma cells via the AMPK/mTOR/autophagy signaling pathway. The results need to be further verified *in vivo*.

PF549

CLINICAL RELEVANCE OF LONG NON CODING RNA IN MULTIPLE MYELOMA: RETROSPECTIVE MONOCENTRIC STUDY

V. Ferla[1,*], A. Pompa[1], L. Agnelli[1,2], F. Rossi[1], F. Guidotti[1,2], A.I. Gregorini[1], S. Fabris[1,2], M. Barbieri[1,2], M. Lionetti[1,2], K. Todoerti[1,2], M. Manzoni[1,2], A. Cortelezzi[1,2], A. Neri[1,2], L. Baldini[1,2]

[1]Hematology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, [2]Department of Oncology and Hemato-Oncology, University of Milan, Milano, Italy

Background: Multiple myeloma (MM) is a malignant proliferation of bone marrow plasma cells (BMPC) characterized by highly heterogeneous genetic background and clinical course. Long non-coding (lnc)-RNA represents the largest class of non-protein coding genes in the human genome. LncRNAs deregulation has been reported to promote tumor formation, progression and metastasis in many types of cancer. Their role in MM is progressively expanding. In previous report, specific lncRNA transcriptional profile has been described in subgroups characterized by molecular/cytogenetics alteration or progressive stages of the disease (Ronchetti *et al.,* 2016).
Aims: This study was aimed at evaluating the correlation between lncRNA expression profiles and clinical variables in MM at diagnosis.
Methods: We retrospectively collected the clinical and laboratory data of 80 MM patients (pts), who have been admitted to our Hematology unit from 2000 to 2014. Complete clinical data were available together with FISH/cytogenetics information and transcriptional profiles generated on GeneChip® Human Gene 1.0st arrays.
Results: The median age at diagnosis was 67 years, 38% of pts had anemia, 11% renal failure, only 4% was hypercalcemic, and 57% showed bone damage. BMPC infiltrate was greater than 60% in half of the cases, while extramedullary localizations occurred in only 2 pts. Del(13) was present in 51% of pts; del(17) in 10.1%; t(4; 14) translocation in 10%; t(11;14) in 25%; t(14;16) or t(14;20) in 7.5%; t(6;14) in 6.3%; 36.6% pts were hyperdiploid, 52.7% had 1q gain and 19% had 1p loss. Fit pts underwent autologous stem cells transplantation (ASCT), whereas others were treated with chemiotherapy, immunomodulatory drugs or Bortezomib. The median overall survival (OS) was 77 months and the time to next treatment (TNT) was 22 months. According to ISS), pts in stage I were 25, in stage II 30, in stage III 23. According to R-ISS, pts in stage I were 14, in stage II 52, and in stage III 12. R-ISS increased the number of patients in category II and effectively stratified pts in term of OS e TNT (p=0.005 and p=0.0003, respectively). We evaluated the association between lncRNA expression, assessed as a continuous variable, and outcome using *globaltest* package in R software, and identified 12 and 1 lncRNAs whose expression showed respectively negative and positive association with OS. Considering TNT, 15 lncRNAs were positively and 1 negatively correlated. In particular, the most significant correlation with poor outcome, in terms of both time to therapy and survival, was found for LIN00599, a lncRNA that was described to regulate the transcription through endogenous competition with microRNAs (specifically miR-4306, miR-185-5p, miR-4644). No correlation was found between lncRNA expression and response to therapy, independently of the type of treatment and the number of previous therapies. Additionally, our data indicated absence of relationship between lncRNA expression and ASCT therapy. Finally, the expression of each of the 13 lncRNAs associated with OS and the 16 associated with TNT was then tested as dichotomic variable in association with ISS and R-ISS. Our analysis revealed that none of the tested lncRNA improved ISS models.
Summary and Conclusions: In our retrospective cohort, representative of the main molecular alterations in MM, we unraveled lncRNA transcripts whose expression could be associated with outcome. However, lncRNA expression did not improve R-ISS staging model, which in our cohort retained the highest prognostic value.

PF550

DRUG SENSITIVITY SCREENING IN MULTIPLE MYELOMA (MM) FOR PRECISION CANCER THERAPY

D.B. Thimiri Govinda Raj[1,*], M. Giliberto[2], A. Cremaschi[2], S.S Skånland[2], A. Gade[3], D. Wang[2], G.E. Tjønnfjord[4], F. Schjesvold[4], L.A. Munthe[2], K. Tasken[1]

[1]Centre for Molecular Medicine Norway (NCMM), Nordic EMBL Partnership, University of Oslo and Oslo University Hospital, [2]Centre for Molecular Medicine Norway (NCMM), Nordic EMBL Partnership, University of Oslo and Oslo University Hospital, [3]NCMM UIO, [4]Department of Haematology, Oslo University Hospital, Oslo, Norway

Background: Chronic Lymphocytic Leukemia (CLL) and Multiple Myeloma (MM) are considered incurable. Although modern treatment regimens prolong survival, CLL and MM eventually relapse. Current challenges include design of optimal treatment for individual patients based on characterization of the tumor and its intratumor heterogeneity as observed by whole genome sequencing. Efficient therapies require a personalized approach that combines targeting malignant plasma cells as well as the support malignant plasma cells receive from the tumor microenvironment. Another major limitation is that there exists no efficient approach to identify the most efficient drugs for each patient and also for different cancer stage. Using our drug sensitivity screening platform, we aim to address the limitation in identifying the efficient drugs for individual patients.

Aims: We aim to establish a drug sensitivity screening platform for MM. We aim to perform drug sensitivity screening with 32 drugs at 5 concentrations to select drug candidates and pathway inhibitors for each patient sample. Selected drug candidates will be further analyzed by bioassays and flow cytometry to assess effects on intracellular signaling (phosphoflow-based approach). We aim to select efficient drug combinations for synergistic effects. We propose to use the drug sensitivity screening platform to identify and validate drug candidates for xenografting and precision medicine clinical trials.
Methods: We have established culture settings that mimic the tumor microenvironment for MM (Wang D. et al Leukemia 2017). MM cells were co-cultured with autologous bone marrow Th cells (wherein CD8+ T cells are depleted) directly in the presence of anti-CD3/28 beads and 100U/ml human IL2 for 2 days. Stimulated MM cells were then isolated using Miltenyi MACS CD138 positive selection. CD138+ MM cells were cultured in 384 well formats with 32 drugs at 5 concentrations for 72 hours (3 days). To define drugs that inhibit malignant plasma cell growth, we use the cell-based assays CellTiter-Glo® luminescent cell viability assay and CellTox™ green cytotoxicity assay as readout. As a quality control, DNA replication was tested by BrdU incorporation and also appropriate cell surface marker staining.
Results: Previous work from our group reported the experimental setting that mimic the tumor microenvironment for MM (Wang D. et al Leukemia 2017). We have performed drug screening on 10 patient samples. We have observed inhibitory effect of proteasome inhibitors on CD138+ MM cells in our drug screening experiment. We have also performed preliminary experiments with drugs that are in clinical trial. We performed drug screening on M2 cell line (patient derived cell line) for 527 drugs at 5 concentrations (Figure 1).

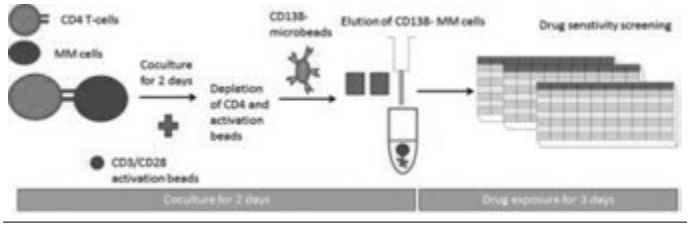

Figure 1.

Summary and Conclusions: Using our established MM drug sensitivity screening approach, we aim to perform drug screening on several patient samples. We aim to use statistical approach to identify synergistic drug effects and validate the drug combinations experimentally. As a future perspective, we would combine machine learning strategies with the experimental drug screening strategies for the precision medicine clinical trials.

PF551

ASSESSMENT OF SELECTED TOLL-LIKE RECEPTORS EXPRESSION ONT AND B LYMPHOCYTES IN PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

E. Grywalska[1,*], M. Pasiarski[2], P. Steckiewicz[2], A. Stelmach-Goldys[3], B. Sosnowska-Pasiarska[4], K. Gosik[1], A. Kowalska[1], B. Garus[2], S. Gozdz[5], J. Rolinski[1]

[1]Department of Clinical Immunology and Immunotherapy, Medical University of Lublin, Lublin, [2]Department of Hematology, Holycross Cancer Center, [3]Department of Hematology, Holycross Cancer Center, [4]Department of Oncocardiology, [5]Department of Clinical Oncology, Holycross Cancer Center, Kielce, Poland

Background: Monoclonal gammopathy of undetermined significance (MGUS) is an asymptomatic plasma cell dyscrasia that is present in more than 3% of the general white population older than age 50 and has an average multiple myeloma progression risk of 1% per year. Toll-like receptors (TLRs) play a protective role against infections, the leading cause of morbidity and mortality in MGUS patients, by recognizing a wide variety of pathogen-associated molecular patterns.
Aims: The aim of this study was to determine the percentages and absolute numbers of lymphocytes B and T with expression of TLR2, TLR4, and TLR9 in peripheral blood of MGUS patients. We analysed relationship between the number of TLR-positive lymphocytes and established prognostic factors in MGUS.
Methods: The study included 40 untreated patients with MGUS. The control group comprised 20 healthy subjects. After isolation of peripheral blood mononuclear cells, the viable cells underwent labeling with fluorochrome-conjugated monoclonal antibodies, and analyzed by flow cytometer.
Results: Compared to healthy individuals, patients with MGUS showed lower frequencies of lymphocytes B with phenotypes CD19+TLR2+ (p=0.000016), CD19+TLR4+ (p=0.0003), and CD19+TLR9+ (p=0.00053), as well as lower frequencies of CD3+TLR2+ (p=0.0034), CD3+TLR4+ (p=0.0021), and CD3+TLR9+ (p=0.00154) lymphocytes T. Lower percentages of TLR+ lymphocytes were associated with higher monoclonal (M) protein levels and decreased concentration of hemoglobin. Moreover, the number of plasmocytes correlated negatively with the number of CD19+TLR9+ (r=-0.654, p=0.002) B cells and CD3+TLR4+ (r=-0.680, p=0.003) T cells.
Summary and Conclusions: This study demonstrates that expression of TLR might represent hallmarks of immunosuppression in MGUS patients, and confirmed the association between negative prognosis and low expression of TLR in MGUS patients. Determination of TLR-positive T and B lymphocytes constitutes valuable diagnostic tool, completing cytometric evaluation of MGUS.
This work was supported by research grants no. DS460 of the Medical University of Lublin and no. UMO-2016/21/B/NZ6/02279 of the Polish National Science Centre.

PF552

DISCOVERY BLOOD PLASMA PROTEOMICS IN ELUCIDATING BIOMARKERS TO DISTINGUISH ONSET OF LETHAL MULTIPLE MYELOMA IN PREMALIGNANT MGUS

L.Y. Moreno[1,*], N.J. Weston-Bell[1], K. Vandyke[2], D. Hewett[2], A. Zannettino[2], S. Garbis[3], S. Sahota[1]

[1]Tumour Immunogenetics Group, Cancer Sciences Academic Unit, University of Southampton, Southampton, United Kingdom, [2]Myeloma Research Laboratory, Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide, Australia, [3]Proteomics Group, Cancer Sciences Academic Unit, University of Southampton, Southampton, United Kingdom

Background: Multiple myeloma (MM) is characterized by accumulation of malignant plasma cells in the bone marrow, and is almost invariably preceded by asymptomatic monoclonal gammopathy of undetermined significance (MGUS). In symptomatic MM, clinical diagnosis is generally based on evidence for hypercalcemia, renal failure, anemia, and bone lesions (CRAB features). Despite global efforts to achieve early detection of malignancy, MM disease is still diagnosed at a late stage with onset of organ failure. Biomarkers that can identify early malignant transformation to MM in MGUS will overcome a major drawback, and allow immediate therapeutic intervention to improve disease outcomes in the absence of morbidity associated with CRAB features. In this, liquid biopsy biomarkers based on blood analysis directly will allow an ease of detection with minimal intervention.
Aims: To address this challenge, we have examined and compared the plasma proteome of 5 IgG MGUS patients, 5 IgG MM patients and 5 Healthy age-matched donors to interrogate potential biomarkers able to distinguish disease states.
Methods: We used multiplex isobaric labelling and three-dimensional liquid chromatography tandem mass spectrometry (TMT-3DLC-MS/MS) based quantitative proteomic analysis of non-depleted plasma. A total of 1095 proteins were commonly detected and fully quantified in all samples using high confidence ($q<0.01$) and 50% of isolation interference in Proteome Discoverer 1.4 software. The differential expression analysis was conducted using Limma.
Results: The expression analysis identified 478 proteins differentially expressed between MM and Healthy, 514 proteins between MGUS and Healthy and 82 proteins between MGUS and MM. 333 dysregulated proteins were commonly identified in MGUS and MM patients compared to Healthy donors. The differentially expressed proteins common to MGUS and MM revealed roles in bone cell and osteoclast development and bone remodelling (ATP6AP1, SPP1, SPP2, CTHRC1, MEPE), immune response and cytokine production (CD97, CFH, CFB, CFD, CCL5, CCL6, CXC16, CD14), cell adhesion (HSPB1, FBN1, FBLN5, SPON2, THBS1, FN1, FGA, POSTN, CD97, DAG1, FSTL3, ICAM1, MSLN), extracellular matrix and structural organization (SPINT1, LTBP2, TIMP1, COL1A2, BMP1, ICAM1, PRSS3, CST3) and cell migration (EFEMP1, CCL18, CCL14, PFN1, CFL1). Moreover, a number of these proteins have been previously identified in diseases with MM-related symptoms, including amyloidosis, kidney failure and secondary malignant neoplasms of the lymph node. Additionally, 31 proteins were found dysregulated *exclusively* in MM compared to MGUS and Healthy, including ECM1, BCHE, HPR, FCN2, FCN3, SLAMF1, C4BPA and C4BPB. These proteins are mainly associated with immune response, vesicle-mediated transport and proteolysis. b2-microglobulin was also identified as upregulated in MM. For MGUS, 26 proteins were identified dysregulated *solely* in the asymptomatic stage, including LGALS3, AMBP, PRDX1 and LCN2. These proteins are related to lymphocyte and

leukocyte aggregation. Importantly, a number of proteins identified as dysregulated in MM and MGUS in this study have been previously reported, including VCAM1, OSTP, extracellular matrix proteins and insulin-like factor binding proteins.

Summary and Conclusions: Our study of the plasma proteome has thus identified a compendium of biomarkers that can now be interrogated further on a more targeted basis to elucidate which specific or combination of plasma proteins demarcate onset of malignant MM. Such biomarkers have enormous potential to progress early detection and management of this lethal disease.

Myeloma and other monoclonal gammopathies – Clinical

PF553

VARIABLES PREDCITVE OF POOR PROGNOSIS IN PATIENTS WITH MAYO STAGE 1 SYSTEMIC LIGHT CHAIN AMYLOIDOSIS

F. Sharpley[1,*], R. Manwani[1], D. Foard[1], S. Mahmood[1], S. Sachchithanantham[1], C. Whelan[1], H. Lachmann[1], J. Gilmore[1], P. Hawkins[1], A. Wechalekar[1]

[1]*National Amyloidosis Centre, UCLH, London, United Kingdom*

Background: Cardiac involvement is the most reliable determinant of prognosis in light chain amyloidosis (AL). Mayo stage 1 patients, defined by normal cardiac biomarkers (N-terminal pro b-type natriuretic peptide (NT-proBNP) <332 ng/L, high-sensitive cardiac troponin T (cTnT) <55 ng/L, and a difference between involved and uninvolved free light chains (dFLC) of <180 mg/L) are still a very variable group with a prognosis ranging from 60-154 months.

Aims: This retrospective analysis of 405 Mayo Stage 1 patients at the National Amyloid Centre, London (UK-NAC) hopes to identify prognostic markers to help further stratify patients.

Methods: The Alchemy database at the UK-NAC was searched for all Mayo Stage 1 systemic AL patients, defined by normal cardiac biomarkers (NT-proBNP <332 ng/L, cTnT 55 ng/L). All Mayo Stage 1 patients assessed at the UK-NAC between 2009-2017 were eligible. Patients with cardiac involvement were excluded. A diagnosis of amyloidosis was confirmed by histology and typing by immunohistochemistry. Baseline assessment of organ function, imaging and biomarker assessments was performed and data collected on treatment regimen. Overall survival (OS) was calculated from date of diagnosis to death or last follow-up and organ involvement was defined according to the international amyloidosis consensus criteria. Survival was calculated by method of Kaplan-Meier and variables predictive of survival by logistic regression analysis. Median values were used to dichotomise continuous variables.

Results: A total of 365 Mayo Stage 1 patients were eligible for analysis. The median patient age was 68 years. The median number of organs involved was 2 (range 1-7); the majority of patients having renal involvement (n=272, 74.5%). Median NT-proBNP at presentation was 152ng/L (range 8-330ng/L), median cTNT 10ng/L (range 3-51ng/L) and median dFLC 61mg/L (range 0.5-3822mg/L). At baseline 36 patients (9.9%) had liver involvement, as defined by ALP 1.5 x upper limit of normal. The median number of treatment lines was one (range 0-5). The most common treatment was bortezomib, (n=236, 64.7%), 48 patients had an autologous stem cell transplant (ASCT) (n=48, 13.2%) and no treatment was given in 25 patients (6.8%). With a median follow up of 34 months (0-105 months), there were 57 deaths. Median OS was not reached. The only variables predictive of early death were troponin (HR 2.340, CI 1.211-4.523, p-0.011) and NT-pro-BNP (HR-2.469, CI 1.399-4.357, p=0.002). Liver involvement using ALP increase above the upper reference limit was significant, (HR-1.1395, 95% CI 1.011-1.284, p=0.032), but not when using the standard definition of liver involvement at ALP 1.5x upper limit. Treatment type had no significant impact on survival, including ASCT (Figure 1).

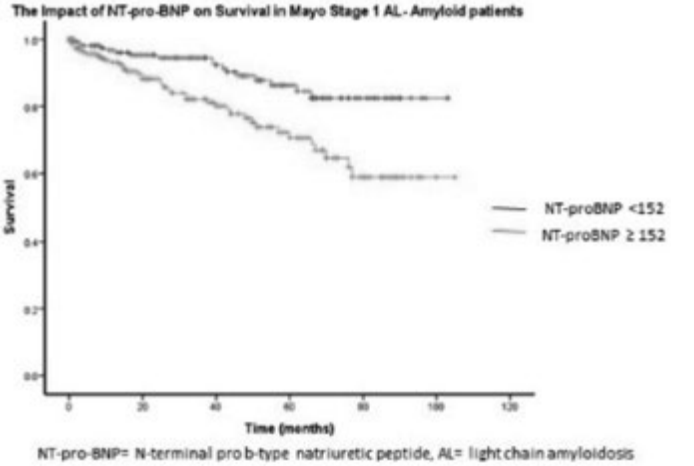

Figure 1.

Summary and Conclusions: In conclusion, this large dataset of Mayo Stage 1 patients adds to data from the Italian/German groups supporting that Mayo Stage 1 amyloid patients are characterised by predominate renal involvement and have a prolonged overall survival. Variables predictive of poor survival in our analysis included: an ALP above the upper limit of normal, NT-pro-BNP and troponin. Cardiac biomarkers appear to offer a prognostic significance even in patients without cardiac involvement by current established criteria. This may reflect early cardiac involvement and potentially offer some insight into the heterogeneity in survival of Mayo Stage 1 patients.

PF554

SINGLE AGENT CARFILZOMIB PROLONGSPFS FOLLOWING TRIPLET THERAPY WITH CYCLOPHOSPHAMIDE & DEXAMETHASONE FOR FIRST RELAPSE/PRIMARY REFRACTORY MULTIPLE MYELOMA: PHASE 2 MUKFIVE STUDY, SECOND ANALYSIS

K. Yong[1,*], S. Hinsley[2], D. Sherratt[2], S. Brown[2], L. Flanagan[2], C. Williams[3], J. Cavenagh[4], M. Kaiser[5], N. Rabin[6], K. Ramasamy[7], M. Garg[8], H. Auner[9], S. Hawkins[10], C. Bygrave[11], R. De Tute[12], G. Morgan[13], F. Davies[13], R. Owen[14]

[1]Haematology, University College London, London, [2]Clinical Trials Research Unit, Leeds University, Leeds, [3]Haematology, Nottingham University Hospitals, Nottingham, [4]Haematology, St. Bartholomew's Hospital, [5]Molecular Pathology, Institute of Cancer Research, [6]Haematology, University College London Hospitals, London, [7]Haematology, University of Oxford, Oxford, [8]Haematology, Leicester Royal Infirmary, Leicester, [9]Haematology, Imperial College London, London, [10]Cancer Centre, Royal Liverpool University Hospital, Liverpool, [11]Haematology, University Hospital Wales, Cardiff, [12]Haematology Malignancy Diagnostic Service, Leeds University Hospital, Leeds, United Kingdom, [13]Myeloma Institute, University of Arkansas, Little Rock, United States, [14]Haematology Malignancy Diagnostic Service, University of Leeds, Leeds, United Kingdom

Background: Carfilzomib (CFZ) is licensed for use in relapsed/refractory (RR) multiple myeloma (MM). In the ENDEAVOR study, carfilzomib was combined with dexamethasone for extended therapy, leading to superior progression-free and overall survival (PFS, OS) when compared with bortezomib and dexamethasone. Today, triplet regimens are standard of care, but administering a triplet regimen indefinitely may be challenging.
Aims: The MUK*five* phase 2 study compared safety and activity of KCD to bortezomib, cyclophosphamide and dexamethasone (VCD), as fixed duration therapy for patients at first relapse, or refractory to one prior line. The study also compared activity and safety of maintenance CFZ *vs* observation after KCD.
Methods: Patients were randomized (R1) in a 2:1 ratio in favor of KCD. Patients in the KCD arm with ≥SD after 6 cycles of KCD were randomized (R2) 1:1 to receive maintenance CFZ or observation. Minimisation factors were response to initial treatment (PR,MR,SD *vs* VGPR,CR) and prior ASCT. There was no maintenance in the VCD arm. See Figure 1 for regimens. Co-primary endpoints were ≥VGPR rate at 24 weeks post R1 (non-inferiority (NI)), and PFS from R2.
Results: 300 patients were randomized, 201 KCD and 99 VCD. Patient and disease features were balanced between arms. First primary endpoint, ≥VGPR at 24 weeks was KCD 40.2%, VCD 31.9% (NI), ORR 84.0% and 68.1% (p=0.0014, superior). 141 patients were eligible for R2, 69 allocated to maintenance CFZ. Arms were balanced for response (≥VGPR:CFZ 58.0%,observation 54.2%,) ECOG, ISS, MRD status (MRD-neg: CFZ 11.6%, observation 13.9%) at end of initial treatment. Median follow-up for patients from R2 was 10.5m (0.9-31.3): 44.3% of patients completed 6 cycles CFZ maintenance, 18% completed 18 cycles. 82.1% of patients had a dose modification, but 88.7% of all cycles were received on time. Median PFS from R2 for CFZ was 11.9m *vs* 5.6m observation (HR 0.59, p=0.009). Safety was as in Table 1. AE's were mild, most were <G3, mainly thrombocytopenia, anaemia, nausea and fatigue. Median follow-up for patients from R1 was 14.0m (0.0-49.4). In PFS analysis of KCD *vs* VCD induction, patients who received maintenance were censored at R2. Median PFS was 11.9 m (80%CI 11.6-12.7) for KCD and 10.2m (9.3-11.3) for VCD; HR=0.89 (80% CI 0.72-1.10).
Summary and Conclusions: Maintenance with single agent CFZ prolongs PFS after triplet therapy with cyclophosphamide and dexamethasone for MM at first relapse or primary refractory to one line. PFS for patients receiving KCD triplet for 6 cycles followed by maintenance CFZ for up to 18 months approximates 18 months, indicating that it is possible to attenuate regimen intensity following initial treatment with triplet regimen while maintaining disease control when using CFZ in the relapse setting.

	KCD: 6 x 28 day cycles	VCD: 8 x 21 day cycles
Carfilzomib	20/36mg/m² IV days 1, 2, 8, 9, 15, 16	
Bortezomib		1.3mg/m² SC days 1, 4, 8, 11
Cyclophosphamide	500mg days 1, 8, 15	500mg days 1, 8, 15
Dexamethasone	40mg weekly	40mg weekly

	Maintenance: 28 day cycles
Carfilzomib	36 mg/m²: Days 1, 2, 15, 16 for 6 months Days 1, 2 for a further 12 months

Figure 1.

Table 1.

Safety	*Maintenance*	*Observation*
Reasons for stopping treatment	Disease progression 65.6%, completed treatment 18.0%, toxicity 4.9%, withdrew consent 6.6%	
Patients in safety population	**67**	**74**
SAEs: n patients | n events	24/67 (35.8%) | 34 (15 treatment related)	6/74 (8.1%) | 6
SAE types: I=Infections, C=Cardiac	I: 18/34 (52.9%); C: 1/34 (2.9%)	I: 1/6 (16.7%); C: 0
SAEs resulting in death	0	1 (16.7%)

PF555

SUBCUTANEOUS DARATUMUMAB IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA: PART 2 UPDATE OF THE OPEN-LABEL, MULTICENTER, DOSE ESCALATION PHASE 1B STUDY (PAVO)

J. San-Miguel[1,*], S. Z. Usmani[2], M.-V. Mateos[3], H. Lokhorst[4], J. Kaufman[5], P. Moreau[6], A. Oriol[7], T. Plesner[8], L. Benboubker[9], K. Liu[10], P. Hellemans[11], T. Masterson[12], P. L. Clemens[12], H. Nahi[13], A. Chari[14]

[1]Clínica Universidad de Navarra-CIMA, IDISNA, CIBERONC, Pamplona, Spain, [2]Department of Hematologic Oncology and Blood Disorders, Levine Cancer Institute/Carolinas HealthCare System, Charlotte, NC, United States, [3]University Hospital of Salamanca/IBSAL, Salamanca, Spain, [4]Department of Hematology, VU University Medical Center, Amsterdam, Netherlands, [5]Hematology and Medical Oncology, Winship Cancer Institute, Emory University, Atlanta, GA, United States, [6]University Hospital of Nantes, Nantes, France, [7]Institut Català d'Oncologia, HGTiP, Barcelona, Spain, [8]Vejle Hospital and University of Southern Denmark, Vejle, Denmark, [9]Service d'Hématologie et Thérapie Cellulaire, Hôpital Bretonneau Centre Hospitalier Régional Universitaire (CHRU), Tours, France, [10]Janssen Research & Development, Raritan, NJ, United States, [11]Janssen Research & Development, Beerse, Belgium, [12]Janssen Research & Development, LLC, Spring House, PA, United States, [13]Karolinska Institute and the Department of Medicine, Division of Hematology, Karolinska University Hospital at Huddinge, Stockholm, Sweden, [14]Tisch Cancer Institute, Mount Sinai School of Medicine, New York, United States

Background: Intravenous (IV) administration of daratumumab (DARA) 16 mg/kg is approved as monotherapy and in combination with standard of care regimens for patients with relapsed or refractory multiple myeloma. The phase 1b PAVO study (NCT02519452) demonstrated that delivery of DARA with recombinant human hyaluronidase enzyme (rHuPH20) by subcutaneous (SC) infusion through a syringe pump (Part 1) or by manual SC injection (Part 2) was well tolerated with an efficacy profile consistent with IV DARA (Chari A, *et al.* ASH 2017; abstract 838).
Aims: We present updated efficacy and safety data from Part 2.
Methods: Eligible patients received ≥2 prior lines of therapy including a proteasome inhibitor and an immunomodulatory drug. In Part 2, patients received a concentrated co-formulation of DARA (DARA SC; 1,800 mg in 15 mL) and rHuPH20 (30,000 U) dose in a single, pre-mixed vial, which was administered in 3 to 5 minutes by manual SC injection. Primary endpoints were C_{trough} of DARA at the end of weekly dosing on Cycle 3 Day 1 and safety. Secondary endpoints included overall response rate, rate of complete response, time to response, and duration of response.
Results: Patients in Part 2 (n=25) had a median age of 68 years and received a median of 3 prior lines of therapy. At a median follow-up of 4.6 months, none discontinued due to treatment-emergent adverse events. Pharmacokinetic analyses indicated that DARA SC had a T_{max} of approximately 72 hours and achieved similar or greater C_{trough} on Cycle 3 Day 1 compared to what has been observed with DARA IV. Most common Grade 3/4 treatment-emergent adverse events (>1 patient) were lymphopenia (16%), thrombocytopenia (8%), and neutropenia (8%). Infusion-related reactions (IRRs) were reported in 3 (12%) patients, all occurring within 6 hours of the first injection. No Grade 4 IRRs or discontinuations due to IRRs occurred. DARA SC injections in the periumbilical area were well tolerated

with reversible erythema observed in 20% of patients. DARA SC achieved an overall response rate of 44%, including 28% of patients achieving a very good partial response or better.
Summary and Conclusions: DARA SC, which enables dosing in 3-5 minutes, was well tolerated with low IRR rates, had an acceptable pharmacokinetic profile, and demonstrated clinical response rates similar to DARA IV. Updated data based on longer follow-up will be presented at the meeting.

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LEVOFLOXACIN PROPHYLAXIS IN NEWLY DIAGNOSED MYELOMA REDUCES FEBRILE EPISODES AND DEATH WITHOUT INCREASING HEALTHCARE ASSOCIATED INFECTIONS: RESULTS FROM THE TEAMM TRIAL

S. Bowcock[1,*], T. Planche[2], G. Iqbal[3], G. Pratt[4], K. Yong[5], J. Wood[3], K. Raynes[3], P. Hawkey [6], J. Dunn[3], M. Drayson[6]

[1]Haematological medicine, King's college hospital, [2]Microbiology, St George's Hospital, London, [3]Warwick Clinical Trials Unit, University of Warwick, Coventry, [4]Haematology, University hospital Birmingham, Birmingham, [5]Haematology, University college London, London, [6]University of Birmingham, Birmingham, United Kingdom

Background: Infection is the biggest single cause of the high early death rate in myeloma. Levofloxacin is effective against the common bacterial infections in myeloma but there is concern about the development of healthcare associated infection (HCAI).
Aims: TEAMM (Tackling EArly Morbidity and Mortality in Myeloma) was a randomised, double-blind, placebo-controlled multi-centre phase III clinical trial assessing the benefits of levofloxacin prophylaxis *versus* the risk of HCAI. Patients were randomised to levofloxacin or placebo for 12 weeks to see if levofloxacin reduced febrile episodes and death. Subjects were regularly screened for carriage of resistant organisms.
Methods: Patients were age>21 years with newly diagnosed myeloma, intention to treat myeloma actively, and were +/- 14 days into anti-myeloma treatment. Patients received 500 mg levofloxacin or placebo tablets once daily for 12 weeks, dose adjusted for renal function. Patients were permitted to continue routine non-bacterial anti-infective prophylaxis, including 3x weekly Co-trimoxazole(CoT) for pneumocystis. Faecal and throat samples were taken 4 weekly to detect carriage of *Clostridium difficile(C diff)*, MRSA and faecal ESBL+ Gram-negative bacteria (ESBLGnB). The primary endpoint was the number of febrile episodes (defined as a temperature of ≥38°C treated with anti-infectives) and or death by any cause in the first 12 weeks using Kaplan-Meier methods with censoring at 12 weeks. Secondary outcomes included site of infection, severe septic episodes and non-febrile infections (treated with anti-infectives). Analysis was on an intention to treat basis.
Results: 977 patients were recruited from 93 UK centres from August 2012-April 2016. Median age 67 years, 63% male, 76% eGFR>50 ml/min, 54% planned autologous stem cell transplantation, 93% ECOG performance status ≤2, 71% with bone disease. The primary endpoint showed a significant benefit for the use of levofloxacin with 95 primary events in 489 (19%) Levofloxacin patients *versus* 134 in 488 (27%) placebo patients: Hazard ratio (HR)=0.66 (95%CI=0.51-0.86, p 0.002). Primary events (febrile episodes, deaths, febrile episodes with death) in Levofloxacin *vs* placebo arms were (87, 4, 4 *vs* 112, 15, 7) respectively. Of the 586 total infections (febrile and non-febrile) there were 257 in 189 Levofloxacin patients *vs* 329 in 214 placebo patients (^{2}=7.55,P-trend=0.006). There was a survival benefit for Levofloxacin *vs* placebo at 12 weeks (^{2}=5.84 p=0.02) but there was no benefit at 52 weeks (p=0.94). *C.diff* carriage at baseline was uncommon (7 of 785 subjects). There was no significant difference between the 2 arms for carriage or infection with *C. diff*, MRSA and ESBLGnB. There was 1 case of invasive *C.diff* (placebo arm) and no *pneumocystis*. Levofloxacin significantly reduced invasive gram-negative infections (6 *vs* 25) but not Gram positive infections (Table 1). CoT (315 patients, determined by centre) significantly reduced the number of febrile episodes and deaths and was additive with the effects of levofloxacin. Cox regression adjusting for baseline factors showed levofloxacin treatment HR 0.66(95%CI 0.51-0.86, p=0.002) and prophylactic CoT HR 0.59(95%CI=0.44-0.81, p=0.0008) to be independent predictors for reduction of febrile episodes and death. Other pre-specified subgroup data will be presented.
Summary and Conclusions: Prophylactic use of 12 weeks levofloxacin in patients with newly diagnosed myeloma significantly reduces febrile episodes and deaths without increasing key HCAI organism carriage or infection. Optimal duration of antibiotic prophylaxis remains to be determined.

Table 1.

Bacteriology of infections			
Species	Levofloxacin	Placebo	Total
Total all organisms isolated	41	68	109
Total gram negative bacteria	6 (19%)	25 (81%)	31
Enterobacteraciae	4	13	17
Pseudomonas	0	5	5
Other	2	7	9
Total gram positive	15 (44%)	19 (56%)	34
Staph aureus	4	5	9
Streptococcus pneumoniae,	0	2	2
Other gram positive	11	12	23
Total other (fungal and viral)	20 (45%)	24 (55%)	44
Site of infections	Lower respiratory tract (49%)		
	Upper respiratory tract (12%)		
	Skin soft tissue (8%)		
	Bloodstream (7%)		
	Urinary tract (7%)		
	Gastrointestinal (3%)		

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PROGNOSTIC SIGNIFICANCE OF MAGNETIC RESONANCE IMAGING BEFORE AND AFTER UPFRONT AUTOLOGOUS TRANSPLANTATION FOR NEWLY DIAGNOSED MULTIPLE MYELOMA – A SUBGROUP ANALYSIS FROM THE GMMG MM5 PHASE III TRIAL

M. Merz[1,*], T. Hielscher[2], A. Seckinger[1], A. Jauch[1], E. K. Mai[1], U. Bertsch[1], M.S. Raab[1], K. Neben[3], H. Salwender[4], I.W. Blau[5], H.-W. Lindemann[6], J. Dürig[7], C. Scheid[8], M. Haenel[9], K. Weisel[10], S. Delorme[2], H.U. Kauczor[1], D. Hose[1], H. Goldschmidt[1], J. Hillengass[11]

[1]University Hospital of Heidelberg, [2]German Cancer Research Center, Heidelberg, [3]Klinikum Mittelbaden, Baden-Baden, [4]Asklepios Klinikum Hamburg-Altona, Hamburg, [5]Charite, Berlin, [6]Krankenhaus Hagen, Hagen, [7]Uniklinikum Essen, Essen, [8]Uniklinikum Köln, Köln, [9]Klinikum Chemnitz, Chemnitz, [10]Uniklinikum Tübingen, Tübingen, Germany, [11]Roswell Park Comprehensive Cancer Center, Buffalo, United States

Background: We analyzed prognostic significance of magnetic resonance imaging (MRI) before and after autologous transplantation for newly diagnosed patients with multiple myeloma enrolled in the prospective, multicenter MM5 phase III trial from the German Speaking Myeloma Multicenter Group (GMMG).
Aims: Identify baseline MRI patterns associated with adverse outcome and imaging minimal radiological disease with MRI after transplantation.
Methods: From July 2010 to October 2012, 502 patients were randomly assigned to a bortezomib-based induction therapy (either PAd or VCD) followed by high dose melphalan and autologous stem cell transplantation (ASCT). After ASCT, patients were treated with a lenalidomide consolidation and maintenance therapy either for 2 years or until complete response (CR). Hundred and sixty-seven patients were recruited at the University Hospital of Heidelberg with 83 patients receiving an MRI before ASCT. A second MRI was performed in 77 patients after ASCT and before lenalidomide consolidation/maintenance therapy (median: 98 days, interquartile range: 20 days). We analyzed prognostic significance of initial bone marrow MRI lesions as well as impact of changes in MRI signal after ASCT. MRI findings were also correlated to baseline characteristics and treatment response.
Results: MRI detected a pathological bone marrow signal either depicted by focal lesions and/or diffuse infiltration in all analyzed patients at baseline. Patients with extramedullary disease (EMD, 25.3% of patients) had a significantly shorter overall survival (OS) than patients without EMD at primary diagnosis (5 year OS 59%, 95% confidence interval (CI) [40%;87%] *vs* 83% [73%;93%], p log-rank: 0.03). Residual focal lesions or diffuse marrow infiltration was detected in 81% (n=62) and 61% (n=47) of patients after ASCT, respectively. A residual diffuse marrow infiltration was associated with shorter progressions-free survival (PFS) in patients without CR (n=55) after ASCT (Median PFS: 26 months 95%CI [24;34] *vs* 54 months [32, not reached], p log-rank: 0.03). In 36% of patients (n=28) residual focal lesions underwent T2 hyperintense signal transformation after ASCT. Patients with T2 hyperintense focal lesions achieved more frequently a (near) complete response (75% *vs* 41%, p=0.005) but had a shorter PFS compared to patients without T2 hyperintense lesions after ASCT (Median PFS: 17 months 95%CI [14;34] *vs* 45 months [29, not reached], p log-rank: 0.014). Patients with T2 hyperintense lesions after ASCT harbored more frequently deletion 13p (62% *vs* 33%, p=0.03) as well as EMD (48% *vs* 7%, p<0.001) and showed more often a medium/high proliferation index as assessed by gene expression profiling (92% *vs* 67%, p=0.03). Even after adjustment for treatment arm, baseline International Staging System stage, tandem ASCT as well as response to treatment, presence of cystic T2 hyperintense

lesions after ASCT remained to have an negative impact on PFS (Hazard Ratio, 95% CI: 2.47 [1.25;4.91], p=0.0097).
Summary and Conclusions: Changes of MRI signal after ASCT are of major prognostic significance in newly diagnosed patients with multiple myeloma treated within the GMMG MM5 trial.

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PHASE 2 STUDY OF VENETOCLAX PLUS CARFILZOMIB AND DEXAMETHASONE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

L.J. Costa[1,*], E. Stadtmauer[2], G. Morgan[3], G. Monohan[4], T. Kovacsovics[5], N. Burwick[6], A. Jakubowiak[7], M. Mobasher[8], K. Freise[9], J.A. Ross[9], J. Pesko[9], W. Munasinghe[9], J. Cordero[9], L. Morris[9], P. Maciag[9], O.F. Bueno[9], S. Kumar[10]

[1]University of Alabama at Birmingham, Birmingham, [2]University of Pennsylvania, Philadelphia, [3]University of Arkansas for Medical Sciences, Little Rock, [4]University of Kentucky, Lexington, [5]Huntsman Cancer Institute, University of Utah, Salt Lake City, [6]VA Puget Sound Health Care System, University of Washington, Seattle, [7]The University of Chicago Medicine, Chicago, [8]Genentech Inc., South San Francisco, [9]AbbVie, Inc., North Chicago, [10]Mayo Clinic, Rochester, United States

Background: The BCL-2 inhibitor venetoclax (VEN) has demonstrated efficacy, as monotherapy and combined with PI bortezomib, in relapsed/refractory (R/R) multiple myeloma (MM).
Aims: We report preliminary data for VEN combined with second generation PI carfilzomib and dexamethasone (VENKd) in R/R MM.
Methods: In this ongoing phase 2, dose escalation study (NCT02899052), pts with R/R MM received VENKd on 28-d cycles: VEN 400 mg/day+K 27 mg/m^2 d1,2,8,9,15,16+ dex 40 mg d1,8,15,22 (Cohort 1), same regimen but with VEN 800 mg/day (Cohort 2), VEN 800 mg/day+K 70 mg/m^2 d1,8,15+dex 40 mg d1,8,15, 22 (Cohort 3/expansion cohort), or VEN 800 mg+K 56 mg/m^2 d1,2,8,9,15,16+dex 40 mg d1,2,8,9,15,16,22,23 (optional Cohort 4; no data available at cutoff). Treatment continued until progressive disease (PD) or unacceptable toxicity.
Results: As of 01Dec2017, 26 pts were enrolled. Median age was 67.5 years (40 – 79), 68% had ISS II/III disease, and 23% had t(11;14). Pts received a median of 1 prior therapy (1 – 3); no pts had prior K exposure, 96% had received prior PI (54% refractory), 62% were IMiD refractory, and 35% double refractory. At data cut off, 23 pts were on therapy for 0.3 – 10 months and 3 pts discontinued the study for PD, physician decision, and death. 85% of pts had an AE, grade 3/4 AEs were neutropenia (15%), hypertension (12%), thrombocytopenia (8%), decreased white blood cells (8%), and nausea (4%). 7 serious AEs occurred, but no dose-limiting toxicities were reported. Maximum tolerated dose was not reached and Cohort 3 is being expanded. VEN pharmacokinetics with Kd were comparable to VEN plus bortezomib and dexamethasone. Of 17 pts evaluated after completing ≥2 cycles, 3 had complete response (CR), 2 very good partial response (VGPR), 3 partial response (PR), 3 stable disease, and 2 PD (awaiting response data for 4 pts). Median time to first response was 1 month. Of 5 evaluable pts with t(11;14) MM, 1 achieved CR, 1 VGPR, 3 PR.
Summary and Conclusions: VENkd is well tolerated with promising preliminary efficacy that supports study in pts with R/R MM. Accrual continues with 34 pts enrolled to date. Updated safety and efficacy results will be available for presentation.

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A NOVEL RISK-STRATIFICATION ALGORITHM FOR RELAPSED MULTIPLE MYELOMA (RMM): ASSESSMENT OF PERFORMANCE AND VALIDATION USING REAL-WORLD PATIENT DATA FROM FRANCE, GERMANY AND THE UNITED KINGDOM

R. Hájek[1,*], M. Delforge[2], M. S. Raab[3], A. Briggs[4], M. Campioni[5], L. DeCosta[6], Z. Szabo[5], S. Kroep[7], W. Bouwmeester[7], S. Gonzalez-Mcquire[5]

[1]Department of Haematooncology, University Hospital Ostrava, Ostrava, Czech Republic, [2]Department of Haematology, University Hospital Leuven, Leuven, Belgium, [3]Department of Internal Medicine, University Hospital Heidelberg, Heidelberg, Germany, [4]Institute of Health and Wellbeing, University of Glasgow, Glasgow, United Kingdom, [5]Amgen (Europe) GmbH, Zug, Switzerland, [6]Amgen Ltd., Uxbridge, United Kingdom, [7]Pharmerit International, Rotterdam, Netherlands

Background: There are no validated tools for risk assessment in RMM. The International Staging System (ISS) and revised ISS (R-ISS) were developed in the setting of newly diagnosed MM. Both tools include parameters that are often not monitored in RMM, and neglect data used define disease severity at first relapse, such as clinical outcomes and safety of first-line (1L) treatment. Therefore, a novel risk-stratification algorithm (RSA) was recently designed to predict risk of death in patients with RMM starting second-line (2L) treatment. The algorithm uses 16 predictors to stratify patients into four risk groups with different survival expectations (group 1: lowest risk–group 4: highest risk), and was the first to combine both frailty assessment and disease aggressiveness into a single score. It was developed using multivariable Cox regression using real-world data on MM from the Czech Registry of Monoclonal Gammopathies (RMG).
Aims: To provide insight into the predictive value of this new RSA and its suitability for use in clinical practice, we validated the RSA and assessed its performance using real-world data from three European countries.
Methods: Patient characteristics and outcomes data from bespoke retrospective chart audits in France, Germany and the United Kingdom (UK) were pooled for this real-world validation dataset. Physicians collected data from all patients with MM who began 2L anti-myeloma treatment in 2013, ensuring sufficient follow-up. The predictive performance of the Cox regression model was assessed using Harrell's concordance index (C-index; the RSA's ability to distinguish patients who died from those who did not); a value in the range 0.7–0.8 indicated accurate discriminative power. The ability of the RSA to discriminate by overall survival (OS) across four risk groups was evaluated using Kaplan–Meier curves and hazard ratios (HRs). The prognostic value of the total, frailty and aggressiveness scores was assessed by fitting three univariate Cox models with OS as dependent and each of the scores as predictor.
Results: Chart data were collected from 998 patients (France, 386; Germany, 344; UK, 268). Half (49.8%) received lenalidomide at 2L, 33.1% received bortezomib, and 3.7% received both agents. The validation dataset had lower mean β2 microglobulin and albumin levels, higher lactate dehydrogenase levels and bone marrow plasma cell count, and less 1L toxicity than the RMG cohort. Nonetheless, assessment of the stratification performance of the novel RSA by means of Kaplan–Meier analysis (Figure 1A) and HR data (Table 1) demonstrated clear discrimination in OS between the four risk groups, with a clear distribution of patients across groups in terms of both frailty and disease aggressiveness (Figure 1B). Mean risk scores were 2.3, 4.6, 9.8 and 27.4 in groups 1–4. The C-index was 0.715 (95% confidence interval: 0.690–0.734). Univariate Cox models showed that the hazards for risk of death associated with each unit increase in the total risk, aggressiveness and frailty scores were 1.018, 1.101 and 1.341, respectively; these results were consistent across the countries.

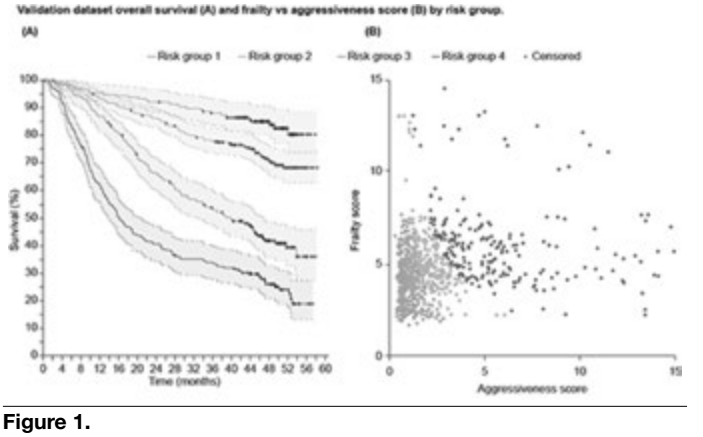

Figure 1.

Table 1.

Overall survival in pooled validation dataset and in Czech dataset.

	Validation dataset			Czech RMG		
Risk group	N (%)	Median OS (95% CI), months	HR (95% CI)	N (%)	Median OS (95% CI), months	HR (95% CI)
Group 1	178 (18%)	NA	Reference	351 (25%)	61.6 (51.7–71.4)	Reference
Group 2	345 (35%)	NA	1.87 (1.23–2.83)	596 (42%)	29.6 (26.6–32.6)	2.24 (1.78–2.83)
Group 3	249 (25%)	39.8 (32.7–46.7)	4.61 (3.09–6.88)	318 (22%)	14.2 (11.3–17.1)	4.30 (3.38–5.49)
Group 4	226 (23%)	16.2 (13.7–21.0)	8.51 (5.73–12.64)	153 (11%)	5.9 (4.4–7.5)	10.88 (8.32–14.23)

2L, second line; CI, confidence interval; HR, hazard ratio; NA, not available; OS, overall survival; RMG, Registry of Monoclonal Gammopathies.

Summary and Conclusions: Validation of the novel RSA in three independent real-world datasets proves it stratifies patients by risk of death. Despite patient-level differences between the development and validation cohorts, the RSA maintained discriminative ability in quantifying risk and stratifying

patients with RMM. This is the first tool that combines frailty and aggressiveness to provide a systematic approach to measuring risk and drivers of risk, which can support treatment decisions in RMM.

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CARFILZOMIB IN COMBINATION WITH BENDAMUSTINE AND DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA: A MULTICENTER PHASE IB/II TRIAL OF THE EUROPEAN MYELOMA NETWORK TRIALIST GROUP (EMNTG)

F. Gay[1,*], M. Offidani[2], A. Günther[3], M. Salvini[1], M. Engelhardt[4], W. Poenisch[5], S. Spada[1], R. Wäsch[4], V. Montefusco[2], F. Patriarca[2], N. Schub[3], S. Gentili[2], E. Angelucci[2], P. Corradini[2], H. Einsele[6], M. Boccadoro[1], P. Sonneveld[7], M. Gramatzki[3]

[1]Myeloma Unit, Division of Hematology, University of Torino, A.O.U. Città della Salute e della Scienza di Torino, Torino, [2]Italian Multiple Myeloma Network, GIMEMA, Italy, [3]Division of Stem Cell Transplantation and Immunotherapy, University of Kiel, Kiel, [4]Department for Hematology and Oncology, University of Freiburg, Freiburg, [5]Department of Hematology, University of Leipzig, Leipzig, [6]Department of Medicine 2, University Hospital of Würzburg, Würzburg, Germany, [7]Department of Hematology, Erasmus MC Cancer Institute, Rotterdam, Netherlands

Background: The prognosis of refractory or relapsed multiple myeloma (RRMM) patients remains poor. The proteasome inhibitor (PI) carfilzomib (K) in combination with dexamethasone (d) showed high activity and an acceptable toxicity profile in heavily pre-treated multiple myeloma (MM) patients. Bendamustine (B) is an active and safe alkylating agent for patients with MM. There is evidence that PIs act synergistically with alkylating agents.
Aims: To evaluate the safety and efficacy of KBd combination, followed by Kd maintenance in RRMM patients.
Methods: Patients ≥18 years of age, with RRMM after failure of ≥2 treatment regimens were included. Phase IB was designed to determine the maximum tolerated dose (MTD) with a 3+3 dose escalation scheme. Treatment consisted of 28-day cycles of Bendamustine 70 mg/m^2 on day 1 and 8, Carfilzomib (escalating dose) on day 1,2,8,9,15,16 and dexamethasone 20 mg/m^2 on day 1,2,8,9,15,16,22,23. After receiving 8 cycles, responding patients continued with maintenance with Kd only on two consecutive days every 14 days until progression. Based on the safety/efficacy profile of the phase I portion of the trial, recommended phase II dose was 27 mg/m2 (Gramatzki M, *et al.* ASH 2016). Primary endpoint of the Phase II portion of the study was the rate of at least very good partial response (VGPR). For the present interim analysis, data cut-off was December 11, 2017.
Results: A total of 63 pts were evaluated (phase I: 13 patients; phase II: 50 patients). Median age (IQR) was 66 years (61-70), median number of prior lines was 4 (range 2-9), 87% of patients were previously exposed to bortezomib and 86% to immunomodulatory drugs; 75% had previously received autologous stem-cell transplantation; 48% of 46 patients evaluable for FISH had high-risk cytogenetic abnormalities (del17 and/or t(4;14) and/or t(14;16)). At data cut-off, 11 patients were still on treatment. The main reason for treatment discontinuation was progressive disease (27 patients). 51% of patients achieved at least a partial response and 32% at least VGPR, including 18% of patients achieving at least a near complete response; 41% achieved a stable disease, with a clinical benefit rate of 92%. After median follow-up of 12.8 months, median progression-free survival (PFS) was 11.6 months, median overall survival (OS) was 24 months. One-year PFS was 35% *vs* 72% for patients with high *vs* standard-risk FISH (HR 2.3; P=0.04). The main grade (G) ≥3 adverse events (AEs) were hematologic: lymphopenia (29%), neutropenia (25%) and thrombocytopenia (22%). The more frequent non-hematologic G≥3 AEs were: pneumonia (13%) and thromboembolic events (10%). Heart failure and acute coronary syndrome were reported in 3% of patients each, hypertension in 2%.
Summary and Conclusions: KBd followed by Kd maintenance was effective in RRMM. Cardiac and vascular AEs need attention and infection prophylaxis is mandatory in this immunosuppressed heavily pretreated population. *The trial is registered at Clinicaltrials.gov: NCT02056756*

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CARFILZOMIB AND DEXAMETHASONE (KD56) *VS* BORTEZOMIB AND DEXAMETHASONE (VD) IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA (RRMM): UPDATED OVERALL SURVIVAL (OS), SAFETY, AND SUBGROUP ANALYSIS OF ENDEAVOR

R.Z. Orlowski[1,*], P. Moreau[2], H. Ludwig[3], A. Oriol Rocafiguera[4], W.J. Chng[5], H. Goldschmidt[6], Z. Yang[7], A.S. Kimball[7], M. Dimopoulos[8]

[1]The University of Texas MD Anderson Cancer Center, Houston, United States, [2]University Hospital Hotel-Dieu, Nantes, France, [3]Wilhelminen Cancer Research Institute, Wilhelminenspital, Vienna, Austria, [4]Institut Catala d'Oncologia, Hospital Germans Trias i Pujol, Barcelona, Spain, [5]National University Cancer Institute, Singapore, Singapore, [6]Heidlberg University, Heidlberg, Germany, [7]Amgen Inc., Thousand Oaks, United States, [8]University Athens School of Medicine, Athens, Greece

Background: The phase 3 ENDEAVOR trial demonstrated significantly improved PFS and OS with Kd56 *vs* Vd in RRMM patients (pts; Dimopoulos *Lancet Oncol* 2016 and 2017).
Aims: We report updated data after additional follow-up.
Methods: Hazard ratios (HRs) and 95% CIs were estimated using stratified or unstratified Cox proportional hazards models for primary and subgroup OS analyses, respectively.
Results: 929 pts were randomized (Kd56, n=464; Vd, n=465). As of 19-Jul-17, median OS was 47.8 (Kd56) *vs* 38.8 (Vd) months (mos; HR, 0.76 [95% CI, 0.633–0.915]; median follow-up, 44.3 *vs* 43.7 mos). OS was longer with Kd56 *vs* Vd within age subgroups (<65 years [yrs]: median, 47.8 *vs* 42.2 mos; HR, 0.79 [95% CI, 0.598–1.031]; 65–74 yrs: median, 49.0 *vs* 36.2 mos; HR, 0.71 [95% CI, 0.520–0.958]; ≥75 yrs: median, 36.1 *vs* 23.9 mos; HR, 0.78 [95% CI, 0.506–1.199]). OS was longer with Kd56 *vs* Vd by prior lines of therapy (1 line: median, 51.3 *vs* 43.7 mos; HR, 0.77 [95% CI, 0.583–1.018]; 2–3 lines: median, 39.5 *vs* 28.4 mos; HR, 0.75 [95% CI, 0.589–0.959]) and prior bortezomib (btz) exposure (prior btz: median, 41.8 *vs* 32.7 mos; HR, 0.85 [95% CI, 0.669–1.082; no prior btz: median, not estimable [NE] *vs* 42.2 mos; HR, 0.66 [95% CI, 0.496–0.875]). OS was longer with Kd56 *vs* Vd for high-risk (median, 28.0 *vs* 22.7 mos; HR, 0.81 [95% CI, 0.580–1.136]) and standard-risk (median, NE *vs* 43.5 mos; HR, 0.79 [95% CI, 0.618–1.009]) cytogenetics pts. 457 (98.7%, Kd56) and 451 (98.9%, Vd) pts had an adverse event (AE); 379 (81.9%, Kd56) and 324 (71.1%, Vd) had a grade ≥3 AE. Exposure-adjusted pt incidences per 100 pt-yrs (95% CI) of AEs were 1352.07 (1233.62–1481.89) for Kd56 and 1754.86 (1600.15–1924.53) for Vd; for grade ≥3 AEs, these values were 162.31 (146.77–179.50) and 175.90 (157.75–196.13). The most common AEs in the Kd arm (≥30% of subjects) were anemia (43.6%), diarrhea (36.7%), pyrexia (32.6%), hypertension (32.4%), fatigue (32.2%) and dyspnea (32.2%).
Summary and Conclusions: With median follow-up of ~44 mos, clinically meaningful OS improvements were observed with Kd56 *vs* Vd, including in all subgroups examined. The Kd56 safety profile was consistent with previous analyses.

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MAINTENANCE AFTER LENALIDOMIDE, BORTEZOMIB, AND DEXAMETHASONE INDUCTION AND TRANSPLANT IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA AND HIGH-RISK CYTOGENETICS: AN ENHANCED MEDICAL RECORD ANALYSIS

R. Fonseca[1,*], K. Parikh[2], B. Ung[2], Q. Ni[2], A. Agarwal[2]

[1]Mayo Clinic, Phoenix, [2]Celgene Corporation, Summit, United States

Background: In the United States and Europe, lenalidomide (LEN or R) was the first drug approved for post–stem cell transplant (SCT) maintenance therapy (MT) in multiple myeloma (MM). Induction with lenalidomide, bortezomib, and dexamethasone (RVD) is common in newly diagnosed MM (NDMM), but real-world evidence of MT with LEN (R-MT) after RVD and SCT is limited, particularly in patients with high-risk cytogenetic abnormalities. Here, we report results of a retrospective observational analysis comparing R-MT *vs* no MT (No-MT) in this setting.
Aims: To compare post-SCT R-MT *vs* No-MT in patients with NDMM treated with RVD induction in a real-word setting.
Methods: This analysis used electronic health records from the Flatiron Health database. Eligible patients diagnosed with MM between Jan 2011 and Dec 2017 and who had ≥2 documented clinical visits were included. NDMM was defined as no anti-myeloma treatment for >14 days before diagnosis. The Index date was defined as the earlier of either the date of SCT+90 days or the start of R-MT. Patients were identified as high risk by fluorescence *in situ* hybridization at diagnosis using international standards (Table 1). Time to next treatment (TTNT), defined as the duration from the index date to the start of a new line of treatment, was analyzed by the Kaplan-Meier method and Cox proportional hazards model.
Results: Among 340 patients who received RVD induction and SCT, 85 had high-risk cytogenetic abnormalities. Of those patients, 41 (48%) received R-MT and 44 (52%) did not (No-MT). Patients in the R-MT group were older than those in the No-MT group (mean age, 62 *vs* 58 yrs; P=.0285), and a higher proportion had an ECOG performance status of 0 (80% *vs*

41%, respectively; P=.0399). More patients receiving R-MT had chromosome 1 abnormalities than did those receiving No-MT, but fewer had del17p13. Median follow-up was 21.9 *vs* 9.7 months. A smaller proportion of patients in the R-MT group advanced to second-line treatment than in the No-MT group (24% *vs* 59%, respectively); these patients had a significantly longer median TTNT than those receiving No-MT (38.8 *vs* 2.8 months, respectively; hazard ratio=3.5 [95% CI, 2.25-5.58]; P <.001).

Table 1.

Genetic Abnormality, %	R-MT (n = 41)	No-MT (n = 44)
Chromosome 1 abnormality	83	64
Del17p13	20	39
t(14;16)	12	9
t(4;14)	10	23
t(14;20)	0	2

No-MT, no maintenance therapy; R-MT, lenalidomide maintenance therapy.

Summary and Conclusions: This analysis demonstrated that patients with NDMM and high-risk cytogenetic abnormalities who received R-MT were less likely to progress to second-line treatment and had a significantly longer TTNT than were patients who received No-MT. These real-world outcomes align with reported clinical trial outcomes, which showed improved progression-free survival with R-MT *vs* placebo or observation in high-risk patients. Although patients with high-risk cytogenetic abnormalities may benefit from the addition of a proteasome inhibitor (PI) to MT, this study showed the benefit of LEN monotherapy. Future studies are needed to determine the impact of adding a PI to MT on tolerability, quality of life, and healthcare costs.

PF563

EUROPEAN POST-APPROVAL SAFETY STUDY IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA: SAFETY IN PATIENTS TREATED WITH POMALIDOMIDE IN A REAL-WORLD SETTING

M. Schmidt-Hieber[1,*], N. Abildgaard[2], B. Gamberi[3], F. Di Raimondo[4], A. Ramirez Payer[5], C. Kyriakou[6], H. Froen[7], A. Izarra[8], E. Kueenburg[9], A. Di Micco[9], B. Rosettani[9], P. Bacon[9], M. Atiba-Davies[9], T. Plesner[10]

[1]Helios Clinic Berlin-Buch, Berlin, Germany, [2]BD Odense University Hospital, Odense, Denmark, [3]Arcispedale Santa Maria Nuova, Reggio Emilia, [4]Department of Hematology, University of Catania, Catania, Italy, [5]H.U. Central de Asturias, Calle Carretera de Rubín, Oviedo, Spain, [6]University College London and Northwick Park Hospitals, London, United Kingdom, [7]Vestre Viken, Drammen, Norway, [8]Norrlands University Hospital, Umea, Sweden, [9]Celgene International, Boudry, Switzerland, [10]Department of Hematology, University of Southern Denmark, Center Little Belt, Vejle Hospital, Vejle, Denmark

Background: The combination of pomalidomide (POM) and dexamethasone (DEX) for the treatment (Tx) of relapsed or refractory multiple myeloma (RRMM) in patients (pts) who have received ≥2 prior Tx regimens, including lenalidomide and bortezomib, was approved in Europe in August 2013. POM+DEX is now a standard Tx for pts with RRMM. These pts are at an increased risk for adverse events (AEs) due to prior exposure to multiple lines of Tx and a high disease burden. The European post-approval safety study (EU PASS; NCT02164955) is an observational, non-interventional registry designed to characterize the safety profile of POM-based Tx in pts with RRMM in a real-world setting.
Aims: To report the incidence of important AEs with POM-based Tx, such as neutropenia, thrombocytopenia, venous thromboembolism (VTE), peripheral neuropathy (PN), and second primary malignancies (SPMs), in pts with RRMM treated with POM according to current clinical practice in a post-marketing setting.
Methods: Pts with symptomatic RRMM initiating POM-based Tx were enrolled and treated at the investigator's discretion. Thromboprophylaxis was administered per local standard practice. AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (v4.0). The study is ongoing and open for recruitment in centers across Europe. Participating pts provided informed consent.
Results: As of January 30, 2018, 530 pts across 100 institutions in 8 European countries were included in the safety population. At the time of this abstract, Tx was ongoing in 260 pts (49.1%). Median age was 70 (range, 37-92) yrs, with 29.4% of pts aged <65 yrs, 42.1% between 65 and 75 yrs of age, and 28.5% aged >75 yrs; 54.5% were male. Median time from diagnosis was 4.7 (range, 0.4-26.0) yrs. Median number of prior Txs was 3 (range, 0-13); 72.8% of pts had ≥3 prior lines. Most pts received prior lenalidomide (98.7%), and 98.1% received prior bortezomib. In 303 pts assessed for Eastern Cooperative Oncology Group performance status, 242 (79.9%) had a good performance status (0 or 1). In this analysis, median Tx duration was 17.7 (range, 0.0-134.6) wks and 3.6% of pts (n=19) discontinued Tx due to AEs. AEs (all grades) occurred in 499 pts (94.2%). PN (all grades) was observed in 21 pts (4.0%), VTE in 8 pts (1.5%), and pulmonary embolism in 3 pts (0.6%). Overall, 346 pts (65.3%) had grade 3/4 AEs, including neutropenia (n=110 [20.8%]), anemia (n=43 [8.1%]), thrombocytopenia (n=40 [7.5%]), and febrile neutropenia (n=18 [3.4%]). Grade 3/4 infections occurred in 127 pts (24.0%); of those, 40.9% were pneumonia. Grade 3/4 acute myocardial infarction occurred in 1 pt (0.2%). Incidence of AEs in pts aged >75 yrs was similar to that in the overall population. The following 20 SPMs were observed in 16 pts (3.0%): 11 nonmelanoma skin cancers (including 7 basal cell carcinomas, 3 squamous cell carcinomas, and 1 Bowen disease), 8 solid tumors (including 3 colorectal cancers, 2 carcinomas of unknown primary [liver and peritoneal metastases], 1 breast cancer, 1 soft tissue carcinoma, and 1 transitional cell carcinoma), and 1 hematologic SPM (myelodysplastic syndrome).
Summary and Conclusions: This ongoing, prospective, non-interventional study in pts with RRMM continues to demonstrate that POM-based Tx is generally well tolerated in the real-world setting, and its overall safety profile is similar to that seen in pivotal trials (Moreau P, *et al.* Eur J Haematol. 2017). Updated data will be presented at the meeting.

PF564

IMPACT OF PRIOR MALIGNANCIES ON THE DEVELOPMENT OF SUBSEQUENT MALIGNANCIES AND SURVIVAL IN MULTIPLE MYELOMA: A NATIONWIDE POPULATION-BASED STUDY IN THE NETHERLANDS

M. Brink[1,*], M.C. Minnema[2], O. Visser[3], A. Broijl[4], N.W. van de Donk[5], P. Sonneveld[4], S. Zweegman[5], A.G. Dinmohamed[1,4,6]

[1]Research, Netherlands Comprehensive Cancer Organisation, [2]Hematology, Cancer Center UMC Utrecht, [3]Registration, Netherlands Comprehensive Cancer Organisation, Utrecht, [4]Hematology, Erasmus MC Cancer Institute, Rotterdam, [5]Hematology, VU University Medical Center, Amsterdam, [6]Public Health, Erasmus University Medical Center, Rotterdam, Netherlands

Background: Following advances in treatment and supportive care, multiple myeloma (MM) survivors are an increasing group of individuals, whom, in turn, are at increased risk of developing subsequent malignancies. Prior malignancies might influence the risk on development of subsequent malignancies and survival in MM patients. Information is limited, only recently a Swedish population-based study (Jonsdottir, *Blood Adv*, 2017) reported on this topic.
Aims: In this nationwide population-based study, the impact of prior malignancies for developing a subsequent malignancy or survival in MM patients in the Netherlands was evaluated.
Methods: We identified all newly diagnosed MM patients between 1994-2012 from the nationwide population-based Netherlands Cancer Registry (NCR). Prior and subsequent malignancies (excluding basal cell carcinoma) relative to MM were identified from the NCR and classified into subgroups according to the third edition of the International Classification of Diseases for Oncology (Figure 1A). Synchronous malignancies diagnosed within a time-interval of 6 months prior to or after MM diagnosis, and malignancies diagnosed from autopsies were excluded. Cox regression models were constructed to calculate hazard ratios (HRs) and associated 95% confidence intervals (CIs). The first and second model assessed the risk of the development of a subsequent malignancy and the risk of death in MM patients with either a prior malignancy diagnosis or not, respectively. We adjusted for sex, age at and year of MM diagnosis. For the first model, all MM patients were followed from the time of MM diagnosis to subsequent malignancy development, death, or end of follow-up (February 1, 2017), whichever came first. For the second model, survival time was defined as the time from MM diagnosis to death or end of follow-up (February 1, 2017), whichever came first. A $P<0.05$ indicated statistical significance.
Results: Our analytic cohort included 17,196 MM patients (median age 70 years; range 22-99; 55% males), of whom 1,619 (9.4%) and 247 (1.4%) had 1 and ≥2 prior malignancies, respectively. Patients with a prior malignancy were younger at MM diagnosis, as compared to those without (median age 67 *vs* 70; $P<0.001$). A total of 196 (10.5%) of 1,866 MM patients with a prior malignancy developed a subsequent malignancy, as compared with 1,393 (9.1%) of 15,330 MM patients without ($P=0.04$). The types of prior and subsequent malignancies are presented in Figure 1A. MM patients with a prior malignancy diagnosis had a 47% increased risk of developing a subsequent malignancy, as compared with MM patients without (HR,

1.47; 95% CI, 1.40-1.55; *P*<0.001). Overall survival of MM patients according to the number of prior malignancies is shown in Fig 1B. MM patients with 1 and ≥2 prior malignancies had, respectively, a 42% (HR, 1.42; 95% CI, 1.34-1.50; *P*<0.001) and 68% (HR, 1.68; 95% CI, 1.47-1.92; *P*<0.001) increased risk of death, as compared with MM patients without.

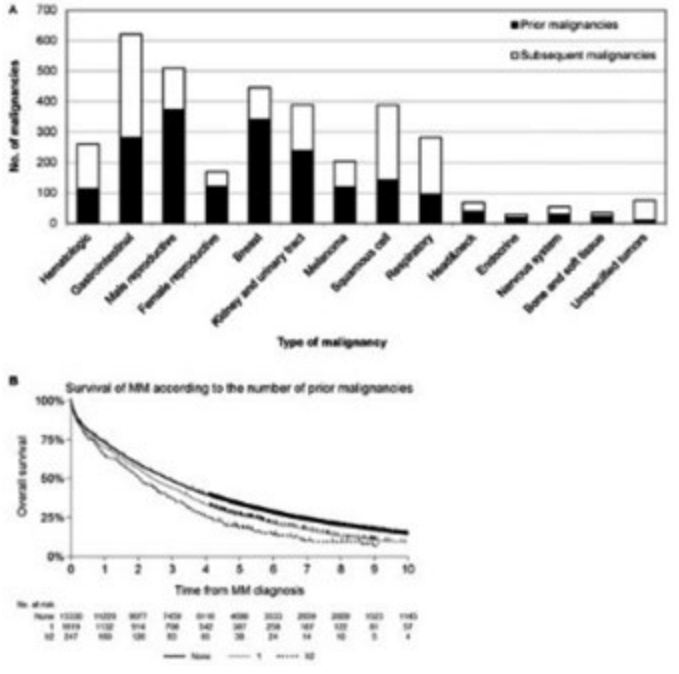


Figure 1.

Summary and Conclusions: We demonstrated that a prior malignancy diagnosis among MM patients increased the risk of developing a subsequent malignancy and negatively influences survival and this was in line with the Swedish study. An explanation for these findings could suggest a role for genetic susceptibility to cancer; however, this usually is associated with young age and observed in ~5% of all cancers only. Another explanation could be the effects of prior cytotoxic treatment. As MM survivorship is expected to increase, these findings may be important to augment cancer surveillance for early detection and appropriate management of subsequent malignancies.

PF565

A PHASE IB STUDY OF ATEZOLIZUMAB (ATEZO) ALONE OR IN COMBINATION WITH LENALIDOMIDE (LEN) IN PATIENTS (PTS) WITH MULTIPLE MYELOMA (MM)

H. Cho[1,*], C. Cole[2], T. Martin[3], J. Zonder[4], J. Fay[5], J. Byon[6], A. Teterina[7], C. Green[6], J. Adamkewicz[6], J.M. Venstrom[6], M. Dhodapkar[6]

[1]Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, [2]Division of Hematology/Oncology, University of Michigan School of Medicine, Ann Arbor, [3]Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, San Francisco, [4]Barbara Ann Karmanos Cancer Institute, Wayne State University, Detroit, [5]Baylor Research Institute, Dallas, [6]Genentech, Inc., South San Francisco, United States, [7]Hoffmann-La Roche Ltd., Mississauga, Canada

Background: Despite treatment (tx) advances, most MM pts relapse, and relapsed/refractory (R/R) disease remains a challenge. Immunomodulatory agent len is approved for MM, and its immune-activating effects make it a candidate for combination with immune checkpoint (PD-L1/PD-1) inhibitors. PD-L1 supports tumor survival via immunosuppression and is expressed on MM and accessory cells in the tumor microenvironment. To date, minimal activity has been seen with anti–PD-1 monotherapy.
Aims: To evaluate the safety and activity of atezo (anti–PD-L1) alone and with len as part of an open-label Ph Ib study of MM pts (NCT02431208).
Methods: R/R MM pts with ≤3 prior therapies were enrolled in Cohorts A and B; Cohort C enrolled pts with measurable disease after ASCT. Pts received atezo 1200 mg IV Q3W (single agent, Cohort A). Cohort B and C pts also received len PO 2 wk on/1 wk off at 10, 15 or 25 mg starting C1D1 (Cohort B) or at 10 mg starting C4D1 (Cohort C). Primary endpoints were safety, ORR and the RP2D of len with atezo. Secondary endpoints included PFS and other efficacy measures. T-cell subsets were measured pre- and post-tx by flow cytometry; IFNγ, IL-2, -6 and -10 were measured in blood and bone marrow aspirate plasma by singleplex immunoassays.
Results: Data cutoff was Nov 6, 2017; 24 pts (Cohort A, n=6; Cohort B, n=9; Cohort C, n=9) were evaluable for safety and activity. Due to a partial clinical hold per the FDA, no Cohort C pt received atezo+len. The majority of pts were alive at data cutoff (median follow-up: 22 mo [Cohort A], 16 mo [Cohort B], 17 mo [Cohort C]). 4 (67%) pts in Cohort A, 3 (33%) in Cohort B and 1 (11%) in Cohort C were >65 y old. High-risk cytogenetic features were present in 4 (67%) pts in Cohort A and 1 (11%) pt each in Cohorts B and C; median (range) prior lines of therapy in Cohorts A, B and C were 2 (1-3), 3 (1-3) and 1 (1-2), respectively. All pts had ≥1 AE; 2 (33%) pts in Cohort A, 5 (56%) in Cohort B and 2 (22%) in Cohort C had Grade 3-4 AEs. Grade 3-4 SAEs occurred in 1 pt per cohort. No Grade 5 AEs were reported. 4 (44%) Cohort B pts and 1 (11%) Cohort C pt had AEs leading to atezo interruption; 4 (44%) Cohort B pts had len dose modification/interruption. No withdrawals from atezo or len due to AEs occurred. ORR was 11% in Cohort B (1 VGPR, atezo+len 15 mg). No Cohort A or C pt achieved ≥PR; 1 Cohort C pt had an MR. Median PFS (95% CI) was 4.9 mo (2.1, 4.9), 3.5 mo (2.9, 16.4) and 6.7 mo (2.8, NE) in Cohorts A, B and C, respectively. In Cohort A, an increase in activated CD8+ T cells (HLA-DR+Ki-67+), immune-activating (IFNγ/IL-2) and -suppressing (IL-6/IL-10) cytokines and PD-L1 MFI on CD8+ T cells was detected in blood by C1D15. Post-ASCT pts (Cohort C) had higher pre-tx IL-2 and an early rise in IL-10 (C2D1) in blood after atezo monotherapy *vs* R/R pts (Cohorts A, B). Addition of len (Cohort B) was associated with increased Tregs (CD4+CD25+CD127lo) in blood (C2D1) and bone marrow (C4D1) and a greater rise in circulating IL-6.
Summary and Conclusions: Atezo alone and with len in MM pts was safe and tolerable. Despite small pt numbers and limited time on study tx, no new safety signals were observed. No single-agent activity was seen with atezo in R/R pts, but results may differ post ASCT. Atezo+len had limited clinical activity regardless of len dose. Biomarker data suggest that atezo promotes T-cell activation as early as C1D14 in blood, but this is insufficient for optimal anti-tumor activity. Addition of len did not enhance immune stimulation or decrease tumor-promoting cytokines. Optimal combination partners for atezo in MM remain to be identified.

PF566

TIME TO PLATEAU AS A PREDICTOR OF SURVIVAL IN NEWLY DIAGNOSED MULTIPLE MYELOMA

P. Mellors[1,*], M. Binder[1], F. Buadi[1], M. Lacy[1], M. Gertz[1], A. Dispenzieri[1], S. Hayman[1], P. Kapoor[1], W. Gonsalves[1], Y. Hwa[1], A. Fonder[1], M. Hobbs[1], T. Kourelis[1], R. Warsame[1], S. Zeldenrust[1], J. Lust[1], N. Leung[1], R. Go[1], R. Kyle[1], V. Rajkumar[1], S. Kumar[1]

[1]Mayo Clinic, Rochester, United States

Background: Response rates in newly diagnosed multiple myeloma have improved dramatically with the introduction of highly effective novel therapies. However, survival in patients achieving optimal responses to initial treatment can vary significantly and new prognostic markers are required to improve risk stratification. Few studies have examined the relationship between the time required to achieve a best response plateau and survival outcomes in the era of novel agents.
Aims: The aim of this study was to characterize the relationship between time to best response plateau (T_{Plat}) and survival in newly diagnosed multiple myeloma treated with novel agents.
Methods: We investigated the relationship between T_{Plat} and survival in 1099 newly diagnosed patients treated with novel agents at our institution from December 2005 to December 2015. T_{Plat} was defined as time from initiation of first-line therapy to best response to first-line therapy. In order to avoid an immortal time bias, overall and progression free survival estimates were calculated from the time of best response to first-line therapy (landmark analysis). The log-rank test was used to compare survival outcomes in subgroups. Multivariable-adjusted proportional hazards regression was used to adjust for known prognostic factors including age, sex, best response to first-line therapy, first-line therapy (immunomodulator, proteasome inhibitor, upfront autologous hematopoietic stem cell transplantation), baseline paraprotein concentration, International Staging System stage, and the presence of cytogenetic high-risk abnormalities.
Results: Six hundred and forty of the 1099 patients (58%) were men. The

median age at diagnosis was 63 years (23 - 89). The five most common fist-line therapies were lenalidomide and dexamethasone (Rd; 37%), bortezomib, cyclophosphamide, and dexamethasone (VCD; 24%), bortezomib, lenalidomide and dexamethasone (VRd; 16%), bortezomib and dexamethasone (Vd; 7%), and ixazomib, cyclophosphamide and dexamethasone (ICd; 4%). The median T_{Plat} was 4.9 months (0.7 - 58.6) and plateau duration was 1.8 years (0.2 - 11.0). Patients who required >120 days to achieve a plateau had improved overall and progression free survival (p <0.001 for both comparisons). This effect was observed both in the patients who underwent autologous hematopoietic stem cell transplantation upfront and those who did not (p <0.001 for both comparisons). The estimates from the multivariable-adjusted regression models are shown in Table 1.

Table 1.

Effect estimates from multivariable-adjusted Cox regression models for the effect of time to plateau on overall and progression free survival.

Model	n	Parameter	HR (95% CI)	p-value
Overall survival landmark (time from best response to event)				
Unadjusted	1099	T_{Plat} > 120 days (vs. ≤ 120)	0.53 (0.43-0.66)	<0.001
Multivariable-adjusted 1	1099	T_{Plat} > 120 days (vs. ≤ 120)	0.59 (0.47-0.75)	<0.001
Multivariable-adjusted 2	1099	T_{Plat} > 120 days (vs. ≤ 120)	0.63 (0.49-0.79)	<0.001
Multivariable-adjusted 3	910	T_{Plat} > 120 days (vs. ≤ 120)	0.58 (0.45-0.76)	<0.001
Multivariable-adjusted 4	634	T_{Plat} > 120 days (vs. ≤ 120)	0.66 (0.48-0.89)	0.007
Progression free survival landmark (time from best response to event)				
Unadjusted	1099	T_{Plat} > 120 days (vs. ≤ 120)	0.62 (0.54-0.72)	<0.001
Multivariable-adjusted 1	1099	T_{Plat} > 120 days (vs. ≤ 120)	0.71 (0.61-0.83)	<0.001
Multivariable-adjusted 2	1099	T_{Plat} > 120 days (vs. ≤ 120)	0.73 (0.62-0.86)	<0.001
Multivariable-adjusted 3	910	T_{Plat} > 120 days (vs. ≤ 120)	0.73 (0.61-0.87)	<0.001
Multivariable-adjusted 4	634	T_{Plat} > 120 days (vs. ≤ 120)	0.76 (0.62-0.94)	0.011

The multivariable-adjusted models included the following baseline characteristics: Model 1 was adjusted for age, sex, and best response during first-line therapy. Model 2 was additionally adjusted for the type of first-line therapy (immunomodulator, proteasome inhibitor, upfront autologous hematopoietic stem cell transplantation). Model 3 was additionally adjusted for the paraprotein concentration (M spike). Model 4 was additionally adjusted for the International Staging System (ISS) stage and the presence of cytogenetic high-risk abnormalities.

Summary and Conclusions: Patients who responded more slowly to first-line therapy (T_{Plat} >120 days) experienced improved survival compared to those responding more rapidly. Patients with a prolonged T_{Plat} could represent an "ongoing responder" phenotype that portends a survival advantage independent of treatment modalities, depth of response, and established biomarkers. It is important to account for these biological differences as we design response adapted therapy approaches in multiple myeloma.

PF567

SAFETY AND EFFICACY OF POMALIDOMIDE+LOW-DOSE DEXAMETHASONE IMMEDIATELY FOLLOWING LENALIDOMIDE-BASED TREATMENT FAILURE IN PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA

D.S. Siegel[1,*], G.J. Schiller[2], K. Song[3], R. Agajanian[4], K. Stockerl-Goldstein[5], H. Kaya[6], M. Sebag[7], C. Samaras[8], E. Malek[9], G. Talamo[10], C.S. Seet[11], J. Mouro[12], F. Zafar[12], W. Chung[12], S. Srinivasan[12], M. Qian[12], A. Agarwal[12], A. Thakurta[12], N. J. Bahlis[13]

[1]John Theurer Cancer Center, Hackensack University Medical Center, Hackensack, [2]David Geffen School of Medicine at UCLA, Los Angeles, United States, [3]Vancouver General Hospital, Vancouver, Canada, [4]The Oncology Institute of Hope and Innovation, Downey, [5]Washington University School of Medicine, St. Louis, [6]Cancer Care Northwest, Spokane, United States, [7]McGill University Health Centre, Montreal, Canada, [8]Cleveland Clinic, [9]University Hospitals Case Medical Center, Cleveland, [10]Penn State Hershey Cancer Institute, Hershey, [11]UCLA Medical Center, Los Angeles, [12]Celgene Corporation, Summit, United States, [13]University of Calgary, Calgary, Canada

Background: Pomalidomide (POM)+low-dose dexamethasone (LoDEX) is a standard of care for the treatment (Tx) of patients (pts) with relapsed and/or refractory multiple myeloma (RRMM). This regimen is being investigated in the MM-014 (NCT01946477) trial as a third-line Tx in pts with RRMM in whom last prior therapy with lenalidomide (LEN) failed (cohort A). As LEN becomes increasingly established in earlier lines of Tx, it is pertinent to demonstrate efficacy in pts who have exhausted the benefit of LEN as their last Tx.
Aims: To present safety and efficacy results of POM+LoDEX as a third-line Tx immediately after LEN in cohort A of the MM-014 trial, including a subset analysis of pts who were previously treated with proteasome inhibitors (PI) and/or bortezomib (BORT).
Methods: Eligibility criteria included age ≥18 y, documented MM, 2 prior Tx lines, and progressive disease (PD) after ≥2 cycles of second-line LEN-based therapy. Pts received 28-d cycles of POM 4 mg/d on d 1-21+LoDEX 40 mg/d (20 mg/d if >75 y) on d 1, 8, 15, and 22, with mandatory thromboprophylaxis. The primary endpoint was overall response rate (≥partial response per modified International Myeloma Working Group criteria). The clinical benefit rate was defined as the percentage of pts achieving complete response, very good partial response, partial response, or minimal response. Key secondary endpoints included progression-free survival (PFS), safety, and second primary malignancies (SPMs). All pts provided informed consent.
Results: A total of 56 pts were enrolled in cohort A. Median age was 68 y, with 62.5% of patients aged >65 y in the intention-to-treat (ITT) population (prior PI, 63.4% [n=41]; prior BORT, 64.1% [n=39]) and 57.1% overall (prior PI, 58.5%; prior BORT, 56.4%) were male. Most pts had an ECOG performance status of 0/1: 92.9%, 95.1%, and 94.9%, respectively. All pts were refractory or relapsed to their most recent LEN-containing regimen, and 91.1%, 92.7%, and 92.3% of pts were LEN refractory, respectively. A majority of patients had prior stem cell transplant: 64.3% overall, 63.4% with prior PI, and 61.5% with prior BORT. Cytogenetic data by FISH were available for 50 pts: 4 were positive for del(17p), 4 for t(4;14), and 2 for t(14;16). Median duration of prior LEN-containing Tx was 23.6 mos (range, 3.5-107.0 mos), and in 60.7% of pts, the most recent LEN dose was 25 mg/d. Median follow-up was 19.0 mos at the data cutoff (10/2/2017). Responses and PFS outcomes are reported in the Table 1. Of the 56 pts in the ITT population, 52 discontinued Tx, 31 (59.6%) due to PD, 7 withdrawal, 5 adverse events (AEs), 3 lack of efficacy, 2 death, and 3 other reasons. The most common grade 3/4 treatment-emergent AEs included anemia (25.0%), neutropenia (10.7%), fatigue (14.3%), and infections (25.0%, including pneumonia [14.3%]). Grade 3/4 pulmonary embolism was reported in 2 pts, and 1 pt had an SPM. Similar safety results were noted in pts with prior exposure to PI or BORT.

Table 1.

Outcome	Cohort A (n = 56)	Prior PI Exposure (n = 41)	Prior BORT Exposure (n = 39)
ORR, %	33.9	31.7	28.2
VGPR	12.5	9.8	7.7
CBR, %	44.6	41.5	38.5
PFS, median, months			
ITT (n = 56)	9.6	7.9	7.9
EE (n = 53)	13.8		

BORT, bortezomib; CBR, clinical benefit rate; EE, efficacy evaluable; ORR, overall response rate; PFS, progression-free survival; PI, proteasome inhibitor; VGPR, very good partial response.

Summary and Conclusions: POM+LoDEX is a safe and effective third-line Tx for pts with RRMM following second-line failure of LEN-based Tx, including pts with prior exposure to PI or BORT. All pts, including those with prior PI or BORT Tx, experienced lower rates of hematologic AEs and longer median PFS than what has been previously reported for other studies using POM+LoDEX in later lines of Tx.

PF568

PRE-TREATMENT CD38 EXPRESSION LEVELS IN MYELOMA CELLS AND CD38-POSITIVE REGULATORY T CELLS AFFECT THE RESPONSE OF RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS TO TREATMENT WITH DARATUMUMAB

A. Kitadate[1,*], H. Kobayashi[1], Y. Abe[1], K. Narita[1], M. Takeuchi[1], K. Matsue[1]

[1]Division of Hematology/Oncology, Department of Medicine, Kameda Medical Center, Kamogawa, Japan

Background: Daratumumab (DARA), a humanized antibody to CD38, is a promising agent for multiple myeloma treatment. DARA targets CD38-expressing myeloma cells and nonplasma cells that express CD38, including CD38-positive regulatory T-cells (Treg). Therefore, DARA has been shown to induce myeloma cell death through several mechanisms, including complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), and immunomodulatory effects.
Aims: To investigate the mechanism of action of DARA, we analyzed baseline CD38 expression and lymphocyte subsets, including CD38-positive Treg cells, before and after DARA treatment.
Methods: We included 28 relapsed/refractory myeloma patients, who were treated with DARA at Kameda Medical Center. Patients who were followed up for more than one cycle of DARA were enrolled in the study. Peripheral blood and bone marrow samples were analyzed before and during therapy. Using flowcytometric analysis, we evaluated CD38 expression (MFI; mean

fluorescence intensity) and lymphocyte subsets (CD4/CD8 T cells, NK cells, and Treg cells). Treg cells were identified as a fraction of the CD4+CD25^{high}-CD127^{dim} population. We also examined CD55 and CD59 expression levels, which can be associated with CDC.

Results: The median age of the patients was 75 years (range: 53-91), and 14 of the patients were male. Twenty-two patients (79%) had received more than 3 prior therapies. Patients received a median of 4 prior lines of therapy (range, 2-11). Twenty-six patients (93%) were refractory to both proteasome inhibitors (PI) and immunomodulatory drugs (IMiDs). Eleven patients (39%) received a PI-based DARA-containing regimen, 14 patients (50%) received an IMiDs-based DARA-containing regimen, and three patients (11%) received other DARA-containing regimens. Seventeen patients (61%) had a partial response (PR) or better (responders), and 11 patients (39%) did not respond (non-responders). We confirmed that pretreatment levels of CD38 MFI were significantly higher in responders than in non-responders (p=0.001). There were no significant differences in the baseline expression levels of CD55 and CD59 in responders and non-responders. Before treatment, the absolute number of CD38-positive Treg cells was significantly higher in responders (median, 10.9/μl; range, 5.1-37.3/μl) than in non-responders (median, 4.8/ L; range, 1.1-12.8/μl, p=0.02), but absolute Treg cell numbers were not associated with DARA response. CD38-positive Treg cells were depleted in all treated patients, but total Treg cell numbers remained relatively stable after DARA treatment. In addition, we observed that absolute CD8-positve T-cell numbers significantly increased after DARA treatment (257.1±150.5 *vs* 379.6±193.6 / L, p=0.003). However, increased numbers of CD8-positive T-cells were not associated with clinical response. The numbers of CD4-positive T-cells significantly decreased after DARA treatment, while CD56-positive NK cells were immediately depleted in all treated patients.

Summary and Conclusions: Pretreatment levels of CD38 MFI could be a predictive marker for response to DARA treatment. Moreover, the frequency of CD38-positive Treg cells present before treatment also could serve as a response marker. These results suggest that depletion of CD38-positive Treg cells could contribute to some immunological mechanisms of DARA. This study provides evidence to support multiple mechanisms of action for DARA including ADCC and immunomodulatory effects.

PF569

UPDATED RESULTS OF A PHASE II STUDY WITH CARFILZOMIB, CYCLOPHOSPHAMIDE AND DEXAMETHASONE (CCD) FOR NEWLY DIAGNOSED TRANSPLANT INELIGIBLE MULTIPLE MYELOMA PATIENTS

A. Larocca[1,*], M.T. Petrucci[2], C. Conticello[2], L. De Paoli[2], M. D'Agostino[1], S. Spada[1], A. Bernardini[1], P. Musto[2], G. Benevolo[2], M. Offidani[2], A. Siniscalchi[2], P. Corradini[2], M. Cavo[2], M. Boccadoro[1], S. Bringhen[1]

[1]Myeloma Unit, Division of Hematology, University of Torino, A.O.U. Città della Salute e della Scienza di Torino, Torino, [2]Italian Multiple Myeloma Network, GIMEMA, Italy

Background: The standard treatment for newly diagnosed transplant-ineligible multiple myeloma (MM) patients includes the combination bortezomib-melphalan-prednisone (VMP) and lenalidomide-dexamethasone (Rd), which are associated with a median PFS of 21 and 26 months, respectively. Carfilzomib is a second-generation, irreversible proteasome inhibitor with significant activity in MM both at diagnosis and at relapse.

Aims: The combination carfilzomib-cyclophosphamide-dexamethasone (CCd) showed high complete response (CR) rates and a good safety profile in elderly newly diagnosed MM patients *(Bringhen S et al., Blood 2014).* Here we report an update after 5 years of follow-up.

Methods: Patients received oral cyclophosphamide (300 mg/m^2 day 1,8,15), oral dexamethasone (40 mg day 1,8,15,22) and iv carfilzomib administered over 30 minutes (20 mg/m^2 day 1,2, and 36 mg/m^2 day 8,9,15,16, cycle 1; 36 mg/m^2 day 1,2,8,9,15,16, cycles 2-9) for 9 28-day cycles, followed by maintenance with iv carfilzomib (36 mg/m^2 day 1,2,15,16) every 28 days until progression/intolerance.

Results: Fifty-eight patients were enrolled; median age was 71 years, 17 (29%) patients were older than 75 years, 23 (40%) had ISS stage III, 18 had unfavorable FISH profile [t(4;14) or t(14;16) or del17p] and 10 (17%) were frail, based on Charlson co-morbidity index, geriatric assessment scores ADL and IADL, and age. Fifty-five patients were evaluable for response. Median duration of induction treatment was 9 cycles. Overall, 94.5% of patients achieved at least PR, 69% at least VGPR, 51% nCR/CR, including 16% stringent-CR. Median time to PR was 1 month. After a median follow-up of 57 months, median PFS was 35.5 months and median OS was not reached; 3-year OS was 72% (Figure 1). The risk of progression or death was higher in patients with ISS stage III *vs* I (HR 2.54; 95%CI 0.98-6.58; p=0.054) and with high-risk chromosomal abnormalities (HR 2.41; 95%CI 1.16-5.00; p=0.02). Grade (G) 4 hematologic adverse events (AEs) included neutropenia (4 patients). G3 or higher non-hematologic AEs were infections (3 patients), cardiac (4 patients), constitutional (2 patients), renal (2 patients) and gastrointestinal complications (1 patient). Peripheral neuropathy was limited in severity to grade 1 or 2 (5 patients). Overall, CCd was well tolerated, 25% of patients required carfilzomib dose reduction and only 14% of patients required drug discontinuation during induction due to AEs. Among patients who completed induction, 43 patients could be evaluated for maintenance treatment. After a median duration of maintenance of 20 months, 100% of patients achieved a PR, 84% at least a VGPR, 60% a nCR/CR, including 21% stringent-CR. Median PFS from the start of maintenance was 33.4 months, median OS from start of maintenance was not reached and 3-year OS was 73%. The most frequent AE (all grades) during maintenance was fever (G1-2 in 10 patients, G3 1 patient), occurring during the evening following the Carfilzomib infusion and not associated with chills, rigors, dyspnea and/or creatinine increase. There was only 1 G3 neutropenia and 1 G2 pericardial effusion. Peripheral neuropathy remained limited (4 patients, only 1 G3).

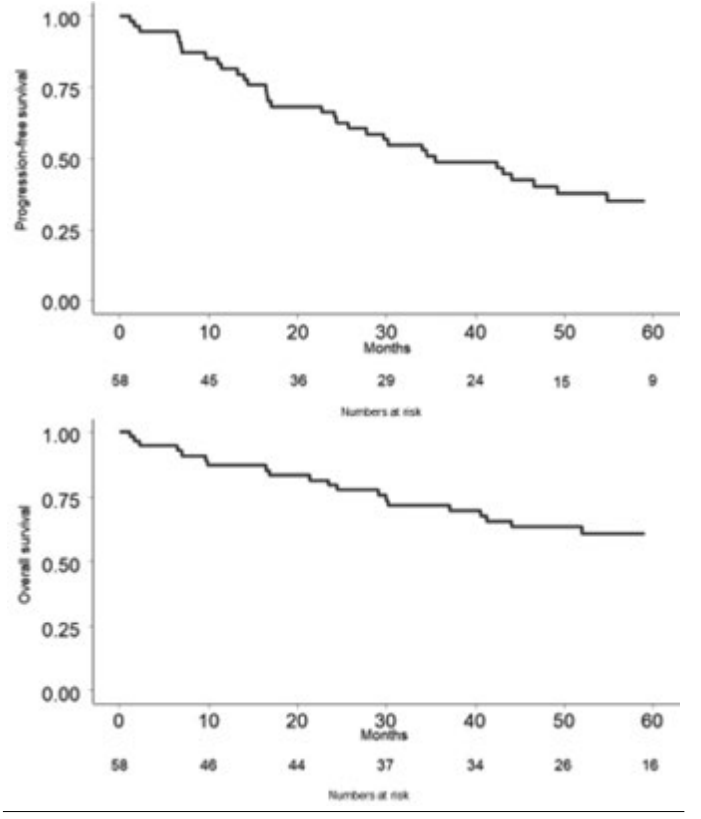


Figure 1.

Summary and Conclusions: CCd is highly active, showing rapid and deep responses that further improve during maintenance. Accordingly, PFS with CCd compares favorably with standard frontline regimens so far available in newly diagnosed MM patients. Maintenance with carfilzomib results into a significant increase in CR rate and a remarkably long PFS, with an acceptable toxicity profile.

PF570

DEVELOPMENT OF A PREDICTIVE MODEL OF MULTIPLE MYELOMA (MM) PATIENT OUTCOMES BASED ON TREATMENT (TX) SEQUENCING USING DATA FROM THE CONNECT® MM PATIENT REGISTRY

S. Jagannath[1,*], R.M. Rifkin[2], C. Gasparetto[3], K. Toomey[4], B.G. Durie[5], J.W. Hardin[6], H. Terebelo[7], L. Wagner[8], M. Narang[9], S. Srinivasan[10], A. YoussefAgha[10], A. Kitali[10], G. Larkins[10], A. Agarwal[10], R. Abonour[11]

[1]Mount Sinai Hospital, New York, NY, [2]US Oncology Research, Rocky Mountain Cancer Centers, Denver, CO, [3]Duke University Medical Center, Durham, NC, [4]Steeplechase Cancer Center, Somerville, NJ, [5]Cedars-Sinai Samuel Oschin Cancer Center, Los Angeles, CA, [6]University of South Carolina, Columbia, SC, [7]Providence Cancer Institute, Southfield, MI, [8]Wake Forest University School of Medicine, Winston-Salem, NC, [9]US Oncology Research, Maryland Oncology Hematology, Colum-

bia, MD, [10]Celgene Corporation, Summit, NJ, [11]Indiana University Simon Cancer Center, Indianapolis, IN, United States

Background: With the approvals of novel agents, the evolution of MM tx over the previous decade has considerably improved patient (pt) outcomes. (Rajkumar SV, 2016). The remitting-relapsing nature of MM and expanding tx options highlight the need to better understand tx sequencing and its impact on outcomes. Effectiveness and durations of response diminish with each successive line of therapy. In the US, pts with newly diagnosed MM (NDMM) are most often treated with lenalidomide (LEN), bortezomib (BORT), or the combination of an immunomodulatory agent and a proteasome inhibitor (PI). Recent randomized phase 3 trials exploring novel drug combinations at relapse may not reflect real-world outcomes, as they are limited by trial eligibility criteria. Connect® MM is a US, multicenter, prospective, observational cohort study designed to examine diagnostic and tx patterns, clinical outcomes and quality of life in pts with NDMM.
Aims: Using Connect MM, develop a novel model to test clinical outcomes in pts based on tx sequencing between agents throughout the MM disease course.
Methods: From 250 community and academic sites, adult pts ≤60 days from MM diagnosis were enrolled: Cohort 1 (n=1493) from Sept 2009 to Dec 2011, and Cohort 2 (n=1518) from Dec 2012 to Apr 2016. Tx transitions were analyzed at the time of disease progression as recorded by the treating physician. Pts were grouped (mutually exclusive) based on tx received before and after disease progression: 1) IMiD® agents (LEN- and POM-containing regimens), 2) PIs (BORT- and carfilzomib-containing regimens), 3) IMiD agent+PI, 4) Gap (pts off tx for ≥90 days), 5) Other (non-IMiD agent/PI regimens). Per common clinical practice, transitions could occur between any of the groups. Transitions were included for up to the 6th line of therapy and each pt could have >1 transition. Median and 3-y overall survival (OS) were estimated for each transition from the start of post-progression therapy until death or censoring using stratified covariate adjusted survival curves (Zhang 2007). Covariates included cohort, line at transition, duration of pre-transition therapy, age group, calcium, creatinine, hemoglobin, bone involvement, and transplant in first line.
Results: As of Feb 2017, a total of 1523 tx transitions at progression had occurred in 856 pts. Fewer tx transitions occurred with each successive line of therapy: 856 (line 1 to line 2); 385 (line 2 to line 3); 175 (line 3 to line 4); and lower numbers for subsequent lines. Frequencies of tx transitions between groups are shown in the Table 1. Median OS was longest for pts who transitioned from an IMiD agent to an IMiD agent+PI regimen (n=66 transitions; 61.9 mo; 3-yr OS: 71%) or from an IMiD agent+PI to another IMiD agent+PI regimen (n=41 transitions; 61.9 mo; 3-yr OS: 68%). Median PFS and OS were longest for front-line therapy, and decreased with each subsequent line of therapy.

Table 1.

		To Regimen, n (%) [Median OS, mo; 3-y OS rate]			
		PI	IMiD Agent	IMiD Agent + PI	Others/ Gap
From Regimen	PI (n = 514)	145 (28) [34.6; 0.48]	135 (26) [43.7; 0.6]	90 (18) [43.4; 0.61]	144 (28)
	IMiD Agent (n = 399)	198 (50) [37.1; 0.51]	67 (17) [41.5; 0.66]	66 (17) [61.9; 0.71]	68 (17)
	IMiD Agent + PI (n = 191)	57 (30) [35; 0.49]	34 (18) [NR; 0.53]	41 (21) [61.9; 0.68]	59 (31)
	Others/Gap (n = 419)	177 (42)	123 (29)	63 (15)	56 (13)
	Total (N = 1523)	577 (38)	359 (24)	260 (17)	327 (22)

Summary and Conclusions: Using the largely community-based Connect MM registry, we have developed a model which describes pt outcomes based on specific tx sequences. While not statistically tested, analyses showed that starting with an IMiD agent±PI regimen and then transitioning to an IMiD agent+PI after progression resulted in the best outcome for pts. Continued IMiD agent use at the time of progression may provide persistent immune modulation function, even in pts who have previously progressed on an IMiD agent-based regimen. This model may provide useful information on optimal tx sequencing and contribution of novel agents to clinical practice.

PF571

EXTERNAL VALIDATION OF THE MULTIPLE MYELOMA (MM) RISK-STRATIFICATION ALGORITHM IN A REAL-WORLD GREEK DATA SET

E. Terpos[1,*], M.A. Dimopoulos[1], E. Kastritis[1], M. Roussou[1], W. Bouwmeester[2], S. Kroep[2], L. DeCosta[3], M. Campioni[4], Z. Szabo[4], S. Gonzalez-Mcquire[4]

[1]Department of Clinical Therapeutics, National and Kapodistrian, University of Athens, School of Medicine, Athens, Greece, [2]Pharmerit International, Rotterdam, Netherlands, [3]Amgen Ltd, Uxbridge, United Kingdom, [4]Amgen (Europe) GmbH, Zug, Switzerland

Background: MM is heterogeneous, with varying drivers of progression, prognosis and response. There is a need for tools to help physicians evaluate MM prognosis at first relapse. A novel risk-stratification algorithm (RSA) was developed using real-world data from the Czech Registry of Monoclonal Gammopathies (RMG – Development Cohort (DC)) for patients with relapsed MM initiating second-line (2L) therapy. The RSA used 16 predictors from routine clinical practice to estimate risk scores: age; Eastern Cooperative Oncology Group performance status; extramedullary disease; new bone lesions; refractory status; severe toxicities at first line; time to next treatment; serum β-2 microglobulin and lactate dehydrogenase at diagnosis and at relapse; bone marrow plasma cell count, thrombocyte count, calcium and albumin at relapse; cytogenetic abnormalities at diagnosis. Scores were used to stratify patients into four risk groups (1 (lowest risk) – 4 (highest risk)), based on overall survival (OS) expectations. The RSA also provides a frailty and aggressiveness score to define drivers of risk.
Aims: External validation of the RSA is needed to ensure replicability of results in external data sets that can vary in terms of treatment patterns and demographics. This analysis aimed to assess validity of the RSA in a real-world data set in Greece.
Methods: To assess RSA validity, data from a single center registry between June 2000 to October 2017 were analyzed. All patients with MM initiating 2L treatment during this period were included. Data for one of the RSA predictors (new bone lesions at 1st relapse) were not available; bone lesion data were randomly assigned by applying the incidence of bone lesions observed in the DC. Missing values for other predictors were imputed five times using multiple imputation and the third imputation was selected for the base case analysis. A sensitivity analysis of five imputed data sets, pooled using Rubin's rules, and scenario analyses for the bone lesion data were performed.
Results: Data from 232 patients were analyzed. Patient characteristics showed differences from the DC which resulted in different distribution of risk groups in the two data sets. The median OS from initiation of 2L was 62.3 months. Median OS was not reached in group 1 and median OS in groups 2–4 was 74.9, 33.7 and 10.8 months, respectively. There was a clear discrimination of OS between the four risk groups; however, the confidence intervals (CIs) of the hazard ratios (HRs) overlapped (Figure 1). The frailty and aggressiveness scores across the four groups showed similar distribution to the DC, with a trend towards lower frailty-driven risk and similar aggressiveness-driven risk. The c-index was 0.77 (95% CI: 0.72–0.82); a value in the range 0.7–0.8 had been predetermined to identify accurate discriminative power. The R^2 value for the Greek data was 0.43.

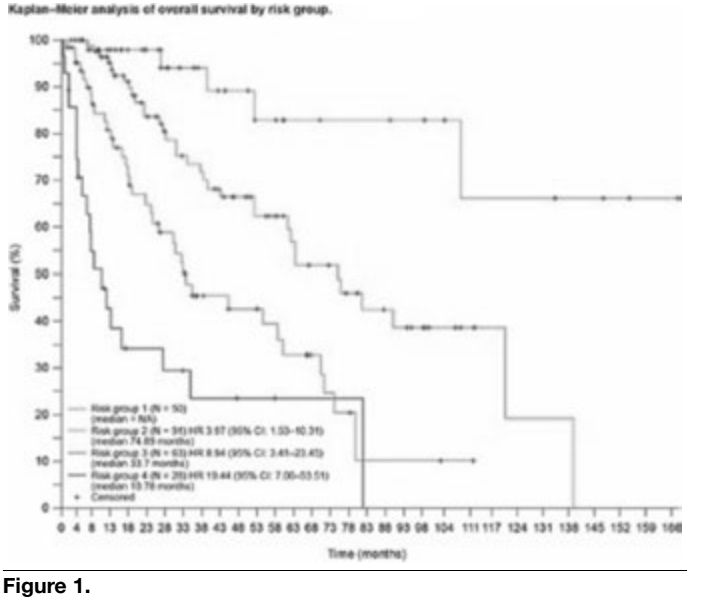


Figure 1.

Summary and Conclusions: The RSA was effective in quantifying risk and stratifying patients with relapsed MM, using long-term follow-up real-world data from a single center. The analysis is limited by a small sample size and the need for imputation of missing values, which can result in wider CIs than expected. Given the uncertainty in all analyses comparing risk groups, the HRs estimated by Rubin's rules gave results consistent with the base-case analysis. The RSA could be used as stratification criteria in clinical

trials for patients with relapsed MM. It may also help identify patient severity and drivers of risk to tailor management strategies, based on identifying both frailty and aggressiveness.

PF572

RANDOMIZED PHASE II STUDY TO OPTIMIZE MELPHALAN, PREDNISOLONE AND BORTEZOMIB (MPB) IN TRANSPLANT-INELIGIBLE NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM): JAPAN CLINICAL ONCOLOGY GROUP STUDY (JCOG1105)

D. Maruyama[1,*], S. Iida[2], G. Ogawa[3], S. Kusumoto[2], S. Fukuhara[1], N. Fukuhara[4], S. Seo[5], K. Miyazaki[6], M. Yoshimitsu[7], J. Kuroda[8], N. Tsukamoto[9], H. Tsujimura[10], A. Hangaishi[11], T. Yamauchi[12], T. Utsumi[13], I. Mizuno[14], Y. Takamatsu[15], Y. Nagata[16], K. Minauchi[17], E. Ohtsuka[18], I. Hanamura[19], T. Tokunaga[20], S. Yoshida[21], S. Yamasaki[22], Y. Suehiro[23], Y. Kamiyama[24], K. Miyamoto[3], Y. Watanabe[3], K. Tsukasaki[5], H. Nagai[20]

[1]Hematology, National Cancer Center Hospital, Tokyo, [2]Hematology and Oncology, Nagoya City University Hospital, Nagoya, [3]JCOG Data Center/Operating Office, National Cancer Center Hospital, Tokyo, [4]Hematology and Rheumatology, Tohoku University Hospital, Sendai, [5]Hematology, National Cancer Center Hospital East, Kashiwa, [6]Hematology and Oncology, Mie University Graduate School of Medicine, Tsu, [7]Hematology and Immunology, Kagoshima University Hospital, Kagoshima, [8]Hematology and Oncology, Kyoto Prefectural University of Medicine, Kyoto, [9]Hematology, Gunma University, Maebashi, [10]Hematology-Oncology, Chiba Cancer Center, Chiba, [11]Hematology, NTT Medical Center Tokyo, Tokyo, [12]Hematology and Oncology, University of Fukui, Fukui, [13]Hematology, Shiga General Hospital, Moriyama, [14]Hematology, Hyogo Cancer Center, Akashi, [15]Medical Oncology, Hematology and Infectious Diseases, Fukuoka University Hospital, Fukuoka, [16]Internal Medicine III, Hamamatsu University School of Medicine, Hamamatsu, [17]Hematology, Sapporo Hokuyu Hospital, Sapporo, [18]Hematology, Oita Prefectural Hospital, Oita, [19]Hematology, Aichi Medical University, Nagakute, [20]Hematology, National Hospital Organization Nagoya Medical Center, Nagoya, [21]Hematology, National Hospital Organization Nagasaki Medical Center, Ohmura, [22]Hematology and Clinical Research Institute, National Hospital Organization Kyushu Medical Center, [23]Hematology, National Hospital Organization Kyushu Cancer Center, Fukuoka, [24]Clinical Oncology and Hematology, The Jikei University School of Medicine, Tokyo, Japan

Background: Although the MPB regimen was established by the randomized phase III study comparing MPB with melphalan plus prednisolone (MP) (VISTA study) in patients (pts) with transplant-ineligible NDMM, the "intensive" MPB schedule was associated with a number of notable adverse events. Therefore, further refinement of the MPB regimen through a well-designed clinical trial was desired.
Aims: The objective of this randomized phase II study was to compare two less intensive modified MPB regimens for selecting a more optimal regimen in transplant-ineligible NDMM (UMIN000011180).
Methods: Pts with NDMM who were not candidates for stem cell transplantation because of age (65-79 years) or refusal (20-64 years) were eligible. Pts were randomly assigned 1:1 to Arm A (known as PETHEMA/GEM05 MPB) consisting of one cycle of subcutaneous (SC) or intravenous (IV) bortezomib at 1.3 mg/m² given twice-weekly plus 9 mg/m² of oral melphalan and 60 mg/m² of prednisolone on days 1–4 of a 6-week cycle, followed by 8 cycles of 4 weekly doses of bortezomib plus the same doses of MP of a 5-week cycle, or Arm B (further less intensive MPB) consisting of 9 cycles of SC or IV bortezomib at 1.3 mg/m² given in 3 weekly doses plus 7 mg/m² of melphalan and 60 mg/m² of oral prednisolone on days 1–4 of a 4-week cycle. The primary endpoint was the complete response rate (%CR). The planned sample size was 45 pts in each arm to achieve 85% probability for selecting the regimen with a higher%CR in one arm (30%) than in the other (20%) based on the Simon's selection design.
Results: Between July 2013 and April 2016, a total of 91 pts were randomized to Arm A (45 pts) and Arm B (46 pts), and their median (range) ages were 72 (65-79) and 72 (65-78) years. Efficacy analysis was performed for all 88 eligible pts. In both arms, only 1 pt each received bortezomib maintenance after 9 cycles of MPB. Median%planned and cumulative doses of bortezomib were 88% and 45.8 mg/m² in Arm A, and 100% and 35.1 mg/m² in Arm B. The%CR as a primary endpoint in Arms A and B was 18.6% (95% CI, 8.4-33.4) and 6.7% (95% CI, 1.4-18.3). The overall response rate was 79.1% (95% CI, 64.0-90.0) in Arm A and 73.3% (95% CI, 58.1-85.4) in Arm B. As of the data cut-off, the median follow-up of all pts was 26 months (range, 7-47). The 2-year and median progression-free survival (PFS) were 58.1% (95% CI, 41.1-71.7) and 2.5 years in Arm A, and 31.7% (17.8-46.6) and 1.4 years in Arm B, with a hazard ratio in Arm B to Arm A of 1.93 (95% CI, 1.09-3.42) (Figure 1). The 2-year overall survival was 90.2% (95% CI, 75.9-96.2) in Arm A and 92.2% (95% CI, 77.4-97.5) in Arm B. The most common grade 3/4 hematologic toxicities were leukocytopenia (73.3% *vs* 30.4%), neutropenia (64.4% *vs* 28.3%), and thrombocytopenia (35.6% *vs* 10.9%). Sensory peripheral neuropathy of grade 2/3/4 was observed in 24.4/2.2/0% in Arm A and 8.7/0/0% in Arm B, and all grades of diarrhea, nausea, fever and rash occurred frequently in Arm A. Only 1 treatment-related death due to pneumonitis was observed in Arm B.

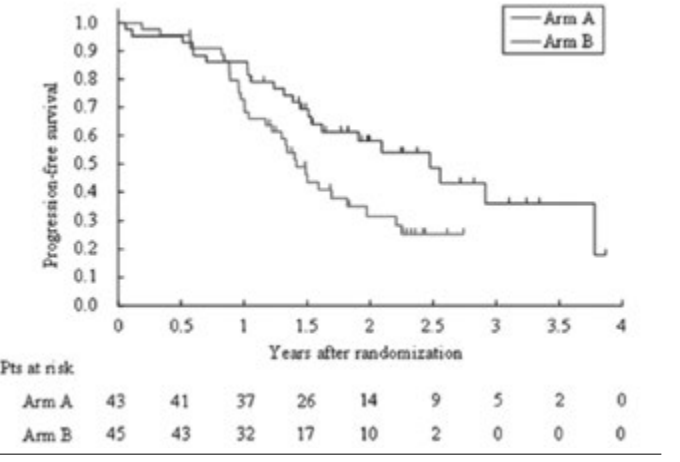


Figure 1.

Summary and Conclusions: Arm A had a higher%CR and superiorPFS with more frequent but manageable toxicities than those in Arm B. These results proposed that the intensive twice-weekly dosing in the first cycle and high cumulative dose of bortezomib were important in maximizing the efficacy of the modified MPB regimen.

PF573

REAL-WORLD TREATMENT PATTERNS AND PROGRESSION-FREE SURVIVAL (PFS) AMONG PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) RECEIVING POMALIDOMIDE-BASED REGIMENS

R. Vij[1,*], C. Chen[2], C. Davis[2], H. Kim[3], H. Goldschmidt[4], B. Durie[5], D. Kuter[6]

[1]Washington University School of Medicine, St Louis, [2]Bristol-Myers Squibb, Princeton, [3]PAREXEL, Billerica, United States, [4]Heidelberg University Hospital, Heidelberg, Germany, [5]Cedars-Sinai Medical Center, Los Angeles, [6]Massachusetts General Hospital Cancer Center, Boston, United States

Background: Despite therapeutic advances such as novel immunomodulatory drugs (IMiDs), most patients (pts) with MM still relapse. The second-generation IMiD pomalidomide (pom), combined with dexamethasone (Pd), has shown efficacy in RRMM clinical trials, including in pts refractory to the standard-of-care IMiD lenalidomide and proteasome inhibitor (PI) bortezomib, as well as in pts with high-risk cytogenetics (Dimopoulos MA *et al. Leukemia* 2014). These data led to EU and US approval of Pd for pts with RRMM and ≥2 prior therapies (Tx), including lenalidomide and bortezomib (or other PI in the US), and with disease progression on their last prior Tx (or within 60 d of completion in the US). However, real-world data on Tx patterns and outcomes in pts who receive pom-based Tx are lacking.
Aims: To assess real-world Tx patterns and outcomes in pts with RRMM receiving Pd or a Pd triplet Tx.
Methods: PREAMBLE (NCT01838512) is an ongoing observational study exploring real-world Tx outcomes in MM. This analysis included pts ≥18 y of age, with RRMM and ≥1 prior Tx, whose first pom-based Tx was Pd or a Pd triplet received at any time during RRMM Tx. PFS was evaluated using Kaplan–Meier methods. Hazard ratios (HRs) were calculated using uni- and multivariate Cox regression; covariates for adjustment were selected using stepwise regression. 'Novel agents' included monoclonal antibodies and histone deacetylase inhibitors. Informed consent was obtained for all pts.
Results: At database lock (Nov 30, 2017), 240 pts were identified who started with Pd (Pd group; n=179, 75%) or a Pd triplet (Pd triplet group; n=61, 25%); median follow-up was 15 mo. Baseline characteristics were generally similar between the groups; however, pts in Europe were more

likely to receive Pd (66%) than Pd triplets (46%), whereas pts in North America were more likely to receive Pd triplets (54%) than Pd (34%). Overall median age at initiating pom was 67 y; at enrollment, 73% had refractory MM and 24% had stage III disease. Comorbidities were similar between groups. In the Pd group, median (range) number of prior Tx was 3 (1–14), and in the Pd triplet group, 2 (1–9); overall, 50% received ≥3 prior Tx. In prior Tx, pts in the Pd *vs* Pd triplet group were more likely to have received an IMiD (52% *vs* 28%; p=0.0011), whereas pts in the Pd triplet group were more likely to have received an IMiD+PI Tx (26% *vs* 7%; p<0.0001) or novel agent (15% *vs* 3%; p=0.0018). A greater proportion of pts finished Pd than Pd triplets (85% *vs* 66%; p=0.0011); median duration of Tx was similar between groups (3.8 *vs* 3.3 mo). In subsequent Tx, pts in the Pd triplet group were more likely to receive a novel agent than Pd-group pts (15% [6/40] *vs* 2% [2/131]); similar proportions in each group (Pd *vs* Pd triplet) received PIs (46% *vs* 45%), IMiDs (47% *vs* 33%), or IMiD+PIs (6% *vs* 8%). PFS was longer in pts who received a Pd triplet than pts who received Pd (Figure 1). In univariate PFS analysis, HR (Pd triplet over Pd) was 0.57 (95% CI 0.28, 1.19; p=0.134), but multivariate analysis revealed a significant PFS benefit for pts who received a Pd triplet *vs* Pd (HR: 0.46 [95% CI 0.21, 0.96]; p=0.041).

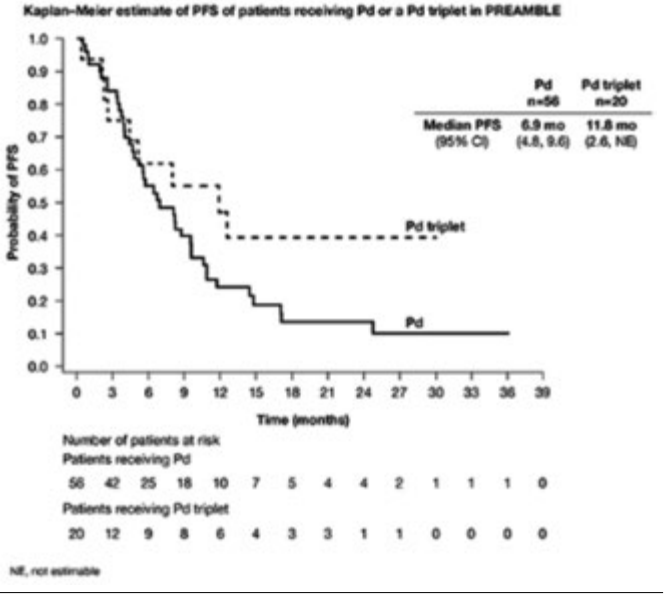


Figure 1.

Summary and Conclusions: 50% of pts who received Pd or a Pd triplet in PREAMBLE received ≥3 prior Tx. Real-world data in RRMM suggest Pd triplets may be linked to improved PFS *vs* Pd, and may represent an alternative Tx strategy. Ongoing additional analyses will evaluate outcomes from specific Pd triplet regimens.

PF574

TIME TO FIRST ONSET OF TREATMENT-EMERGENT ADVERSE EVENTS (TEAES) OF INTEREST: A POST-HOC ANALYSIS FROM ASPIRE AND ENDEAVOR TRIALS

M.-V. Mateos[1,*], S. Knop[2], M. Delforge[3], L. DeCosta[4], F. Suzan[5], Z. Szabo[5], K. Wang[6], J. Mikhael[7]

[1]Hematology Service, University Hospital Salamanca, Salamanca, Spain, [2]Wuerzburg University Medical Center, Wuerzburg, Germany, [3]Department of Hematology, University Hospital (UZ) Leuven, Leuven, Belgium, [4]Global Biostatistical Science, Amgen Ltd., Uxbridge, United Kingdom, [5]Amgen (Europe) GmbH, Zug, Switzerland, [6]Global Safety, Amgen Inc., South San Fransisco, CA, [7]International Myeloma Foundation, North Hollywood, CA, United States

Background: In the ASPIRE and ENDEAVOR phase 3 trials, carfilzomib (K) in combination with lenalidomide and dexamethasone (KRd), or with dexamethasone alone (Kd), respectively, was investigated in patients with relapsed and/or refractory multiple myeloma (RRMM). An increased incidence of cardiac and vascular adverse events (AEs) was shown in K arms *versus* the comparator arms of these trials. Cardiac events typically occurred early (<5 cycles) in the course of K therapy. However, the underlying mechanism leading to AEs has not been established. Such exploration is complex due to multiple confounding factors such as the potential dose effect, disease burden, patient characteristics, co-medications, and comorbidity.

Aims: To describe time to first onset (TTFO) of TEAEs within each trial, beyond their incidences and severity reported earlier.

Methods: This post-hoc analysis describes the median TTFO of TEAEs of all grades or grade 3 or more (Gr3+) from the ASPIRE or ENDEAVOR trials. TTFO was calculated from start of treatment to the time when an AE first occurred. Repeated events occurring in a subject were excluded from this analysis. TTFO patterns were assessed within study; due to the differences in design and patient population, no cross-study comparison was performed.

Results: Results and cumulative incidence are presented in Figure 1 in the form of boxplots of TEAE Gr3+ grouped into 4 AE classes (hematological AEs, cardiac AEs, vascular AEs and non-hematological AEs). Median treatment duration in ENDEAVOR was 48 weeks for Kd and 27 weeks for bortezomib with dexamethasone (Vd) arm; in ASPIRE, it was 72 weeks for K in the KRd arm and 57 weeks for lenalidomide plus dexamethasone (Rd) arm. Hematological AEs Gr3+ occurred within 2-3.5 months (mos) in ASPIRE and within 1-2.5 mos in ENDEAVOR, and no differences in the median TTFO between arms of each trial were observed. Median TTFOs of cardiac AEs were earlier in KRd *vs* Rd subjects (cardiac failure [CF]: 4.5 mos for KRd, 9.3 mos for Rd; ischemic heart disease [IHD]: 4.9 mos for KRd, 9.1 mos for Rd). The reverse pattern was seen in ENDEAVOR, with longer median TTFOs and broader ranges among Kd *vs* Vd subjects (CF: 7.6 mos for Kd, 4.6 mos for Vd; IHD: 1.4 mos for Kd, 0.8 mos for Vd). KRd subjects had an earlier onset of vascular AEs *vs* Rd subjects, especially hypertension (HT: median TTFO of 7.3 mos in KRd *vs* 13.6 mos in Rd), whereas in ENDEAVOR, it was longer in Kd than Vd subjects (8.8 *vs* 3.0 mos), while no difference was observed for thrombo-embolic events. Median TTFOs of dyspnea were similar between treatment arms within each study. The median TTFO of acute renal failure (ARF) was longer for KRd subjects *vs* Rd (13.3 *vs* 7.6 mos, respectively), and the difference was even more pronounced in ENDEAVOR (Kd 7.2 *vs* Vd 1.6 mos). While the main limitation of this Gr3+ analysis was the small number of subjects (<10) for some TEAEs, TTFOs of all-grade TEAEs (not shown) generally showed a comparable pattern to that of Gr3+ TEAEs.

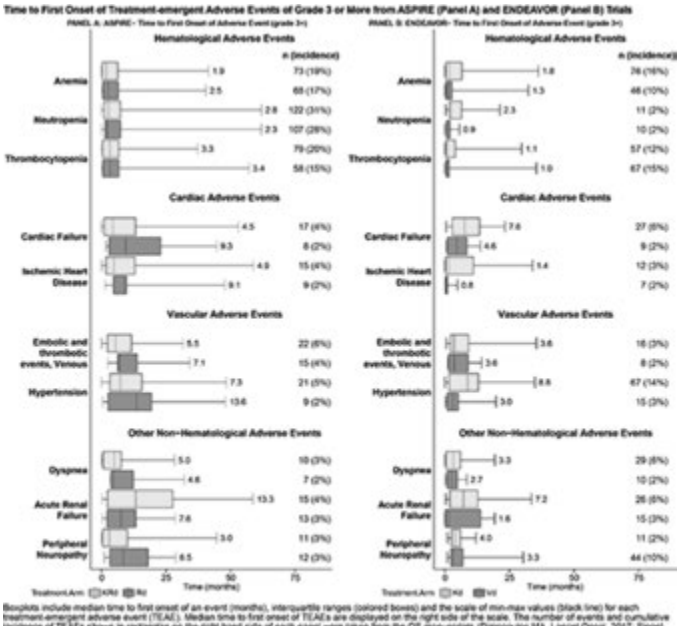


Figure 1.

Summary and Conclusions: CF and HT had a marked earlier onset in KRd *vs* Rd subjects and a later onset in Kd *vs* Vd subjects, although their incidence was higher in K *vs* comparator arms. The largest variations in TTFO patterns were seen for ARF that had a later appearance among K subjects. By contrast, hematological AEs were similar in all 4 arms. In this descriptive analysis, variations in TTFO of a TEAE between arms of a trial may suggest multiple mechanisms without any easily expected timing of toxicities (*e.g.*, cardiac events). The longer exposure to K *vs* Rd or Vd in these studies may also have accounted for TTFO variations.

PF575

SMOLDERING MYELOMA AFTER IMWG UPDATE: FACTORS PREDICTIVE OF EVOLUTION, ROLE OF ADVANCED IMAGING AND OF CLINICAL JUDGEMENT. A REAL-LIFE RETROSPECTIVE EXPERIENCE

L. Torti[1,*], A.M. Morelli[1], F. Bacci[2], P. Di Bartolomeo[1]

[1]Clinical Hematology, Department of Hematology, Transfusion Medicine and Biotechnology, "Spirito Santo" Civic Hospital, Pescara, [2]Section of Hematopathology, Department of Hematology and Oncological Sciences, S.Orsola Malpighi Hospital, Bologna, Italy

Background: Smoldering myeloma(SMM)is a heterogeneous clinical entity where a watch-and wait-approach has been standard of care up to now.Recently it has been demonstrated that a subset of high-risk cases can benefit of early-treatment.IMWG revised diagnostic-criteria adding markers as Myeloma-Defining-Events,allowing earlier intervention.We aimed to study diagnostic-workup and prognostic-factors predicting progression.We also focus on early-treatment.
Aims: We retrospectively reviewed data from 139 smoldering myeloma patients diagnosed from 1998 to 2016 in order to assess risk-profile and evolution in symptomatic-myeloma.Our aim was to define risk-factors and an effective followup-strategy.We also tried to understand when is right-time to start therapy comparing old-CRAB with new-MDE-events.
Methods: We evaluated 139 SMM-patients consecutively diagnosed at our centre in median-time of 18 years(median followup 13 years,range 2-19).
Results: We have found 62 low-risk SMM, 46 high-risk SMM and 31 ultra-high-risk-SMM in a retrospective single-center analysis.62 patients have evolved in myeloma requiring treatment and respectively:25/31 of the ultrahigh-risk-group progressed in a medium-time-span of 11months (range 6-39)(Figure 1AB), 23/46 of the high-risk-group ended in symptomatic myeloma with a medium time of 32 months(range 24-72)from diagnosis and only 10/62 of the low-risk-series after 94-median-months(range 39-140).In the low-risk evolved patients an important risk-factor for evolution was the amount of monoclonal-protein over time:most of patients evolved (8/10) have shown an evolving type SMM. In the high-risk-patients the most powerfull predictive-factors of progression were an unfavorable cytogenetic-picture (17 patients with a prevalence of amplification of 1q in 11 patients) (Figure 1C), focal lesions in MRI or PETCT (17 and 15 patients) and LDH-values (high in 13 patients).Among MDEs the most meaningful biomarker was FLCratio>100 (21/31 patients) followed by MRI-lesions (7/31 patients) and lastly by bone-marrow-plasma cell involvement greater than 60% (3/31 patients) (Figure 1AB). Between ultrahigh risk SMM 21 were diagnosed before 2014 IMWG update through a retrospective analysis and 10 after.Of the first group 18 patients have evolved in MM requiring therapy, median time of 11 months (Figure 1D).

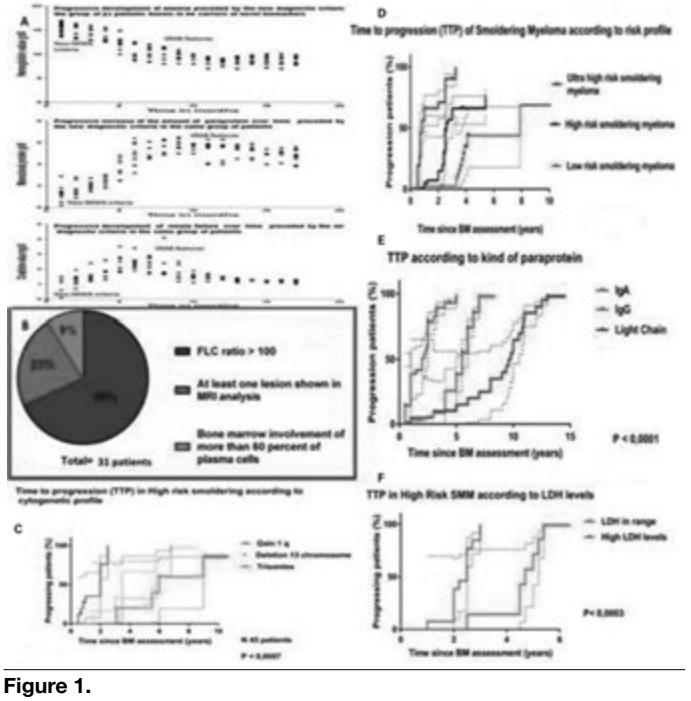


Figure 1.

Conversely in the second-group not all patients started therapy. In fact 3 young patients were not treated until now and continued a close-observation with a monthly-followup. They presented stable-monoclonal-protein and laboratory profile, excellent clinical condition.They presented only one slim-CRAB for at least 9 months(respectively plasma-cell-involvement>60% and in 2 patients FLCratio>100).Here earlier-treatment could not be beneficial but might instead results in greater toxicity.Other 7 presented deterioration of clinical-conditions with at least two-slimCRAB(respectively FLC ratio>100 and lesions on MRI)not preexisting.They showed an evolving-type-SMM.Biological-behavior in this subgroup was very aggressive and they started treatment.They are under therapy with a good-disease-control.Clinical-judgement must help physicians in making decisions.
Summary and Conclusions: Risk of SMM progression is not uniform and several-markers(cytogenetic,evolving type,lesions on advanced imaging,kind of paraprotein as described here (Figure 1E), LDH-levels (Figure 1F) are usefull in clinical-practice to predict evolution.Novel biomarkers will be available in the future to plan followup and to identify group benefiting of early-treatment.

PF576

SAFETY OUTCOMES IN PATIENTS (PTS) WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) TREATED WITH LENALIDOMIDE FOR ≤24 MONTHS *VS* >24 MONTHS IN A EUROPEAN POST-APPROVAL SAFETY STUDY (EU PASS)

G. Semenzato[1,*], D. Kyriakou[2], P. Moreau[3], X. Leleu[4], I. Wolfgang Blau[5], A. Symeonidis[6], A. Bosi[7], E. Kueenburg[8], A. Di Micco[8], B. Rosettani[8], S. Peters[8], P. Bacon[8], B. Gamberi[9]

[1]Azienda Ospedaliera di Padova, Padova, Italy, [2]University Hospital of Larissa, Larissa, Greece, [3]CHU Nantes Hôtel Dieu, Nantes, [4]Hôpital La Miletrie, Poitiers, France, [5]Charité - Campus Benjamin Franklin, Berlin, Germany, [6]University of Patras Medical School, Rion of Patras, Greece, [7]AOU Careggi, Firenze, Italy, [8]Celgene International, Boudry, Switzerland, [9]Arcispedale S. Maria Nuova, Reggio Emilia, Italy

Background: EU PASS is a large, multicenter, observational study evaluating the safety and tolerability of lenalidomide (LEN)+dexamethasone (DEX) *vs* other agents in patients (pts) with RRMM in a real-world setting.
Aims: To compare the safety and tolerability of treatment (Tx) with LEN in pts treated for ≤24 mos *vs* pts treated for >24 mos.
Methods: EU PASS enrolled pts with RRMM who had received ≥1 prior therapy. Pts who initiated Tx with LEN+DEX were in the LEN cohort; pts who initiated a non–LEN-based Tx were in the background cohort. All pts provided informed consent. Off-label use of LEN was allowed. Adverse events (AEs) were graded per the National Cancer Institute Common Terminology Criteria for Adverse Events v3.0; treatment-emergent AEs (TEAEs) of special interest were analyzed using standardized Medical Dictionary for Regulatory Activities queries. Pts were followed up for ≤36 mos after Tx discontinuation to monitor for second primary malignancies (SPMs) per a July 2011 protocol amendment. From August 2008 to July 2014, 3630 pts were included in the safety population. A subgroup analysis was performed on the LEN cohort to compare the safety of LEN in pts treated for ≤24 mos *vs* pts treated for >24 mos.
Results: Of the 2151 pts receiving LEN, 1847 (85.9%) were treated for ≤24 mos and 304 (14.1%) for >24 mos. Demographics were balanced between the 2 groups. The median age was 70.0 yrs in the ≤24-mos group and 69.0 yrs in the >24-mos group; 53.0% and 56.6% of the pts were male. In both groups, ≈ one-quarter of pts were aged ≥76 yrs. The 2 groups had a similar median time from diagnosis to enrollment (≤24 mos: 2.8 yrs; >24 mos: 3.5 yrs). In both groups, >90% of pts experienced ≥1 TEAE; ≥1 grade 3/4 TEAE occurred in 57.2% and 61.5% of pts in the ≤24-mos and >24-mos groups, respectively. More pts discontinued due to a TEAE in the ≤24-mos group *vs* the >24-mos group (36.2% *vs* 22.4%, respectively). A lower percentage of pts had a dose reduction due to a TEAE (20.7% *vs* 44.1%) in the ≤24-mos *vs* the >24-mos group, respectively. Median times to first dose reduction (9.4 *vs* 34.9 wks) and first dose interruption (7.9 *vs* 20.4 wks) were shorter in the ≤24-mos group. The incidences of grade 3/4 neutropenia and venous thromboembolism (VTE) were lower and the incidence of grade 3/4 thrombocytopenia was higher in the ≤24-mos than in the >24-mos group. The incidences of other grade 3/4 TEAEs of special interest were similar between the 2 groups (Table 1). The rate of SPMs was lower in the ≤24-mos than in the >24-mos group (2.8% *vs* 9.5%, respectively). A lower frequency of solid tumor SPMs was observed in the ≤24-mos than in the >24-mos group (1.5% *vs* 3.0%, respectively). The 2 groups had a similar frequency of hematologic SPMs (0.6% *vs* 0.7%). However, the incidence rate per 100 person-years for SPMs was higher in the ≤24-mos than in the >24-mos group (4.61 *vs* 2.59, respectively).
Summary and Conclusions: There were no new safety signals with long-term (>24 mos) LEN Tx. Higher rates of grade 3/4 neutropenia and VTE

were observed in the pts with >24 mos of LEN Tx, whereas the rates of other grade 3/4 TEAEs of special interest did not increase notably. Pts in the >24-mos group maintained LEN Tx at starting-dose levels longer than did pts in the ≤24-mos group. The SPM incidence rate per 100 person-years did not increase with long-term LEN Tx. These results from a real-world setting show that long-term LEN Tx is generally safe and well-tolerated in pts with RRMM.

Table 1.

Grade 3/4 TEAEs of Special Interest by SMQ, n (%)	≤ 24 Months of LEN Treatment n = 1847	> 24 Months of LEN Treatment n = 304
Neutropenia	348 (18.8)	84 (27.6)
Acute and opportunistic infections	262 (14.2)	46 (15.1)
Thrombocytopenia	199 (10.8)	17 (5.6)
Renal failure	77 (4.2)	11 (3.6)
Venous thromboembolism	49 (2.7)	14 (4.6)
Neuropathy	24 (1.3)	6 (2.0)
Cardiac failure	22 (1.2)	6 (2.0)
Bleeding events	21 (1.1)	4 (1.3)
Cardiac arrhythmias	17 (0.9)	6 (2.0)
Rash	8 (0.4)	0 (0)
Hypothyroidism	1 (0.1)	0 (0)

LEN, lenalidomide; SMQ, standardized Medical Dictionary for Regulatory Activities query; TEAE, treatment-emergent adverse event.

PF577

CLINICAL CHARACTERISTICS AND OUTCOMES OF OLIGOSECRETORY AND NON-SECRETORY MULTIPLE MYELOMA

M. Migkou[1], Y. Cohen [2], E. Kastritis[1,*], M. Gavriatopoulou[1], E. Eleutherakis-Papaiakovou[1], E. Terpos[1], D. Fotiou[1], M. Roussou[1], D.C. Ziogas[1], E. Terpos[1], I. Avivi[2], M.A. Dimopoulos[1]

[1]Department of Clinical Therapeutics, National and Kapodistrian University of Athens, Athens, Greece, [2]Department of Hematology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

Background: In multiple Myeloma (MM), clonal plasma cells secrete monoclonal immunoglobulins (MIg) which are identified and measured in the serum and/or urine. Typically, the amount of MIg is large enough to be followed serially with relative accuracy to allow disease evaluation. However, in a subset of patients, secreted MIg in serum and urine is low, below what is considered as "measurable" for precise serial evaluation (oligo-secretory MM). In addition, a small subset of patients has no detectable MIgs by conventional immunofixation in serum and urine (non-secretory MM).
Aims: Our aim was to evaluate the characteristics and outcomes of patients with oligo- and non-secretory myeloma, in a large dataset of patients diagnosed and treated with contemporary tools and regimens.
Methods: The study included 852 consecutive patients with complete baseline data on serum and urine PEP, diagnosed and treated in the Department of Clinical Therapeutics (Athens, Greece) and in the Hematology Department at Tel Aviv Medical center (TA, Israel). Oligosecretory disease was defined as both serum MIg<1 g/dL and urine MIg<200 mg/24h. Non-secretory disease was defined as negative immunofixation in both serum and urine.
Results: We identified 100 (11.7%) patients with oligo/non-secretory MM, including 20 (2.3%) with non-secretory MM. Compared to patients with secretory MM, patients with oligo/non-secretory MM were younger (p=0.03), less anemic (p<0.001), had less often renal dysfunction (p=0.05) and less extensive bone marrow infiltration (p=0.001). Bone disease was similar in frequency (p=0.22) and extent (p=0.13), but hypercalcemia was less common (p=0.002). Oligo/non-secretory patients were more often ISS-1 (55% *vs* 23%) than ISS-2 (16% *vs* 34%) or ISS-3 (29% *vs* 43%)(p<0.001). In patients with available FISH, high risk cytogenetics were slightly less common in oligo/non-secretory MM (16% *vs* 22%, p=0.26). Disposition in R-ISS stages -1, -2 and -3 was 31%, 53% & 16% *vs* 15%, 66% & 19% respectively (p=0.011). Patients with oligo/non-secretory MM, were treated more often with bortezomib (84% *vs* 60%) (p=0.001). Median follow up is 4 years and 4-year OS for patients with oligo-/non-secretory disease was 64% *vs* 58% for secretory MM (p=0.034). However, after adjusting for known prognostic factors in multivariate analysis, oligo/non-secretory MM was not associated with a different survival (p>0.3). Restricted analysis, focusing on patients with non-secretory disease, revealed that these patients were younger (p=0.02) and less anemic (p=0.008) compared to other MM patients, though the degree of marrow plasma cell infiltration was similar. Non secretory patients had more often an elevated LDH (p=0.052), however, they were more often ISS-1 (61% *vs* 25%) than ISS-2 (5% *vs* 33%) or ISS-3 (33% *vs* 42%) (p=0.002). Lytic bone disease (85% *vs* 75%, p=0.32) and hypercalcemia were similar (16% in both groups). Only 1 of 9 patients with non-secretory MM had high risk cytogenetics. FLCs were available in 17 patients with non-secretory MM: only 3 had normal FLC ratio; the others had abnormal FLC ratio and 9/14 had iFLC ≥100 mg/L. Patients with non-secretory myeloma had similar survival with the other patients (4-year OS: 53% *vs* 59%, p=0.68).
Summary and Conclusions: About 12% of MM patients present with oligo- or non-secretory disease at diagnosis and they have different characteristics but, in the era of the contemporary therapies, similar outcome to other MM patients. With the use of FLCs more than 60% of them patients with non-secretory disease can be followed for response and eligible for participation in clinical trials.

PF578

SERUM NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN INDEPENDENTLY PREDICTS FOR RENAL RESPONSE IN MYELOMA PATIENTS WITH SEVERE RENAL IMPAIRMENT

E. Terpos[1], M. Roussou[1], I. Papassotiriou[2], M. Gavriatopoulou[1], E. Eleutherakis-Papaiakovou[1], A. Margeli[2], D. Fotiou[1], N. Kanellias[1], M. Migkou[1], G.-P. Papassotoriou[2], E. Kastritis[1,*], M.A. Dimopoulos[1]

[1]Department of Clinical Therapeutics, National and Kapodistrian University of Athens, School of Medicine, [2]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece

Background: Severe renal impairment (RI) is a common complication of multiple myeloma (MM). Immediate anti-MM therapy can restore renal function in several patients, but we lack biomarkers that could predict renal outcomes. Neutrophil gelatinase-associated lipocalin (NGAL) is one of the earliest and most robust markers of acute kidney injury while serum Cystatin C (CysC) reflects renal function more accurately than creatinine and correlates with both tumor burden and renal function in MM.
Aims: To evaluate serum NGAL and CysC in MM patients with severe RI (eGFR<30 ml/min/1.73m^2, by CKD-EPI formula), including those on dialysis, as biomarkers for prediction of renal responses.
Methods: NGAL and CysC were measured in the same frozen serum sample collected before any therapy was given. Serum NGAL was measured using ELISA (BioPorto Diagnostics A/S, Gentofte, Denmark). CysC was measured using a latex particle-enhanced nephelometric immunoassay (Dade Behring-Siemens Healthcare Diagnostics, Liederbach, Germany). Patients on dialysis received dialysis with regular membranes. IMWG renal response criteria were used.
Results: The analysis included 82 newly diagnosed MM patients with severe RI treated and followed at the Department of Clinical Therapeutics (Athens, Greece). Median creatinine was 5 mg/dl (range 2-10), median eGFR was 11.3 ml/min/1.73 m^2 (1.3-29.8) and dialysis was required in 32 (39%) patients. The median age was 71 years, median involved FLC was 5225 mg/L, hypercalcemia was found in 24%, LDH ≥ULN in 41% and high-risk cytogenetics in 27% while 98% were ISS-3 and 56% were R-ISS-3. Treatment was bortezomib-based in all patients (in 23% VD and in 77% a triplet). Median NGAL levels were 191.5 ng/mL (range 20-550) and of CysC were 3.42 mg/L (1.1-7.8) and were strongly correlated (R^2=0.421, p<0.001); also both strongly correlated with eGFR (for CysC: R^2=0.43, p<0.001 and for NGAL: R^2=0.225, p<0.001) and both were higher in patients requiring dialysis *vs* those not in dialysis (median NGAL:308 *vs* 153 ng/mL, p<0.001, median CysC:4.99 *vs* 2.73 mg/L, p=0.001). Renal response (Rrenal) was achieved by 60% (including 50% major Rrenal) and 34% discontinued dialysis. Median time to Rrenal was 1 month and median time to dialysis independence was 2 months. Lower levels of NGAL (p=0.009) and CysC (p=0.014) were associated with higher probability of major Rrenal among patients with severe RI but not on dialysis, but baseline eGFR was not (p=0.346). None was associated with dialysis independence among those requiring dialysis. By ROC analysis, in patients with severe RI but not on dialysis, NGAL <130 ng/ml was strongly associated with major Rrenal (86% *vs* 24% at 3 months, p<0.001; Figure 1). Regarding CysC, levels <2.6 mg/L were associated with higher probability and shorter time to major Rrenal (p=0.012). Both NGAL and CysC had no predictive value for patients under dialysis. In multivariate analysis performed in patients not on dialysis, that included age, NGAL, CysC and eGFR, only NGAL<130 ng/ml was significantly associated with major Rrenal (HR 5, 95% CI 2-18, p=0.01).
Summary and Conclusions: Serum levels of NGAL were strong predictors of major Rrenal in MM patients with severe RI,not on dialysis. Serum NGAL could identify MM patients with severe RI who should be treated with more aggressive therapies, that could include high cut-off dialysis membranes and more effective and rapidly acting antimyeloma regimens.

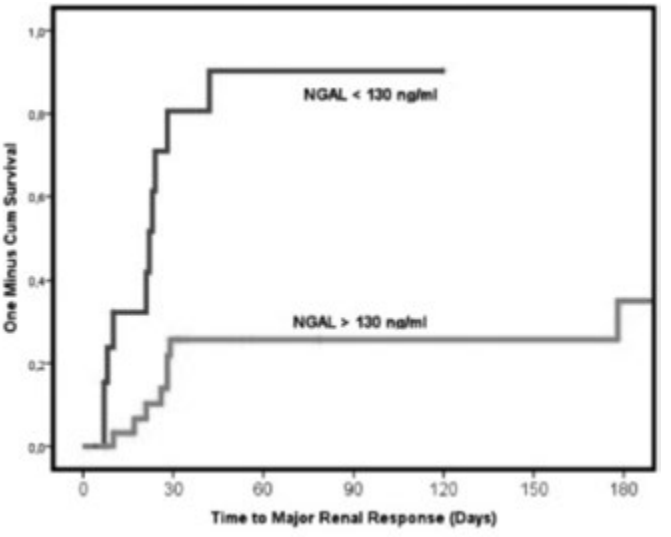


Figure 1.

PF579

DARATUMUMAB, CARFILZOMIB, AND DEXAMETHASONE (D-KD) IN LENALIDOMIDE-REFRACTORY PATIENTS WITH RELAPSED MULTIPLE MYELOMA (MM): SUBGROUP ANALYSIS OF MMY1001

P. Moreau[1,*], J. Martinez-Lopez[2], M.-V. Mateos[3], J. Bladé[4], S. Lonial[5], L. Benboubker[6], A. Oriol[7], B. Arnulf[8], P. Rodriguez-Otero[9], L. Pineiro[10], A. Jakubowiak[11], C. de Boer[12], J. Wang[13], J. Schecter[13], A. Chari[14]

[1]University Hospital Hôtel-Dieu, Nantes, France, [2]Hospital-12-de-Octubre, Madrid, [3]University Hospital of Salamanca/IBSAL, Salamanca, [4]Hospital Clínic de Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain, [5]Winship Cancer Institute, Emory University, Atlanta, GA, United States, [6]Hôpital Bretonneau, Centre Hospitalier Régional Universitaire (CHRU), Tours, France, [7]Institut Català d'Oncologia and Institut Josep Carreras, Hospital Germans Trias i Pujol, Barcelona, Spain, [8]Hôpital Saint Louis, APHP, Paris, France, [9]Clínica Universidad de Navarra-CIMA, IDISNA, Pamplona, Spain, [10]Texas Oncology-Baylor Charles A. Sammons Cancer Center, Dallas, TX, [11]University of Chicago Medical Center, Chicago, IL, United States, [12]Janssen Biologics, Leiden, Netherlands, [13]Janssen Research & Development, LLC, Raritan, NJ, [14]Tisch Cancer Institute, Mount Sinai School of Medicine, New York, NY, United States

Background: Lenalidomide-refractory patients have poor outcomes, highlighting an unmet medical need. In the phase 1b MMY1001 study (NCT01998971), D-Kd induced deep responses and was well tolerated in patients with relapsed MM.
Aims: We examined the safety and efficacy of D-Kd in lenalidomide-refractory patients.
Methods: In total, 85 carfilzomib-naïve patients with 1-3 prior lines of therapy were enrolled. Patients received carfilzomib (20 mg/m^2 on Cycle 1 Day 1 [C1D1] and 70 mg/m^2 on C1D8+) on Days 1, 8, and 15 of 28-day cycles and dexamethasone 40 mg QW. Daratumumab was given QW C1-C2, Q2W C3-C6, and Q4W thereafter; 10 patients received a standard first dose of daratumumab (16 mg/kg) on C1D1, and 75 patients received a split first dose of daratumumab (8 mg/kg on C1D1 and C1D2). Median infusion times during Week 1 were 7.08 hours (range: 6.5-8.9 h) for patients who received a standard first daratumumab dose and 4.25 hours (range: 3.9-10.6 h) and 4.17 hours (range: 3.9-8.6 h) for Day 1 and Day 2, respectively, among patients who received a split first daratumumab dose. Refractory MM was defined as progression during or within 60 days of completion of the last line of therapy.
Results: Among lenalidomide-refractory patients (n=51) in the MMY1001 D-Kd arm, median age was 66 years (range 38-85 years), and 92% had an Eastern Cooperative Oncology Group status ≤1. Patients had received a median of 2 (range 1-4) prior lines of therapy; 98% had received bortezomib, 18% had received pomalidomide, 43% were refractory to bortezomib, and 18% were refractory to pomalidomide. In total, 20 patients (39%) discontinued due to progressive disease (26%), adverse events (AEs; 6%), patient withdrawal (6%), or physician decision (2%). The most common hematologic grade 3/4 treatment-emergent AEs (TEAEs; ≥10%) were thrombocytopenia (37%), anemia (29%), neutropenia (28%), and lymphopenia (26%). Infusion-related reactions were observed in 37% of patients (43% for standard first daratumumab dose; 36% for split first daratumumab dose); none were grade 3/4. With 8.3 months of median follow up, median progression-free survival (PFS) was 14.1 months (95% CI 9.4-not estimable); the 12-month PFS rate was 69% (95% CI 49-82). Overall response rate (ORR) and minimal residual disease (MRD)-negative rates are summarized in the Table 1. Median time to MRD negativity (10^{-5}) was 5.1 months.

Table 1.

%	Lenalidomide refractory	All
ORR*	81	86
Stringent complete response	8	6
Complete response	4	14
Very good partial response	56	53
Partial response	13	14
MRD-negative rate		
10^{-4}	6	9
10^{-5}	2	5
10^{-6}	0	2

*Among response-evaluable patients who received >2 cycles or discontinued treatment.

Summary and Conclusions: The combination of daratumumab and weekly Kd was well tolerated and demonstrated promising efficacy in lenalidomide-refractory patients. Updated data will be presented.

PF580

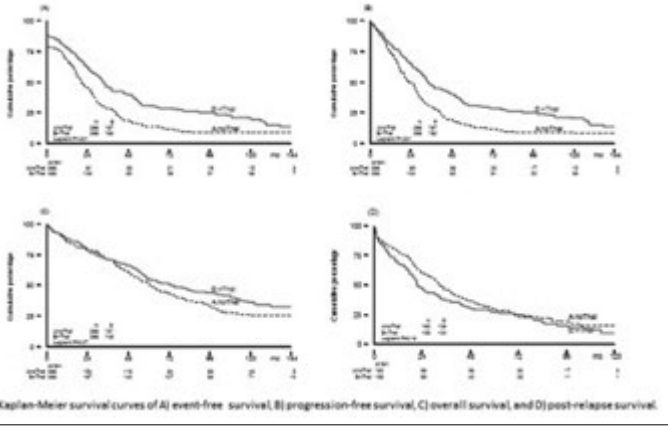
LONG-TERM RESULTS FROM THE HOVON-50 STUDY: IMPROVED SURVIVAL WITH THALIDOMIDE BEFORE AND AFTER TRANSPLANT IN NEWLY DIAGNOSED MULTIPLE MYELOMA

N. Van De Donk[1,*], B. van der Holt[2], M. Minnema[3], E. Vellenga[4], S. Croockewit[5], M.J. Kersten[6], P. von dem Borne[7], P. Ypma[8], R. Schaafsma[9], O. de Weerdt[10], S. Klein[11], M. Delforge[12], M.-D. Levin[13], G. Bos[14], K.-S. Jie[15], H. Sinnige[16], J. Coenen[17], E. de Waal[18], S. Zweegman[1], P. Sonneveld[19], H. Lokhorst[1]

[1]Hematology, VUMC, Amsterdam, [2]HOVON data center, Erasmus MC Cancer Institute, Rotterdam, [3]Hematology, UMC Utrecht Cancer Center, Utrecht, [4]Hematology, University Medical Center Groningen, Groningen, [5]Hematology, Radboud University Medical Center, Nijmegen, [6]Hematology, Academic Medical Center, Amsterdam, [7]Hematology, Leiden University Medical Center, Leiden, [8]Hematology, Haga Hospital, , Den Haag, [9]Hematology, Medisch Spectrum Twente, Enschede, [10]Hematology, Antonius Ziekenhuis, Nieuwegein, Nieuwegein, [11]Internal Medicine, Meander Medical Center, Amersfoort, Netherlands, [12]Hematology, University of Leuven, Leuven, Belgium, [13]Internal Medicine, Albert Schweitzer Hospital, Dordrecht, [14]Hematology, Maastricht University Medical Center, Maastricht, [15]Hematology, Zuyderland Medical Center, Sittard, [16]Hematology, Jeroen Bosch Hospital, , Den Bosch, [17]Hematology, Isala, Zwolle, [18]Hematology, Medisch Centrum Leeuwarden, Leeuwarden, [19]Hematology, Erasmus Medical Center, Rotterdam, Netherlands

Background: The HOVON-50 phase 3 trial compared induction therapy with thalidomide+adriamycine+dexamethasone (TAD) followed by high-dose melphalan (HDM)/autologous stem cell transplantation (auto-SCT) and maintenance therapy with thalidomide until progression (arm A) to induction therapy with vincristine+adriamycine+dexamethasone (VAD) followed by HDM/auto-SCT and interferon-α until progression (arm B) in newly diagnosed multiple myeloma (MM) patients. In 2010 we showed that patients treated in the thalidomide arm had an improved response rate, as well as event-free survival (EFS) and progression-free survival (PFS), compared to the control arm. However, after a median follow-up of 52 months no benefit in terms of overall survival (OS) was observed. Long-term data are needed to determine the sustainability of the initially observed results, and to evaluate possible long-term side effects such as second primary malignancies (SPMs).
Aims: Final analysis with an extended follow-up of median 129 months to evaluate the impact of the first novel agent thalidomide on EFS,PFS, and OS, as well as SPMs in transplant-eligible MM patients.
Methods: A total of 556 MM patients were enrolled in the HOVON-50 trial. EFS was determined from the date of randomization until induction failure (less than PR), progression, or death, whichever came first.PFS was calculated from randomization until progression, relapse, or death, whichever came first. OS was measured from randomization until death from any cause. Cox regression analysis was used to evaluate the impact of treatment arm on survival when adjusted for baseline characteristics. For the current analysis, patients who received an allogeneic stem cell transplantation (allo-SCT; 94 patients) after HDM were censored for EFS,PFS, and OS at the date of allo-SCT. We will denote these three survival endpoints as EFSc,PFSc, and OSc.
Results: The best response achieved on protocol after long-term follow-up was slightly improved when compared to the initial analysis, and remained

significantly higher in the patients randomized to thalidomide (CR rate: 32% *versus* 24%; *P*=0.021). EFSc and PFSc remained significantly prolonged in the thalidomide arm, compared to the control arm (EFSc: hazard ratio (HR)=0.66, 95% confidence interval (CI): 0.54-0.81, *P*<0.0001; PFSc: HR=0.64, 95% CI: 0.52-0.79, *P*<0.0001). With long-term follow-up, we now also observed a superior OSc in the thalidomide arm, when adjusted for covariates in multivariate analysis (HR=0.79, 95% CI: 0.62-0.99, *P*=0.042). Elevated LDH was independently associated with impaired EFSc (HR=0.63, 95% CI: 0.48-0.84, *P*=0.001), PFSc (HR=0.62, 95% CI: 0.47-0.82, *P*<0.001), and OSc (HR=0.41, 95% CI: 0.29-0.58, *P*<0.001). Furthermore, female sex was associated with improved PFSc, while OSc was adversely affected by WHO performance status and ISS stage. Achievement of a CR, included as a time-dependent co-variate, was associated with a significantly enhanced EFSc, PFSc, and OSc. Survival after progression did not differ between both arms. Also the incidence of second primary malignancies (SPMs) was similar in both arms (4% at 5 years and 6-8% at 10 years after randomization) (Figure 1).



Kaplan-Meier survival curves of A) event-free survival, B) progression-free survival, C) overall survival, and D) post-relapse survival.

Figure 1.

Summary and Conclusions: With prolonged follow-up there is not only an EFSc and PFSc benefit for thalidomide before and after HDM/auto-SCT, but when adjusted for covariates, also an OSc benefit, without an increased risk of SPMs. Our data indicate, that thalidomide-based treatment is still a valid treatment option for transplant eligible MM patients in countries without access to proteasome inhibitors or next generation immunomodulatory drugs.

PF581

THE HORIZON STUDY: A PRELIMINARY REPORT ON EFFICACY AND SAFETY OF MELFLUFEN IN LATE STAGE RELAPSED-REFRACTORY MYELOMA (RRMM) PATIENTS REFRACTORY TO POMALIDOMIDE AND/OR DARATUMUMAB

M.V. Mateos[1,*], A. Oriol Rocafiguera[2], P. Rodríguez Otero[3], J. Bladé[4], A. Larocca[5], J.S. Moreb[6], A. Alegre[7], M. Cavo[8], H. Hassoun[9], A. Mazumder[10], C. Maisel[11], A. Paner[12], N. Gabrail[13], K. Halka[14], J. Zonder[15], E.M. Ocio[1], C. Byrne[16], J. Harmenberg[16], E. Nordstrom[16], S. Thuresson[16], H. Zubair[16], P. Richardson[17]

[1]Hospital Clinico Universitario de Salamanca, Salamanca, [2]ICO Badalona -Hospital Germans Trias i Pujol, Badalona, [3]Clinica Universidad de Navarra, Pamplona, [4]Servicio de Onco-Hematologia, Hospital Clinica de Barcelona, Barcelona, Spain, [5]Ematologia U., A.O.U. Città della Salute e della Scienza di Torino-S.C., Torino, Italy, [6]UF Health Shands Cancer Hospital, Gainesville, FL, United States, [7]Hospital Universitario La Princesa, Madrid, Spain, [8]Policlinico S. Orsola Malphigi, Bologna, Italy, [9]Memorial Sloan Kettering Cancer Center, New York, [10]Hudson Valley Hematology & Oncology Associates RLLP, Hawthorne, NY, [11]Baylor Scott & White Charles A. Sammons Cancer Center, Dallas, TX, [12]Rush University Medical Center, Chicago, IL, [13]Gabrail Cancer Center Research, Canton, OH, [14]Scott & White Memorial Hospital and Clinic, Temple, TX, [15]Karmanos Cancer Institute, Detroit, MI, United States, [16]Oncopeptides AB, Stockholm, Sweden, [17]Harvard Medical School, Dana-Farber Cancer Institute, Boston, MA, United States

Background: Melflufen is a next generation alkylator, belonging to the novel class of Peptidase Enhanced Cytotoxics (PEnCs) with a unique mechanism of action designed for efficient targeting of tumor cells. Melflufen provides a peptidase enhanced therapy with an alkylating payload, and triggers rapid, robust and irreversible DNA damage. The effect of melflufen is exerted through alkylation of DNA. The lipophilicity of melflufen leads to rapid and extensive distribution into cells where it is readily metabolized by intracellular peptidases (often over-expressed in malignant cells) into hydrophilic alkylating metabolites leading to 50-fold enrichment of these metabolites in MM cells. In addition, melflufen has potent anti-angiogenic properties.
This is a report on early efficacy of melflufen in RRMM patients that have been exposed to proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs) and are refractory to pomalidomide (pom) and/or daratumumab (dara). A completed Phase 1/2 study of melflufen+dexamethasone (dex) (O-12-M1, NCT01897714) has been previously reported.
Aims: To study the efficacy and safety of melflufen in combination with low dose dex in patients that are refractory to pom and/or dara (NCT02963493).
Methods: Melflufen 40 mg is given i.v. on Day 1 of each 28-day cycle, with dex 40 mg weekly (20 mg for ≥75yrs), in RRMM patients refractory to pom and/or dara with measurable disease and at least 2 prior lines of therapy including an IMiD and a PI. Response is investigator assessed at each cycle by IMWG criteria. The primary objective is overall response rate (ORR). Patients receive treatment until there is disease progression or unacceptable toxicity.
Results: As of 06 Feb 2018, 47 patients (16 ongoing) received a total of 126 doses (1-9 cycles) of melflufen. Median time since diagnosis was 6.6 years (1-16). Median number of prior therapies was 6 (3-12). 100% of patients received prior PIs and IMiDs with 81% refractory to a PI, 98% refractory to an IMiD and 79% refractory to both. 87% had received prior alkylators (incl. 77% prior ASCT) and 74% received prior monoclonal antibodies. 91% were refractory to pom, 66% to dara and 57% to both pom and dara. Of the 47 patients, treatment was ongoing in 16 (34%) and was discontinued in 31 (66%) due to PD (49%), AEs (9%) and other reasons (9%). Treatment-related G3/4 AEs were reported in 31 patients (66%); with those occurring in >10% of the patients included thrombocytopenia (53%), neutropenia (45%) and anemia (21%). Only 2 patients (4%) had a treatment-related G3/4 febrile neutropenia. No single non-hematologic AE occurred in more than 1 patient.
42 patients received ≥1 dose of melflufen and had at least 1 assessment of response and were included in the efficacy analysis. 2 patients (5%) achieved VGPR and 8 (19%) achieved PR for an ORR of 24%. 5 patients achieved MR for a CBR of 36%. 19 patients (45%) had maintained SD as their best response.
Summary and Conclusions: Despite the recent advances in therapy for MM the disease remains incurable. Late stage RRMM patients refractory to pom and/or dara have limited treatment options. Melflufen is well tolerated with few treatment discontinuations due to AEs and has encouraging results with an ORR of 24% and CBR 36% where most other conventional therapies have failed. Recruitment to this Phase 2 study and to the Phase 3 Ocean trial (NCT03151811) is ongoing.

PF582

PHASE I-B STUDY OF ISATUXIMAB+CARFILZOMIB IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA (RRMM)

J. Richter[1], S. Wong[2], A. Chari[3], N. Shah[2], S. Jagannath[3], H.J. Cho[3], N. Biran[1], J. Wolf[2], S. Parekh[3], P. Munster[2], D. Madduri[3], F. Campana[4], T. Martin[2,*]

[1]Medicine, Hackensack University, Hackensack, [2]Medicine, UCSF, San Francisco, [3]Medicine, Mount Sinai Hospital, New York, [4]Oncology, Sanofi-Oncology, Boston, United States

Background: Isatuximab is an anti-CD38 mAb with potent anti-myeloma (MM) activity as monotherapy or in combination with lenalidomide (Len)+dexamethasone (d) in RRMM. Carfilzomib is a next generation proteasome inhibitor approved for use in RRMM as a single agent or in combination with d or Len+d. The hypothesis is that the combination of isatuximab (ISA) and carfilzomib (CFL) may demonstrate potent synergy for the treatment of RRMM.
Aims: The primary objective was to determine the maximum tolerated dose (MTD) of ISA+CFL in RRMM. Secondary objectives included assessment of safety, PK, immunogenicity, and efficacy (per IMWG response criteria (ORR)). (NCT-02332850)
Methods: Eligible patients (pts) had disease progression after 2 prior lines, an ECOG <3, and adequate organ function. Therapy included ISA IV every 2 weeks (Q2W), or weekly (QW) for 4 doses then Q2W together with CFL (20mg/m2 Day 1, 2=>27 mg/m2: Day 8, 9, 15, 16 Q28d and for all subsequent CFL doses). Dosing levels (DL) included: ISA 10 mg/kg Q2W, ISA 10 mg/kg QW x 4 then Q2W and ISA20 mg/kg QW x 4 then Q2W in a 3+3 dose escalation (DE) design. An expansion cohort (EC) of 18 pts was enrolled at ISA 10 mg/kg QW x 4 then Q2W (DL2).
Results: 15 pts have been treated in DE and 18 in the EC. The median age of the 33 pts was 61 (range 39-79) yrs, with a median # of prior lines of 3

(range 2-8). All pts were IMid and PI exposed: 26/29 Len refractory (Refr), 21/29 Bort Refr, 13/29 Pom Refr and 8/11 CFL Refr. Median follow-up is 7m (1 – 27m). 29 pts are evaluable for overall response (ORR). The ORR is 69% (1 sCR, 7 VGPR, 12 PR) and clinical benefit rate is 86% (5 MR). The median progression free survival has not been reached. Disposition: 15 patients have progressed (PD, with 4 deaths from PD), 1 pt withdrew after 27 cycles (for treatment holiday), and 17 remain on therapy. The median # of cycles given is 4 (range 1-27). No DLT or unexpected toxicity has been observed and no patients stopped therapy due to toxicity. The most frequent occurring treatment emergent adverse events (TEAEs-all grades, incidence ≥15%), were thrombocytopenia (66%), pain (back, chest wall, pelvis: 60%), upper respiratory infection (56%), diarrhea (40%), fatigue (40%), anemia (33%), cough (33%), elevated creatinine (30%), nausea (30%), neutropenia (27%), headache (27%), dyspnea (16.7%) and fever (16.7%). Serious AEs were infrequent (SAE=9 events: 4 fever, 3 URI, 1 sinus tachycardia, 1 hypotension) and overall <5% of AEs were Gr 3/4. Infusion reactions (IRs) were the most common ISA-related AEs. Seventeen IRs were reported in 16/32 pts (50%: Gr 1 (9)+Gr 2 (8)).

Summary and Conclusions: Combining ISA and CFL appears safe; with no unexpected toxicity and AEs consistent with the toxicity profile of the individual agents. Encouraging anti-MM activity was seen at all DLs in predominantly refractory patients with an ORR of 69% and CBR of 84%. Although an MTD was not reached at ISA doses of up to 20 mg/kg, the ISA dose of 10 mg/Kg QW x 4 then Q2W has been selected for combination studies. PK and PD studies support the use of ISA at 10 mg/kg when used in combination and 2 Phase III combination studies are underway in RRMM: IKEMA- ISA+CFL+d *versus* CFL+d (NCT03275285) and ICARIA- ISA+Pom +d *versus* Pom+d (NCT02990338).

PF583

IMPACT OF BASELINE RENAL FUNCTION ON EFFICACY AND SAFETY OF DARATUMUMAB PLUS BORTEZOMIB-MELPHALAN-PREDNISONE (VMP) IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS INELIGIBLE FOR TRANSPLANTATION (ALCYONE)

M. Cavo[1,*], M. A. Dimopoulos[2], J. San-Miguel[3], A. Jakubowiak[4], K. Suzuki[5], S.-S. Yoon[6], M. Cook[7], M. Boccadoro[8], P.J. Ho[9], L. Pour[10], S. Knop[11], C. Doyen[12], A. Crepaldi[13], T. Masszi[14], J. Blade[15], J. Wang[16], S. Wroblewski[17], W. Deraedt[18], M. Qi[17], M.-V. Mateos[19]

[1]"Seràgnoli" Institute of Hematology, Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy, [2]National and Kapodistrian University of Athens, Athens, Greece, [3]Clínica Universidad de Navarra-CIMA, IDISNA, CIBERONC, Pamplona, Spain, [4]University of Chicago Medical Center, Chicago, IL, United States, [5]Japanese Red Cross Medical Center, Department of Hematology, Tokyo, Japan, [6]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea, Republic Of, [7]University Hospitals Birmingham NHS Trust, Birmingham, United Kingdom, [8]Myeloma Unit, Division of Hematology, University of Torino, Torino, Italy, [9]Institute of Haematology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia, [10]University Hospital Brno, Brno, Czech Republic, [11]Würzburg University Medical Center, Würzburg, Germany, [12]Université Catholique de Louvain (UcL) CHU UCL Namur, Yvoir, Belgium, [13]Clínica de Tratamento E, Cuiaba, Brazil, [14]Department of Haematology and Stem Cell Transplantation, St László Hospital, 3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary, [15]Servei d'Hematologia, Hospital Clínic de Barcelona, Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain, [16]Janssen Research & Development, Raritan, NJ, [17]Janssen Research & Development, Spring House, PA, United States, [18]Janssen Research & Development, Beerse, Belgium, [19]University Hospital of Salamanca/IBSAL, Salamanca, Spain

Background: In the phase 3 ALCYONE study, daratumumab (D) in combination with VMP (D-VMP) prolonged progression-free survival *versus* VMP alone, and exhibited favorable tolerability in patients with newly diagnosed multiple myeloma.

Aims: We conducted a subgroup analysis of the efficacy and safety of D-VMP *versus* VMP according to baseline creatinine clearance (CrCl; ≤60 mL/min [moderately impaired] and >60 mL/min).

Methods: Randomized patients were ineligible for high-dose chemotherapy with autologous stem cell transplantation and had baseline CrCl ≥40 mL/min. Up to nine 6-week VMP cycles (V 1.3 mg/m^2 subcutaneously on Days 1, 4, 8, 11, 22, 25, 29, 32 in Cycle 1 and Days 1, 8, 22, 29 in Cycles 2-9; M 9 mg/m^2 orally and P 60 mg/m^2 orally on Days 1-4 in Cycles 1-9) with or without D (16 mg/kg intravenously weekly for Cycle 1, every 3 weeks for Cycles 2-9, and every 4 weeks for Cycles 10+ [post VMP-treatment phase] until progression) were received. Minimal residual disease at 10^{-5}threshold was evaluated using clonoSEQ® (Adaptive Biotechnologies).

Results: Among 706 (350 D-VMP; 356 VMP) randomized patients, 295 (150 D-VMP; 145 VMP) patients had baseline CrCl ≤60 mL/min and 411 (200 D-VMP; 211 VMP) patients had baseline CrCl >60 mL/min. For D-VMP *vs* VMP, the median duration of study treatment was 15.3 months *vs* 12.0 months for the ≤60 mL/min subgroup and 14.5 months *vs* 12.0 months for >60 mL/min subgroup, respectively.

D-VMP prolonged progression-free survival *versus* VMP in ≤60 mL/min (median not reached [NR] *vs* 16.9 months; hazard ratio [HR] 0.36; 95% confidence interval [CI] 0.24-0.56) and >60 mL/min (median NR *vs* 18.3 months; HR 0.63; 95% CI 0.45-0.88) subgroups after median follow-up of 16.5 months. Overall response rate benefit was maintained for D-VMP *vs* VMP in the ≤60 mL/min (89% *vs* 73%; ≥complete response [CR]: 43% *vs* 24%) and >60 mL/min (92% *vs* 74%; ≥CR: 43% *vs* 25%) subgroups. Similar findings were observed with minimal residual disease-negative rates in the ≤60 mL/min (25% *vs* 8%; odds ratio [OR] 4.13; 95% CI 2.02-8.46) and >60 mL/min (20% *vs* 5%; OR 4.55; 95% CI 2.26-9.14) subgroups.

Safety findings for D-VMP *versus* VMP are summarized in the Table 1. In the ≤60 mL/min and >60 mL/min subgroups, infusion reactions were observed in 27% (Grade 3/4: 5%/0.7%) and 29% (Grade 3/4: 4%/0.5%), respectively.

Table 1.

	CrCl ≤60 mL/min		CrCl >60 mL/min	
Grade 3/4, %	D-VMP	V MP	D-VMP	V MP
Most common (≥10%) treatment-emergent adverse events				
Neutropenia	47	38	35	39
Thrombocytopenia	43	42	28	34
Anemia	21	29	12	13
Pneumonia	15	6	9	2
Peripheral sensory neuropathy	2	4	1	4

Summary and Conclusions: D in combination with VMP prolongs progression-free survival, induces deep responses, and demonstrates acceptable tolerability regardless of baseline renal function. NCT02195479.

PF584

UPDATE OF THE PHASE 2 STUDY OF CARFILZOMIB, THALIDOMIDE, AND LOW-DOSE DEXAMETHASONE AS INDUCTION/CONSOLIDATION IN NEWLY DIAGNOSED, TRANSPLANT ELIGIBLE PATIENTS WITH MULTIPLE MYELOMA, CARTHADEX TRIAL

R. Wester[1,*], B. van der Holt[1], E. Asselbergs[1], S. Zweegman[2], M.J. Kersten[3], E. Vellenga[4], M. van Marwijk Kooy[5], O. de Weerdt[6], M. Minnema[7], S. Lonergan[1], A. Palumbo[8], H. Lokhorst[2], A. Broijl[1], K. Iskander[9], P. Sonneveld[1]

[1]Hematology, Erasmus MC Cancer Institute, Rotterdam, [2]Hematology, VU University Medical Center, [3]Hematology, Academic Medical Center, Amsterdam, [4]Hematology, Univerisity Medical Center Groningen, Groningen, [5]Hematology, Isala Clinics, Zwolle, [6]Hematology, St. Antonius Hospital, Nieuwegein, [7]Hematology, UMC Utrecht Cancer Center, Utrecht, Netherlands, [8]Hematology, University of Turin, Torino, Italy, [9]Amgen, Thousand Oaks, United States

Background: This phase 2 dose escalation trial investigates the combination of Carfilzomib with Thalidomide and Dexamethasone (KTd) for induction and consolidation in transplant-eligible patients with newly diagnosed multiple myeloma (NDMM). We report the response, progression-free survival (PFS), overall survival (OS) and safety of 4 dose levels of Carfilzomib.

Aims: To investigate the efficacy of KTd for induction and consolidation therapy in NDMM.

Methods: Transplant-eligible patients aged between 18 and 65 years with NDMM were enrolled in this phase 2 trial. Patients were treated with 4 cycles of KTd. The dose of Carfilzomib in the first dose level was 20 mg/m^2 i.v. on days 1, 2 followed by 27 mg/m^2 on days 8, 9, 15, 16 of cycle 1 and on days 1, 2, 8, 9, 15 and 16 of cycles 2 to 4. Thalidomide dose was 200 mg orally on days 1-28 and Dexamethasone dose was 40 mg orally once per week. Carfilzomib was escalated to 20/36 mg/m^2, 20/45 mg/m^2, and 20/56 mg/m^2, in dose level 2,3 and 4 respectively. Induction was followed by stem cell harvest after Cyclophosphamide priming (2 to 4 mg/m^2) and G-CSF. Hereafter patients received high-dose Melphalan (200mg/m^2) and autologous stem cell transplantation followed by consolidation treatment with 4 cycles of KTd in the same schedule except a lower dose of Thalidomide (50mg). Primary endpoint was response after induction and overall, specifically complete response (CR) and very good partial response (VGPR). Secondary endpoints were PFS, OS and safety.

Results: Between September 16, 2010 and January 14, 2014, 111 patients

were enrolled with a median age of 58 years and a median follow-up of 73, 62, 55 and 49 months, in dose level 1-4 respectively. For all dose levels overall response rate was 94%. (s)CR rate after induction was 18%, ≥VGPR rate was 65%, and ≥PR rate was 93%. After HDM (s)CR rate increased to 31% and after consolidation to 63%. (s)CR and VGPR were higher after consolidation in dose levels 2-4 *versus* dose level 1, although not statistically significant (Fisher's exact p=0.17 and p=0.10, respectively). In all patients PFS at 48 months was 58% (95% CI, 48% to 67%). PFS per dose level was 59% (95% CI, 44% to 72%), 50% (95% CI, 27% to 69%), 65% (95% CI, 40% to 82%) and 55% (95% CI, 31% to 73%) in dose level 1,2, 3 and 4 respectively, and in dose levels 2-4 together PFS was 57% (95% CI 43% to 68%). PFS in dose level 1 *versus* dose level 2-4 was comparable (logrank p=0.89) (Figure 1A). In all patients OS at 48 months was 79% (95% CI, 70% to 85%). OS per dose level was 75% (95% CI, 61% to 85%), 75% (95% CI, 50% to 89%), 85% (95% CI, 61% to 95%) and 85% (95% CI, 60% to 95%) respectively. In dose levels 2-4 together OS was 82% (95% CI, 69% to 89%). OS in dose level 1 *versus* dose level 2-4 was comparable (logrank p=0.53) (Figure 1B). Safety analysis of all 111 patients showed grade 3/4 non-hematological toxicity mainly consisting of infections (11%), respiratory disorders (8%) and vascular disorders (9%). Cardiac adverse events were limited and included heart failure (n=3, 2 at 27 mg/m^2, 1 at 56mg/m^2), dyspnea (n=1 at 27mg/m^2) and chest pain (n=1 at 45mg/m^2).

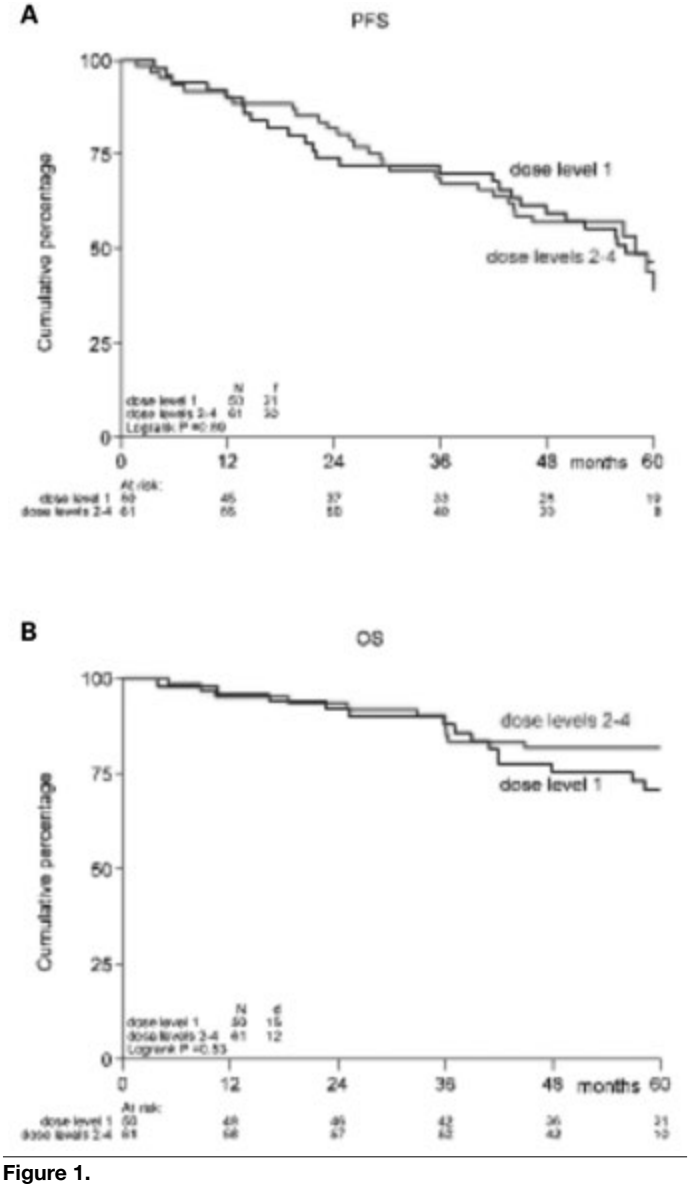


Figure 1.

Summary and Conclusions: KTd is an effective regimen with higher response rates in dose level 2-4 *versus* dose level 1. PFS and OS between dose level 1 and dose levels 2-4 were comparable. This study was registered at www.trialregister.nl as #NTR2422 and is supported by Amgen.

PF585

CLINICAL CHARACTERISTICS OF NEWLY DIAGNOSED PATIENTS WITH AL AMYLOIDOSIS WITH "NON-MEASURABLE" FREE LIGHT CHAINS: PROGNOSTIC IMPLICATIONS AND RESPONSE EVALUATION

M. Gavriatopoulou[1], M. Migkou[1], I. Dialoupi[1], E. Eleutherakis-Papaiakovou[1], D.C. Ziogas[1], N. Kanellias[1], D. Fotiou[1], E. Terpos[1], T. Bagratouni[1], A. Papathoma[2], M. Spyropoulou-Vlachou[2], M.A. Dimopoulos[1], E. Kastritis[1,*]

[1]Department of Clinical Therapeutics, National and Kapodistrian University of Athens, [2]Immunology Unit, "Alexandra" Hospital, Athens, Greece

Background: Current criteria for the assessment of hematologic response in patients with AL amyloidosis are based on the measurement of the difference between the involved and uninvolved serum FLCs (dFLC). These criteria have been validated but require that dFLC should be ≥50 mg/L at baseline in order to assess hematologic responses. However, patients with dFLCs <50 mg/L are excluded from clinical trials as having "non-measurable" disease although the natural history of their disease may not differ from that of patients with "measurable dFLCs". Three different centres have published data indicating that patients with dFLC <50 mg/L may have a more favourable outcome, less cardiac involvement and more often renal involvement.
Aims: Our aim was to evaluate whether patients with light chain (AL) amyloidosis and "non-measurable" dFLCs (i.e with dFLC <50 mg/dL) have different clinical characteristics or outcome than patients with measurable dFLCs
Methods: The analysis included 244 patients with AL amyloidosis treated in a single centre (Department of Clinical Therapeutics, Athens, Greece). Organ involvement was defined according to the 2005 ISA criteria. Hematologic complete response (hemCR) was defined as a negative serum and urine immunofixation and a normal FLC ratio. Serum FLC concentration was measured using the FREELITE assay (The Binding Site, Birmingham, UK).
Results: Forty nine (20%) patients had dFLC <50 mg/L at the time of diagnosis and before initiation of any therapy. These patients had more often renal involvement (84% *vs* 64%, p=0.031), but had less often cardiac involvement (47% *vs* 72%, p=0.004), peripheral nerve (8% *vs* 25%, p=0.021) or soft tissue involvement (8% *vs* 23%, p=0.032). However, median eGFR was lower in patients with dFLC <50 mg/L (48 *vs* 77 ml/min/1.73 m2, p=0.005) and median BM infiltration was 10% *vs* 15% (p<0.001) while distribution per Mayo stage was 29% , 50% & 21% *vs* 17% , 49% & 34% for stage-1, -2, & -3 respectively (p=0.09). There was no difference in the treatments that were given (bortezomib based in 67% *vs* 59% respectively) or ASCT (8% *vs* 6%) (all p>0.5). The median OS was significantly longer for those with dFLC <50 mg/L (1-year OS: 82% *vs* 61%, 3-year OS: 82% *vs* 45%, 5-year OS: 70% *vs* 35%, p=0.001). In multivariate analysis for OS, dFLC<50 mg/L was associated with a 75% reduction in the risk of death (HR: 0.25, 95% CI 0.12-0.56, p=0.001) independently of cardiac involvement and Mayo stage. Regarding renal survival, we found no significant difference in rates of progression to dialysis for the two groups, with and without dFLC <50 mg/L. Among patients with dFLC <50 mgL but with dFLC ≥20 mg/L, the reduction of dFLC at 3 months landmark to <10 mg/L was associated with better OS (3 years OS 100% *vs* 80%).
Summary and Conclusions: About one fifth of newly diagnosed patients with AL amyloidosis have low level dFLC ("non-measurable" by current criteria) and this is associated with significantly better outcome, independently of other standard prognostic factors. This parameter should be included in the standard staging and risk assessment of patients with AL amyloidosis. Furthermore, patients with dFC <50 mg/L should be included in clinical trials as they benefit from reduction of their FLCs to <10 mg/L, and this criterion should be accounted for in the hematologic response criteria.

PF586

SELINEXOR COMBINED WITH POMALIDOMIDE AND LOW DOSE DEXAMETHASONE (SPD) IN A RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENT POPULATION

C. Chen[1,*], H. Sutherland[2], R. Kotb[3], M. Sebag[4], D. White[5], W. Bensinger[6], C. Gasparetto[7], R. LeBlanc[8], C. Venner[9], S. Lentzsch[10], G. Schiller[11], B. Lipe[12], A. Del Col[13], M. Kauffman[14], S. Shacham[14], J. Jeha[14], J.-R. Saint-Martin[14], J. Shah[14], N. Bahlis[15]

[1]Princess Margaret Cancer Center, Toronto, [2]Vancouver General Hospital, Vancouver, [3]Cancer Care Manitoba, Manitoba, [4]Royal Victoria Hospital, Montreal, [5]Dalhousie University and QEII Health Sciences Center, Halifax, Canada, [6]Swedish Cancer Center, Seattle, [7]Duke University Cancer Center, Durham, United States, [8]Hôpital Maisonneuve-Rosemont, Montreal, [9]Cross Cancer Institute, Edmonton, Canada, [10]Columbia University, New York, [11]UCLA Ronald Reagan Medical Center, Los Angeles, [12]University of Rochester Medical College, New York, United States, [13]Myeloma Canada, Laval,

Canada, [14]Karyopharm Therapeutics, Newton, United States, [15]Southern Alberta Cancer Research Institute, Calgary, Canada

Background: The nuclear export protein exportin 1 (XPO1) is overexpressed in a wide variety of cancers including multiple myeloma (MM). Selinexor is a first-in-class Selective Inhibitor of Nuclear Export (SINE) compound that binds and inactivates XPO1. Selinexor forces nuclear retention and reactivation of cell cycle regulators such as p53, IkB, and Rb. Pomalidomide/dexamethasone (Pd) is approved in relapsed/refractory MM (RRMM) with an overall response rate (ORR) of 30% and progression-free survival (PFS) rate of 3.6 months in patients (pts) having received a prior proteasome inhibitor (PI) and IMiD. Strategies to improve the ORR and PFS are needed. In murine MM models, the combination of selinexor with IMiDs shows synergistic anti-MM activity and good tolerability.
Aims: This Ph 1b/2 (NCT02343042), dose escalation study was designed to determine the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) for the safety, tolerability and efficacy of the combination of selinexor, pomalidomide, and low dose dex (SPd) in pts with RRMM.
Methods: Pts with RRMM who received ≥2 prior therapies including lenalidomide (len) and a PI were enrolled. Selinexor was evaluated in 2 different dosing schedules of once-weekly (QW, 60; 80 mg) or twice-weekly (BIW, 60; 80 mg), with pomalidomide (pom) 4 mg PO daily, and dexamethasone (dex) 20 mg BIW or 40 mg QW.
Results: As of Feb 27th 2018, 33 pts (16 male / 17 female) have been enrolled.The median age is 61 years, with a median of 4 (range, 2 – 9) prior treatment regimens. Thirty-one patients were IMiD refractory (20 len, 11 pom/len). Six dose limiting DLTs were observed: G3 fatigue (60 mg BIW, pom 4 mg), G3 febrile neutropenia (FN) (60 mg BIW, pom 3 mg), G3 FN and G4 neutropenia (ANC) (80 mg QW, pom 4), G3 thrombocytopenia (PLT) (80 mg QW, pom 3 mg) and 4 missed doses in Cycle 1 due to symptomatic hyponatremia (80 mg BIW, pom 4 mg). Enrollment on selinexor 80 mg QW, pom 3 mg is ongoing. Common treatment related Grade 1/2 adverse events (AEs) include: nausea (48%), fatigue (42%), anorexia (45%) and diarrhea (30%). Grade 3/4 AEs include: ANC (55%), PLT (30%), and anemia (27%). Twenty-seven pts were evaluable for response. Responses rates can be seen in Table 1. Median PFS is 11.6 months with a median follow up of 7.7 months.

Table 1. SPd – Response rates in evaluable patients.

Prior Therapy Status	N*	ORR	CBR
Pom Naïve & Len Refractory or Relapsed	19	12 (63%)	14 (74%)
Pom & Len Refractory	8	3 (38%)	5 (63%)

Overall Response Rate= VGPR + PR, Clinical Benefit Rate= VGPR + PR + MR (*Includes 1 unconfirmed PR and 1 unconfirmed MR)

Summary and Conclusions: Conclusions – Enrollment is ongoing to evaluating once weekly selinexor in combination with Pd. This all oral combination of selinexor, pom and dex (SPd) has significant clinical activity with an ORR 63% in pom naive pts with heavily pretreated MM compared to previously published data of 30% ORR. No unexpected adverse events were noted. Phase 1 dose escalation of the combination of SPd is ongoing to define the RP2D. SPd appears active and supports further clinical development in RRMM.

PF587

ABSENCE OF BONE DISEASE IS AN INDEPENDENT FAVORABLE PROGNOSTIC FACTOR OF OVERALL SURVIVAL IN SYMPTOMATIC MULTIPLE MYELOMA PATIENTS TREATED WITH NOVEL AGENTS

E. Katodritou[1,*], T. Triantafyllou[1], V. Palaska[1], N. Drougos[1], E. Verrou[1], K. Psianou[1], K. Loga[1], K. Kokoviadou[2], K. Keramidioti[1], N. Karampatzakis[1], E. Giannopoulou[1], V. Gastari[1], C. Chadjiaggelidou[1], D. Mallouri[3], G. Kaiafa[4], A. Kioumi[2], A. Anagnostopoulos[3], P. Konstantinidou[1], E. Terpos[5]

[1]Hematology, Theaganion Cancer Hospital, [2]Hematology, Papageorgiou General Hospital, [3]Hematology and Bone Marrow Transplantation Unit, George Papanikolaou General Hospital, [4]Hematology, AHEPA University Hospital, THESSALONIKI, [5]Clinical Therapeutics, National and Kapodistrian University, ATHENS, Greece

Background: Bone disease is a common clinical manifestation in Multiple Myeloma (MM). However, a remarkable number of patients are diagnosed with symptomatic MM without evidence of bone disease. The prognostic role of absence of bone disease has not been investigated.
Aims: We evaluated the clinical characteristics of patients presenting without bone lesions and we examined the possible prognostic significance of bone disease absence in symptomatic MM patients treated with novel agents.
Methods: We studied the medical records of patients with symptomatic MM defined by conventional criteria (*i.e.* anemia and/or hypercalcemia/and or renal insufficiency and/or lytic bone lesions) diagnosed and treated upfront with novel agent combinations. We compared those with symptomatic disease excluding bone lesions (group A) with those who had documented bone disease by conventional X-ray imaging (group B); computer tomography was performed per protocol, according to clinical indications. Patients characteristics between the 2 groups were compared with standard methods. Progression-free survival (PFS) and overall survival (OS) were plotted with the Kaplan Meier curve. Prognostic factors for OS were evaluated with cox regression; p<0.05 was considered as statistically significant.
Results: Three-hundred thirty-eight consecutive symptomatic MM patients were evaluated (M/F: 179/159, median age 67, range: 34-88, IgG: 184, IgA: 89, light-chain: 54, non-secretory: 11, ISS1: 101, ISS2: 105, ISS3: 132); 96/338 (28%) presented at diagnosis without bone disease. Patients characteristics such as median age, β2 microglobulin, lactate dehydrogenase (LDH), hemoglobin, platelets, albumin, creatinine, calcium, International staging system (ISS), revised-ISS (R-ISS), type of MM and high-risk cytogenetics were well balanced between groups. Patients with bone disease had more frequent worse performance status according to Eastern Cooperative Oncology Group (ECOG scale 3/4: 40% *vs* 22%, respectively; p=0.003). All patients were treated with novel agents in 1st line, and 20% underwent autologous transplantation. Type of first or second line treatment did not differ between the 2 groups; no difference in response rates or progression-free survival (PFS) after induction therapy was observed; PFS2 was marginally longer in patients without bone disease (49 months *vs* 36 months; p=0.058). After a median follow up of 71 months (95% CI: 61-80), the median OS for patients without bone disease was 65 months (95% CI: 46-84) *vs* 45 (95% CI: 37-53) for those with documented bone disease (log rank p<0.001); In the univariate analysis, ISS, R-ISS, high risk cytogenetics, estimated glomerular filtration rate (eGFR) <40ml/min/1.73m^2, LDH ≥300U/L and absence of bone lesions were predicted for OS (p<0.05 for all parameters); absence of bone lesions, and R-ISS were significant predictors for OS in the multivariate analysis (HzR: 0.64, p= 0.03, R-ISS1-3: HzR: 0.28, p<0.001,R-ISS2-3 HzR: 0.48, p=0.007, respectively) (Figure 1).

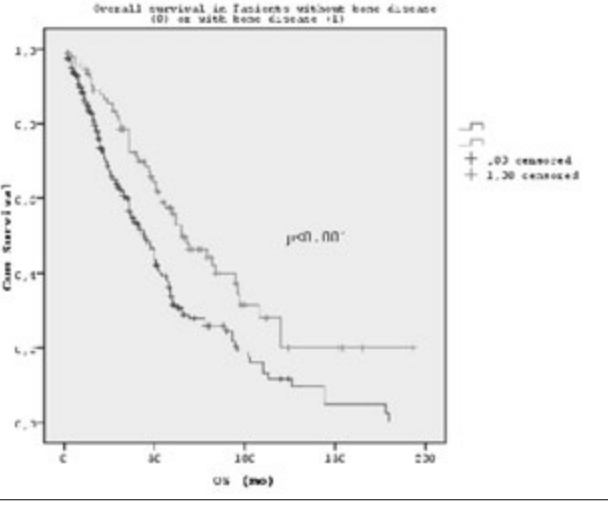


Figure 1.

Summary and Conclusions: Based on our analysis the absence of bone disease at diagnosis of symptomatic MM patients is one of the most powerful prognostic factors for OS, leading to a 36% reduction of death probability; of note, patients without bone disease exhibited marginally longer PFS2, suggesting that they may display a more sensitive first relapse. Further investigation is needed to confirm this finding, using modern imaging for the definition of bone disease in order to integrate this marker, in possible future prognostic models.

PF588

CORRELATION OF 11C-METHIONINE-PET AND FDG-PET IN MULTIPLE MYELOMA WITH MRD ASSESSMENT BY FLOWCYTOMETRY

M. Marcos[1,*], M.I. Morales[2], S. Villar[1], R. Figueroa[3], L.E. Tamariz[1], P. Rodríguez[1], A. Alfonso[1], R. Lecumberri[1], F. Prosper[1], C. Panizo[1], B. Paiva[4], M.J. García-Velloso[2], J. San Miguel[1]

[1]Hematology, [2]Nuclear Medicine, Clínica Universidad de Navarra, Pamplona, [3]Hema-

tology, Clínica Universidad de Navarra, Madrid, [4]Cytometry, Centro de Investigación médica aplicada Universidad de Navarra, Pamplona, Spain

Background: In 2016, the International Myeloma Working Group (IMWG) updated their criteria for response assessment in multiple myeloma (MM) and for the first time, 18FDG-PET/CT was included as a tool to define a imaging/metabolic complete response. However, standarization of 18FDG-PET/CT has not been yet achieved and both lack of specificity and sensitivity are important concerns. For this reason, other trazers such as 11C-methionine (MET) has recently emerged as a potentially more sensitive marker to evaluate tumor burden and disease activity in MM patients.
Aims: The purpose of this study is to evaluate the performance of 11C-MET-PET compared to 18FDG-PET. First, we have evaluated the correlation between both metabolic trazers (MET and FDG) and second, we will correlate the PET results with immunophenotypic response using multiparametric flow citometry (MFC) applying the EUROFLOW protocol (MRD 10^{-6}).
Methods: Between September 2016 and February 2018, both MET and FDG-PET were performed for staging or restaging in a total of 53 patients (61.8% male, range 11-78 years old): plasmacytoma (n=9), smoldering MM (SMM, n=3), and symptomatic MM (n=41). In 37 patients PET and MFC exams were simultaneously performed: 7 cases at diagnosis, 13 patients were in stringent complete response (sCR), 4 were in very good partial response (VGPR), two in partial response (PR), one with stable disease (SE) and 10 with progressive disease (PD). Nine out of 37 patients (23.4%) were MRD negative by using MFC. We compared frequency in dicothomous variables by Fisher exact test or Chi Squared depending on expected values.
Results: We found that MET-PET was more sensitive than FDG-PET: MET-PET was positive (detected focal lesions) in 38/52 subjects (73.1%), whereas FDG-PET/CT showed lesions in only 27 patients (51.9%; p<0.0001).
17 patients were studied at diagnosis or relapse (all with BM infiltration confirmed by MFC) and 20 cases were analyze after treatment for MRD assessment. At time of diagnosis/PD, we were able to detect focal lessions in all patients (sensitivity 100%) with PET-MET; however, FDG-PET negativity was observed in two over 20 (10%) patients. As above mentioned, at response evaluation, 20 patients were simultaneously studied for MRD by MFC and PET, upon correlating MET-PET and MFC, discordant results were found in 7/20 (35%, p=ns): MET-PET was positive with MRD negative in 4 (20%), the opposite (negative MET-PET and positive MRD) was observed in 3 patients (15%). The comparison between FDG-PET and MFC showed that 8/20 (40%, p=ns) patients had discordant results: FDG-PET positive and MRD negative occurred in only one case (5%) while 7 (35%) patients had FDG-PET negative and MRD positive.
Summary and Conclusions: This study demonstrates that MET has higher sensitivity in comparison to standard FDG to detect myelomatous disease both at diagnosis and at the time of MRD. Moreover, MET showed to be positive in 4/7 negative FDG cases that remained as MRD positive. Overall, these results confirm the complementarity and the value of high sensitive technologies to evaluate myeloma response. Further analysis will contribute to elucidate if MET has also a better predictive value as compared to FDG in the longterm follow-up.

PF589

TRANSLOCATION (14;16) POSITIVE MULTIPLE MYELOMA PATIENTS: CLINICAL FEATURES AND SURVIVAL OUTCOMES OF A HIGH-RISK POPULATION.

R. Mina[1,2,*], N. Joseph[3], F. Gay[1], A. Nooka[2], E. Kastritis[4], S. Spada[1], M.T. Petrucci[5], J.L. Kaufman[2], V. Montefusco[5], M. Gavriatopulous[4], F. Patriarca[5], M. Gilestro[1], L.H. Boise[2], C. Conticello[5], S. Grandi[1], M. Roussou[4], N. Giuliani[5], A. M. Liberati[5], D. Almaula[3], M. Offidani[5], R. Ribolla[5], D.L. Jaye[6], L. De Paoli[5], E. Terpos[4], S. Lonial[2], M. Boccadoro[1], M.A. Dimopoulos[4]

[1]Myeloma Unit, Division of Hematology, University of Torino, Torino, Italy, [2]Winship Cancer Institute/ Hematology and Medical Oncology, [3]Emory University, atlanta, United States, [4]Department of Clinical Therapeutics, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece, [5]Italian Multiple Myeloma Network, GIMEMA, Italy, [6]Department of Pathology, Emory University, Atlanta, , atlanta, United States

Background: Multiple myeloma (MM) is characterized by recurrent chromosomal abnormalities (CA); among those involving IgH genes, t(4;14), t(14;16) and t(14;20) have been associated with shorter survival. However, t(14;16) incidence is less than 5% in newly-diagnosed (NDMM) patients, so that the clinical features and outcomes of these patients have not been thoroughly described.
Aims: We retrospectively reviewed databases of patients treated in the context of Italian and EMN clinical trials (n=76), as well as patients treated at Winship Cancer Institute (WCI) at Emory University, USA (n=29), and the Department of Clinical Therapeutics, National and Kapodistrian University (NKU) of Athens, Greece (n=18).
Methods: Patients diagnosed between December 2006 and March 2017, carrying t(14;16) detected by FISH at diagnosis, were pooled. Baseline characteristics, response to 1st line treatment and survival outcomes were analysed.
Results: A total of 123 patients were evaluated. Median age at diagnosis was 66 years (range 38-87 years); 49% of patients presented with haemoglobin ≤10 g/dl and 11% with platelets ≤100 x10^9/L. Lactate dehydrogenase (LDH) levels above the upper limit of normal (ULN), hypercalcemia and creatinine values >2 mg/dl were observed in 19%, 17% and 13% of the patients, respectively and 43% of patients had ISS and R-ISS stage 3 disease.Eighty-two% of patients had at least 1 additional unfavourable CA, including del13q (71%), del17p (22%) and 1q gain (27%). Interestingly, concomitant t(4;14) or t(11;14) was observed in 11% and 4% of patients, respectively.Induction therapy consisted of an immunomodulatory drug, a proteasome inhibitor or a combination of both in 43%, 29% and 28% of patients, respectively. One fourth of the patients received consolidation with autologous transplantation in first remission (ASCT-1) and 43% of patients received maintenance therapy. Median progression-free survival (PFS) and overall survival (OS) for the entire cohort were 19 and 53 months, respectively (Figure 1). Among ASCT-eligible patients, those who received ASCT-1 had longer median PFS (31 *vs* 10 months; HR: 0.35, p=0.003) and OS (58 *vs* 34 months; HR 0.48, p=0.04) as compared to those who did not. Maintenance therapy significantly prolonged median PFS (36 *vs* 19 months, HR:0.56; p=0.03) as compared to no maintenance. The best response to 1st line treatment was ≥partial response (PR) in 83% of patients, with 26% of ≥complete remission (CR). Patients achieving ≥CR had prolonged PFS (HR:0.29, p<0.001) as compared to patients in PR/very-good PR. In the multivariate analysis, baseline hypercalcemia and beta-2-microglobulin >ULN were associated with significantly shorter PFS (HR: 2.3, p=0.02; HR: 2.7, p=0.001, respectively) and OS (HR: 5.4, p<0.001; HR: 1.9, p=0.02, respectively), while ASCT-1 (HR:0.3, p=0.001) and maintenance therapy (HR:0.43, p=0.07) confirmed their advantage in terms of PFS.

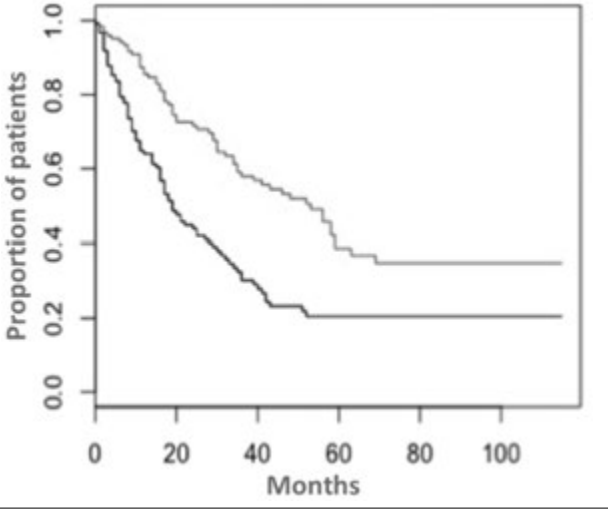


Figure 1.

Summary and Conclusions: In the largest cohort of t(14:16) patients described to date, t(14;16) characterizes a group of high-risk MM patients, presenting with frequent concurrent additional adverse CAs and advanced stage disease (R-ISS3), that result into a poor PFS and OS. Treatment intensification with ASCT in first remission and maintenance therapy prolonged survival of patients with t(14;16). Novel approaches and clinical trials are needed to improve outcomes of this high risk population.

PF590

THE SWEDISH MYELOMA REGISTRY: INCREASED SURVIVAL OVER TIME IN PATIENTS 66-80 YEARS OLD, DIAGNOSED 2008-2016

C.H. Blimark[1,*], A. Genell[2], B. Björkstrand[3], K. Carlson[4], K. Forsberg[5], G. Juliusson[6], B. Lauri[7], O. LInder[8], H. Nahi[3], R. Svensson[9], L. Veskovski[10], I. Turesson[11]

[1]Department of Hematology, Sahlgrenska University Hospital, Institution of Internal Medicine, Sahlgrenska Academy at University of Gothenburg,, [2]Regional Cancer Center

West, Western Sweden Health Care Region, Gothenburg, [3]Department of Hematology, Karolinska Institutet, Stockholm, [4]Department of Hematology, Uppsala University Hospital, Uppsala, [5]Department of Hematology, Umeå University Hospital, Umeå, [6]Hematology/Transplantation, Stem cell center, Skåne University Hospital, Lund, [7]Department of Internal medicine, Sunnerbyn sjukhus, Luleå, [8]Department of Hematology, Örebro University Hospital, Örebro, [9]Department of Hematology, Linköping University Hospital, Linköping, [10]Department of Hematology, Borås Hospital, Borås, [11]Department of Hematology, Skåne University Hospital, Malmö, Sweden

Background: The Swedish Myeloma Registry (SMR) is a prospective observational register documenting real-world management and outcomes in multiple myeloma (MM), smoldering multiple myeloma (SMM), solitary skeletal plasmacytoma (SSP), extramedullary plasmacytoma (EMP), and plasma-cell-leukemia (PCL) since 2008.
Aims: We present data with focus on treatment, response and survival.
Methods: Data were collected through report-sheets at diagnosis to all clinicians diagnosing any of the diagnoses in the register and, for MM, a year later after follow-up on initial treatment and complications.Using diagnostic data 2008-2016 and follow-up data on MM-patients diagnosed 2008-2015, we estimated baseline-characteristics and relative survival (RS). End of follow-up was at death or November 1, 2017, when data was extracted from the register.
Results: We present baseline-data on 6000 patients (coverage 98% compared to the mandatory Swedish Cancer Registry) and, at data from 1-year-follow-up on 4262 MM patients. The median age was 71 years (range 19-98), and 57% men and 43% women, twenty-four per cent were 80 years and above at diagnosis. In first line treatment, the use of high dose Melphalan and stem cell support (ASCT) was stable in the cohort 65 years and younger (ca 80%), and increasing in the patients 66-70 years (from 23 to 35%), the newer drugs bortezomib, thalidomide and lenaldomide were used in 85%, 76%, and 41% in the age cohorts <=65 years, 66-80 years, and 80+ years, respectively. In the study period 2008 to 2015, the use of the above drugs in 1st line treatment increased from 36% to 98% in the age cohort 65 years and younger, from 46% to 91% in patients 66-80 years, and from 21% to 64% in the cohort above 80 years. In the period 2008-2014, we could note a significant increase in response rates after 1st line treatment, from 60% 2008 to 76% VGPR or better in patients 65 years and younger, in the cohort 66-80 years and 80 and above, the figures were 35% 2008 to 60% 2014 and 24% 2008 to 40% 2014, respectively. (Data for 2015 too few to report). Survival: For MM, SMM, SSP and PCL the median RS (with 95% C.I) was 4.24 (4.06-4.46), 8.21 (6.77-9.08), 9.68 (8.70-NA) and 0.89 (0.65-1.68) years.The median RS for ESP was not reached/not available (NA) during the observed time. In the 3-year RS in all patients, there was a signficant survival benefit for patients diagnosed 2011 or later; 67.9%(95%CI 65.9-69.8) *vs* 63% (95%CI 60.8-64.9). This was most evident in the age-group 66-80 year, where in the periods of diagnosis <=2011 and >2011 the 3-year RS was 61.7 percent (95% C.I. 58.8-64.8 percent) and 70.7 percent (68.0-73.5 percent) (Figure 1).

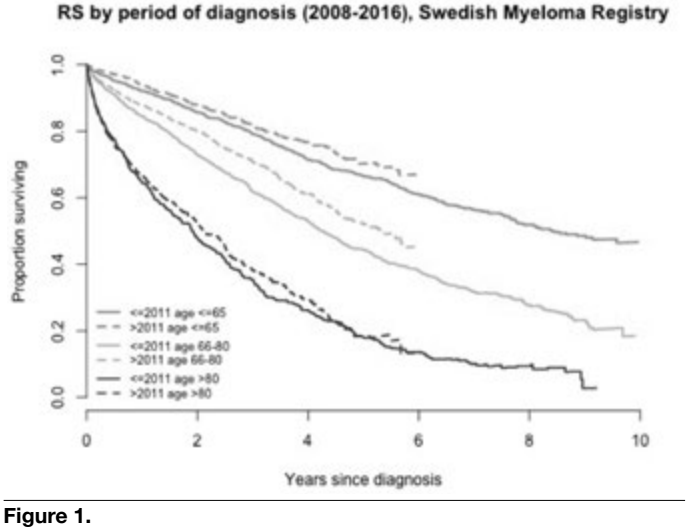


Figure 1.

Summary and Conclusions: In the Swedish Myeloma Registry, survival in MM, SMM and plasmocytomas are encouraging, and our data suggest an increased survival over time, especially in the age-group 66-80 years at diagnosis. We find an increased utilization of newer drugs and increasing response rates after 1st line treatment in the study period. There is still an need for improvement in the treatment of the very elderly. These results supports the need for population-based data as a valuable compliment to randomized clinical trials to monitor the real world outcome and survival in all patients with plasma cell disorders.

PF591

18F-FDG PET/CT AT DIAGNOSIS IS USEFUL FOR DETERMINING THE SURVIVAL OUTCOMES OF PATIENTS WITH MULTIPLE MYELOMA CLASSIFIED AS STAGE II WITH THE REVISED INTERNATIONAL STAGING SYSTEM

S.-H. Jung[1,*], S.-Y. Ahn[1], S.-S. Lee[1], D.-H. Yang[1], J.-S. Ahn[1], H.-J. Kim[1], J.-J. Lee[1]

[1]Chonnam National University Hwasun Hospital, Hwasun-Eup, Korea, Republic Of

Background: Although the Revised International Staging System (R-ISS) was validated in an analysis of an independent cohort of unselected patients with multiple myeloma (MM), stage II problematic increased and included a more heterogeneous population with various risk factors.
Aims: Therefore, we evaluated the prognostic role of 18F-FDG PET/CT at diagnosis in newly diagnosed patients with MM classified as stage II.
Methods: We retrospectively analyzed the records of 168 patients with newly diagnosed MM between February 2012 and March 2017 at Chonnam National University Hwasun Hospital.
Results: Using the R-ISS, 13.1% of patients were R-ISS I, 67.9% were R-ISS II, and 19.0% were R-ISS III. ISS was not prognostic for progression free survival (PFS) and overall survival (OS) when applied to the all patients, but the median PFS and OS were significantly different by the three stages of R-ISS. In total cohort, patients with more than 3 hypermetabolic focal lesion (FL) at baseline PET/CT showed significantly inferior PFS and OS than other patients (PFS, 31.0 months *vs* 16.7 months, P <0.001; OS, not reached *vs* 43.3 months, P=0.006). In addition, patients with extramedullary disease at baseline PET/CT had significantly inferior PFS and OS than those without extramedullary disease (PFS, 24.6 months *vs* 17.2 months, P <0.010; OS, P=0.006). In patients with R-ISS II, 54 patients (47.3%) had more than 3 hypermetabolic FL or extramedullary disease at baseline PET/CT, and the presence of hypermetabolic FLs (>3) or extramedullary disease was significantly associated with shorter PFS and OS (PFS, 31.0 months *vs* 16.4 months, P=0.001; OS, not reached *vs* 43.3 months, P=0.018). In multivariate analysis, the presence of >3 hypermetabolic FLs or extramedullary disease at baseline PET/CT was significantly associated with inferior PFS and OS in patients with R-ISS II (HR 2.439, 95%CI 1.402-4.243, P=0.002).
Summary and Conclusions: In conclusion, PET/CT at diagnosis may be useful for determining the survival outcomes of patients with R-ISS II.

PF592

HAEMATOLOGICAL DIAGNOSIS AND MANAGEMENT IN MONOCLONAL GAMMOPATHY OF RENAL SIGNIFICANCE: SINGLE CENTRE EXPERIENCE

E. Troy-Barnes[1,*], A. Kousios[1], R. Burrell[1], N. Duncan[1], T. Cook[2], H. Auner[3], C. Jakob[3], M. Bua[3], R. Charif[1], C. Roufosse[2], A. Chaidos[3]

[1]Renal Department, [2]Histopathology Department, [3]Department of Haematology, Imperial College Healthcare NHS Trust, London, United Kingdom

Background: Monoclonal Gammopathy of Renal Significance (MGRS) is a recently introduced term to describe kidney lesions caused by monoclonal immunoglobulins produced by B cell or plasma cell clonal disorders, which otherwise do not meet criteria of symptomatic lymphoma or myeloma and have no haematological indication for treatment. The size of the pathogenic clone is often small and occasionally it is undetectable in bone marrow biopsy. Haematological diagnosis could be challenging, but important for treatment decisions. Unlike symptomatic lymphoma and myeloma, treatment objective in MGRS is the preservation of kidney function.
Aims: We describe the spectrum of MGRS diagnoses made at the Hammersmith Hospital between April 2006 and September 2017.
Methods: We reviewed the records of all native kidney biopsies, haematological diagnosis and treatment in the above period.
Results: Out of 4,374 native kidney biopsies, monoclonal immunoglobulin associated lesions were identified in 163 cases (3.7%). After exclusion of symptomatic MM and lymphomas 60 biopsies (1.4%) were consistent with MGRS. The median age at diagnosis was 66 years (range 25-87 years). Renal histological diagnoses included: AIg amyloidosis (n=22, 37%), proliferative glomerulonephritis with monoclonal immunoglobulin deposits (PGNMID) (n=20, 33%), monoclonal immunoglobulin deposition disease

(MIDD) (n=10, 17%), light chain tubulopathy (n=1, 1.7%), type-1 cryoglobulinaemic glomerulonephritis (n=4, 6.7%), fibrillary glomerulopathy (n=2, 3.3%) and C3 glomerulonephritis (n=1, 1.7%). Mean eGFR at presentation was 54ml/min/1.73m². Thirty two patients presented (53%) presented with nephrotic range proteinuria (urine protein: creatinine ratio >300 mg/mmol) and of those 15 patients (47%) had eGFR>60ml/min/1.73m². Haematological diagnosis was possible in bone marrow histology in 49 patients. These included MGUS (n=32, 65%), smouldering myeloma (n=11, 22%), lymphoplasmacytic lymphoma / Waldenstrom's macroglobulinaemia (n=2, 4%), chronic lymphocytic leukaemia (n=2, 4%) and marginal zone lymphoma (n=1, 2%). Almost half of PGNMID cases (n=11) did not have a detectable clonal B cell or plasma cell clone in bone marrow biopsy. MGUS and smouldering myeloma cases were treated with chemotherapy indicated for myeloma. This included bortezomib-based protocols (VCD, VD), high-dose melphalan and autologous stem cell transplantation and cyclophosphamide with steroids with or without thalidomide. PGNMID treatment was heterogenous, including glomerulonephritis protocols (cyclophosphamide, steroids with or without Rituximab), myeloma regimens in confirmed plasma cell histology or monitoring only in cases with stage 1-2 CKD without significant proteinuria.

Summary and Conclusions: MGRS is an increasingly recognised clinical entity since its introduction by the International Kidney Myeloma Group (IKMG) in 2012. Haematological assessment of MGRS must exclude symptomatic myeloma or lymphoma and identify the pathogenic clone to direct specific therapy. Consensus diagnostic and treatment approaches are missing, at least for entities other than AIg amyloidosis. Case studies in our series suggest that rapid and deep haematological response offers the best renal outcome, an observation that follows the treatment paradigm of AIg amyloidosis.

PF593

IXAZOMIB, LENALIDOMIDE AND DEXAMETHASONE FOR RELAPSED AND REFRACTORY MULTIPLE MYELOMA – DATA FROM THE CZECH REGISTRY OF MONOCLONAL GAMMOPATHIES

J. Minarik[1,*], R. Hajek[2], A. Jungova[3], M. Mistrik[4], T. Pika[1], E. Gregora[5], J. Radocha[6], J. Straub[7], L. Pour[8], M. Wrobel[9], L. Brozova[10], J. Bacovsky[1], P. Krhovska[1], K. Hradska[2], P. Jindra[3], P. Pavlicek[5], K. Machalkova[6], I. Spicka[7], M. Stork[8], T. Jelinek[2], H. Plonkova[2], V. Scudla[1], V. Maisnar[6]

[1]Department of Hemato-Oncology, University Hospital Olomouc, Olomouc, [2]Department of Hematooncology, University Hospital Ostrava and Faculty of Medicine University of Ostrava, Ostrava, [3]Hematology and Oncology Department, Charles University Hospital Pilsen, Pilsen, Czech Republic, [4]Department of Hematology and Transfusiology, Faculty of Medicine, University Hospital Bratislava, Bratislava, Slovakia, [5]Department of Internal Medicine and Hematology, 3rd Faculty of Medicine, Charles University and Faculty Hospital Kralovske Vinohrady, Prague, [6]4th Department of Internal Medicine – Hematology, Faculty Hospital and Charles University in Hradec Kralove, Hradec Kralove, [7]1st Medical Department – Clinical Department of Haematology, First Faculty of Medicine and General Teaching Hospital Charles University, Prague, [8]Department of Internal Medicine, Hematology and Oncology, University Hospital Brno and Faculty of Medicine Masaryk University, Brno, [9]Department of Hematology, Hospital Novy Jicin, Novy Jicin, [10] Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Background: The combination of ixazomib, lenalidomide and dexamethasone (IRD) showed in the Tourmaline-MM1 trial an outstanding efficacy in relapsed and refractory multiple myeloma (RRMM).

Aims: The aim of our study was to evaluate the outcomes of IRD in a …real world" setting.

Methods: We analysed data of 118 patients with RRMM who started IRD treatment within a compassionate Named Patient Program between January 2016 and November 2017. The data were acquired from the Czech Registry of Monoclonal Gammopathies (RMG) and included eight hematological centres from the Czech Republic and Slovakia. Median age was 66,5 years (41-84) with M/F ratio 1,2:1 and standard representation of individual M-protein isotypes and ISS stage. Altogether 15,3% had extramedullary disease, 5,9% had elevated creatinine levels above 176umol/l. High-risk cytogenetics, including t(4;14), t(14;16) and del17 was found only in 13 patients, precluding valid statistical analysis. Most patients received IRD for their first relapse (58,5%), followed by second (23,7%) and third relapse (7,6%), some patients being treated in their fourth (5,9%), or higher relapse (4,2%). Most patients received prior bortezomib (94,1%), thalidomide (40,7%) with minor pre-treatment with lenalidomide (16,9%) or carfilzomib (5,9%). 74 patients (62,7%) underwent previous autologous stem cell transplantation (ASCT).

Results: The median follow up was 9,7 months. The therapeutic response was assessed according to IMWG criteria in 76 patients evaluable at the time of data collection. Complete response (CR) was in 11,8%, very good partial response (VGPR) in 15,8%, partial response (PR) in 40,8%, minimal response (MR) in 9,2%, stable disease in 14,5% and progressive disease (PD) in 7,9%. Overall response rate (ORR=PR and better) was 68,4% and Clinical benefit rate (CBR=MR and better) was 77,6%. Median time to response was 1,6 months.The median overall survival (OS) was not reached with 12 month OS 77,4% and 24 month OS 68,8%. Median progression free survival (PFS) was 23,1 months with 12 month PFS 60,3% and 24 month PFS 42%. Patients treated in the first relapse had significantly better PFS (median not reached) than in the second (23,1 months), third (8,7 months) or higher relapse (4,6 months). Patients with extramedullary plasmocytoma (N=18) had worse PFS (median 9,0 months) than the rest of the cohort. Most of the toxicities were grade ≤2. Grade ≥3 reached only neutropenia (37,7%), thrombocytopenia (24,5%), infection (18,9%), anemia (13,2%), other toxicities such as fatigue, neuropathy, exanthema, diarhea and VTE were all <5%.

Summary and Conclusions: We conclude that the fully oral IRD regimen belongs to the most effective novel drug combinations in relapsed or refractory multiple myeloma. It confirmed its efficacy in the "real world" setting. The treatment was safe and well tolerated even in elderly population. The introduction of IRD regimen lead to PFS prolongation to nearly 2 years and significant OS improvement. The patients with less pretreatment (*i.e.* in the first or second relapse) had significantly better outcomes than patients with more advanced disease.

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PF594

ALLOGRAFT IN MULTIPLE MYELOMA: EXPERIENCE OF MULTIPLE MYELOMA GIMEMA LAZIO GROUP

F. Vozella[1,*], A. P. Iori[1], R. Cerretti[2], A. Severino[3], P. Chiusolo[4], A. Piciocchi[5], S. Grammatico[1], L. Cesini[1], E. Mariggiò[1], F. Fazio[1], L. Cudillo[2], L. Franceschini[2], T. Za[4], S. Sica[4], M.T. Petrucci[1], V. De Stefano[4], L. De Rosa[3], W. Arcese[2], R. Foà[1]

[1]Hematology, Sapienza University of Rome, [2]Hematology, Tor Vergata University of Rome, [3]Hematology, San Camillo Hospital, [4]Hematology, Cattolica del Sacro Cuore University of Rome, [5]Hematology, Gimema Data Center, Rome, Italy

Background: In the era of new drugs prognosis of patients (pts) with multiple myeloma (MM) has significantly improved. New or old drugs, followed by single or tandem autologous stem cell transplant, is the standard of care for pts with newly diagnosed MM. In the last three years MM received the most drug approvals for any one malignancy, both in the United States as well as in Europe. Nevertheless, MM is still considered to be an incurable disease and current therapies can only slow disease progression, prolong survival, and minimize symptoms. In fact, the majority of pts with MM will relapse or become refractory and the remission duration in relapsed MM decreases with each regimen.

Aims: If the role of autologous stem cell transplantation in MM has been confirmed by many trials, allogeneic hematopoietic cell transplantation (HCT) is less commonly used due to high treatment related mortality (TRM) and worsening of the quality of life. As a result of this issues, it is still unclear how to best utilize this potent and effective treatment modality. We report the experience of Multiple Myeloma GIMEMA Lazio Group in 70 pts with newly diagnosed (38) or relapsed/refractory (32) MM who underwent HCT between February 1985 and February 2017.

Methods: The median age was 45.7 years (range, 32.1 - 67.1), 43 men and 27 women. Median age at HCT was 48.6 years (range, 32.6 - 65.8), median time from diagnosis to HCT was 16.8 months (range, 3 - 130.6). As induction treatment 43/70 pts received old drugs, *i.e.* vincristine, doxorubicin and dexamethasone (VAD; n=33) or melphalan and prednisone (MP; n=10), while 27/70 pts were treated with novel agents, *i.e.* velcade-based (n=13) or IMiD-based regimens (n=14). Among newly diagnosed MM, HCT was performed as frontline therapy in 24/38 pts and in a tandem autologous/allogeneic in 14/38 pts. No differences in terms of OS and PFS were found between HCT "frontline *vs* relapse", p=0.72 and p=0.34 respectively, neither between induction treatment with "old *vs* new drugs", p=0.72 and p=0.15 respectively. After induction, 9 pts (13%) achieved complete response (CR), 8 pts (11.3%) achieved a very good partial response (VGPR), 41 pts (58.6%) achieved partial response (PR), 1 patient (1.4%) maintained a stable disease (SD) and 11 pts (15.7%) performed HCT in progression disease (PD).

Results: Overall, 65 pts (93%) achieved a response (CR; n=32, VGPR; n=2, PR; n=31), 2 pts (2.8%) showed PD during or immediately after HCT, 3 pts (4.2%) died for TRM. More in detail a CR was observed in 7/9 pts who underwent HCT in CR, 4/8 in VGPR, 17/41 in PR and 4/11 in PD. Among 65 pts who obtained a response, 35 pts (54%) presented a disease recurrence

in 48% and 54% at 5 and 10 years, respectively. TRM was 14% and 18% at 5 and 10 years, respectively. Acute and chronic GVHD occurred in 37 and 36 pts respectively. Overall survival (OS) and progression-free survival (PFS) at 10 years was 43.1% (range, 32.3 - 57.4) and 25.1% (range, 16.5 - 38.2) respectively (Figure 1).

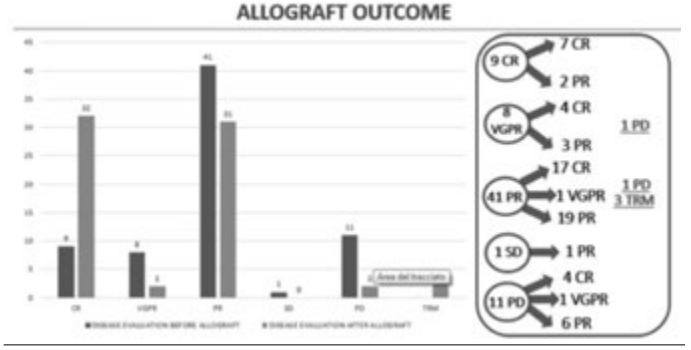

Figure 1.

Summary and Conclusions: Autologous stem cell transplant remains the standard of care for young MM pts but it's not curative. Our retrospective analysis presents some methodological limitations but it shows that the role of HCT is not clear. Physicians should evaluate its combination in prospective trials for young high-risk or relapsed/refractory pts (not beyond the first relapse), in a tandem autologous/allogeneic transplant, with an induction and a possible maintenance based on new drugs.

PF595

CAN STANDARDISATION BE ACHIEVED? ASSESSMENT OF PLASMA CELL MYELOMA MINIMAL RESIDUAL DISEASE TESTING BY FLOW CYTOMETRY: AN INTERNATIONAL INTER-LABORATORY STUDY

S. Scott[1,2,*], M. Fletcher[3], L. Whitby[3], C. Yuan[4], L. Burgos [5], S. Mazzucchelli[6], P. Lin[7], R. de Tute[8], P. Dorwal[9], P. Wallace[10], P. Tembhare[11], M. Arroz[12], D. Barnett[2,3], A. Chantry[2]

[1]UKNEQAS for Leucocyte Immunophenotyping, UK NEQAS for Leucocyte Immunophenotyping, [2]Department of Oncology and Metabolism, Faculty of Medicine, Dentistry and Health, University of Sheffield, [3]UKNEQAS for Leucocyte Immunophenotyping, Sheffield Teaching Hospitals, Sheffield, United Kingdom, [4]Clinical Flow Cytometry Laboratory, Laboratory of Pathology, CCR, NCI, NIH, Bethesda, United States, [5]Centro De Investigaciones Medicas Aplicadas (CIMA), Clinica Universidad De Navarra, Pamplona, Spain, [6]Ematology and Citofluorimetry Department, Synlab Suisse SA, Lugano, Switzerland, [7]Department of Hematopathology, MD Anderson Cancer Center, Houston, United States, [8]HMDS, Department of Haematology, St, James's Institute of Oncology, Leeds, United Kingdom, [9]Flow cytometry Laboratory, Waikato Hospital, Hamilton, New Zealand, [10]Department of Flow and Image Cytometry, Roswell Park Cancer Institute, Buffalo, United States, [11]Hematopathology Laboratory, Tata Memorial Center, Mumbai, India, [12]Flow Cytometry Laboratory, Department of Clinical Pathology, CHLO S. Francisco Xavier Hospital, Lisbon, Portugal

Background: Minimal Residual Disease (MRD) testing has been established as a common primary endpoint in Plasma Cell Myeloma (PCM) clinical trials, and is increasingly being used to manage patients with the disease. Much effort has gone into standardising MRD testing by flow cytometry (FC) on PCM patients.
Aims: To assess the implementation of consensus guidelines for technical and reporting aspects of MRD testing by FC in PCM patients in an inter-laboratory study.
Methods: Ten laboratories, adhering to consensus guidelines, were included in this study: 4x US, 1x Spain, 1x Switzerland, 1x Portugal, 1x India, 1x UK and 1x New Zealand. A dilution series of 4 samples were sent to each laboratory. Samples contained stabilised bone marrow cells from a PCM patient mixed with a stabilised peripheral blood stem cell harvest to mimic a PCM patient after induction chemotherapy/stem cell transplant. Samples were manufactured to contain 0% malignant plasma cells (PC) (Sample A), and then approximately 0.1% (Sample B), 0.01% (Sample C), 0.001% (Sample D), 0.0001% (Sample E).
Results: Eight laboratories returned results. All, detected malignant PCs at levels of 0.1%, 0.01% and 0.001% malignant PCs. 6/8 laboratories detected malignant PCs at a level of 0.0001%. 1/8 laboratories erroneously detected a malignant PC population in sample A (0% level). Quantitative data returned by laboratories showed good consensus for detecting malignant PCs at levels of 0.1% (Coefficient of Variation (CV)=28%) and 0.01% (41%) but with greater variation at the levels of 0.001% (CV=153.7%) and 0.0001% (CV=233.1%). Of the laboratories that detected a malignant PC population in all 4 relevant samples (n=6), all showed good assay linearity (R2=0.9986-1.0000; slope value 0.7843-1.3830); however, examples of post analytical (calculation error) and systematic error were identified.Additionally, the median results calculated for laboratories using the Beckman Coulter Navios flow cytometer (n=3) were consistently 1.6 fold higher than laboratories using the Becton Dickinson FacsCanto for samples B-D with a statistically significant difference for sample B (P=0.0336) and sample C (P=0.0398) identified. Only 2 participants using the Beckman Coulter Navios quantified the sample E so we were unable to calculate statistics for this sample. Analysis of marker staining classification (Absent, Weak, Moderate, Strong) was shown to be unreliable with no consensus identified (Figure 1).

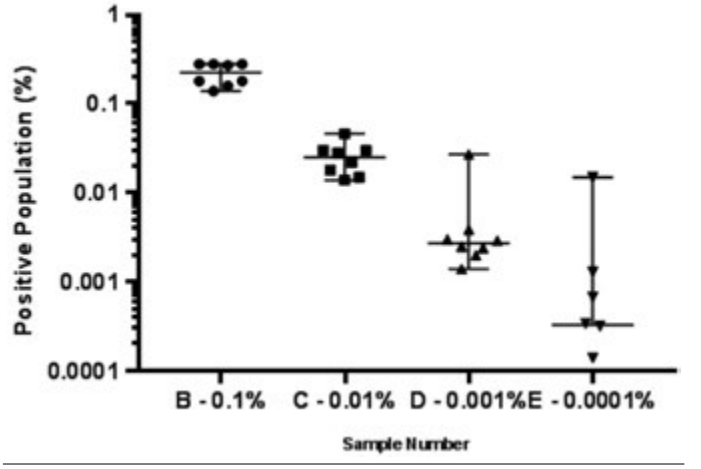

Figure 1.

Summary and Conclusions: In this study, we found that laboratories could reliably detect MRD in PCM patients using FC down to the levels required by current clinical trials (0.01%). Importantly, we have shown that even at levels of 0.001% malignant PCs, well standardised FC assays can attain good consensus important in future clinical trials, thus providing reassurance that PCM MRD testing by flow cytometry is fit-for-purpose when performed in line with international guidelines. Furthermore, we have shown that of the cohort 6/8 laboratories detected a malignant PC population at 0.0001% in line with the theoretical limit of detection of molecular methods of MRD detection. Participant assays showed good linearity down to 0.0001%, allowing the possibility of predicting survival based on log reduction in malignant PC populations in future clinical trials. The consistently different results seen when comparing laboratories using the Beckman Coulter Navios and the Becton Dickinson FacsCanto flow cytometers must be considered when assessing results from clinical trials.

PF596

MP0250 IN COMBINATION WITH BORTEZOMIB AND DEXAMETHASONE IN PATIENTS WITH RELAPSED-AND-REFRACTORY MULTIPLE MYELOMA: FIRST SAFETY AND EARLY EFFICACY ANALYSIS OF MP0250-CP201

M. Szarejko[1,*], S. Knop[2], M. Schreder[2], H. Goldschmidt[3], M.S. Raab[3], A. Jurczyszyn[4], N. Grzasko[5], J. Duerig[6], B. Gamberi[7], A. Vacca[8], R. Ria[8], J. Haunschild [9], C. Zitt[9], F. Hermann[9], K. Dawson[9], A. Harstrick[9], J. Acosta[9], G. Lemaillet[9], S. Bringhen[10]

[1]Medical - Clinical Development, University Clinical Centre, Gdansk, Poland, [2]Department of Medicine II, University Hospital Wurzburg, Wurzburg, [3]Hematology, Oncology and Rheumtology, Heidelberg University Hospital, Heilderberg, Germany, [4]Clinical Department of Hematology, University Hospital, Krakow, [5]Department of Hematology, Centre of Oncology of Lublin Region, Lublin, Poland, [6]Department of Hematology, University Hospital Essen, Essen, Germany, [7]Hematology Complex Operative Unit, Senior Hospital Santa Maria Nuova, Reggio Emilia, [8]Deparment of Biomedical Sciences and Human Oncology, University of Bari Medical School, Bari, Italy, [9]Molecular Partners AG, Zurich Schlieren, Switzerland, [10]Department of Oncology and hematology, Clinical trials in onco-hematology and multiple myeloma, City of Health and Science of Turin, Turin, Italy

Background: Upregulation of both the vascular endothelial growth factor

(VEGF) and hepatocyte growth factor (HGF) pathways are implicated in tumor survival, growth, angiogenesis, and loss of response to therapy and linked to poor prognosis in relapsed-and-refractory multiple myeloma (RRMM). MP0250 is a first-in-class, tri-specific multi-DARPin® drug candidate neutralizing VEGF-A and HGF as well as binding to human serum albumin to increase plasma half-life. MP0250 shows activity in multiple preclinical tumor models including a MM model in which it enhances the effects of bortezomib in terms of M protein production and bone lysis.
Aims: To determine the safety, tolerability, and efficacy of MP0250 in combination with bortezomib and dexamethasone (dex) and the recommended dose for further development in MM.
Methods: This trial (NCT03136653) is recruiting adults ≥18 years of age with RRMM who have progressed after at least two prior treatment regimens including bortezomib and an immunomodulatory drug (IMiD). A dose-escalation phase (part 1) consisting of two cohorts will define a safe dose of the combination of bortezomib+dex and MP0250 followed by a dose expansion phase. Up to 40 patients will be enrolled. Patients will receive treatment until there is documented disease progression or unacceptable toxicity. The primary endpoint is efficacy in terms of overall response rate (ORR) per International Myeloma Working Group (IMWG) criteria. Secondary endpoints include safety, immunogenicity, progression free survival (PFS) and duration of response (DOR). Exploratory endpoints include Pharmacokinetics and potential biomarkers. The safety analysis set (SAF) is defined as patients who have received at least 1 dose of combination of MP0250 plus bortezomib+dex.
Results: As of 19 February 2018, 8 patients have been treated with 8 mg/kg MP0250 in cohort 1 and were included in the SAF, with the last patient enrolled on 02 January 2018. Median time from initial diagnosis to first dose of MP0250 was 4.8 years (range, 1-10). Median number of prior therapies was 3 (range, 2-5). All 8 patients had been exposed to IMiDs and proteasome inhibitors (PI) and 37.5% were considered PI refractory. The most frequent drug-related grade ≥3 AEs included thrombocytopenia in 3 pts (37.5%) and hypertension in 2 pts (25%). Grade 3 proteinuria and transient grade 3 liver enzyme elevation was seen in 1 patient each (12.5%). One dose-limiting toxicity has been reported in cohort 1 (grade 3 hypertension). There were no infusion-related reactions. All patients treated at the 8 mg/kg dose were evaluable for response assessment. At last follow-up, 5 (62.5%) patients had achieved a partial response. Pharmacokinetic data obtained from the first 3 patients are in agreement with results obtained in the phase 1 study with MP0250.
Summary and Conclusions: Early data from patients treated in cohort 1 with MP0250 plus bortezomib+dex show an acceptable safety profile and promising activity in RRMM patients. Enrollment is ongoing and updated data from cohorts 1 (8 mg/kg) and 2 (12 mg/kg) will be presented.

PF597

STATIN USE IMPROVES MYELOMA-SPECIFIC SURVIVAL AND IS SAFE TO USE IN LYMPHOMA: A POPULATION-BASED STUDY OF 20,464 PATIENTS WITH LYMPHOID MALIGNANCIES DIAGNOSED IN SWEDEN 2007-2013

E. Brånvall[1,*], S. Eloranta[1], S. Ekberg[1], B.M. Birmann[2], K.E. Smedby[3]

[1]Department of Medicine, Solna, Unit for Clinical Epidemiology, Karolinska Institutet, Stockholm, Sweden, [2]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's hospital and Harvard Medical School, Boston, United States, [3]Department of Medicine, Unit for Clinical Epidemiology, Karolinska Institutet, Stockholm, Sweden

Background: Reduced cancer-specific mortality with statin use has been described among patients with several cancer forms, as well as in a few studies of lymphoid neoplasms including multiple myeloma (MM). Statins inhibit cholesterol- and isoprenoid biosynthesis, and *in vitro* data suggest several downstream effect mechanisms inhibiting cancer cell signaling and growth. There is also *in vitro* evidence of inhibited MIP-1a expression in MM, suggesting a potential protective role in MM progression. In lymphoma however, *in vitro* data suggest statins might inhibit rituximab binding to CD20, and concern has been raised that statins could impair rituximab treatment efficacy.
Aims: We aimed to assess if statin use around the time of diagnosis and initial treatment of a lymphoid neoplasm alters lymphoma/MM-specific survival overall or in subtypes.
Methods: Using the Swedish population-based national health care registers: the Cancer Register, Cause-of-Death Register, Prescribed Drug Register, LISA database, and the National Lymphoma Quality Register, we identified all incident diagnoses of major lymphoid neoplasms (non-Hodgkin lymphoma (NHL) subtypes including chronic lymphocytic leukemia (CLL) and MM) from January 1st 2007 until Dec 31st 2013. Persons with any dispensing of statins during a 6-month period before or after diagnosis were considered statin users before or after diagnosis respectively. We used Cox regression models with time-lagged statin exposure in order to avoid reverse causation. Models were adjusted for age at diagnosis, sex, year of diagnosis, education level, and concomitant medication with anticoagulants, diuretics, beta-blockers, ACE inhibitors, calcium blockers and anti-diabetics to capture comorbidity. NHL models were also adjusted for International Prognostic Index score and active lymphoma treatment (yes/no). Follicular lymphoma was divided in treated *versus* not treated patients, in order to assess effect modification in rituximab-treated patients. Statin dose intensity was classified according to the American College of Cardiology/American Heart Association statin guidelines into high, moderate and low intensity therapy where simvastatin, pravastatin, fluvastatin, atorvastatin and rosuvastatin were classified based on average mg/day dispensed during the 6-month period before diagnosis.
Results: There were 20,464 patients with incident lymphoid neoplasms: 12,865 with NHL, 3,279 with CLL and 4,323 with MM. Overall, 20% used statins at the time of diagnosis. Simvastatin accounted for 83% of the statin dispensings; 68% used moderate-intensity and only 4% used high-intensity statin therapy. Pre-diagnosis statin use was not associated with altered lymphoma-specific death among patients with NHL (HR: 0.95 (95% CI: 0.85-1.07)), major NHL subtypes, or CLL. Among patients with MM, statin use was associated with a statistically significant reduced MM-specific mortality (HR 0.81 (95% CI: 0.70-0.95)) (Figure 1). Results were similar for statin use after diagnosis (MM HR 0.73 (95% CI: 0.60-0.89)). There was no evidence of an association between statin dose-intensity and lymphoma/MM-specific death.

Figure 1.

Summary and Conclusions: In this large population-based cohort of lymphoid neoplasm patients, statins were safe to use during lymphoma treatment and were associated with an improved disease-specific survival in MM. Additional analyses of statin use and dose-intensity throughout follow-up and lymphoma/MM-specific outcomes are in progress and will be reported at a later stage.

PF598

A PROSPECTIVE ANALYSIS ON THE FREQUENCY AND PREDICTIVE VALUE OF MINIMAL RESIDUAL DISEASE ASSESSMENT BY FLOW CYTOMETRY AND PET-CT IMAGING AMONG MYELOMA PATIENTS ON/OFF MAINTENANCE

M. Beksac[1,*], G. Cengiz Seval[2], U. Sahin[3], M. Merter[4], S. Ipek[1], E. Ozkan[5], K. Dalva[2]

[1]Hematology, [2]Ankara University School of Medicine, [3]Yildirim Beyazit University Yenimahalle Education and Research Hospital, [4]Firat University School of Medicine, [5]Nuclear medicine, Ankara University School of Medicine, Ankara, Turkey

Background: The IMWG uniform response criteria have been revised to include assessment of marrow and extra-marrow minimal residual disease (MRD) by functional imaging and multi-color flow cytometry (MFC) and/or molecular methods (Kumar et al Lancet 2016).
Aims: The aim of this study was to evaluate MRD by PET CT imaging and MFC among patients who stopped treatment following achievement of immunological complete remission (CR) and completion of induction or first relapse.

Methods: In this ongoing study 62 patients in CR-1 (n:47),CR-2(n:2) or VGPR (n:13) as defined by IMWG criteria and not on any anti-myeloma treatment excluding bisphosphonates (n:2 Denosumab) or Lenalidomide (n:10), Bortezomib (n:2) and Panobinostat-Bortezomib-Dexamethasone (n:1) maintenance were enrolled (Table 1). Bone marrow EDTA anti-coagulated samples have been collected between Nov 2016- February 2018. Patients had immunological evaluation every three months; Flow-MRD and PET CT evaluation at CR staging repeated if found positive. Except for 3 patients, all have received autologous stem cell transplants (ASCT). MRD assessment by MFC using these antibodies CD27PC7, $CD56^{PC5}$, $CD19^{ECD}$, $CD38^{A750}$, $CD138^{APC}$, $CD45^{KO}$, $CD38^{FITC}$, $CD81^{PE}$, $CD117^{PC7}$ Rabbit Immunogloblin Fraction (DAKO X0903) Polyclonal Rabbit Anti-Human Kappa Chains /FITC, Rabbit F(ab')2 (DAKO F0434)and Anti-Human Lambda Chains /PE, Rabbit F(ab')2 (DAKO R0437) analyzed by the Beckman Coulter Cytometer. Flow MRD negativity was accepted as levels clonal plasma cells below the threshold of 10^{-6} Progression Free Survival (PFS) analysis was calculated using the SPSS (IBM SPSS Statistics 21; IBM Corp., Chicago, IL) statistical tool kit.
Results: Among all patients MRD was found to be negative in 34/62 of the cases. Fifteen of these cases were either in the early postASCT/maintenance period or on maintenance with IMID/PI /Denosumab. Except for two, all patients who had stopped treatment in CR due to toxicity or re-imbursement reasons were found to be MRD positive. PET-CT was able to detect residual disease in four cases with a Flow MRD negative result. A strong correlation between Flow and PET results (p=0.01). FCM analysis was repeated with 6 months intervals among 12 patients. MRD positivity was associated with biochemical (4/62) and clinical (4/62) progression within a median follow-up of six months. No progression could be documented within the FCM and PET (-) MRD group. Three-years PFS analysis revealed simultaneous either FCM/PET MRD (+) (n=2) to exert a delay in progression compared to MRD (+) with both methods (n=2) (89.5%±7.1% *vs* 75.0%±21.7%; p:0.88) (Figure 1).

Table 1.

General characteristics of patients	
Age (median, range)	61 (37-80)
Gender, male/female (n)	37 / 25
Chain type, IgA/IgG/light chain (n)	12 / 39 / 11
ISS I/II/III (n)	16/ 32 / 14
FISH, negative/high risk/others/unknown (n)	19 / 10 / 2 / 11
ASCT +/- (n)	52 / 10
Last treatment before MRD, Bortezomib-based/IMiDs/ASCT/others (n)	8 / 18 / 7 / 15
Time from CR to MRD assessment (median, range)	23,5 (4,5-121,7)
Maintenance treatment +/- (n)	15 / 47
Post MRD follow-up period, months (median, range)	6.1 (1-20)

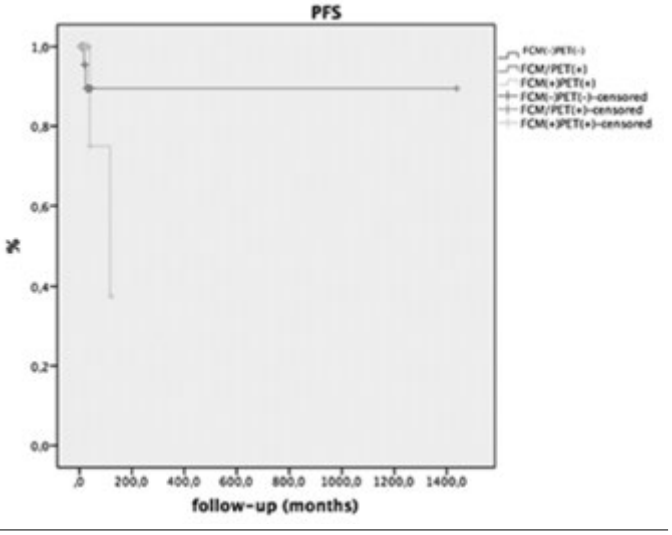

Figure 1.

Summary and Conclusions: In this ongoing prospective study among patients who are on/off treatment, MFC was able to detect MRD in the majority of patients at levels 10^{-6}. With additional patients and follow-up we will be able to observe the impact of Flow-MRD on progression kinetics more accurately.

PF599

EARLY RELAPSE IS A POWERFUL INDEPENDENT NEGATIVE PREDICTOR FOR OVERALL SURVIVAL IN MULTIPLE MYELOMA PATIENTS TREATED WITH NOVEL AGENTS

E. Katodritou[1,*], V. Palaska[1], T. Triantafyllou[1], K. Kokoviadou[2], E. Verrou[1], N. Drougos[1], K. Loga[1], K. Psianou[1], A. Adamoudi[1], E. Fytopoulou[1], E. Giannopoulou[1], N. Karampatzakis[1], I. Batsis[3], K. Keramidioti[1], S. Georgiadou[1], M. Varthaliti[1], V. Gastari[1], C. Chadjiaggelidou[1], G. Kaiafa[4], A. Kioumi[2], I. Sakellari[3], A. Anagnostopoulos[3], P. Konstantinidou[1], E. Terpos[5]

[1]Hematology, Theaganion Cancer Hospital, [2]Hematology, Papageorgiou Genaral Hospital, [3]Hematology and Bone Marrow Transplantation, Papanikolaou General Hospital, [4]Hematology, Ahepa University Hospital, Thessaloniki, [5]Department of Clinical Theraputics, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece

Background: Novel agents have improved outcomes of Multiple Myeloma (MM) patients yet some patients display early relapse (ER), defined as relapse occurred within 12 months from starting initial therapy.
Aims: The aim of the study was to examine the clinical characteristics related to ER, the impact of ER on survival and possible factors predicting ER, in patients treated upfront with novel agents.
Methods: We reviewed the medical files of patients with symptomatic MM treated with novel agents; eligible for analysis were considered patients who achieved at least partial response (PR) after initial therapy and lived for >12 months. Patients with ER were compared with those who either progressed or continued to response beyond 12 months. Patients characteristics between the 2 groups were compared with standard methods. Progression-free survival (PFS) and overall survival (OS) were plotted with the Kaplan Meier curve. Prognostic factors for ER and OS were evaluated with binary logistic and cox regression analysis, respectively; p<0.05 was considered as statistically significant.
Results: Two hundred and fifty-six consecutive symptomatic MM patients were evaluated (M/F: 129/127, median age 66, range: 37-88, IgG: 138, IgA: 68, light-chain: 41, non-secretory: 9, ISS1: 81, ISS2: 79, ISS3: 96); ER occurred in 22/256 patients (8%). Median age at diagnosis did not differ between the 2 groups; ER patients had more frequently lower platelets, higher β2 microglobulin (β2M), higher bone marrow plasma cell (BMPC) infiltration and advanced ISS stage, compared to others (p<0.05); The number of patients with high risk cytogenetics did not differ between groups (p>0.05); 45% of patients received upfront IMiD-based therapies and 55% bortezomib-based therapies. There was no significant difference in treatments applied in each group either initially or at first relapse; 60/256 (23%) patients underwent autologous stem cell transplantation after induction; patients with ER displayed less frequent complete response (CR) compared to others (18% *vs* 47%; p=0.01). The median time to progression for ER patients was 7 months (95% CI: 5-8.7 months) *vs* 35 months (95% CI: 30-39 months) for the remaining patients (p<0.001); PFS2 was significantly shorter in patients with ER compared with the rest (49 months *vs* 19 months; p<0.001). After a median follow up of 76 months (95% CI: 69-82), the median OS for patients with ER was 25 months (95% CI: 12-37) *vs* 57 months (95% CI: 51-63) for those who continued to respond beyond 12 months (p<0.001); ER and high-risk cytogenetics were the most powerful negative predictors of OS in the multivariate cox regression analysis (p<0.001; HzR: 3.7 and p=0.009; HzR: 1.9). In the univariate logistic regression analysis, platelets <100.000/ L, hemoglobin <10g/dL, β2M>5.5mg/dL and bone marrow (BMPC) plasma cells >50%, were independent predictors for ER (p<0.05); platelets <100.000/ L and BMPC >50% were significant predictors for ER in the multivariate logistic regression analysis (OR: 4.9, p= 0.01 and 3.6, p=0.03, respectively).
Summary and Conclusions: Our data demonstrate that despite the use of novel agents, ER remains a clinical issue and correlates significantly with parameters related to high tumor burden. Platelets <100.000/ L and BMPC>50% were independent predictors for ER; ER led to significantly shorter PFS2 and a 4-fold increase of the probability of death, suggesting that patients displaying ER should be treated with an aggressive therapeutic approach in the early lines of myeloma therapy.

PF600

SENSIVITY AND CLINICAL VALUE OF POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY AND WHOLE BODY MAGNETIC RESONANCE IMAGING TO ASSESS BONE DISEASE IN NEWLY DIAGNOSED MULTIPLE MYELOMA

E. Fernandez-Poveda[1,*], V. Cabanas-Perianes[1], M.-J. Moreno-Belmonte[1], M. Blanquer-Blanquer[1], F. Guzman-Aroca[1], B. Torregrosa-Sala[1],

D. Abellán-Rivero[1], A. Lopez-Sanchez[1], M. Berenguer-Piqueras[1], F. Labbadia[1], R. Perez-Lopez[1], J.-J. Cerezo-Manchado[1], M. Moya-Arnao[1], A. Martinez-Marin[1], B. Navarro-Almenzar[1], J.-M. Moraleda-Jimenez[1]

[1]Hospital Clinico Uiniversitario Virgen de la Arrixaca, Murcia, Spain

Background: Bone disease extension in symptomatic multiple myeloma has prognostic implications. However, new image methods are necessary to establish the diagnosis according to the updated International Myeloma Working Group criteria (IMWG).
Aims: Our aim was to compare the sensitivities of whole body magnetic resonance imaging (WB-MRI) and 18-fluoro-deoxyglucose positron emission tomography/computered tomography (PET/CT) for the detection of skeletal lesions, and to determine if additional lesions detected by MRI are of clinical relevance.
Second aim was to detect differences in the assessment of extramedullary disease and bone fractures.
Methods: MRI and PET/CT were performed in the initial work-up to 54 patients diagnosed with symptomatic multiple myeloma at our institution between 2013 and 2017. Any focal lesion seen on PET-CT ≥5 mm in size or >1 focal lesion in WB-MRI ≥5 mm was considered a myeloma defining event (MDE) according the IMWG. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of PET-CT to detect MDE, extramedullary disease and fractures at diagnosis were assessed. PET-CT was compared to WB-MRI using Cohen`s quadratic-weighted Kappa.
Results: Baseline characteristics of the 54 patients are shown in Table 1.
PET-CT showed a very low PPV (59.4%) and moderate NPV (81.8%) to detect MDE compared to WB-MRI with a low concordance rate (Kappa 0.39, p=0.003). However, PET-TC findings matched those of WB-MRI in the evaluation of extramedullary disease at diagnosis with a Kappa score =1 (p<0.001). Our data showed a high PPV (100%) and low NPV (72,5%) of PET-TC to detect fractures, with high level of disagreement between the two techniques (Kappa score 0.23, p=0.009).

Table 1.

Baseline Characteristics					
Gender	n	%	Durie-Salmon	n	%
Female	29	53,7	IA	11	20,4
Male	25	46,3	IB	2	3,7
			IIA	15	27,8
Age			IIB	0	0
mean (range)	63,9 (39-81)		IIIA	21	38,9
median	64,5		IIIB	5	9,3
MM	n	%	ISS	n	%
IgG K	17	31,5	I	27	50
IgG L	13	24,1	II	16	29,6
IgA K	7	13	III	11	20,4
IgA L	4	7,4	R-ISS	n	%
BJ K	5	9,3	I	26	48,1
BJ L	4	7,4	II	24	44,4
Others	4	7,4	III	4	7,5
ECOG	n	%	High-risk genetic*	n	%
0	10	18,5	Positive	4	7,5
1	24	44,4	Negative	50	92,5
2	17	31,5			
3	3	5,6	* t (4;14), t (14;16), P53 deletion		

ISS: International staging system
R-ISS: Revised international staging system

Summary and Conclusions: Our data indicate that WB-MRI is a better technique than PET-TC to detect myeloma defining events. Although extramedullary disease is equally assessed by both techniques, PET-TC is less useful in the diagnosis of bone fractures. Overall WB-MRI seems to be more useful than PET-TC in newly diagnosed myeloma multiple patients.

Myeloproliferative neoplasms – Biology & Translational Research

PF601

ANTI-SLAMF7 ANTIBODY SUPPRESSES FIBROCYTE DIFFERENTIATION AS A POTENT THERAPEUTIC AGENT FOR MYELOFIBROSIS

T. Maekawa[1,*], Y. Osawa[1], T. Izumi[1], S. Nagao[1], K. Takano[1], Y. Okada[1], N. Tachi[1], M. Teramoto[1], T. Kawamura[1], T. Horiuchi[1], R. Saga[1], S. Kato[1], T. Yamamura[1], J. Watanabe[1], A. Kobayashi[1], S. Kobayashi[1], K. Sato[1], M. Hashimoto[2], S. Suzu[2], F. Kimura[1]

[1]Division of Hematology, Department of Internal Medicine, National Defense Medical College, Tokorozawa, [2]Center for AIDS Research, Kumamoto University, Kumamoto, Japan

Background: Myelofibrosis (MF) occurrence can be attributed to various pathogenic mechanisms, such as elevated circulating cytokine levels, cellular interactions and genetic mutations. A recent study showed that the neoplastic clone of fibrocytes, spindle-shaped fibroblast-like blood cells derived from monocyte lineage, was essential in primary MF pathogenesis and that serum amyloid P (PRM-151), which suppressed fibrocyte differentiation, markedly improved survival and MF in a murine xenograft model. Using a romiplostim (Rom)-induced murine MF model, we demonstrated that fibrocyte differentiation could be directly induced by myeloproliferative leukaemia protein (MPL; TPO receptor) activation and lead to progression of MF. Fibrocytes are now one of the possible therapeutic targets for MF. We previously demonstrated using DNA microarray analysis that in comparison with macrophages, human fibrocytes expressed SLAMF7. Elotuzumab (Elo) is an anti-SLAMF7 antibody recently used for treating relapsed/refractory multiple myeloma, with encouraging results and promising safety. Here, we try to elucidate the efficacy of Elo as a therapeutic agent for MF.
Aims: This study aimed to investigate the effects of Elo against human fibrocyte differentiation promoting MF *in vitro* and *in vivo*.
Methods: We compared the SLAMF7 expression in monocytes of the peripheral blood between patients with MF and healthy donors. We cultured human peripheral blood mononuclear cells (PBMCs) with or without Elo *in vitro* to evaluate whether Elo suppressed fibrocyte differentiation and calculated the spindle-shaped cells ratio. Then, we cultured human PBMCs by combining Elo and natural killer (NK) cells, Rom, ruxolitinib or IFNα2 to evaluate their interactions. To evaluate the efficiency of Elo for a Rom-induced murine MF model, we transplanted human peripheral blood or cord blood stem cells into NOG mice with 2.5-Gy radiation, and administered 1 mg/kg Rom once a week after 8-12 weeks of transplantation. Humanised NOG mice were sacrificed 1 to 2 weeks after Rom administration, and MF grade of bone marrow and spleen weight were examined.
Results: In comparison to healthy donors, the SLAMF7 expression in monocytes of the peripheral blood was significantly increased in patients with MF. While Elo independently inhibited fibrocyte differentiation from human PBMCs, NK cells augmented the inhibitory effect. Fifty and 100 g/mL of Elo showed comparable inhibitory effect for fibrocyte differentiation *in vitro*. Elo represented the inhibitory effect even after Rom administration, and addition of ruxolitinib diminished the inhibitory effect of Elo on fibrocyte differentiation. IFNα2 demonstrated the severe cytotoxic effect. In humanised NOG mice, Elo administration suppressed MF development. Amelioration of splenomegaly and anaemia was also observed in mice treated with Elo and Rom than Rom alone.
Summary and Conclusions: The SLAMF7 expression was elevated in monocytes of patients with MF. Anti-SLAMF7 antibody Elo administration significantly suppressed fibrocyte differentiation *in vitro*, and ameliorated MF *in vivo*. Elo could be a potential therapeutic agent for MF, and this effect of Elo may be partly dependent on the JAK–STAT pathway.

PF602

HETEROZYGOUS CONSTITUTIVE CALR DEL52 KNOCK-IN MICE SHOW A TRANSPLANTABLE ET-PHENOTYPE WHILE THE HOMOZYGOUS STATE IS LETHAL DUE TO A HEART DEFECT

T. Balligand[1,*], J.-P. Defour[1], Y. Achouri[2], G. Gaudray[1], S.N. Constantinescu[1]

[1]Ludwig Cancer Research / de Duve Institute, [2]de Duve Institute, Woluwe Saint-Lambert, Belgium

Background: Frame-shifting mutations (-1/+2) of the human calreticulin (*CALR)* gene are responsible for 25-30% of cases of essential thombocythemia (ET) and primary myelofibrosis. The mutant CALR proteins are now known

to activate the thrombopoietin receptor and therefore induce cytokine-independent hyper-proliferation of megakaryocyte progenitors (Chachoua *et al.*; Marty *et al.*; Elf *et al.*; Araki *et al.*). We have already shown that CRISPR-Cas9 engineered homologous mutations in the murine *Calr* gene are able to induce an identical oncogenic effect *in vitro* in a mouse-derived cell-line at an endogenous level of expression and that murine CALR mutants induce persistent JAK2 signaling in the presence of TpoR (Balligand *et al.*).
Aims: We aimed to generate a mouse model of myeloproliferative neoplasms (MPNs) that would be physio-pathologically relevant to the development process of the disease.
Methods: We microinjected CRISPR/Cas9 constructs for introducing del52 *Calr* along with a small oligoDNA as repair template into fertilized B6D2 mouse zygotes. Allele burden analysis was carried out by PCR amplification of the exon 9 of the *Calr* gene before analysis using a GeneScan machine.
Results: We observe that our *Calr* del52/WT mice develop an ET-phenotype characterized by a 1.5-fold increase of platelet levels in peripheral blood as soon as 11 weeks of age, compared to *Calr* WT/WT littermates. Total white blood cell levels showed a 2-fold increase starting at 52 weeks of age. Total red blood cell levels were unchanged across the two groups. Anatomohistopathology shows a significant 1.4-fold increase in megakaryocyte size and a significant 1.5-fold increase in megakaryocyte numbers in the bone marrow. No hematopoiesis was evident in the spleen with the absence of splenomegaly. Transplantation of different ratios of *Calr* del52/WT and *Calr* WT/WT bone marrow in lethally irradiated wild-type B6D2 mice reproduces a significant 1.5-fold increase in platelet level that starts at 14 weeks post-transplantation (Figure 1A). No differences in WBC or RBC levels were observed until 60 weeks of age. We monitored the allele burden for the *Calr* del52 mutation and observed an increasing trend in the mice reconstituted with 80% or with 50% of *Calr* del52/WT bone marrow that became statistically significant starting at 150 days post transplantation, effectively showing that the *Calr* del52/WT hematopoietic population is achieving dominance over the *Calr* WT/WT population (Figure 1B). Finally, we could not obtain any *Calr* del52/del52 pups by crossing our mice, showing that the homozygous state of the mutation is lethal during embryogenesis. We show that *Calr* del52/del52 embryos have heart development defects characterized by a thinning of the ventricular walls much like what has been described with the *Calr* knock-out model of Michalak (*J Cell Biol. 1999 Mar 08;144(5):857-68*). This brings evidence that the loss of the C-terminus of the protein, which has an important calcium-buffering role, is sufficient to reproduce the heart development defects observed with loss of the full protein.

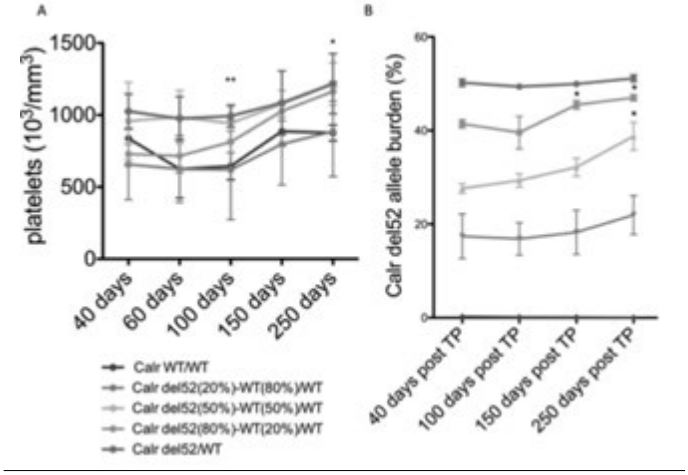

Figure 1.

Summary and Conclusions: We report successful creation of a mouse model of the type-1 mutated *Calr* induced MPNs, that shows an ET-phenotype that is transplantable with evidence of a slow expanding dominance over co-transplanted wild-type bone marrow. We also show that total loss of the C-terminus of the calreticulin protein is sufficient to disrupt critical calcium signaling that is important for heart embryogenesis by reproducing the phenotype observed in the total absence of the calreticulin protein.

PF603

PHARMACOLOGIC TARGETING OF NEOPLASTIC STEM CELLS IN HUMAN MAST CELL LEUKEMIA

G. Eisenwort[1,2,*], B. Peter[1,2], I. Sadovnik[2], J. Schwaab[3], M. Jawhar[3], A. Keller[2], G. Stefanzl[2], K. Blatt[1,2], G. Hoermann[2], M. Bilban[2], M. Willmann[1,4], C. Winding[4], W.R. Sperr[1,2], M. Arock[5], T. Rülicke[1,4], A. Reiter[3], P. Valent[1,2]

[1]Ludwig Boltzmann Cluster Oncology, [2]Medical University of Vienna, Vienna, Austria, [3]University Hospital Mannheim, Mannheim, Germany, [4]University of Veterinary Medicine, Vienna, Austria, [5]Ecole Normale Supérieure de Cachan, Cachan, France

Background: Leukemic stem cells (LSC) are increasingly recognized as new promising targets of potentially curative therapies in various forms of leukemia. Systemic mastocytosis (SM) is a rare hematopoietic neoplasm characterized by an abnormal expansion of mast cells (MCs) in the bone marrow (BM) and other organs. Whereas patients with indolent SM (ISM) have a normal life-expectancy, patients with advanced forms of SM have a grave prognosis. MC leukemia (MCL), the rare leukemic variant of advanced SM, is defined by a rapid expansion of immature MCs in various hematopoietic organs and a poor prognosis with short survival times. The role of LSC in the development and progression of aggressive SM (ASM) and MCL is poorly understood.
Aims: We have recently been able to show that the putative MCL LSC reside within a $CD34^+$ fraction of the neoplastic clone. The aims of the present study were to characterize the cell surface phenotype, frequencies, and functional properties of LSC in patients with advanced SM and MCL and to explore the effects of various targeted drugs on growth and survival of these cells.
Methods: LSC were purified from BM mononuclear cells by flow sorting and injected intravenously in NOD-SCID-IL-$2Rg^{-/-}$mice exhibiting human membrane-bound SCF (NSG_{SCF}). Engraftment was assessed by analysing the murine BM for the presence (percentage) of human $CD117^+$ cells. Phenotyping studies were performed on whole BM after erythrocyte lysis. Effects of targeted drugs on survival of LSC were assessed by multi-color flow cytometry and Annexin-V and DAPI staining.
Results: We found that the NSG_{SCF}-engrafting leukemia-initiating LSC in MCL reside in a $CD34^+/CD38^-$ fraction of the clone, similarly to LSC in CML and normal hematopoietic stem cells. As assessed by cell-dilution experiments, 0.0003-0.0045% of all $CD45^+$ cells and as little as 500 $CD34^+$ cells were found to induce robust MCL-like engraftment in NSG_{SCF} mice. Phenotypic assessment of LSCs from patients with MCL and ASM by flow cytometry and gene chip analysis revealed a distinct phenotype. Compared to $CD34^+/CD38^-$hematopoietic stem cells from the BM of healthy donors, MCL LSCs expressed higher levels of CD30 (Ki-1) but lower levels of CD117 (KIT). In 3/10 MCL patients (30%) and 1/11 ASM patients (9%) MCL LSC expressed elevated levels of IL-1RAP and in 1/12 MCL patients (8%) and 3/16 ASM patients (19%) LSC expressed elevated levels of CD52 (CAMPATH-1). Furthermore, CD371 (CLL-1) and CD33 (Siglec-3) were overexpressed on $CD34^+/CD38^+$ progenitor cells in MCL compared to healthy BM. Application of the KIT-targeting drug midostaurin or the CD33-targeting drug gemtuzumab-ozogamicin (GO) as single agent was not able to induce apoptosis in primary MCL LSC. However, a combination of these two agents produced a synergistic apoptosis-inducing effect on LSC. Moreover, pre-incubation with GO or a combination of GO and Midostaurin were found to inhibit the engraftment of MCL LSC in NSG_{SCF} mice. Finally, we were able to demonstrate that treatment of MCL or ASM patients with midostaurin is able to reduce the numbers (percentage) of $CD34^+/CD38^-$cells in the BM of these patients, although LSC were never completely eliminated.
Summary and Conclusions: Together, we have characterized the phenotype of MCL LSC and identified clinically relevant molecular targets in these cells. Since targeted drugs are indeed able to counteract growth of MCL LCS, our studies may have clinical implications and may help develop curative treatment approaches in this fatal leukemia.

PF604

THE PHENOTYPIC PROFILE OF ACUTE MYELOID LEUKEMIA SECONDARY TO CHRONIC MYELOPROLIFERATIVE NEOPLASMS: RESULTS OF A MULTI-CENTER STUDY

F. Mannelli[1,*], P. Guglielmelli[2], S. Bencini[3], M.I. Bonetti[3], G. Rotunno[2], A. Pacilli[2], M. Benelli[4], S. Bellesi[5], S. Betti[5], E. Rumi[6], G. Finazzi[7], F. Buccisano[8], E. Borlenghi[9], F. Lo Coco[8], G. Rossi[9], M. Cazzola[6], A. Rambaldi[7], A. Bosi[3], A.M. Vannucchi[2]

[1]CRIMM - Hematology, Careggi Hospital, [2]CRIMM - Hematology, [3]Hematology, University of Florence, Florence, [4]Bioinformatics, Prato Hospital, Prato, [5]Hematology, Catholic University, Rome, [6]Hematology, Pavia University, Pavia, [7]Hematology, Bergamo Hospital, Bergamo, [8]Hematology, Tor Vergata University, Rome, [9]Hematology, Brescia Hospital, Brescia, Italy

Background: The mechanisms of evolution of myeloproliferative neoplasms

(MPN) into secondary acute myeloid leukemia (sAML) are not fully understood. Molecular studies described heterogeneous leukemogenesis patterns depending on persistence or loss of the MPN driver mutation in the predominant clone. Mutations of *de novo* AML are typically absent in sAML, conversely enriched in TP53 mutations; in 20-30% of sAML, preexisting MPN-related driver mutations are lost at evolution. Data on phenotypic features of sAML are scarce.
Aims: We studied a large set of sAML by a systematic phenotypic study aimed at highlighting distinct features and potentially getting insight into leukemogenesis.
Methods: Inclusion required sAML diagnosis according to WHO and availability of a pre-defined minimum set of phenotypic data. Flow cytometry FCS files were sent to coordinating center (Florence) for central analysis. Major cell compartments were identified by FSC/SSC, reactivity for CD45/CD34 and lineage-specific antigens; a total of 34 parameters were expressed as mean intensity and percentage of positivity. FSC/SSC and antigen intensity were normalized to internal T lymphocytes and negative controls. A cohort of 114 *de novo* AML cases observed in Florence was analyzed in parallel.
Results: From 2007 to 2017, 103 sAML pts were enrolled, including 32 (31.1%) with sAML after PMF, 69 (67.0%) after PV/ET and 2 after unclassifiable MPN. Median age at evolution was 66y (43-90 y); time from MPN diagnosis to sAML was 10.4y (0.3-27.1y). As per driver mutation status at sAML, 58 had *JAK2*V617F, 19 *CALR* mutation, 4 *MPL* mutation; 12 patients were triple-negative, including 6 who converted to *JAK2*-wt sAML; data were not available in 10 cases. The median distribution of compartments was: blasts 24.7%, granulocytic cells 22.9%, monocytic 14.6%, erythroid 5.5%, eosinophil 0.5%, basophil 1.2% and dendritic cells 2.6%. The comparison between *JAK2+* and *CALR+* subsets highlighted a higher involvement of monocytic lineage in the former (28.5% *vs* 3.1%; p=0.010) without other meaningful difference in phenotypic parameters. In the comparison between *de novo* and sAML, several parameters were found to differ significantly, either for blasts and maturing cells. Blasts from sAML displayed higher CD34 expression (96% *vs* 64%; p<0.0001), as expected from different incidence in NPM1+ cases (0.9% *vs* 26.3%), correlating with reduced CD34 positivity. Granulocytic cells were more represented in sAML (22.9% *vs* 15.0%; p=0.0031), consistent with the underlying MPN. Noteworthy, dendritic cells were relatively expanded in sAML (2.6% *vs* 0.51%; p<0.0001) and represented the predominant population within leukemic clone in 12 (11.7%) sAML cases *vs* 3 (2.6%; p=0.0135) in *de novo*. By hierarchical clustering analysis of 44 sAML cases where extensive genetic data were available we observed a strong tendency for sAML to segregate from *de novo* AML (Figure 1; p<0.0001). This allowed also the identification of smaller clusters sharing genotypic abnormalities beyond driver mutations (*i.e.* TP53); their backward analysis highlighted a recurrent phenotypic signature with multiple cell populations identified by APS tool (Figure 1).

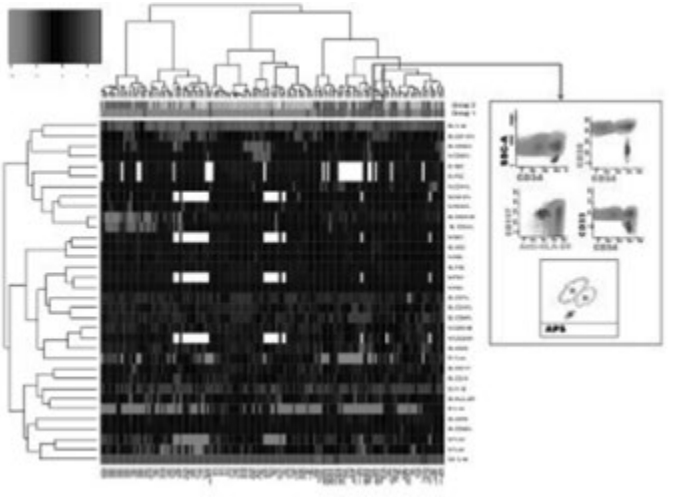

Figure 1.

Summary and Conclusions: A systematic analysis of phenotypic profile of sAML revealed common features distinct from *de novo* AML, largely independent from MPN driver mutation status, involving both immature and maturing cell compartments. Some findings (*i.e.* dendritic cell expansion, enrichment for genotypes in unique phenotypic clusters) deserve further studies and might be relevant for understanding mechanisms of clonal progression and leukemogenesis.

PF605

POTENT ACTIVITY OF AZACITIDINE ON LEUKEMIA-INITIATING CELLS IN A XENOGRAFT MODEL OF JUVENILE MYELOMONOCYTIC LEUKEMIA

M. Erlacher[1,*], C.F. Krombholz[1], S. Sushree[1], K. Aumann[2], C. Niemeyer[1], C. Flotho[1]

[1]Division of Pediatric Hematology and Oncology, [2]Institute of Pathology, University Medical Center Freiburg, Freiburg, Germany

Background: Juvenile myelomonocytic leukemia (JMML) is an aggressive myeloproliferative neoplasm of early childhood, often with fatal outcome. Hematopoietic stem cell transplantation is required in most cases. The driving force of JMML is deregulated activation of the RAS signaling pathway. In addition, epigenetic modifications are disease drivers and affect clinical picture and prognosis. In line with this, the clinical efficacy of the DNA-demethylating agent azacitidine was demonstrated in a retrospective case series, which led to an ongoing trial for prospective evaluation of azacitidine in children with JMML (ClinicalTrials.gov NCT02447666).
Aims: We used our previously developed xenotransplantation model to gain further insight into the antileukemic activity of azacitidine in JMML.
Methods: Newborn Rag2-/-gc-/- mice were sublethally irradiated and transplanted with 1x10^6 primary JMML cells. Upon stable xenologous engraftment, mice were treated with azacitidine (3mg/kg/day i.p.), cytarabine (20mg/kg/day i.p.) or saline. Recipient mice received 2 or more treatment cycles (*i.e.* 5 days treatment+9 days of recovery) and were then analyzed in detail by flow cytometry and histopathology. Human leukemic cells were extracted from bone marrow and subjected to methylome analysis (Infinium 450K arrays). Reactivation of endogenous retroviral elements was analyzed by qRT-PCR.
Results: Xenotransplantation of primary JMML cells reproduced a characteristic JMML phenotype with chronic disease course. Epigenomes were highly comparable between patient samples and human cells isolated from recipient mice 8 weeks after transplantation. After two treatment cycles, both azacitidine and cytarabine strongly reduced JMML infiltration in all organs analyzed. However, only azacitidine but not cytarabine depleted early CD34+ stem and progenitor cells within the human leukemia population. Accordingly, human cells isolated from azacitidine-treated mice did not initiate leukemia in secondary recipients while leukemia cells isolated from cytarabine or mock-treated animals were serially transplantable. When recipient mice treated with two cycles of azacitidine or cytarabine, were analyzed 9 weeks after cessation of treatment, partial recovery of leukemia was observed in all mice but the suppressive effect was more sustained in the azacitidine group. The strong anti-leukemic effects of azacitidine *in vivo* were accompanied by dramatic global DNA demethylation with a complete loss of fully methylated CpG sites. As a result, the profiles of azacitidine-treated JMML cells resembled more healthy human CD34+ cells than mock-treated JMML. In addition, azacitidine led to reexpression of endogenous retroviral sequences. Cytarabine neither induced epigenetic changes nor reactivated endogenous retroviral elements in JMML cells.
Summary and Conclusions: Both cytarabine and azacitidine repress JMML in xenograft mice. However, only azacitidine but not cytarabine efficiently targets leukemia-initiating cells and abrogates their retransplantation capacity. Cellular effects of azacitidine include dramatic global DNA demethylation and reactivation of endogenous retroviral sequences.
In sum, azacitidine-mediated reversal of aberrant DNA methylation patterns present in JMML results in efficient suppression of leukemia growth in xenograft mice. Most importantly, azacitidine depletes CD34+ leukemic stem cells *in vivo* and abrogates their retransplantation capacity.

PF606

PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS REVEALED CALCIUM/CALMODULIN DEPENDENT PROTEIN KINASE 2 AS A POTENTIAL THERAPEUTIC TARGET OF MYELOFIBROSIS

M. Miyauchi[1,*], K. Taoka[1], Y. Masamoto[1], S. Yamazaki[1], S. Arai[1], M. Kurokawa[1]

[1]Department of Hematology and Oncology, The University of Tokyo, Graduate School of Medicine, Tokyo, Japan

Background: Myelofibrosis (MF) is a myeloproliferative neoplasm characterized by megakaryocytic atypia, fibrosis in bone marrow, and extramedullary hematopoiesis. Although mutational analyses reveal that about 90% of patients with primary MF harbor JAK2, CALR or MPL mutation that constitutively activates JAK-STAT pathway, the outcome of JAK2 inhibitor treatment for MF patients still remains to be improved. However,

only a limited number of humanized disease-models are currently available to develop a novel therapeutic strategy for MF. We have previously reported that induced pluripotent stem cells (iPSCs) established from MF patients (MF-iPSCs) by using retroviral vectors recapitulated the disease phenotype (Hosoi *et al.* 2014), indicating that MF-iPSCs are one of the useful humanized disease-models of MF.
Aims: The aim of the present study is to identify novel therapeutic targets of MF.
Methods: We have established iPSCs from three independent MF patients harboring a JAK2 V617F, CALR type 1, or CALR type 2 mutations by using integration-free episomal vectors and confirmed that established MF-iPSCs harbored the identical mutations to parental cells. After hematopoietic differentiation, we obtained hematopoietic progenitor cells (HPCs) immunophenotypically defined as CD34+/CD43+ cells for further analyses.
Results: Viability assays revealed that JAK2 inhibitors (Ruxolitinib and HSP90) impaired cell survival of MF-HPCs compared to normal-HPCs, suggesting that MF-HPCs also reproduce drug-sensitivity. To investigate therapeutic targets of MF, we optimized culture condition of HPCs to perform compound screening. By the use of small chemical library containing 192 compounds and two MF-iPSCs harboring a JAK2 or CALR mutation, we identified KN93, calcium/calmodulin dependent protein kinase (CAMK) 2 inhibitor, as a compound which inhibited the viability of MF-HPCs from all three patients in dose-dependent manner, compared to normal-HPCs. For validation, we used Trifluoperazine (TFP), another CAMK inhibitor and TFP had similar inhibitory effects on MF-HPCs, indicating that CAMK2 is a candidate of therapeutic target for MF. To address the efficacy of CAMK2 inhibition in other models mimicking MF, we used Ba/F3 mouse cell line ectopically expressing MPL W515L mutant (Ba/F3_MPLmu; IC50 of ruxolitinib=112 nM), along with ruxolitinib-resistant Ba/F3_MPLmu (Ba/F3_MPLmu_R; IC50 of ruxolitinib=642 nM) cells established through one-month exposure to ruxolitinib. KN93 and TFP decreased cell growth, induced apoptosis, and suppressed the phosphorylation of Stat5, a major signaling event in MF, in both Ba/F3_MPLmu_R and Ba/F3_MPLmu cells. Moreover, combination of CAMK2 inhibitors and ruxolitinib induced apoptosis and inhibited the phosphorylation of Stat5 more efficiently than single agent, suggesting that CAMK2 inhibitors and ruxolitinib exhibited a cooperative effect against MF cells. To address the effectiveness of CAMK2 inhibition in primary samples, we used CD34+ cells isolated from peripheral blood mononuclear cells of MF patients. Although either KN93 or ruxolitinib inhibited the colony forming capacity of primary MF cells with similar efficiency, combination of KN93 with ruxolitinib showed cooperative inhibition. Taken together, these findings indicate that CAMK2 would be a therapeutic target of MF patients.
Summary and Conclusions: In conclusion, we established MF-iPSCs form three independent patients harboring a JAK2 V617F, CALR type 1, or CALR type 2 mutations. Compound screening assay of MF-HPCs with small chemical library identified CAMK2 as a potential therapeutic target of MF.

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MUTATIONAL MECHANISMS OF EZH2 INACTIVATION IN MYELOID NEOPLASMS

A. Chase[1,*], J. Score[1], F. Lin[1], C. Bryant[2], K. Waghorn[1], A. Villasante[3], T. Ernst[4], N. Cross[1]

[1]University of Southampton, Salisbury, [2]University of Southampton, Southampton, United Kingdom, [3]Department of Biomedical Engineering, Columbia University, New York, United States, [4]Universitätsklinikum Jena, Jena, Germany

Background: The polycomb repressive complex 2 (PRC2) component EZH2 catalyses the trimethylation of histone H3 lysine 27 (H3K27), a mark associated with transcriptional repression. Inactivating EZH2 mutations are seen in myeloid malignancies and we have previously shown that missense mutations within the catalytic SET domain and neighbouring CXC domain can result in loss of catalytic activity (See Table 1, mutations 17, 18, 19). The significance of missense mutations outside of these regions however is unclear. The majority of missense mutations outside of the SET/CXC domains fall within domains DI and DII suggesting that they affect function.
Aims: To determine the functional consequences of non-SET/CXC domain missense mutations.
Methods: (1) The PRC2 complex was isolated by immunoprecipitation from Sf9 insect cells transfected with EED, SUZ12 and wild type (WT) or mutant FLAG-EZH2. PRC2 histone methylation activity was assessed in an *in vitro* reaction with histones as substrate and S-adenosyl methionine as a methyl donor. (2) A murine Ezh2-null induced pluripotent stem (iPS) cell line, EZH2 Δ/Δ clone 10 (Villasante *et al.*, Cell Cycle 2011;10:1488-98) was transiently transfected with WT or mutant EZH2. H3K27 methylation was then measured by immunostaining with anti-H3K27me3 followed by flow cytometry. (3) Splicing was assessed by PCR on cDNA extracted from patient leukocytes using primers in flanking exons. Exon 8 mutations were further assessed using the pSliceExpress minigene system with transfection into HEK293 and HeLa cells.
Results: The *in vitro* methylation assay on isolated PRC2 and histones demonstrated non-SET/CXC domain mutations (See Table 1, mutations 4, 6, 8) retained H3K27 trimethylation activity. Since in the cellular context PRC2 interacts with accessory proteins which may differentially interact with WT and mutant EZH2 to affect function, we developed an EZH2-null iPS cell-based assay. As expected, transfection of WT EZH2 into the EZH2-null iPS cell line resulted in gain of H3K27 methylation whilst transfection of EZH2 with catalytically inactive SET domain mutations (muts. 18, 19) showed no change. Transfection with domain DI and DII mutations showed differential effects with all DI mutations showing complete or partial loss of methylation activity (muts. 1-5) whilst the majority of DII mutations retained full activity (muts. 8-12, 14). Using RT-PCR we then examined the effect of missense mutations on splicing. Single mutations in exons 5, 6, 7 and 14 (muts. 1, 6, 7, 16) had no effect on splicing whilst 4/4 exon 8 mutations, encoding most of DII, resulted in complete skipping of exon 8 or partial loss of exon 8 due to use of a cryptic splice site (muts. 10, 11, 12, 14). Aberrant splicing was confirmed using a minigene assay for all four exon 8 mutations plus a further three exon 8 mutations for which cDNA was not available (muts. 8, 9, 13).

Table 1.

EZH2 mutation details and assay results.

Mutation No.	Variant (NM_004456)	Exon	Domain	In-vitro histone methylation assay	Cell-based methylation assay	Splicing
1	c.384A>C p.(L128F)	5	DI	ND	Inactive	Normal
2	c.394C>T p.(P132S)	5	DI	ND	Inactive	ND
3	c.401T>A p.(M134K)	5	DI	ND	Inactive	ND
4	c.434T>G p.(F145C)	5	DI	Active	Inactive	ND
5	c.439A>G p.(K156E)	5	DI	ND	Intermediate	ND
6	c.574G>A p.(D192N)	6	Between DI and DII	Active	Inactive	Normal
7	c.727A>C p.(E243Q)	7	DII	ND	ND	Normal
8	c.730T>G p.(Y244D)	8	DII	Active	Intermediate	Abnormal
9	c.745G>A p.(E249K	8	DII	ND	Active	Abnormal
10	c.754C>G p.(L252V)	8	DII	ND	Active	Abnormal
11	c.763G>A p.(A255T)	8	DII	ND	Active	Abnormal
12	c.863G>A p.(R288Q)	8	DII	ND	Active	Abnormal
13	c.890A>G p.(H297R)	8	DII	ND	ND	Abnormal
14	c.893G>T p.(R298L)	8	DII	ND	Active	Abnormal
15	c.1033G>A p.(A345T)	10	Between DII and CXC	ND	Active	ND
16	c.1583G>A p.(C528Y)	14	CXC	ND	ND	Normal
17	c.1728C>G p(C576W)	15	CXC	Inactive[1]	ND	ND
18	c.2068C>T p.(R690C)	18	SET	Inactive[1]	Inactive	ND
19	c.2191T>G p.(Y731D)	19	SET	Inactive[1]	Inactive	ND

(1) Data published in Ernst et al, Nat. Genet. 2010, 42(8):722-6.

Summary and Conclusions: The majority of EZH2 missense mutations outside of the SET/CXC domains lie within domains DI and DII. A combination of histone methylation and splicing assays has shown these to have differential functional mechanisms. DI mutations affect catalytic activity of the PRC2 complex in a cellular context whilst the majority of DII mutations fall within exon 8 and affect splicing. The pathogenic consequences of DI and DII missense mutations is therefore consistent with our current understanding of EZH2 as a tumour suppressor gene in myeloid malignancy

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MUTANT CALRETICULINS ASSOCIATED WITH MYELOPROLIFERATIVE NEOPLASM RETAIN *IN-VITRO* CHAPERONE ACTIVITY

A. Roy[1,*], D. Colau[1], C. Pecquet[1], E. Leroy[1], I. Chachoua[1], E. Fertig[2], S.N. Constantinescu[1]

[1]Signal Transduction Unit, Ludwig Institute for Cancer Research, Universite catholique de Louvain, Brussels, Belgium, [2]Laboratorul de Patologie Ultrastructurala, Institutul National Victor Babes, Bucharest, Romania

Background: Around 15% of myeloproliferative neoplasms have recently been linked to calreticulin (CALR) mutations. The mutant CALRs induce aberrant, persistent JAK2 dependent-thrombopoietin receptor (TpoR)/MPL

signaling and autonomous proliferation of Ba/F3 TpoR cells. The dependence on TpoR and JAK2 explains the clinical observation of thrombocytosis and the megakaryocyte hyperplasia in the bone marrow. However, the mutant CALRs may have other biological consequences that are yet to be unraveled. CALR is an endoplasmic reticulum (ER) resident molecular chaperone that helps in polypeptide folding through lectin-dependent and peptide-dependent mechanisms. Literature also indicates that mutations in CALR can affect its chaperone activity. We examined HLA Class I surface expression in UT7-TpoR cells expressing mutant CALRs (CALR del52 and CALR ins5) and found decreased cell-surface levels when compared to UT7-TpoR-CALR WT cells. We asked whether this is the result of impaired chaperone activity by the mutant CALRs.
Aims: In this study, we analyzed the *in-vitro* chaperone activity of the two CALR mutants – type I (CALR del52) and type II (CALR ins5).
Methods: Recombinant CALR WT, CALR del52 and Malate Dehydrogenase as well as purified IgY and pre-formed HLA-A2 complex were used to study the *in-vitro* chaperone activity of CALR. Ba/F3 cells expressing either CALR WT, CALR del52 or CALR ins5 was used for immunoprecipitation assays. Immunofluorescence staining of the ER followed by confocal microscopic observation was used to determine the morphology of ER.
Results: We performed an *in-vitro* chaperone activity assay using recombinant CALR WT and CALR del52 with purified HLA-A2 complex. In this assay, CALR del52 was observed to be as efficient a chaperone as CALR WT. Moreover, we used two other substrates- Malate Dehydrogenase and IgY to differentiate between peptide-dependent and lectin-dependent chaperone activity. Again, no significant difference was observed between CALR WT and CALR del52 when they were used with their substrates at 1:1 stoichiometry. Significantly, CALR del52 itself showed slightly reduced *in vitro* thermal stability when compared to CALR WT. CALR in living cells forms complexes with other ER resident chaperones. Using immunoprecipitation, we observed the presence of CALR del52 and CALR ins5 in complexes containing calnexin, BiP and PDIA3. Finally, expression of the mutant CALRs did not induce ER stress and gross ER morphology remained unchanged.
Summary and Conclusions: Our results indicate that CALR del52 and CALR ins5 retain when equivalent protein levels are used their chaperone activity. CALR mutants exit the ER due in part due to the lack of the KDEL signal. Of interest, we established that the mutant CALRs exhibit reduced cellular expression when compared to CALR WT. Thus, although mutant CALRs retains chaperone activity, the end result is a decreased absolute level of chaperone activity. We suggest that patient cells may have insufficient CALR chaperone activity in the ER, due to lower total cellular CALR expression and not to due to an impaired chaperone activity, although masking of the N-terminus by yet to be identified proteins cannot be completely ruled in the case of mutated CALRs. Further investigations are underway to understand how this affects myeloproliferation and potential immunogenicity of the new C-terminal tail.

PF609

SDF1 ALPHA-INDUCED CHEMOTAXIS OF JAK2-V617F POSITIVE CELLS IS DEPENDENT ON BRUTON'S TYROSINE KINASE AND PHOSPHOLIPASE C GAMMA 1

S.C. Nimmagadda[1,*], S. Frey[1], P. Müller[1], D. Wolleschak[1], B. Edelmann[1], T. Fischer[1]

[1]Department for Hematology and Oncology, University Clinic of Magdeburg, Magdeburg, Germany

Background: Myeloproliferative neoplasms (MPNs) including polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) are clonal myeloid disorders and are often accompanied by JAK2-V617F mutation. The most common symptoms of MPN patients are leukocytosis, increased risk of thrombosis, splenomegaly and an inflammatory response syndrome.
Aims: Aberrantly active p65 regulates Burton's tyrosine kinase (BTK) expression in multiple myeloma and in acute myeloid leukemia cells. p65 transcriptionally regulates expression of CXCL10 in JAK2-V617F cells. These observations led us to investigate if JAK2-V617F kinase induces BTK expression (via activated p65) and activation and further characterize its physiological relevance.
Methods: 32D myeloid and BaF3 lymphoid progenitor cells expressing EPO-R and JAK2-WT or JAK2-V617F (32D and BaF3 JAK2-WT/ -V617F) and primary granulocytes isolated from JAK2-V617F positive MPN patients or healthy donors (HD) were used. Cells were treated with inhibitors against, JAK1/2 (ruxolitinib), BTK (ibrutinib) and PLC 1 (U17322) or PLC 1 shRNAs and analyzed by immunoblotting, promoter, chemotaxis, and RhoA GTPase assays.
Results: BTK and p65 are constitutively active in 32D and BaF3 JAK2-V617F cells compared to JAK2-WT cells. Of note, BTK was overexpressed in 32D JAK2-V617F cells. The observations, p65 activation, and BTK overexpression led us to hypothesize a role for activated p65 in BTK expression and perform luciferase-based BTK promoter assays. 32D JAK2-V617F cells indeed demonstrated elevated luciferase activity in comparison to JAK2-WT (control) cells. Confirming a role for JAK2-V617F dependent p65 activity on BTK promoter, ruxolitinib treatment negatively affected BTK promoter activity and protein expression. Ibrutinib treatment further demonstrated that BTK lies upstream of PLC 1. Growing evidence supports a role for BTK and chemokine SDF1α in cell migration. We, therefore, used SDF1α as a chemoattractant and performed chemotaxis assays. Basal migration of 32D JAK2-V617F cells was significantly higher as compared to control cells. SDF1α further enhanced migration of 32D JAK2-V617F cells, indicating a collaboration between JAK2-V617F signaling and SDF1α induced chemotaxis. Inhibitors targeting JAK1/2 (ruxolitinib), BTK (ibrutinib), and PLC (U17322) suppressed SDF1α-induced chemotaxis in both 32D and BaF3 JAK2-V617F cells. Knockdown of PLC 1, the downstream target of the JAK2 V617F-BTK signaling confirmed chemotaxis data using inhibitors. Interestingly, granulocytes isolated from MPN patients in comparison to HD demonstrated increased basal migration. SDF1α treatment further enhanced migration in MPN granulocytes. Clinically achievable concentrations of ibrutinib (0.5 μM) significantly reduced SDF1α-stimulated migration in MPN granulocytes. Since SDF1α and BTK family kinases are important in the regulation of Rho GTPases, we evaluated RhoA GTPase activity. Ibrutinib treatment negatively affected basal and SDF1α induced RhoA GTPase activity, indicating RhoA GTPase as one of the downstream targets of the JAK2-BTK signaling axis.
Summary and Conclusions: Here, we describe the molecular basis for JAK2-V617F-induced abnormal and SDF1α-induced cell migration via BTK, PLC 1, and RhoA GTPase. The data provide a rationale to further investigate the contribution of these molecules in abnormal cell motility of JAK2-V617F myeloid progenitors from bone marrow to peripheral blood and to extramedullary organs. Future studies are warranted to further understand molecular mechanisms and clinical potential of these targets.

PF610

HDAC INHIBITION BY VALPROIC ACID DECREASES JAK2V617F LEVELS IN MYELOPROLIFERATIVE NEOPLASMS VIA UP-REGULATION OF MIR-101, *IN VIVO* AND *IN VITRO*

E. De Marinis[1,*], N. Cenfra[2], D. Liberati[3], M. C. Scerpa[1], F. Pagano[1], A. Quattrocchi[1], S. Tomassini[2], M. Gentile[1], A. Ceccherelli[1], A. Cicalini[1], F. Fantasia[1], G. Cimino[2], C. Nervi[1]

[1]Department of Medical-Surgical Sciences and Biotechnologies, University La Sapienza, Latina, [2]Department of Cellular Biotechnology and Hematology, University La Sapienza, Rome, [3]Department of Medical-Surgical Sciences and Biotechnologies, Psychiatry Unit, University La Sapienza, Latina, Italy

Background: Epigenetic-modifying drugs, such as the histone-deacetylase inhibitors (HDACi) are active in myeloproliferative neoplasms (MPN) patients bearing JAK2V617F mutation. However, the long term-tolerability and toxicity of these agents are important concerns. Valproic acid (VPA) is a safe and widely used neurologic agent that acts as a potent HDACi. VPA targets normal and neoplastic hematopoietic stem cells, but its activity in MPNs remains to be determined.
Aims: The present study aims to investigate the efficacy of VPA in MPNs and the molecular mechanisms driving its effect in MPN patients carrying JAK2V617F mutation, *in vitro* and *in vivo*.
Methods: Peripheral blood (PB) buffy coat specimens were collected from healthy donors or patients with MPN. Furthermore, a JAK2V617F+ Essential Thrombocythemia (ET) patient was treated *per os* with 1500mg VPA/die and blood samples were collected periodically. Primary cells, myeloid JAK2WT K562 cells, and JAK2V617F+ HEL, SET2 and UKE1 cells were employed and tested by: i) Trypan blue exclusion, annexinV and propidium iodide staining for cell growth and apoptosis analysis; ii) Western blot and qRT-PCR (Applied Biosystem 7000) to evaluate proteins and mRNA levels; ChIP assay to investigate gene promoters status. Clonogenic assays from peripheral blood mononuclear cells were performed using MethoCult™ (Stem Cell Technologies); Colony genotyping for JAK2 performed by qPCR.
Results: Clinical response, without toxicity, and a significant reduction of JAK2V617F mutant allele burden have been observed over the time of treatment with VPA (>80 months) in a patient with a diagnosis of JAK2V617F+ ET, intolerant to hydroxyurea, who received VPA *per os* for a bipolar disorder. Furthermore, VPA treatment significantly suppressed JAK2V617F+ MPN patients derived BFU-E colonies growth, with a major effect on mutat-

ed clones. VPA treatment also arrested cell growth and induced apoptosis in myeloid cell lines carrying JAK2V617F but not in JAK2WT cells. In JAK2V617F+ cells, VPA inhibited phospho-STAT5 levels and decreased the expression levels of JAK2 and chromatin remodeling proteins EZH2 and DNMT3a. Strikingly, either JAK2, EZH2 or DNMT3a are miR-101 post-transcriptional targets. We found that miR-101 and its precursors pri-miR-101-1 and pri-miR-101-2 expression levels increased time-dependently upon VPA treatment in the ET JAK2V617F+ patient undergoing treatment with VPA and in JAK2V617F-mutated cells. Indeed, in JAK2V617F+ cells, VPA promoted the recruitment of RNA polymerase II and H3K4me3 enrichment at primiR-101-2 promoter, thus increasing its transcriptional activity and miR-101 production. In turn, miR-101 induces the post-transcriptional silencing of JAK2, thus affecting the JAK2/STAT signaling pathway, and of chromatin modifiers EZH2 and DNMT3A.

Summary and Conclusions: Overall, our results relate the changes induced by VPA on chromatin accessibility of pri-miR-101 promoters and modulation of miR-101 activity with its clinical efficacy and molecular response of a JAK2V617F+ ET patient and JAK2V617F+ primary myeloid cells and cell lines. miRNAs can be involved in HDACi mechanisms acting as additional molecular determinants for epigenetic regulation, re-establishing the correct pattern of gene expression. These mechanisms may contribute to the VPA efficacy in JAK2V617F+ MPNs.

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RESULTS FROM 48-WEEK FOLLOW-UP OF THE EXPAND STUDY: A PHASE 1B, OPEN-LABEL, DOSE-FINDING STUDY OF RUXOLITINIB IN PATIENTS WITH MYELOFIBROSIS AND LOW PLATELET COUNTS (50-99 × 10^9/L) AT BASELINE

A.M. Vannucchi[1,*], P. te Boekhorst[2], C.N. Harrison[3], G. He[4], M. Caramella[5], D. Niederwieser[6], F. Boyer-Perrard[7], M. Duan[8], N. Francillard[9], B. Molloy[10], M. Wroclawska[10], H. Gisslinger[11]

[1]Center for Research and Innovation of Myeloproliferative Neoplasms, Azienda Ospedaliero-Universitaria Careggi, University of Florence, Florence, Italy, [2]Erasmus MC/Centrumlocatie, Rotterdam, Netherlands, [3]Guy's and St Thomas' NHS Foundation Trust, Guy's Hospital, London, United Kingdom, [4]No.1 Hospital of Nanjing Med., Nanjing, Jiangsu, China, [5]ASST Grande Osp Metropolitano, Milano, Italy, [6]University of Leipzig, Leipzig, Germany, [7]Centre Hospitalier Universitaire d'Angers, Angers, France, [8]Peking Union Medical College Hospital, Beijing, China, [9]Novartis Pharma S.A.S, Rueil Malmaison, France, [10]Novartis AG, Basel, Switzerland, [11]Department of Medicine I, Division of Hematology and Hemostaseology, Medical University of Vienna, Vienna, Austria

Background: Ruxolitinib (RUX, JAK inhibitor) was approved by FDA and EMA for patients (pts) with myelofibrosis (MF) with baseline (BL) platelet (PLT) counts ≥50 × 10^9/L. However, the recommended starting dose of RUX in pts with BL PLT counts 50 to <100 × 10^9/L is 5 mg twice daily (bid), followed by a dose titration. The purpose of the EXPAND study was to gather further information on pts with BL PLT counts ≥50 × 10^9/L and <100 × 10^9/L. The results from the dose-escalation (DE) phase of the EXPAND study established 10 mg bid as the maximum safe starting dose (MSSD) for both cohorts of pts with PLT counts of 75 to 99 × 10^9/L and 50 to 74 × 10^9/L (Vannucchi. *Blood.* 2015;126:2817). Here, we report the results from 48-week (wk) follow-up of the EXPAND study.

Aims: To evaluate 10 mg bid as a safe starting dose of RUX in MF pts with low PLT counts.

Methods: EXPAND (NCT01317875) is an open-label, phase 1b, dose-finding study in MF pts with BL PLT counts of 50 to 99 × 10^9/L. The study consists of 2 phases: DE and safety expansion (SE). In the DE phase, pts were assigned to stratum 1 (S1 [N=27; 75-99 × 10^9/L]) or stratum 2 (S2 [N=19; 50-74 × 10^9/L]) based on their BL PLT counts with the primary objective of determining the MSSD (incidence rate of dose-limiting toxicity [DLT]). The key secondary objectives included safety (adverse events [AEs]) and efficacy (spleen response [SR]: proportion of pts achieving ≥50% of reduction in palpable spleen length); the key exploratory objectives included PROs (change in TSS [modified MFSAFv2.0 diary]). After determination of the MSSD, additional pts were enrolled in the SE phase.

Results: Overall, 69 pts were enrolled (S1=44; S2=25; age ≥65 years=62%, male=48%, primary MF=74%, high-risk MF=41%, JAK2 mutation positive=80%). Median exposure to RUX was 51.43 wks in S1 and 67.43 wks in S2. At wk 48, 14 pts in S1 and 3 pts in S2 were still receiving RUX treatment (Tx). At data cutoff, primary reasons for end of Tx were AEs (S1=11 pts; S2=7 pts), Tx duration completed (S1=7 pts; S2=6 pts), physician decision (S1=4 pts; S2=3 pts), disease progression (S1=4 pts; S2=1 pt), and deaths (S1=0; S2=3 pts). Reported AEs were consistent with known safety profile of RUX (Table 1).

Table 1.

AEs (All Grades, in ≥20% of pts in Either Stratum [Regardless of Study Drug Relationship])

Preferred Term	Stratum 1 (N=44)		Stratum 2 (N=25)	
	All Grades	Grade 3 or 4	All Grades	Grade 3 or 4
Thrombocytopenia	28 (63.6)	22 (50.0)	19 (76.0)	19 (76.0)
Anemia	23 (52.3)	14 (31.8)	12 (48.0)	6 (24.0)
Diarrhea	13 (29.5)	2 (4.5)	8 (32.0)	0
Pyrexia	9 (20.5)	0	7 (28.0)	2 (8.0)
Asthenia	8 (18.2)	3 (6.8)	7 (28.0)	2 (8.0)
Cough	4 (9.1)	0	10 (40.0)	0
Abdominal pain	8 (18.2)	0	5 (20.0)	0
Headache	6 (13.6)	0	6 (24.0)	0
Back pain	6 (13.6)	1 (2.3)	5 (20.0)	1 (4.0)
Epistaxis	10 (22.7)	1 (2.3)	1 (4.0)	0
Nasopharyngitis	5 (11.4)	0	6 (24.0)	0
Nausea	5 (11.4)	0	5 (20.0)	0
Edema peripheral	5 (11.4)	3 (6.8)	5 (20.0)	0
Pain in extremity	4 (9.1)	0	6 (24.0)	0
Hypocalcemia	3 (6.8)	0	6 (24.0)	0
Hypertension	2 (4.5)	2 (4.5)	5 (20.0)	0

Grade (G) 3 or 4 AEs were reported in 57/69 pts. AEs (regardless of study drug relationship) led to Tx discontinuation in 13 pts in S1 and 10 pts in S2.

Overall, 44/69 pts had AEs that required dose adjustment/Tx interruption (most common reason: thrombocytopenia [S1=16; S2=17]). Thrombocytopenia (G4) was the only DLT (related to study drug) reported in both strata at 10 mg bid (S1=1 pt; S2=2 pts). A G4 worsening from BL in PLTs was seen in 4/44 pts in S1 and 8/25 pts in S2. Reasons for on-Tx death included AML (1), cardiac arrest (1), unknown (1; not suspected to be related to study drug) in S1 and complications after GI ulcer (1), multiple organ failure (1) in S2. At wk 48, 7/22 pts in S1 and 5/14 pts in S2 achieved SR; while majority of pts achieved SR at any time point in both strata (S1=22/43 pts; S2=17/25 pts [Figure 1]). Improvement in TSS was observed at MSSD in both strata (mean change from BL at wk 24: S1 [N=20], −7.7; S2 [N=18], −3.9).

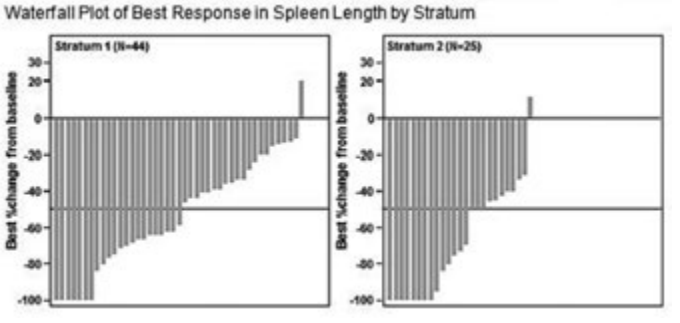

Figure 1.

Summary and Conclusions: In pts with low PLT counts, starting dose of 10 mg bid was safe, generally well tolerated, and consistent with known safety profile of RUX, with only 3 reported cases of DLTs. RUX Tx also provided spleen reduction and TSS benefit in majority of pts. Study results confirmed that 10-mg bid dose is suitable for use in low-PLT population of pts with MF.

PF612

AVAPRITINIB (BLU-285), A SELECTIVE KIT INHIBITOR, IS ASSOCIATED WITH HIGH RESPONSE RATE AND TOLERABLE SAFETY PROFILE IN ADVANCED SYSTEMIC MASTOCYTOSIS (ADVSM): RESULTS OF A PHASE 1 STUDY

M.W. Deininger[1], J. Gotlib[2], W.A. Robinson[3], D.H. Radia[4], M.W. Drummond[5], A.T. Quiery[6], E. Hexner[7], S. Verstovsek[8], H. Shi[9], O. Schmidt-Kittler[9], M. Tugnait[9], M.G. Conlan[9,*], D.J. DeAngelo[10]

[1]Department of Internal Medicine and the Huntsman Cancer Institute (HCI), University of Utah, Salt Lake City, [2]Division of Hematology, Stanford Cancer Institute/Stanford University School of Medicine, Stanford, [3]Medical Oncology, University of Colorado Denver, Aurora, United States, [4]Hematology, Guys and St Thomas NHS Foundation, London, [5]Hematology, Beatson Cancer Centre, Glasgow, United Kingdom, [6]Hematology and Oncology, University of Michigan, Ann Arbor, [7]Medicine/Abramson Cancer Center, University of Pennsylvania, Philadelphia, [8]Leukemia, Division of Cancer Medicine, University of Texas MD Anderson Cancer Center, Houston, [9]Blueprint Medicines, Cambridge, [10]Hematology/Oncology, Dana-Faber Cancer Institute, Harvard Medical School, Boston, United States

Background: AdvSM is a life-threatening disease of neoplastic mast cells (MCs) associated with bone marrow (BM) involvement and organ damage. Approximately 90-95% of patients (pts) exhibit the *KIT* D816V mutation, which results in ligand-independent proliferation and increased survival of MCs. Avapritinib (AVA) is a potent and selective tyrosine kinase inhibitor with subnanomolar activity against KIT D816V.
Aims: This 2-part Phase 1 study was designed to determine the recommended Phase 2 dose (RP2D) of AVA in pts with AdvSM (Part 1) and to assess preliminary efficacy (Part 2).
Methods: Part 1 used a standard 3+3 dose escalation design, with enrichment of cohorts after higher dose levels were cleared for safety. Adult pts with AdvSM (aggressive SM [ASM], SM with an associated hematologic neoplasm [SM-AHN] or mast cell leukemia [MCL]), or other myeloid neoplasm were eligible. Part 2 will enroll approx 35 pts with AdvSM treated at the RP2D. In both study parts, there were no restrictions on prior therapies and pts were required to have a "C-finding" of organ damage based on World Health Organization (WHO) criteria but not on modified (m) International Working Group Myeloproliferative Neoplasms Research and European Competence Network on Mastocytosis (IWG-MRT-ECNM) response criteria. AVA was administered orally once daily, continuously, in 28-day cycles (C), starting at 30 mg. Efficacy assessments, including BM biopsy and liver and spleen imaging for volume assessment, were performed on day 1 of C3, C7, C11, C18 and then every 6 mo; serum tryptase and *KIT* D816V mutant allele fraction (MAF) and other myeloid gene mutations were assessed at central laboratories. Response was assessed using local BM results by m-IWG-MRT-ECNM response criteria with confirmation of response 12 wks after initial response.
Results: With a data cutoff of 04/10/17, a total of 32 pts were treated in Part 1 (17 ASM, 9 SM-AHN, 3 MCL, 2 smoldering SM [SSM], 1 other) at doses between 30 mg and 400 mg daily. *KIT* D816V was present in 88% of pts; 69% had prior SM treatment (4 with midostaurin). Median baseline BM MCs was 25% (range, 5-95%) and serum tryptase was 170 ng/mL (range, 41-1414). No maximum tolerated dose was reached; the RP2D was 300 mg daily. The most common adverse events (AEs) were periorbital edema (59%), fatigue (41%), peripheral edema (34%), and nausea, anemia, and thrombocytopenia (28% each). Only 1 Grade 3 or 4 AE was reported in ≥1 pt (neutropenia, 13%). Dose was reduced in 41% of pts; 2 pts discontinued, none due to AE. Among all 32 pts, normalization (complete response) or ≥50% reduction from baseline (partial response), respectively, was observed for BM MCs (15/26, 58%; 6/26, 23%), tryptase (15/25, 60%; 10/25, 40%), and splenomegaly (6/11, 55%: 4/11, 36%). *KIT* D816V MAF decreased by ≥50% in 19/26 (73%). Improvement in cutaneous mastocytosis was observed in 13/15 pts (87%). 18 pts had an evaluable C-finding at baseline per m-IWG-MRT-ECNM criteria (35% splenomegaly, 28% cytopenias, 19% abnormal liver function, 13% weight loss and/or ascites, 6% hypoalbuminemia); best overall response rates (ORR), confirmed and unconfirmed, are shown in the Table 1. Responses were observed at all dose levels. Median duration of treatment is 9 mo. Part 2 of the study has started with 17 pts treated as of 31/01/18.

Table 1.

Best response n (%)	ASM (n=7)	SM-AHN (n=8)	MCL (n=3)	Overall (n=72)
Overall response rate (Complete response + partial response + clinical improvement)	6 (86)	5 (63)	2 (67)	13 (72)
Complete response	2 (29)	0	0	2 (11)
Partial response	3 (43)	4 (50)	1 (33)	8 (44)
Clinical improvement	1 (14)	1 (13)	1 (33)	3 (17)
Stable disease	1 (14)	3 (38)	1 (33)	5 (28)

Summary and Conclusions: Avapritinib has a tolerable safety profile and is highly active in all subtypes of AdvSM, with an ORR of 72% by m-IWG-MRT-ECNM criteria and improvement in all measures of MC burden in pts with AdvSM and SSM. These data support further evaluation of avapritinib across the spectrum of SM.

PF613

DURABILITY OF SPLEEN RESPONSE MAY AFFECT OUTCOME OF RUXOLITINIB-TREATED PATIENTS WITH MYELOFIBROSIS: RESULTS FROM A MULTICENTRE STUDY ON 284 PATIENTS

F. Palandri[1,*], M. Nicolosi[2], G. Palumbo[3], M. Bonifacio[4], M. Breccia[5], R. Latagliata[5], B. Martino[6], N. Polverelli[7], E. Abruzzese[8], M. Tiribelli[9], M. Bergamaschi[10], A. Tieghi[11], A. Iurlo[12], N. Sgherza[13], F. Cavzzini[14], A. Isidori[15], G. Binotto[16], A. Ibatici[17], M. Crugnola[18], F. Heidel[19], C. Bosi[20], D. Bartoletti[1], L. Scaffidi[4], U. Vitolo[2], F. Aversa[18], M. Trawinska[21], R.M. Lemoli[22], D. Penna[9], D. Cattaneo[12], A. Cuneo[23], F. Soci[11], G. Semenzato[16], G. Auteri[24], L. Catani[24], M. Cavo[24], N. Vianelli[24], G. Benevolo[25]

[1]Institute of Hematology "L. and A. Seràgnoli", Sant'Orsola-Malpighi University Hospital, Bologna, Bologna, [2]Division of Hematology, Città della Salute e della Scienza Hospital, Torino, Torino, [3]Division of Hematology, AOU "Policlinico-V. Emanuele", University of Catania, Catania, [4]Department of Medicine, Section of Hematology, University of Verona, Verona, [5]Division of Cellular Biotechnologies and Hematology, University Sapienza, Roma, [6]Division of Hematology, Azienda Ospedaliera 'Bianchi

Melacrino Morelli', Reggio Calabria, [7]Unit of Blood Diseases and Stem Cells Transplantation, Department of Clinical and Experimental Sciences, University of Brescia, ASST Spedali Civili of Brescia, Brescia, [8]Division of Hematology, Ospedale S. Eugenio, Rome, [9]Division of Hematology and BMT, Azienda Sanitaria Universitaria Integrata di Udine, Udine, [10]Clinic of Hematology, Department of Internal Medicine (DiMI), IRCCS AOU San Martino-IST, Genova, [11]Division of Hematology, Azienda Ospedaliera-IRCSS Arcispedale Santa Maria Nuova, Reggio Emilia, [12]Hematology Division, IRCCS Ca' Granda - Maggiore Policlinico Hospital Foundation, University of Milan, Milan, [13]Division of Hematology, Casa Sollievo Sofferenza, , San Giovanni Rotondo, [14]Division of Hematology, University of, Ferrara, [15]Hematology and Stem Cell Transplant Center, AORMN Hospital, Pearo, [16]Unit of Hematology and Clinical Immunology, University of Padova, Padova, [17]Division of Hematology and Bone Marrow Transplant, IRCCS San Martino-IST, Genova, Genova, [18]Division of Hematology, Azienda Ospedaliero-Universitaria di Parma, Parma, [19]Internal Medicine II, Hematology and Oncology, Friedrich-Schiller-University Medical Center, Jena, Germany, [20]Division of Hematology, Piacenza, Piacenza, [21]Division of Hematology, Ospedale S. Eugenio, Roma, [22]Clinic of Hematology, Department of Internal Medicine (DiMI), IRCCS AOU San Martino-IST, Genova, Genova, [23]Division of Hematology, University of Ferrara, Ferrara, [24]Institute of Hematology "L. and A. Seràgnoli", Sant'Orsola-Malpighi University Hospital, Bologna, [25]Division of Hematology, Città della Salute e della Scienza Hospital, Torino, Italy

Background: Ruxolitinib (RUX) is a JAK1/2 inhibitor able to control myelofibrosis (MF)-related splenomegaly, with around 60-80% of patients (pts) achieving a reduction in spleen length by at least 25%. The achievement of a single spleen response was not found to correlate with survival in previous analysis. Also, no data are available on the dynamics of spleen response over time and on the impact of RUX-response feature on outcome.
Aims: To report the dynamics of spleen response on outcome of 284 evaluable MF pts treated with RUX according to prescribing information.
Methods: A clinical database was created in 23 European Hematology Centres including retrospective data of 462 MF pts treated with RUX from Jan 2011 to July 2017. 284 pts (61.5%) received at least 4 evaluations of spleen length by palpation for a period of at least 12 months and were included in the analysis. Spleen response was defined as reduction of spleen length ³25% by palpation. Overall Survival (OS) was calculated from the start of RUX. Progression-free survival included progression to acute leukemia (AL) and death, whichever came first. Event-free survival (EFS) included also RUX discontinuation for any cause.
Results: A total of 284 (52.1% primary, 47.9% post Polycythemia Vera/Essential Thrombocythemia) MF-patients were analyzed. After a median follow-up of 25.8 months from RUX-start, 48 deaths, 18 progressions to AL, and 85 RUX discontinuation were recorded. Overall, 195 pts (68.7%) achieved a spleen response at 6 months. By landmark analysis at 6 months, OS of pts in spleen response at 6 months was comparable to non-responders at that time-point, after adjustment for DIPSS risk (p=0.12), while spleen responders had significantly better PFS and EFS (p=0.02, p=0.005 respectively) (Figure 1a,b,c). Pts were classified into three groups of response: a) pts with stable response (SR) at all evaluations; b) pts with fluctuating results (FR); c) pts with no response (NR). Compared to pts with FR/NR, pts with stable response were less frequently at high DIPSS risk (OR 0.36, 95% CI 0.14-0.94, p=0.04), carried less frequently a large (>10 cm) splenomegaly (OR 0.20, 95% CI 0.09-0.45, p<0.001), and started RUX more frequently <2 yrs from MF diagnosis (OR 0.47, 95% CI 0.26-0.83, p=0.01). Among the three groups of response, we observed 172 (60.6%) SR, 50 (17.6%) FR, and 62 (21.8%) NR. Using a Cox model adjusted for DIPSS category, OS and PFS were significantly worse for pts with NR compared to pts with SR/FR (p=0.04 and p=0.05) (Figure 1d,e). 3y-EFS according to different group of response was 73.2%, 58.3% and 45.2% for SR, FR and NR, respectively (p=0.004) (Figure 1f).

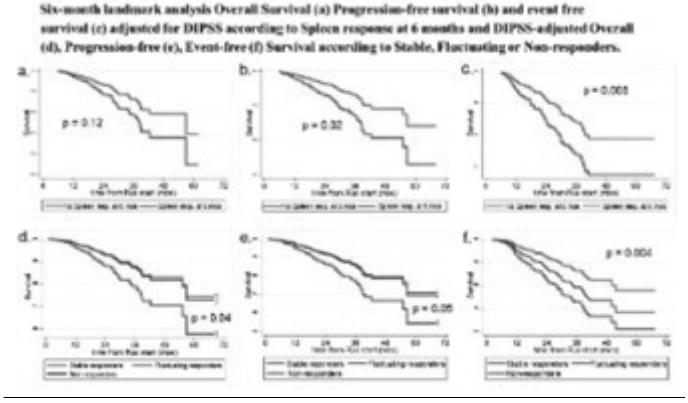

Figure 1.

Summary and Conclusions: This large study failed to detect a direct correlation between spleen response at a specific time-point (6-month) and OS. Additionally, the absence of a spleen response for a period ≥12 months significantly correlated with worse OS and PFS. Noteworthy, achieving a FR correlated with lower EFS, mainly due to a higher rate of drug discontinuation, but did not hamper OS and PFS compared to stable responders. Overall, the study shows that around 40% of pts did not achieve a stable response to RUX, and points out that the dynamics of response, more than a single evaluation of response, correlates with OS.

PF614

DEVELOPMENT OF A PROGNOSTICALLY RELEVANT 'CACHEXIA INDEX' IN PRIMARY MYELOFIBROSIS USING SERUM ALBUMIN AND CHOLESTEROL LEVELS

M. Nicolosi[1,*], D. Penna[1], M. Mudireddy[1], N. Szuber[1], T. Lasho[1], C. Hanson[1], R. Ketterling[1], N. Gangat[1], A. Pardanani[1], A. Tefferi[1]

[1]Hematology, Mayo Clinic, Rochester, United States

Background: "constitutional symptoms" is a consistent risk variable in current prognostic models in primary myelofibrosis (PMF), including the international prognostic scoring system (IPSS), dynamic IPSS (DIPSS), DIPSS-plus and the recently unveiled mutation-enhanced IPSS (MIPSS70 and MIPSS70-plus) (*JCO. 2018;36:310*). The individual components of "constitutional symptoms" are poorly standardized, subjectively influenced and their accuracy is difficult to ascertain. Constituional symptoms in PMF reflect the hypercatabolic/cachectic state of the disease and are thus amenable to more objective quantification using laboratory biomarkers of cachexia.
Aims: To develop a new 'cachexia index' for PMF.
Methods: Patient histories were retrospectively reviewed and serum albumin or cholesterol levels were collected at time of diagnosis or first referral to our instituion. Receiver operating characteristic (ROC) plots were employed in order to determine serum cholesterol and albumin levels that were prognostically most discriminative.

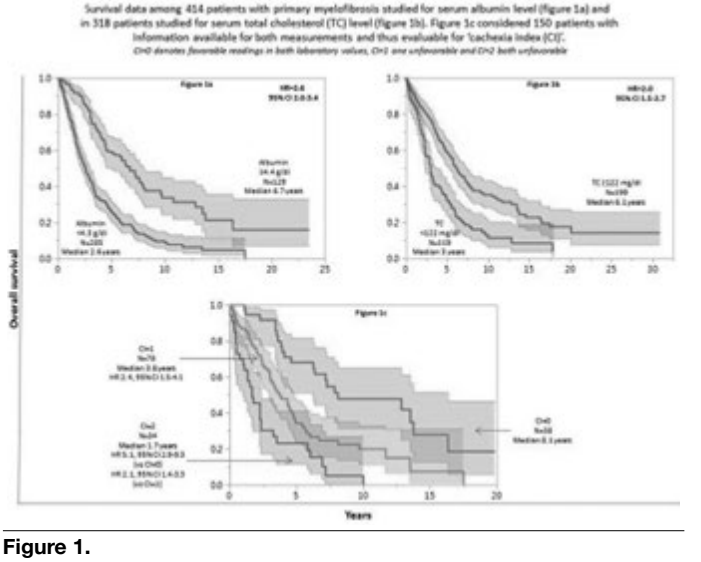

Figure 1.

Results: Among 1,109 consecutive patients with PMF reviewed, serum albumin level was recorded in 484 patients and total cholesterol (TC) in 374; 179 patients had information on both. Patients with serum albumin level of <4.3 g/dl were older and universally displayed prognostically adverse clinical features; by contrast, differences in cytogenetic or mutation profiles were not apparent. Patients with serum TC of <122 mg/dl were also more likely to display adverse features but, in addition, displayed male preponderance and significant clustering with the presence of *JAK2* and *ASXL1* mutations. In age-adjusted univariate analysis, lower levels of serum albumin (p<0.001), TC (p<0.001), high-density lipoprotein (HDL) (p<0.001) and low-density lipoprotein (LDL) (p<0.001) cholesterol, but not triglycerides (p=0.6), were associated with shortened survival; only the first two retained their significance on multivariable analysis. Subsequent ROC analysis resulted in TC level of 122 mg/dl and serum albumin level of 4.3 g/dl as the most optimal cutoff values for survival prediction. Serum albumin level of <4.3 g/dl and TC level of <122 mg/dl both remained significant during multivariable analysis that included DIPSS,

DIPSS-plus or the revised cytogenetic risk stratification; their respective HRs (95% CI) were 1.8 (1.2-2.8) and 1.8 (1.2-2.8) with DIPSS, 1.7 (1.1-2.6) and 1.7 (1.1-2.5) with DIPSS-plus and 2.8 (1.8-4.4) and 2.2 (1.4-3.4) with cytogenetic risk. Additional multivariable analysis that included the individual components of DIPSS/DIPSS-plus resulted in complete loss of significance for constitutional symptoms and circulating blasts ≥1%. The two laboratory parameters were consequently used to develop three-tiered "cachexia index (CI)": a score of "0" (CI-0), "1" (CI-1) or "2" (CI-2) was assigned depending on the absence or presence of one or two unfavorable readings, respectively. The PMF CI eclipsed "constitutional symptoms" in its prediction of inferior survival; HRs (95% CI) were 4.9 (2.7-9.1) for CI-2, 2.4 (1.5-4.1) for CI-1 and 1.2 (0.7-1.8) for constitutional symptoms. PMF CI was also prognostically independent of DIPSS, DIPSS-plus and the revised cytogenetic risk stratification (Figure 1).

Summary and Conclusions: We have developed an operational 'cachexia index' for PMF using two simple and widely available laboratory tests: serum albumin and cholesterol levels. The PMF cachexia index overtook the prognostic contribution of 'constitutional symptoms' and remained significant in the context of all contemporary prognostic models.

PF615

PATIENTS WITH LOW JAK2 ALLELE BURDEN HAVE A HIGH RATE OF CO-OCCURRENT CALR MUTATIONS BEARING AN EFFECT ON DISEASE PHENOTYPE IN ESSENTIAL THROMBOCYTHEMIA AND PRIMARY MYELOFIBROSIS.

B. Mora[1,*], M. Maffioli[1], D. Barraco[1], M. Merli[1], D. Caramazza[1], L. Elli[2], R. Accetta[2], A. Bussini[2], F. Pallotti[23], S. Uccella[4], R. Casalone[2], T. Giorgino[5], F. Passamonti[13]

[1]Hematology, Department of Specialist Medicine, Ospedale di Circolo, ASST Sette Laghi, Varese, Italy, [2]Cytogenetics and Medical Genetics Laboratory, ASST Sette Laghi, Varese, Italy, Varese, [3]Department of Medicine and Surgery, University of Insubria, Varese, Italy, [4]Department of Medicine and Surgery, Unit of Pathology, University of Insubria, Varese, Italy, [5]Biophysics Institute, National Research Council of Italy, Milano, Italy

Background: Co-occurrence of driver mutations (*JAK2*, *CALR*, *MPL*) in myeloproliferative neoplasms has been described in 10-15% of patients with low (<5%) *JAK2*V617F allelic burden (*JAK2* AB). Information on the biologic correlates of co-existing driver mutations and differences with "single mutated" cases is scant.

Aims: The primary objective of this study is to assess the frequency of "double mutated" cases in our Institutional dataset of essential thrombocythemia (ET) and primary myelofibrosis (PMF) with low *JAK2* AB. In addition, clinical relevance of co-existing mutations is investigated.

Methods: Diagnosis of ET and PMF was performed from 2004 to January 2018, reviewed according to the last WHO criteria. Informed consent was obtained from all patients. *JAK2* AB was evaluated with allele specific quantitative PCR (sensitivity threshold: 0.001%); *CALR* mutation screening was performed with fragment length analysis, followed by Sanger sequencing in case of positive results. *MPL* mutations were detected by Sanger sequencing. Differences between covariates were evaluated with Wilcoxon rank-sum and Fisher's exact tests.

Results: Within our cohort of 463 molecularly annotated patients (313 ET and 150 PMF), *JAK2*V617F mutated cases were 206 (65.8%) and 102 (68%), respectively. *JAK2* AB was available in 237 cases (160 ET and 77 PMF). We categorized patients into three groups on the basis of the *JAK2* AB: low (<5%), intermediate (5-10%) and high (>10%). Then, we searched for *CALR* and *MPL* mutations (Table 1).

Table 1.

JAK2 AB, % (n. pts)	*CALR* mutational status, n. pts			*MPL* mutational status, n. pts			Double mutated, n. pts
	Mutated	Wild type	Not done	Mutated	Wild type	Not done	
Low, <5% (25)	7	17	1	1	15	9	8
Intermediate, 5-10% (24)	0	24	0	0	0	24	0
High, >10% (188)	0	70	118	0	12	176	0

In the group of 25 low (<5%) *JAK2* AB mutated cases, 7 patients showed *CALR* mutation (4 ET and 3 PMF) and one PMF case resulted *MPL* mutated. We then evaluated all patients with intermediate (5-10%) *JAK2* AB for *CALR*, without finding any mutated case. Finally, a random sample of patients with high (>10%) *JAK2* AB was screened for *CALR* and *MPL*, finding no co-occurence. Hence, we found an imbalance in *CALR-JAK2* co-mutations in the low *JAK2* AB group respect to other *JAK2* AB groups (~ 30% *vs* 0%, *P* <.0001). Within the set of low *JAK2* AB, *CALR-JAK2* co-mutated *versus* single-mutated cases showed significantly higher hemoglobin level (median value, 14.8 *vs* 12.5 g/dL; *P*=0.01), and platelet count (median value, 956 *vs* 531 x 10^9/L; *P*=0.002) at diagnosis. The effect on hemoglobin was mostly evident in PMF (Figure 1a), while that on platelets was independent from diagnosis (Figure 1b). No differences were found as for leukocyte count, blast count, spleen size, symptoms and age at diagnosis; neither imbalance was evident in vascular events and second malignancies (pre, at, and post diagnosis), and survival.

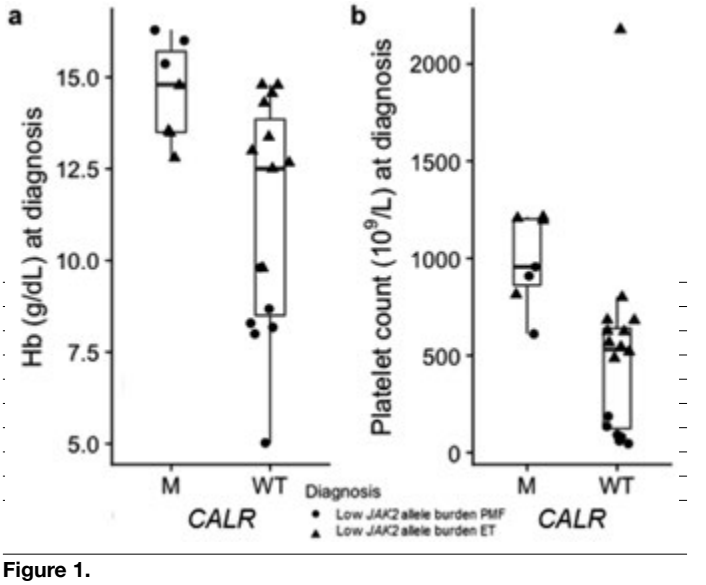

Figure 1.

Summary and Conclusions: In our dataset of 237 *JAK2* mutated ET and PMF patients, ~ 30% of low (<5%) *JAK2* AB mutated patients also harbor other driver mutations. These patients have a more myeloproliferative phenotype respect to single mutated ones (higher values of hemoglobin and platelets). Our data supports the relevance of investigating *CALR* mutational status in low *JAK2* allele burden ET and PMF patients.

PF616

PREDICTORS OF RESPONSE TO RUXOLITINIB (RUX) IN PATIENTS (PTS) WITH MYELOFIBROSIS (MF) IN THE PHASE 3B EXPANDED-ACCESS JUMP STUDY

V. Gupta[1,*], M. Griesshammer [2], B. Martino[3], L. Foltz[4], R. Tavares[5], H.K. Al-Ali[6], P. Giraldo[7], P. Guglielmelli[8], E. Lomaia[9], C. Bouard[10], J. Perez Ronco[10], R. Tiwari[11], P. Raanani[12]

[1]Princess Margaret Cancer Centre, Toronto, Ontario, Canada, [2]Johannes Wesling Clinic, Minden, Germany, [3]Azienda Ospedaliera Bianchi-Melacrino'-Morelli, Reggio Calabria, Italy, [4]St. Paul's Hospital, University of British Columbia, Vancouver, British Columbia, Canada, [5]Universidade Federal de Goiania, Goiania, Brazil, [6]University Hospital of Halle, Halle (Saale), Germany, [7]Miguel Servet University Hospital and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Zaragoza, Spain, [8]CRIMM, Center for Research and Innovation of Myeloproliferative Neoplasms, AOU Careggi, Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy, [9]Almazov National Medical Research Centre, St. Petersburg, Russian Federation, [10]Novartis Pharma AG, Basel, Switzerland, [11]Novartis Healthcare Pvt. Ltd., Hyderabad, India, [12]Davidoff Cancer Center, Rabin Medical Center, Petah Tikva, Israel

Background: RUX is a potent JAK1/JAK2 inhibitor that leads to reductions in splenomegaly and symptoms and improved overall survival in pts with MF. Although most pts with MF benefit from RUX treatment, response to RUX may vary among pts. JUMP is the largest phase 3b, expanded-access trial in MF in countries with no access to RUX outside a clinical trial. Analysis of this large, prospective, multinational database may allow for the identification of clinical factors associated with a response to RUX.

Aims: To analyze the impact of baseline pt characteristics and dose on spleen and symptom response in pts with MF in the JUMP study.

Methods: Pts were treated for up to 24 months after the last pt's first visit (23 December 2014) unless discontinuation criteria were met. Eligible pts had IPSS intermediate (Int)-2– or high-risk MF, with or without splenomegaly, or Int-1–risk MF with a palpable spleen (≥5 cm). Starting doses were based on baseline platelet (PLT) count (5 mg bid [≥50 to

<100×10⁹/L], 15 mg bid [100-200×10⁹/L], or 20 mg bid [>200×10⁹/L]) and could be titrated during treatment. Spleen response was evaluated according to IWG-MRT criteria. Symptom responses were evaluated using the FACT-Lymphoma total score (FACT-Lym TS) and FACIT-Fatigue scales.
Results: Data were collected from 2233 pts treated at 279 clinical sites across 26 countries. Overall, 54.5% of pts were male and 59.4% had primary MF. Median baseline characteristics were age, 67 y (range, 18-89 y; ≥65 y, 59.7%); spleen length, 12 cm; time since diagnosis, 25.8 mo; hemoglobin (Hb), 106 g/L (<100 g/L, 38.3%); and PLT count, 254×10⁹/L (<100×10⁹/L, 6.2%). Mean FACT-Lym TS and FACIT-Fatigue scores were 113.9 and 32.7, respectively. Median RUX exposure was 12.4 mo. Improvements in splenomegaly and symptoms were seen in all pt subgroups. Overall, 34.3% of evaluable pts (672/1960) achieved a spleen response by IWG-MRT criteria at wk 24. In univariate analysis, baseline factors associated with lower spleen response were IPSS Int-2/high risk, Hb <10 g/dL, PLT count <100×10⁹/L, time since MF diagnosis >2 y, prior treatment, and blasts ≥1%. Two factors remained significant by multivariate analysis: IPSS Int-2/high risk and prior treatment. Additionally, spleen response was correlated with a higher RUX dose, with pts treated with ≥10 mg bid in the first 12 wk having better response rates (39.7% *vs* 25.3%, adjusted OR, 0.58 [95% CI, 0.37-0.91]). Among evaluable pts, 53.7% (753/1403) achieved a symptom response on the FACT-Lym TS scale; 49.1% (709/1443) achieved a response on the FACIT-Fatigue scale. In univariate analysis, only a RUX dose titrated to <10 mg bid during the first 12 wk of therapy negatively correlated with symptom response (FACT-Lym TS, 44.7% *vs* 54.9%; OR, 0.66 [95% CI, 0.50-0.89]; FACIT-Fatigue, 42.0% *vs* 50.3%; OR, 0.72 [95% CI, 0.54-0.95]). No associations were seen by multivariate analysis (Figure 1).

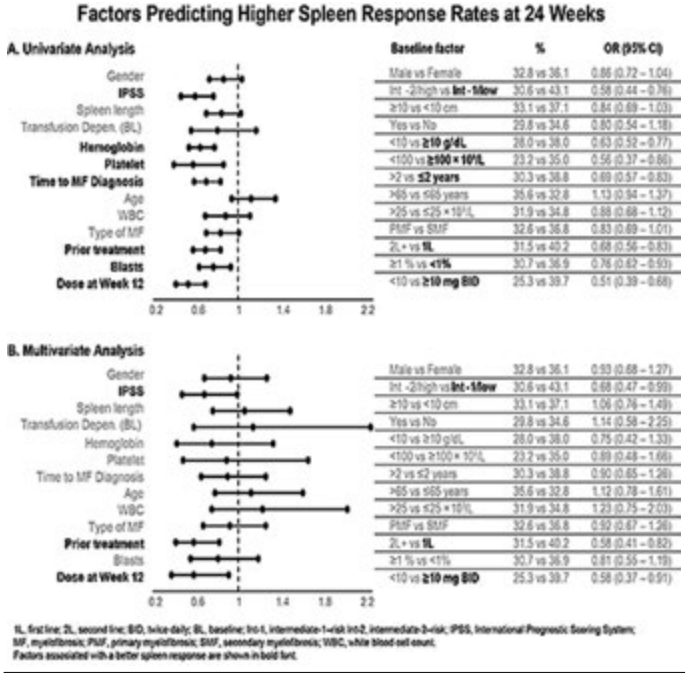

Figure 1.

Summary and Conclusions: This study used factors available in routine clinical practice to identify pts likely to have a better response to RUX. Less-advanced disease, dose ≥10 mg bid, and RUX as first-line therapy were the independent factors associated with higher spleen response rates. No predictive factors were identified for symptom response. These findings suggest that treating pts with RUX earlier in the disease course and at higher doses may lead to greater splenic responses.

PF617

HIGH RISK OF RECURRENT VENOUS THROMBOEMBOLISM IN BCR-ABL-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS AFTER TERMINATION OF ANTICOAGULATION

K. Wille[1,*], P. Sadjadian[1], T. Becker[1], K. Vera[1], A. Horstmann[1], M. Griesshammer[1]

[1]Universitiy Clinic for Hematology, Oncology, Hemostaseology and Palliative Care, Johannes Wesling Medical Center Minden, Minden, Germany

Background: Venous thromboembolism (VTE) is a major burden in patients with *BCR-ABL*-negative myeloproliferative neoplasms (MPN). In addition to cytoreductive treatment anticoagulation is mandatory, but optimal duration of anticoagulation is a matter of debate.
Aims: In this analysis we included 526 MPN patients seen in our center since 2006.
Methods: A VTE associated to MPN was defined as a venous thrombosis at any site, which occurred at least 5 years before MPN diagnosis or thereafter.
Results: In total, 82 of 526 MPN patients (15.6%) had a MPN associated VTE. Median age at first VTE was 52.5 years (range 23-81). During follow up time of 3781 years 110 VTE events (VTEs) have occurred with an event rate of 2.9% per patient/year. 44.5% (49 of 110) of all VTEs appeared before or at MPN diagnosis and 51.8% (57 of 110) occurred at "unusual" sites like splanchnic or cerebral veins. MPN patients with VTEs were significantly more frequent *JAK2* positive (p<0.001) or were diagnosed as polycythemia vera (p=0.004). MPN patients without VTEs had a higher rate of *CALR* positivity (p=0.022). Total follow up time after first VTE was 382 years with 27 VTEs accounting for a recurrence rate of 7.1% per patient/year. In 43 of 75 MPN patients with VTEs (57.3%), prophylactic anticoagulation was terminated after a median time of 12 months (range 1-204) and 19 patients (44.2%) had a VTE recurrence after a median of 13 months (range 4-168). In contrast, only three of 32 patients with ongoing anticoagulation had a VTE recurrence (p=0.001).
Summary and Conclusions: Thus, termination of prophylactic anticoagulation was associated with a significant higher risk of VTE recurrence. Our data suggest that in MPN patients with VTEs a prolonged duration of anticoagulation may be beneficial.

PF618

RESULTS FROM ONGOING PHASE 1/2 TRIAL OF SL-401 IN PATIENTS WITH INTERMEDIATE OR HIGH RISK RELAPSED/REFRACTORY MYELOFIBROSIS

N. Pemmaraju[1,*], V. Gupta[2], G. Schiller[3], S. Lee[4], A. Yacoub[5], H. Ali[6], M. Talpaz[7], M. Sardone[8], H. Wysowskyj[8], S. Shemesh[8], J. Chen[8], C. Brooks[8], T. Goswami[8], V. Dunn[8], A. Pardanani[9], A. Tefferi[9], S. Verstovsek[1], J. Khoury[1], M. Patnaik[9]

[1]The University of Texas MD Anderson Cancer Center, Houston, United States, [2]Princess Margaret Cancer Centre, Toronto, Canada, [3]UCLA, Los Angeles, [4]Weill Cornell Medical Center, New York, [5]Kansas University Cancer Center, Westwood, [6]City of Hope, Duarte, [7]University of Michigan Health System, Ann Arbor, [8]Stemline Therapeutics, New York, [9]Mayo Clinic, Rochester, United States

Background: Myelofibrosis (MF) patients that have failed or are intolerant to JAK inhibitors (JAKi) have no standard treatment options. SL-401 is a novel targeted therapy directed to the interleukin-3 receptor-α (CD123), a target expressed on a variety of malignancies, including certain myeloproliferative neoplasms (MPNs) such as MF. CD123 is also expressed on plasmacytoid dendritic cells (pDCs), which can reside in the tumor microenvironment and may play a role in promoting growth of MPN and other malignancies. Accordingly, a therapy directed at CD123-expressing malignant cells and/or neighboring pDCs may offer a novel therapeutic approach. SL-401 demonstrates high levels of clinical activity against blastic plasmacytoid dendritic cell neoplasm (BPDCN), a CD123+ malignancy derived from pDCs, and is being evaluated in Phase 1/2 trials of other cancers.
Aims: Primary objectives include assessment of safety, determining optimal dose/regimen, and evaluating efficacy outcomes.
Methods: This multicenter, 2-stage Phase 1/2 trial is enrolling MF patients that were relapsed, refractory, or unable to tolerate JAKi. In the Stage 1 dose escalation cohort (completed), SL-401 was dosed as a daily IV infusion at 7, 9, and 12 mcg/kg/day, on days 1-3 every 21 days (cycle 1-4), every 28 days (cycles 5-7), and every 42 days (cycles 8+). In Stage 2 (ongoing), the optimal dose determined in Stage 1 (12 mcg/kg) is being employed.
Results: As of 2/1/18, 14 patients (pts) with MF were treated with SL-401 (n=6 as 2nd-line; n=8 as 3rd-line, and beyond; with JAKi being the most commonly administered prior therapy). Median age 69 yrs (range 55-81); 71% female. DIPPS Plus risk group was intermediate-1 in 7% (1/14), intermediate-2 in 57% (8/14), and high in 36% (5/14) of pts. Median platelet count 56K/uL (range 19-232). 79% (11/14) of pts had baseline platelets <100K/uL, of which 4 pts (29%) had platelets <50K/uL. 79% (11/14) of pts had baseline splenomegaly defined as 5 cm below costal margin (BCM) by physical exam. Most common treatment-related adverse events (TRAEs), all grades, were thrombocytopenia, hypoalbuminemia and dizziness (all 29%). Most common ≥grade 3 TRAEs were anemia (21%) and thrombocytopenia (14%). Capillary leak syndrome

reported in 1 pt (7%; grade 3). 55% (6/11) of pts with baseline splenomegaly had spleen reductions 25% (range 29-100%), including 3 pts (27%) with spleen reductions 35%, by physical exam: baseline to best response of 5 to 0 cm (100%), 19 to 10 cm (47%), 35 to 19 cm (46%), 30 to 20 cm (33%), 17 to 12 cm (29%), and 14 to 10 cm (29%) BCM. 5 spleen reductions occurred in pts with platelets <100K/uL, including 1 pt with a platelet count <50K/uL (100% spleen reduction [5 to 0 cm]). 75% (9/12) of evaluable pts experienced reductions in total symptom score (TSS) (range: 25%>100%), of which 4 pts (33%) had ≥50% TSS reduction. Median overall survival has not been reached (median follow-up 6.5 mos; range 0.1-20.6+ mos).

Summary and Conclusions: Single agent SL-401 was well-tolerated and demonstrated spleen reductions and symptom improvement in relapsed/refractory MF pts, including in some pts with thrombocytopenia. Given CD123 expression on certain myeloid neoplastic cells and tumor microenvironmental pDCs, SL-401 may offer a novel targeted approach in MF. Enrollment continues, and updated safety and efficacy data will be presented. Registration-directed designs are being evaluated. Clinical trial information: NCT02268253.

PF619

MUTATIONAL LANDSCAPE AND ITS IMPACT ON OUTCOMES IN ACCELERATED AND BLAST PHASE OF MYELOPROLIFERATIVE NEOPLASMS

C. Mcnamara[1,*], T. Panzarella[2], J. Kennedy [1], A. Arruda[1], J. Claudio[1], J. Ho[1], G. Daher-Reyes[1], R. Al Blooshi[1], N. Siddiq[1], R. Devlin[1], T. Stockley[3], M. Sukhai[3], N. Kanwar[3], H. Tsui[4], S. Chan[1], D. Maze[1], A. Schimmer[1], A. Schuh[1], H. Sibai[1], K. Yee[1], M. Minden[1], S. Kamel-Reid[3], V. Gupta[1]

[1]Department of Medical Oncology and Hematology, [2]Department of Biostatistics, [3]Advanced Molecular Diagnostics Laboratory, Princess Margaret Cancer Centre, [4]Department of Pathology, Toronto General Hospital, Toronto, Canada

Background: There is a paucity of data regarding the impact of mutations on outcomes in accelerated phase (AP) and blast phase (BP) of myeloproliferative neoplasms (MPN). Moreover, it is unknown whether mutational status affects survival as seen in chronic phase.

Aims: To comprehensively describe the mutational spectrum of patients with AP/BP of MPN and correlate molecular profile with baseline clinical features and outcomes.

Methods: All patients with Philadelphia negative MPN in AP/ BP and assessed at Princess Margaret Cancer Centre between January 1998 and April 2017 were identified by cross referencing the MPN and leukemia databases. Targeted sequencing with a 54 myeloid gene panel was performed. The primary endpoint was overall survival defined from the time of transformation to AP/ BP until death or last follow-up. Overall survival endpoints were estimated using the Kaplan-Meier method and log rank test. For univariate and multivariable analyses, overall survival endpoints were fit using the Cox proportional hazards model.

Results: All patients with MPN in AP/BP and with an available molecular sample (n=122; AP=14; BP=108) were included in the study. Treatment consisted of intensive therapy (induction chemotherapy and/or transplant) (n=44), less intensive (azacitidine/ low dose cytarabine/ clinical trial) (n=27) or best supportive care (BSC) (n=51). *JAK2V617F* was the most common mutation occurring in 55% of subjects, *CALR* in 13% and *MPL* in 6%. Thirty-two (26%) patients were triple negative at the time of AP/BP. Frequently mutated genes included: *ASXL1* (30%), *TET2* (25%), *SRSF2* (22%), *RUNX1* (21%) and *TP53* (17%). Mutations in 1, 2, 3 and ≥4 genes were seen in 18 (15%), 16 (13%), 30 (25%) and 56 (46%) patients respectively. *SETBP1* mutation was enriched in triple negative patients (25% *vs* 3%, p=0.002). Apart from LDH, there was no significant difference in the baseline clinical and laboratory variables and overall survival (OS) between *JAK2/ CALR/ MPL* mutated and triple negative patients in AP/ BP. Median OS for the entire cohort was 5.8 months. The 2 year OS was 18%, 15% and 9% for the intensive, less intensive and BSC groups respectively. *TP53* was the only individual mutation to correlate with shorter OS (HR 1.91, 95% CI: 1.02-3.56, *p*=0.04; Figure 1). Number of mutations was also significant with ≥4 mutations associated with worse survival (HR 5.51 95% CI: 2.50-12.15 p<0.0001). Other factors significant on the univariate and multi-variable analysis and associated with shorter OS included: lower albumin level, increased percentage of blasts in peripheral blood and ≥3 cytogenetic abnormalities.

Summary and Conclusions: *TP53* mutation and ≥4 mutations predict shorter survival in patient with AP/BP of MPN. Mutational profile along with cytogenetics can help in selecting patients who may benefit most from curative approach. This is an area of unmet clinical need and alternative treatment strategies are urgently needed.

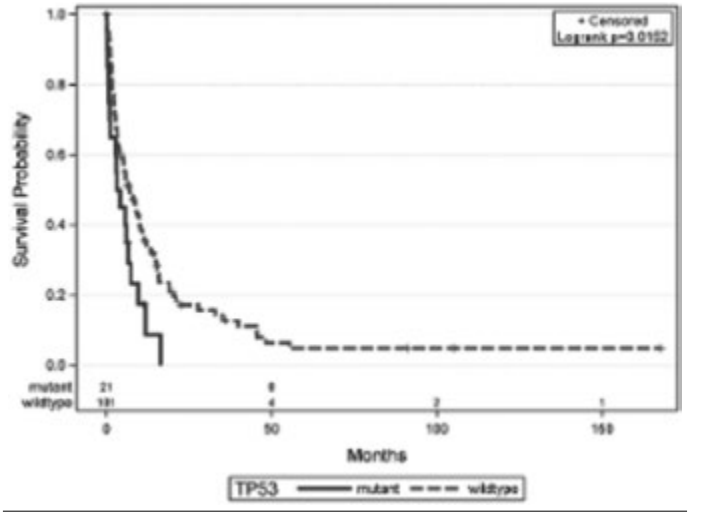

Figure 1.

PF620

SAFETY AND EFFICACY OF COMBINATION THERAPY OF INTERFERON ALPHA-2 AND RUXOLITINIB IN POLYCYTHEMIA VERA AND LOW-/INTERMEDIATE-1-RISK MYELOFIBROSIS – A ONE YEAR FOLLOW-UP UPDATE OF A PHASE II STUDY

S.U. Mikkelsen[1,2,*], L. Kjær[1], M.E. Bjørn[1], T.A. Knudsen[1], A.L. Sørensen[1], V. Skov[1], C.B.L. Andersen[1], O.W. Bjerrum[2], N. Brochmann[1], D. El Fassi[3,4], T.A. Kruse[5], T.S. Larsen[6], H.T. Mourits-Andersen[7], C.H. Nielsen[4], N. Pallisgaard[8], M. Thomassen[5], H.C. Hasselbalch[1]

[1]Department of Hematology, Zealand University Hospital, Roskilde, [2]Department of Hematology, Rigshospitalet, [3]Department of Hematology, Herlev University Hospital, [4]Institute for Inflammation Research, Rigshospitalet, Copenhagen, [5]Department of Clinical Genetics, [6]Department of Hematology, Odense University Hospital, Odense, [7]Department of Hematology, South-West Jutlandic Hospital, Esbjerg, [8]Department of Pathology, Zealand University Hospital, Roskilde, Denmark

Background: Interferon-α2 (IFNα2) reduces elevated blood cell counts and splenomegaly in pts with myeloproliferative neoplasms (MPN), and has demonstrated the potential to restore polyclonal hematopoiesis. In a subset of pts, long-term treatment with IFNα2 may induce 'minimal residual disease' defined by normal blood cell counts, normal bone marrow morphology, and low clonal allelic burden (<1%). Its use is limited by inflammation-mediated toxicity, leading to treatment discontinuation in 20-30% of pts. Ruxolitinib (Rux), a potent anti-inflammatory agent, has shown benefit in pts with myelofibrosis (MF) and polycythemia vera (PV) in regard to reducing splenomegaly and symptoms. However, in the majority of pts Rux does not markedly reduce the mutant allelic burden. Combination therapy (CT) with these two agents may be more efficacious than monotherapy with either, potentially improving tolerability of IFNα2 as well.

Aims: To investigate the safety and efficacy of CT with IFNα2 and Rux in MPN pts.

Methods: The COMBI study (#EudraCT2013-003295-12) is a prospective, open-label, single-arm, multicenter phase II study of CT with pegylated IFNα2 (PEG-IFNα2) and Rux in MPN pts (off-label use). Initial therapy was PEG-IFNα2a 45 g or PEG-IFNα2b 35 g once weekly SC and Rux 20 mg BID PO. Planned treatment duration is 24 months. Enrollment is completed, and we report the results from 12 months of follow-up in the 50 enrolled pts. Of these, 32 pts had PV, and 18 pts had low-/intermediate-1-risk MF (primary MF, n=13; post-PV MF, n=4; post-essential thrombocythemia, n=1). The majority of the pts (n=47) were resistant and/or intolerant to IFNα2 monotherapy. Objectives included remission (ELN and IWG-MRT 2013 revised criteria encompassing histologic, hematologic, and clinical responses), toxicity, complete hematologic response (CHR), and molecular response. All pts provided written informed consent.

Results: Partial remission (PR) and sustained CHR were achieved in 9% and 44% of PV pts, respectively. In MF pts, complete or partial remission was achieved in 39%, and sustained CHR in 58%. The median *JAK2*V617F allelic burden declined significantly from 47% (range, 1.8-97%) to 23.5% (range, 0.73-89%; *P*<.0001) in PV pts, and from 45% (range, 0.1-97%) to 18% (range, 0.08-95%; *P*<.0001) in MF pts. Hematologic toxicity was the most common adverse event (AE) and was managed by dose reduction. The most common non-hematologic AEs were arthralgia and/or myalgia (n=40, in 24

pts), symptoms related to PEG-IFNα2 injection (flu-like symptoms, injection site reactions; n=36, in 24 pts), and gastrointestinal symptoms (nausea, abdominal pain, dyspepsia, diarrhea; n=31, in 27 pts). Thirty-seven serious adverse events (SAEs) were recorded in 23 pts. Four SAEs; angina pectoris, herpes zoster (n=2), and anemia/thrombocytopenia, were considered causally related to study therapy. One thromboembolic event (ischemic stroke) was recorded in a PMF pt. The discontinuation rate was 20% (Table 1).

Table 1.

Summary and Conclusions: We conclude that CT with IFNα2 and Rux is feasible and efficacious in pts with low-/intermediate-1-risk MF and, to a lesser extent, in pts with PV including in pts who were unresponsive or intolerant to IFNα2 monotherapy. A high number of SAEs was reported which in many cases reflected short hospitalizations (<24 hrs) elicited by signs of infection. These preliminary results encourage phase III studies as well as a study with CT in newly diagnosed MPN pts.

PF621

BONE MARROW MORPHOLOGY IN PATIENTS WITH POLYCYTHEMIA VERA RESISTANT TO FIRST AND SECOND LINE THERAPIES

H.M. Kvasnicka[1,*], G.L. Frank[1], M. Lemp[1], F. Lang[2], J. Thiele[3]

[1]Senckenberg Institute of Pathology, [2]Department of Medicine, Hematology/Oncology, University of Frankfurt, Frankfurt, [3]Institute of Pathology, University of Cologne, Cologne, Germany

Background: Resistance to first and/or second line therapies in polycythemia vera (PV) is frequently associated with an aggressive disease course and incresed risk of progression to Post-PV myelofibrosis (PPMF). In this regard, treatment failure to Hydroxyurea (HU) as the most frequently used cytoreductive agent is seen in about 15–20% of patients (pts).
Aims: This study was conducted to gain insight into the morphological and clinical heterogeneity of inadequately controlled PV, in particular after standard long-term HU therapy.
Methods: More than 500 consecutively collected cases with PV were screened in this observational study. Only pts with available bone marrow (BM) trephine biopsies at this disease stage were selected. PPMF was defined according to the WHO 2016 criteria and HU resistance was assessed according to ELN. BM trephines were evaluated for grade of BM fibrosis, cellularity, amount of CD34+ progenitors and morphology of megakaryocytes including the occurrence of myelodysplastic features associated with disease progression. Hematological and clinical data were collected at corresponding time points. The total cumulative HU dosage, the average dosage per years, as well as the highest used dosage to control disease were calculated.
Results: 61 cases (32 males, 29 females, median age 66 years) were analyzed. Median time after diagnosis was 8.3 years (range 2.7 – 20 years), median treatment time with first-line cytoreductive agents (HU in 89.3%) was 6 years (range 1 – 13 years years). Only 60.7% (37/61) of cases presented at this time point with a BM fibrosis grade ≥2, *i.e.* PPMF. 9 cases (14.8%) revealed a BM fibrosis grade of 1, whereas an uncontrolled myeloproliferation defined by increased platelet and/or leukocyte counts was found in 15 cases (24.6%). In 5 patients (8.2%) a blast phase was diagnosed at the time of treatment failure which was not associated with progressive fibrosis. Cases with grade 1 fibrosis or uncontrolled myelo-proliferation had higher hemoglobin levels, lower leukocytes count, and higher platelet counts at time of treatment failure as compared to the PPMF group. Classical PPMF more often was presenting with relevant cytopenia and erythrocyte transfusion dependency. MDS-like progression characterized by highly atypical megakaryocytes was observed in 15 pts (24.6%). There was no difference in the amount of CD34+ progenitors between classical PPMF and cases presenting with either grade 1 fibrosis or uncontrolled myeloproliferation. Pts presenting with MDS-like BM features (Figure 1) received a significantly higher total HU dosage (median 4,500 *vs* 721 gram, p=0.018) and showed a longer median treatment time with HU (8 *vs* 4 years), whereas the average annual dosage showed no significant difference.

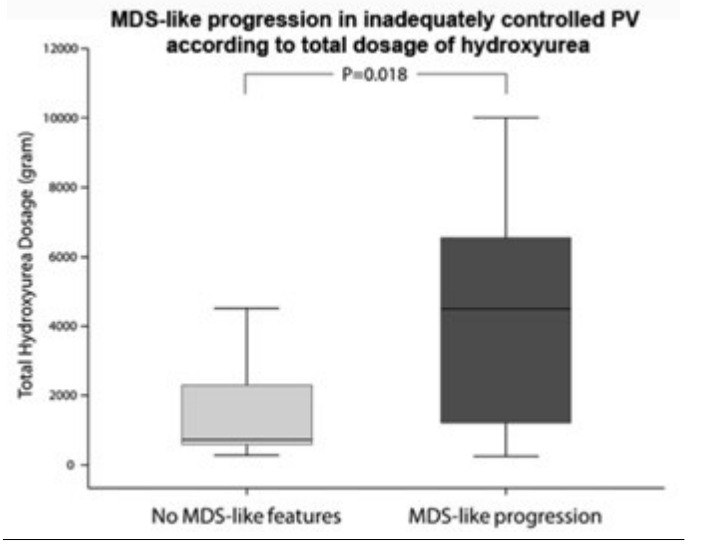

Figure 1.

Summary and Conclusions: Progression of inadequately controlled PV represents a biological spectrum and is not restricted by degree of BM fibrosis as in classical WHO defined PPMF, but can also be defined by resistance to cytoreductive agents like HU. In clinical practice it is important to recognize that high dosages of HU and long treatment duration are more often seen in the important subgroup presenting with MDS-like features.

PF622

WHO 2016 CLASSIFICATION IN 80 CHILDREN AND ADOLESCENTS WITH CHRONIC MYELOPROLIFERATIVE NEOPLASMS

M. Santopietro[1,*], G. Palumbo[1], M.L. Moleti[1], L. Cardarelli[1], L. Rizzo[1], F. Malaspina[1], C. Presicce[1], S.M. Orlando[2], R. Foà[1], F. Giona[1]

[1]Hematology, Department of Cellular Biotechnologies and Hematology, Sapienza University of Rome-Azienda Policlinico Umberto I, [2]Gimema Foundation, Rome, Italy

Background: The first classification of chronic myeloproliferative neoplasms (MPNs) was proposed in 1951 by Dameshek and since then it has been modified several times. The last classification of *BCR-ABL1*-negative MPNs, defined in 2016 by the World Health Organization (WHO), considers morphologic bone marrow (BM) features and driver mutations ($JAK2^{V617F}$, JAK2 exon 12, CALR and MPL) as major criteria. Thus, the WHO 2016 criteria better define MPNs as polycythemia vera (PV), essential thrombocythemia (ET), prefibrotic/early primary myelofibrosis (PMF) (pre-PMF) and overt PMF, making the identification of masked PV (mPV) and "true" JAK2-mutated ET easier. Our experience has suggested that MPNs in children and adolescents are characterized by subtypes that differ from those found in adults and require specific diagnostic criteria.
Aims: The present study was carried out in order to (a) retrospectively review the diagnosis of MPNs in patients aged <20 years based on the WHO 2016 classification and adapted in accordance with our experience in this category of patients, and (b) analyze their clinical and biologic features according to both the initial diagnosis criteria and the adapted WHO 2016 criteria.
Methods: Out of 93 patients aged <20 years with a diagnosis of MPNs carried out from 1980 to 2015, 80 (median age: 14.6 years) with bone marrow (BM) evaluation and available molecular analysis ($JAK2^{V617F}$, JAK2 exon 12, CALR, MPL, EPO-R and HIF) were considered. These patients were divided into different subgroups according to the criteria of their initial diagnosis (PVSG 1975, 1986, 1996, WHO 2001 and 2008): ET, PV, PMF. The subgroups were then retrospectively revised according to the WHO 2016 criteria into: ET, mPV, PV, pre-PMF, overt PMF. Patients with *HIF* mutations and/or criteria of familial erythrocytosis were defined as FE. Patients with MPL^{S505A} mutation were diagnosed as hereditary thrombocytosis (HT).
Results: As shown in Figure 1, of the 56 patients with an initial diagnosis of

ET, 16 (28.5%) had HT due to a *MPL*^{S505A} mutation, 3 (5.4%) were classified, according to the WHO 2016 classification, as mPV and 2 (3.6%) as PMF. Two out of 8 patients, with an initial diagnosis of PV, had FE due to a HIF2α mutation. Considering the adapted WHO 2016 classification, JAK2^{V617F} mutation was found in 28.5% and 44% of ET and PV patients, respectively. CALR mutations and *MPL*$^{W515\_P518>KT}$ were found in 26% and 2.7% of ET patients. Of 23 HT patients, 87% showed *MPL*^{S505A} and 13% *MPL*^{V501A}. Considering both the classification at initial diagnosis and the adapted WHO 2016 criteria, hemoglobin and hematocrit values were significantly higher in FE and slightly lower in PV patients (p<0.0001) and platelet counts were higher in ET and slightly lower in HT patients (p <0.0001) compared to other subgroups. Regarding the other features at diagnosis, leukocyte counts were significantly higher in PMF and ET patients (p= 0.0032), and the presence of medullary fibrosis (≥2 WHO grading) became significant in PMF patients (p= 0.0052), only according to the adapted WHO classification.

Diagnosis at the onset of the disease	N. of patients (%)	WHO 2016 classification (adapted)						
		PV	mPV	FE	ET	HT	pre-PMF	Overt PMF
PV	8 (10%)	6 (75%)	-	2 (25%)	-	-	-	-
FE	8 (10%)	-	-	8 (100%)	-	-	-	-
ET	56 (70%)	-	3 (5.4%)	-	35 (62.5%)	16 (28.5%)	1 (1.8%)	1 (1.8%)
HT	7 (8.75%)	-	-	-	-	7	-	-
PMF	1 (1.25%)	-	-	-	-	-	1	-
N. of patients (%)	80	6 (7.5%)	3 (3.75%)	10 (12.5%)	35 (43.75%)	23 (28.75%)	2 (2.5%)	1 (1.25%)

Figure 1.

Summary and Conclusions: The WHO 2016 criteria are able to identify mPV, pre-PMF and overt PMF in children and adolescents with MPNs also. On the whole, 43%, 56% and 75% of ET, PV and PMF, respectively, did not show gene driver mutations. Hereditary forms can be observed in a significant proportion (41%) of young patients with MPNs. Taken together, our observations suggest that molecular analysis to identify inherited molecular defects must be performed in children and adolescents with MPNs.

PF623

SAFETY AND EFFICACY OF RUXOLITINIB (RUX) IN PATIENTS (PTS) WITH DIPSS LOW-RISK MYELOFIBROSIS (MF) IN THE PHASE 3B EXPANDED-ACCESS JUMP STUDY

P. Guglielmelli[1,*], P. Raanani[2], R. Tavares[3], L. Foltz[4], B. Martino[5], E. Lomaia[6], M. Griesshammer[7], H.K. Al-Ali[8], V. Gupta[9], C. Bouard[10], J. Perez Ronco[10], R. Tiwari[11], P. Giraldo[12]

[1]CRIMM, Center for Research and Innovation of Myeloproliferative Neoplasms, AOU Careggi, Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy, [2]Davidoff Cancer Center, Rabin Medical Center, Petah Tikva, Israel, [3]Universidade Federal de Goiania, Goiania, Brazil, [4]St. Paul's Hospital, University of British Columbia, Vancouver, British Columbia, Canada, [5]Azienda Ospedaliera Bianchi-Melacrino'-Morelli, Reggio Calabria, Italy, [6]Almazov National Medical Research Centre, St. Petersburg, Russian Federation, [7]Johannes Wesling Clinic, Minden, [8]University Hospital of Halle, Halle (Saale), Germany, [9]Princess Margaret Cancer Centre, Toronto, Ontario, Canada, [10]Novartis Pharma AG, Basel, Switzerland, [11]Novartis Healthcare Pvt. Ltd., Hyderabad, India, [12]Miguel Servet University Hospital and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Zaragoza, Spain

Background: RUX, a potent JAK1/JAK2 inhibitor, led to reductions in splenomegaly and symptoms and improved overall survival in pts with intermediate (Int)-2– and high-risk MF according to the International Prognostic Scoring System (IPSS) in the phase 3 COMFORT studies. Similar findings were observed in pts with Int-1–risk MF in the JUMP study, the largest phase 3b, expanded-access trial with RUX in countries with no access to RUX outside a clinical trial. However, data on RUX in pts with low-risk disease are limited.
Aims: To assess the safety and efficacy of RUX in a cohort of pts with Dynamic IPSS (DIPSS) low-risk MF in the JUMP study.
Methods: DIPSS scores were determined using pt characteristics at baseline, and pts with low-risk MF were included. Starting doses were based on baseline platelet (PLT) count (5 mg bid [≥50 to <100×10^9/L], 15 mg bid [100-200×10^9/L], or 20 mg bid [>200×10^9/L]) and could be titrated during treatment. The primary endpoint was RUX safety and tolerability. Changes in palpable spleen length and symptom scores were also assessed.
Results: Based on available data, 60 pts (primary MF, 53%) were determined to have DIPSS low-risk disease; 63% were male. Median baseline characteristics were age, 56.5 y (range, 29-65 y); time since diagnosis, 40.8 mo; hemoglobin, 124.5 g/L; and PLT count, 263.5×10^9/L. Mean spleen length was 13.4 cm (SD, 7.37 cm). Most pts had completed treatment per protocol (68.3%). Reasons for treatment discontinuation included adverse events (AEs; 13.3%), physician decision (5.0%), disease progression (3.3%), and death (1.7%). Overall, 63.3% of pts had dose modifications (AEs, 51.7%); 26.7% had temporary interruptions (AEs, 23.3%). Median exposure was 25.8 mo. The mean average daily dose was 30.4 mg (SD, 10.97 mg). The most common hematologic grade 3/4 AEs were anemia (20.0%) and thrombocytopenia (6.7%); 1 pt discontinued due to anemia (grade 1/2). AEs (all-grade, grade 3/4) in >10% of pts included pyrexia (18.3%, 0%), diarrhea (16.7%, 1.7%), headache (15.0%, 0%), asthenia (13.3%, 5.0%), fatigue (13.3%, 0%), cough (13.3%, 0%), and increased weight (11.7%, 0%). Infections in >2 pts were influenza (10.0%), nasopharyngitis (8.3%), respiratory tract infection (5.0%), and herpes zoster (5.0%). At wk 24, 67.4% of pts (31/46) had a ≥50% reduction from baseline in spleen length, and 10.9% (5/46) had 25%>50% reductions; rates were similar at wk 48 (61.0% [25/41] and 17.1% [7/41]). Best response in spleen length by wk 72 is shown (Figure 1). Most pts (82.1%) achieved a ≥50% reduction at any time. Median time to a ≥50% reduction in spleen length was 8.0 wk (range, 3.1-60.3 wk), and the estimated probability of maintaining a response was 0.92 (95% CI, 0.78-0.97) at 72 wk. Pts also experienced significant improvements in symptoms. From wk 4 to 48, 17.3%>21.4% and 36.5%>42.6% of pts achieved a clinically meaningful response on the FACT-Lymphoma total score and FACIT-Fatigue, respectively.

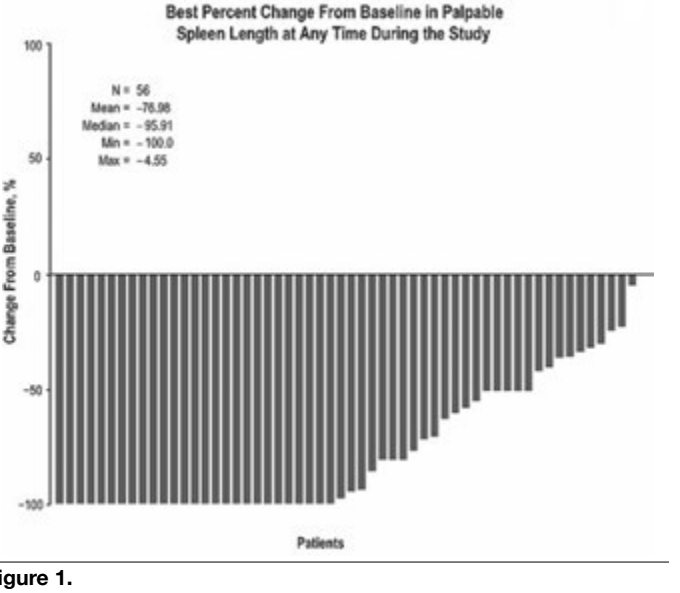

Figure 1.

Summary and Conclusions: RUX demonstrated a safety and AE profile consistent with previous reports. Interestingly, rates of hematologic AEs and discontinuations due to AEs were lower than in the overall JUMP population, despite longer exposure to RUX (25.8 *vs* 12.4 mo). Additionally, low-risk pts achieved slightly greater spleen size reductions, with 92% of pts maintaining a response for >1 y. Symptom improvement was also consistent with that seen in pts with higher-risk disease. These findings suggest that earlier RUX treatment may lead to greater benefits in pts with MF.

PF624

PRIMARY THERAPY AND SURVIVAL IN PRIMARY MYELOFIBROSIS: A NATIONWIDE POPULATION-BASED ANALYSIS AMONG 1,445 PATIENTS DIAGNOSED IN THE NETHERLANDS BETWEEN 2001 AND 2015

S. Slot[1], O. Visser[2], M. Schaap[3], J. Janssen[4], P. te Boekhorst[5], S. Zweegman[1], A. Dinmohamed[5,6,7,*]

[1]Hematology, VU University Medical Center, Amsterdam, [2]Registration, Netherlands

Comprehensive Cancer Organisation (IKNL), Utrecht, [3]Hematology, Radboud University Medical Center Nijmegen, Nijmegen, [4]Hematology, VU University Medical Center, Utrecht, [5]Hematology, Erasmus MC Cancer Institute, Rotterdam, [6]Research, Netherlands Comprehensive Cancer Organisation (IKNL), Utrecht, [7]Public Health, Erasmus University Medical Center, Rotterdam, Netherlands

Background: Primary myelofibrosis (pMF) is a chronic myeloproliferative neoplasm that is incurable in the vast majority of patients. It remains largely unclear how current management strategies impacted outcome of pMF patients at the population level.
Aims: The aim of this nationwide, population-study was to assess trends in primary therapy and survival among pMF patients diagnosed during a 15-year period in the Netherlands.
Methods: We selected all 1,445 pMF patients diagnosed between 2001-2015 (median age 70 years; range 20-95 years; 63% males) from the nationwide Netherlands Cancer Registry (NCR) with survival follow-up through December 31, 2016. Patients were categorized into 2 periods (2001-2007 and 2008-2015) and 3 age groups (≤60, 61-70, and >70). Data on therapy—chemotherapy (CTx), non-CTx therapies (eg, immunomodulatory drugs [IMiDs] and ruxolitinib), and allogeneic stem cell transplantation (alloSCT)—initiated within 9 months since diagnosis were available in the NCR. Data on the International Prognostic Scoring System (IPSS) and the exact therapeutic regimens were available for the period 2014-2015. Relative survival (RS) was calculated as a measure of disease-specific survival. $P<0.05$ indicates statistical significance.
Results: Slightly more patients received non-CTx therapies over time—albeit that increase was modest, namely from 2% to 8% ($P=0.028$), 4% to 12% ($P=0.007$), and 3% to 9% ($P=0.001$) across all 3 age groups (Figure 1). There were no statistically significant changes over time in the application of CTx and alloSCT across the 3 age groups (Figure 1). Of note, the age range of alloSCT recipients was 32-68 years.Data of 256 patients diagnosed during 2014-2015 showed that 136 (53%) patients received no anti-neoplastic therapy, of whom 24/43 (56%), 47/82 (57%), 33/65 (51%), and 17/42 (40%) had low, int-1, int-2, and high risk IPSS, respectively. In 24/256 (9%) patients, the IPSS was unknown. The distribution across the 4 IPSS groups among the 120 (47%) treated patients were 19 (16%), 35 (29%), 32 (27%), and 25 (21%), respectively. All 59 CTx recipients received hydroxyurea (HU), except one who received melfalan. HU was applied in 14/19 (74%), 20/35 (57%), 14/32 (44%) and 5/25 (20%) patients across the 4 IPSS groups, respectively. Most of the 49 non-CTx recipients received ruxolitinib (70%), followed by IMiDs (14%), interferon-α (12%), and momelotinib (4%). Ruxolitinib was applied in 5/19 (26%), 7/35 (20%), 11/32 (34%), and 8/25 (32%) patients across the 4 IPSS groups, respectively. Ten of 12 alloSCT recipients had high risk IPSS. Five-year RS (95% confidence intervals) did not improve over time across 3 age groups and was 76% (67%>83%), 49% (39%>58%), and 31% (24%>39%) in 2001-2007, as compared with 80% (72%>86%), 53% (46%>60%), and 39% (33%>46%) in 2008-2015, respectively ($P>0.05$ for all comparisons; Figure 1). There was a hint that RS reached a plateau after 6 years since diagnosis for patients age ≤60 in 2008-2015.

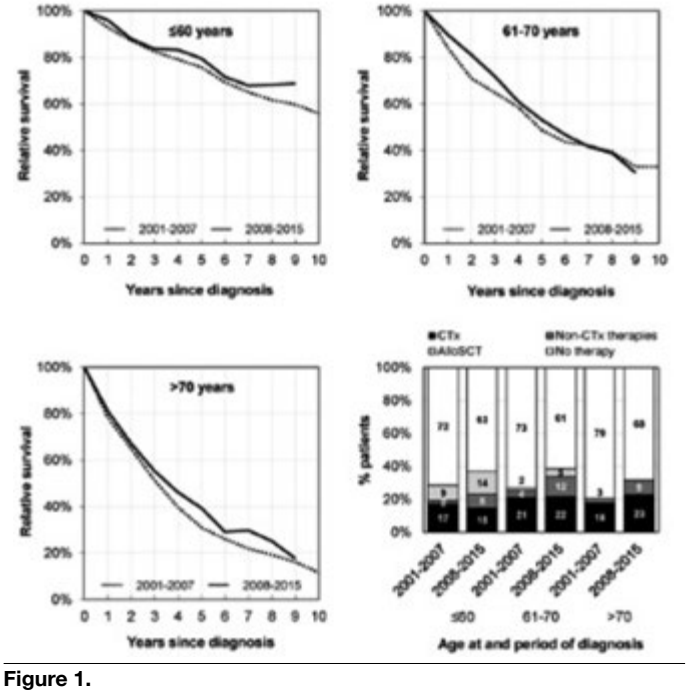

Figure 1.

Summary and Conclusions: We show that pMF patients have excess mortality—as compared to the general population—which did not significantly decline over time, irrespective of age. This might suggest that current treatment practices are inadequate to improve outcome for most patients. Future population-based research is needed to assess the impact of improved management (eg, broader application of reduced-intensity conditioning alloSCT among younger, transplant-eligible patients) and novel agents (eg, wider use of ruxolitinib that was approved for routine use in the Netherlands since 2014) on the outcome of pMF patients managed in daily practice.

PF625

RUXOLITINIB COMPARED WITH BEST AVAILABLE THERAPY FOR POLYCYTHAEMIA VERA PATIENTS RESISTANT OR INTOLERANT TO HYDROXYCARBAMIDE IN MAJIC - AN INVESTIGATOR-LED RANDOMISED TRIAL

C. Harrison[1,*], M.F. McMullin[2], A. Panchal[3], C. Yap[3], S. Fox[3], R. Bishop[3], N. Curto-Garcia[1], J. Coppell[4], J. Laurie[5], M. Garg[6], J. Ewing[7], S. Knapper[8], J. Crowe[9], Z. Maung[10], A. Godfrey[11], N. Panoskaltsis[12], R. Scherber[13], H. Geyer[14], A. Dueck[15], R. Mesa[16], A. Mead[17]

[1]Guy's & St Thomas' NHS Foundation Trust, London, [2]Queen's University, Belfast, [3]Cancer Research UK Clinical Trials Unit, University of Birmingham, Birmingham, [4]Royal Devon & Exeter NHS Foundation Trust, Exeter, [5]Worthing Hospital, Western Sussex NHS Foundation Trust, Worthing, [6]University Hospital of Leicester, Leicester, [7]Birmingham Heart of England NHS Foundation Trust, Birmingham, [8]School of Medicine, Cardiff University, Cardiff, [9]Royal United Hospital Bath NHS Trust, Bath, [10]Western General Hospital, Lothian Health Board, Edinburgh, [11]Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, [12]Department of Haematology, London North West Healthcare NHS Trust, London, United Kingdom, [13]Department of Haematology & Oncology, Oregon Health & Sciences University, Portland, [14]Division of Hospital Medicine, [15]Section of Biostatistics, [16]Division of Hematology & Medical Oncology, Mayo Clinic, Scottsdale, United States, [17]MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom

Background: Polycythaemia vera (PV) is a myeloproliferative neoplasm driven by *JAK2* mutation & is associated with thrombotic/haemorrhagic events, systemic symptoms & disease transformation. Hydroxycarbamide (HC) is the standard first-line treatment for high-risk PV; however, symptom control is poor & »25% of patients become HC resistant/intolerant (RES/INT). Ruxolitinib (RUX) a JAK1/2 inhibitor has significant clinical benefits in myelofibrosis (MF) & in selected HC RES/INT PV patients. The role of RUX for many patients with HC RES/INT PV encountered in routine clinical practice remains unclear.
Aims: To evaluate efficacy of RUX compared to Best Available Therapy (BAT) treatment in a "real-world" setting of PV patients who met modified European LeukaemiaNet (ELN) criteria for HC RES/INT.
Methods: We conducted a randomised, phase II, trial of RUX *vs* BAT for patients with HC RES/INT PV. Primary outcome was achievement of complete haematological response, CR within 1 year (according to ELN guidelines); secondary outcomes included partial haematological response, safety, thrombosis, haemorrhage, transformation (all adjudicated by central review), as well as symptom & quality of life assessment. Patients were recruited over 48 months (2012-2016) & randomised to receive 5-10mg bd of RUX (determined by baseline platelet count) or BAT, with stratification by gender & planned follow-up of 5 years without crossover within the trial. Patients eligible for modified intention to treat (mITT) analysis were those who commenced study treatment & had at least one response assessment. Patient reported outcomes were assessed using EQ5D, MDASI & MPN Symptom Assessment Form (MPN10).
Results: Overall 190 patients were recruited, 182 (107 male) were eligible for mITT analysis, 93 (51%) & 89 (49%) in RUX & BAT arms respectively, mean age 64.6yrs & HC RES/INT (55.4%/44.5%). Baseline characteristics at randomisation were balanced. The primary outcome was achieved in 46 (49.5%) of the patients in the RUX arm *vs* 24 (27.0%) in the BAT arm (p= 0.0009). Partial response occurred in 44 (47.3%) & 59 (66.3%) of those treated with RUX & BAT respectively. Median dose of RUX received was 10mg bd & median follow-up was 2.6 years. Both speed of attaining response (Figure 1A) & duration of overall response (Figure 1B) were also significantly superior for RUX treated patients. Mean MPN-10 TSS, itching, fatigue, night sweats, early satiety, weight loss, bone pain, inactivity, & concertation during the first 12 months were all significantly lower for RUX *vs* BAT (all p<0.05). In RUX treated patients, 12 thrombotic events were experienced by 10 patients & 9 haemorrhagic events by 9 patients; for BAT, 12 thrombotic events occurred in 10 patients & 9 haemorrhagic events in 8 patients. There was no difference in overall survival but a trend to

improved transformation-free survival with RUX was observed (Figure 1C). No new pattern of safety events was noted: grade 3 anaemia occurred in 6.5% of RUX patients *vs* 1.1% in the BAT arm, grade 4 thrombocytopenia in 1.1% of RUX *vs* 0% of BAT patients respectively, & grade 3/4 infections occurred in 8.6%/2.2% of RUX arm *vs* 2.2%/3.4% in BAT arm.

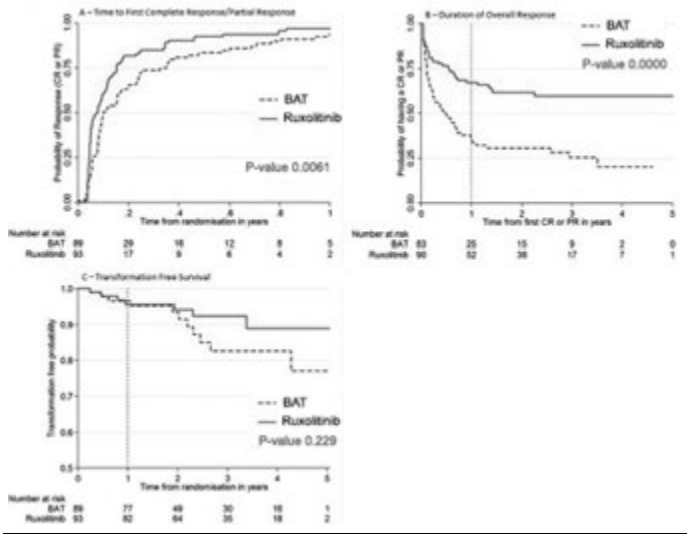

Figure 1.

Summary and Conclusions: Clinically & statistically significant improvement in complete haematological responses within 1 year occurred in RUX compared to BAT treated patients. Responses were more rapidly achieved & were more durable with RUX than BAT & associated with improved symptoms. Our data show RUX is superior to BAT in a "real world" setting of HC RES/INT PV & for the first time that RUX may improve TFS.

PF626

RESULTS FROM ONGOING PHASE 1/2 TRIAL OF SL-401 IN PATIENTS WITH RELAPSED/REFRACTORY CMML

M. Patnaik[1,*], H. Ali[2], V. Gupta[3], G. Schiller[4], S. Lee[5], A. Yacoub[6], M. Talpaz[7], M. Sardone[8], H. Wysowskyj[8], S. Shemesh[8], J. Chen[8], C. Brooks[8], T. Goswami[8], A. Pardanani[1], A. Tefferi[1], S. Verstovsek[9], J. Khoury[9], N. Pemmaraju[9]

[1]Mayo Clinic, Rochester, [2]City of Hope, Duarte, United States, [3]Princess Margaret Cancer Centre, Toronto, Canada, [4]UCLA, Los Angeles, [5]Weill Cornell Medical Center, New York, [6]Kansas University Cancer Center, Westwood, [7]University of Michigan Health System, Ann Arbor, [8]Stemline Therapeutics, New York, [9]The University of Texas MD Anderson Cancer Center, Houston, United States

Background: The outcomes for chronic myelomonocytic leukemia (CMML) patients are poor, with overall response rates (ORR) of ~16% for hypomethylating agents (HMA) in first-line registration studies, and median overall survival (OS) of ~4-7 months in the relapsed/refractory setting. SL-401 is a novel targeted therapy directed to the interleukin-3 receptor-α (CD123), a target expressed on a variety of malignancies, including certain myeloproliferative neoplasms (MPNs) such as CMML. CD123 is also expressed on plasmacytoid dendritic cells (pDCs), which can reside in the tumor microenvironment and may play a role in promoting growth of MPN and other malignancies. Accordingly, a therapy directed at CD123-expressing malignant cells and/or their neighboring pDCs may offer a novel therapeutic approach. SL-401 has demonstrated high levels of clinical activity against blastic plasmacytoid dendritic cell neoplasm (BPDCN), a CD123+ malignancy derived from pDCs, and is also being evaluated in Phase 1/2 trials of other cancers.
Aims: Primary objectives include assessment of safety, determining optimal dose/regimen, and evaluating efficacy outcomes in patients with relapsed/refractory CMML.
Methods: This multicenter, single-arm 2-stage Phase 1/2 trial is enrolling patients with MPN including relapsed/refractory CMML. In the Stage 1 dose escalation cohort (completed), SL-401 was dosed as a daily IV infusion at 7, 9, and 12 mcg/kg/day, on days 1-3 every 21 days (cycle 1-4), every 28 days (cycles 5-7), and every 42 days (cycles 8+). In Stage 2 (ongoing), the dose determined to be optimal in Stage 1 (12 mcg/kg) is being employed.
Results: As of 2/27/18, 14 CMML patients (CMML-1 [n=8]; CMML-2 [n=6]) received SL-401. 12 patients were second-line and 2 patients were third-line, with HMAs being the most commonly administered prior therapy. Median age was 70 years (range 42-80); 71% patients were male. Of the 13 patients with cytogenetic risk categories available, 5 patients (38%) were high risk, 5 patients (38%) were intermediate risk, 2 patients (15%) were low risk, and 1 patient (8%) was other. At baseline, median bone marrow (BM) blasts were 8% (range 0-18%); 57% of patients had splenomegaly (range: 2 to 20 cm palpable below costal margin (BCM) by physical exam). Most common treatment-related adverse events (TRAEs), all grades, were hypoalbuminemia and vomiting (36%), thrombocytopenia, fatigue, edema and nausea (each 29%). Most common ≥grade 3 TRAEs were thrombocytopenia (29%) and hypotension (14%). Capillary leak syndrome was reported in 3 patients (21%; all grade 2). 71% (5/7) of patients with baseline splenomegaly had 50% reduction in spleen size by physical exam: baseline 5, 4, 2, and 2 cm all reduced to 0 cm (100%) BCM; baseline 20 cm reduced to 10 cm (50%) BCM. 17% (2/12) of evaluable patients had BM complete response (BMCR), including 1 CR (15 months on treatment) and 1 BMCR (4+ months on treatment, ongoing).
Summary and Conclusions: Single agent SL-401 was well-tolerated and demonstrated 71% (5/7) rate of spleen reductions and a 17% (2/12) rate of BMCR (1 CR and 1 BMCR) in relapsed/refractory CMML, an area of unmet medical need. Given CD123 expression on certain myeloid neoplastic cells and pDCs in the tumor microenvironment, SL-401 may offer a novel targeted approach for CMML patients. Enrollment continues, and updated safety and efficacy data will be presented. Registrational designs are being evaluated. Clinical trial information: NCT02268253.

PF627

DABRAFENIB IN PEDIATRIC PATIENTS WITH BRAF V600–POSITIVE LANGERHANS CELL HISTIOCYTOSIS

J.A. Whitlock[1], A. Broniscer[2], S.R. Ambati[3], J.R. Hansford[4], T.R. Hummel[5], S. Leary[6], L.V. Marshall[7], V. Shen[8], M.W. Russo[9], K. Dasgupta[10], L. Tseng[9], B. Mookerjee[9], D. Hargrave[11,*]

[1]University of Toronto, The Hospital for Sick Children, Toronto, Canada, [2]St. Jude Children's Research Hospital, Memphis, [3]Memorial Sloan Kettering Cancer Center, New York, United States, [4]The Royal Children's Hospital, Murdoch Children's Research Institute, University of Melbourne, Melbourne, Australia, [5]Cancer and Blood Diseases Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, [6]Cancer and Blood Disorders Center, Seattle Children's Hospital, Seattle, United States, [7]The Royal Marsden Hospital and The Institute of Cancer Research, Sutton, United Kingdom, [8]Children's Hospital of Orange County, Orange, [9]Novartis Pharmaceuticals Corporation, East Hanover, United States, [10]Novartis Healthcare Pvt. Ltd., Hyderabad, India, [11]Great Ormond Street Hospital for Children, London, United Kingdom

Background: Langerhans cell histiocytosis (LCH) is a rare tumor disorder associated with varied clinical manifestations and morbidities. LCH often occurs early in life, and there are limited effective therapies. *BRAF* V600 alterations (which constitutively activate the MAPK pathway) are oncogenic in many tumor types and are present in ≈ 57% of patients with LCH.
Aims: Here we report the safety and preliminary efficacy of the BRAF inhibitor dabrafenib in a phase I/IIa multicohort study in pediatric patients with *BRAF* V600–positive LCH (NCT01677741; BRF116013).
Methods: Patients aged 1 to <18 years with *BRAF* V600–positive LCH and refractory, recurrent, or progressive disease following ≥1 standard therapy were treated with dabrafenib (2 equal daily doses) in the dose-escalation (ESC) cohort (3.75 or 4.5 mg/kg/day) or in the disease-expansion (EXP) cohort at the recommended doses of 5.25 mg/kg/day (patients aged <12 y) and 4.5 mg/kg/day (patients aged ≥12 y). Primary objectives were safety and tolerability, and secondary objectives included tumor response, disease activity, and pharmacokinetics. Tumor response was reported by investigator assessment using Histiocyte Society criteria (HSC), and disease activity was measured using the Donadieu total scoring system (DTS).
Results: At the data cutoff for this interim analysis (September 12, 2017), 13 patients (enrolled from October 2013 to September 2016) had been treated with dabrafenib across the ESC (n=2) and EXP (n=11) cohorts. Median age was 3 years (range, 1-11 years), and prior therapies included chemotherapy (n=13), biologics, hormonal therapy, and immunotherapy (n=1 each). Overall, median duration of dabrafenib exposure was 92 weeks (range, 31-206 weeks), and 9 of 13 patients (69%) had treatment ongoing at data cutoff. The most frequent treatment-related adverse events were vomiting (46%), blood creatinine increase, dry skin, and melanocytic nevus (each 31%). One patient experienced treatment-related serious adverse events (headache, pyrexia, somnolence, and vomiting) that resolved without sequelae following dose interruption. No on-treatment deaths occurred. The overall response rate was 77% (10 of 13 patients; 5 patients with complete resolution [38%] and 5 with regression [38%]). Nine patients were continuing in response at

data cutoff (duration of response: range, 145 to 1236 days [both ongoing] with a minimum follow-up of ≈ 1 year). Three patients (23%) had stable disease. Twelve of 13 patients were on treatment and progression free at 12 months. One patient discontinued treatment at 31 weeks due to an adverse event (grade 2 creatinine increase), as required by protocol, while in complete remission. At screening, 8 patients (62%) had a DTS ≥1. Following treatment with dabrafenib, all but one patient (score unchanged) achieved a reduction in DTS, with 6 of 8 scores (75%) declining to 0.

Summary and Conclusions: Dabrafenib demonstrated a manageable safety profile in pediatric patients with *BRAF* V600–positive LCH. Observed responses and improvements in disease activity with dabrafenib in the majority of patients merit further investigation in this patient population.

PF628

COMPARISON OF RUXOLITINIB AND REAL-WORLD BEST AVAILABLE THERAPY IN TERMS OF OVERALL SURVIVAL AND THROMBOSIS IN PATIENTS WITH POLYCYTHEMIA VERA WHO ARE RESISTANT OR INTOLERANT TO HYDROXYUREA

A. Alvarez-Larran[1,*], S. Verstovsek[2], M. Pérez-Encinas[3], J.-J. Kiladjian[4], F. Ferrer-Marín[5], A.M. Vannucchi[6], J. Martínez López[7], C. Besses[8], C. Cameron[9], A. Situ[9], A. Charter[10], J.C. Hernández-Boluda[11]

[1]Hematology Department, Hospital Clínic, Villarroel 170, 08036, Barcelona, Spain, [2]The University of Texas MD Anderson Cancer Center, Houston, Texas, United States, [3]Hematology Deparment, Hospital Clínico Universitario de Santiago de Compostela, Santiago de Compostela, Spain, [4]Hôpital Saint-Louis et Université Paris Diderot, Paris, France, [5]Hematology and Medical Oncology Unit, Hospital Morales-Meseguer, Centro de Hemodonacion, IMIB-Murcia, CIBERER, UCAM, Spain, [6]Center of Research and Innovation of Myeloproliferative Neoplasms AOU Careggi, University of Florence, Florence, Italy, [7]Hematology Department, Hospital 12 de Octubre, Madrid, [8]Hematology Department, Hospital del Mar, Barcelona, Spain, [9]Cornerstone Research Group, Burlington, Ontario, Canada, [10]Novartis Pharma AG, Basel, Switzerland, [11]Hematology Department, Hospital Clínico Universitario, INCLIVA, Avd. Blasco Ibáñez 17, 46010, Valencia, Spain

Background: Ruxolitinib is a Janus kinase (JAK) 1 and 2 inhibitor approved for the treatment of patients with hydroxyurea-resistant/intolerant polycythemia vera (PV). In randomized controlled trials, ruxolitinib proved superior to best available therapy (BAT) in achieving hematocrit control without phlebotomy and reducing symptoms. However, comparison of overall survival (OS) and rate of thrombosis has been complicated due to a high degree of patient crossover from BAT to ruxolitinib in these trials.

Aims: To compare OS and thrombosis for ruxolitinib *versus* real-world BAT in patients with hydroxyurea (HU) resistant/intolerant PV.

Methods: Propensity score matching (PSM) was conducted using individual patient data (IPD) for patients treated in the ruxolitinib arm from the Phase III RESPONSE trial, and IPD for real-world patients treated with BAT from the Grupo Español de Enfermedades Mieloproliferativas Crónicas Filadelfia Negativas (GEMFIN) registry. The distribution of therapies for patients in the matched real-world BAT group were as follows: hydroxyurea (44%), busulfan (10%), radioactive phosphorus (2%), interferon (6%), anagrelide (12%), other therapy (11%) or no cytoreductive therapy (26%) (Some patients in the real-world BAT group were treated with multiple therapies). Eligible patients had resistance or intolerance to HU according to the modified European Leukemia Net criteria (Barosi G, *et al.* Br J Haematol 2010). Covariates selected for calculating propensity scores (PS) included age, sex, history of thrombosis at time of resistance/intolerance, cytopenia at the lowest HU dose and *JAK2* mutational status. PSM was performed using caliper matching with a caliper width of 20% of the standard deviation of the logit-transformed PS, using sampling without replacement. Hazard ratios (HRs) were estimated from Cox proportional hazards models.

Results: Prior to performing PSM, there were statistically significant differences between ruxolitinib (n=110) and real-world BAT (n=191) in terms of age, sex, and cytopenia at the lowest HU dose or JAK2 mutational status. Before PSM, patients treated with ruxolitinib had significantly longer OS (HR=0.27 [0.12–0.62]) and a lower risk of thrombosis (HR=0.18 [0.05–0.61]) than patients treated with real-world BAT. The matched ruxolitinib and BAT cohorts formed after PSM (n=89 and 92 in each cohort for OS and thrombosis, respectively) were balanced for all covariates included in calculating PS. After PSM, ruxolitinib maintained a significantly prolonged OS (HR=0.28 [0.11–0.72]) and a lower risk of thrombosis (HR=0.21 [0.06–0.76]) compared with real-world BAT (Figure 1).

Summary and Conclusions: PSM analysis showed that patients resistant/intolerant to HU and treated with ruxolitinib in the RESPONSE trial had a significantly reduced risk of thrombosis and mortality compared to those with matched characteristics receiving BAT in the GEMFIN registry. Future studies are needed to validate these findings, and also assess comparative efficacy for other outcomes such as AML/MF/MDS. However, our study further supports the use of ruxolitinib in patients with resistance/intolerance to HU.

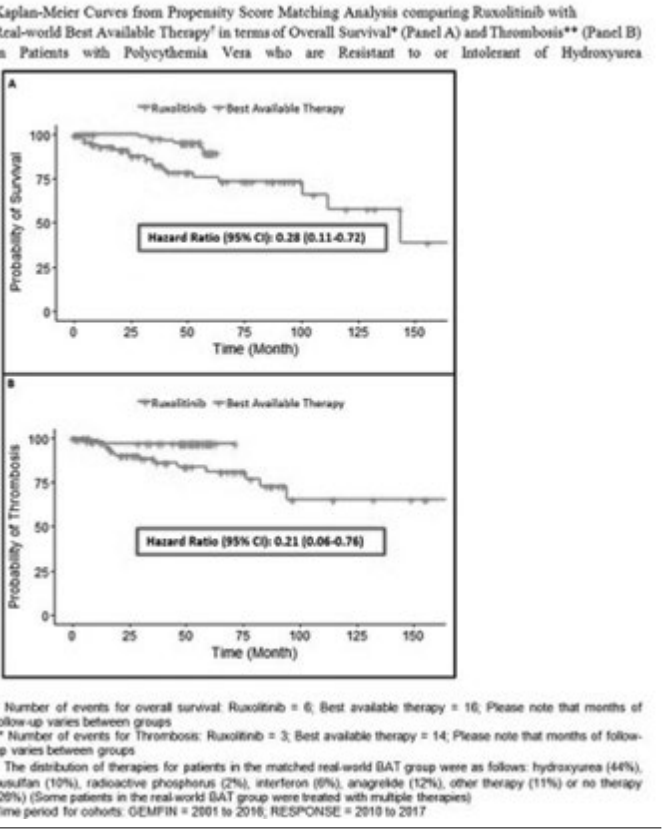

Figure 1.

PF629

RELATIONSHIP BETWEEN MPN PAIN AND EMOTIONAL HEALTH: AN ANALYSIS BY THE MPN QUALITY OF LIFE INTERNATIONAL STUDY GROUP

H. Geyer[1], R. Scherber[2,*], J. Palmer[1], A. Dueck[1], M. Girardo[1], L. Padrnos[1], R. Mesa[3]

[1]Mayo Clinic, Phoenix, [2]OHSU, Portland, [3]University of San Antonio, San Antonio, Texas, United States

Background: Debilitating pain is a frequent complaint in myeloproliferative neoplasms (MPNs). It is well recognized that close relationships exist between pain and expressions of emotional health including cognitive performance, social engagement and physical function. Uncontrolled pain has furthermore been associated with the development of depression and anxiety. The degree to which severe pain relates to the emotional health of MPN patients remains underexplored.

Aims: We aimed to analyze the relationship between MPN pain (bone pain or abdominal pain) and neurocognitive complaints, along with other MPN symptoms, demographic features and patient functionality.

Methods: Data was collected among an international cohort of MPN patients including MF, ET and PV. Subjects completed the EORTC-QLQC30, Brief Fatigue Inventory (BFI) and Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF). Pain was assessed using the MPN-SAF quantitative questions related to 'bone pain' and 'abdominal pain' on a 0 (all reported symptoms absent) to 100 (all reported symptoms worst-imaginable) scale. Patients with mean pain scores of 4 and above were considered 'Severe Pain' whereas those under that value were considered 'Limited Pain'. Chi-square tests were used for categorical variables and two sample t-test were used for continuous variables.

Results: ***Demographics.*** A total of 2321 subjects with MPNs (ET 874, PV 953, MF 486) were prospectively enrolled and administered the MPN-SAF, BFI and EORTC QLQ-C30. Patient demographics including median age (61) and gender (M 46.8%) were within expected range. In comparison to Limited Pain patients, all Severe Pain patients demonstrated significant reductions in

mean EORTC QLQC30 scores for functional domains (Figure 1, all p<0.0001). They were also more likely to describe feeling irritable (32.5% *vs* 11.4%, p<0.0001) and depressed (33.2% *vs* 10.4%, p<0.0001; TSS 4.3 *vs* 1.8, p<0.0001) and have compromised relations with other people (TSS 3.9 *vs* 1.6, p<0.0001). Severe Pain patients also described difficulty with concentration (TSS 4.6 *vs* 1.8, p<0.0001), sleeping (TSS 4.9 *vs* 2.3, p<0.0001) and unintentional weight loss (2.3 *vs* 0.9, p<0.0001) while demonstrating marked reductions in overall quality of life (TSS 4.0 *vs* 6.0, p<0.0001).

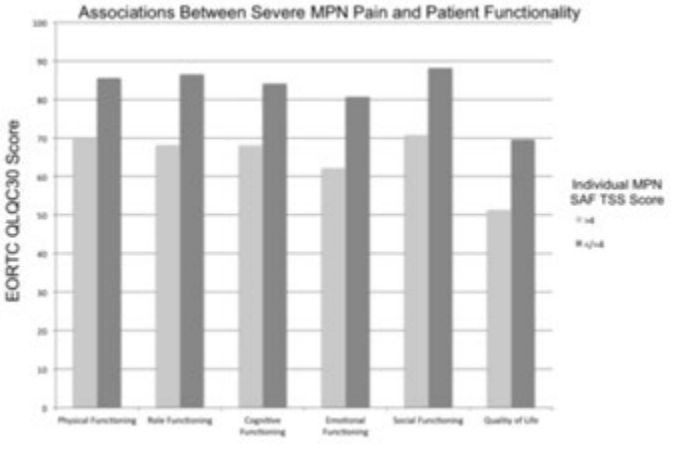

Figure 1.

Summary and Conclusions: The presence of severe abdominal or bone pain in MPN patients is significantly associated with compromised emotional health. Previous studies have demonstrated that MPN disease burden does not necessarily correlate with symptom severity, suggesting that Severe Pain patients may experience significant mental health afflictions even with low-risk disease. Appropriate measures for screening and management of MPN pain should be further investigated.

PF630

ANALYSIS OF IPSET-THROMBOSIS *VS* R-IPSET-THROMBOSIS IN A POPULATION OF 776 ESSENTIAL THROMBOCYTHEMIA PATIENTS - A MULTICENTER RETROSPECTIVE STUDY

M. Sobas[1,*], I. Andrasiak[2], K. Lewandowski[3], M. Narkun[3], J. Czyż[4], L. Szukalski[4], P. Nierychlewska[4], A. Czyż[1], A. Gołos[5], A. Masternak[6], A. Abuin Blanco[7], M. Perez Encinas[7], M.T. Gomez Casares[8], M.S. Noya Pereira[9], J. Marczyk[1], K. Roczek[1], T. Wróbel[1]

[1]Hematology, Medical University, [2]WroMedica Research Center, Wrocław, [3]University of Medical Science, Poznań, [4]University Hospital, Bydgoszcz, [5]Institute of Hematology, Warsaw, [6]Szpital Wojewódzki, Opole, Poland, [7]University Hospital, Santiago de Compostela, [8]Hospital Universitario Dr. Negrin, Gran Canaria, [9]Hospital Complejo Hospitalario Universitario, A Coruna, Spain

Background: The main morbidity of essential thrombocythemia (ET) are thrombotic complications. Classical risk score includes age>60 yrs and history of thrombosis separates patients into high and low-risk groups. Recently, JAK2 mutation and cardiovascular (CV) risk factors were additionally included in a 3-tiered (Low, Intermediate and High) International Prognostic Score of Thrombosis (IPSET-thrombosis). New revised IPSET-thrombosis (R-IPSET-thrombosis), based on thrombosis history, age>60 yrs and JAK2+, separates patients in four group: very low (no factors), low (JAK2+), intermediate (age>60 yrs) and high (history of thrombosis or age>60 yrs plus JAK2+).
Aims: To compare IPSET-thrombosis and R-IPSET-thrombosis in a population of 776 ET patients
Methods: We performed a retrospective, multicenter (5 Polish and 3 Spanish) study. Diagnosis was made according to WHO 2016 criteria. Clinical and analytical data were collected. Patients were assessed according to the IPSET-thrombosis and R-IPSET-thrombosis. Thrombosis free survival (TFS) was calculated from diagnosis and analyzed for each risk score.
Results: Out of 776 patients, 500 were female (64%), 392 were older >60 yrs (50.5%) and 451/770 (57%) presented CV factors at diagnosis. Mutation JAK2 was present in 65% of ET patients, and mutation CALR was present in 72% of ET JAK2- patients. Thrombosis before diagnosis occured in 13,5% and after diagnosis in 9%. Distribution of risk groups according to IPSET-thrombosis and R-IPSET-thrombosis is presented in a Table 1. The median follow-up period was 58 months (range 3-354). The potential prognostic factors: age >60 yrs, CV factors, platelet and white blood cell (WBC) count, JAK2 and CALR mutation, antiagregation/anticoagulation treatment were evaluated for TFS in univariate analysis. The following factors were found to be significant: CALR mutational status, age >60 yrs, the presence of CV factors, and high platelet count. In multivariate analysis, TFS was significantly impacted only by CALR mutational status (HR 0.39; p=0.06) and age >60 yrs (HR 1.2; p=0.035). Interestingly, we observed a significant association between JAK2+ and thrombosis incidence before diagnosis (25.3% *vs* 12.9%; p<0.001), however we did not find such association after diagnosis. According to IPSET-thrombosis, a 15 years – TFS for low *vs* intermediate *vs* high risk group was 91% (95%; CI 84-98) *vs* 87% (95%; CI 80-94) *vs* 72% (95%; CI 63-81), respectively (p=0.008). There were no significant differences in a TFS for a different risk groups assessed according to R-IPSET-thrombosis.

Table 1.

	R-IPSET-thrombosis				
IPSET-thrombosis	Very Low	Low	Intermediate	High	Total
Low	146	0	33	0	179
Intermediate	0	115	82	3	200
High	0	84	1	312	397
Total	146	199	116	315	776

Summary and Conclusions: Our results confirm that CALR mutation status and age influence TFS. However, we are not able to confirm the superiority of R-IPSET-thrombosis *vs* IPSET-thrombosis. We think that it could be related to the relatively short follow-up period and the possible influence of antiagregation/anticoagulation treatment in JAK2+ patients, who developed thrombotic complications before diagnosis and received anticoagulation/antiagregation treatment thereafter.

PF631

SKEWED RATIO BETWEEN TYPE 1 AND TYPE 2 CALR MUTATIONS IN ESSENTIAL THROMBOCYTOSIS PATIENTS WITH CONCOMITANT JAK2 V617F MUTATION

A. Aggerholm[1,*], L. Mors Haunstrup[1], M. Tang Severinsen[2], L. Hyldahl Ebbesen[1]

[1]Hematology, Aarhus University Hospital, Aarhus C, [2]Hematology, Aalborg University Hospital, Aalborg, Denmark

Background: Most patients with essential thrombocytosis (ET) and primary myelofibrosis (PMF) can be molecularly characterized by mutations in either Janus kinase 2 (JAK2, 60-65%), Calreticulin (CALR, 20-25%) or thrombopoitin receptor (MPL, 5%) and mutations in these genes are overall believed to be mutually exclusive. Several studies have analyzed the clinical and hematological features of ET and PMF patients according to mutational status. Younger age, high platelet counts but low risk of thrombosis has been associated with CALR mutated ET and PMF compared to JAK2 mutated cases. Numerous different indel mutations in exon 9 of CALR have been found with Type 1 (52 bp deletion) and Type 2 (5 bp insertion) as the most common variants. Recent reports indicate that Type 1 mutations are associated with PMF or ET with an increased risk of myelofibrotic transformation, while Type 2 mutations are associated with ET, low risk of thrombosis and indolent clinical course. Even though, overall believed to be mutually exclusive recent reports have shown concomitant mutations of JAK2 V617F and CALR in some MPN patients.
Aims: To ensure adequate molecular characterization for aiding clinicians providing more precise diagnoses and prognostication in patients with PMF and ET, we addressed the issue of double mutants in our patients. We retrospectively analysed for CALR mutation in all MPN patients initially analysed for JAK2 V617F between January 2012 and April 30th 2015 for whom allele frequencies of the JAK2 mutation was between 0.01% and 5%. 136 patients were positive for JAK2 V617F with allele frequencies in this range. In addition, 46 patients with JAK2 V617F allele frequencies between 6% and 10% were analysed.
Methods: The JAK2 V617F analysis was performed with allele specific qPCR with sensitivity at 0.01% and CALR exon 9 mutations were analysed by fragment length analysis with sensitivity at 2%.
Results: We identified 15 patients with concomitant CALR mutation within the group of MPN patients with JAK2 V617F allele frequencies below 5%, while none of 46 patients with JAK2 V617F allele frequencies above 5% had a CALR mutation. Ten patients were diagnosed with ET, three with PMF and two patients were MPN-unclassifiable. We identified five Type 1 and ten Type 2 CALR mutations with allele frequencies in the range 9 - 44%. Median age of all patients was 70 y (40 – 76) and all except two

patients with PMF had thrombocytosis. Median age of patients with ET was 69.5 y (40 – 75) and median thrombocyte count was 1195 (715 – 1755). None of the ET patients progressed to myelofibrosis (median follow-up 89 m, 40-204). In the ET group 3 patients were positive for CALR mutation Type 1 and seven were positive for Type 2. Two of 3 ET patients with Type 1 mutation experienced thrombosis two years prior to diagnosis (DVT and non-stemi, respectively), while only one patient with Type 2 mutation had thrombosis (DVT, 2 y post diagnosis).

Summary and Conclusions: Screening of 136 patients with low allele frequencies of JAK2 V617F (<6%) revealed 15 patients with a concomitant CALR mutation. In our cohort of MPN patients, the ET diagnosis was overrepresented among patients with concomitant JAK2 V617F and CALR mutation. We observed more patients with CALR Type 2 mutation than Type 1, which is contrary to previous reports. Our data indicate an association between double mutation in JAK2 V617F and CALR, an ET diagnosis, older age and indolent disease. These data stress the importance of extended molecular analyses in MPN patients with low JAK2 V617F allele frequencies.

Non-Hodgkin lymphoma – Biology & Translational Research

PF632

CLONAL B-CELL LYMPHOCYTOSIS OF MARGINAL ZONE ORIGIN: NEW INSIGHTS INTO A NOVEL AND STILL ENIGMATIC ENTITY

A. Xochelli[1,*], R. Walewska[2], C. Kalpadakis[3], A. Ferrer Del Alamo[4], D. Rossi[5], G. Tapia[6], A. Gardiner[2], M. Joao Baptista[7], F. Sole[8], T. Vassilakopoulos[9], G. Kanellis[10], S. Sachanas[11], M. Angelopoulou[9], A. Athanasiadou[12], N. Stavroyianni[12], A. Anagnostopoulos[12], T. Papadaki[13], B. Espinet[4], G. Gaidano[14], G. Pangalis[11], K. Stamatopoulos[1], D. Oscier[2]

[1]Institute of Applied Biosciences, CERTH, Thessaloniki, Greece, [2]Department of Haematology, Royal Bournemouth Hospital, Bournemouth, United Kingdom, [3]Department of Haematology, University of Crete, Heraklion, Greece, [4]Department of Pathology, Hospital del Mar, Hospital del Mar Research Institute (IMIM), Barcelona, Spain, [5]Hematology, Oncology Institute of Southern Switzerland and Institute of Oncology Research, Bellinzona, Switzerland, [6]Department of Pathology, Hospital Universitari Germans Trias i Pujol, Universitat Autonoma de Barcelona, Badalona, Barcelona, Spain, [7]Department of Hematology, ICO-Hospital Universitari Germans Trias i Pujol, Josep Carreras Leukaemia Research Institute, Universitat Autonoma de Barcelona, Badalona, Barcelona, Spain, [8]Josep Carreras Leukemia Research Institute, Campus Badalona, Barcelona, Spain, [9]Department of Haematology, University of Athens, School of Medicine, Athens, Greece, [10]Hematopathology Department, Evangelismos Hospital, Athens, Greece, [11]Department of Haematology, Athens Medical Center-Psychikon Branch, Athens, Greece, [12]Hematology Department and HCT Unit, G. Papanicolaou Hospital, Thessaloniki, Greece, [13]Hematopathology Department, University of Athens, School of Medicine, Athens, Greece, [14]Division of Haematology, Department of Translational Medicine, University of Eastern Piedmont, Novara, Italy

Background: Clonal B cell lymphocytosis of marginal zone origin (CBL-MZ) is used to characterize cases with isolated clonal B lymphoproliferation and immunophenotypic features suggestive of MZ derivation.

Aims: To further investigate this diagnostic conundrum so as to reach to definitive conclusions regarding its precise nature, diagnostic criteria and management.

Methods: In extension of our previous publication of the largest cohort of CBL-MZ thus far described, (Group A; n=102), here we update outcome data for Group A, describe an additional series of CBL-MZ cases (Group B), consolidated through a multi-institutional collaboration within the International Splenic Lymphoma Study Group, and compare Groups A and B. Cases included in the study were selected according to the following criteria: clonal lymphocytosis regardless of clonal size; Matutes score ≤2; absence of lymphadenopathy, organomegaly or concurrent cytopenias; and, absence of any diagnostic hallmark of a well-defined lymphoma.

Results: Overall, 58 novel cases (males/females: 33/25) were included in the study with a median age at diagnosis of 73 years and a median lymphocyte count of 6.3x10^9/l (range: 1.5-36 x10^9/l); 15/46 (32.6%) cases with available data exhibited paraproteinemia. Histopathological examination of the bone marrow biopsy (BMB) was performed in 27/58 cases. The BM infiltration ranged from 7-70%; in most cases, BMB examination disclosed mixed patterns of neoplastic lymphocytic infiltration from mostly small and medium-sized B cells. Some lymphocytes exhibited villi but this was not a consistent finding. Of 15 cases tested, 3 (20%) were found positive for the *MYD88*^{L265P} mutation and were all positive for paraproteinemia (IgM). Karyotype data was available in 34 cases; 15/34 (44%) had an abnormal karyotype and in 4 (11.7%) the karyotype was complex. Deletion 7q32 was observed in 2 patients and trisomy12/dup(12q) and i(17q) in 3 patients. Regarding immunogenetics, the IGHV4-34 and IGHV3-23 genes predominated (3/26, 11.5% each) whereas the IGHV1-2 gene was utilized by a single case; 4/26 rearrangements (15.4%) carried IGHV genes with no somatic hypermutations (SHM), while the remainder (22/26, 84.6%) exhibited some impact of SHM. With a median follow-up of 4 years, only 3 cases exhibited clinical or histopathologic evidence of a known lymphoma: a single case developed diffuse large B cell lymphoma of the skin and 2 cases developed splenomegaly and thus, can be considered as splenic MZ lymphoma or SMZL/leukemia unclassifiable (SMZL/SLLU). Turning to Group A, with a median follow-up of 6 years, a total of 22/102 (21.5%) cases evolved: 16 developed splenomegaly (most of them requiring splenectomy or treatment), likely representing SMZL/SLLU, and 1 each were diagnosed with nodal MZ lymphoma, gastric MALT lymphoma, lymphoplasmacytic lymphoma, DLCBL of the skin, nodal DLBCL and follicular lymphoma grade 1 (clonal relationship was confirmed in the gastric MALT lymphoma case). Groups A *vs* Group B differed significantly only regarding clinical evolution (p=0.02)

that was lower in Group B, likely due to the fact that 16/58 Group B cases were newly diagnosed with a follow-up of less than 2 years.
Summary and Conclusions: Overall, our present findings highlight a rather consistent biological profile for CBL-MZ, while also offering further supportive evidence for its overall indolent clinical nature. Admittedly, open questions remain especially regarding the heterogeneity in clinical outcome and can only be addressed within large collaborative groups and multidisciplinary approaches.

PF633

BUILDING PRECLINICAL MOUSE MODELS FOR MANTLE CELL LYMPHOMA

T. Pieters[1,*], S. T'Sas [1], F. Matthijssens[1], J. Morscio [1], L. Reunes[1], B. Lintermans[1], J. Van Dorpe[2], G. Berx[3], J. Haigh[4], S. Goossens[1], P. Van Vlierberghe[1]

[1]Department for Pediatrics, Center for Medical Genetics, Ghent University, [2]Department of Pathology, Ghent University and Ghent University Hospital, Ghent, [3]Department for Biomedical Molecular Biology, Ghent University, Zwijnaarde, Belgium, [4]Australian Centre for Blood Diseases, Department of Clinical Haematology, Monash University and Alfred Health Alfred Centre, Melbourne, Australia

Background: Mantle cell lymphoma (MCL) is a highly aggressive subtype of B-cell lymphoma that is characterized by a poor response to current treatment regimens. Most MCLs carry a prototypical translocation, t(11;14), which juxtaposes the *CCND1* gene towards the immunoglobulin heavy chain *(IGH)* locus, resulting in cyclin D1 overexpression. Strikingly, MCL has not been recapitulated in transgenic mouse models of Ccnd1 overexpression alone. Notably, a subset of MCL patients are cyclin D1 negative but instead overexpress cyclin D2 (encoded by *CCND2*) as a consequence of recurrent genomic rearrangements involving the *CCND2* locus. These *CCND2* rearrangements were acknowledged in the revised 2016 WHO classification of lymphoid neoplasms and hints towards a putative role for Cyclin D2 in the etiology of MCL.
Aims: We wanted to evaluate if cyclin D2 could act as a bona fide oncogene in the pathogenesis of MCL. Furthermore, we aimed to model MCL in mice and use them as a preclinical tool to identify novel therapeutic options for MCL patients.
Methods: To identify the role of Cyclin D2 in the formation of MCL *in vivo*, we developed a conditional R26-driven Ccnd2 overexpression mouse model. For this, we used a targeting vector that contained a floxed stop cassette followed by the *Ccnd2* gene and a EGFP/luciferase reporter, which was subsequently targeted in mESCs using recombinase-mediated cassette exchange (RMCE, Figure 1A).

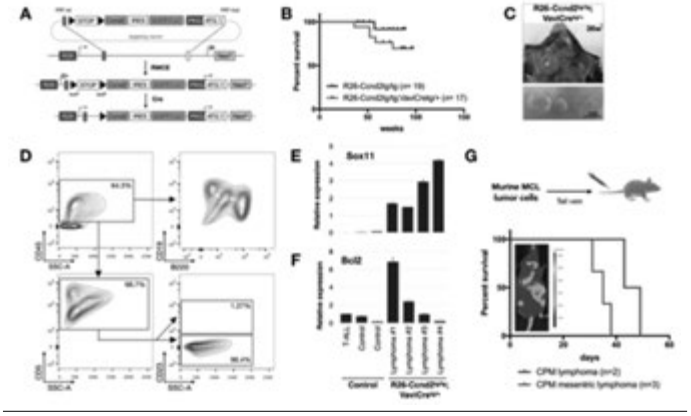

Figure 1.

Results: We found that hematopoietic-specific Ccnd2 activation (using VaviCre) is sufficient to drive MCL formation in mice. Moreover, we could show that this murine model recapitulates several clinical features of MCL patients, including CD19+, CD5+, CD23- immunophenotype, clonal tumor expansion, lack of somatic hypermutation, high expression of Sox11 and Bcl-2, and infiltration in spleen, liver and GI tract (Figure 1B-F). Detailed molecular analysis of these murine MCLs using shallow sequencing and whole exome sequencing revealed genetic aberrations similar to these found in MCL patients, including a somatic deletion of Crfl2. Furthermore, these murine MLC tumors were transplantable and homed to sites within the lymphatic system, but unfortunately did not show aggressive growth *in vivo*. Finally, we tested putative synergism between Ccnd2 overexpression and other cooperating genetic lesions that occur in human MCL, such as loss of p53. To this end, we generated mice with B-cell specific Cyclin D2 overexpression and p53 deletion. These mice also formed MCL-like tumors and developed "full blown" MCL within weeks upon their transplantation into immunocompromised mice (Figure 1G). Importantly, the transplanted tumor cells are luciferase-positive and are therefore suitable for *in vivo* drug testing using bioluminescence. As proof-of-principle, we tested the efficacy of known MCL drugs, such as Ibrutinib and Venetoclax, alone or in combination in our model system.
Summary and Conclusions: In conclusion, we generated novel cyclin-D2-driven MCL mouse models that can be used for preclinical drug screening and will hopefully lead to a better outcome for MCL patients.

PF634

MYC-MEDIATED DOWNREGULATION OF MIR-150 CONTRIBUTES TO THE TRANSFORMATION OF FOLLICULAR LYMPHOMA BY UPREGULATING FOXP1 LEVELS

K. Musilova[1,2,*], J. Devan[1], K. Cerna[1,2], V. Seda[1,2], G. Pavlasova[1,2], R. Pytlik[3], V. Prochazka[4], Z. Prouzova[5], M. Trbusek[2], L. Zlamalikova[6], K. Liskova[6], L. Kruzova[4], M. Jarosova[2], C. Kornauth[7], I. Simonitsch-Klupp[7], A.I. Schiefer[7], O. Merkel[7], P. Burda[8], K. Machova Polakova[8], H. Mocikova[9], L. Kren[6], J. Mayer[2], M. Trneny[10], C.S. Zent[11], A. Evans[11], A. Janikova[2], M. Mraz[1,2]

[1]Molecular Medicine, CEITEC MU, [2]Dept. of Internal Medicine - Hematology and Oncology, University Hospital Brno and Faculty of Medicine Masaryk University, Brno, [3]First Department of Medicine - Dept. of Hematology, General University Hospital in Prague and Charles University, Prague, [4]Department of Hemato-Oncology, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc, Olomouc, [5]Department of Pathology and Molecular Medicine, Second Faculty of Medicine, Charles University, Prague, [6]Department of Pathology, University Hospital Brno, Brno, Czech Republic, [7]Department of Clinical Pathology, Medical University Vienna, Vienna, Austria, [8]Institute of Hematology and Blood Transfusion, [9]Internal Clinic of Haematology, 3rd Faculty of Medicine, Charles University in Prague and University Hospital Kralovske Vinohrady, [10]First Department of Medicine - Dept. of Hematology, Charles University, Prague, Czech Republic, [11]Department of Pathology and Laboratory Medicine and Medicine, University of Rochester Medical Center, School of Medicine and Dentistry, Rochester, NY, United States

Background: Recent studies revealed number of genomic aberrations associated with transformation of follicular lymphoma (FL) to diffuse large B-cell lymphoma (DLBCL) including frequent aberrations activating MYC. However, the precise molecular mechanisms underlying this process are largely unclear.
Aims: We aim to understand the role of miRNAs in the process of high-grade transformation of FL (tFL).
Methods: We performed a global miRNA expression analysis in 16 paired primary samples from FL patients before and after transformation to DLBCL. The gene expression/IHC studies were performed on samples from an additional 10 paired FL-tFL and 85 FL.
Results: *miR-150* was uniformly down-modulated (~3.5-fold) in all examined tFL (n=26, 13 paired samples). We showed that elevated levels of MYC protein are responsible for repressing *miR-150* in tFL, and rare MYC-positive FL had significantly lower *miR-150* expression compared to MYC-negative FL cases. Additionally, samples of CLL with MYC aberration had lower *miR-150* levels. We have shown that silencing of MYC leads to up-regulation of miR-150 in B cells, and MYC over-expression leads to repression of *miR-150*. This was not dependent on LIN28A/B proteins, which are induced by MYC and impair the *miR-150* maturation in myeloid cells. The LIN28A/B protein was absent in all examined B cell lines and primary samples. Our data from chromatin-IP for MYC show that MYC regulates *miR-150* expression directly by binding in the region upstream of *MIR150* transcription start site. Interestingly, low-level *miR-150* expression predicted a substantially shorter overall survival (OS) in FL in a univariate and multivariate analyses (median 9.1 yrs *vs* not reached; P=0.008; HR 3.5 [CI: 1.3-9.2]). *miR-150* was also significantly lower in patients with early death or relapse within 2 years from diagnosis, which suggests that measuring *miR-150* levels could be useful for identifying the ~20% of FL patients with the most aggressive course (Casulo *et al.*, 2015). We showed that low *miR-150* levels in FL and tFL lead to upregulation of its target FOXP1, which is a positive regulator of B cell activation and BCR signalling. The high-level FOXP1 expression associated with high Ki67 proliferation index and shorter OS in FL (median 33 months *vs* not reached; P=0.03; HR 5.0 [CI: 1.1-22.6]. However, *miR-150* likely regulates also other targets that influence cell cycle alone or together with FOXP1.
Summary and Conclusions: We have shown for the first time that miRNAs are involved in FL transformation. *miR-150* is transcriptionally repressed by MYC in transformed FL and aggressive FL, and this leads to higher

FOXP1 levels. The low levels of *miR-150* and high levels of its target, FOXP1, are associated with shorter OS and early relapse in FL, and this suggest that *miR-150* could serve as a promising biomarker.
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PF635

THE AXL TYROSINE KINASE INHIBITOR, BGB324, INDUCES CELL DEATH IN MANTLE CELL LYMPHOMA

P. Gelebart[1,*], J. Han[2], I. Karlsen[2], M. Eriksen Gjerstad[2], L. Helgeland[3], F. Baran-Marszak[4], B. Papp[4], E. Mc Cormack[2]

[1]Clinical Science, [2]University of Bergen, Bergen, Norway, [3]Pathology, University of Bergen, Bergen, Norway, [4]INSERM U978, Université Paris 13, Bobigny, France

Background: Mantle cell lymphoma (MCL) is an aggressive B-cell non-Hodgkin lymphoma with poor overall survival.Thus, there is an urgent need to develop and identify new therapeutic target for MCL.
Aims: In this study, we report that the tyrosine kinase receptor AXL is consistently expressed in MCL. MCL cells express the AXL isoform 1 and 2 as well as the more recently describe isoform 3. In the view of previously describe oncogenic function of AXL in several solid and hematopoietic cancer, we hypothesized that AXL may be involved in the deregulation of cell signaling observed in MCL.
Methods: MCL cell lines or patient cells were treated with BGB324 or vehicle for a period of 2 days. Analysis of cell proliferation was assessed by trypan blue exclusion and MTA assays. Cell cycle was investigated by a flow cytometry and apoptosis induction was monitored by caspase activation. For the *in vivo* study, NSG mice were injected with Jeko-1 luciferase MCL cells and treated with vehicle (N=8) or BGB324 (N=8). Tumor development was monitored by an imaging approach (IVIS).
Results: We first demonstrated the expression of AXL at the mRNA and protein level in six MCL cell lines as well as in 7 patient samples examined using reverse transcription polymerase chain reaction and western blot approaches. To confirm the transcript identity, we cloned and sequenced the different AXL isoforms. We also found that AXL was constitutively activated in MCL cells using different phopho-AXL antibodies. Importantly, pharmacologic inhibition of AXL activity, with BGB324, resulted in a decrease in cell proliferation, cell cycle arrest and cell apoptosis of MCL cell lines and patient cells *in vitro*. Moreover, treatment of MCL cells lines with BGB324 led to a significant decrease in cyclin D1, b-catenin expression as well as a decrease in AKT and NF-kB activity. *In vitro*, the inhibition of BTK, by ibrutinib, and AXL show synergetic effect in induction of MCL cell apoptosis. *In vivo*, in a xenograft mouse model of MCL, treatment with BGB324 suppresses the growth of MCL cells with a higher efficacy than ibrutinib.
Summary and Conclusions: Taken together these data seem to support the concept that AXL contributes to the pathogenesis of MCL and that BGB324, an AXL inhibitor already engage in clinical trial, may hold promises for treating MCL as a single agent or in combinatory treatment with BTK inhibitor.

PF636

MODELLING MYD88 P.L265P DRIVEN LYMPHOMA

G. Knittel[1,*], T. Rehkämper[1], R. Flümann[1], P. Nieper[1], F. Beleggia[1], C. Fritz[1], A. Da Palma-Gueirrero[1], P. Pfeiffer[1], A. Klatt[1], L. Frenzel[1], R. Büttner[1], C. Reinhardt[1]

[1]University Hospital of Cologne, Köln, Germany

Background: The adaptor protein MYD88 is critical to relay activation of Toll-like receptor signaling to NF-kB activation. MYD88 mutations, particularly the p.L265P mutation, have been described in numerous distinct B cell malignancies, indluding diffuse large B cell lymphoma (DLBCL). 29% of activated B cell (ABC)-type DLBCL, which is characterized by constitutive activation of the NF-kB pathway, carry the p.L265P mutation. In addition, ABC-DLBCL frequently displays focal copy number gains affecting BCL2.
Aims: Here, we aimed to investigate the role of the Myd88p.L265P point mutation in lymphomagenesis by generating mouse models harbouring this recurrent mutation and to use these models for therapy development.
Methods: We generated a novel mouse model (termed Myd88c-p.L252P), in which Cre-mediated recombination, specifically in B cells, leads to the conditional expression of Myd88p.L252P (the orthologous position of the human MYD88p.L265P mutation) from the endogenous locus. More precisely, the endogenous exons 2 to 6 of Myd88 (which consists of 6 exons) were flanked by loxP-sites. Downstream of the last exon, a second set of exons 2 to 6 was inserted, harboring the point mutation p.L252P. Cre-mediated recombination leads to the excision of the endogenous exons 2 to 6 and expression of the inserted, mutated set of exons. This system very closely mimicks the situation observed in the clinic, as it allows for the heterozygous expression of Myd88p.L252P from the endogenous locus.
Additionally, we generated a BCL2-overexpression allele, which mimicks recurrent chromosomal amplifications of the BCL2 locus in DLBCL.
Results: BCL2 overexpression and MYD88 p.L265P strongly cooperate in generating a hyper-reactive state of the B cell compartment in double-mutant animals. This hyper-reactive state is characterized both by the loss of self-tolerance and an increased response to stimulation by foreign antigen. Double-mutant animals ultimately develop clonal lesions mainly in the mesenterial area, presumably out of the enhanced pool of activated B cells. These lesions histologically resemble DLBCL with plasmacytic features. Cells isolated from these tumors can be cultivated *ex vivo* and are transplantable, hence provide a useful tool for the investigation of new treatment strategies.
Summary and Conclusions: In summary, we generated a mouse model that enables Cre-mediated expression of Myd88p.L252P from the endogenous locus. Specifically in combination with BCL-2 overexpression, B cell-specific expression of Myd88p.L252P results in the development of an aggressive lymphoma with plasmoblastic features, most reminiscent of ABC-type DLBCL. These tumors are sensitive to BCL-2 inhibition by ABT-199.

PF637

CD3 BISPECIFIC ANTIBODY SCREEN IDENTIFIES CD20 AS THE MOST EFFICIENT TARGET FOR ELIMINATION OF B CELL MALIGNANCIES; PRE-CLINICAL EVALUATION OF DUOBODY-CD3XCD20

P. Engelberts[1,2,*], I. Hiemsta[1], J. Meesters[1], B. De Jong[1], T. Salcedo[1], S. Verploegen[1], E. van den Brink[1], D. Schuurhuis[1], S. Horbach[1], A. Labrijn[1], P. Parren[2], J. Schuurman[1], E. Breij[1]

[1]Genmab, Genmab, Utrecht, [2]Hematology and blood Transfusion, Leiden University Medical Center, Leiden, Netherlands

Background: The field of immuno-oncology is evolving rapidly through the addition of CD3 bispecific antibody (BsAb) molecules to its arsenal. While such T-cell retargeting bsAb have shown clinical activity in lymphoid malignancies, it is not known which B cell targets are optimally targeted using CD3 BsAb.
Aims: (i) To screen B cell targets suitable for CD3 BsAb targeting, using Genmab's proprietary bispecific antibody platform, (ii) preclinical development of a highly potent CD3 BsAb to target B cell malignancies in the clinic.
Methods: To identify the most potent B cell-targeting CD3 BsAb, an *in vitro* functional screen was performed with a panel of CD3 bispecific antibody molecules containing tumor-targeting Fab-arms recognizing the medium-to-highly expressed B-cell surface antigens CD19, CD20, CD22, CD24, CD37, CD70, CD79b, CD138 and HLA-DR. Target binding capacity *in vitro* was assessed using flow cytometry and the cytotoxic potential towards B cell lymphoma cell lines was tested using chrome-release assays. *In vitro* T cell activation (determined by the upregulation of CD69, CD25, PD-1) and T cell-mediated cytotoxicity (measured as the reduction in number of remaining tumor cells) of the most potent CD3-BsAb, a CD3xCD20 BsAb designated DuoBody-CD3xCD20, were measured by flow cytometry. Anti-tumor activity *in vivo* was measured using cell line-derived xenografts in humanized mouse models. Finally, the capacity of DuoBody-CD3xCD20 to induce depletion of normal B cells from peripheral blood and lymphoid structures after intravenous or subcutaneous administration in cynomolgus monkeys was assessed as part of the non-clinical safety studies.
Results: Functional screening of a panel of B cell-targeting CD3 BsAbs *in vitro* identified multiple targets that could be used to induce T-cell mediated cytotoxicity towards malignant B cells. No correlation between target expression and BsAb-induced T cell-mediated cytotoxicity could be found. CD20-targeting BsAbs were among the most efficient CD3 BsAbs. Specifically, one of the CD3xCD20 BsAbs showed extraordinary potency and outperformed the other CD20-targeting BsAb as well as CD3 BsAbs against all other B cell targets tested. This BsAb, designated DuoBody-CD3xCD20, was shown *in vitro* to only activate T cells in the presence of B cells and to

induce potent T-cell-mediated cytotoxicity of B cell lymphoma cell lines with EC_{50} values in the low picomolar range. DuoBody-CD3xCD20-dependent T cell-activation and T cell-mediated cytotoxicity resulted in tumor growth inhibition in xenograft models *in vivo*, both in prophylactic and therapeutic settings. Non-clinical safety studies with DuoBody-CD3xCD20 in cynomolgus monkeys showed potent, long-lasting B cell depletion from both peripheral blood and lymphoid organs. Comparison of intravenous and subcutaneous administration demonstrated lower peak cytokine levels after subcutaneous antibody administration, while bioavailability and the capacity to deplete B cells were comparable to the intravenous route.
Summary and Conclusions: DuoBody-CD3xCD20 was identified as the most potent B cell-targeting CD3 BsAb in a panel of BsAbs targeting 9 different B cell targets. In non-clinical safety studies, DuoBody-CD3xCD20 showed efficient depletion of B cells and an acceptable safety profile. The clinical safety of DuoBody-CD3xCD20 (GEN3013) in patients with B cell malignancies will be assessed in a first-in-human study.

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TG-1701 A NOVEL, ORALLY AVAILABLE, AND COVALENTLY-BOUND BTK INHIBITOR

E. Normant[1,*], L. Gorelik[2], R. Shmeis[1], H. Le[1], R. Nisch[1], H.P. Miskin[1], P. Sportelli[1], M.S. Weiss[1]

[1]TG Therapeutics, Inc., [2]Fortress Biotech, New York, NY, United States

Background: Targeting Bruton's tyrosine kinase (BTK), an essential component of the BCR signaling pathway, has been demonstrated to be an effective treatment option for B-cell lymphomas and autoimmune diseases. However, new BTK inhibitors are needed to allow for better safety and efficacy as a single agent and in combination with other agents.
Aims: Herein we present TG-1701, a novel, orally available and covalently-bound BTK inhibitor that exhibits unique pharmacologic properties compared to prior BTK inhibitors.
Methods: TG-1701 was evaluated and compared to ibrutinib and/or acalabrutinib in numerous enzyme based, cell-based, and animal models
Results: TG-1701 and ibrutinib have comparable IC50s against BTK (3 nM and 1 nM respectively). TG-1701 exhibits superior selectivity to BTK compared to ibrutinib in *in vitro* whole kinome screening (DiscoverX, San Diego, CA) (Table 1).

Table 1.

IC50 (nM)	BTK	HER2	ITK	HER4	CSK	EGFR
TG-1701	3	> 3000	> 3000	147	347	270
ibrutinib	1	36	62	4	57	2

In addition, TG-1701 is 61-fold less active on EGFR compared to BTK with a Kd of 270 nM and 4.4 nM respectively. Ibrutinib, however, is only 6.7-fold less active on EGFR compared to BTK with a Kd of 2 nM and 0.3 nM respectively. TG-1701 inhibited the growth of the follicular lymphoma DOHH-2, mantle cell lymphoma Mino and DLBCL SU-DHL-6 cell lines with GI50% of 369, 449 and 313 nM respectively. TG-1701 inhibited IgM-activated BCR pathway in DOHH-2 cells, in particular the phosphorylation of BTK, PLCy2 and ERK1/2. In a cell-based assay, TG-1701 blocked IgM-dependent CD69 expression, adhesion of JEKO cells to VCAM-1, and CXCL12-dependent migration. A fluorescent BTK-occupancy assay was developed and validated *in vivo*, in the spleen of mice, where BTK was found to be completely occupied after administration of a single dose of TG-1701 at 12.5 mg/kg. *In vivo*, the anti-tumor efficacy of TG-1701 was assessed in several lymphoma xenograft models, *e.g.* SU-DHL-6, Mino, and OCI-Ly10 ABC-DLBCL, where TG-1701 showed potent anti-tumor activity equivalent to or greater than ibrutinib and similar to the recently approved BTK inhibitor, acalabrutinib. In addition, the pharmacodynamic profile of TG-1701 allows for a once a day dosing.
Summary and Conclusions: TG-1701 is a novel and highly-selective, irreversible BTK inhibitor with potent *in vitro* and *in vivo* activity. TG-1701 is currently being tested in a phase 1 dose escalation study.

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DASATINIB ENHANCES ANTHRACYCLIN-BASED CHEMOTHERAPY IN PRE-CLINICAL MODELS OF T-CELL LYMPHOMA

C. Carniti[1,*], M. Magni[1], G. Biancon[1], P. Quaranta[1], P. Corradini[12]

[1]Medical Oncology and Hematology Department, Fondazione IRCCS Istituto Nazionale Tumori, [2]Department of Oncology and Hemato-oncology, Università degli Studi di Milano, Milano, Italy

Background: Although new treatment approaches for T-cell lymphomas (TCLs) have been studied over the past several years, anthracycline-based chemotherapy programs, cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) or CHOEP (CHOP+Etoposide), remain the only form of effective chemotherapy for patients with newly diagnosed T-cell lymphomas. We previously reported that stem cell transplantation (SCT) is a curative option for T-cell Lymphoma patients but 25-30% of patients do not become transplant eligible due to primary refractory or early progressive disease and have very poor prognosis and few therapeutic options.
Aims: In this study, we investigated the mechanisms associated with different responses to CHOEP in preclinical models of T-cell lymphomas. Results of these analyses prompted us to investigate whether dasatinib (DA), a pan-tyrosine kinase inhibitor, could enhance response to CHOEP in T-cell lymphoma experimental models.
Methods: Six different T-cell lymphoma and leukemia cell lines (Jurkat, HD-MAR-2, Karpas 299, Sup-T1, HH and OCI-Ly12) representative of TCL heterogeneity, were incubated with escalating doses of CHOEP. Gene expression profiling (GEP) and western blotting (WB) analysis were performed to assess effects of treatments. Escalating doses of DA were used in order to calculate the concentration of drug able to inhibit 50% of proliferation (IC_{50}) for every cell line. Suboptimal concentrations (IC_{20}) of CHOEP and DA were used to assess the efficacy of the combination. Analysis of cell viability, cell cycle distribution, apoptosis and mitochondrial depolarization were performed using flow cytometry. ANOVA one-way test was adopted to establish if drug combinations significantly reduced proliferation. The *in vivo* effect of the combination was tested in xenograft models and tumor growth assessed.
Results: CHOEP treatment induced concentration and time-dependent growth inhibition in all cell lines, with the most sensitive cells being HH and the least sensitive being HD-MAR-2 (5 fold more resistant). To gain insights into the molecular mechanisms of action of CHOEP, GEP analysis was conducted. CHOEP treatment induced a marked upregulation of genes encoding for Src family kinases (SFKs). WB analysis confirmed that SFKs are activated upon CHOEP treatment thus suggesting the rationale for combining a tyrosine kinase inhibitor to potentiate CHOEP activity. Accordingly, the addition of DA to CHOEP significantly inhibited cell proliferation in SUP-T1, Jurkat, HD-MAR-2, OCI-Ly12 and HH cells (mean inhibition: CHOEP 20,56%±4 range 14% - 26%; DA 19%±2%, range 14% - 28%; DA-CHOEP 45%±8% range 32% - 56%; mean±SD, $p \leq 0.001$) but not in Karpas 299 cell line (CHOEP 22%±3%; DA 26%±2%; DA-CHOEP 35%±2%, mean±SD, p:ns). The antiproliferative effect of the DA-CHOEP combination was related to a significant increase in cell death, associated with severe mitochondrial depolarization. In xenograft models obtained by the subcutaneous inoculation of SUP-T1 and OCI-Ly12 cell lines in NOD/SCID mice, the DA-CHOEP combination strongly reduced tumor weights when compared to DA and CHOEP used alone (DA-CHOEP tumor growth inhibition, TGI= 98%; DA TGI=38.9%; CHOEP TGI=77.4% for SUP-T1 cell line and DA-CHOEP TGI: 74.5%; DA TGI 27.5%; CHOEP TGI: 11.5% for OCI-Ly12 cell line).
Summary and Conclusions: This study provides the first preclinical evidence supporting that dasatinib could potentiate CHOEP efficacy in TCLs irrespective of histologies and of CHOEP sensitivity *per se*.

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ELEVATED SERUM IL-8, IL-10, IL-22, AND MCP-1 ARE ASSOCIATED WITH INFERIOR CLINICAL OUTCOMES IN PATIENTS WITH PERIPHERAL T-CELL LYMPHOMA

J. H. Yi[1,*], W.S. Kim[2], S.J. Kim[2]

[1]Hematology-Oncology, Chung-Ang university Hospital, [2]Hematology-Oncology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic Of

Background: Cytokines are one of the major non-cellular component of tumor microenvironment, and they act as messengers between malignant cells and the cellular component of tumor microenvironment.
Aims: While the role of cytokines in Hodgkin's lymphomas or B-cell lymphomas has been widely investigated, little is known about in case of PTCL. We evaluated how the pre-treatment serum level of various cytokines impact on clinical outcomes in patients with PTCL.
Methods: Patients out of our prospective cohort studies (NCT#00822731, NCT#01877109) who had PTCL and whose serum samples were collected for measurement of cytokines before receiving any kinds of treatments were included in the analysis. We measured the levels of eotaxin, GROα, IFN-

α&γ, IL-1α,1β,1RA,2,4,5,6,7,8,9,10,12p70,13,15,17α,18,21,22,23,27, and 31, IP-10, MCP-1, MIP-1α&β, RANTES, SDF1α, TNFα&β in triplicate with the Procarta cytokine profiling kit (Panomics, CA, USA). The endpoints were OS and CR rate.

Results: A total of 137 patients were included, and the median age was 57 years (17 - 85 years). The histologic subtypes were as follows: PTCL-NOS(N=55, 40%), AITL(N=44, 32%), ALCL(N=30, 22%), others(N=8). In the whole cohort, with the median overall survival being 63.7 months, we found that expression of IL-22, serum level of IL-10 above 3.8 pg/mL, serum level of MCP-1 above 300 pg/mL, and serum level of IL-8 above 40 pg/mL negatively affect clinical outcomes in terms of complete response rate and the overall survival. Compared to other subtypes, patients with AITL were more likely to have higher level of IL-10 (p=.003), IL-22 (p=.013) and MCP-1 (p=.042), reflecting the inflammatory milieu of the disease. Buth the impact on clinical outcomes were greater in ALCL patients as most of the OS differences were produced in these patients. In patients with PTCL-NOS, increased IFN-γ (>1.0 pg/mL) and expression of IL-13 was associated with adverse outcomes (Figure 1).

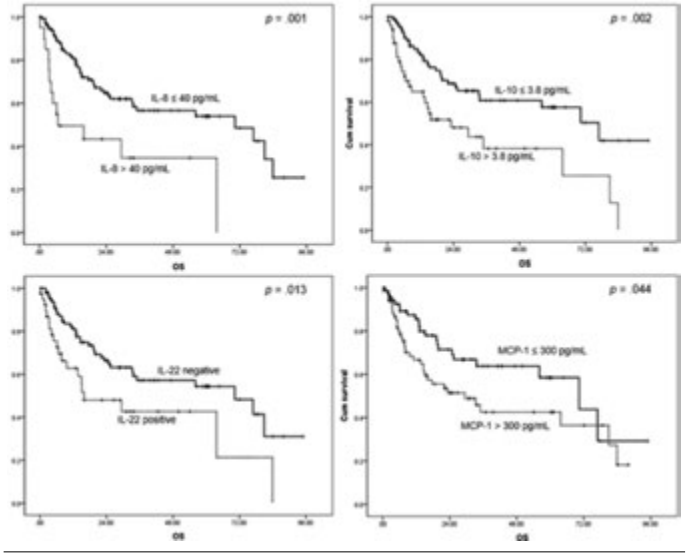

Figure 1.

Summary and Conclusions: The current study demonstrated that several cytokines related to JAK pathway (IL-10, 22) or tumor microenvironment (MCP-1, IL-8) have prognostic relevance, which is an important finding in understanding the pathogenesis of PTCL and suggesting the future direction of treatment.

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BIOMARKER ANALYSES OF PATIENTS WITH RELAPSED DIFFUSE LARGE B-CELL LYMPHOMA, FOLLICULAR LYMPHOMA, OR RICHTER'S TRANSFORMATION TREATED WITH IBRUTINIB+ NIVOLUMAB IN THE PHASE 1/2A LYM1002 STUDY

B. Hodkinson[1], M. Schaffer[1], J. Brody[2], C. Carpio[3], A. López-Guillermo[4], D. Ben-Yehuda[5], B. Ferhanoglu[6], A. Nagler[7], M. Ozcan[8], D. Buglio[9], I. Avivi[10], M. Streit[1], J. Alvarez[1], R. Ceulemans[11], B. Kharabi Masouleh[11], N. Fourneau[11], W. Jurczak[12], S. Balasubramanian[1], A. Younes[13,*]

[1]Janssen Research & Development, Spring House, PA, [2]Division of Hematology and Medical Oncology, Icahn School of Medicine at Mount Sinai, New York, NY, United States, [3]Department of Hematology, University Hospital Vall d'Hebron, [4]Department of Hematology, Hospital Clinic, University of Barcelona, Barcelona, Spain, [5]Department of Hematology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel, [6]Department of Internal Medicine, Koc University, Istanbul, Turkey, [7]Chaim Sheba Medical Center, Tel-Hashomer, Israel, [8]Department of Hematology, Ankara University School of Medicine, Ankara, Turkey, [9]Bristol-Myers Squibb, Lawrenceville, NJ, United States, [10]Department of Hematology and Bone Marrow Transplantation, Tel Aviv Medical Center, Tel Aviv, Israel, [11]Janssen Research & Development, Beerse, Belgium, [12]Department of Hematology, Jagiellonian University, Krakow, Poland, [13]Lymphoma Department, Memorial Sloan Kettering Cancer Center, New York, NY, United States

Background: Ibrutinib (ibru) is a first-in-class, oral, covalent Bruton's tyrosine kinase inhibitor approved in various B-cell non-Hodgkin's lymphomas (NHL). Preclinical research suggested synergistic antitumor activity due to T-cell activation and Th1 polarization when ibru was combined with programmed cell death protein-1 (PD-1) pathway inhibitors. A phase 1/2a study (LYM1002, EudraCT 2014-005191-28) evaluating ibru/nivolumab (nivo) combination demonstrated acceptable safety and efficacy comparable to single-agent ibru in multiple B-cell malignancies; clinical response in Richter's transformation (Richter's) exceeded expectation (overall response rate [ORR] 30.0% for follicular lymphoma [FL], 35.6% for diffuse large B-cell lymphoma [DLBCL], and 60.0% for Richter's) (Younes, *et al. Blood.* 2017;130:833).

Aims: To identify predictive and mechanistic biomarkers correlated with response to ibru+nivo combination in patients (pts) with DLBCL, FL, or Richter's.

Methods: LYM1002 was an open-label, single-arm study in which NHL pts received intravenous nivo (3 mg/kg) on a 14-day cycle with ibru 560 mg once daily. Responders were defined as pts who achieved complete response (CR) or partial response (PR) except blood lymphocyte count control. PD-L1 levels were measured by immunohistochemistry (IHC) staining with Dako 28-8 antibody and gene expression profiling (GEP) using AffyMetrix HG-U133+2 arrays in baseline tumor biopsy tissue. PD-L1 elevation was defined as ≥5% tumor cells (IHC) or expression above the median (GEP). Proportions of 22 distinct immune cell types were evaluated by GEP.

Results: 39 pts with DLBCL, 35 with FL, and 20 with Richter's were evaluable for biomarkers. DLBCL pts with elevated PD-L1 by IHC had a trend toward increased ORR (62.5% *vs* 18.8%, *p*=0.06), as well as significantly increased CR (37.5% *vs* 0; *p*=0.03). There was also a trend toward improved progression-free survival in DLBCL pts (n=23), as well as in germinal center B-cell (GCB)-DLBCL subtype (n=13) pts with elevated PD-L1 compared with those without elevation (Figure 1). This trend was not noted in FL pts; only 3 FL pts were positive for PD-L1 by IHC and only 1 had elevated PD-L1. In Richter's, 12/16 evaluable pts responded, but only 3/15 pts with IHC data had elevated PD-L1 levels; all 3 achieved PR. For immune cell analysis, GEP data were available in 14 responders (7 each for DLBCL and FL) and 22 nonresponders (12 for DLBCL, 10 for FL). Regarding the levels of 22 distinct immune cell types in responders *versus* nonresponders, results were generally more consistent between all DLBCL and the GCB subtype, but were substantially different between DLBCL and FL. Increased numbers of cytotoxic CD8 T cells, M1 macrophages, and resting dendritic cells were significantly associated with response in DLBCL but not FL; however, in FL, increased γ-δ-T cells were associated with response.

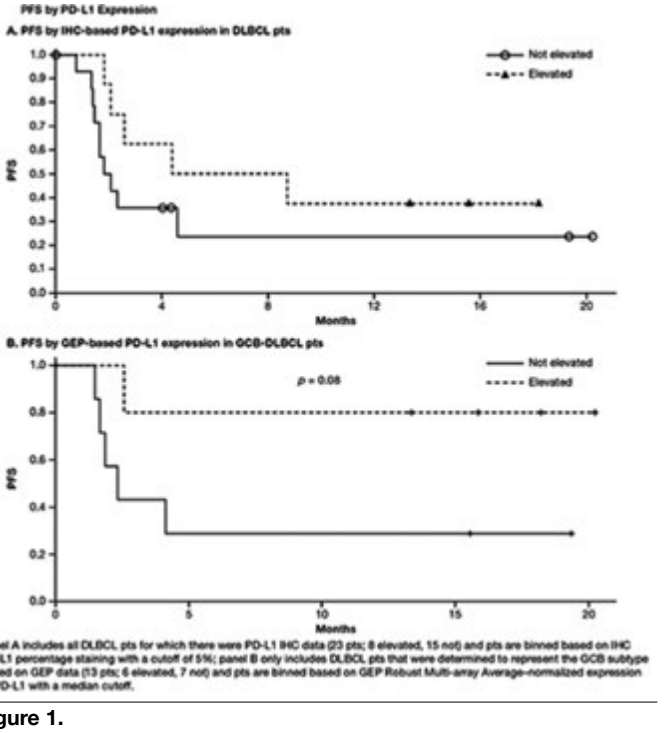

Figure 1.

Summary and Conclusions: In this study, DLBCL pts with elevated PD-L1 expression showed a trend toward better response and survival with ibru+nivo treatment, although pt number was small and significance was reached only for CR. PD-L1 elevation was low in FL and Richter's, although the few pts with elevated PD-L1 in Richter's had a response. Histology-dependent variations in tumor infiltrating immune cells at baseline suggest that differences in microenvironment are critical to the differential ibru+nivo response in DLBCL and FL. Our findings may support the identification of

pts who benefit most from ibru+nivo and suggest development of novel combination therapies to enhance ibru clinical activity.

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SYSTEMS BIOLOGY ANALYSIS OF RESPONSIVENESS OF NON-HODGKIN LYMPHOMA B-CELL LINES TO CD37 TARGETING RADIOIMMUNOTHERAPY

K.B. Melhus[1], E. Gaudio[2], L. Cascione[2], A.H. Repetto-Llamazares[1], J. Dahle[1], S. Patzke[1,3,*], F. Bertoni[2]

[1]Research & Development, Nordic Nanovector ASA, Oslo, Norway, [2]Institute of Oncology Research, Università della Svizzera italiana, Bellinzona, Switzerland, [3]Radiation Biology, Institute for Cancer Research, OUH Norwegian Radium Hospital, Oslo, Norway

Background: CD37 is an internalizing transmembrane glycoprotein widely expressed on mature B-cells and B-cell malignancies. The next generation anti-CD37 radioimmunoconjugate (RIC) ^{177}Lu-lilotomab satetraxetan (Betalutin®), containing the beta-emitting radionuclide lutetium-177, is currently being tested as one time injection therapy in a clinical phase 2b trial for follicular lymphoma (FL) and phase 1 trial for diffuse large B-cell lymphoma (DLBCL).
Aims: The present work is the first to explore intrinsic genetic factors and expression features that correlate with the responsiveness of well characterized B-cell lymphoma cell lines to treatment with ^{177}Lu-lilotomab satetraxetan.
Methods: Fifty five biologically well characterized human lymphoma cell lines (including 7 activated B-cell-like (ABC) and 20 germinal center-like (GCB) DLBCL cell lines) were treated for 18 hours with increasing doses of ^{177}Lu-lilotomab satetraxetan (from 0 to 20 µg/ml; 600 MBq/mg), washed, and seeded on micro-well plates. Cell viability was assessed by flow cytometry directly after treatment and dose-response profiles were determined six days post seeding using a CyQuant direct cell proliferation kit. Cell lines were grouped by dose-response-curve profiles and IC50 values into subgroups with different sensitivity to ^{177}Lu-lilotomab satetraxetan. Correlation analysis of sensitivity subgroups to genetic aberrations and baseline gene/protein expression was performed using appropriate statistical tools as previously described (PMID: 29066507).
Results: Hierarchical clustering analysis of dose-response curves of inhibition of proliferation identified five major groups.The most sensitive cell lines showed >80% growth inhibition at <1 µg/ml of ^{177}Lu-lilotomab satetraxetan (mostly DLBCLs) and most resistant cell lines showed <50% growth inhibition even at 20 µg/ml ^{177}Lu-lilotomab satetraxetan (mostly cutaneous T cell lymphoma). Sensitivity was not directly correlated to CD37 mRNA expression. The median IC50 of ABC-DLBCLs was 1.2 µg/ml, with only U2932 and RIVA being treatment resistant cell lines. The median IC50 of GCB-DLBCLs was 5.2 µg/ml, showing bi-modal distribution in groups of 10 cell lines below and above the median IC50. Treatment resistance in GCB-DLBCLs was not inversely correlated to CD37 mRNA or protein expression and neither correlated to aggressiveness associated mutations in *TP53*, or translocation/amplification of *MYC* or *BCL2* loci. Differential gene expression analysis between response groups of GCB-DLBCL cell lines identified 80 modulated genes, 40 of them (50%) with elevated base-line expression in sensitive cell lines and 40 (50%) of them with elevated base-line expression in resistance cell lines. Gene set enrichment analysis revealed increased expression of cytoskeleton and metabolism regulatory genes in treatment sensitive and increased expression of inflammation and survival regulatory genes in treatment resistant GCB-DLBCL cell lines. Further bioinformatic analysis including cell lines of other indications as well as additional genome wide copy number variation, methylation, RNA sequencing and miRNA datasets is currently ongoing.
Summary and Conclusions: ^{177}Lu-lilotomab satetraxetan shows promising activity against not only GCB-DLBCL cell lines, but also several ABC-DLBCL cell lines. Resistance/sensitivity is not predicted by genetic hallmarks of lymphoma aggressiveness. Initiated correlation analysis of global genetic/transcriptomic/proteomic markers will prove valuable for identification of clinically relevant biomarker(s) and putative co-targetable conveyors of treatment resistance.

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CBL-MZ IS NOT A SINGLE BIOLOGICAL ENTITY: EVIDENCE FROM GENOMIC ANALYSIS AND PROLONGED CLINICAL FOLLOW UP

J. Strefford[1,*], H. Parker[1], N. McIver Brown[2], Z. Davis[2], M. Rose-Zerilli[1], A. Xochelli[3], J. Gibson[4], M. Parry[1], R. Walewska[2], D. Oscier[2]

[1]Cancer Sciences Unit, Faculty of Medicine, University of Southampton, Southampton, [2]Department of Molecular Pathology, Royal Bournemouth Hospital, Bournemouth, United Kingdom, [3]Institute of Applied Biosciences, Centre for Research and Technology Hellas, Thessaloniki, Greece, [4]Biological Sciences, Faculty of Natural and Environmental Sciences, Southampton, United Kingdom

Background: The term clonal B cell lymphocytosis of marginal zone origin (CBL-MZ) has recently been suggested for asymptomatic individuals whose routine blood count shows a persistent modest lymphocytosis. However, it remains unclear if CBLMZ is the precursor to one or several well defined WHO entities and what factors predict for disease progression.
Aims: To address this, we performed a genomic analysis of a well-characterized CBL-MZ cohort with long follow-up.
Methods: 37 patients (M/F ratio 1.2:1, median age at presentation was 73.2 years [range 47.8 – 95.5 years]) who fulfilled the diagnostic criteria for CBL-MZ and in whom clinical, morphological, immunophenotypic, immunogenetic and cytogenetic data was available, were followed up for a median of 9.6 years (range 2.5-22.4 years). All cases were screened for the presence of somatic mutations in KLF2, NOTCH2, CCND3, BCOR, MAP2K1, BRAF V600E, MYD88, TNFAIP3 and TP53 using a bespoke Haloplex Target Enrichment system (Agilent Technologies). DNA from
buccal cells [n=22] was used to confirm the somatic origin of variants identified in these genes.
Results: 9/37 (23.6%) cases showed evidence of progressive disease with a median time to progression of 69 months. Five cases evolved to splenic marginal zone, two to splenic lymphoma/leukemia unclassifiable and one each to lymphoplasmacytic lymphoma, and MALT lymphoma. Fifteen genomic mutations, involving all candidate genes screened apart from BCOR and BRAF V600E, were identified in 12 cases. The most frequent mutations involved MYD88, CCND3 and TP53 in five, three and three cases respectively, but the pattern and incidence of mutations differed from that reported in any well-defined WHO disorder. While neither cytogenetic nor immunogenetic data correlated with the natural history of CBL-MZ, 5/9 patients with progressive disease had one or more mutations
compared to 6/28 with stable disease (p=0.03).
Summary and Conclusions: Our clinical outcome data indicate that CBL-MZ usually pursues a stable course but the higher rate of progression in the current study compared to previous studies probably reflects the longer follow-up and reinforces the need for long term clinical management. CBLMZ can evolve into several well-defined WHO disorders especially those of MZ origin. The genomic data is consistent with this observation as although the genomic abnormalities in CBL-MZ overlap with those found in any of the well-defined entities to which it could evolve, the incidence of mutations is lower and does not mirror any specific disease. In summary, both the clinical outcome and genomic data indicate that CBL-MZ is not a single biological entity.

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HETEROGENEOUS MUTATIONS AT RHOA EXON 2 REVEALED BY TARGETED SEQUENCING OF CELL FREE DNA AND PERIPHERAL BLOOD MONONUCLEAR CELL DNA IN PERIPHERAL T-CELL LYMPHOMA

B. Ottolini[1], N. Nawaz[1], S. Mamand[1], R.L. Allchin[1], P. Fields[2], R. Dillon[3], M.J. Ahearne[1], S.D. Wagner[1,*]

[1]Leicester Cancer Research Centre, University of Leicester, Leicester, [2]Department of Haematology, Guys and St. Thomas' NHS Trust, [3]Department of Medical and Molecular Genetics, King's College London, London, United Kingdom

Background: Angioimmunoblastic T-cell lymphoma (AITL) and ~20% of peripheral T-cell lymphoma-not otherwise specified (PTCL-NOS) are likely to be derived from normal follicular helper T-cells. These Tfh lymphoma cases share genetic abnormalities including a point mutations leading to RHOA^{G17V}. The new genetics has potential to provide biomarkers for diagnosis and to guide treatment but this approach is limited because adequate tissue biopsies can be difficult to obtain.
Aims: To determine the usefulness of cell free DNA (cfDNA) or peripheral blood mononuclear cell (PBMNC) DNA to detect the mutational burden associated with peripheral T-cell lymphoma.
Methods: Whole exome sequencing (WES) and targeted sequencing (using a 60 amplicon panel of 12 genes and covering 12 kb) were carried out on lymph node tissue, cell free DNA and PBMNC DNA. Validation was by droplet digital PCR (ddPCR).
Results: WES of PBMNC DNA from two patients with PTCL showed a novel point mutation leading to RHOA^{F25L} (patient 1, 32% (59/124 reads) and patient 2, 26% (43/121 reads)) without other detectable RHOA mutations. Targeted sequencing (mean read depth 3554x) of cfDNA and PBMNC DNA in 16 patients with AITL and PTCL-NOS demonstrated mutations in

TET2, DNMT3A, IDH2, STAT3, JAK2 and P53 as previously reported in PTCL. Focusing on RHOA exon 2 we found four recurrent point mutations. These included G17V (median variant allele frequency (VAF) 5.9 (range 0.8 to 48.7)) but also F25L (median VAF 35.6, range 0.6 to 95.1) (similar frequency to that obtained by WES) and mutations reported in other cancers *i.e.* C16stop (median VAF 8.9, range 1.0 to 56.2) and G14V (median VAF 2.0, range 0.8 to 26.7). The two dominant RHOA variants (F25L and G17V) were validated by ddPCR assays and Sanger sequencing. *In silico* prediction suggested that F25L perturbed a pi-stack interaction with F171 to disrupt alpha-helix conformation and this conclusion was supported by the phenotype prediction programs, PolyPhen2 (score 1.0, probably damaging) and SIFT (0.02, deleterious). Is $RHOA^{F25L}$ detectable within lymphoma tissue? Of those patients with circulating $RHOA^{F25L}$ targeted sequencing of lymphoma tissue detected the mutation in only 50% of patients and at relatively low frequency (median VAF 4.5%, range 0.5-10.1%). Further sequencing from multiple tissue cores from two patients confirmed that mutations were not homogenous within tissue but were spatially separated. Therefore, RHOA exon 2 mutation in PTCL is more complex than previously believed. Mutations vary in frequency between lymph node and blood and also within lymph nodes. Finally, we analysed the mutational load in cfDNA in serial samples from a small number of patients and showed changes in the dominance of specific RHOA mutations associated with response and relapse and suggesting differential expansion of subclones during treatment.

Summary and Conclusions: Analysis of RHOA mutations in blood revealed a spectrum quite different to that observed in lymphoma tissue. Our findings, using PBMNC DNA and cfDNA, are likely to be the representation in the blood of intra-tumour heterogeneity. The data suggests differential contribution of mutations present in the tissues to those detectable in the circulation although the mechanisms underlying this need to be explored in future work. Blood-based detection of RHOA mutations could transform clinical practice impacting diagnostic pathways and predicting treatment response.

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PROGRAMMED DEATH-1 PATHWAY UP-REGULATION BY LENALIDOMIDE IN ACTIVATED LOW-GRADE LYMPHOMA CELLS

S. Bossio[1], A.G. Recchia[1], L. De Stefano[1], A. Palummo[1], F. Storino[1], N. Caruso[2], M. Gentile[2], E. Vigna[2], C. Stellitano[3], D. Fenoglio[4], A. Gulino[5], G. Filaci[4], M. Manzoni[6], F. Fais[7], A. Neri[6], B. Martino[8], G. Cutrona[9], P. Tassone[10], C. Tripodo[11], M. Ferrarini[12], F. Morabito[1,*]

[1]Unità di Ricerca Biotecnologica, Fondazione Amelia Scorza-AIL, Aprigliano (CS), [2]Department of Onco-Hematology, Hematology Unit, A.O. of Cosenza, Cosenza, [3]Hematology Unit,, Azienda Ospedaliera "Bianchi Melacrino Morelli", Reggio Calabria, [4]Centre of Excellence for Biomedical Research and Department of Internal Medicine, University of Genoa, Genoa, [5]Department of Health Science, Human Pathology Section, Tumor Immunology Unit, University of Palermo School of Medicine Palermo, Palermo, [6]Department of Oncology and Hemato-Oncology and Hematology Unit, University of Milano and Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milano, [7]Molecular Pathology Unit and Department of Experimental Medicine, IRCCS-A.O.U. San Martino-IST and University of Genova, Genoa, [8]Hematology Unit, Azienda Ospedaliera "Bianchi Melacrino Morelli", Reggio Calabria, [9]9Molecular Pathology Unit, IRCCS-A.O.U. San Martino-IST, Genoa, [10]Department of Experimental and Clinical Medicine, University of Catanzaro Magna Graecia, Catanzaro, [11]Department of Oncology and Hemato-Oncology and Hematology Unit, University of Palermo School of Medicine Palermo, Palermo, [12]Dipartmento di Medicine Sperimentale, University of Genoa, Genoa, Italy

Background: Programmed death-1 (PD1) is expressed on T cells, B cells, and other immune effector cells and interacts with the PD1 ligand (PDL1) expressed on a wide variety of tumors. The PD1/PDL1 cross-link induces negative signals to the T cell, provides a T-cell exhaustion state and, ultimately, provokes neoplastic tolerance. PD1 is expressed on Hodgkin lymphoma (HL) and T-cells of B-cell non-HL. The tumor microenvironment (TME) is essential for supporting proliferation and survival of lymphoma cells and in resisting the effects of chemotherapy. Interrupting the signaling pathways mediated by cells or humoral factors might enhance the effects of chemotherapy and suggests that the TME is a target for therapy. Lenalidomide (LEN) is an oral immunomodulator (IMID) with direct antineoplastic activity and immunologic effects, including stimulation of T cell– and NK cell–mediated cytotoxicity in experimental models. Preclinical findings indicate that combination of IMIDs with immune checkpoint inhibitors may promote therapeutic synergy and long-term antitumor immunity to improve clinical outcome.

Aims: 1) To characterize the PD1, PDL1 and the lesser-known PDL2, phenotype in peripheral neoplastic CD19+ lymphocytes and T-cell subsets in patients with low-grade B-cell lymphoma; 2) to evaluate the role of the TME in supporting the PD1 axis; and 3) to determine whether LEN influences PD1 or cognate ligand expression.

Methods: Samples obtained from peripheral blood of patients with low grade lymphoma in leukemic phase attending participating Hematology Units in Southern Italy were used to determine PD1, PDL1, PDL2 phenotype by flow-cytometry (FC). Autologous activated T-cells (AAT) were obtained by *in vitro* co-culture of patient T-cells with anti-CD3/CD28 beads, rIL2, and with PBMCs. In selected experiments LEN (Celgene) was added to cell cultures.

Results: Twenty-eight cases of lymphoma were evaluated for PD1, PDL1 and PDL2 expression on malignant B- and T-cells by FC. The expression of PDL1 was practically undetectable, while PD1 and PDL2 were similarly expressed on B-cells. Significantly higher PD1 expression compared with very low levels of ligands were detected in CD4+ and CD8+ cells. Co-culture of lymphoma cells with AAT cells showed consistent formation of B/T-cell clusters. PD1 and PDLs expression significantly increased in AAT co-culture on B-cells. PD1 expression on CD3+ cells was unaffected by AAT, although the expression of both ligands increased significantly. Closer analysis of T-cell subsets showed that PD1 expression increased significantly following co-culture experiments only in CD4+ cells. Lymphoma-AAT co-culture experiments (n=4) indicated that LEN (0.5/1 uM) did not negatively influence the formation of AAT clusters. After 48 h of co-culture, the expression of CD19+CD5-PDL1+ cells increased in 4/4 cases following LEN treatment while, PDL2 expression remained unchanged. CD3+ cells showed a significant increase in PD1 expression by LEN, while the expression of both ligands remained unaffected. Evaluation of activated T-cell subsets showed similar results, with the exception of stronger induction of PD1 and PDL1 expression by LEN in CD8+ cells.

Summary and Conclusions: Our results suggest the potential involvement of the PD1/PDLs-axis in lymphoma patients. In particular, LEN further induces the expression of PD1 in CD8+ and CD4+ cells and may contribute to reactivate PD1 signaling under treatment. The PD1 pathway may be potentially targeted to overcome both the intrinsic and LEN-induced exhaustion phenotype.

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THE EFFECT OF DUVELISIB, A DUAL INHIBITOR OF PI3K-DELTA,GAMA ON COMPONENTS OF THE TUMOR MICROENVIRONMENT IN PREVIOUSLY UNTREATED FOLLICULAR LYMPHOMA

C. Casulo[1,*], A. Goy[2], K. Van Eygen[3], J. Sancho[4], S. Mercadal[5], R. Johnson[6], S. DeVos[7], K.-K. Bouabdallah[8], K. Sprott[9], N. Le[9], J. Pachter[9], D. Weaver[9]

[1]Wilmot Cancer Institute, University of Rochester Medical School, Rochester, [2]Hackensack University Medical Center, Hackensack, United States, [3]AZ Groeninge, Kortrijk, Belgium, [4]Hospital Universitario Germans Trias i Pujol, Badalona, [5]Department of Hematology, ICO L'Hospitalet-Hospital Duran i Reynals, L'Hospitalet de Ll., Barcelona, Spain, [6]St. James's Univ. Hospital Trust, Leeds, [7]Division of Hematology/Oncology, Ronald Reagan UCLA Medical Center, Los Angeles, United States, [8]University Hospital of Bordeaux, CHU Haut-Leveque, Bordeaux, France, [9]Verastem, Inc., Needham, United States

Background: Duvelisib (DUV), an oral dual inhibitor of PI3K-d,g, is clinically active in hematologic malignancies, including follicular lymphoma (FL), CLL, and T cell lymphoma (Flinn, 2017). PI3K-d inhibition directly targets malignant cells and PI3K-g inhibition disrupts the supportive tumor microenvironment (TME). In the CONTEMPO trial (NCT02391545), previously untreated FL patients (pts) treated with DUV+rituxumab had an ORR of 93% (36% CRR) and pts treated with DUV+obinutuzumab had an ORR of 89% (41% CRR). In both treatment arms chemokines reflective of the tumor microenvironment were inhibited (Casulo, 2016).

Aims: Herein we examine the effects of DUV on the TME via *in vitro* assays utilizing whole blood obtained from pts with treatment-naive follicular lymphoma.

Methods: *Ex vivo* whole blood assays were conducted from healthy volunteers and 32 FL pts enrolled in CONTEMPO [pre-and post-DUV]. *Ex vivo* and *in vitro* PI3K-g assays [(fMLP-stim monocyte and CXCL12-stim human T cell pAKT(S473), murine bone marrow monocyte migration, and macrophage polarization quantified by ARG1 expression] and PI3K-d assays [LPS-stimulated monocyte pAKT(S473)] with PI3K-d-selective [idelalisib (IDELA), TGR-1202, IPI-3063] and PI3K-g-selective [IPI-549] inhibitors were compared.

Results: DUV (IC_{50} 0.4 ±0.1 mM) and IDELA (IC_{50} 1.0±0.2 mM) potently inhibited LPS-induced human monocytes via PI3K-d compared with the PI3K-g selective IPI-549 (IC_{50} 12±0.5mM). For TGR-1202, the IC_{50} (25±8 mM) was below the RP2D clinical exposure. DUV (IC_{50} 0.5±0.2 mM) and IPI-549 (IC_{50} 1.6 ±0.2 mM) potently inhibited PI3K-g dependent fMLP-stimulated human monocytes compared to IDELA (IC_{50} 9.4±2.3) and TGR-1202 (IC_{50} 55±16 mM). In FL pts treated with DUV, these PI3K-g and PI3K-δ selective assays were inhibited 1-4 hours post treatment. Consistent with a PI3K-g mechanism, both DUV and IPI-549 inhibited macrophage polarization to M2, reduced CXCL12-induced macrophage migration (DUV IC_{50} 51 nM; IPI-549 IC_{50} 85 nM), and blocked CXCL12-induced T cell migration (DUV EC_{50} 128±39 nM; IPI-549 EC_{50} 17±17nM), which was not observed with PI3K-δ inhibitor IPI-3063 (IC_{50} 630±71nM).
Summary and Conclusions: The disruption of PI3K-d,g function in FL pts treated with DUV supports its inhibition of the tumor microenvironment through cancer-supportive macrophages and T cells.

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INCORPORATION OF GENE EXPRESSION PROFILING FOR CELL-OF-ORIGIN DETERMINATION USING FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUE SECTIONS IN ROUTINE WORK-UP OF DIFFUSE LARGE B-CELL LYMPHOMA CASES

R.S. Robetorye[1,*], C. Ramsower[1], A. Rosenthal[1], T. Yip[1], A. Wendel Spiczka[1], B. Glinsmann-Gibson[1], L. Rimsza[1]

[1]Department of Laboratory Medicine & Pathology, Mayo Clinic Arizona, Phoenix, United States

Background: Diffuse large B-cell lymphomas (DLBCL) represent a clinically heterogeneous group that is classified together based on similarities in morphology and immunophenotype. However, it was discovered that gene expression profiling (GEP) could classify DLBCL into distinct molecular subgroups based on cell-of-origin (COO), including germinal center B-cell type (GCB), activated B-cell type (ABC), and unclassified (UNC) type. COO assignment of DLBCL has important biological and prognostic significance, as well as potential therapeutic implications, with the ongoing development of selective agents for treatment of specific DLBCL subtypes.
Aims: Here, we describe the development of a clinical GEP assay (Lymph2Cx testing) to perform COO assignment in the routine work-up of DLBCL using FFPE tissue sections and summarize the results of the first 98 clinical DLBCL cases analyzed by the laboratory.
Methods: Lymph2Cx COO analysis was performed on 98 clinical DLBCL cases. RNA was extracted from macrodissected FFPE tissue sections and hybridized to the 20 fluorescently-labeled gene probes in the Lymph2Cx panel, including 8 genes overexpressed in ABC, 7 genes overexpressed in GCB, and 5 housekeeping genes. Probe/RNA complexes were purified on a NanoString® nCounter® Prep Station and transferred to a NanoString nCounter® Digital Analyzer for quantification. The counts were processed using the National Cancer Institute's Lymphoma/Leukemia Molecular Profiling Project Lymph2Cx DLBCL COO Classifier (patented algorithm) for subtyping. Minimum laboratory time required to perform the Lymph2Cx assay is approximately 28 hours/2.4 lab days.
Results: We have successfully analyzed 98 DLBCL cases from 66 male and 32 female patients using the Lymph2Cx assay and detected 46 GCB, 35 ABC, and 17 UNC cases. 85 of the 98 total cases were analyzed by immunohistochemistry using the Hans algorithm, and included 53 GCB and 32 non-GCB cases, with an overall concordance rate of 76%. 96 of the 98 DLBCL cases were also analyzed by FISH and detected 9 cases of high-grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements, including 6 cases with genetic double-hit type rearrangements and 3 triple-hit cases. Lymph2Cx analysis of these 9 cases detected 6 GCB type, 2 ABC type, and 1 UNC type lymphomas. The 98 DLBCL cases subjected to Lymph2Cx analysis included 37 nodal and 61 extranodal lymphomas. Most of the 17 total UNC cases were comprised of extranodal lymphomas, suggesting that UNC lymphomas may arise more frequently in extranodal sites. In addition, ABC cases occurred 2 times or more frequently at extranodal sites *versus* nodal locations in both male and female patients.
Summary and Conclusions: In summary, we have demonstrated that the Lymph2Cx COO assay can be performed relatively rapidly in the clinical laboratory and can be incorporated into the routine workflow for the workup of DLBCL cases. The assay is highly robust and reproducible and can provide valuable biological, prognostic, and potential therapeutic information for DLBCL patients.

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DIFFUSE LARGE B CELL LYMPHOMA WITH CONCORDANT BONE MARROW INVOLVEMENT. UTILITY OF FLOW CYTOMETRY, CLINICAL AND BIOLOGICAL EVALUATION

L. Morais[1,*], C. Castañon[1], S. Alonso-Alvarez[1], M. Alcoceba[2], M. Garcia-Alvarez[2], O. Blanco[2], E. Perez[2], M. Baile[2], O. Lopez[2], M.B. Vidriales[2], E. Colado[1], A. Fonseca[1], M.D. Caballero[2], M. Gonzalez[2], N. Gutierrez[2], C. Nicolas[1], A. Martin[2]

[1]Hematology, HUCA, Oviedo, [2]Hematology, HUSAL, Salamanca, Spain

Background: Bone Marrow (BM) involvement by aggressive lymphoma is associated with poor prognosis.
Aims: We present the update of a Spanish series, and a prospective independent validation series, in which biological characteristics and prognostic of BM involvement have been analyzed.
Methods: Retrospective control series included 232 patients diagnosed of DLBCL (1999-2014) at HUSAL. Validation series includes patients diagnosed of DLBCL from 1/Jan/2016-31/Dec/2017 at HUCA. In both cohorts BM trephine biopsies were classified as "no involvement", "concordant" (DLBCL BM involvement) & "discordant" infiltration (low grade lymphoma BM involvement). Multyparameter Flow cytometry (MFC) analysis of parallel BM aspirates was performed, for improving tumoral clone detection and for phenotypically characterizing discordant cases. We analyzed *MYC, BCL2* and *BCL6* in DLBCL samples by FISH. Cumulative incidence of central nervous system (CNS) relapse was analyzed.
Results: ***Biology and clinical description*** In the control series 31 patients (13%) had concordant and 21 (9%) discordant BM involvement. MFC identified clonal lymphocytic populations in other 15 patients in whom histological studies were not diagnostic. In the validation series, 32 patients have been included; 7 (21%) with concordant and 1 (3%) with discordant BM involvement. MFC confirmed BM involvement in these patients and detected 2 extra cases: 1 DLBCL phenotype & 1 with low grade lymphoma BM involvement. MFC also accounted for 22% of the total of BM infiltration diagnoses. Thus, MFC allowed to observe phenotypes suggestive of different indolent lymphoproliferative disorders (Table 1). FISH analysis showed in both concordant BM groups a high percentage of *MYC* rearrangements:5/17 patients analyzed (29%) and 3/7 (43%) in the control and the validation series respectively. In both cohorts concordant BM disease was statistically associated with higher LDH values at diagnosis and higher risk IPI. ***CNS relapse*** 5-year-cumulative incidence of CNS relapse (occult and clinical leptomeningeal infiltration) in the control series was 33% in the concordant group, 6% in the discordant group,1% in non-infiltrated group (p<0.001). Concordant BM infiltration had an independent association with CNS relapse (HR:11.2;95%CI:2-61.2;p=0.006). In the validation series, we confirmed that all patients with concordant BM fulfilled high risk criteria for CNS relapse (CNS-IPI). Prophylaxis was recommended. No evidence of CNS relapse has been observed, maybe due to systematic CNS prophylaxis and intensive treatment. Three patients with concordant BM received frontline autologous stem cell transplantation.

Table 1.

Bone marrow histological and phenotypic description in patients diagnosed of DLBCL

	MFC									
Histology		CLL	FL	MZL	LPL	DLBCL	Composite	I	No
	Low grade (D)	4	4	2	-	1	1	5	4
	High grade (C)	-	-	-	1	2 1	6	-	-	9
	Unspecified lymphoid infiltrate	3	4	1	2	1	1	1	1	11	3	15

Bold: Hospital Universitario Central de Asturias
Regular: Hospital Universitario de Salamanca
CLL: Chronic lymphoid leukemia; FL: Folicular lymphoma; MZL-Marginal Zone Lymphoma; LPL: Linfoplasmocytic lymphoma; DLBCL: Diffuse large B cell lymphoma; I: Inespecific; D: Discordant; C:Concordant

Summary and Conclusions: Concordant BM infiltration in DLBCL defines a subtype of aggressive lymphoma, associated with high LDH, IPI, and a high rate of *MYC* translocations, which might explain the adverse prognosis recently reported. Concordant BM DLBCL patients were associated with a high

incidence of CNS relapse, reproducing coetaneous bibliography. CNS prophylaxis and aggressive therapy in this group should be considered. MFC helps to identify concordant BM cases at the moment of diagnosis of DLBCL, and it should be considered as part of BM initial evaluation in DLBCL in order to identify these patients.

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DUVELISIB INHIBITION OF CHEMOKINES IN PATIENTS WITH CLL AND INHL

D. Weaver[1,*], K. Sprott[1], J. Pachter[1], N. Le[1], M. Davids[2], M. Montillo[3], Z. Nagy[4], A. Illes[5], G. Etienne[6], J. Delgado[7], B. Kuss[8], C. Tam[9], Z. Gasztonyi[10], F. Offner[11], S. Lunin[12], F. Bosch[13], P. Hillmen[14], N. Lamanna[15], S. Stilgenbauer[16], P. Luigi Zinzani[17], I. Flinn[18], J. Brown[2]

[1]Verastem, Inc., Needham, [2]Dana-Farber Cancer Institute, Boston, United States, [3]Niguarda Cancer Center, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy, [4]1st Department of Internal Medicine, Semmelweis University, Budapest, [5]Department of Hematology, Institute for Medicine, University of Debrecen, Debrecen, Hungary, [6]Hematology Department, Institut Bergonie, Bordeaux, France, [7]Hospital Clinic, Barcelona, Spain, [8]Flinders Medical Centre (FMC), Bedford Park, [9]Peter MacCallum Cancer Centre, Melbourne, Australia, [10]Dep. of Internal Medicine and Hematology, Petz Aladár County Hospital, Győr, Hungary, [11]Hematology, University Hospital Ghent, Gent, Belgium, [12]Florida Cancer Specialists, Venice, United States, [13]Department of Hematology, University Hospital Vall d'Hebron, Barcelona, Spain, [14]St. James's Institute of Oncology, The Leeds Teaching Hospitals, Leeds, United Kingdom, [15]New York Presbyterian, Columbia University Medical Center, New York, United States, [16]Department III of Internal Medicine, University Hospital Ulm, Ulm, Germany, [17]Institute of Hematology Serágnoli, University of Bologna, Bologna, Italy, [18]Sarah Cannon Research Institute, Nashville, United States

Background: Duvelisib (IPI-145) (DUV) is an oral dual inhibitor of phosphoinositide 3-kinase (PI3K)-δ and PI3K-γ being developed to treat B-cell malignancies. PI3K-δ inhibition directly targets proliferation and survival of malignant leukemia and lymphoma cells, while PI3K-γ inhibition modulates the tumor microenvironment (TME) through key support cells, including tumor-associated macrophages, nurse-like stroma and T cells, and via soluble factors stimulating tumor growth, survival and migration. The Phase 3 DUO study in relapsed/refractory (RR) CLL/SLL and the Phase 2 DYNAMO study in RR iNHL both met their primary endpoints (Flinn, ASH 2017; Zinzani EHA 2017).
Aims: Herein we present the effect of DUV on cytokines known to be involved in the TME in pts with RR CLL and RR iNHL.
Methods: DUO (NCT02004522) pts were randomized to DUV (n=160) or ofatumumab (OFA) (n=159). DYNAMO (NCT01882803) pts (n=129) received DUV. Serum from baseline and C2D1 was used for correlative studies of 24 chemokines, cytokines and serum factors. Bonferroni-Holm adjustment for multiple comparisons was applied.
Results: In DUO, CCL1, CCL17, CXCL9, CXCL10, CXCL11, and IL-10 were reduced in pts treated with DUV (median% inhibition=43.8%) but not in those treated with OFA ($p \leq 0.0009$). Eight chemokines were reduced in both treatment arms, but the level of reduction was significantly greater for DUV pts (median% inhibition, DUV 64.6% *vs* OFA 26.8%; $p \leq 0.001$). Many of the chemokines inhibited following DUV treatment are associated with the TME, including TNFα, IL-10, IL2Rα, IL12P40, CCL1, CCL17, CCL19, CXCL9, CXCL10, CXCL11, and CXCL13. In DYNAMO, 13 corresponding chemokines were also inhibited ($p \leq 0.008$), including TME factors. Reductions occurred rapidly (by C2D1) in both studies. In DUO, there was a correlation between duration of response and reduction (highest quartile) of the following chemokines: CCL17 (19.4 mo. Q4 *vs* 10.4 mo. Q1-3), CXCL11 (14.9 mo. Q4 *vs* 10.9 mo. Q1-3) IL-6 (16.6 mo. Q4 *vs* 10.9 mo. Q1-3), TRAIL (24 mo. Q4 *vs* 10.3 mo. Q1-3), VEGF_D (24 mo. Q4 *vs* 10.9 mo. Q1-3), TPO (14.9 mo. Q4 *vs* 10.9 mo. Q1-3).
Summary and Conclusions: Pts with CLL and iNHL treated with DUV monotherapy showed significant reduction of chemokines potentially derived from the tumor cells and TME. Further investigation of the effects of DUV on TME pharmacodynamic markers is warranted.

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ACCURATE GCB/ABC CLASSIFICATION OF *DE NOVO* DIFFUSE LARGE B-CELL LYMPHOMA WITH ABERRANT CD10+ MUM1+ PHENOTYPE BY RT-MLPA

O. Laghmari[1], Y. Le Bris[2,*], A. Menard[3], A. Bonnet[4], A. Moreau[5], W. El Alami Thomas[6], A.-H. Pavageau[7], H. Maisonneuve[8], P. Ruminy[9], P. Guerzider[10], C. Copie-Bergman[11], T. Molina[12], M.-C. Bene[3], S. Le Gouill[4], C. Bossard[1]

[1]Pathology Department, [2]Hematology Biology, [3]Department of Hematology Biology, [4]Department of Hematology, [5]Department of Pathology, University Hospital Hotel Dieu, [6]Department of Pathology, Institut d'Histopathologie (IHP), Nantes, [7]Department of Pathology, [8]Department of Hematology, Centre Hospitalier de Vendée, La Roche sur Yon, [9]University Hospital, INSERM U1245, Rouen, [10]Department of Pathology, Centre Hospitalier de Saint Nazaire, Saint Nazaire, [11]Department of Pathology, University Hospital Henri Mondor, Créteil, [12]Department of Pathology, University Hospital Necker, Paris Descartes University, Paris, France

Background: The cell-of-origin (COO) determination of diffuse large B cell lymphoma (DLBCL) into germinal center B-cell like (GCB), and non-GCB (activated B-cell like (ABC) and unclassifiable) subgroups based on the Hans algorithm, the most commonly used immunohistochemical algorithm, remains an approximate approach compared with gene-expression profiling. The determination of this prognostic criterion needs to be improved in routine diagnosis practice.
Aims: The aim of this study was to verify at the mRNA level the COO classification of a subgroup of GCB DLBCL according to Hans algorithm but featuring an aberrant CD10+ MUM1+ phenotype. We addressed this issue using the RT-MLPA assay, a robust and sensitive method already successfully tested for the COO classification on archival paraffin-embedded formalin-fixed (FFPE) tissues.
Methods: We retrospectively identified 62 CD10+ GCB DLBCL according to Hans classifier from 255 *de novo* DLBCL with available FFPE tissue treated in our institution between 2006 and 2016, including 22 CD10+ MUM1+ (8.6% of DLBCL) and 40 CD10+ MUM1- cases. RNA was extracted from FFPE tissue from all 62 CD10+ GCB DLBCL and the RT-MLPA assay evaluated the expression of 14 genes to differentiate ABC from GCB molecular subtypes (Mareschal *et al.* JMD 2015).
Results: Interpretable expression profiles were obtained for 54 of 62 cases (87%, 33 CD10+ MUM1- and 21 CD10+ MUM1+). The 22 CD10+ MUM1+ DLBCL did not significantly differ from the 40 CD10+ MUM1- DLBCL in terms of main clinicopathological criteria (age, gender, stage, performance status, IPI, serum lactate deshydrogenase, overall and progression-free survivals. There was no difference either on immunohistochemical expression of BCL6, BCL2, cMYC, CD5 with 27% of double-expressor lymphomas in both subgroups. However, while the majority of CD10+ MUM1- DLBCL (29/33; 88%) were classified in the GCB subtype by RT-MLPA (4/33 unclassified), only a minority of CD10+ MUM1+ DLBCL (7/21; 33%) were classified in this molecular subtype and 10/21 (48%) were reclassified in the ABC subtype (4/21 unclassified). Interestingly, after molecular reclassification, the proportion of double-expressor lymphomas became more important in the ABC subtype (5/10, 50%) than in the GCB one (12/36, 33%), and patients with a GCB subtype featured a better progression-free survival than those with a ABC subtype (72% *vs* 60% respectively; p=0.5), in accordance with the literature. Finally, when we re-evaluated the percentage of MUM1+ tumor cells in the CD10+ MUM1+ subgroup, 8/9 cases reclassified in the ABC subtype harbored ≥80% of MUM1+ tumor cells (compared with only 2 cases with a maximum of 80% of MUM1+ tumor cells among the 6 cases classified in the GCB subtype).
Summary and Conclusions: This study shows that 67% of CD10+ MUM1+ DLBCL are misclassified in the GC subgroup according to the Hans algorithm since 48% of them were reclassified in ABC subtype by RT-MLPA. Consequently, the COO classification of CD10+ MUM1+ DLBCL based on Hans algorithm should be interpreted with caution in routine diagnosis. A high proportion of MUM1+ tumor cells by immunohistochemistry (>80%) could identify the majority of these non GC CD10+ MUM1+ DLBCL, and help to precise their COO classification.

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IMMUNOGLOBULIN KAPPA DELETING ELEMENT (IGK-KDE) REARRANGEMENTS AS POSSIBLE TARGET FOR MINIMAL RESIDUAL DISEASE (MRD) EVALUATION IN MANTLE CELL LYMPHOMA (MCL)

I. Della Starza[1,*], L.A. De Novi[1], M. Cavalli[1], N. Novelli[1], E. Genuardi[2], B. Mantoan[2], D. Drandi[2], M. Ferrante[2], L. Monitillo[2], D. Barbero[2], E. Ciabatti[3], S. Grassi[4], R. Bomben[5], M. Degan[5], V. Gattei[5], S. Galimberti[3], A. Di Rocco[1], M. Martelli[1], S. Cortelazzo[6], A. Guarini[7], R. Foà[1], M. Ladetto[8], S. Ferrero[2], I. Del Giudice[1]

[1]Department of Cellular Biotechnologies and Hematology, Hematology, Sapienza University, Rome, [2]Department of Molecular Biotechnologies and Health Sciences, Hematology, Turin University, Turin, [3]Department of Oncology, Hematology, Santa

Chiara Hospital, Pisa, Pisa, [4]Department of Medical Biotechnologies, Siena University, Siena, [5]Experimental OncoHematology, National Cancer Institute, Aviano, Aviano, [6]Medical Oncology and Hematology Division, Hematology, Istituto Clinico Humanitas Gavazzeni, Bergamo, Bergamo, [7]Department of Molecular Medicine, Sapienza University, Rome, [8]Division of Hematology, Azienda Ospedaliera SS Antonio e Biagio e Cesare Arrigo, Alessandria, Alessandria, Italy

Background: Minimal residual disease (MRD) assessment is of high clinical relevance in patients (pts) with mantle cell lymphoma (MCL). The real-time quantitative PCR (RQ-PCR) is currently the most sensitive, standardized and broadly applied method. However, in mature B-cell malignancies, the presence of somatic hypermutation (SHM) in VDJH rearrangements leads to frequent mismatches between primers, probes and the target, thus impairing tumor cells quantification. By contrast, in the immunoglobulin kappa-deleting-element (IGK-Kde) rearrangements, the JK-CK intron intervening sequences deletion removes the IGK enhancer, essential for the SHM process.
Aims: To evaluate the applicability of *IGK*-Kde rearrangements as targets for MRD RQ-PCR detection in MCL, to compare them to *IGH* and *BCL1/IGH*, and to perform a comparative RQ-PCR/digital-droplet-PCR (ddPCR) analysis.
Methods: Molecular IGK screening was performed (Pongers-Willemse, 1999) on two cohorts: the first from Turin (19 pts enrolled in the FIL-MCL0208 trial, NCT02354313), the second from Rome (17 outpts). The PCR products were examined by heteroduplex analysis (Langerak, 1997). The germline probes/primers and the clone specific primers were designed using two drawing strategies (Della Starza, 2014). RQ-PCR analyses followed the EuroMRD Consortium guidelines (van der Velden, 2007). The ddPCR was performed as published (Drandi, 2015; Cavalli, 2017).
Results: Of 36 MCL diagnostic BM samples from 36 pts, 3 showed poorly amplifiable DNA, so a total of 33 cases underwent IGK screening. Overall, 25/33 MCL cases were positive for at least one conventional target (*IGH* or *BCL-1/IGH*) and 8/33 were marker negative: 26/33 (79%) pts resulted positive for a *IGK* rearrangement, showing a 75% (6/8) target recovery for negative cases. Combining sequence analysis-molecular cloning, 19/26 (73%) suitable sequences for the ASO primer design were obtained and in 14/19 (74%) a useful primer for MRD monitoring was achieved. However, the analysis showed a high rate of non-specific amplification of normal mononuclear cells DNA (9/14 cases: 64%), thus hampering the sensitivity of the assay [71% (10/14) cases reached a sensitivity ≥10-4]. Based on the material availability, the MRD analysis was performed in 30 follow-up (FU) samples from 10 pts: a 100% concordance rate with both *BCL-1/IGH* and *IGH* was observed. [SF1] Finally, a comparative RQ-PCR/ddPCR analysis, carried out on 24 FU samples from 8 pts, showed a 79% (19/24) concordance. The discrepancies fell in the remaining 5 (21%), of which 4/5 resulted RQ-PCR positive not quantifiable and ddPCR negative. In this subgroup, no clinical relapses were observed. On the contrary, in 1/5 FU the ddPCR quantified the disease and the RQ-PCR was negative, but the FU is too short for a clinical correlation.
Summary and Conclusions: *IGK-Kde* rearrangements can be found in 79% of our MCL, representing a target marker in 75% of *no marker* cases. MRD RQ-PCR monitoring is possible in 54% (14/26) of cases, showing 100% concordance with the conventional targets. However, owing to the frequently observed background amplification, the sensitivity of this assay is lower in MCL compared with acute lymphoblastic leukemia (Van der Velden, 2002), and in line with multiple myeloma (Puig, 2012). Also in this setting ddPCR has a good concordance with RQ-PCR and it might help to identify false positive/negative results. From a clinical perspective, we suggest that *IGK-Kde* MRD monitoring deserves further evaluation in larger series of MCL pts as a supplementary tool that might integrate the standardized MRD strategies.

Platelets disorders

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CIRCULATING MICRORNAS (MIRNAS) IN ITP PATIENTS BEFORE AND AFTER TREATMENT WITH THROMBOPOIETIN RECEPTOR AGONISTS (TPO-RAS)–DIAGNOSTIC AND PROGNOSTIC VALUE

L. Garabet[1,2,*], W. Ghanima[1], A. Rangberg[1], C. Furlund Nystrand[1], R. Teruel-Montoya[3], C. Martinez[3], M.L. Lozano[3], J. Bussel[4], P.M. Sandset[5], C. Monceyron Jonassen[1]

[1]Østfold Hospital Trust, Kalnes, [2]Akershus University Hospital, Lørenskog, Norway, [3]Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, Murcia, Spain, [4]New York Presbyterian Hospital, Weill Cornell Medical Center, New York, United States, [5]University of Oslo, Oslo, Norway

Background: Immune thrombocytopenia (ITP) is an autoimmune disease diagnosed clinically by isolated thrombocytopenia and exclusion of underlying diseases; there is no specific diagnostic test for ITP. TPO-RAs are effective treatment for ITP with response rates of 40-90%. MiRNAs are small, noncoding RNAs involved in post-transcriptional regulation of gene expression. Dysregulated expression of miRNAs is associated with different diseases such as cancer and autoimmune diseases. Circulating miRNAs are stable, easy to measure, and considered as potential disease biomarkers.
Aims: To characterize circulating miRNAs in ITP patients before and after treatment with TPO-RAs, and explore their diagnostic and prognostic value in ITP.
Methods: Ten miRNAs were selected for validation from our previous miRNA screening study where microRNA PCR panel was used to profile 179 miRNAs in 8 ITP patients before and after TPO-RAs and 8 controls. Validation was performed using droplet digital PCR (ddPCR), a technology allowing for absolute quantification of miRNAs, in 23 ITP patients before, 2 and 6 weeks after TPO-RA-treatment and 22 controls. The 10 miRNAs were: miR-423-5p, miR-26a-5p, miR-16-2-3p, miR-590-5p, miR-199a-5p, miR-382-5p, miR-195-5p, miR-92b-3p, miR-221-3p and miR-33a-5p.
Results: Mean age of patients was 58 years (controls 47 yrs), and 56% females (controls 59%); 22/23 patients had chronic ITP (cITP) with mean platelet count $38.7 \cdot 10^9$/L. Ten patients were on steroids and 3 on immune suppressants. Eltrombopag was initiated in 15 patients and romiplostim in 8. We identified 3 differentially expressed miRNAs in ITP patients compared to controls: miR-199a-5p (p=0.0001), miR-33a-5p (p=0.0002) and miR-195-5p (p=0.035). miR-199a-5p was down-regulated, while miR33a-5p and miR-195-5p were up-regulated; miR-195-5p did not change during TPO-RA-treatment and remained higher than in controls. ROC curve analysis comparing ITP patients and controls showed that area under the curve (AUC) for miR-195-5p, miR-33a-5p and miR-199a-5p was 0.66 (95% CI 0.49-0.81), 0.799 (0.66-0.93) and 0.814 (0.68-0.94) respectively. Logistic regression was used to determine the best combination of miRNAs to diagnose ITP; the combination of miR-33a-5p and miR-199a-5p provided a significantly increased AUC of 0.93 (0.86-0.99).
During TPO-RA-treatment, 6 of 10 miRNAs showed significant changes: miR-199a-5p (p=0.001), miR-33a-5p (p=0.003), miR-382-5p (p=0.004), miR-92b-3p (p=0.005), miR-26a-5p (p=0.008) and miR-221-3p (p=0.023). Of those, miR-33a-5p decreased 6 weeks after treatment, while the remaining 5 miRNAs increased after 2 weeks of treatment. Regression analysis revealed that pre-treatment levels of miR-199a-5p and miR-221-3p predicted platelet count 6 weeks after TPO-RAs (p=0.002, r^2 =0.40, and p=0.015, r^2=0.26, respectively) (Figure 1).

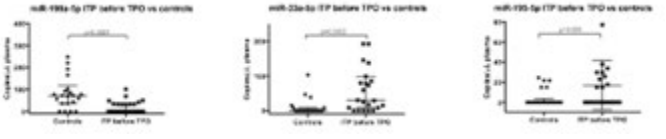

Figure 1.

Summary and Conclusions: Combining miR-199a-5p and miR-33a-5p demonstrated very good discrimination ability between ITP patients and controls and thus appears to be of diagnostic value in ITP. The unique expression of miR-195-5p being persistently upregulated in ITP patients regardless of TPO-RA-treatment and increased platelet count, may indicate a role in the pathophysiology of cITP. Finally, we propose that miR-199a-5p and miR-221-3p are candidate miRNAs to predict response to TPO-RAs. With the potential roles of these miRNAs in the diagnosis and pathophysiology of ITP, and apparent predictive ability of response to treatment with TPO-RAs, larger studies are needed to further explore and confirm the roles of miRNAs in ITP.

PF653

A SINGLE-ARM, OPEN-LABEL, LONG-TERM EFFICACY AND SAFETY STUDY OF SUBCUTANEOUS (SC) ROMIPLOSTIM IN CHILDREN WITH IMMUNE THROMBOCYTOPENIA (ITP)

J. Grainger[1], J. Bussel[2], M. Tarantino[3,4,*], N. Cooper[5], D. Beam[6], J. Despotovic[7], A. Maschan[8], N. Carpenter[9], M. Eisen[10], J. Park[10]

[1]University of Manchester, Manchester, United Kingdom, [2]Weill Cornell Medicine, New York, [3]The Bleeding and Clotting Disorders Institute, [4]University of Illinois College of Medicine-Peoria, Peoria, United States, [5]Imperial College, London, United Kingdom, [6]Cook Children's Medical Center, Fort Worth, [7]Texas Children's Hematology Center, Houston, United States, [8]Dmitry Rogachev National Research Center for Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation, [9]Amgen Ltd., Uxbridge, United Kingdom, [10]Amgen Inc., Thousand Oaks, United States

Background: Romiplostim was evaluated in children with ITP in phase 1/2 and 3 studies.
Aims: Here, we evaluate children with ITP receiving open-label SC romiplostim for ≤3 years.
Methods: Eligible children had ITP for ≥6 months, ≥1 prior ITP therapy, and screening platelet count ≤30×10^9/L or uncontrolled bleeding. Weekly dosing from 1-10 g/kg targeted platelet counts of 50-200×10^9/L. In Europe, bone marrows biopsies were evaluated at baseline and after 1 or 2 years (cohorts 1 or 2) and/or at end of treatment (if patients discontinued early).
Results: As of 20 Mar 2017, 203 patients received ≥1 dose. Baseline median (min-max) age was 10 (1-17) years, ITP duration 1.8 (0.5-13.8) years, and platelet count 14 (2-265)×10^9/L; 10 patients (5%) had had prior splenectomy. The median (Q1, Q3)% of time with a platelet response (platelet count ≥50×10^9/L; no rescue medications in the past 4 weeks) in months 0-6 was 50% (17%, 83%) (primary endpoint). Over the course of the study, 88% (179/203) of patients had a platelet response (Figure 1). From week 12 on, median platelet counts were >50×10^9/L. Four patients maintained platelet counts ≥50×10^9/L with no ITP medications (including romiplostim) for ≥24 weeks, starting 12, 14, 47, and 49 weeks after starting romiplostim. Fifty-two (26%) patients received rescue medications and 3 patients had splenectomy on study. Median (min-max) treatment duration was 53 (8-119) weeks, for a total of 226 patient-years. Median (min-max) average weekly dose over the course of the study was 6.9 (0.2-9.5) µg/kg. The median dose was 9 g/kg at 1 year (n=106) and 10 g/kg at 2 years (n=17) (Figure 1).

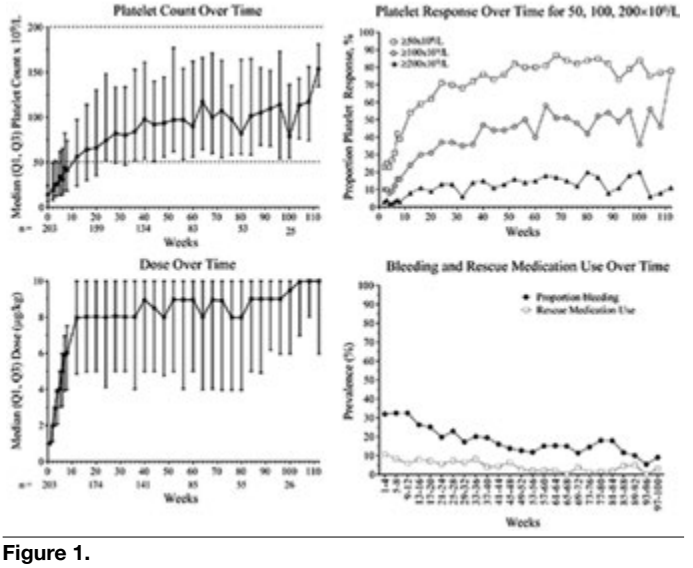

Figure 1.

Most (63%) patients initiated self-administration. Sixty-four patients (31%) discontinued treatment, most frequently for lack of efficacy (n=38), patient request (n=7), and adverse event (AE) (n=7). Forty-one (20%) patients had serious AEs (SAEs) including epistaxis (5%) and decreased platelet count (3%). Five patients had treatment-related SAEs: 2 headaches, 2 abdominal pain, and 1 each of presyncope and neutralizing antibodies (Ab). Bleeding was seen in 62% of patients and decreased over time. CTCAE grade ≥3 bleeding was seen in 17 patients (8%) and included epistaxis (4%), ecchymosis (1%), and contusion (1%); 2 patients had grade 4 bleeding events of "ITP". There were 6 cases of neutralizing Ab to romiplostim (of 201 patients tested), but none to TPO; 5/6 discontinued due to Ab, 5/6 had continued elevated platelet counts and in 2/6 cases Ab were not found on retesting. For cohort 1, of 30 patients with baseline bone marrow biopsies [all with modified Bauermeister scores of grade 0 (no reticulin), 1 (fine fibers), or 2 (fine fiber network)], 27 had evaluable on-study biopsies scheduled for 1 year; 1 patient had an increase from grade 0 to 2, 4 patients had an increase in 1 grade, 1 patient had a decrease in 2 grades, and 3 had a decrease in 1 grade. There were no findings of collagen or abnormalities.
Summary and Conclusions: In this interim datacut of an ongoing open-label study of romiplostim in children with ITP for ≥6 months, 88% of children had a platelet response at some point on study. Median platelet counts were >50×10^9/L from week 12 on, likely due to the time to escalate to the relatively high median dose. Overall, the median dose was 6.9 g/kg; the median romiplostim dose over time reached 10 g/kg. No new safety signals were observed over 226 patient-years. Future datacuts will provide more information on long-term efficacy and safety.

PF654

THE BURDEN OF DISEASE AND IMPACT OF IMMUNE THROMBOCYTOPENIA (ITP) ON PATIENT QUALITY OF LIFE AND PRODUCTIVITY: RESULTS FROM THE ITP WORLD IMPACT SURVEY (I-WISH)

N. Cooper[1,*], W. Ghanima[2], D. Provan[3], D. Arnold[4], M. Michel[5], C. Santoro[6], A. Kruse[7], S. Watson[8], M. Morgan[8], M. Hou[9], Y. Tomiyama[10], S. Laborde[11], B. Lovrencic[12], J. Waller[13], G. Taylor-Stokes[13], D. Judge[13], M. Stankovic[14], J. Bussel[15]

[1]Hammersmith Hospital, Imperial College London, London, United Kingdom, [2]Department of Medicine, Ostfold Hospital Trust, Kalnes, Norway, [3]Barts and The School of Medicine and Dentistry, London, United Kingdom, [4]Division of Medicine, McMaster University, Ontario, Canada, [5]Henri Mondor University Hospital, Créteil, France, [6]Department of Cellular Biotechnologies and Haematology, "Sapienza" University of Rome, Rome, Italy, [7]Platelet Disorder Support Association, Cleveland, United States, [8]ITP Support Association, Bedfordshire, United Kingdom, [9]Department of Hematology, Shandong University, Jinan, China, [10]Osaka University Hospital, Osaka, Japan, [11]O'Ctyo, Saint Loubes, France, [12]Italian Association Immune Thrombocytopenic Purpura, Caprino Veronese, Italy, [13]Bespoke Team, Adelphi Real World, Macclesfield, United Kingdom, [14]Novartis, Basel, Switzerland, [15]Division of Hematology/Oncology, Weill Cornell Medical College, New York, United States

Background: ITP is defined by isolated thrombocytopenia, with the diagnosis relying on exclusion of other causes of thrombocytopenia. Incidence rates range from 10-70 cases per 1,000,000 per year. Previous studies have highlighted fatigue as an important morbidity; however limited data are available on the overall impact of ITP on the patient's quality of life (QoL).
Aims: The I-WISh survey aims to understand the impact of ITP on patient (pt) QoL using a global patient and physician sampling frame. Here, we present the interim data from the pt survey.
Methods: I-WISh is a cross-sectional survey of ITP pts and physicians in 14 countries. Pts were recruited via physicians and pt support groups. Pts completed a 30-minute online survey providing information on demographics, diagnosis experience, symptoms, impact on daily living and emotional well-being, QoL and their treatment and management. Survey materials were designed and endorsed by a steering committee, including expert clinicians and pt advocacy leads specialising in ITP.
Results: 260 pts, 70% female, with a mean age (SD) of 55.3 (15.071) years completed the survey. Mean length of time since ITP diagnosis (SD, range) was 12.0 (11.3, 0.1-60) years. Pts reported their current health state using a 7-point Likert scale (7=excellent health), 67% of patients reported a score ≥5 and 11% a score ≤3. Mean time (SD) from initial presentation to a health care professional and ITP diagnosis was approximately 15.1 (77.5) weeks. Of all pts, 15% felt they experienced a delay in their ITP diagnosis and 65% wanted more support during their diagnosis. Initial presentation was made to a General Practitioner by 69% of pts, whereas diagnosis was by a haematologist for 81% of pts. The most frequently reported symptoms at diagnosis were bruising (of the skin/mucous membranes) (73%), fatigue (68%) and petechiae (65%); at the time of survey completion, they were fatigue (59%), bruising (31%) and petechiae (27%). Bruising, fatigue and petechiae were reported as the most severe symptoms when scored ≥5 on a 7-point Likert scale (7=worst imaginable) (57%, 71% and 48% at diagnosis; 22%, 64% and 30% at time of survey completion; respectively). ITP significantly impacted QoL and daily living; 44% of patients reported that ITP impacted their energy levels more than half the time; 28% said ITP had a negative impact on their normal capacity to exercise more than half the time; 21% reported that ITP impacted their undertaking of daily tasks more than half the time. 36% of pts stated ITP had a high impact on their emotional well-being (a score of ≥5 on a 7-point Likert scale, where 7 is a great deal). Additionally, pts reported a high impact of ITP on their work, with

28% of pts having reduced their hours at work due to ITP. Patients said "healthy blood counts" (79%) was their main treatment goal, followed by "increasing my energy levels" (55%) and "preventing episodes of worsening of my ITP" (46%).
Summary and Conclusions: This interim data analysis demonstrates the multifaceted burden of ITP on pts' lives. There was high symptom burden, and a negative impact on emotional well-being and pt's ability to work. Fatigue was the most severe symptom and pts indicated a desire to increase their energy levels, suggesting patients are primarily concerned with the impact of ITP on their QoL. These results highlight a need to further explore and define overall disease burden of ITP and to consider how these findings should affect the management of ITP in these patients.

PF655

FINAL SAFETY AND EFFICACY DATA OF LONG-TERM OPEN-LABEL DOSING OF SUBCUTANEOUS (SC) ROMIPLOSTIM IN CHILDREN WITH IMMUNE THROMBOCYTOPENIA (ITP)

M. Tarantino[12,*], J. Bussel[3], V. Blanchette[4], D. Beam[5], J. Roy[6,7], J. Despotovic[8], A. Raj[9], N. Carpenter[10], B. Mehta[11], M. Eisen[11]

[1]University of Illinois College of Medicine-Peoria, [2]The Bleeding and Clotting Disorders Institute, Peoria, [3]Weill Cornell Medicine, New York, United States, [4]Hospital for Sick Children, Toronto, Canada, [5]Cook Children's Medical Center, Fort Worth, United States, [6]The University of Queensland, Saint Lucia, [7]Children's Health Queensland & Pathology Queensland, South Brisbane, Australia, [8]Texas Children's Hematology Center, Houston, [9]Pediatric Cancer and Blood Disorders Clinic, Louisville, United States, [10]Amgen Ltd., Uxbridge, United Kingdom, [11]Amgen Inc., Thousand Oaks, United States

Background: Children with ITP for ≥6 months who completed a romiplostim phase 1/2 or 3 study could enter an open-label extension.
Aims: Final extension data for these children with ITP treated with romiplostim are described here.
Methods: All patients received weekly SC romiplostim, adjusted weekly by 1 µg/kg/week from 1–10 µg/kg to target platelet counts of 50–200×10^9/L. Incidence of adverse events (AEs) was the primary endpoint.
Results: Median (min–max) treatment for the 65 patients was 135 (5–363) weeks for a total of 182 patient-years, or 2.8 years / patient. Baseline median (min–max) age was 11 (3–18) years; 56% were female; 9.1% had prior splenectomy. Median (min–max) average weekly dose was 4.8 (0.1–10.0) µg/kg, including dose escalation; 20 patients started on 1 g/kg. All 65 patients received their doses per protocol >90% of the time; 21 missed ≥1 dose for noncompliance for a total of 65 times. Reasons for discontinuing romiplostim (n=28, 42%) included consent withdrawn (n=10), other therapy (n=6), and AE (n=2) (asthenia, headache, dehydration, and vomiting in one patient and anxiety in the other; per investigators, none were treatment related). Fifty-four serious AEs occurred in 19 patients but were treatment related in only one patient (concurrent grade 4 thrombocytopenia, grade 3 epistaxis, and grade 2 anemia). Bleeding AEs occurred in 57 patients; 3 were deemed treatment related (injection site hemorrhage, injection site bruising, and epistaxis). No thrombotic events were reported. Bone marrow biopsies were performed for 2 patients with additional cytopenias; both had iron-deficiency anemia. Upon leaving the study to receive other therapy, one patient had anti-romiplostim neutralizing antibody (Ab) which was absent on retesting 3 and 6 months later. No patients had anti-TPO neutralizing Ab. Median platelet counts were >50×10^9/L from week 2 on and >100×10^9/L from weeks 24-260 (Figure 1).Nearly all (94%, 61/65) patients had ≥1 platelet response (platelet counts ≥50×10^9/L, excluding counts ≤4 weeks after rescue medication). Most (72%, 47/65) patients had a platelet response ≥75% of the time and 58% (38/65) did ≥90% of the time. Sixty (92%) patients (or caregivers) self-administered romiplostim. Twenty-three (35%) patients received rescue medications; usage was highest in the first few months. Fifteen (23%) patients had treatment-free periods of platelet counts ≥50×10^9/L for ≥24 weeks (here also called remission; Table 1, Figure 1); these patients (9 girls, 6 boys) had had ITP for a median (min–max) of 3.5 (1.3–13) years, none had prior splenectomy, and had received romiplostim for 2.1 (0.7–6) years. All 15 of these patients had platelet counts over 100×10^9/L for ≥3 months and 12/15 for ≥6 months. The median (min–max) duration of being ≥100×10^9/L for these 15 patients was 42 (13–109) weeks. Of baseline characteristics such as sex, platelet counts, ITP duration, and number of past ITP treatments (1, 2, 3, >3), only age <6 years was predictive of developing treatment-free periods ≥24 weeks (p=0.0035).
Summary and Conclusions: Seven years of data from this open-label extension study of romiplostim in children with ITP show that >90% of children achieved a platelet response with romiplostim. Romiplostim was mostly well tolerated. Importantly, 23% of these patients with longstanding ITP (median 3.5 years) were able to discontinue all ITP medications (including romiplostim) for at least 6 months.

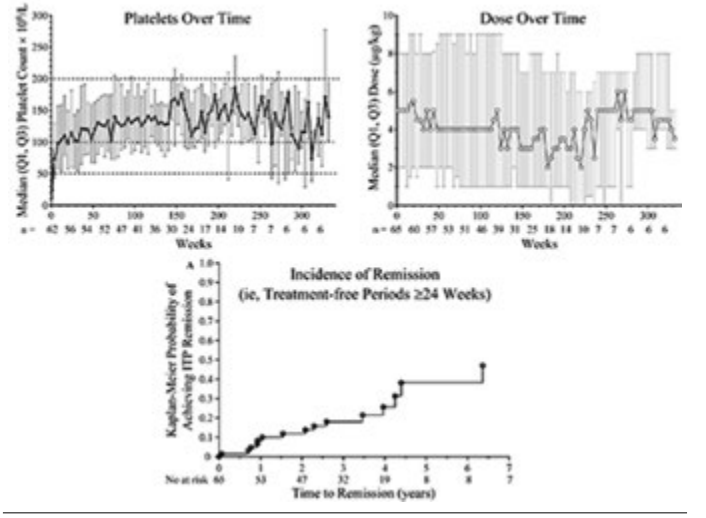

Figure 1.

Table 1.

Treatment-free periods of ≥24 weeks with platelet counts ≥50×10^9/L (ie, remission)

Patient number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age at study end, years	17	8	12	9	6	13	19	8	8	9	5	5	7	15	15
Race	W	B	W	W	W	W	W	W	B	A	W	B	H	W	W
ITP Rx, #, prior to studies	3	6	4	2	2	3	4	5	2	1	2	1	4	4	3
Baseline platelet count (×10^9/L)*	10	7	5	19	7	82	17	22	26	29	30	1	11	10	7
Romiplostim, years[1]	6	4.4	4.2	0.7	2.1	3.9	3.5	1.6	0.8	0.9	0.7	0.9	2.3	2.3	1.1
Maximum dose, µg/kg	10	8	9	5	10	2	2	1	3	1	2	1	9	4	10
ITP, years[2]	7.1	5.5	5.1	1.3	4.3	11	13	2.6	1.4	2.3	2.9	3.5	3.5	5.1	2.6
ITP remission, years	1.1	2.1	1.1	1.6	1.0	0.8	0.9	1.7	2.1	1.1	0.6*	0.6	0.8	0.6	0.4†

A, Asian; B, Black; H, Hispanic/Latino; Rx, therapy; W, white. [1]At start of parent study (not extension). [2]At remission start.
*Remission ended before study end. †This patient met remission criteria for 0.4 years on study and ≥0.5 years post study.

PF656

PREEMPTIVE RITUXIMAB HAS A FAVORABLE BENEFIT-RISK BALANCE IN THE PREVENTION OF LONG-TERM RELAPSES IN IMMUNE-MEDIATED THROMBOTIC THROMBOCYTOPENIC PURPURA

M. Jestin[1,*], Y. Benhamou[2], F. Provôt[3], L. Galicier[4], M. Hié[5], P. Poullin[6], S. Saheb[7], C. Deligny[8], S. Girault[9], T. Kanouni[10], A. Charvet Rumpler[11], M. Hamidou[12], A. Veyradier[13], P. Coppo[14]

[1]Hematology, Hôpital Saint-Antoine, PARIS, [2]Internal medicine, CHU, ROUEN, [3]Nephrology, CHRU, Lille, [4]Clinical immunology, Saint Louis, [5]Internal medicine, CHU La Pitié Salpétrière, Paris, [6]Hemapheresis, CHU La Timone, Marseille, [7]Hemapheresis, CHU La Pitié Salpétrière, Paris, [8]Internal medicine, CHU, Fort-de-France, [9]Hematology, CHU Dupuytren, Limoges, [10]Hematology, CHU, Montpellier, [11]Hematology, CHRU Jean Minjoz, Besançon, [12]Internal medicine, CHU, Nantes, [13]Biological hematology, Hôpital Lariboisière, [14]Hematology, Saint Antoine, Paris, France

Background: Immune thrombotic thrombocytopenic purpura (iTTP) is a fulminant and spontaneously fatal disease. Up to 50% of patients finally experience one or several relapses. Persistently undetectable ADAMTS13 activity in patients otherwise in remission represents a reliable early predictor of full clinical relapse (CR). Preemptive rituximab infusions prevent CR in iTTP by maintaining ADAMTS13 activity within normal values. However, the long-term outcome of these patients and the potential adverse events of this strategy need further investigations.
Aims: To evaluate the feasibility, efficacy and safety of preemptive rituximab in patients with iTTP in clinical remission but with a persistently undetectable ADAMTS13 activity.
Methods: We report the long-term outcome of 92 patients with iTTP in remission treated preemptively with rituximab for a biological relapse (BR) *i.e.* a severe ADAMTS13 deficiency (activity <10%) identified during follow-up, without CR. ADAMTS13 activity was systematically measured every 3 months. We compared in these patients the median number of iTTP episodes and the median cumulative incidence of annual relapse that occurred before and after preemptive rituximab. We also compared patients treated with preemptive rituximab to a distinct group of patients with a persistent severe ADAMTS13 deficiency but managed before the era of rituximab.

Results: Before rituximab, thirty-seven patients had experienced >1 episode of iTTP (median 3, IQR 2-3), and the median cumulated incidence of relapse was 0.33 episode/year (IQR, 0.23-0.66). Following preemptive rituximab, the median cumulated incidence of relapse in the whole population dramatically decreased (0 episode/year [IQR, 0-1.32], p<0.001) after a 35.8-month follow-up (IQR, 23.3-68). ADAMTS13 activity recovery was sustained in 34 patients (37%) following a single course of preemptive rituximab (comprising 1 to 4 infusions at 375mg/m^2/infusion), after a 31.5-month follow-up (IQR, 18-65). Forty-five patients (49%) had at least one new BR after initial improvement, which usually improved with further courses of rituximab. In these patients, the median time between 2 courses was 17.5 months (IQR, 12.6-25). Thirteen patients (14%) had a persistently undetectable ADAMTS13 activity after a first course of rituximab. Among them, 10 patients received repeated courses of rituximab and retreatment allowed ADAMTS13 improvement in 6 cases. Finally, only 7 patients/92 (7.6%) were refractory to preemptive rituximab. In total, 14 patients/92 (15%) experienced a CR after a median follow-up of 29 months (IQR, 20-57). Nineteen patients (20.7%) experienced benign adverse effects attributed to rituximab. From a historical group of 23 iTTP with a persistently undetectable ADAMTS13 activity and managed before the era of rituximab, 74% clinically relapsed after a 7-year follow-up (IQR, 5-11). These patients experienced significantly more relapses than patients treated with rituximab (p <0.001) (Figure 1).

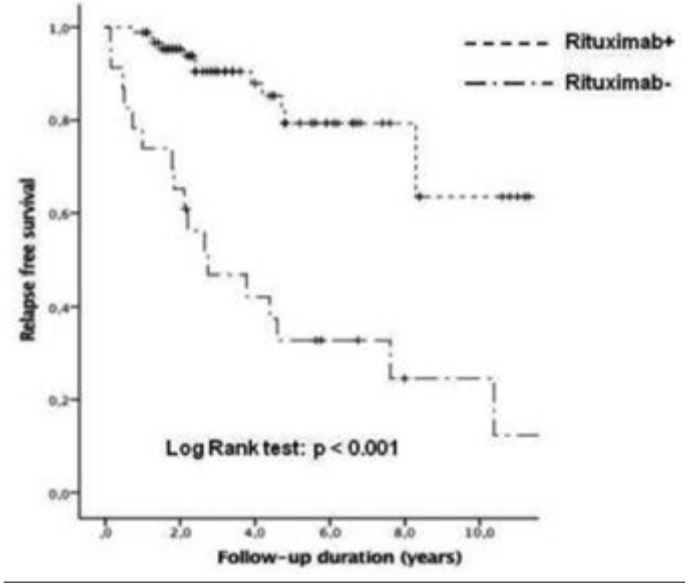

Figure 1.

Summary and Conclusions: In conclusion, a persistently undetectable ADAMTS13 activity in iTTP otherwise in remission is associated with a high relapse rate; in these patients, rituximab prevents CR by maintaining a detectable ADAMTS13 activity with an advantageous risk-benefit balance. However, half of patients require repeated courses to maintain a normal ADAMTS13 activity.

PF657

AVATROMBOPAG DEMONSTRATES SUPERIORITY TO PLACEBO FOR THE TREATMENT OF CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA IN A PHASE 3, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

W. Jurczak[1,*], K. Chojnowski[2], J. Mayer[3], B. Jamieson[4], W. Tian[4], L.F. Allen[4]

[1]Jagiellonian University, Kraków, [2]Medical University of Łódź, Lodz, Poland, [3]University Hospital Brno, Brno, Czech Republic, [4]Dova Pharmaceuticals, Durham, United States

Background: Avatrombopag (AVA) is an oral, 2nd generation thrombopoietin receptor agonist (TPO-RA) currently in development for the treatment of thrombocytopenia. Given the need for chronic administration, it is important to minimize treatment-related toxicities in patients with ITP. The once daily oral dosing of AVA, which can be taken with food, has no signal for hepatotoxicity, and a reliable pharmacokinetic-pharmacodynamic response profile due to limited metabolism of the parent molecule may provide advantages for its use in patients with ITP.

Aims: This trial investigated the efficacy and safety of AVA *versus* placebo (PBO) for the treatment of patients withchronic ITP in a multicenter, multinational, randomized, parallel group, PBO-controlled Phase 3 trial(NCT01438840).

Methods: Adult patients (age ≥18 years) with chronic ITP (Baseline platelet count <30 X 109/L) were randomized 2:1 to 20 mg/day AVA or PBO over the 6-month study period. The primary efficacy endpoint was the number of cumulative weeks with a platelet response defined as a plateletcount ≥50 x 109/L, in the absence of rescue therapy. Secondary endpoints included platelet response at Day8, and the proportion of patients with a reduction in concomitant ITP medications from Baseline.

Results: In total, 49 patients were included in the full analysis. The AVA treatment group included 32 patients; 22 (68.8%)completed the study. In the PBO group, which included 17 patients, only one (5.9%) completed the study. AVA was superior to PBO in the mean cumulative number of weeks with a platelet count ≥50 x 109/L duringthe 6-month treatment period (12.4 weeks *vs* 0 weeks; P<0.0001). Day 8 platelet responses were higher for patients who received AVA, 65.6% compared with 0% for PBO (P<0.0001). A durable platelet response was observed in more AVA-treated patients (34.3%) compared with PBO-treated patients (0%; P=0.009). Treatment emergent AEs (TEAEs) were reported by 31 patients (96.9%) in the AVA-treated group compared with 10 patients (58.5%) in the PBO-treated group; the incidence of TEAEs, Grade 3/4 TEAEs, and serious AEs were similar in the two treatment groups when adjusted for treatment exposure. The most commonly reported TEAEs included headache, contusion, upper respiratory tract infection, arthralgia, and epistaxis.

Summary and Conclusions: AVA is novel 2nd generation TPO-RA that has been shown to be well tolerated and superior to PBO in patients with chronic ITP may potentially provide an additional treatment option for patients with refractory chronic ITP.

PF658

A PHASE 1B, OPEN-LABEL, DOSE-ESCALATION STUDY OF PRTX-100, A HIGHLY PURIFIED FORM OF STAPHYLOCOCCAL PROTEIN A (SPA), IN ADULT PATIENTS WITH PERSISTENT/CHRONIC IMMUNE THROMBOCYTOPENIA (ITP)

N. Cooper[1,*], A. Newland[2], R. Kazmi[3], M. Scully[4], S. Audia[5], J.-F. Viallard[6], M. Hamidou[7], J.B. McClain[8], R. Francovitch[8], M. Marc[9]

[1]Haematology, Imperial College, Hammersmith Hospital, [2]Haematology, Queen Mary, University of London, London, [3]Haematology, University Hospitals Southampton NHS Foundation Trust, Southampton, [4]Haematology, University College London, London, United Kingdom, [5]Internal Medicine and Immunology, CHU Dijon, Dijon, [6]Internal Medicine, CHU Bordeaux, Bordeaux, [7]Internal Medicine, Hôtel Dieu University Hospital, Nantes, France, [8]Protalex, Inc., Florham Park, United States, [9]Internal Medicine, Henri Mondor University Hospital, AP-HP, Université Paris-Est Créteil, Créteil, France

Background: ITP is a rare autoimmune bleeding disorder characterized by isolated thrombocytopenia caused by antibody-dependent platelet destruction and impaired platelet production. Various therapies (eg glucocorticoids, IV immunoglobulin, and thrombopoietin receptor agonists) are available but are limited by inadequate efficacy, side effects and/or cost. PRTX-100 is a highly purified form of SpA that binds to human B-lymphocytes and monocytes and modulates immune processes. Preclinical data indicate that PRTX-100 may have the potential to treat ITP by reducing immune-mediated platelet destruction (Kapur *et al*., Br J Haematol 2017).

Aims: We present safety and efficacy data from the first four dosing cohorts of patients with refractory ITP enrolled in a phase 1b open-label study (PRTX-100-203).

Methods: Adults with persistent or chronic ITP who had received at least one prior ITP treatment and had either a platelet count <30,000/μL (if not receiving any ITP therapy) or <50,000/μL (if receiving a constant dose of permitted ITP treatment) were eligible. PRTX-100 was administered via a 30-min infusion (60 min if total dose >500 μg) on Days 1, 8, 15 and 22 in a standard 3+3 dose-escalation study design. Starting dose was 3 μg/kg with subsequent dose increases to 6, 12, 18 and 24 μg/kg. Primary objective: to characterize safety of up to five dose levels of PRTX-100. Safety analyses: adverse events (AEs), serious AEs, infusion reactions, clinical laboratory tests, vital signs, physical findings and electrocardiograms. Efficacy endpoints include platelet response (increased platelet count ≥30,000/μL and at least doubling of baseline count in patients with a baseline count <30,000/μL; or, in patients with a baseline count ≥30,000/μL and <50,000/μL, an increase in count to ≥50,000/μL and at least a doubling of baseline count or an increase to >100,000/μL). Secondary objectives include immunogenicity and pharmacokinetics.

Results: Data are available from 13 patients enrolled in the first four dosing cohorts: 3 µg/kg (n=3), 6 µg/kg (n=4), 12 µg/kg (n=3), and 18 µg/kg (n=3). There were 6 women and 7 men (10 Caucasian, 1 Asian, 2 other) with an age range of 21 to 81 years and most had a splenectomy. Two patients in the 6 µg/kg cohort discontinued the study (1 due to a serious unrelated grade 4 worsening of ITP after receiving 2 doses of PRTX-100; 1 due to non-compliance with study visits after receiving 3 doses of PRTX-100). All 11 remaining patients received 4 doses of PRTX-100. Two serious or higher-grade AEs were seen in this group: unrelated grade 4 mouth bleeding (n=1), unrelated grade 3 axonal neuropathy (n=1). Two grade 1 infusion reactions occurred: itching rash at the infusion site (n=1); pruritus (n=1). Laboratory events: grade 3/4 hyperglycaemia, grade 3 lymphocytopenia, and grade 4 abnormal urine glucose (n=1 patent); grade 3 hypophosphatemia and grade 4 abnormal urine glucose (n=1); grade 3 cholesterol increase (n=1). Five patients had increased platelet counts as early as Day 3. Two patients had a protocol-defined platelet response at the 3 µg/kg and 18 µg/kg doses (see Figure 1). A further 5 patients had an increase in their counts, although not to the level of response.

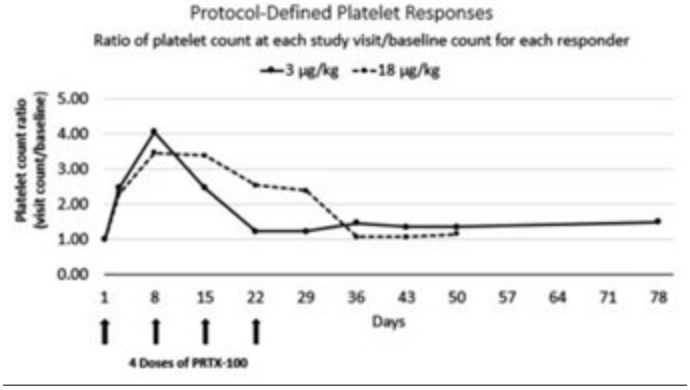

Figure 1.

Summary and Conclusions: Data from the first four cohorts of patients treated with PRTX-100 demonstrate an acceptable safety profile. Platelet counts were elevated in several patients and two patients so far have achieved a platelet response. Enrolment into the last dosing cohort (24 µg/kg) is ongoing and updated data will be included in any presentation.

PF659

RITUXIMAB IN ADULT PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA: STANDARD DOSE *VERSUS* LOW DOSE

S. Datta[1,*], S. Kalantri[1], S. Saha[1], N. Sinha[1], S.K. Samanta[1], S.S. Ray[1], M. Bhattacharyya[1]

[1]Clinical Haematology, Institute of Haematology and Transfusion Medicine; Medical College Hospital, Kolkata, India, Kolkata, India

Background: Rituximab 375 mg/m^2 weekly for 4 weeks has significant activity in adults with primary immune thrombocytopenia (ITP). Several evidences support that lower dose of rituximab (100 mgweekly for 4 weeks) is also effective in this setting. A randomized trial comparing standard dose and low dose rituximab in ITP is sparse in the literature.
Aims: To compare the efficacy of standard dose rituximab with that of low dose rituximab in previously treated adult patients with primary immune thrombocytopenia.
Methods: Fifty previously treated adult patients with primary immune thrombocytopenia were randomized to receive standard dose (375 mg/m^2 weekly for 4 weeks) or low dose (100 mg flat dose weekly for 4 weeks) rituximab in this single-center open-labeled trial conducted between August 2015 and August 2017. Platelet counts were measured weekly for the first month and then monthly. Patients with steroid dependency or active bleeding were allowed to continue steroids at the minimal effective dosage sufficient to maintain a safe number of platelets. However, only patients in whom steroid discontinuation was possible during or soon after rituximab therapy were considered responders.
Results: Each treatment arm had twenty-five patients. At one month, in standard dose (SD) arm Overall response (OR) and complete response (CR) rates were 60% (15 patients) and 36% (9 patients), respectively. In low dose (LD) arm OR and CR rates were 56% (14 patients) and 28% (7 patients) respectively. The difference of initial response between two treatment arm was not statistically significant (p=0.829). At six months standard-dose rituximab arm showed CR and OR rates of 28% (7 patients) and 44% (11 patients). Low-dose rituximab arm had CR and OR rates of 20% (5 patients) and 40% (10 patients). There was no statistically significant difference in response at six months between the two treatment groups (p=0.921). At one year, six patients (24%) of SD rituximab arm were in CR and rest of responders relapsed. In LD rituximab arm, at one year, CR and OR rates were 8% (2 patients) and 12% (3 patients), respectively (p=0.339). Relapse rates among responders at six months were 26.7% (4/15) and 28.6% (4/14) in SD and LD rituximab arm, respectively. At one year, relapse rates were 53.3% (8/15) in SD rituximab arm and 57.1% (8/14) in LD rituximab arm (p=0.837). Relapse rates after achieving CR were 22% in SD rituximab arm and 28% in LD rituximab arm. The relapse rates were very high in patients who achieved R (Response =platelet count ≥30 ×10^9 /L to <100 ×10^9 /L), 100% in SD arm and 85% in LD arm. The median duration of follow-up in non-relapse patients was 55 weeks (Range: 35-86 weeks) in SD arm and 47 weeks (Range: 34-98 weeks) in LD arm (Table 1).

Table 1.

Response after rituximab therapy

		Standard dose rituximab	Low dose rituximab	*P*
Patients (n)		25	25	-
One month	OR (%)	15 (60)	14 (56)	0.829
	CR (%)	9 (36)	7 (28)	
	R (%)	6(24)	7 (28)	
Six months	OR (%)	11 (44)	10 (40)	0.921
	CR (%)	7 (28)	5 (25)	
	R (%)	4(16)	5 (20)	
One year	OR (%)	6 (24)	3 (12)	0.339
	CR (%)	6 (24)	2 (8)	
	R (%)	0(0)	1 (4)	
Median time to response, in weeks (range)		2 (1-8)	3 (1-14)	0.246
Median time to CR, in weeks (range)		4 (2-24)	4 (2-20)	0.787
Relapse Rates in responders (%)		8 (53.3)	8 (57.1)	0.837
Relapse after CR (%)		2 (22.2)	2 (28.6)	0.979
Relapse after R (%)		6 (100)	6 (85.7)	
Median follow up in non relapse population, in weeks (range)		55 (35-86)	47 (34-98)	0.136

Summary and Conclusions: Low-dose rituximab has similar response rate and relapse rate compared to standard dose rituximab in previously treated adult patients with primary immune thrombocytopenia.

PF660

SAFETY PROFILE OF CAPLACIZUMAB DURING THE PHASE III HERCULES STUDY: FREQUENCY OF ADVERSE EVENTS ACCORDING TO STUDY PERIOD

P. Coppo[1,*], S. Cataland[2], F. Peyvandi[3], M. Scully[4], P. Knöbl[5], J.A. Kremer Hovinga[6], A. Metjian[7], J. de la Rubia[8], K. Pavenski[9], F. Callewaert[10], D. Biswas[10], H. De Winter[10], R. K. Zeldin[10]

[1]Department of Hematology, Saint-Antoine University Hospital, Paris, France, [2]Division of Hematology, Department of Internal Medicine, The Ohio State University, Columbus, United States, [3]Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Milan, Italy, [4]Department of Haematology, University College London Hospitals NHS Trust, London, United Kingdom, [5]Department of Medicine 1, Division of Hematology and Hemostasis, Vienna University Hospital, Vienna, Austria, [6]University Clinic of Hematology and Central Hematology Laboratory, Bern University Hospital, Inselspital, Bern, Switzerland, [7]Division of Hematology, Duke University School of Medicine, Durham, United States, [8]Hematology Department, Universidad Católica de Valencia Hospital Doctor Peset, Valencia, Spain, [9]Department of Laboratory Medicine and Pathobiology, St. Michael's Hospital/Research Institute, Toronto, Canada, [10]Clinical Development, Ablynx NV, Zwijnaarde, Belgium

Background: Acquired (immune-mediated) thrombotic thrombocytopenic purpura (aTTP) is a life-threatening thrombotic microangiopathy, characterized by thrombocytopenia, hemolytic anemia, and organ ischemia. Results of the Phase 3 HERCULES study in aTTP patients demonstrated that caplacizumab reduces the time to platelet count response, the incidence of patients experiencing either aTTP-related death, recurrence of aTTP, or a major thromboembolic event during study drug treatment, recurrence of aTTP during the overall study, refractoriness to therapy, and healthcare resource utilization (Scully *et al.*, Blood 2017 130:LBA-1). Mucocutaneous bleeding was the most frequently reported adverse event (AE).
Aims: To analyze the frequency of treatment-emergent AEs (TEAE) according to timing of occurrence during the Phase III HERCULES study.

Methods: All reported TEAEs were classified according to the Medical Dictionary for Regulatory Activities Version 20.0. TEAEs were analyzed according to the following study periods: double-blind (DB) daily plasma exchange (PE) treatment period; DB post-daily PE treatment period; and the treatment-free follow-up (FU) period. Analysis was performed on the study's safety population (*i.e.*, all patients who received at least 1 administration of study drug).
Results: The safety population consisted of 71 caplacizumab-treated and 73 placebo-treated patients. The median (min; max) duration of DB study drug treatment was 35 (1; 65) days for the caplacizumab group and 23 (2; 66) days for the placebo group (*i.e.*, shorter in the placebo group due to the switch to open-label caplacizumab treatment for those with a recurrence, which may have impacted the incidence of TEAEs in the two groups). During the overall study period, at least one TEAE was reported in 69 patients (97.2%) in the caplacizumab group and 71 patients (97.3%) in the placebo group. Of these, TEAEs were reported in 57/71 (80.3%) *vs* 56/73 (76.7%) patients during the DB daily PE period, and in 52/65 (80.0%) *vs* 57/64 (89.1%) patients during the DB post-daily PE period, in the caplacizumab *vs* placebo group respectively. In the caplacizumab group, the most common reported TEAEs during the DB daily PE period were: epistaxis [9 patients (12.7%)], gingival bleeding [8 patients (11.3%)], and urticaria [11 patients (15.5%)], while during the post-daily PE period epistaxis [15 patients (23.1%)], headache [11 patients (16.9%)], and gingival bleeding [5 patients (7.7%)] were the most frequently reported TEAEs. In the placebo group, the most common TEAEs during the DB daily PE period were hypokalemia [11 patients (15.1%)], and insomnia, anxiety, chest pain, bruising, urticaria and pruritus (each in 5 patients (6.8%)], while during the DB post-daily PE period, TTP [28 patients(43.8%)], contusion [7 patients (10.9%)] and headache [5 patients (7.8%)] were the most frequently reported TEAEs. In the 28-day FU period, TEAEs were reported in 36/66 (54.5%) patients in the caplacizumab and 18/39 (46.2%) patients in the placebo group. The most commonly reported TEAEs in the caplacizumab group during the FU period were headache [5 patients (7.6%)] and TTP [6 patients (9.1%)]. During the FU period, all other TEAEs were reported in <5% of patients.
Summary and Conclusions: The safety profile of caplacizumab was generally favorable. In line with its pharmacology, treatment with caplacizumab was associated with an increased risk of mucocutaneous bleeding. These events were reported with a similar frequency during the daily PE period (*i.e.*, when platelet counts are low) and the post-daily PE period (*i.e.*, when platelet counts are normalized).

PF661

LOW-DOSE RITUXIMAB PLUS PREDNISOLONE YIELDS HIGHER SUSTAINED RESPONSE RATES THAN PREDNISOLONE MONOTHERAPY AS FRONTLINE THERAPY IN ADULT PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA

S. Datta[1,*], S. Kalantri[1], S. Saha[1], N. Sinha[1], S.K. Samanta[1], S.S. Ray[1], M. Bhattacharyya[1]

[1]Clinical Haematology, Institute of Haematology and Transfusion Medicine; Medical College Hospital, Kolkata, India, Kolkata, India

Background: Prednisolone is most widely used first-line therapy for treatment of newly diagnosed ITP. But most of the adults with ITP relapse after initial response to steroid. To intensify frontline therapy, Rituximab (375 mg/m^2, weekly for four weeks) in combination with high dose dexamethasone (HDD), was used in newly diagnosed ITP and showed higher long-term remission rates compared with dexamethasone alone. Subsequently, non-randomized trials used low-dose rituximab (100 mg, weekly for four weeks) in combination with HDD in treatment naïve adult patients with ITP. To date, there have been no randomized trials comparing low-dose rituximab plus prednisolone with prednisolone alone as frontline therapy in adult patients with ITP.
Aims: In this randomized trial we compared efficacy and safety of Low-dose rituximab plus prednisolone with prednisolone alone in newly diagnosed adult patients with ITP.
Methods: In this study, we enrolled newly diagnosed treatment naïve adult (≥18 years) ITP patients with symptomatic bleeding manifestations between February 2016 to October 2017. Total 60 patients were evaluated for eligibility and at randomization 8 patient were excluded from the study (pregnant=1, HIV positive=1, HBs Ag positive=1, ANA positive=1, three patient received IVIg, one received high dose methyl prednisolone). Fifty-two patients were randomly assigned to two treatment arms. Twenty-six patients received prednisolone 1 mg/kg for 4 weeks followed by tapering of prednisolone over another 4 weeks. Other twenty-six patients received additional rituximab 100 mg weekly, four doses.
Results: The initial responses were measured at one month. In Rituximab-Prednisolone arm 18 (69.2%) patients achieved CR and 3 (11.5%) achieved R, mounting an Overall response (OR) to 80.7%. In prednisolone arm, OR, CR, and R were achieved by 18 (69.2%), 13(50%), and 5(19.2%) patients, respectively. There was no statistically significant difference in initial response between two cohorts (p=0.368). At six months, in Rituximab-Prednisolone arm CR, R and OR were 65.4%, 11.5%, and 76.9%, respectively. Prednisolone arm showed CR, R, and OR of 26.9%, 11.5%, and 38.4%, respectively (p=0.013). One year response data was available for 19 patients of RP arm and for all the 26 patients of Prednisolone arm. At one year, Rituximab-Prednisolone arm and Prednisolone arm showed CR rates of 52.6% (10/19) and 15.4% (4/26), respectively (p=0.008). At three months, five patients (27.8%) of Prednisolone arm relapsed, whereas no patient of Rituximab-Prednisolone arm relapsed at 3 months (p=0.01). At six months, only one patient (4.8%) of Rituximab-Prednisolone arm relapsed, whereas 8 patients (44.4%) of Prednisolone arm relapsed at 6 months (p=0.003). One year relapse data were available for 14 patients of the Rituximab-Prednisolone arm and for all the 18 patients of Prednisolone arm. At one year, 4 patients (28.6%) of the Rituximab-Prednisolone arm and 14 patients (77.8%) of Prednisolone arm relapsed (p=0.002). Eleven patients (42.3%) of the Rituximab-Prednisolone arm and 10 (38.5%) patients of Prednisolone arm experienced adverse effects. All the adverse effects were grade 1 or grade 2 (Table 1).

Table 1.

Response rates at different point-of-time

		Rituximab-Prednisolone	Prednisolone	p
One month	CR (%)	18 (69.2)	13 (50)	0.368
	R (%)	3 (11.5)	5 (19.2)	
	OR (%)	21 (80.7)	18 (69.2)	
Three months	CR (%)	18 (69.2)	8 (30.8)	0.019
	R (%)	3 (11.5)	5 (19.2)	
	OR (%)	21 (80.7)	13 (50)	
	Relapse (%)	0/21 (0)	5/18 (27.8)	0.01
Six months	CR (%)	17 (65.4)	7 (26.9)	0.013
	R (%)	3 (11.5)	3 (11.5)	
	OR (%)	20 (76.9)	10 (38.4)	
	Relapse (%)	1/21 (4.8)	8/18 (44.4)	0.003
Nine months	CR (%)	15/24 (62.5)	5/26 (19.2)	0.005
	R (%)	2/24 (8.3)	2/26 (7.7)	
	OR (%)	17/24 (70.8)	7/26 (26.9)	
	Relapse (%)	2/19 (10.5)	11/18 (61.1)	0.001
One year	CR (%)	10/19 (52.6)	4/26 (15.4)	0.008
	R (%)	0/19 (0.0)	0/26 (0.0)	
	OR (%)	10/19 (52.6)	4/26 (15.4)	
	Relapse (%)	4/14 (28.6)	14/18 (77.8)	0.002

Summary and Conclusions: The combination of low-dose Rituximab plus Prednisolone as frontline therapy for adult patients with newly diagnosed primary immune thrombocytopenia is well tolerated and induces a higher long-term response and lower incidence of relapse than Prednisolone alone.

PF662

EXTENDED FOLLOW UP OF PATIENTS TREATED IN THE RITUXIMAB AS SECOND-LINE TREATMENT FOR ADULT IMMUNE THROMBOCYTOPENIA – THE RITP STUDY

E. Tjønnfjord[1], P.A. Holme[2], B. Darne[3], A. Khelif[4], A. Waage[5], W. Ghanima[6,*]

[1]Hematology, [2]Hematolgy, Rikshospitalet, OUS, Oslo, Norway, [3]Monitoring Force, Paris, France, [4]Hematology, CHU Farhat Hached, Sousse, Tunisia, [5]Hematology, St. Olavs Hospital, Trondheim, [6]Hematology, Sykehus Østfold, Kalnes, Sarpsborg, Norway

Background: Immune thrombocytopenia (ITP) is characterized by immune-mediated destruction of platelets and suboptimal production. **RITP** was a randomized placebo-controlled trial in which ITP patients who failed to achieve adequate response to steroids were randomized to receive rituximab or placebo plus standards of care (Ghanima *et al.* Lancet 2015; 385:1653-61). Study endpoints were treatment failure (splenectomy or meeting criteria for splenectomy), response rates and duration of response with a follow-up of 18 months. Apart from longer duration of response in the rituximab arm, the study showed no significant differences the other outcomes.
Aims: To provide long-term rates of splenectomy, death and duration of response based on extended open follow-up after completion of the RITP study.
Methods: Platelet counts, splenectomy status, ITP medication and death (after completion of RITP) were retrospectively collected. Response was defined as platelet count of ≥30X10 /L or at least a doubling of the platelet count from baseline without administration of any platelet increasing therapy except stable or decreasing doses of prednisone or prednisolone during the past 4 weeks;

or ≥100X10 /L for complete response (CR) after week 4 from first study drug administration. Relapse was defined as platelet count <30X10^9/L or reinstitution of ITP medication if this proceeded date of available platelet count. The study was approved by the ethics committees in Norway and Tunisia.
Results: Extended follow-up data were acquired from 90 of 109 patients participating in RITP. Median duration of follow-up from randomization to last observation was 72 months (IQR 62-82). Overall (double-blind and follow-up periods), 35 patients underwent splenectomy (13 in the rituximab; 22 in the placebo arm) with a no significant trend towards longer time to splenectomy in the rituximab arm (p=0.11) (Figure 1A). Eleven patients (10%) died during the extended follow-up: 5 in the rituximab and 6 in the placebo arms. Seventy-six of 109 patients achieved response (40 in the rituximab; 36 in the placebo arms) including complete response (28 in the rituximab; 21 in the placebo arms) during the RITP study. Figure 1B shows probability of first relapse in responding patients. Median duration of response, was 8.2 (5.2-16.7) after response and 17 (8-34) months after CR, in the rituximab arm, and 1.8 (1.3-3.7) and 11 (4.5- not reached) months respectively in the placebo arm. The difference in the duration of responses was not statistically significant (p=0.09). Notably, most of the relapses occurred during first 18 months after the administration of study medication. During the extended follow-up, 2 more patients in the rituximab arm relapsed after achieving response and 4 after achieving CR.

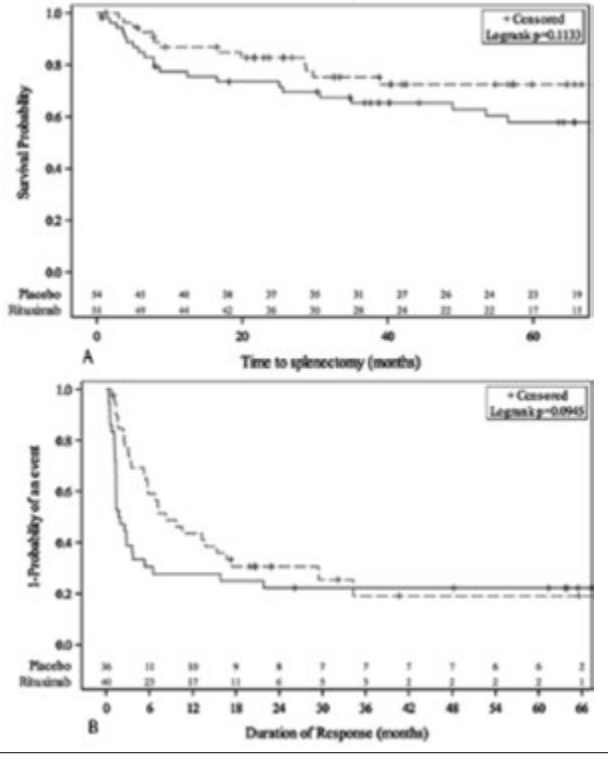

Figure 1.

Summary and Conclusions: To our knowledge, the data provided here represent the longest single study follow-up reported on the effect of rituximab in ITP. Although rituximab seemed to yield longer duration of response and CR, the effect was clearly transient and the two curves cross after 34 months. Around 20% of patients in both arms are still responders at 6 years. Interestingly a trend towards lower splenectomy rate was found in rituximab treated patients. The retrospective collection of data in the follow-up represent a limitation of the study. In the future, efforts should be made to augment the initial response to rituximab and to prolong the duration of response. Our ongoing PROLONG study will determine if the response can be prolonged by administration of maintenance therapy with rituximab.

PF663

EPIDEMIOLOGY AND MANAGEMENT OF PRIMARY IMMUNE THROMBOCYTOPENIA: REAL WORLD DATA FROM THE UK ITP REGISTRY

A. Zaidi[1,*], C. Gracie[2], U. Doobaree[2], D. Provan[2], M. Vickie[1]

[1]Haematology, Royal London Hospital, BartsHealth NHS Trust, [2]Department of Immunobiology, Queen Mary University of London, London, United Kingdom

Background: Immune thrombocytopenia (ITP) is a rare bleeding diathesis with an autoimmune background. Treatment practices vary considerably, there is a lack of epidemiological data and few large-scale randomised clinical trials to guide management.
Aims: We reviewed the UK Adult ITP registry, consisting of 89 sites across the United Kingdom who recruit cases of primary ITP, to describe real world characteristics of the population and evaluate management of ITP.
Methods: All clinical data from 2010- Jan 2018 was analysed for the diagnostic workup, bleeding symptoms, lines of treatment and clinical outcomes.
Results: 2920 patients (57% female) were entered into the registry in the time period. Median age at diagnosis was 50y (IQ range 35-66). The median platelet count at diagnosis was 19 x10^9/L (IQR 5 - 57). 66% had bleeding symptoms (18.3% - 1 episode; 14.8% - 2 episodes; 32.8% ≥3 episodes) whereas 34% (995 patient) never had any bleeding. The commonest bleeding sites were: subcutaneous/ soft tissue (58.8%), oral (13.3%), epistaxis (11.6%) and gynaecological (4.9%). Intracranial haemorrhage occurred in 0.65% (0.44% non-traumatic) and all occurred within 6 months of diagnosis.Median Hb at diagnosis was 14.6g/L (IQR 12.4-14.6) , WCC 7.2 x10^9/L (IQR 5.5-9.9), neutrophils 4.4 x10^9/L. Median PT was 11.7s (IQR 10.9- 14.5) and APTT was 28.5s (IQR 25-31.8). 1171 patient had Antinuclear antibodies (ANA) sent, of these 14.6% were positive. 270 had Direct Antiglobulin Test (DAT) testing of which 2.59% were positive. Lupus anticoagulant testing was positive in 13.8% (n=577). Insufficient data was recorded for bone marrow testing to draw conclusions. Twenty seven percent (787) never required therapy for ITP. 21.6% (631) received 1 line of therapy, 14.6% received 2 lines and and 19.9%% were treated with ≥3 lines of therapy. The most frequent treatment was steroids (65% prednisolone, 4.11% methylprednisolone and 9.25% dexamethasone) followed by IVIG (31.47%), rituximab (17.25%), MMF (11.7%), romiplostim (9.6%) and eltrombopag (5.99%). Splenectomy was performed in 9.83%.Outcomes and follow-up (6 months, 1 year, 5 years) are shown in Table 1. We compared partial response (PR, platelet count 30-100x10^9/L) and complete response (CR, platelet count >100 x10^9/L) in those who required no therapy, 1 and ≥2 lines of treatment over time.The proportion of patients in the total cohort with severe thrombocytopenia (platelet <10 x10^9/L) at 1 year was 3.1% and at 5 years, 2.7%. PR and CR rates one year from diagnosis were 44.7% and 41.9% respectively with 83.6% having a Platelet count >30 x10^9/L at 5 years. A greater percentage of patients were in PR/CR in those who were untreated *vs* those who required ≥2 lines of therapy (96% *vs* 79%). 1.37% of patients still had bleeding symptoms after 5 years.

Table 1.

Outcomes, bleeding symptoms and Platelet count at Diagnosis and up to 5 years from diagnosis for Overall cohort, Comparing untreated patients'yy those receiving1 line of therapy or 2+ lines of therapy

	Total Cohort				No Treatment				1 Line Of therapy				≥2 lines of therapy			
	Diagnosis	6 mo	1 Yr	5 Yr	Diag	6 mo	1 Yr	5 Yr	Diag	6 mo	1 Yr	5 Yr	Diag	6 mo	1 Yr	5 Yr
Median Platelet Count	19	91	82	76	68	83	79	85	16	126	106	66	11	87	78	73
% Patients with Platelets < 10 x10^9/L	37	3	3.1	2.7	11.8	0.45	0	1.8	38.5	1.2	0.6	0	48	4.5	4.9	3.4
% Patients with Platelets 11-30 x10^9/L	24	9.2	10.3	14	13.2	7.2	5.9	1.8	20.3	3.5	3.1	10	26	13.2	14	17.6
% Patients with Platelets 31-100 x10^9/L (PR)	33	43.3	44.7	44	61.3	43.9	40	41	36.8	34.1	43.6	37.5	23	40.4	40	38.2
% Patients with Platelets >100 x10^9/L (CR)	1	44.5	41.9	39	15	30.5	34	36	4.3	61.2	42.8	32.5	4.2	42.7	41	40.8
% Patients with Bleeding symptoms	29.7	4.25	3.42	1.4												
% Bleeding events requiring transfusion	11.5	1.7	0.7	0												

Summary and Conclusions: These data describe characteristics and outcome for patients with primary ITP in the UK population. Although limited by being observational and retrospective, they demonstrate that approximately 1/3 patients with primary ITP do not need treatment and currently, steroids and IVIG are the most common treatments used. Approximately 20% of patients require additional treatment beyond this including immunosuppressive therapy and thrombopoeitin agonists. Approximately 10% require splenectomy. Longer term follow up of upto 5 years from diagnosis shows more than 80% of patients will be at least PR (platelet count >30 x10^9/L) and fewer than 2% of patients will have ongoing bleeding symptoms.

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PREDICTORS OF SUCCESS FOR SPLENECTOMY IN CHILDHOOD AUTOIMMUNE CYTOPENIA

C. Dichamp[1], V. Li Thiao Te[1], H. Fernandes[2], Y. Bertrand[3], T. Leblanc[4], G. Leverger[5], V. Barlogis[6], W. Abouchahla[7], M. Pasquet[8], S. Bayart[9], N. Cheikh[10], C. Paillard[11], D. Plantaz[12], Y. Perel[2], N. Aladjidi[2,*]

[1]Pediatric Oncohematology Unit, University Hospital of Amiens, Amiens, [2]Pediatric Oncohematology Unit / CEREVANCE / CIC1401, Inserm CICP, University Hospital of Bordeaux, Bordeaux, [3]Pediatric Oncohematology Unit, University Hospital of Lyon, Lyon, [4]Pediatric Hematology Unit, APHP - Hospital Robert Debré, [5]Pediatric Onco-Hematology Unit, APHP - Hospital Armand Trousseau, Paris, [6]Pediatric Hematology

Unit, University Hospital of Marseille, Marseille, [7]Pediatrics, University Hospital of Lille, Lille, [8]Pediatric Oncohematology Unit, University Hospital of Toulouse, Toulouse, [9]Pediatric Hematology Unit, University Hospital of Rennes, Rennes, [10]Pediatric OncoHematology Unit, University Hospital of Besançon, Besançon, [11]Pediatric Hematology Unit, University Hospital of Strasbourg, Strasbourg, [12]Pediatrics, University Hospital of Grenoble, Grenoble, France

Background: Chronic immunologic thrombocytopenic purpura (cITP), autoimmune hemolytic anemia (AIHA) and Evans syndrome (ES) in children are rare diseases, characterized in 20-70% of cases by recurrent outbreaks and treatment dependence. In children with cITP, as in adults, splenectomy remains one of the second-line treatments with the higher curative potential. Its role in rarest AIHA and ES is less known. The long-term risks and the potential spontaneous recovery of those diseases limit its use
Aims: The objective of this study is to identify predictive factors for successful splenectomy in the treatment of childhood autoimmune cytopenia (AIC).
Methods: In this national observational prospective cohort of children with AIC, all children who had a splenectomy before the age of 18 and followed for more than 12 months were analyzed. The failure of splenectomy was defined as the initiation of a subsequent second-line treatment. A uni and multivariate study of factors associated with failure was performed.
Results: Among the 1345 children included in the cohort, 196 were splenectomized for AIC, and 156 met the inclusion criteria: diagnosis of AIC between 1986 and 2014 ; 124 cITP (80%), 23 AIHA (15%) and 9 ES (5%). The sex ratio was 1, the median age at diagnosis was 7.6 years (0.6-17.4), the median age at splenectomy was 11 years (0.8-17.7) with a median time of 27 months (0,2-162,2) between diagnosis and splenectomy. The cytopenia was primary in 54 patients (48%) and associated with various immunological abnormalities (secondary) in 63 patients (45%). The mean number of pre-splenectomy treatments was 1 (0-11). With a median follow-up of 4.9 years (1-24) since the procedure, splenectomy was successful in 103 patients (66%), and 53% of the whole patients are in complete, sustained remission without relapses or pulses at the latest follow-up. The 53 patients (34%) in the failure group received further second-line treatments in a median delay of 1.5 years (0.01-19). Predictors of splenectomy failure in univariate analysis were the target AIHA or ES *vs* cITP (OR: 2.14, CI 95% [1.20, 3.81], p 0.01), and a context of secondary AIC (OR: 2.99, CI 95% [1.65, 5.43], p: 0.00003). In multivariate analysis, the context of secondary AIC remained significantly associated with failure (OR: 2.99, CI 95% [1.59, 5.67], p: 0.0007). During the follow-up, 19 patients (12%) had severe infections, 9 (6%) had thromboses, and 7 (4%) had died (median age 7, all had secondary cytopenia and had received multiple second lines).
Summary and Conclusions: For the first time long-term and comparative data on splenectomy for childhood AIC are provided. The benefit-risk ratio of the procedure seems lower in ES and AIHA than in cITP, in contexts where AIC are associated with other immunological abnormalities. The indication for splenectomy should be discussed on a case-by-case basis, and the best strategy to delay or avoid it, using alternative medical approaches, remains to be established.

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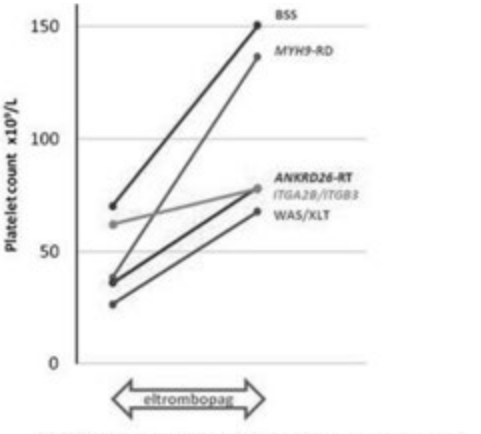
SHORT- AND LONG-TERM ELTROMBOPAG FOR THE TREATMENT OF INHERITED THROMBOCYTOPENIAS: A PHASE 2 CLINICAL TRIAL

C. Zaninetti[1,*], T. Fierro[2], A. Bertomoro[3], M.A. Alberelli[4], D. Veneri[5], V. Bozzi[1], S. Barozzi[1], P. Noris [1], F. Melazzini [1], E. De Candia[4], P. Minuz[5], F. Fabris[3], P. Gresele [2], C.L. Balduini[1], A. Pecci[1]

[1]Department of Internal Medicine, IRCCS Policlinico San Matteo Foundation, and University of Pavia, Pavia, [2]Department of Medicine, Section of Internal and Cardiovascular Medicine, University of Perugia, Perugia, [3]Department of Medicine-DIMED, University of Padova, Padova, [4]Department of Internal Medicine, Policlinico Gemelli Hospital, and Catholic University of Sacred Heart of Rome, Roma, [5]Department of Internal Medicine, University of Verona, Verona, Italy

Background: Inherited thrombocytopenias (ITs) are a group of rare disorders characterized by low platelet count (PC) and variable risk of bleeding. Until recently, platelet transfusion was the only effective measure for increasing PC. Thrombopoietin-receptor agonists represent an appealing therapeutic option to increase PC in patients with IT, but clinical data are limited.
Aims: To ascertain whether and in which forms of IT (a) short-term eltrombopag can transiently increase PC and reduce bleeding tendency, and (b) long-term eltrombopag can stably reduce bleeding symptoms in patients with clinically relevant spontaneous hemorrhages.
Methods: Patients initially received eltrombopag 50 mg/day for 3 weeks. Then, treatment was stopped in subjects who obtained a PC ≥100 x10^9/L, or continued at 75 mg/day for 3 further weeks (Phase 1). Major response was defined by the achievement of PC ≥100 x10^9/L with no bleeding tendency, minor response by the achievement of PC at least two times higher than the baseline value and reduction of bleeding tendency. Patients with clinically relevant bleeding symptoms at baseline (score ≥2 of the WHO bleeding scale), who completed the Phase 1 without side effects obtaining reduction of bleeding, entered the 16-week-long Phase 2. They initially received eltrombopag 25 mg/day and were re-evaluated for dose adjustments every 4 weeks. Major and minor responses were defined by a complete or a partial remission of bleeding, respectively (NCT02422394). All patients provided written informed consent for the study.
Results: We enrolled 24 patients (mean age 41 years, 58% males) affected with 5 forms of IT: *MYH9*-related disease (*MYH9*-RD, n=9), *ANKRD26*-related thrombocytopenia (*ANKRD26*-RT, n=9), X-linked thrombocytopenia/Wiskott-Aldrich syndrome (XLT/WAS, n=3), monoallelic Bernard-Soulier Syndrome (mBSS, n=2), and *ITGA2B*/*ITGB3*-related thrombocytopenia (*ITGA2B/ITGB3*-RT, n=1). Mean PC at baseline was 40 x10^9/L. All patients entered the Phase 1 and 23 of them completed the treatment. Eleven subjects (48%) obtained a major response, 10 (43%) a minor response, and 2 (9%) did not respond. The mean PC at the end of Phase 1 was 105 x10^9/L; the mean increase of PC with respect to baseline in responders was 70 x10^9/L. Among the 12 patients with spontaneous bleeding at baseline, 10 (83%) obtained complete remission of hemorrhages, 1 (8.5%) obtained reduction, and 1 (8.5%) did not obtain improvement. Four patients entered the Phase 2 (*MYH9*-RD, n=2; WAS, n=1; *ITGA2B/ITGB3*-RT, n=1) and 3 completed the treatment plan. All of them obtained a minor response associated with a stable increase of PC throughout the treatment period. Response to eltrombopag was associated with a stable improvement of quality of life in all the 3 cases. No major adverse events were recorded during the Phase 1 of the study. In one patient with WAS, treatment was discontinued during the Phase 2 because of exacerbation of a pre-existing cutaneous eczema; no other relevant adverse events were recorded during the Phase 2 (Figure 1).



Mean increase of platelet count obtained during the Phase 1 of the trial according to the different forms of IT.

Figure 1.

Summary and Conclusions: Short-term eltrombopag is safe and effective in transiently increasing PC in patients with *MYH9*-RD, *ANKRD26*-RT, XLT/WAS, and mBSS. Although the extent of platelet response was different between the different disorders, eltrombopag is expected to avoid or strongly limit the use of platelet transfusions in preparation for most surgical procedures in all these ITs. Long-term eltrombopag was safe and effective in reducing bleeding symptoms and improving quality of life in patients with *MYH9*-RD, WAS and *ITGA2B/ITGB3*-RT.

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PHASE II, MULTIPLE-DOSE STUDY OF ANTI-FCRN ANTIBODY, ROZANOLIXIZUMAB (UCB7665), IN PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA: SECOND INTERIM ANALYSIS

T. Robak[1,*], I. Jarque[2], V. Musteata[3], P. Kiessling[4], U. Massow[4], J. Higginson[5], R. Snipes[6], S. Jolles[7]

[1]Medical University of Lodz, Lodz, Poland, [2]Hospital Universitario y Politécnico La Fe, Valencia, Spain, [3]Institute of Oncology, Chisinau, Moldova, Republic of, [4]UCB Pharma, Monheim, Germany, [5]UCB Pharma, Slough, United Kingdom, [6]UCB Pharma, Raleigh, United States, [7]University Hospital of Wales, Cardiff, United Kingdom

Background: Rozanolixizumab is a humanised, high-affinity, anti-human neonatal Fc receptor (FcRn) monoclonal antibody, developed with the aim

of reducing levels of pathogenic immunoglobulin G (IgG) in autoimmune and alloimmune diseases.

Aims: Report the safety, tolerability and efficacy from an ongoing Phase II, open-label study of rozanolixizumab in patients (pts) with primary immune thrombocytopenia (ITP) (NCT02718716). Interim analysis (8th Data Monitoring Committee review) data cut-off: 27 November 2017.

Methods: Eligibility criteria included: ≥18 years (y) of age; diagnosis of primary ITP for ≥3 months prior to screening; platelet count <30x10^9/L at screening and <35x10^9/L at baseline; current, or history of, peripheral blood smear consistent with ITP. Eligible pts received rozanolixizumab SC at 5x4 mg/kg or 3x7 mg/kg, weekly; the higher dose group was enrolled after review of pt safety data in the lower dose group.

Results: A total of 30 pts received rozanolixizumab: 4 mg/kg (n=15), 7 mg/kg (n=15). Median age was 55 y (range 20–86); 4 mg/kg, 66 y (21–86) and 7 mg/kg, 54 y (20–73). Median duration of disease at baseline was 6.5 y (range 0.3–36); 4 mg/kg, 7.1 y (1.7–29) and 7 mg/kg, 5.2 y (0.3–36). Of 27 pts who received prior ITP therapies, median number of therapies received was 4 (range 1–15); 4 mg/kg, 4 (1–15) and 7 mg/kg, 2 (1–12). Most common prior therapies: azathioprine (11/30 [37%] pts; 4 mg/kg, 40%; 7 mg/kg, 33%, romiplostim (10/30 [33%] pts; 4 mg/kg, 40%; 7 mg/kg, 27%), immunoglobulins (9/30 [30%] pts; 4 mg/kg, 27%; 7 mg/kg, 33%).

Overall, 21/30 (70%) pts reported ≥1 TEAE (66 TEAEs combined total): 12/15 (80%) pts in the 4 mg/kg group and 9/15 (60%) pts in the 7 mg/kg group. There was one serious TEAE (bleeding from genital tract, also classified as severe in intensity [CTCAE ≥Grade 3]) in the 4 mg/kg group, deemed unrelated to study medication by the investigator; all other TEAEs reported were mild/moderate (Grade 1/2). Two pts reported TEAEs (Grade 1) deemed related to study medication by the investigator: injection site reaction (4 mg/kg group) and headache (7 mg/kg group); the TEAEs did not interfere with subsequent dosing. The most frequently reported TEAE was headache (Grade 1): 3/15 (20%) pts in the 4 mg/kg group and 6/15 (40%) pts in the 7 mg/kg group (Table 1). No clinically relevant changes were observed in all other haematology, coagulation, clinical chemistry (including albumin), urinalysis, ECGs, vital signs or liver function tests. Changes were observed in total protein, as expected. No deaths or treatment discontinuations due to TEAEs were reported. At the interim analysis, maximum mean decreases in total IgG concentration were observed at Day 29 for rozanolixizumab 4 mg/kg (mean decrease from baseline 43.6%, range 21.9–68.6) and at Day 22 for rozanolixizumab 7 mg/kg (49.9%, 29.5–65.5). Clinically relevant improvements in platelet counts (count ≥50x10^9/L) were reported for 5/15 pts (33.3%) in the 4 mg/kg group (overall maximum value range: 52x10^9–198x10^9/L) and 5/15 pts (33.3%) in the 7 mg/kg group (52x10^9–133x10^9/L).

Table 1.

TEAEs occurring in >2 patients in total by preferred term

MedDRA (v20.0) Preferred term	Rozanolixizumab SC 5x4 mg/kg N=15 n (%)	Rozanolixizumab SC 3x7 mg/kg N=15 n (%)	All patients N=30 n (%)
Headache	3 (20)	6 (40)	9 (30)
Diarrhoea	1 (7)	2 (13)	3 (10)
Influenza-like illness	2 (13)	1 (7)	3 (10)

CTCAE, Common Terminology Criteria for Adverse Events; MedDRA, Medical Dictionary for Regulatory Activities; n, number of patients reporting at least 1 TEAE at the time of interim analysis; SC, subcutaneously; TEAE, treatment-emergent adverse event

Summary and Conclusions: From data available to date, multiple dosing with rozanolixizumab SC 4 mg/kg and 7 mg/kg has been well tolerated in pts with ITP, and initial platelet responses have been observed in both dose groups. The safety profile for rozanolixizumab SC reported in this trial is in line with the safety profile reported in the first in human trial (NCT02220153) with healthy participants receiving rozanolixizumab SC.[1]

[1]Kiessling *Sci Trans Med* 2017;9(414)eean1208

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SWI IDENTIFIES BRAIN MICROHAEMORRHAGES IN ASYMPTOMATIC PATIENTS WITH IMMUNE THROMBOCYTOPENIA (ITP)

C. Vladescu[1], G. Adams[12,*], M. Morrison[34], D. Paul[1], T. Young[1], M. Atta[1], A. Luqmani[1], A. Waldman[45], N. Cooper[12]

[1]Department of Haematology, Hammersmith Hospital, [2]Department of Medicine, Imperial College, London, United Kingdom, [3]Department of Radiology and Biomedical Imaging, UCSF, San Francisco, United States, [4]Division of Brain Sciences, Imperial College, London, [5]Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, United Kingdom

Background: Immune thrombocytopenia (ITP) is an autoimmune disease characterised by a platelet count <100 x 10^9/L. Treatment is aimed at stopping bleeding and avoiding serious bleeding; options include steroids, immune-modulating drugs, thrombopoietin receptor agonists and splenectomy. Despite low platelet counts, many patients remain asymptomatic and morbidity and mortality is frequently from toxicities of treatment. There is therefore a trend to reduce the platelet target for treatment. Currently, there is no identifiable platelet count below which serious bleeds are known to occur, nor any method in place to assess whether patients develop internal bleeding at low platelet counts. Susceptibility-weighted imaging (SWI) is an MRI technique that exploits phase shifts due to magnetic susceptibility perturbation to generate tissue contrast; it is exquisitely sensitive to the presence of blood products, and is increasingly used in clinical practice to identify very small bleeds in the brain (microbleeds).

Aims: We propose the use of SWI to stratify ITP patients at risk of asymptomatic brain haemorrhage.

Methods: 44 adult ITP patients (median age=37.95, range=18 to 87) with at least one platelet count <30x10^9/L were recruited to a prospective MR study between 2014 and 2018 at the Hammersmith Hospital. No patient had neurological deficit at the time of neuroimaging. A dedicated MRI protocol including SWI was acquired at 3T (Siemens Verio, Erlangen, De; VB19). Microbleeds were detected with the aid of a semi-automated intensity-based detection algorithm and verified by an experienced neuroradiologist blinded to clinical details. Platelet count, bleeding scores, age of patient, duration of disease and treatment history was compared between patients with and without detectable microhaemorrhage.

Results: Intraparenchymal microhaemorrhages (of less than 3 mm diameter) were identified in 19 of 44 patients (43%) by semi-automated algorithm and 11 by visual inspection only. The majority had less than 5 microbleeds. These can occur in normal individuals with advancing age. Only 4 patients (9%) had greater than 5 microbleeds. Three of these patients had multi-refractory disease, two with disease of greater than 21 years. One patient had a head injury at a platelet count of 1 sustaining frontal bleeds. The fourth patient had not been treated for 10 years following diagnosis as a child. None had apparent neurological deficits. Bleeds were more likely in patients with lower platelet counts in an univariate model (p value 0.0094) as well as in logistic multivariate regression model (p value 0.046) when adjusted for age, gender and bleeding scores. Notably, severe thrombocytopenia was not always associated with microbleeds; 15 of 31 patients with counts less than 10 x 10^9/L did not have detectable microbleeds (Figure 1).

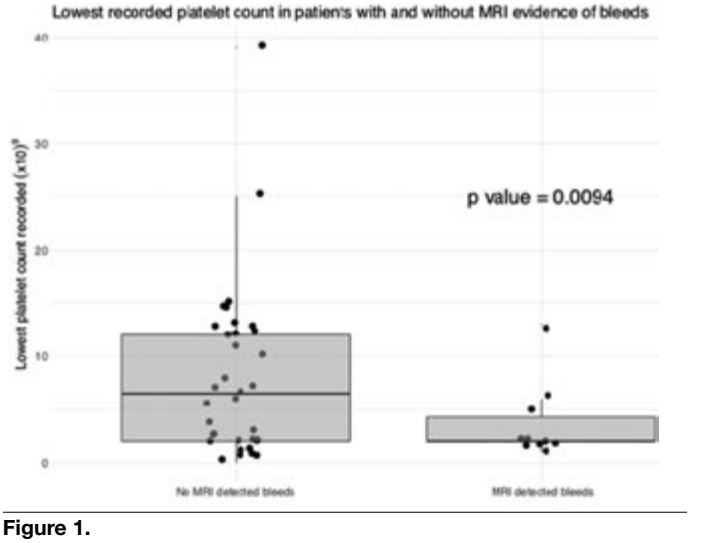

Figure 1.

Summary and Conclusions: ITP is associated with cerebral microhaemorrhage, which appears related to lower platelet count and refractory disease. There was no correlation with bleeding symptoms, although this requires further evaluation. The results of this preliminary study have potentially important implications for risk stratification and management of patients with ITP. They also provide proof of principle for using MRI to identify risk factors and functional consequences of haemorrhage in ITP and other thrombocytopenic disorders.

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DEFINING TREATMENT SUCCESS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA RECEIVING THROMBOPOIETIN RECEPTOR AGONIST THERAPY: RESULTS FROM A MODIFIED DELPHI CONSENSUS PANEL

C.A. Bradbury[1,*], D. Provan[2], N. Cooper[3], J. Grainger[4], Q. Hill[5], J. Thachil[6], J.-P. Westwood[7], N. Ramscar[8], A. Roy[9]

[1]University of Bristol, Bristol, [2]Barts and the London School of Medicine, [3]Hammersmith Hospital, London, [4]Royal Manchester Children's Hospital, Manchester, [5]The Leeds Teaching Hospital, Leeds, [6]Central Manchester University Hospital, Manchester, [7]University College Hospital London, [8]Novartis UK, London, United Kingdom, [9]Novartis Pharmaceuticals, East Hanover, United States

Background: Immune thrombocytopenia (ITP) is an autoimmune disorder causing reduced platelet counts <100,000/μL, with a range of potential bleeding consequences. Major bleeding episodes are less common when platelet counts are >30,000/μL, and treatment is generally reserved for patients with platelet counts <30,000/μL, bleeding, or a high risk of bleeding such as those undergoing surgery. Experimental and observational studies have suggested that thrombopoietin receptor agonist (TPO-RA) therapy may be tapered with the intent of discontinuation in patients with adequate response to treatment; however, there are no specific criteria for identifying treatment success, remission, and appropriate tapering strategies.
Aims: To gain consensus on parameters for defining sufficient TPO-RA treatment success in patients with ITP such that a tapering and discontinuation approach may be considered.
Methods: A consensus meeting using the modified Delphi method was held among UK ITP experts. Experts participated in a structured technology-based platform that captured individual uninfluenced opinion, followed by a panel debate and discussion in which consensus was sought. Expert panel participants responded to questionnaires in advance of the consensus meeting. Questionnaire topics focused on the appropriateness of the TPO-RA tapering and discontinuation approach; identifying suitable patients; outcomes to be expected from discontinuation; duration of remission; and evidence needed to further clarify treatment approaches and recommendations.
Results: Seven ITP experts participated in the June 2017 consensus meeting. Expert questionnaire responses indicated a high level of agreement that tapering of TPO-RA can be a rational approach to treatment and that in some patients who are deemed clinically improved and feel well, discontinuation of treatment is warranted. Experts agreed that a tapering approach may be considered after 6 months for patients with an adequate treatment response who are less likely to encounter a high-risk bleeding scenario, and after at least 12 months for those with a less clear treatment response who may need more time and continued monitoring. Regarding the definition of adequate treatment response, experts agreed that at a minimum, patients should have a stable platelet count of >50,000/μL without bleeding problems in the last 6 months. Similarly, experts agreed that tapering and discontinuation should not be continued if the platelet count fell below 30,000/μL or if patients experienced persistently worse quality of life due to anxiety, fatigue, or other humanistic factors. It was agreed that at present there are no clear predictive factors to identify those patients suitable for tapering. Also, consensus was reached on the need for prospective research based on clear eligibility criteria and tapering protocols.
Summary and Conclusions: This modified Delphi panel gained consensus on the potential merit of tapering and discontinuing TPO-RA therapy in patients with ITP. Platelet counts should remain consistently >50,000/μL without hospital encounters for at least 6–12 months depending on the patient. There is an important need for adequate new evidence regarding TPO-RA tapering and discontinuation.

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MYH9-RELATED THROMBOCYTOPENIA: FOUR NOVEL VARIANTS AFFECTING THE TAIL DOMAIN OF THE NON-MUSCLE MYOSIN HEAVY CHAIN IIA ASSOCIATE WITH A MILD CLINICAL EVOLUTION OF THE DISEASE

C. Zaninetti[1,*], D. De Rocco[2], T. Giangregorio[3], V. Bozzi[1], J. Demeter[4], P. Leoni[5], P. Noris[1], S. Ryhänen[6], S. Barozzi[1], A. Savoia[7], A. Pecci[1]

[1]Department of Internal Medicine, IRCCS Policlinico San Matteo Foundation, and University of Pavia, Pavia, [2]Department of Medical Sciences, University of Trieste, [3]Institute for Maternal and Child Health, IRCCS Burlo Garofolo, Trieste, Italy, [4]First Department of Internal Medicine, Division of Hematology, Semmelweis University, Budapest, Hungary, [5]Hematology Clinic, Department of Clinical and Molecular Sciences, Università Politecnica delle Marche, Ancona, Italy, [6]University Central Hospital, University of Helsinki, Helsinki, Finland, [7]Department of Medical Sciences, Institute for Maternal and Child Health, IRCCS Burlo Garofolo, and University of Trieste, Trieste, Italy

Background: MYH9-related disease (MYH9-RD) is an autosomal-dominant syndromic thrombocytopenia caused by mutations in MYH9, the gene encoding the heavy chain of non-muscle myosin IIA (NMMHC-IIA). Patients present congenital thrombocytopenia, giant platelets, and inclusions of NMMHC-IIA in leukocytes, and have a variable risk of developing one or more non-congenital manifestations, namely proteinuric nephropathy often evolving to end-stage kidney failure, sensorineural deafness, presenile cataracts, and/or abnormalities of liver enzymes. NMMHC-IIA comprises two domains: the N-terminal globular head domain (HD), which binds actin and hydrolyzes ATP generating mechanical force, and the C-terminal tail domain (TD) mainly involved in the assembly of the myosin molecule. The TD is composed of a long coiled-coil region and a short non-helical tailpiece. The incidence and the severity of the non-congenital manifestations of MYH9-RD are predicted by the causative MYH9 mutation. Globally, variants affecting the HD of NMMHC-IIA correlate with a more severe clinical picture than those involving the TD. Moreover, recent studies demonstrated that different alterations of the TD associate with remarkably different disease evolution.
Aims: To enlarge the spectrum of mutations responsible for MYH9-RD and assess the prognostic significance of novel causative variants.
Methods: Nine subjects belonging to 4 Caucasian pedigrees, referred to our Institution because of chronic thrombocytopenia, were included in the study. All patients provided written informed consent. Diagnosis of MYH9-RD was confirmed in all the cases by the identification of NMMHC-IIA leukocyte inclusions through immunofluorescence analysis of blood smears. MYH9 variants identified in probands were tested in all the available relatives. Non-congenital features of the disorder were searched in all patients independently of the presence of a symptomatic disease.
Results: We identified a novel MYH9 mutation in each family. Two variants (c.3486G>C and c.4261G>A) lead to aminoacid substitutions in the coiled-coil region of the TD of NMMHC-IIA (p.Arg1162Ser and p.Glu1421Lys, respectively). The other two mutations are a splicing variant (c.5765+2T>G) and a single nucleotide deletion (c.5806del) both resulting in frameshift alterations of the non-helical tailpiece (p.Arg1922Argfs*43, and p.Arg1936Glyfs*12, respectively). The variants segregate with the phenotype within the respective families and are not reported in public databases. Bioinformatic prediction tools suggest that both missense mutations are likely to be pathogenic. Overall, patients have moderate thrombocytopenia with mild or absent bleeding symptoms. In 5 out of 9 subjects, audiometric examination disclosed a moderate, bilateral and symmetric hearing defect involving the middle and high tones. In 4 of such cases, the age at onset of deafness was above 40 years. Only one patient presents kidney damage at the stage of proteinuric nephropathy not associated with renal failure. Cataract was detected in only one case as well.
Summary and Conclusions: We report 4 novel variants affecting the TD of NMMHC-IIA and responsible for MYH9-RD in 4 families. Characterization of phenotypes of affected individuals showed that all of these mutations are associated with a mild clinical evolution of the disease.

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REAL-WORLD TAPERING AND DISCONTINUATION OF THROMBOPOIETIN RECEPTOR AGONIST THERAPY IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA: A SURVEY OF HEMATOLOGISTS IN THE UNITED KINGDOM

N. Cooper[1,*], Q. Hill[2], J. Grainger[3], J.-P. Westwood[4], C. Bradbury[5], D. Provan[6], J. Thachil[7], N. Ramscar[8], A. Roy[9]

[1]Hammersmith Hospital, London, [2]The Leeds Teaching Hospital, Leeds, [3]Royal Manchester Children's Hospital, Manchester, [4]University College London Hospital, London, [5]University of Bristol, Bristol, [6]Barts and the London School of Medicine, London, [7]Central Manchester University Hospital, Manchester, [8]Novartis UK, London, United Kingdom, [9]Novartis Pharmaceuticals, East Hanover, United States

Background: Treatment of immune thrombocytopenia (ITP) with a thrombopoietin receptor agonist (TPO-RA) may improve platelet count, preventing major bleeding episodes and improving quality of life in 80–90% of patients. Many patients require continuous TPO-RA therapy in order to maintain adequate platelet levels. However, recent evidence suggests that some patients may have a durable remission of disease even after discontinuation of TPO-RA therapy. Observational and case studies of TPO-RA tapering and discontinuation have presented a range of practices and patient responses across many different countries and settings.

Aims: To better understand TPO-RA tapering practices and experience among UK practitioners treating patients with ITP.
Methods: Hematologists or hematologist/oncologists practicing in the UK were recruited using a panel methodology and invited to participate in a short survey regarding appropriateness of and experience with tapering and discontinuation of TPO-RAs in patients with ITP. A total of 495 physicians were approached, with a top threshold target of 60 survey completers, based on feasibility and time period (1 week) for response. The survey requested information related to how the practitioners determine appropriateness of a tapering strategy; how many of their patients have undergone a tapering strategy; and the reasons and modalities of treatment reinitiation after TPO-RA discontinuation based on their most recent 3 cases. Responses were collected and descriptive assessments conducted.
Results: Forty-nine hematologists (9.8%) completed the survey, having been in practice for an average of 12 years and managing an average of 47 patients with ITP over the past 6 months. Respondents were most often based in academic/teaching hospitals (84%) and reported spending most of their time (86%) in direct patient care. TPO-RA tapering and discontinuation practices were considered to be appropriate in 30–34% of patients with ITP overall. Less than half of practitioners applied these practices in patients determined to be well-controlled and appropriate candidates for TPO-RA tapering and discontinuation (35% for eltrombopag, 47% for romiplostim). Stable platelet count was the most common reason for tapering and discontinuing treatment (58–76%), followed by a lack of or reduced risk of bleeding (17–24%). Overall, 29–35% of patients reinitiated treatment after an average of 86–106 days in treatment-free remission. The biggest driver of the decision to reinitiate treatment was platelet count (100%), followed by clinical symptoms (67–75%). Patients who reinitiated treatment did so with TPO-RAs (85%), other pharmacological treatment (11%), surgery (6%), and/or another nonpharmacological treatment (2%).
Summary and Conclusions: Survey respondents discontinued TPO-RA therapy in approximately one-third of patients with ITP; patients had to have well-controlled ITP to be suitable candidates for this strategy. Respondents relied on both laboratory values and clinical symptoms when determining patient suitability for discontinuation and reinitiation of treatment. The majority of patients who discontinued did not require further treatment. Approximately one-third of patients reinitiated therapy, most frequently with TPO-RAs. Tapering and discontinuation practices among a sample of UK hematologists suggested a willingness to try in patients considered appropriate by the clinician. Larger observational studies are needed to better understand how this strategy is considered and applied in real-world clinical practice.

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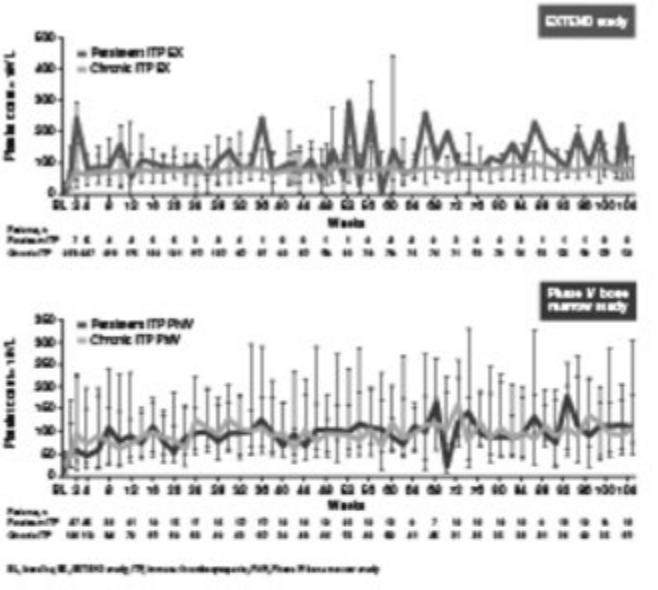
ELTROMBOPAG TREATMENT IN PATIENTS WITH PERSISTENT *VERSUS* CHRONIC IMMUNE THROMBOCYTOPENIA: COMPARISON OF EFFICACY AND SAFETY RESULTS FROM THE PHASE III EXTEND STUDY AND A PHASE IV STUDY

R.S. Wong[1,*], O. Meyer[2], T. Shamsi[3], R.K. Brynes[4], M.N. Saleh[5], A. Khelif[6], A. Orazi[7], Z. Aziz[8], A. Melikyan[9], O. Ocak Arikan[10], J. Maier[10], J.B. Bussel[11]

[1]Sir YK Pao Centre for Cancer & Department of Medicine and Therapeutics, Chinese University of Hong Kong, Shatin, NT, Hong Kong, [2]Institute of Transfusion Medicine, Charité-Universitätsmedizin Berlin, Berlin, Germany, [3]Research Department, National Institute of Blood Disease and Bone Marrow Transplantation, Karachi, Pakistan, [4]Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, CA, [5]Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, United States, [6]Hôpital Farhat Hached, Sousse, Tunisia, [7]Department of Pathology, Weill Cornell Medical College, New York, United States, [8]Clinical Oncology, Hameed Latif Hospital, Lahore, Pakistan, [9]National Research Center for Hematology, Moscow, Russian Federation, [10]Novartis Pharma AG, Basel, Switzerland, [11]Pediatric Hematology/Oncology, Weill Cornell Medicine, New York, United States

Background: Primary immune thrombocytopenia (ITP), an acquired autoimmune-mediated disorder characterized by isolated thrombocytopenia, is classified as persistent between 3 and 12 months from diagnosis (perITP), and chronic (cITP) if ongoing for more than 12 months. Eltrombopag (EPAG) is an oral thrombopoietin receptor agonist approved for previously treated cITP patients ≥1 year old. To compare EPAG in perITP *vs* cITP, subanalyses were performed of EXTEND, a Phase III, open-label, extension study of long-term efficacy and safety of EPAG in adults with ITP ≥6 months who had participated in prior EPAG studies (Wong *et al. Blood* 2017;130:2527–2536), and a 2-year Phase IV, open-label, bone marrow safety study of EPAG in adults with ITP ≥6 months (Brynes *et al. Acta Haematol* 2017;137:66–72; Wong *et al. Blood* 2017;130:abst 3628).
Aims: Describe effects of EPAG on platelet counts and long-term safety in subsets of patients with perITP (ITP ≥6 months) or cITP (ITP ≥12 months).
Methods: Patients on EXTEND and the Phase IV study started EPAG at 50 mg/day, titrated to 25–75 mg/day or less often as required, based on a platelet count target range ≥50–200x10^9/L.
Results: In EXTEND, 7/265 (3%) patients had perITP and 258 (97%) patients had cITP at baseline. Median (range) treatment duration was 2.3 years (56 days–3.7 years) and 2.5 years (10 days–8.8 years); mean (range) daily dose was 32.0 (7–74) mg/day and 50.4 (1–75) mg/day. In the Phase IV study, 37/161 (23%) had perITP and 124 (77%) had cITP at baseline. Median (range) treatment duration was 2.0 years (31 days–2.1 years) and 2.0 years (21 days–2.2 years); mean (range) daily dose was 48.8 (11–75) mg/day and 48.8 (5–75) mg/day. Median platelet counts in all subsets of both studies increased to ≥50x10^9/L within 2 weeks (Figure 1A). In EXTEND, 6/7 (86%) perITP and 230/258 (89%) cITP patients achieved a platelet count ≥50x10^9/L without rescue therapy. In the Phase IV study, platelet counts ≥50x10^9/L were achieved by 31/37 (84%) perITP and 109/124 (88%) cITP patients, without rescue therapy. During EXTEND, 5/7 (71%) and 148/258 (57%) patients maintained platelets continuously ≥50x10^9/L for ≥28 weeks whilst on treatment; in the Phase IV study, this was achieved by 19/37 (51%) and 62/124 (50%) patients. During EXTEND, serious AEs occurred in 1/7 (14%) perITP patient and 86/258 (33%) cITP patients (Figure 1B). During the Phase IV study, serious AEs occurred in 12/37 (32%) perITP and in 29/124 (23%) cITP patients (Figure 1B).

A. Median platelet count over time to week 104 in persistent ITP and chronic ITP patients during EXTEND and Phase IV studies



B. Most frequently occurring adverse events (≥15% in any group) and serious adverse events (≥5% in any group), regardless of relationship to study drug during EXTEND and Phase IV studies

	EXTEND		Phase IV study	
AE, n (%) (≥15% in any group)	Persistent ITP (N=7)	Chronic ITP (N=258)	Persistent ITP (N=37)	Chronic ITP (N=124)
Any event	7 (100)	240 (93)	32 (86)	108 (87)
Headache	2 (29)	75 (29)	8 (22)	23 (19)
Upper respiratory tract infection	2 (29)	61 (24)	4 (11)	17 (14)
Nasopharyngitis	1 (14)	69 (27)	4 (11)	13 (10)
Fatigue	1 (14)	44 (17)	6 (16)	11 (9)
Nausea	1 (14)	28 (11)	6 (16)	15 (12)
Cough	1 (14)	29 (11)	6 (16)	17 (14)
Diarrhea	–	41 (16)	4 (11)	18 (15)
Arthralgia	–	41 (16)	8 (22)	14 (11)
Dyspepsia	–	13 (5)	7 (19)	9 (7)
Influenza-like illness	–	24 (9)	6 (16)	9 (7)
Vertigo	–	6 (2)	6 (16)	6 (5)
Serious AE, n (%) (≥5% in any group)				
Any event	1 (14)	86 (33)	12 (32)	29 (23)
Cataract	–	14 (5)	–	–
Nausea	–	–	2 (5)	–
Fatigue	–	–	2 (5)	–
Deaths (on treatment, +1 day), n (%)				
Any event	–	1 (<1)	–	3 (2)
Adenocarcinoma	–	1 (<1)	–	–
Cerebral hemorrhage	–	–	–	2 (2)
Acute respiratory distress syndrome	–	–	–	1 (1)

Figure 1.

Summary and Conclusions: In both studies, the overall effects of EPAG on platelet counts were similar in perITP and cITP patients; the rate of AEs and serious AEs were as expected for eltrombopag treatment in ITP. However, results should be interpreted with caution because of the relatively small number of perITP patients (only a total of 44 between both studies). Nonetheless, our study indicates that EPAG has the potential to be an effective treatment option for patients with perITP. Further investigation of outcomes in perITP patients would be of interest, with larger patient numbers, to confirm responses and advance our understanding of this patient population. Although not a focus of these analyses, moving forward it would also be of interest to explore outcomes in perITP patients with a 3–<6 month duration of ITP.

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PERFORMANCE OF THE ISTH BLEEDING ASSESSMENT TOOL IN PREDICTING THE PRESENCE OF INHERITED PLATELET FUNCTION DISORDERS

M. Blaauwgeers[1,*], I. van Asten[2], A. Huisman[2], S. Korporaal[2], M. Coppens[3], M. Kruip[4], E. Beckers[5], J. Eikenboom[6], R. Tamminga[7], R. Urbanus[2], R. Schutgens[1]

[1]Van Creveldkliniek, [2]Clinical Chemistry and Hematology, UMC Utrecht, Utrecht, [3]AMC, Amsterdam, [4]Erasmus MC, Rotterdam, [5]Maastricht UMC, Maastricht, [6]LUMC, Leiden, [7]UMCG, Groningen, Netherlands

Background: The ISTH bleeding assessment tool (ISTH-BAT) was developed to standardize the documentation of bleeding symptoms, to guide further laboratory investigation and to help diagnose patients with possible bleeding disorders. Previous studies have shown that the BAT score was low in healthy volunteers and elevated in participants with clinical evidence of a bleeding disorder, but very few studies have assessed the clinical utility of the ISTH-BAT in predicting the presence of a platelet defect in patients with an increased bleeding tendency.
Aims: To evaluate the performance of the ISTH-BAT bleeding score (BS) in predicting the presence of platelet defects in patients with an increased bleeding tendency and (suspected) inherited platelet function disorders (PFDs).
Methods: Adult patients with an increased bleeding tendency, suspected for having a PFD, were included in a nationwide cross-sectional study on PFDs ('Thrombocytopathy in the Netherlands'). All participants gave written informed consent. The ISTH-BAT was administered by a physician prior to platelet function testing consisting of light transmission aggregometry (LTA), platelet nucleotide content analysis and flow cytometry. In addition, genetic analysis was performed on a 89-gene NGS platform.
Results: A total of 165 patients were enrolled in the study, of whom 135 were female (82%). Ninety-eight patients (59%) had an objective platelet defect based on abnormal results on LTA, platelet nucleotide content, flow cytometry and/or genetic analysis.The ISTH-BAT score was not significantly different between patients with an objective platelet function defect (median 10; interquartile range 7-15) and those with normal results (median 10; interquartile range 8-13) (p=0.78). The score was alsno not significantly different between men (median 9; interquartile range 5-13) and women (median 10; interquartile range 7-14) (p=0.07). The ISTH-BAT score could not discriminate between patients with and without a platelet function defect based on platelet function testing (area under the ROC curve=0.487 [95% CI 0.399-0.575]).
Summary and Conclusions: Although the ISTH-BAT is a useful tool for systematically documenting bleeding symptoms in patients with excessive bleeding, the BS obtained from the ISTH-BAT is unable to predict the presence of a platelet defect in patients with (suspected) platelet function disorders.

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SPLENECTOMY IN COMPARISON WITH RITUXIMAB AS A SECOND LINE TREATMENT FOR RELAPSED OR REFRACTORY ITP

A. Boban[1,*], M. Morić-Perić[2], S. Zupančić-Šalek[3], D. Pulanić[3], S. Bašić-Kinda[4], M. Vodanović[4], I. Radman[4], R. Serventi-Seiwerth[4], N. Duraković[1], I. Zekanović[2], K. Benček[5], I. Aurer[1]

[1]Division of Haematology, Department of Internal Medicine, University Hospital Center Zagreb, School of Medicine University of Zagreb, Zagreb, [2]Haematology Unit, Department of Internal Medicine, Zadar General Hospital, Zadar, [3]Division of Haematology, Department of Internal Medicine, University Hospital Center Zagreb, School of Medicine University of Zagreb, School of Medicine Josip Juraj Strossmayer University of Osijek, [4]Division of Haematology, Department of Internal Medicine, University Hospital Centre Zagreb, [5]Division of Haematology, Department of Internal Medicine, School of Medicine University of Zagreb, Zagreb, Croatia

Background: Splenectomy has been used for the treatment of immune thromboctopenia (ITP) for more than a hundred years. However, the position of splenectomy in treating ITP has been questioned in the era of the new second line therapies, namely rituximab, anti-CD20 monoclonal antibody, and recently introduced thrombopoietin-receptor agonists.
Aims: The aim of this single-centre retrospective study was to re-evaluate the role of splenectomy as the second-line therapy for ITP.
Methods: We have retrospectively analysed efficacy and safety of splenectomy and rituximab used as the second line treatment of patients with relapsed/refractory ITP from our centre treated between 2000 and 2018. Patients with secondary ITP were excluded from the analysis.
Results: During the evaluation period, 48 patients with relapsed, refractory or corticosteroid dependent ITP received rituximab or had splenectomy as a second-line treatment. Twenty-four patients (50%) had splenectomy (19 female (79%), median age 38 (6-71) years) and 24 patients (50%) were treated with rituximab (12 female (50%), median age 30 (7-68) years). Median time from diagnosis of ITP and splenectomy was 2 (1-12) years and 1.5 (0.25-24) years for rituximab. Median follow-up period for splenectomy was 4 (0.5-16) years and 2 (0.3-10) years for rituximab.
Following splenectomy, eighteen patients (75%) achieved remission, while 12 patients (50%) achieved remission after rituximab. Relapse was observed in one patient 15 years after splenectomy, and in three patients following rituximab treatment, 12, 17 and 36 months later. Regarding side effects, one patient developed deep venous thrombosis related to splenectomy, whereas two patients had severe CMV infections after rituximab treatment.
Summary and Conclusions: The obtained results confirm that splenectomy is very efficient as a second line treatment of relapsed, refractory or corticosteroid-dependent ITP. Splenectomy is even demonstrating superiority in efficacy when compared with rituximab treatment in this indication. Complications following splenectomy and the rate of relapse were lower than among patients receiving rituximab. However, as splenectomy is an invasive procedure, further analysis is needed to identify a subset of patients that would have the most benefit from it.

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IMMUNE THROMBOCYTOPENIA PURPURA (ITP) PATIENT JOURNEY – ROUTINE CLINICAL CARE FOR CHRONIC ITP IN DENMARK, 2009-2015

A. Ording[1], N. Kristensen[1], H. Frederiksen[2,*], M. Nørgaard[1], N. Alam[3], S. Bahmanyar[4], W. Ghanima[56], H. Toft Sørensen[1], C.F. Christiansen[1]

[1]Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, [2]Department of Haematology, Odense University Hospital, Odense, Denmark, [3]Centre for Observational Research, Amgen Ltd., Uxbridge, United Kingdom, [4]Clinical Epidemiology Unit & Center for Pharmacoepidemiology, Karolinska Institutet, Stockholm, Sweden, [5]Department of Hematology, Institute of Clinical Medicine, University of Oslo, Oslo, [6]Department of Research, Østfold hospital, Sarpsborg, Norway

Background: Immune thrombocytopenia (ITP) is an autoimmune disease characterized by decreased production and increased peripheral destruction of platelets. Treatment is recommended for patients with persistent/recurrent bleeding and/or platelet count <30 x10^9/L.
Aims: We aimed to examine the clinical characteristics of routine clinical treatment of patients with chronic ITP (cITP) in Denmark.
Methods: This cohort study included all Danish patients aged ≥18 years diagnosed with incident cITP from April 1, 2009 to December 31, 2015 as reported to the Nordic Country Patient Registry for Romiplostim, which collects data on all cITP patients in Denmark. cITP was defined as ≥2 ITP diagnoses made >6 months apart, as recorded in the Danish National Patient Registry, and confirmed by medical record review. The date of the second ITP diagnosis was denoted cITP diagnosis date (index). Patients with secondary ITP were excluded. Patient distribution by nadir platelet count within 90 days before index and the percentage of patients who experienced ≥1 bleeding-related hospitalization within one year before index were reported. The overall proportion of patients treated with ITP drugs, splenectomy, and supportive treatments (including tranexamic acid and platelet transfusion) between first ITP diagnosis and index was estimated. Patients were followed for 12 months after their index date, with censoring at emigration or death. We calculated the 1-year cumulative risk of treatment initiation, with death as a competing risk, for patients previously untreated as of the index date.
Results: The study included 964 cITP patients (57% female, median age 58 years, interquartile range (IQR): 36-71). Median time from first ITP diagnosis to index was 7 months (IQR: 6-10). Nearly half (n=470, 48%) were treated between first diagnosis and index; of these 470 patients, 43% had a record of treatment with corticosteroids, 18% with rituximab, 12% with supportive treatment, 12% with IVIg, 3.7% with splenectomy, and 3.1%

with TPO-RA. For the 494 patients who did not receive any treatment prior to index date, the lowest platelet count within 90 days before index was <50×10^9/L for 20% of all patients; between 50 and 150 for 44%; and ≥150 for 7.1% (missing for 29%). For those treated prior to index date, corresponding proportions were 28%, 24%, 34% (missing for 14%), respectively. Bleeding events within one year prior to index date was reported for 32% of those who were treated, and for 26% of those not treated before index. Of the 494 patients not receiving therapy between first ITP diagnosis and index 9.2% (95% confidence interval (CI): 7.7, 11.0) (n=43) patients initiated therapy in the subsequent 12 months. The 12-month risk of therapy initiation for those unexposed to a given therapy prior to cITP diagnosis was: 6.3% (95% CI: 5.1, 7.7) for corticosteroids, 5.3% (95%CI: 4.4, 6.3) for rituximab, 3.1% (2.4, 4.0) for IVIg, 1.7% (95% CI: 1.2, 2.3) for TPO-RA, and 3.7% (2.9, 4.5) for splenectomy.
Summary and Conclusions: Nearly half of all patients diagnosed with cITP in routine clinical practice in Denmark had been treated for ITP before their cITP diagnosis; 3.7% of these patients with treatment before cITP were splenectomized and 3.1% were treated with TPO-RA. In the subsequent 12 months post-diagnosis, 9.2% of previously untreated patients commenced therapy.

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DISEASE MANAGEMENT OF PATIENTS WITH IMMUNE THROMBOCYTOPENIA - RESULTS OF A REPRESENTATIVE RETROSPECTIVE SURVEY IN GERMANY

A.S. Kubasch[1,*], J. Kisro[2], J. Heßling[3], H. Schulz[4], H.-J. Hurtz[5], M. Klausmann[6], A. Ehrnsperger[7], U. Platzbecker[1]

[1]Medical Clinic and Policlinic I, University Hospital Carl Gustav Carus, TU Dresden, Dresden, [2]Lübecker onkologische Schwerpunktpraxis, Lübeck, [3]Onkologie am Segelfliegerdamm, Berlin, [4]Praxis internistischer Onkologie und Hämatologie, Frechen, [5]Onkolog. Gemeinschaftspraxis und Tagesklinik, Halle, [6]Gemeinschaftspraxis Dr. Klausmann, Aschaffenburg, [7]Novartis Pharma GmbH, Nuernberg, Germany

Background: Due to a better understanding of the disease pathology, treatment options for patients with immune thrombocytopenia (ITP) have increased over the last years. However, only few data exist on the real-life management of patients with ITP in Germany.
Aims: The current analysis of a national survey was undertaken to describe the diagnostic and treatment patterns of patients with ITP managed by hematologists in routine care.
Methods: A retrospective data collection using questionnaires was performed by 26 hematology practices distributed all over Germany. From 02/2016 until 12/2017, all patients diagnosed with ITP were documented. Documentation included patient characteristics, patient history and laboratory as well as clinical parameters at diagnosis and present, and previous and current therapeutic strategies. Next to the overall evaluation, patients were furthermore grouped by platelet count at diagnosis and results were compared between 4 groups: 1) 0-10 x 10^9/l; 2) 11-30 x 10^9/l; 3) 31-50 x 10^9/l; 4) 51-100 x 10^9/l.
Results: Data of 1,038 patients could be evaluated and a preliminary analysis was carried out. Overall, 47% of patients were female and 53% male. At diagnosis, more than half of the patients (56%) were >60 years. ITP was mainly diagnosed more than 3 years before the analysis (3-5 years: 28%; >5 years: 32%), and most patients (71%) suffered from primary ITP. An incidental finding was the main reason that led to the diagnosis of ITP (41%). Only 5% of all patients underwent splenectomy. The main strategies applied during 1st line treatment were steroids (45%) as well as "watch and wait" (40%), followed by intravenous immunoglobulins (IVIG; 6%). Multiple other treatment options were given to patients during 2nd and 3rd line therapy. Next to steroids and IVIGs, thrombopoietin receptor agonists (TPO-RAs) were increasingly used as 2nd (19%) or 3rd line treatment (26%).
Summary and Conclusions: According to the data analysis, steroids and a "watch and wait" strategy dominated 1st line treatment of patients with ITP. Most patients receive treatment with steroids as their first option. Only a minority of patients underwent splenectomy, which is probably due to better 2nd line therapies and an increased awareness of late remissions in ITP. A final analysis of the data will be presented at the conference, including data on subgroups.

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PREDICTIVE FACTORS FOR SUCCESSFUL SPLENECTOMY OUTCOME – SYSTEMATIC REVIEW

M. Mitrovic[1,2,*], D. Antic[1,2], M. Virijevic[1,2], Z. Pravdic[1], N. Suvajdzic[1,2]

[1]Clinic of Hematology, Clinical Center of Serbia, [2]Faculty of Medicine, University of Belgrade, Belgrade, Serbia

Background: Splenectomy may lead to a good response in 60-80% of adults with corticosteroid refractory immune thrombocytopenia (ITP). However, in the era of novel drugs the appropriate selection of patients for splenectomy is essential for optimization of the treatment outcome. Accordingly, it is important to identify both pre- and post-operative parameters predictable for the splenectomy outcome.
Aims: We analysed the large number of publications addressing this issues, in order to make systematic review of predictive factors for outcome of splenectomy in corticosteroid refractory ITP.
Methods: The Medline database was searched from January 1,1966, to January 1, 2018. using keywords: "splenectomy," "spleen and remov," "spleen and extract", "thrombocytopenia," "thrombocytopenic purpura," "ITP" or "AITP" as well as "predictor". The search was limited to English-language articles. The bibliographies of all retrieved articles were searched for additional relevant articles. Retrieved articles were selected for review if they reported ≥15 consecutive splenectomized ITP patients. Articles that reported data on children were included only if it could be determined that 75% or more of the patients were ≥14 years old.
Results: The literature search identified 410 articles; 335 articles did not meet our selection criteria and were not reviewed. We selected 75 articles with 6151 patients analizing three groups according to the type of response to the splenectomy: predictors of initial response (50 articles), predictors of sustained remission (35 articles) and predictors of relapse (11 articles).
Predictors of initial response: Age, duration of illness before splenectomy, platelet sequestration site, response to steroides and intravenous immunoglobulins (IVIg), preoperative and postoperative platelet count (PC), spleen size/weight were predictive in 62.5% (20/32), 14% (3/21), 63.3% (7/11), 42.9% (9/21), 20% (2/10), 50% (4/8), 100% (17/17), 20% (1/5) articles, respectively. Neither sex nor antiplatelet antibodies were predictive in analysed articles (19/19 and 11/11 articles).
Predictors of sustained remission: Age, duration of illness before splenectomy, platelet sequestration site, response to steroides and IVIg, preoperative and postoperative PC were predictive in 34.8% (8/15), 5.9% (1/17), 62.5% (5/8), 21.7% (5/23), 25% (1/4),71.4% (5/7), 70.6% (12/17) articles, respectively. Sex, spleen size/weight and antiplatelet antibodies were non-predictive in analysed articles (18/18, 2/2 and 6/6 articles).
Predictors of relapse: Age, response to steroides and IVIg, postoperative PC, spleen size/weight were predictive in 28.5% (2/7), 50% (2/4), 100% (1/1), 92.3% (12/13) and 2/6 (33.3%) articles, respectively. Sex, duration of illness before splenectomy, preoperative PC and antiplatelet antibodies were non-predictive in analysed articles (8/8, 5/5, preoperative PC 3/3 and 3/3 articles, respectively).
Summary and Conclusions: The reviewed articles used diverse criteria to evaluate patients' characteristics and to report outcomes. However, a comprehensive analysis of all published reports for predictive parameters for the outcome of splenectomy showed that the most reliabe predictor is postoperative PC. On the other hand, among preoperative variables, younger age and platelet sequestration site were related with response.

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CARDIOVASCULAR AND BLEEDING OUTCOMES IN A NORDIC COHORT OF ADULT PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA (CITP)

K. Adelborg[1,*], N.R. Kristensen[1], M. Nørgaard[1], S. Bahmanyar[2], W. Ghanima[3], K. Cetin[4], H.T. Sørensen[1], C.F. Christiansen[1]

[1]Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark, [2]Clinical Epidemiology Unit & Center for Pharmacoepidemiology, Karolinska Institutet, Stockholm, Sweden, [3]Department of Medicine, Østfold Hospital Trust, Sarpsborg, Norway, [4]Center for Observational Research, Amgen Inc, Thousand Oaks, United States

Background: ITP is a rare condition associated with low platelet counts and an increased tendency to bleed. There is a paucity of real-world data on cardiovascular and bleeding outcomes according to platelet count levels in these patients, as well as how these events influence mortality.
Aims: To examine the incidence of cardiovascular events and bleeding in association with platelet count levels and the prognostic impact of these events in a population-based cohort of cITP patients.
Methods: Using data from the Nordic Country Patient Registry for Romiplostim, we studied adults diagnosed with cITP (1996-2015), defined as ITP lasting a duration of >12 months. Incidence of cardiovascular events and bleeding requiring inpatient, outpatient, or emergency room hospital contact (via cumulative incidence risk function with death as a competing risk) and all-cause mortality (via Kaplan-Meier method) were estimated. We constructed matched (age, sex, country, ITP duration) comparison cohorts of ITP patients without these events, and hazard ratios were com-

puted through Cox proportional hazard regression to examine the prognostic impact of cardiovascular events and bleeding.
Results: Among 3,584 cITP patients (median age 58; 58% women), absolute 1- and 5-year rates were 1.9% and 5.8% for arterial cardiovascular events, 1.2% and 3.2% for venous thromboembolism, 7.5% and 17.2% for bleeding, and 3.5% and 15.2% for all-cause mortality. After adjusting for important demographic and clinical factors, rates of cardiovascular events were similar across baseline platelet counts (measured within 90 days prior to cITP diagnosis), while patients with baseline platelet counts <50×10^9/L had more than 2-fold higher 1-year rates of bleeding and all-cause mortality than patients with baseline platelet counts in the normal range (150-249x10^9/L). Occurrence of arterial cardiovascular events, venous thromboembolism, and bleeding were associated with subsequent 7-fold, 6-fold, and 3-fold increased 1-year all-cause mortality, respectively, compared to the matched comparison cohorts.
Summary and Conclusions: In patients with cITP, rates of cardiovascular events were low; rates of bleeding and all-cause mortality were somewhat higher. Cardiovascular events occurred across all platelet count levels, while low platelet counts were associated with an increased risk of bleeding and all-cause mortality. Cardiovascular and bleeding events were strong adverse prognostic factors for all-cause mortality.

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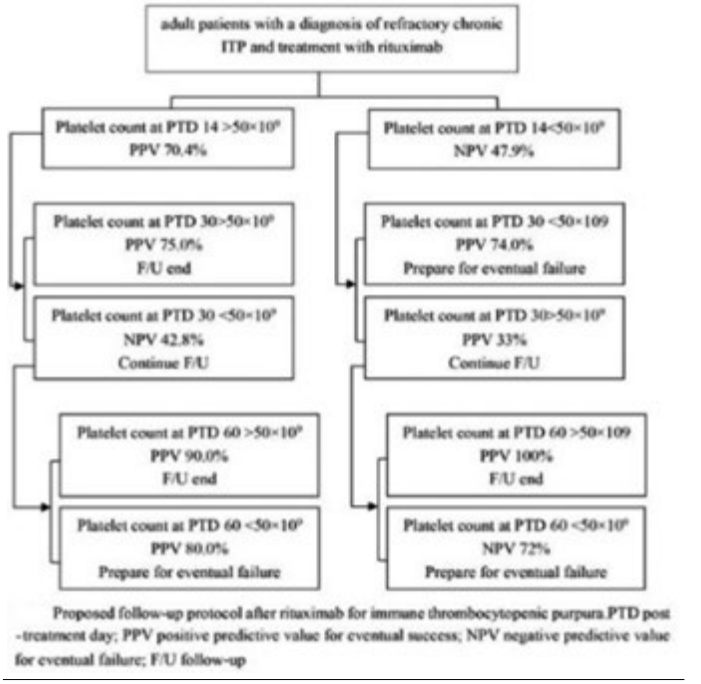
PLATELET COUNT EVOLUTION AS A PREDICTOR OF OUTCOME AFTER RITUXIMAB TREATMENT IN CHRONIC IMMUNE THROMBOCYTOPENIA

S. Wang[1,*], Y. Nie[2], M. Ju[1], T. Sun[1], H. Li[1], D. Zhang[1], L. Zhang[1], R. Yang[1]

[1]Institute of Hematology & Blood Disease Hospital, CAMS & PUMC, China, [2]Sinous Diagnostics, Tianjin, China

Background: Primary immune thrombocytopenia (ITP) is an autoimmune disorder in which a patient's immune system is activated by platelet-specific autoantigens produced by autoreactive B cells. Most adults with acute ITP will achieve an initial platelet count response to corticosteroid-based treatments; however, relapses are common within the first year and additional treatments are frequently required . Of the many available second-line alternatives, the anti-CD20 antibody rituximab are widely used.It depletes B cells, especially the memory B cell subset, and reverses regulatory T-cell dysfunction. Rituximab has been shown to provide initial response rates of about 60%; however, sustained responses lasting for more than 2 years occur in substantially fewer than 60% of patients. In this study, we want to present a prediction tool to identify eventual refractory ITP cases would aid patient counseling and management planning. The purpose of this study was to determine if responsive and refractory patients after treatment with Rituximab for ITP differ in terms of platelet count evolution, and to set a cut-off value for predicting response at the earliest possible time after treatment with Rituximab.
Aims: To determine whether platelet count evolution differs between patients with a successful or unsuccessful result after rituximab treatment in chronic ITP and to identify biological and clinical predictors of response.
Methods: A retrospective study was conducted in 103 chronic ITP patients hospitalized in our medical center between January 2012 and December 2014.Successes were defned to have a fnal platelet count of ≥50×10^9/L.Failures were defned to have a fnal platelet count of <50×10^9/L.The sensitivity, specificity, positive predictive value (PPV), negative predictive value(NPV) of platelet count in different time points were analyzed for the predictor of treatment response. Optimal cutoff values were established using ROC analysis.
Results: 1. Archived records of 103 consecutive patients that underwent Rituximab for ITP were reviewed. Multivariate analysis revealed that a lower absolute CD41$^+$megakaryocytes count <150 at diagnosis was an independent risk factor for treatment failure. (P=0.003; 95% confidence interval, 1.784~15.471; odds ratio, 5.253). long-term response (LTR) was higher in CD41$^+$megakaryocytes count >150 group *vs* <150 group patients.(P=0.001; 50.6% *vs* 17.5% after 48 months). 2. Successes and failures were found to have significantly different platelet counts from three days after treatment(PTD 3) with rituximab[74.4±104.1 ×10^9/L *vs* (37.3±37.4)×10^9/L,P=0.005], and remained different thereafter, with increasing significance[64.8±70.4 *vs* (31.2±24.9)×10^9/L, P=0.001 in PTD 14. In both groups, the different platelet counts stopped fluctuating and became raised gradually in PTD 60 107.1±62.0 ×10^9/L *vs* (22.6±18.4)×10^9/L, P<0.001 . 3. Patients were divided further using a optimal cut-off platelet count of 50×10^9/L on PTD 14, PTD 30, and PTD 60, and PPV and NPV values were calculated for predicting eventual success and failure (Figure 1).



Proposed follow-up protocol after rituximab for immune thrombocytopenic purpura.PTD post -treatment day; PPV positive predictive value for eventual success; NPV negative predictive value for eventual failure; F/U follow-up

Figure 1.

Summary and Conclusions: Response can be predicted by obtaining platelet counts at 14 days, 1 month, and 2 month after rituximab. We present a protocol that guides patient monitoring and management planning.

PF679

SUSTAINED PLATELET COUNTS ≥100X109/L DURING ELTROMBOPAG TREATMENT IN PATIENTS WITH PERSISTENT/CHRONIC IMMUNE THROMBOCYTOPENIA (ITP): RESULTS FROM A 2-YEAR, PHASE IV, OPEN-LABEL STUDY

T.S. Shamsi[1,*], R.S. Wong[2], A. Orazi[3], Z. Aziz[4], A. Melikyan[5], M. Stankovic[6], J. Maier[6], R.K. Brynes[7]

[1]Research Department, NIBD & BMT, Karachi, Pakistan, [2]Sir YK Pao Centre for Cancer and Department of Medicine and Therapeutics, Chinese University of Hong Kong, Shatin, NT, Hong Kong, [3]Department of Pathology, Weill Cornell Medical College, New York, United States, [4]Clinical Oncology, Hameed Latif Hospital, Lahore, Pakistan, [5]National Research Center for Hematology, Moscow, Russian Federation, [6]Novartis Pharma AG, Basel, Switzerland, [7]Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, CA, United States

Background: Primary ITP is an acquired disorder of autoimmune-directed platelet reduction and impaired platelet production, defined as platelet counts <100x10^9/L in the absence of other causes of thrombocytopenia. The oral thrombopoietin receptor agonist (TPO-RA) eltrombopag (EPAG), approved for use in pts with previously treated (eg corticosteroids) chronic ITP aged ≥1 yr, has proven efficacy in increasing and maintaining platelet counts ≥50x10^9/L. However, few studies have evaluated how many pts achieve a platelet count ≥100x10^9/L with EPAG treatment. Here we report effects on platelet count during a Phase IV, open-label bone marrow safety study of EPAG in adults with ITP ≥6 months (Brynes *et al. Acta Haematol* 2017;137:66–72).
Aims: Evaluate effects on platelet counts, with a focus on counts ≥100x10^9/L, and long-term safety, during 2 yrs of EPAG treatment.
Methods: Adults ≥18 yrs old with ITP ≥6 months were enrolled in this safety study of the effects of EPAG on bone marrow fibrosis (NCT01098487). Pts with prior exposure to EPAG or romiplostim were eligible but treatment must have been completed ≥6 months before screening. Pts treated with any other TPO-RA were not eligible. All pts started EPAG at 50 mg/day (25 mg/day for East Asian pts), titrated to 25–75 mg dose as required to maintain platelet counts within the clinically indicated range. Platelet counts were prospectively recorded; weekly until pts reached a stable dose, then every 4 wks during the study.
Results: 162 pts (median [range] age 42 yrs [18–80]) were enrolled; 50% Caucasian, 49% Asian; 23% persistent (n=37) and newly diagnosed (n=1) ITP, 77% chronic ITP (n=124); 23% were splenectomized. 70% of pts had

received prior ITP therapy before enrollment; 57% were receiving ITP therapy at study initiation (baseline [BL]). 77% and 57% had platelet counts ≤50x10^9/L or <30x10^9/L at BL. Median daily EPAG dose was 49.7 mg (range 5–75); median treatment duration was 104 wks (range 2.4–112.6). 72% of pts attended all study visits and completed the study; 61% of pts completed ≥24 months of EPAG treatment. 80% (130/162) of pts achieved platelet counts ≥100x10^9/L at least once during EPAG treatment without rescue medication; 9% (14/162) had ≥100x10^9/L at BL. 51% (61/120) of those pts still on treatment maintained platelet counts ≥100x10^9/L for ≥13 wks without rescue therapy (Figure 1). 87% (141/162) of pts achieved platelet counts ≥50x10^9/L at least once during EPAG treatment without rescue medication; 23% had ≥50x10^9/L at BL. 51% (62/122) of those pts still on treatment maintained platelet counts ≥50x10^9/L for ≥43 wks without rescue therapy. Adverse events (AEs; any grade) were reported in 87% (141/162) of pts (most frequently headache; 19%; cough, arthralgia, diarrhea; 14% each). AEs considered drug-related occurred in 37% (60/162); 13% (21/162) discontinued because of an AE, including increased alanine aminotransferase (n=3), cerebral hemorrhage, hepatitis toxic, and increased blood bilirubin (n=2 each). Six (4%) pts experienced thromboembolic events (transverse sinus thrombosis, cerebral venous thrombosis, transient ischemic attack, deep vein thrombosis, pulmonary embolism, and thrombophlebitis).

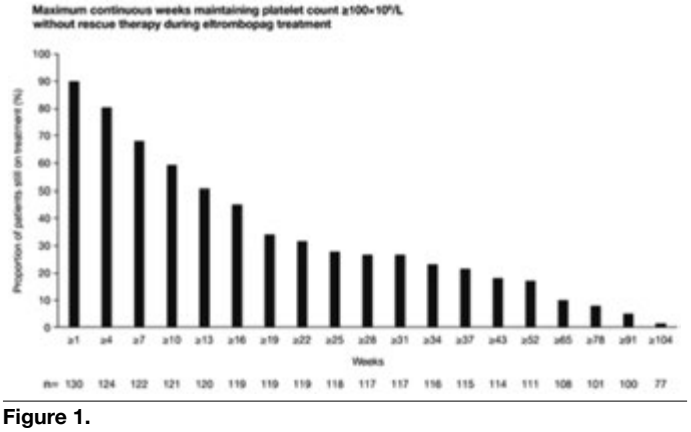


Figure 1.

Summary and Conclusions: During 2 yrs of EPAG, 80% of pts experienced at least one platelet count ≥100x10^9/L without rescue therapy, and 51% of pts still receiving EPAG maintained this platelet count for ≥13 wks. Reported AEs were consistent with the known EPAG safety profile, or underlying disease. These results further support the use of EPAG as an effective and well-tolerated treatment for pts with ITP.

PF680

LONG-TERM EFFICACY AND SAFETY OF ROMIPLOSTIM FOR TREATMENT OF CHRONIC IMMUNE THROMBOCYTOPENIA

E. Arshanskaya[1], S. Semochkin[2,*], V. Ivanova[1], V. Ptushkin[1,2]

[1]City Clinical Hospital n.a. S.P. Botkin, [2]Pirogov Russian National Research Medical University, Moscow, Russian Federation

Background: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by transient or persistent decrease in platelet count due to decreased production and increased peripheral destruction of platelets secondary to antiplatelet antibodies. About 10% of patients do not respond to standard treatment including splenectomy. A new method of treatment for ITP is the use of thrombopoietin (TPO) mimetics to increase the rate of platelet production. Activation of the TPO receptor that is present on megakaryocytes and platelets leads to increased thrombopoiesis. Romiplostim is the first thrombopoietic agent that has been shown to be a highly effective treatment for both splenectomized and nonsplenectomized ITP patients.
Aims: The primary aims of the study were to determine the efficacy and safety of long-term romiplostim for treatment of chronic ITP on the patient's own population.
Methods: Seventy-five adult chronic ITP patients who were refractory to previous ITP treatments (platelet count <30.0x10^9/L) were enrolled in the study in the period from 01.2012 to 12.2015. Romiplostim was initiated at 1-3 mg/kg per week. The dose was then adjusted as needed based on the patient's platelet count weekly. The efficacy was evaluated based on platelet responses and the percentage of patients able to lower or discontinue their doses of concurrent ITP medications. A primary platelet response defined as a platelet count ≥50.0x10^9/L with at least the doubling of the baseline value, without any rescue intervention during the preceding 4 weeks. A complete response considered if achieved at a platelet level of ≥100.0 x 10^9/L. A sustained response diagnosed if the platelet count was 50.0 x 10^9/L for at least 6 months. This analysis performed as of 31 August 2017.
Results: The median age was 57 (range, 18.2-79.5) years. The median number of prior ITP treatments was one (range, 1-4). Seventy-two (96%) patients had previously been treated with glucocorticosteroids, per 6 (8%) patients with eltrombopag and rituximab, and 11 (16%) patients had undergone a splenectomy. The median ITP duration before eltrombopag treatment was 2.8 (range, 0.7-49.2) years. The median platelet count at baseline was 19.0 (range, 1.0-28.0) x 10^9/L. A primary platelet response to romiplostim was observed in 65 (87%) patients, complete platelet response in 39 (52%), and sustained platelet response in 38 (51%). Forty-eight (64%) patients continued treatment with romiplostim at the time of the current analysis with a median duration of therapy of 29.1 (range, 1.0-91.8) months. Two (2.7%) patients received a romiplostim shortly before performing surgical interventions. The mean starting dose of romiplostim was 2.1±1.0 g/kg, and the final dose - 4.6±2.1 g/kg. The reasons for the completion of the therapy were ineffectiveness in 11 (14.7%) patients, adverse events in 6 (8%) and achieving a sustained response, which allowed gradual withdrawal of treatment in 6 (8%) patients. An alternative TPO receptor agonist (eltrombopag) was used in 12 (16%) cases. Episodes of bleeding occurred in 35 (47%) cases, including ≥3 grade in 5 (7%). The need for a short-term appointment of glucocorticosteroid drugs occurred in 33 (44%) and *t*ransfusions of thromboconcentrates in 6 (8%) patients. The most common adverse events (all severity) were headache in 11 (14.7%), nasal bleeding in 10 (13.3%), arthralgia in 4 (5.3%), hypertension in 2 (2.7%) and thrombotic complications in 3 (4%) patients.
Summary and Conclusions: Our study confirms the high frequency of sustained platelet response (50.7%) on romiplostim and the safety of long-term use.

PF681

ITP SECONDARY TO ALEMTUZUMAB-INDUCED RENAL TRANSPLANT: OUTCOME OF 31 PATIENTS

E. Lucchini[1,2,*], A. Luqmani[3], M. Atta[3], S. Deplano[3], M. Layton[3], C. Vladescu[3], D. Paul[3], R. Charif[4], N. Cooper[2,3]

[1]Clinica Ematologica, University of Udine, Udine, Italy, [2]Department of Medicine, Imperial College, [3]Department of Hematology, [4]Nephrology, Hammersmith Hospital, London, United Kingdom

Background: Alemtuzumab (alem), a monoclonal antibody against CD52, causes profound depletion of T and B cells and as well as monocytes, NK and dendritic cells. Its effect is long-lasting; B cells recover after 12 months and T cells recover after more than 36 months. It is indicated for the treatment of multiple sclerosis and is used in chronic lymphocytic leukemia and in several solid organ transplantations, including renal, as induction therapy for the prevention of early rejection. Autoimmune complications after alem therapy include autoimmune thyroid disorders (17-26%), immune thrombocytopenia (ITP) (1-2%) and glomerulopathy (1%). In our experience at Hammersmith Hospital (HH) the incidence of ITP in alem-induced renal transplant patients (pts) is 1-2%.
Aims: To report the outcome of pts developing ITP after alem-induced renal transplants.
Methods: We retrospectively analyzed 31 pts attending the hematology clinic at HH, London, with ITP requiring treatment after an alem-induced renal transplant between 2002 and 2016. ITP was defined as a rapid onset of isolated thrombocytopenia (platelet count <100x10^9/L) with no other explanation. Pts with a platelet count <30x10^9/L or requiring a higher platelet count for other indications were treated. Response (R) was defined as platelet count >=30x10^9/L and at least doubling of baseline count. Complete response (CR) was defined as platelet count >=100x10^9/L.
Results: 21 (67.7%) pts were males, median age at transplant was 38.5 years and median age at ITP diagnosis was 52 years. 42% were deceased-donor transplants, 58% were living-donor transplants. All pts received tacrolimus as post-transplant immunosuppression, 6 (20%) in association with mycophenolate mofetil (MMF). Median time from renal transplant to ITP development was 33 months (range 1-161), median lowest platelet count was 5.5 x 10^9/L. 18 pts (58%) reported bleeding symptoms at diagnosis. One patient achieved spontaneous CR. Of the remaining 30 pts, 27 (90%) received steroids, IVIG or both as first-line treatment; 3 received additional therapy (IVIG+rituximab (rtx); IVIG+romiplostim (romi); rtx alone). The overall response (R) to first line therapy was 63% (53% achieving CR). After a median follow-up of 34 months (range 1-97), 11 pts (36.6%) are still in CR without further therapy. 19 pts required second line

therapy, ten required 3 lines, five 4 lines, two 5 lines and one 6 lines. All eventually responded: 4 to IVIG +/- steroids; 7 to thrombopoietin receptor-agonists (TPORA); 4 to rtx; 2 to MMF; 2 to combination therapy. 7 pts require ongoing treatment (TPORA/MMF). 16 pts (53%) experienced adverse events: 4 steroid-induced diabetes, 1 knee osteonecrosis, 3 thrombosis, 1 VZV encephalitis, 5 malignancies (3 PTLD and 2 solid tumors), 2 deaths from infectious complications (Table 1).

Table 1.

Treatment	1st line (n=30)	2nd line (n=19)	3rd line (n=10)	>3 lines (n=8)	response rate	relapse rate	adverse events (n=16)
TPO	0	2	4	4	80%	12.5%	2**
Steroids	12	3	2	0	64.7%	27.2%	7
IVIG	4	3	1	0	25%	100%	0
steroids + IVIG	11	3	1	0	73.3%	36.3%	4
RTX	1	5	1	0	71.4%	0%	0
TPO + RTX +/- steroids + IVIG	0	1	0	2	66.6%	50%	1**
MMF	0	0	0	2	100%	0%	0
RTX + IVIG +/- steroids	1	1	0	0	100%	50%	1*
TPO + IVIG +/- steroids	1	0	1	0	100%	50%	1*
MMF + romi + RTX	0	1	0	0	100%	0%	0

*deaths in patients who received multiple lines of treatment before infectious events
**thrombosis

Summary and Conclusions: ITP is a non-negligible complication in pts who received alem as induction therapy for renal transplant. Most pts responded to first-line therapy and long-term response rates (RR) were similar to those expected with primary ITP (36.6%). This is a particularly frail subset of pts due to their chronic immunosuppression, with an increased risk of infections and malignancies, and many pts have an increased thrombotic risk. Balancing risks and benefits of each treatment remains the biggest challenge: steroid-sparing regimens should be preferred given the high toxicity in this cohort. TPORA and rtx had high RR and acceptable side effects, with risks of thrombosis or infection the primary factors driving the treatment choice. Further studies are needed to determine the best treatment pathway for these pts.

PF682

AN OBSERVATIONAL, NON-INTERVENTIONAL CLINICAL PRACTICE STUDY OF ROMIPLOSTIM IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA (ITP) – PLATON FINAL RESULTS

G. Mihaylov[1,*], B. Skopec[2], Z. Sninska[3], N. Tzvekov[4], D. Niepel[5], K. Bjorklof[6], V. Ivanushkin[7], P. Cernelc[2]

[1]Clinic of Hematology, Specialized Hospital for Active Treatment of Hematology Diseases AD, Sofia, Bulgaria, [2]Department of Hematology, University Medical Center Ljubljana, Ljubljana, Slovenia, [3]Deprtment of Hematoloty and Blood Transfusion, University Hospital St. Cyril and Metod, Bratislava, Slovakia, [4]Clinic of Hematology, University Multiprofile Hospital for Active Treatment "Dr. Georgi Stranski" EAD, Pleven, Bulgaria, [5]Global Medical Affairs, Amgen (Europe) GmbH, [6]EU Medical Affairs, Amgen (Europe North East), Zug, Switzerland, [7]Department of Biostatistics, Quartesian, Kharkov, Ukraine

Background: The thrombopoietin-receptor agonist romiplostim (rom) is indicated for adult chronic ITP patients (pts) who are refractory to other treatments (SmPC 2018). At the time of study, rom was approved for second line treatment of either non-splenectomized (splen) pts or pts with a contra-indication for splenectomy.
Aims: The aim of this study was to assess the use of rom in routine clinical practice in Bulgaria, Czech Republic, Slovenia, Slovakia, Romania and Russia.
Methods: This international single-arm, observational, non-interventional study enrolled adults with ITP, who had received ≥1 rom application. A period of 6 months prior to rom initiation was documented retrospectively from pt medical charts; the observation period started with rom initiation with a planned duration of 2 years. All pts provided written informed consent. Assessed parameters included pt demographics, rom application, dosage, adverse drug reactions (ADRs), reason of discontinuation, concomitant ITP therapies, clinically relevant bleeding events, and hospitalizations. Presented is the final analysis.

Results: Of 104 enrolled pts, 100 were analyzed (56% female, median age at diagnosis 45 [interquartile range, IQR: 26.5, 57.5] years, 36% splen, 64% non-splen). Of these, 73% completed the study, 27% discontinued prematurely (13 were lost to follow-up, 5 withdrew consent, 3 died [unrelated to rom], 4 stopped due to administrative reasons, 1 for severe ADR [thrombosis], 1 for other reasons). The median time from ITP diagnosis to rom initiation was 1.9 years (IQR: 0.3, 6.7); in pts with prior bleeding, the median time from start of bleeding to initiation of rom was 48 days (IQR: 25.0, 111.0). 49% had received ≥3 prior ITP therapies. During the 6 months before rom initiation the observation-adjusted event rate per 100 pt years was 131 (95% CI: 97.6, 171.4) for bleeding (122 [81.9, 175.6] in non-splen, 143 [90.7, 214.8] in splen pts) and 104 (95% CI: 86.9, 123.0) for ITP-related inpatient hospitalizations (145 [118.1, 175.8] in non-splen, 54 [36.5, 76.3] in splen pts). The median platelet count at rom start was 19 (IQR: 7.5, 42.0) x 10^9/L, increasing to 55 (IQR: 19.0, 100.0) x 10^9/L after 1 week and remaining above 50 x 10^9/L for the entire observation period (Figure 1). Pts received a median rom dose of 2.6 (IQR: 1.1, 4.5) µg/kg/week and a median of 60 (IQR: 14.5, 88.5) injections during a median duration of exposure of 23 (IQR: 6.7; 24.2) months. The observation-adjusted event rate per 100 pt years after rom initiation was 42 (95% CI: 32.9, 52.8) for bleeding (33 [22.8, 45.1] in non-splen, 58 [41.1, 80.6] in splen pts, no grade 3/4 events) and 47 (95% CI: 37.5, 58.6) for ITP-related inpatient hospitalizations (42 [30.5, 55.6] in non-splen, 57 [39.8, 78.7] in splen pts). 13% required splenectomy (*i.e.* 20% of non-splen) after initiation of rom. 4 pts experienced a total of 16 ADRs, with 1 pt experiencing 2 serious ADRs (thrombosis, dysphagia). At the end of study, 25% were in remission (per physician assessment), 45% had active, managed disease, 18% had active, untreated disease, and 3% had died (melanoma malignum, perianal abscess surgery, pneumonia, all unrelated to rom). For 9% ITP status was not reported.

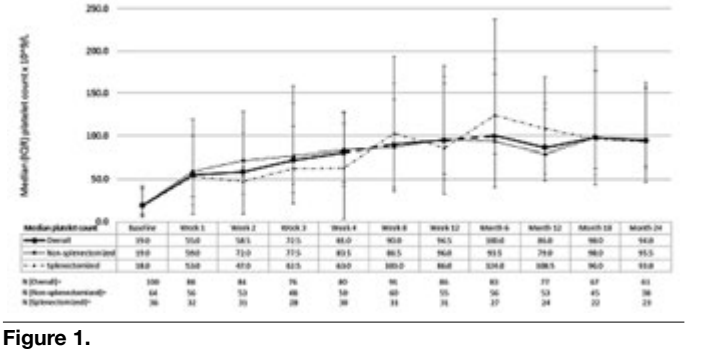


Figure 1.

Summary and Conclusions: In this study, rom increased platelet counts and reduced observation-adjusted rates of bleeding and hospitalizations. 25 pts achieved a remission and 13 pts required splenectomy after initiation of rom. Rom was generally well tolerated.

PF683

COMBINED/CONCOMITANT THERAPY FOR RAPID RECOVERY IN PATIENTS WITH REFRACTORY/RECURRENT IMMUNE THROMBOCYTOPENIA (ITP) AND MODERATE/SEVERE BLEEDING: AN OBSERVATIONAL STUDY

M. Napolitano[1,*], S. Raso[1], M. Santoro[2], A. Passannanti[1], L. LoCoco[3], M.R. Lanza Cariccio[2], S. Mancuso[1], S. Siragusa[1]

[1]Haematology Department, [2]Università di Palermo, [3]Central Laboratory, University Hospital "P. Giaccone", Palermo, Italy

Background: Sequential multiple agents treatment is frequent in patients with refractory/recurrent immune thrombocytopenia (ITP). So far, no data have been published about the use of combined/concomitant therapy in ITP patients in whom a fast increase in platelets count is needed due to severe thrombocytopenia and moderate/severe active bleeding. Primary aim of the current study was to evaluate the outcome of combined/concomitant treatment of adult patients with recurrent/refractory immune thrombocytopenia (ITP) and active moderate/severe bleeding.
Aims: Primary aim of the current study was to evaluate the outcome of combined/concomitant treatment of adult patients with recurrent/refractory immune thrombocytopenia (ITP) and active moderate/severe bleeding.
Methods: We have prospectively evaluated up to 12 months patients admitted to our Hematology Unit from January 2014 to January 2016 with a diagnosis of refractory/recurrent ITP, platelets count <10.000/mmc and active moder-

ate/severe bleeding. Combined/concomitant treatment was defined by a combination of two or more of the following agents, given at the same time: steroids, immunoglobulins, anti-CD20, Romiplostim. A historical cohort of patients diagnosed with relapsed/refractory ITP and treated with single agent was studied to evaluate any difference in 12 months prognosis.
Results: Overall, 18 patients (8F, 10M, mean age 50 years, range:18-87years), received a combined/concomitant treatment: 10 (55.5%) with two agents (High dose immunoglobulins,1g x 2 plus steroids, prednisone 1mg/kg for four weeks), 6 (33.3%) with three agents (Immunoglobulins, prednisone and Rituximab 375mg/mq weekly x 4 weeks) and 2 (11.1%) with four agents (Immunoglobulins, prednisone,Rituximab and Romiplostim). The overall response rate was 75%, with 60% of complete response, defined according to International criteria.2Median time to response was of four weeks; response rate was higher for recurrent (8/18) than refractory ITP (10/18). At Hospital admission three cases of life-threatening bleedings (2 gastro-intestinal and 1 intra-cerebral) were observed in 3 patients, they did not cause death and were succesfully managed with systemic haemostatic agents.Treatment-related adverse events were not reported in any case. Sequelae from intra-cerebral bleeding were observed in one case. After an analysis of the historical cohort, a faster complete response was reached (median response of 5.6 weeks *vs* 4 weeks, p=0.01) in the study group. There was no difference among groups at the12 months follow-up.
Summary and Conclusions: Combined/concomitant multi agent therapy may be a valid option for ITP patients in whom a rapid recovery from severe thrombocytopenia is needed.

Quality of life, palliative care, ethics and health economics

PF684

BRENTUXIMAB VEDOTIN IN RELAPSED/REFRACTORY SYSTEMIC ANAPLASTIC LARGE-CELL LYMPHOMA: A COST-EFFECTIVENESS ANALYSIS

G. Kay[1], C. Parker[1], E. Benson[2], T. Podkonjak[2], R. Selby[3], B. Wang[4], L.-I. Cheng[3,*]

[1]ICON Health Economics, ICON plc, Abingdon, [2]Takeda UK Ltd, Buckinghamshire, United Kingdom, [3]Global Patient Access, Takeda Oncology, [4]Oncology Statistics, Takeda Pharmaceuticals, Cambridge, United States

Background: Systemic anaplastic large cell lymphoma (sALCL) is a peripheral T-cell CD30+ non-Hodgkin lymphoma. CHOP chemotherapy is recommended for newly-diagnosed patients; however, 40-65% of patients will experience relapse/have refractory disease (R/R) after frontline treatment. High-dose therapy and autologous stem-cell transplantation (ASCT) can induce long-term remission in 30-40% of these patients. In 2012, the antibody-drug conjugate Brentuximab vedotin (ADCETRIS) was granted a European marketing authorisation for patients with R/R sALCL based on results from the pivotal Phase 2 study (SG035-0004; NCT00866047).
Aims: To evaluate the cost-effectiveness of brentuximab vedotin in patients with R/R sALCL using a UK payer perspective.
Methods: A partitioned survival model comprising three health states (progression-free survival [PFS], post-progression survival, and death) was developed. The relevant comparator was chemotherapy; this was implemented as a composite of five frequently used regimens (ICE, ESHAP, DHAP, GDP, and Gem-P) to reflect clinical practice. Patients achieving an adequate response to salvage therapy were assumed to undergo ASCT or allogeneic stem cell transplant (alloSCT). Data were obtained from 5-year follow-up from SG035-0004, a systematic literature review and clinical expert opinion. Clinical outcomes (PFS and overall survival [OS]) for brentuximab vedotin, ASCT and alloSCT were extrapolated over lifetime using cure modelling to reflect their curative potential. Clinical outcomes for chemotherapy were based on long-term follow-up for 89 patients in the British Columbia Cancer Agency Lymphoid Cancer database reported by Mak *et al.* (2013). Brentuximab vedotin and chemotherapy were compared naively given SG035-0004 was a single-arm study. Resource use included drug acquisition and administration; ASCT and alloSCT; adverse events; and long-term follow-up. Costs were based on a UK National Health Service (NHS) and Personal Social Services perspective. Sensitivity analyses included: varying the PFS and OS hazards for chemotherapy, using a self-control comparison to inform chemotherapy PFS and varying the rate of ASCT and alloSCT in both treatment arms. Probabilistic sensitivity analyses were also conducted.
Results: In the absence of ASCT/alloSCT, patients who received brentuximab vedotin experienced an additional 7.8 years mean PFS and additional 13.8 years mean OS compared to chemotherapy. Including ASCT/alloSCT in both arms yielded additional PFS and OS for brentuximab vedotin of 7.1 years and 11.9 years respectively. The base case incremental cost-effectiveness ratio (ICER) for brentuximab vedotin was £18,728 per quality-adjusted life-year (QALY) based on a drug acquisition cost of £2,500/vial. ICERs for brentuximab vedotin generated by the deterministic sensitivity analysis ranged between £13,000 and £27,000 per QALY. The probabilities of cost-effectiveness were 77% and 97% at decision thresholds of £30,000 and £50,000 per QALY respectively.
Summary and Conclusions: The ICER for brentuximab vedotin was below the threshold of £30,000 per QALY in the base case and all deterministic sensitivity analyses. As such, brentuximab vedotin is a cost-effective treatment for patients with R/R sALCL.

PF685

REAL-WORLD OUTCOMES ASSOCIATED WITH LENALIDOMIDE, BORTEZOMIB, AND DEXAMETHASONE *VERSUS* BORTEZOMIB AND DEXAMETHASONE IN THE TREATMENT OF NEWLY DIAGNOSED MULTIPLE MYELOMA WITHOUT STEM CELL TRANSPLANT

K. Parikh[1,*], Q. Ni[1], B. Ung[1], A. Agarwal[1], A. Chari[2]

[1]Celgene Corporation, Summit, United States, [2]Mount Sinai Hospital, New York, United States

Background: Recently, the use of triplet regimens featuring 2 novel agents, such as lenalidomide, bortezomib, and dexamethasone (RVd), has increased

for the treatment of multiple myeloma (MM) in the USA. Doublet regimens, such as bortezomib and dexamethasone (Vd), are still a first-line (1L) option for patients (pts) with newly diagnosed MM (NDMM), especially for those who are elderly and/or frail.
Aims: Using a large administrative claims database, this study aimed to evaluate real-world clinical and economic outcomes among pts with NDMM initiating RVd or Vd who had not received a stem cell transplant (SCT).
Methods: From the Truven MarketScan® data set, we identified adult pts with ≥2 claims for MM (International Classification of Diseases, Ninth/Tenth Revision, Clinical Modification codes: 203.0x and C90.0x, respectively) 30 days apart and ≥1 claim for a treatment of interest (index date) during the identification period (01Jan2011 to 31Dec2016). Pts were required to have continuous enrollment for 12 months pre- and post-index date, in addition to ≥1 full cycle of therapy with a valid 1L regimen. Pts were excluded if they had evidence of a prior MM diagnosis, treatment, or receipt of autologous SCT. Propensity score matching (PSM) was utilized to create balanced cohorts. The propensity score (the likelihood of receiving RVd) was estimated from a logistic regression model including age, gender, health plan, payer type, region, index year, medical cost and hospitalizations during baseline, and baseline clinical characteristics. A subgroup analysis of elderly pts (aged >70 years) was also conducted. Time to next treatment (TTNT) was defined as the duration from the index date until the initiation of a second-line therapy. Per patient per month (PPPM) total healthcare costs were calculated for the duration of the index treatment. Kaplan-Meier graphs were used to evaluate TTNT, whereas Wilcoxon rank-sum tests were utilized after PSM to assess differences in PPPM costs.
Results: 1,850 MM pts with ≥1 full cycle of a valid 1L regimen were identified in the database. Of these, 248 received RVd and 579 received Vd as 1L therapy. After PSM, 203 pts remained in each cohort (RVd and Vd) and no significant differences in demographics, clinical characteristics, or pre-medical events were observed. Pts initiating RVd induction therapy had a significantly longer median TTNT (Figure 1) and fewer RVd pts progressed to a second line of therapy (39.4% *vs* 49.3%; *P*=0.045) compared with Vd pts. Similar trends were observed in the elderly subgroup, with RVd pts having a significantly longer TTNT compared with Vd pts (not reached *vs* 34.4 months). Over the duration of the index treatment (time to treatment discontinuation [months] was 13.4 RVd *vs* 6.0 Vd), RVd pts had significantly higher total healthcare costs ($19,776 *vs* $15,028; *P* <0.0001) and pharmacy costs ($8,953 *vs* $517; *P* <0.0001) compared with Vd pts. However, they had significantly lower outpatient costs ($9,313 RVd *vs* $12,469 Vd; *P* <0.0001).

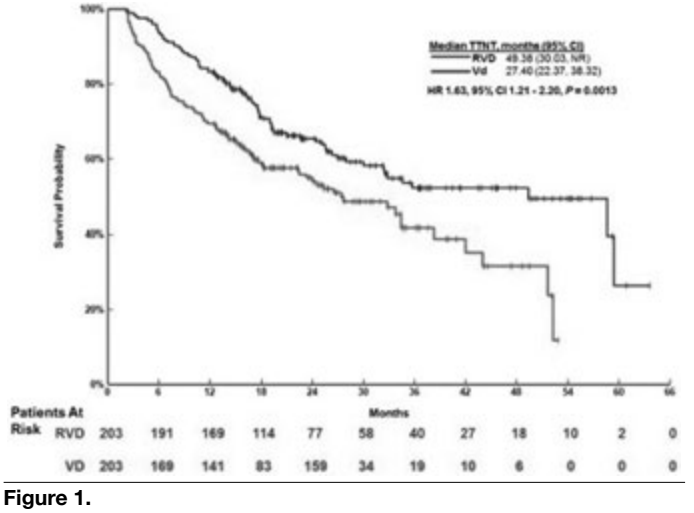


Figure 1.

Summary and Conclusions: This retrospective analysis of pts with NDMM, who had not received a SCT, initiating RVd induction therapy found that they had significantly longer TTNT and were less likely to progress compared with pts with NDMM treated with Vd. A subgroup analysis found that elderly pts (aged >70 years) saw similar benefits. While on therapy, RVd pts incurred higher total healthcare and pharmacy costs, but had fewer outpatient costs.

PF686

CPX-351 FOR THE TREATMENT OF NEWLY DIAGNOSED, THERAPY-RELATED ACUTE MYELOID LEUKEMIA (TAML) OR AML WITH MYELODYSPLASIA-RELATED CHANGES (AML-MRC): NUMBER NEEDED TO TREAT (NNT) TO PREVENT ONE DEATH

A.C. Louie[1,*], K.F. Villa[1], R.J. Ryan[1]

[1]*Jazz Pharmaceuticals, Inc., Palo Alto, CA, United States*

Background: CPX-351, a liposomal co-encapsulation of cytarabine and daunorubicin at a synergistic 5:1 molar ratio, is approved in the United States for the treatment of adults with newly diagnosed tAML or AML-MRC. In a randomized, phase 3 study of CPX-351 *versus* conventional cytarabine/daunorubicin (7+3), CPX-351 significantly prolonged survival and improved remission rates compared with 7+3 in adults aged 60-75 years with newly diagnosed tAML or AML-MRC.
Aims: The current analysis calculated the NNT to prevent 1 death at 2 years as a measure of efficacy of CPX-351 compared with a conventional 7+3 regimen.
Methods: In the phase 3 study (NCT01696084), patients were randomized 1:1 to receive 1-2 induction cycles with CPX-351 (100 units/m^2 [cytarabine 100 mg/m^2+daunorubicin 44 mg/m^2] on Days 1, 3, and 5 [2nd induction: Days 1 and 3]) or 7+3 (cytarabine 100 mg/m^2/day continuously for 7 days [2nd induction: 5 days]+daunorubicin 60 mg/m^2 on Days 1-3 [2nd induction: Days 1-2]). Responders could receive up to 2 consolidation cycles. The study's primary endpoint was overall survival; secondary endpoints included remission rate, duration of remission, and event-free survival. The NNT to prevent 1 death at 2 years with CPX-351 *versus* 7+3 was calculated as the reciprocal of the absolute risk reduction (1/ARR), where ARR equaled the control (conventional 7+3) death rate minus experimental (CPX-351) death rate.
Results: A total of 153 and 156 patients were randomized to receive CPX-351 and 7+3, respectively. Patient characteristics were generally balanced between cohorts; median age was 67.8 (range: 4.2) in the CPX-351 arm and 67.7 (range: 4.1) in the 7+3 arm. Median overall survival was 9.56 months in the CPX-351 arm and 5.95 months in the 7+3 arm (hazard ratio=0.69 [95% CI, 0.52-0.90]; 1-sided *P*=0.003). By 2 years, 84% of patients in the 7+3 arm had died *versus* 67% in the CPX-351 arm, for an NNT of 6 (1/(0.84 – 0.67)). Thus, on average, for every 6 patients treated with CPX-351, 1 death would be prevented over 2 years compared with 7+3. The safety profile of CPX-351 in this study was consistent with the known profile of conventional 7+3. The most common grade ≥3 adverse events in the CPX-351 and 7+3 arms were febrile neutropenia (68% *vs* 71%), pneumonia (20% *vs* 15%) and hypoxia (13% *vs* 15%).
Summary and Conclusions: In this randomized, phase 3 study, CPX-351 prolonged overall survival *versus* 7+3. Based on this survival benefit, the NNT to prevent 1 death at 2 years with CPX-351 *versus* 7+3 was 6 patients. The safety profile of CPX-351 was comparable to that of the conventional cytarabine/daunorubicin 7+3 regimen. These data support the treatment benefit of CPX-351 in adults with newly diagnosed tAML or AML-MRC.

PF687

QUALITY OF LIFE IN NEWLY DIAGNOSED AND RELAPSED DANISH MULTIPLE MYELOMA PATIENTS - A NATIONAL LONGITUDINAL STUDY OF DANISH MYELOMA STUDY GROUP

L.K. Nielsen[1,2,*], M. Jarden[3], C.L. Andersen[4], H. Frederiksen[1,2], H. Gregersen[5], A. Klostergaard[6], M.S. Steffensen[7], P.T. Pedersen[8], B. Broch[9], M. Frederiksen[10], B.A. Jensen[4], C. Helleberg[11], A.K. Mylin[3], N. Abildgaard[1,2]

[1]*Quality of Life Research Centre, Department of Haematology, Odense University Hospital,* [2]*Odense Patient data Explorative Network, Odense University Hospital, Odense,* [3]*Department of Haematology, Copenhagen University Hospital, Copenhagen,* [4]*Department of Haematology, Zealand University Hospital, Roskilde,* [5]*Department of Haematology, Aalborg University Hospital, Aalborg,* [6]*Department of Haematology, Aarhus University Hospital, Aarhus,* [7]*Department of Haematology, Regional Hospital West Jutland, Holstebro,* [8]*Department of Haematology, South West Jutland Hospital, Esbjerg,* [9]*Department of Haematology, Vejle Hospital, Vejle,* [10]*Department of Haematology, Hospital of Southern Jutland, Aabenraa,* [11]*Department of Haematology, Herlev Hospital, Herlev, Denmark*

Background: Multiple myeloma (MM) patients experience many symptoms and consequently report reduced health-related quality of life (QoL) compared to patients with other haematological malignancies or solid cancers. The prognosis of MM has improved markedly due to the introduction of new and more effective, but also potentially toxic therapies. The study, "Quality of life in Danish multiple myeloma patients" (QoL MM study) was initiated in late 2016 to investigate QoL in MM patients from diagnosis to late, advanced disease and throughout different treatments. With inclusion of the general population of MM patients the study offers "real world

data" on QoL in MM patients. This is the first analysis of data from the ongoing QoL MM study.
Aims: To compare QoL between patients with treatment-demanding relapsed or progressive MM (RMM) and patients with newly diagnosed, symptomatic MM (NDMM).
Methods: The QoL MM study is a prospective, national and observational study with additional retrospective data collection from Danish nationwide registers. All haematological departments in Denmark participate in the study. NDMM and RMM patients are eligible for study entry at the time of symptomatic or treatment demanding myeloma. Exclusion criteria are inability to understand the Danish language, or a mental or psychiatric disorder that prevents the patient from completing a questionnaire. Four validated QoL instruments: the European Organisation For Research And Treatment Of Cancer Quality Of Life Questionnaire (QLQ-C30), the Multiple Myeloma module (MY20), the Chemotherapy-Induced peripheral neuropathy questionnaire (CIPN20), and the Short-form health survey version 2-4-week recall (SF12v2) are used, starting at baseline prior to initiated therapy followed by every 4 weeks for 6 months and hereafter every 3 months until 24 months. Data on prior and initiated anti-myeloma treatments are collected from the Danish National Patient Registry. We report mean baseline score differences between the two groups adjusted for earlier identified confounding of gender, age and comorbidity. Data interpretation was based on both statistically significant and clinically relevant differences.
Results: The 1 December 2017, 239 patients had been screened and 144 patients included; 80 NDMM and 64 RMM patients. Of the NDMM patients, 21% were elderly (≥76 years) and 56% were Intermediate Fit or Frail according to the International Myeloma Working Group myeloma frailty score. 22% of the RMM patients were elderly and 31% were Intermediate Fit or Frail. 39% of the RMM patients were included at third or later relapse. For global QoL, physical, role, emotional function and future perspectives the RMM patients reported statistically significant better functioning of moderate or large clinical relevance compared to the NDMM patients. Also, for fatigue, pain, appetite loss and constipation the RMM patients reported statistically significant fewer symptoms of moderate or large clinical relevance compared to patients with NDMM. For none of the domains, the NDMM patients reported a clinical relevant better score than the RMM patients (Table 1).

Table 1.

Mean baseline scores, p-values for confounder adjusted between group comparisons and size of clinical relevant difference

Quality of life domains	NDMM Mean baseline scores (SD)	RMM Mean baseline scores (SD)	*p-value*	Clinically relevant difference
EORTC QLQ-C30				
Global quality of life	50.9 (26.9)	61.7 (19.8)	*0.006*[1]	Moderate
Physical functioning	65.2 (28.5)	76.9 (19.0)	*0.002*[1]	Moderate
Role functioning	48.1 (38.9)	66.9 (27.6)	*0.001*[1]	Large
Emotional functioning	68.0 (22.9)	81.4 (15.4)	*<0.001*[1]	Moderate
Fatigue	47.1 (30.2)	36.3 (21.8)	*0.007*	Moderate
Pain	51.7 (37.1)	30.2 (27.5)	*<0.001*	Large
Appetite loss	25.3 (30.8)	14.1 (21.3)	*0.022*[1]	Moderate
Constipation	19.4 (26.5)	9.9 (20.3)	*0.008*[1]	Moderate
EORTC QLQ-MY20				
Future Perspectives	47.8 (26.8)	62.8 (27.7)	*0.002*	Moderate

SD; standard deviation, [1]The regression analysis made is based on residuals without normal distribution.

Summary and Conclusions: RMM patients with treatment demanding disease report clinically relevant better scores than NDMM patients for nine QoL domains. The findings could be assigned to effective disease monitoring of known MM patients, but also to a psychological reaction caused by the cancer diagnosis in NDMM patients. Through the longitudinal follow-up, our study will address the impact on QoL during and after anti-myeloma treatments.

PF688

A QUALITATIVE ANALYSIS OF PSYCHOLOGICAL HEALTH MEASURES IN SURVIVORS OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA AFTER BEING TREATED WITH BFM-ALL PROTOCOLS

A. Iqbal[1,*], T. Kaur[2], S. Mukherjee[3], M. Bhattacharyya[1]

[1]Clinical Hematology, Institute of Haematology and Transfusion Medicine, [2]Clinical Psychology, Cankids India, [3]Clinical Hematology, NRS Medical College and Hospital, Kolkata, India

Background: Pediatric ALL is one of the curable childhood cancers. The remission rates after the intensive phase of all BFM-ALL trials approach 90% across risk categories and relapse rates are acceptable. The psychosocial consequences of successful treatment are an increasing concern as these children step into adolescence and adulthood. These concerns are amplified in countries like India because of the lack of social support groups, social stigma associated with cancer and because the treatment may also lead to significant life compromises like drop from school and vocational training.
Aims: - To analyze the psychosocial health of the participants objectively using a standard survey instrument
- To understand the social factors responsible for psychosocial illhealth in different dimensions
Methods: 104 survivors of pediatric ALL across 4 biggest centres catering to childhood cancers in Kolkata were analysed using a survey instrument called PedsQL™ generic core scale The 18 points PedsQL™ generic core measuring system encompasses 5 dimensions (general satisfaction, inclusion of family, communication, emotional needs, and academic and social attendance) as delineated to be measures of psychological health by the World Health Organisation. Each dimension had 3-4 questions and responses were recorded on a 5 point scale of 0-4. The survey was conducted by physical interview after prior appointment. The instrument was made available in English, Hindi and Bengali for participants' convenience. All the participants were documented cases of childhood ALL who had completed the entire therapy using one of the BFM protocols (BFM 90, 95 and 2002) and were treatment free for more than 5 years. The appointments and resource personnel were organised by an NGO called Cankids India.
Results: The participants were between 7 – 26 years of age. The median time from completion of therapy to participation was 7.1 years. Participants were grouped in three categories according to the age at interview: less than 10 years of age (A), 10-18yrs of age (B), and >18yrs (C). A used parent proxy report while B and C were analysed using self and parent report. In a scale of 1, the cumulative median score across all age groups was 0.70. Maximum score was found in C (0.8) followed by A (0.75), and B (0.66). Across the dimensions of psychological health, the lowest score was obtained in 'inclusion of family' in the A group and 'academics and social attendance' in B and C groups. Father's occupation, history of major treatment related comorbidities, treatment protocol violation, and absence of family support groups are found to be the main factors associated with lower scores while the specific protocol, age at treatment initiation, and use of cranial RT were not associated with adverse psychological health.
Summary and Conclusions: Psychological ill-health as a result of intensive, long duration treatment used in childhood ALL is profound, and its impact is maximum in the adolescent age group. Identification of age category specific factors is important to curb their effects and implement specific psychological and vocational measures.

PF689

COMBINED VISIBLE AND INFRA RED LOW LEVEL LASER THERAPY FOR PREVENTION OF ORAL MUCOSITIS IN PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION

F. Tabeie[1], M. Tabarraei[2,*], A. Tavakoli[2], L. Izadi[3]

[1]Nuclear medicine, [2]hematology oncology, Shahid Beheshti university of medical sciences and heath services, [3]Chemistry, Elm o sanat university, Tehran, Iran, Islamic Republic Of

Background: Oral mucositis (OM) is known to bea side effect of hematopoietic stem cell transplantation (HSCT).
Aims: The purpose of this study was to evaluate the efficacy of prophylactic combined visible and infra red low level laser therapy (LLLT) to reduce the incidence and sevierity of oral mucositis in patients undergoing hematopoietic stem cell transplantation (HSCT).
Methods: Fifty patients were randomly assigned into two LLLT and control groups. In LLLT group 25 patients received prophylactic LLLT prior to HSCT in addition to standard care for oral mucositis including administration of topical antimicrobial agents and mouth washes. LLLT were performed intraorally three times a week with a combined irradiation of 630 nm visible and 780 nm infra red laser with duration of 40 seconds for each lasers to the tongue and soft palate. The laser parameters used for each lasers were frequency of 100 Hz, power of 100 mW and an energy of 2.5 J. Patients were evaluated after the completion of HSCT for incidence and severity of oral mocositis. The control group underwent the same protocol for LLLT group, but with zerro intensity of lasers.
Results: Incidence of oral mocositis in LLLT and control groups were 25.9% and 70% respectively which was significantly lower in the LLLT group (p

= 0.003) and also mucositis scores were significantly lower in the LLLT group than in the control group (p = 0.004).
Summary and Conclusions: This study indicated that a combined protocol of visible and infra red application of LLLT can reduce the incidence and severity of oral mucositis in patients undergoing HSCT.

PF690

DETERMINANTS AND CLINICAL SIGNIFICANCE OF MUSCULOSKELETAL SYMPTOMS IN PATIENTS WITH CHRONIC GRAFT-*VERSUS*-HOST DISEASE

A. Zelic Kerep[1,2,*], F. Pirsl[1], S. Steinberg[3], S. Mitchell[4], L. Curtis[1], E. Cowen[5], D. Pichard[5], G. Joe[6], L. Comis[6], A. Im[7], A. Berger[8], L. Parsons-Wendell[1], M. Kenyon[1], J. Baruffaldi[1], S. Raja[9], J. Nashed[1], D. Pulanic[2,10,11], K. Baird[12], R. Gress[1], S. Pavletic[1]

[1]Experimental Transplantation and Immunology Branch, Center for Cancer Research (CCR), National Cancer Institute (NCI), National Institutes of Health, Bethesda, United States, [2]Division of hematology, University Hospital Zagreb, Zagreb, Croatia, [3]Biostatistics and Data Management Section, Center for Cancer Research, National Cancer Institute, [4]Outcomes Research Branch, Division of Cancer Control and Population Sciences, CCR, NCI, [5]Dermatology Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, [6]Rehabilitation Medicine Department, Clinical Center, National Institutes of Health, Bethesda, [7]Division of Hematology/Oncology, University of Pittsburgh Medica Center, Pittsburgh, [8]Department of Pain and Palliative Care, Clinical Center, [9]Center for Cancer Research, National Institutes of Health, Bethesda, United States, [10]School of Medicine, University of Zagreb, Zagreb, [11]Faculty of Medicine, University of Osijek, Osijek, Croatia, [12]Pediatric Oncology Branch, CCR, NCI, Bethesda, United States

Background: Muscle cramps, arthralgia and myalgia are common in chronic graft-*versus*-host disease (cGVHD) patients. However, the prevalence and associated factors have not been sufficiently investigated.
Aims: To determine the frequency of muscle cramps, joint and muscle aches and associated factors in cGVHD.
Methods: Data from 334 adult patients (pts) who participated in the NCI natural history study of cGVHD (NCT00092235) from 10/2004 to 5/2016 were analyzed. Five-point Lee cGVHD Symptom Scale (LSS) reported muscle cramps and joint and muscle aches was dichotomized (less symptom bother – 0,1,2; severe symptom bother – 3,4) and tested for associations with: Short Form 36 (SF36, physical (PS) and mental subscale (MS)), 2-minute walk test (2MW), grip strength, joint range of motion, and Human Activity Profile (HAP, maximum and adjusted (AAS)), clinical and laboratory data. Univariate analysis was followed by multivariable logistic regression (MLR) model identified by backward selection on a training set (n=167) and evaluated on the testing (n=167) set to determine its joint ability to predict the symptom category.
Results: In this cohort (median age 49 years, 72% severe NIH global severity - GSS), 75.4% reported joint and muscle aches (36.8% severe), 74.3% muscle cramps (33.5% severe), and 19.5% of pts reported having severe bother of both symptoms (LSS 3-4). In univariate analysis severe muscle cramps were associated with reduced SF36 PS and MS ($p<0.005$), Lee modified total score (total score reduced by the scores for muscle cramps, joint and muscle aches and muscle weakness) and a strong trend ($0.005<p<0.05$) towards association with skin sclerosis, LSS skin thickening (ST), itchy skin, limited joint movement (LJM), shortness of breath with exercise and loss of energy (LOE). MLR (Table 1) jointly identified variables as being at best, moderately predictive in both training and testing sets. Muscle cramps severity did not show associations with concomitant calcineurin inhibitor treatment, level of magnesium, use of statins and antidiabetics. In univariate analysis, severe joint and muscle aches were significantly associated ($p<0.005$) with reduced 2MW, HAP AAS, SF36 PS and MS, Karnofsky performance status, and with greater LSS for skin rash, ST, LJM, LOE, need to sleep more, depression, anxiety, difficulty in sleeping and decreased chloride. Factors jointly identified by MLR (Table 1) were reasonably predictive in both training and testing sets. There was no association with serum anti-CCP or rheumatoid factor. Muscle cramps and joint and muscle aches were not associated with NIH GSS, intensity of immunosuppression, or with the clinicians` therapeutic intent at the time of enrollment (active *vs* non-active).
Summary and Conclusions: These results show a notable burden of muscle cramps and joint and muscle aches in pts severely affected by cGVHD. Both symptoms of interest in this study did not show an association with NIH GSS, which indicates that these symptoms require special consideration when assessing disease severity and therapeutic response. A significant association with a host of potential etiological factors (calcineurin inhibitors, diabetes, serum auto-antibodies, statins) has not been identified, implying cGVHD as an etiological factor. Muscle weakness was also analyzed, however, numerous variables showed significant or a strong trend towards significance of association, likely indicating the complexity of this endpoint.

Table 1.

MUSCLE CRAMPS	JOINT AND MUSCLE ACHES
• *Limited joint movement*, **p=0.012**, OR 1.3 • *Shortness of breath with exercise* **p=0.05** OR 1.3 • *Itchy skin* **p=0.008**, OR 1.4	• *Short Form 36 Physical subscale*, **p=0.02**, OR 0.9 • *Need to avoid certain foods due to mouth pain*, **p=0.008**, OR 1.6 • *Limited joint movement*, p<0.0001, OR 3.0 • *Depression*, **p=0.01**, OR 1.6
• 50% of patients with severe muscle cramps identified in an independent set of data	• 56.9% of patient with severe aches identified in an independent set of data
• 75.9% of patients who did not have severe muscle cramps were correctly identified in independent data set	• 84.5% of patients who did not have severe aches were correctly identified in independent data set

Multivariable logistic regression results for muscle cramps and joint and muscle aches.

PF691

IMPACT OF CLINICAL AND SOCIAL FACTORS ON QUALITY OF LIFE (QOL) IN PATIENTS (PTS) WITH TRANSFUSION-DEPENDENT (TDT) AND NON-TRANSFUSION-DEPENDENT (NTDT) BETA-THALASSEMIA: A MULTICENTER STUDY

V. Viprakasit[1,*], M.D. Cappellini[2], A. Kattamis[3], P. Sutcharitchan[4], D. Mahmoud[5], J. Pariseau[5], A. Laadem[5], A. Khan[6], X.H. Hu[7], A. Taher[8]

[1]Siriraj Hospital, Mahidol University, Bangkok, Thailand, [2]Ca Granda Foundation, Ospedale Policlinico IRCCS, University of Milan, Milan, Italy, [3]Aghia Sophia Children's Hospital, Athens, Greece, [4]Chulalongkorn University and King Chulalongkorn Memorial Hospital, Bangkok, Thailand, [5]Celgene Corporation, Summit, [6]Nathan S. Kline Institute for Psychiatric Research, Orangeburg, [7]Formerly of Celgene Corporation, Summit, United States, [8]American University of Beirut Medical Center, Beirut, Lebanon

Background: Life expectancy for pts with β-thalassemia has increased significantly in recent years. Improved survival is accompanied by considerable ongoing healthcare needs related to this chronic condition; therefore, QoL has emerged as a primary focus of comprehensive pt care.
Aims: To use data from a multicenter study to investigate the clinical and social factors that impact the difference in QoL outcomes between pts with TDT and NTDT β-thalassemia in the routine clinical care setting.
Methods: Adult pts with β-thalassemia were prospectively enrolled in an observational study from centers in Italy, Greece, Lebanon, and Thailand. All pts completed Short Form 36 Health Survey version 2 (SF-36v2) and Functional Assessment of Cancer Therapy (FACT)-Anemia (An) questionnaires at baseline, and then once every 3 weeks using a hand-held electronic device; this analysis evaluated QoL in pts with TDT and NTDT at study entry. Transfusion dependence was defined as receiving ≥6 red blood cell units in the 24 weeks prior to study entry with no transfusion-free period for ≥35 days during that time.
Results: In total, 102 pts were enrolled, of which 52 had TDT and 50 had NTDT. The mean age of pts was 31.2 years and 70 (68.6%) were female. On average, pts with TDT were 3.6 years younger (P=0.06) and had moderately higher hemoglobin levels at baseline (8.8 *vs* 8.2 g/dL; P=0.02). Of 102 pts enrolled, 65 (64%) were of White ethnicity (39 NTDT, 26 TDT) and 37 (36%) were of Asian ethnicity (11 NTDT, 26 TDT). Older pts (≥30 years) and pts with a long diagnosis history (≥25 years) had worse QoL *versus* younger pts and pts with a short diagnosis history (P <0.05). Currently married pts had better QoL (P <0.05). Pts transfused at baseline (100% of TDT pts; 10% of NTDT pts) had better QoL than non-transfused pts. Pts with NTDT reported lower QoL scores on all SF-36v2 domains and summary scores, except for Role-Physical. On average, pts with NTDT experienced lower QoL *versus* their TDT counterparts on the domains of General Health (39.5 *vs* 44.0; P=0.01), Vitality (49.3 *vs* 53.7; P=0.01), and Mental Health (46.8 *vs* 50.8; P=0.01), and in the Mental Component Summary Score (46.5 *vs* 50.8; P=0.01). Similarly, pts with NTDT reported lower FACT-An questionnaire QoL scores on all domains; statistically significant differences were observed for Emotional Well-Being (18.5 *vs* 20.0; P=0.02), Functional Well-Being (20.0 *vs* 23.2; P <0.01), and FACT-General (82.9 *vs* 89.4; P=0.01). Pts recruited by the Thai center reported higher QoL scores on the Functional Well-Being (23.11 *vs* 20.74; P <0.05) and Fatigue Scale domains (41.75 *vs* 38.27; P <0.05) compared with pts from centers in Italy, Greece, and Lebanon.

Summary and Conclusions: In the routine clinical care setting, there are critical unmet medical needs for pts with NTDT as they reported lower QoL scores compared with pts with TDT, as captured by two health questionnaires (SF-36v2 and FACT-An), across all domains except one. There is a need for new interventions to treat pts with NTDT and reduce their burden of disease. Significant differences between pt populations from different geographical locations were identified, suggesting social factors had an impact on difference in QoL between pts with TDT and NTDT. Pts from Thailand reported higher QoL scores for domains on the FACT-An questionnaire *versus* pts from centers in Italy, Greece, and Lebanon; half of the pts with TDT were from the Thai center.

PF692

RAPID AND COMPREHENSIVE GENOMIC CHARACTERIZATION OF HEMATOLOGICAL MALIGNANCIES USING A HYBRID-CAPTURE TARGETED SEQUENCING PANEL

M. Gruber[1,2,*], D. Alpar[3], G. Hoermann[4,5], N. Fortelny[1], B. Erguener[3], L. Muellauer[6], G. Greiner[4], H. Esterbauer[4], M. Schuster[3], I. Simonitsch-Klupp[6], M. Gurbisz[4], R. Kain[6], O. Wagner[4], U. Jaeger[2], C. Bock[1]

[1]CeMM Research Center for Molecular Medicine, [2]Department of Internal Medicine I, Division of Hematology and Hemostaseology, Medical University of Vienna, [3]Biomedical Sequencing Facility, CeMM Research Center for Molecular Medicine, [4]Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria, [5]MLL Munich Leukemia Laboratory, Munich, Germany, [6]Department of Pathology, Medical University of Vienna, Vienna, Austria

Background: Next-generation sequencing (NGS) fuels our knowledge of the genomic landscape of malignancies. Translating this into clinical management warrants comprehensive genomic characterization of tumor samples. To determine the disease-relevant genome of hematological malignancies, multiple assays are currently applied in parallel.
Aims: We here introduce a single hybrid-capture-based NGS workflow for the comprehensive characterization of clinically relevant alterations, comprising copy number aberrations (CNAs), insertions and deletions (InDels), structural rearrangements, single nucleotide variants (SNVs), T or B cell receptor (*TCR* and *BCR*) rearrangements and to assess *IGHV* somatic hypermutation rate.
Methods: Based on curation of literature and public databases, loci of 400 genes, 83 non-coding regions and all *BCR* and *TCR* segments were selected for customized Nextera panel design (Illumina). Fresh or FFPE-derived tumor samples from 26 patients with various hematologic malignancies and 7 reference standards (Horizon) were assessed. Libraries were prepared using Nextera Rapid Capture or an optimized TruSeq protocol and sequenced with 75bp (FFPE) or 150bp (fresh samples) paired-end protocols on HiSeq 3/4000 instruments (Illumina).
We identified SNVs and InDels by aligning sequencing reads to the reference genome, followed by somatic variant calling against a set of healthy controls. Pathogenic mutations were ranked based on low Exome Aggregation Consortium (ExAC)-frequencies in normal populations. CNAs were discovered based on genome-wide read-depth of on- and off-target reads, structural rearrangements were detected using svABA. *BCR* and *TCR* rearrangements were identified by MiXCR, followed by SPAdes for assembly of consensus sequences to determine somatic hypermutation rate using IMGT. Genomic profiles were manually curated for pathogenic relevance using database and literature research. Finally, results were compared with corresponding current diagnostic procedures
Results: Coverage of ≥100x was obtained in 98% percent of the target region, with mean target coverage of 750x-2000x across samples. Within reference standards, we detected all mutations at variant allele frequencies (VAF) of ≥2% and 93% at VAF <2%. Among patient samples, we found 71 putatively pathogenic SNVs (0-6/sample) and 42 InDels (0-4/sample). In addition, we identified 24 (0-3/sample) chromosomal and subchromosomal amplifications and deletions, confirming and extending FISH results in all samples that were provided with at least 25% tumor purity. We also found 5 (0-2/sample) large chromosomal rearrangements (*e.g. IGK-BCL2*) and 49 clonal B cell and T cell receptor rearrangements. In the latter, we identified clonal V(D)J rearrangements, and somatic hypermutation rate in the *IGHV* segment highly correlated with results of Sanger sequencing (r^2=0.9989). In summary, our panel could independently reproduce all results obtained by various conventional methods and detected numerous additional variants of potential pathogenic or therapeutic relevance (Figure 1).
Summary and Conclusions: We introduce a NGS-based assay for highly comprehensive and versatile characterization of hematological patient samples. For all specimens with ≥25% tumor cell purity and appropriate DNA quality, we were able to obtain detailed genomic profiles including somatic SNVs, CNAs, clonality assessment and *IGHV* mutation rate. Our panel therefore represents a powerful tool to facilitate an efficient and streamlined patient stratification with the goal to improve personalized treatment strategies.

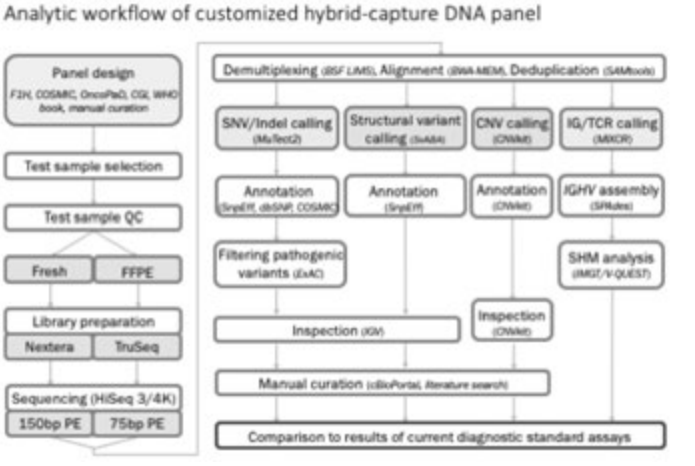


Figure 1.

PF693

UNTANGLING HOSPITALIZATIONS IN SYSTEMIC AMYLOIDOSIS: PATIENT CHARACTERISTICS, ECONOMIC COST AND CLINICAL OUTCOMES

T.P. Quock[1,*], T. Yan [2], R. Tieu[2], A. D'Souza[3], M. Broder[2]

[1]Prothena Biosciences Inc, South San Francisco, [2]Partnership for Health Analytic Research, LLC, Beverly Hills, [3]Medical College of Wisconsin, Milwaukee, United States

Background: The amyloidoses are a group of rare protein-folding disorders characterized by extracellular tissue deposition of misfolded and aggregated autologous proteins as ß-pleated sheet fibrils. Although many organs can be affected, the heart is frequently involved in two forms of systemic amyloidosis: light chain (AL) and transthyretin (ATTR) amyloidosis. Patients with systemic amyloidosis frequently require hospital care.
Aims: To understand patient characteristics, healthcare resource utilization, costs, and clinical outcomes associated with systemic amyloidosis treated in US hospitals.
Methods: This retrospective analysis used 2014-2016 data from the Premier Perspective® Database. The study population comprised of hospitalized patients aged ≥18 years with ≥1 inpatient claim consistent with systemic amyloidosis [International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM) codes: 277.30, 277.39; International Classification of Diseases, 10th Revision, Clinical Modification (ICD-10-CM) codes: E85.4x, E85.8x, E85.9x] in any diagnosis field; the first qualifying hospitalization was included. Patients with evidence of other types of amyloidosis (ICD-9-CM codes: 277.31; ICD-10-CM codes: E85.0x-E85.3x) or chronic inflammatory diseases that may lead to other types of amyloidosis were excluded. Study outcomes included hospitalization costs (in 2016 USD), length of stay (LOS), intensive care unit (ICU) use, and mortality.
Results: 7,533 patients were admitted to the hospital with a diagnosis consistent with systemic amyloidosis; mean (SD) age was 72.8 (11.7) years, 46.3% were female, 68.0% were White, and 78.0% had Medicare. The mean (SD) Charlson Comorbidity Index was 3.2 (2.1), and 59.5% of patients had either, or both, cardiac involvement or renal disease. Among patients with cardiac involvement, 74.6% had congestive heart failure. For all amyloidosis-related admissions, the mean (SD) total hospitalization cost was $18,110.70 (25,245.78) and mean (SD) LOS was 7.4 (9.9) days. 90.1% of patients had urgent/emergent admission. During the hospital stay, 30.7% of patients were admitted to the ICU, with a mean (SD) ICU LOS of 4.1 (5.4) days. In-hospital mortality was 6.7%.
Summary and Conclusions: Disease burden and hospital costs associated with AL or ATTR amyloidosis are high. Based on hospital discharge records, almost 60% of the patients had cardiac and/or renal disease. Mean hospitalization costs were >$18,000 per patient and many patients were admitted to ICU. New therapies aimed at improving organ response have the potential to reduce disease burden and to yield substantial cost savings.

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ERECTILE DISFUNCTION IN HEMATOLOGICAL MALIGNANCIES

F. Krstanoska[1,*]

[1]General Hospital Slovenj Gradec, Slovenj Gradec, Slovenia

Background: Erectile disfunction (ED) is a sexual disfunction, usually in men over forty years old. It is defined as a disability to get an erection enough hard for penetration and to sustain that erection until the the end of intercourse, it is present in most of the cases in last 6 months and make the individual stress and unsatisfaction. Erectile disfunction can be primary (from the beginig of sexual life) or secondary (become later after normal sexual activity).

Aims: The aim of the study in 2016 - 2017 is to find prevalence and severity of erectile disfunction in male patients with hematology malignancy diseases: chronic lymfocytic leukemia, multiple myeloma and myeloproliferative neoplasm at diagnosis and after 1 year of treatment. Patients were classified into three groups based on diagnosis, the WHO performance status, hemoglobin level, renal function, present or absent another vascular disease (cerebral stroke, peripheral occlusive disease, coronary disease and diabetes mellitus).
Methods: All the patients answered at diagnosis questionary 5 item version of the International index of erectile function IIEF- 5 with specificity 0,88 and sensitivity 0,97. After one year of therapy, survivors answered the same 5 questions, each of them has 0 - 5 point and max 25 points. The score depends on severity of erectile disfunction and classified like severe ED 1-7points, moderate 8- 11, mild to moderate 12 -16, mild 17 - 21, normal erectile function 22 - 25 points. The study includes 60 male patients, 20 with chronic lymphatic leukemia, 20 with multiple myeloma and 20 with myeloproliferative neoplasm.
Results: At the diagnosis in group with CLL the average age was 75 years old, 55% of patients had severe ED, moderate 35% and 10% had mild ED. After one year of treatment the results were - 3 patients died, 70% had severe ED and 30% patients had moderate ED. In patients with multiple myeloma at diagnosis the average age of men was 55 years old, the results were: severe ED 30%; mild ED 45%, mild 20%, no ED 5%. After one year patients had: severe ED 50% , moderate 40%, mild 10%.Group of myeloproliferative neoplasm included patients with chronic myeloid leukemia and policitemia rubra vera, average age 65 y, the prevalence at diagnosis: severe ED 70%, moderate 20%, mild 10%.After one year 5 patients died, the others had: severe ED 50%, moderate 20%, mild 30%
Summary and Conclusions: In our patients with hematological malignancy at diagnosis only one patient had not erectile disfunction. After therapy for one year the number of patients with ED raised, only in group with myeloproliferative diseases due to chemotherapeutic agens an supportive care declines severe ED from 70 to 50%,in this group 5 patients died in that group become smaller for 25%. In the group with MM 70% patients after chemotherapy and autologous stem cell transpltion were in complete remission, still after one year the severe ED was found in 70% of them. The possible reason for erectile disfunction in hematological malignancy are: chemoimunotherapy, anemia, renal disease, fatigue, psychological stress, depression, isolation and absence of or fear of partner after the confirmation of malignant disease.

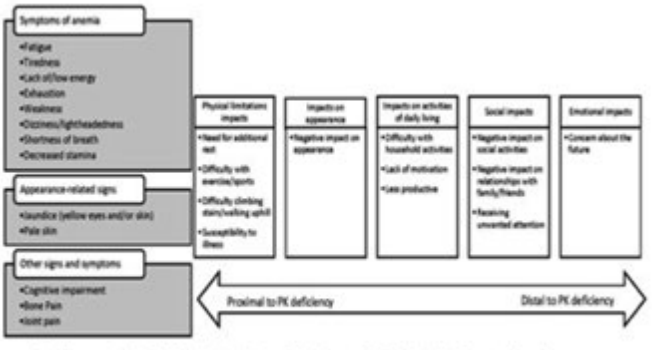
PF695

THE BURDEN OF DISEASE IN PYRUVATE KINASE DEFICIENCY: PATIENTS' PERCEPTION OF THE IMPACT ON HEALTH-RELATED QUALITY OF LIFE

S. Salek[1,*], J. Cohen[2], S. Egan[3], T. Wells[4], B. Witherspoon[4], A. Ryan[4], S. Bodie[5], R. Klaassen[6], R. Grace[7]

[1]School of Life and Medical Sciences, University of Hertfordshire, Hatfield, United Kingdom, [2]Patient Advocate, Wrentham, [3]Endpoint Outcomes, Long Beach, [4]Endpoint Outcomes, Boston, [5]Agios Pharmaceuticals, Inc., Cambridge, United States, [6]Division of Hematology/Oncology, Children's Hospital of Eastern Ontario, Ottawa, Canada, [7]Pediatric Hematology/Oncology, Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Boston, United States

Background: Pyruvate kinase (PK) deficiency is a rare hemolytic anemia caused by mutations in the *PKLR* gene. Though the clinical features of PK deficiency in individual patients can vary substantially, common signs and symptoms may include: fatigue, dyspnea, jaundice, and splenomegaly. Due to the rarity of PK deficiency, an understanding of its burden on patients' lives remains limited and there is no published research that describes the overall experience of living with PK deficiency.
Aims: The aim of this qualitative study was to explore how signs and symptoms of PK deficiency impact adult patients' health-related quality of life (HRQoL).
Methods: Interviews were conducted with twenty-one adults with PK deficiency and symptomatic anemia in the United States, the Netherlands, and Germany. All transcripts were coded using processes guided by established qualitative research methods, including grounded theory and constant comparison method. Conceptual saturation was achieved for nearly all concepts.
Results: Participants were a mean age of 38.9 (19-58), 52% female, and 86% splenectomized. The most common signs and symptoms reported were yellow eyes (90.5%), tiredness (85.7%), yellow skin (81.0%), fatigue (71.4%), low energy (61.9%), and shortness of breath (61.9%). Fifty-nine impacts of PK deficiency were reported and divided into nine major themes: physical, appearance, social, emotional, activities of daily living (ADL), leisure activities, work (or school), sleep, and cognitive deficit. The most commonly reported impacts were a need for additional rest (85.7%), difficulty with exercise or sports (76.2%), susceptibility to illness (66.7%), negative impact on appearance (61.9%) receiving unwanted attention (57.1%), and negative impact on social activities (52.4%). A conceptual model was developed to demonstrate the profound impact that signs and symptoms of PK deficiency have on dimensions of patients' HRQoL (Figure 1).



Conceptual model for pyruvate kinase (PK) deficiency showing impacts found to be most directly related (proximal) to a participants' symptom experience.

Figure 1.

Summary and Conclusions: This is the first study that provides patient perspective on the burden of living with PK deficiency. The development of tools to capture patient-report of signs and symptoms of PK deficiency and their impact on HRQoL will lead to greater appreciation of the challenges that living with PK deficiency presents, and allow examination of the effects of future treatments and pharmacologic interventions on HRQoL of patients with PK deficiency.

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RELEVANT REDUCTION OF POMALIDOMIDE-RELATED NEUTROPENIA BY INTENSIVE USE OF MYELOID GROWTH FACTOR

M.S. Parisi[1,*], V. Calafiore[1], E. Martino[1], V. Del Fabro[1], A. Romano[1], G. Sapienza[1], C. Conticello[1], F. Di Raimondo[1]

[1]hHmatology, Policlinico Universitario, Catania, Italy

Background: Neutropenia and complications of neutropenia such as febrile neutropenia (FN) or pneumonia, are one of major hematological toxicity associated with cancer chemotherapy. It often results in drug dose reduction or delay or interruption, thus leading to morbidity, mortality, and high costs of patient management. Granulocyte colony-stimulating factor (G-CSF) is a myeloid growth factor able to reduce the risk of neutropenia and its complications, enabling safe and effective chemotherapy dose intensity. Hematological and not hematological toxicity of immunomodulatory drug pomalidomide has been studied in association with dexamethasone particularly in three main clinical trials (MM02, MM03 and MM10) in relapsed refractory multiple myeloma (RRMM) patients. The most frequent hematological adverse event of grade 3/4 is neutropenia (between 48% and 50%), followed by anemia (22% to 32%) and thrombocytopenia (between 19 and 31%). Among non-hematological grade 3/4 adverse events pulmonary infections have an incidence between 10% and 20%. In the same clinical trials the frequency of dose reduction was about 30% and treatment was temporary interrupted in about 60%, of patients, while it was definitively interrupted in about 5% (3-6%) of patients.
Aims: In order to limit Pomalidomide and Dexametasone (PomaD) reduction and/or interruption and prevent neutropenia-related adverse events we tried to use G-CSF prophylaxis in our RRMM patients.

Methods: Here we describe the analysis of a total of 51 RRMM patients treated with PomaD. G-CSF was used (mean dose 60 MU) one week after starting pomalidomide therapy when leukocyte counts were ≤2.5· 109/L and neutrophils ≤1.5· 109/L. Median age was 68 years (range 42-78), median number of prior therapies was 4 (range 2-7) and the majority of patient (33/51) were treated for almost 6 cycles (overall treatment duration mean 7.7 months). We decided to maintain a >2.0· 109/L leukocyte count with ANC >1.5· 109/L in attempt to reduce the incidence of neutropenia and its complications.
Results: In our cohort of patients, hematological adverse events occurred at a reduced rate. In particular, neutropenia was present only in 9% of cases, anemia in 5,5% of cases and thrombocytopenia in 3,5% of cases. Reduced frequency of neutropenia among our patients should be attributable to the use of prophylaxis with G-CSF one week after starting of PomaD in that patients with leukocyte counts ≤2.5· 109/L and ANC ≤1.0· 109/L. In this way, we maintained for almost 6 cycles a median of >3.5· 109 leukocyte count (mean 4· 109/L) with a median of neutrophils >1.5 · 109/L (mean 2· 109/L). Table 1 describes mean and median of leukocyte count and ANC for every cycle till the sixth in 33/51 RRMM patients that were treated for almost six cycles with PomaD. We also obtain a significant reduction of the incidence of FN, present only in 11% of patients. In addition, in this setting of prophylaxed patients, the dose of pomalidomide was reduced only in 12.5% and temporary interrupted in less than 20%.

Table 1.

	pre-treatment	I cycle	II cycle	III cycle	IV cycle	V cycle	VI cycle	Total
Median of leukocyte count· 10^9/L	3,76	3,94	4,04	4,1	4	4,28	4,12	4,04
Median of ANC · 10^9/L	2,04	1,95	1,62	1,86	1,91	1,79	1,71	1,86
Mean of leukocyte count · 10^9/L	4,11	4,26	4,07	4,17	4,26	4,23	4,02	4,16
Mean of ANC· 10^9/L	2,42	2,46	1,79	2,04	2,06	2,03	1,86	2,09

Summary and Conclusions: Our data suggest that intensification of G-CSF prophylaxis in RRMM patients during PomaD therapy can reduce frequency of serious adverse events and enable full dosage of pomalidomide with limited reduction or interruption of this drug.

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REAL WORLD EVIDENCE BASED COST-EFFECTIVENESS ANALYSES (CEA) OF NILOTINIB *VERSUS* IMATINIB AS FIRST-LINE TREATMENT IN THE MANAGEMENT OF CHRONIC MYELOID LEUKEMIA (CML) IN CHINA

H. Zhu[1], S. Chen[2], J. Xuan[3], J. Wu[4], Y. Zhang[5], Y. Song[5,*], J. Luo[6], J. Tan[2], C. Liu[7], S. Qu[8], T. Xiong[8], J. Ming[8]

[1]West China Hospital of Sichuan University, Chengdu, [2]The First Affiliated Hospital of Soochow University, Suzhou, [3]Sun-Yat-Sen University, Guangzhou, [4]Tianjin University, Tianjin, [5]Henan Cancer Hospital, Zhengzhou, [6]The Second Hospital of Hebei Medical University, Shijiazhuang, [7]Novartis Pharmaceuticals (China) Oncology, Beijing, [8]IQVIA, Shanghai, China

Background: Nilotinib (NIL) is the only second-generation TKI recommended in Chinese guidelines for first-line treatment of CML-CP [1]. Discontinuation of TKI therapy (treatment-free remission [TFR]) in CML-CP patients with a sustained deep molecular response is a treatment goal that is recommended in the Chinese guidelines [1]. TFR is an approved indication for NIL based on two registration studies (ENESTop: NCT01698905; ENESTfreedom: NCT01784068) demonstrating a favourable risk-benefit ratio for this approach.
Aims: The aim of this study was to understand the impact of TFR on clinical outcomes and costs using a CEA model comparing NIL and IM as first-line treatments. This has not been previously evaluated in China.
Methods: Real world clinical and cost data were obtained from top-tier hospitals in China. A Markov model based on a previously published structure [2] was applied to simulate and evaluate the long term clinical and economic outcomes associated with different CML treatments (NIL or IM). Treatment discontinuation was modelled on the ELN (2013) and Chinese guidelines (2016), and time to first $MR^{4.5}$and sustained first $MR^{4.5}$were based on the results of ENESTnd (NCT00471497). TFR data were extracted from ENESTfreedom, and the frequency of molecular monitoring was based on the FDA approved recommendations.
Results: The incremental cost-effectiveness ratio (ICER) was dominant for TAS. The cost-savings were primarily driven by reduced drug cost associated with NIL first line TFR, and more patients in the NIL arm remaining on first line treatment, which was less costly than the second line treatment (*i.e.*, dasatinib). A sensitivity analysis was conducted varying key model assumptions (*e.g.* treatment benefits and costs), nonetheless NIL remained dominant.
Summary and Conclusions: The RWE and CEA support that first line NIL use can result in greater opportunity for patients to be eligible for TFR, reduced treatment costs and is a cost-effective option for patients when TFR is considered.

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PF698

LIVING AND COPING WITH CHRONIC LYMPHOCYTIC LEUKEMIA: A QUALITATIVE STUDY OF GREEK PATIENTS' EXPERIENCES ON BEHALF OF ERIC, THE EUROPEAN RESEARCH INITIATIVE ON CLL

C. Karamanidou[1], A. Xochelli[1,*], P. Ghia[2], P. Panayiotidis[3], K. Stamatopoulos[1]

[1]Institute of Applied Biosciences, CERTH, Thessaloniki, Greece, [2]Division of Experimental Oncology, IRCCS San Raffaele Scientific Institute and Università Vita-Salute San Raffaele, Milan, Italy, [3]First Department of Propaedeutic Medicine, University of Athens, Athens, Greece

Background: Chronic illnesses require complex management that includes patients' active participation and engagement in care. Chronic lymphocytic leukemia (CLL) is a paradigmatic chronic hematologic malignancy whose management and nature are hard for both patients to understand and physicians to explain. Therefore, understanding CLL patients' perspective is an essential first step towards communicating effectively, responding to needs and promoting active engagement in care thus enhancing quality of life (QoL).
Aims: To explore the illness experience of Greek CLL patients within the context of ERIC's CLL patient empowerment program.
Methods: An in-depth qualitative study with semi-structured interviews was conducted with CLL patients from all over Greece (n=30; both treated and untreated). Data collection was considered as complete by the investigators when saturation was reached *i.e.* no new themes emerged. Interpretative Phenomenological Analysis (IPA) was performed separately by a health psychologist and a hematologist with a 98% inter-rater reliability score. IPA looks for patterns of convergence and divergence across cases. Main themes are generated from all participants' accounts but an idiographic approach is maintained so that individual variations within a theme are not lost.
Results: Seven main themes emerged from the participants' accounts. (i) *Diagnosis*: The announcement of the word leukemia and its association with an aggressive disease cause patients concern, insecurity, depression, anxiety as well as worry for loved ones. (ii) The *illness*: Most patients consider themselves fortunate to be diagnosed with 'the good leukemia'. However, CLL still causes uncertainty in patients who are baffled by the lack of symptoms and anxious about the efficacy, availability and cost of therapy. (iii) *Causes:* Patients attribute their illness to environmental factors *e.g.* exposure to chemicals; lifestyle factors *e.g.* consumption of certain foods; but also emotional factors like sadness or stress after negative life experiences. (iv) *Life with CLL:* Changes are experienced by patients on a social, professional, emotional and personal level. Most view their illness experience as an important life lesson and hold a positive outlook on life. (v) *Relationship with physician:* Patients clearly appreciate their physician, whom they regard as "a friend", "a family member" and sometimes referred to him/her as "god". They emphasize the need for a holistic approach including practical and emotional support, empathy, truth, trust, and equality (vi) *Coping and self-management:* Patients use a spectrum of strategies to cope with their illness and manage their emotions. These include active coping strategies such as information seeking, lifestyle changes, creative hobbies or volunteering; also, emotion-focused coping strategies such as humor, acceptance, positive thinking, social support etc. (vii) *Past and current needs:* A variety of elements mentioned would have helped patients

throughout the course of the illness namely, reliable information regarding the disease and treatment, peer contact, experience exchange, psychological support and emergency preparedness.
Summary and Conclusions: This study's findings have captured an insider's perspective into the CLL illness experience and identified patient needs throughout the illness course. More studies are ongoing within ERIC at an international level so as to identify CLL patient needs in different healthcare contexts that will guide and inform the design of CLL specific patient empowerment programs.

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COMPARISON OF THE PAPER-BASED AND ELECTRONIC VERSION OF A NEW PATIENT-REPORTED OUTCOME MEASURE IN HAEMATOLOGICAL MALIGNANCY FOR USE IN CLINICAL PRACTICE, HM-PRO: AN EQUIVALENCE STUDY

P. Goswami[1,*], T. Ionova[2], E. Oliva[3], M. Salek[1]

[1]Life and Medical Sciences, University Of Hertfordshire, Hatfield, United Kingdom, [2]University Clinic, St.Petersburg State University and Multinational Centre for Quality of Life Research, St Petersburg, Russian Federation, [3]Haematology Unit, Grande Ospedale Metropolitano, Reggio Calabria, Italy

Background: The use of quality of life (QoL) information in patients with haematological malignancies (HM) in routine clinical practice is of value to evaluate risks/benefits of treatment, capture unmet patient's needs, and facilitate physician-patient communication. The new developed HM-PRO is a specific instrument for such use. It is undergoing assessment to meet the minimum standards set out by the FDA for PRO instruments. The use of an electronic patient-reported outcome version not only provides a quick and mostly reliable assessment of QoL but also would enhance the integrity and accuracy of data and is also incited by regulatory authorities.
Aims: To assess the equivalence between paper-based & electronic application of the newly developed HM-PRO.
Methods: Following ISPOR ePRO Guidelines, patients diagnosed with different HM at different stages of disease or treatments were recruited from inpatient/outpatients setting of 7 secondary care hospitals in the UK. The paper-based and electronic versions of the HM-PRO were completed with a 30-minute interval to minimise crossover effect. Relevant treatment & demographic data were collected. Instrument version and order effects were tested on total score of the two parts (Part A: Quality of life & Part B: Signs & symptoms) of HM-PRO in a 2-way ANOVA with patients as random effects. Intra-class correlation coefficients (ICC, 95% CI) and Spearman's rank correlation coefficients were used to evaluate test-retest reliability and reproducibility. 10 patients were randomly selected for cognitive interviewing and content analysis of transcribed interviews was carried out qualitatively.
Results: 193 patients with different HM (ALL-12; AML-28; CLL-17; CML-13; MM-33; INHL-16; ANHL-22; HL-14; MDS-15; and MPN- 23), and median time since diagnosis of 1.7 years (IQR 0.002-25.8) were recruited into the study (Table 1).

Table 1.

Demographic characteristics (n=193), HM-PRO scores, test-retest reliability and reproducibility

Characteristic			Median	IQR
Age (Years)			66.5	17.9-89.1
Time since diagnosis (years)			1.7	0.002 – 25.8
			n	%
Sex		Male	118	61.5
		Female	72	37.5
Inpatient/outpatient		Inpatients	13	6.8
		Outpatient	180	93.8

Domain/Scale (no. Of Items)	Paper mean score (SD)	IQR	Electronic mean score (SD)	IQR	Mean Difference	$r(r_s)$	ICC (95% CI)	P Value*
Physical Well-being (7)	4.80 (3.7)	2-8	4.94 (3.7)	2-8	0.14	0.95 [0.95]	0.97 [0.97-0.98]	0.17
Social Well-being (3)	1.48 (1.6)	0-2	1.50 (1.6)	0-3	0.02	0.88 [0.88]	0.94 [0.92-0.96]	0.45
Emotional Behaviour (11)	8.08 (4.9)	4-11	8.19 (5.0)	4-12	0.10	0.95 [0.95]	0.98 [0.97-0.98]	0.40
Eating & Drinking (2)	1.42 (1.3)	0-2	1.41 (1.4)	0-2	0.02	0.92 [0.93]	0.96 [0.95-0.97]	0.52
Part A (QoL) (23)	15.61 (9.6)	7.5-23	15.89 (9.6)	8-23	0.28	0.97[0.97]	0.98 [0.98 -0.99]	0.07
Part B (Signs and Symptoms) (17)	7.49 (5.2)	4-10	7.64 (5.2)	4-10	0.15	0.96 [0.96]	0.98 [0.97-0.98]	0.22

a – Wilcoxon signed rank test
r – Pearson's correlation coefficient
r_s – Spearman's rank correlation coefficient

A total of 192 cases were used to assess effects of the type of the questionnaire and the order of the administration. No interactions between the type of questionnaire and administration were found for both Part A (*P=0.95*) and Part B *P=0.72*) respectively. Similarly, there was no effect of the type of the questionnaire Part A *(P=0.76*)& Part B (*P=0.78*) and of the order of administration, Part A (*P=0.75*) and Part B (*P=0.058*). The reliability indices (Table 1) were in the acceptable range, with Spearman's correlations greater than 0.9, and ICC ranging from 0.94 to 0.98. The Wilcoxon signs rank test for paired samples showed that the mean scores for each domain and the scale were not statistically different, even without Bonferroni correction for multiple testing. With respect to cognitive interviews, all the 10 patients were able to understand the items in the way they were intended and were able to respond to both electronic and paper version of the instrument spontaneously and without any difficulty.The difference between the completion time for paper (6.4 min) and electronic (7.3 min) versions of the instrument was not statistically significant (P=0.11).
Summary and Conclusions: There were no significant differences between Part-A & Part-B scores of the HM-PRO for paper-based and electronic application. Also, instrument version and administration order effects were not significant. The electronic application possesses good reliability & face validity with minor modifications to its format compared to the original paper-based version.

PF700

A SEER-MEDICARE ANALYSIS OF NOVEL TREATMENT USE AND ASSOCIATED OUTCOMES AMONG AFRICAN AMERICAN PATIENTS WITH MULTIPLE MYELOMA

S. Ailawadhi[1,*], Z. Clancy[2], Z.-Y. Zhou[3], W. Tang[3], C. Cheung[3], Z. Zhou[3], J. Xie[3], K. Parikh[2]

[1]Mayo Clinic Florida, Jacksonville, [2]Celgene Corporation, Summit, [3]Analysis Group, Inc., Boston, United States

Background: Multiple myeloma (MM) is the most common blood cancer among African Americans (AA). Recent advances in novel therapies and autologous stem cell transplants have increased treatment options for patients with MM and improved overall survival (OS).
Aims: To compare clinical and economic outcomes in AA patients with newly diagnosed MM who received novel *vs* non-novel agents as first-line treatment for MM.
Methods: Patients with MM in the Surveillance, Epidemiology, and End Results (SEER)–Medicare database, which links the Medicare claims database and the National Cancer Institute's SEER database at the patient level, from 2007 to 2013 were included. Continuous Medicare enrollment for 6 months before (baseline) and after the date of first-line treatment initiation (index date) was required, except in the case of patient death. Recipients of novel agents (eg, lenalidomide, pomalidomide, bortezomib, or carfilzomib) and non-novel agents were identified based on the first-line treatment patients received. OS, myeloma-specific survival (MSS), and healthcare costs were compared between AA patients who received novel agents and AA patients who received non-novel agents. Multivariable regression models were used to control for baseline characteristics. Among AA patients who received novel agents, outcomes were further compared between those who received lenalidomide plus a steroid agent (Rd) *vs* bortezomib plus a steroid agent (Vd) as first-line treatment. Steroid agents included dexamethasone and prednisone.
Results: Among the 657 AA patients included, 398 (61%) received novel agents as first-line treatment. This proportion was significantly lower compared with Caucasian patients (66%; P=.02). AA patients who received novel agents were significantly younger than those who received non-novel agents (mean, 70 *vs* 73 years; P <.001). Both median OS (3.1 *vs* 1.8 years; P <.001) and MSS (not reached *vs* 4.8 years; P <.01) were significantly longer in AA patients who received novel agents than in those who received non-novel agents. After adjusting for baseline characteristics, the risk of all-cause death remained lower in the group of AA patients who received novel agents (adjusted hazard ratio [HR]=0.77; P=.01). AA patients who received non-novel agents had numerically higher monthly medical costs than did those who received novel agents (adjusted mean difference, $951), which were driven by significantly higher inpatient ($2571) and emergency room ($38) costs (both P <.05). Total healthcare costs (medical plus pharmacy) were comparable between the 2 cohorts. Among AA patients receiving novel agents, 102 (26%) and 147 (37%) received first-line treatment with Rd and Vd, respectively. Duration of treatment (DOT) was longer with Rd than with Vd (median, 4.6 *vs* 4.0 months; adjusted HR=0.72; P=.04). A trend of longer time to next-line treatment initiation or death (a proxy of progression-free survival) was observed among those who received Rd (median, 17.6 *vs* 12.1 months; P=.06).
Summary and Conclusions: AA patients with MM who received novel

agents as first-line treatment for MM had significantly longer OS and MSS than did AA patients who received non-novel agents. Among AA patients who received novel agents, the use of Rd as first-line treatment was associated with longer DOT. There was also a trend toward longer time to next-line treatment initiation or death with first-line Rd *vs* Vd. These results suggest that increasing the use of regimens with novel agents, particularly Rd, may improve outcomes among AA patients with MM.

PF701

USE OF PATIENT-CENTRED OUTCOMES IN THE MANAGEMENT OF ANEMIA: A SYSTEMATIC REVIEW

P. Staibano[1,2,*], I. Perelman[2,3], J. Lombardi[12], A. Davis[4], A. Tinmouth[2,4], M. Carrier[2,4], C. Stevenson[2], E. Saidenberg[2,4]

[1]Faculty of Medicine, [2]Ottawa Hospital Research Institute, [3]School of Epidemiology and Public Health, University of Ottawa, [4]The Ottawa Hospital, Ottawa, Canada

Background: Patient-centred outcomes (PCOs) evaluate the impact of disease on patient wellbeing and are critical for managing chronic disease and understanding the impact of medical therapies from a patient perspective. Anemia is a common consequence of many acute and chronic illnesses. Therapies for anemia, including iron supplementation, erythropoietin-stimulating agents (ESAs), and transfusion, improve hematological parameters and survival. The efficacy of anemia therapies has been widely studied, however the impact of anemia and its management on PCOs remains largely unknown.
Aims: To characterize and assess the literature addressing the usage of PCOs to investigate the effect of anemia management on PCOs in patients with various underlying medical conditions.
Methods: We conducted a search of the PUBMED, EMBASE, PsychInfo, and CINAHL databases for studies published until January 2017. Eligibility criteria included full-text observational cohorts, case series (N >10), and clinical trials published in English. Inclusion criteria were any studies with patients with anemia who were undergoing any treatment for anemia, and measured at least one PCO before and after anemia intervention. Risk of bias was assessed for all studies and all studies were reviewed by two or more independent reviewers. Descriptive statistics were employed to analyze study characteristics, PCO tool use, the quality of PCO reporting, and to assess the effect of anemia treatment on patient wellbeing from a PCO perspective.
Results: Of the 3224 articles identified in the initial search, 130 eligible articles were included in the qualitative synthesis. We report that 62% of studies were RCTs, of which 44% were open-label, and 30% were observational cohort studies. Most studies (46%) assessed anaemia in oncology patients. Other studies included renal, hematologic, cardiac, and gastrointestinal patients. Most studies (78%) investigated ESAs as the anaemia treatment, while 21% investigated either iron supplementation or transfusion. Only 25% of studies reported a PCO as a primary endpoint. A wide variety of PCO tools were used, the FACT/FACIT questionnaires being the most common tools (33%), followed by the linear analogue scale (18%), and the SF-36 (14%). Moreover, 53% of studies utilized one PCO tool, whereas 47% utilized two or more PCO tools. The quality of PCO reporting varied greatly across studies, with some presenting all PCO subscales in great detail, while other studies had incomplete or limited reporting of PCOs. Among the studies reporting hemoglobin outcomes, 68% reported an increase in patient haemoglobin levels. Of these studies, 51% also reported an improvement in PCOs. Moreover, 7% of studies did not report an improvement in haemoglobin; of these studies, 63% reported an improvement in PCOs while 38% did not.
Summary and Conclusions: Studies of PCOs in anemia have largely focused on oncology patients administered ESAs. Other anemic patient groups and other anemia treatments are less well studied from a patient-centred perspective. While the majority of studies were RCTs, a large subset was open-label, only employed a single PCO tool, and did not report PCOs as a primary endpoint. Only half the studies reported a concordant improvement in both haemoglobin levels and PCOs following anemia treatment. Future studies should standardize the use and reporting of PCOs to better understand the patient-centred impact of treatments for anemia.

PF702

COST UTILITY ANALYSIS OF THE USE OF LETERMOVIR FOR THE PROPHYLAXIS OF CYTOMEGALOVIRUS IN PATIENTS UNDERGOING ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANT – TWO SCENARIO ANALYSES FROM ITALY

F. Ciceri[1,2,*], U. Restelli[3], D. Croce[3], V. Pacelli[3], C. Girmenia[4]

[1]Hematology and BMT Unit, IRCCS San Raffaele Scientific Institute, [2]University Vita-Salute San Raffaele, Milan, [3]Center for Health Economics, Social and Health Care Management, LIUC - Università Cattaneo, Castellanza, [4]Department of Haematology, Oncology and Dermatology, Azienda Policlinico Umberto I - Sapienza University, Rome, Italy

Background: Cytomegalovirus (CMV) is a common infection that can be asymptomatic during its latency phase. Immune system dysfunctions, as hematologic malignancies and hematopoietic stem cell transplant (HSCT), solid organ transplantation, and HIV infection, can lead to CMV disease. Current standard of care for CMV infections is represented by preemptive strategy, based on antiviral treatment triggered by CMV detection in blood by different methods. Letermovir is an orphan drug recently approved by European Medicines Agency for CMV prophylaxis in adults CMV seropositive recipients (R+) undergoing allogeneic HSCT.
Aims: The analysis aimed at estimating, from the Italian National Health Service (NHS) perspective, the cost-effectiveness of the use of letermovir CMV prophylaxis in adult R+ patients receiving an allogenic HSCT, compared with a no-prophylaxis strategy assuming preemptive antiviral administration in both groups.
Methods: A cost-effectiveness decision analytic model was adapted to the Italian context in a lifetime horizon, considering costs and effectiveness (quality adjusted life years - QALYs) of two different strategies: use of letermovir prophylaxis for CMV infection followed by preemptive in patients experiencing CMV reactivation despite prophylaxis *vs* no-prophylaxis (current standard practice). The effectiveness of letermovir was based on a phase III clinical study and estimated in the model considering the incidence of clinically significant CMV infection, CMV disease, CMV-related hospitalisations, opportunistic infections, graft-*versus*-host disease (GVHD), and mortality. Utility values were derived from the results of a clinical study and refers to the mean EQ-5D index at week 14, 24 post-transplant in the two scenarios letermovir and no-letermovir arms and in the post-trial period. The direct medical costs considered (2017) are related to drugs and to the clinical events reported upon. Due to lack of cost data published in literature related to the Italian context, the cost of each event was estimated considering its management as in real clinical practice, considering the opinion of two Italian experts of two distinct stem cell transplantation programs. Both costs and QALYs were discounted at a 3% annual rate.
Results: CMV prophylaxis would lead to per capita incremental costs between 13,500 € and 14,300 €, and to per capita incremental QALYs of 0,432. The incremental cost effectiveness ratio (ICER) is between 31,400 and 33,100 €/QALY. In a hypothetical cohort of 1,000 patients, CMV prophylaxis would lead to a reduction of significant CMV infections (-240; -60.0%), of cases of CMV disease (-4.8; -66.7%), and of CMV-related hospital admissions (-45.7; -59.8%). Probabilistic sensitivity analysis showed a percentage of ICERs below a 40,000 €/QALY threshold of 61.8% and 67.2%.
Summary and Conclusions: Letermovir use is cost-effective for the Italian NHS with an ICER consistently below the threshold of 40,000 €/QALY identified by the Italian Health Economics Association. Its use for CMV prophylaxis in adults R+ patients undergoing a HSCT would represent a substantial step forward, compared with current standard of care, for patients management in terms of better outcomes.

PF703

ERN-EUROBLOODNET: THE EUROPEAN REFERENCE NETWORK IN RARE HEMATOLOGICAL DISEASES. 1ST YEAR OF IMPLEMENTATION

M.D.M. Mañú Pereira[1,*], V. Gutierrez Valle[1], M. Pellegrini[2], B. Gulbis[3], P. Fenaux[2]

[1]Josep Carreras Leukaemia Research Institute / Hôpital St. Louis, Barcelona / Paris, Spain, [2]Hôpital St. Louis, Paris, France, [3]Hôpital ERASME-CUB, LHUB-ULB, Brussels, Belgium

Background: Due to the scarcity of patients and knowledge, rare diseases, affecting less than 1 in 2000 individuals, are the area in public health in which joint efforts among European Member States is most crucial. ERN-EuroBloodNet, the ERN in Rare Hematological Diseases (RHD), results from a joint effort of the European Network on Rare and Congenital Anaemias (ENERCA), the European Hematology Association (EHA), and European hematology patient organisations represented in both the EURORDIS European Patient Advocacy Groups (ePAGS) and the EHA Patient Organisations Workgroup. ERN-EuroBloodNet gathers 66 highly skilled and multidisciplinary healthcare teams in 15 Member States. Aimed at facing the challenges of RHD, it gathers also advanced specialized medical equipment and infrastructures which will facilitate concentration of resources for the design, validation and implementation of high-quality and cost-effective services.
Aims: ERN-EuroBloodNet's main goal is to improve the healthcare and

overall quality of life of patients with a rare hematological disease: 1) Give equal access to highly specialized healthcare delivery across Europe 2) Promote the best practices in prevention, diagnosis and safe clinical care across Europe 3) Disseminate cutting-edge knowledge and facilitate continuous medical education 4) Provide inter-professional consultation by sharing of expertise and safe exchange of clinical information 5) Foster European cooperation in highly specialized procedures for diagnosis, promotion of clinical trials and innovative treatments and research.
Methods: RHD are covered in two main thematic groups: non-oncological and oncological diseases that have been divided into 4 and 2 sub-thematic areas, respectively *i.e.* 1) Rare red blood cell defects 2) Bone marrow failure (BMF) and hematopoietic disorders 3) Rare Bleeding-Coagulation disorders and related diseases and 4) Haemochromatosis and hereditary iron metabolism disorders; 1) Myeloid malignancies and 2) Lymphoid malignancies. Methods and tasks aiming to achieve ERN-EuroBloodNet specific objectives have been splitted into five categories of Transversal Field of action (TFA): 1)Cross border health 2)Best practices 3)Continuing medical education 4)Telemedicine 5)Clinical trials and research.
Results: Based on common unmet needs to all RHDs identified during the 1st year of the network, activities developed were focussed to map the existing expertise, services and facilities available for RHD across ERN-EuroBloodNet Members as starting point for network objectives deployment in the consecutive phases.
Summary and Conclusions: Based on the results, the annual programme for the second year of ERN-EuroBloodNet is structured in two main branches: a) Expanding and exploiting the dynamic European repository of ERN-EuroBloodNet members profiles, diseases covered, existing guidelines for RHD, patient registries implemented at the regional, national or EU level, on-going clinical trials and collaborative research initiatives launched in the first period of the network. b) Establishing an educational programme identifying areas requiring continuing medical education in order to contribute to solving inequalities among Member States in the delivery of best care, contributing also to the consolidation of the Clinical Patients Management System by the promotion of its use among ERN-EuroBloodNet members as the space for inter professional consultation of complex cases within the ERN.

PF704

PALLIATIVE CARE INDICATION IN NON-HODGKIN'S LYMPHOMA PATIENTS: A COMPARISON TO SOLID TUMOR PATIENTS

L. Morais[1,*], E. Fernandez[2], L. Fernandez[2], S. Garcia[2], S. Alonso-Alvarez[1], E. Colado[1], T. Bernal[1,2]

[1]Servicio de Hematología, Hospital Universitario Central de Asturias, [2]Departamento de Medicina, Universidad de Oviedo, Oviedo, Spain

Background: The prognosis of diffuse large B cell lymphoma (DLBCL) in patients who are not candidates for autologous stem cell transplant (SCT) is poor, with an expected median overall survival of 6 months. According to actual end-of-life care (EOL) standards, this population should receive early palliative care (PC) integrated with standard oncologic care when relapse is documented. Conversely, recent evidence shows an excess of aggressiveness in this population compared with the EOL care in solid neoplasms. The reasons for this difference remain to be determined.
Aims: To compare EOL care in DLBCL and stage-IV lung cancer in a tertiary care center and identify factors related to late or no PC referral.
Methods: Consecutive relapsed DLBCL not candidates for SCT and stage-IV lung cancer patients were studied. Data are shown as percentage or median (interquartilic range). Univariant comparisons of the main indicators of EOL aggressiveness between the 2 cohorts were performed using Chi-square or Wilcoxon tests as appropriate. Overall survival was calculated from the date of relapse of lymphoma and the date of lung cancer diagnosis until last follow up or death using the Kaplan-Meier method.
Results: From 1st October 2004 to 1stAugust2017, 51 patients with relapsed DLBCL not candidates to autologous SCT and 98 stage-IV lung cancer patients were recorded. Median age at diagnosis was 73 (69-76) and 63 (59-71) years respectively (p<0.001). Male sex was more frequent in lung cancer (68% *vs* 47%, p=0.02). ECOG and number of comorbidities according to the Charlson comorbidity index were similar in the two cohorts. Ninety-two percent lymphoma patients were treated with R-CHOP as first line therapy. At relapse, all but 2 lymphoma patients received chemotherapy again, with no patient included in a clinical trial. Survival for lymphoma patients was significantly lower than for lung cancer patients (8[4-13] *vs* 12[9-19] months respectively, p=0.047). Discussion between patients and physician about prognosis was documented in 8% DLBCL patients as opposed to 85% lung cancer patients (p<0.001). Patients with lymphoma spent more days in hospital as compared to lung cancer patients (23[14-49] *vs* 14[6-24] days, p<0.001). Referral to PC Unit was documented in 14% DLBCL compared to 48% lung cancer patients (p<0.0001). There were no differences in the percentage of patients receiving chemotherapy in their last 14 days of life between cohorts. More lymphoma patients were transfusion dependent in their last 2 months of life (28% *vs* 3%, p<0.0001). Lymphoma patients died more frequently in hospital compared to lung cancer patients (63% *vs* 44%) but this difference was not statistically significant (p=0.08).
Summary and Conclusions: Palliative Care is seldom prescribed in relapsed diffuse large B cell lymphoma patients. This may lead to a more aggressive, suboptimal treatment at EOL in this population. Efforts must be made to improve the process of information between patient and physician and to avoid unavailing hospitalizations.

PF705

EVALUATING THE CORRELATION BETWEEN FATIGUE AND QUALITY OF LIFE IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AND TREATED WITH BIOSIMILAR EPOETIN ALFA FOR CHEMOTHERAPY-INDUCED ANEMIA: THE CIROCO STUDY

K. Laribi[1,*], P. Rodon[2], E. Kfoury[3], M. Hacini[4], R.-F. Soumoudronga[5], S. Choquet[6]

[1]Centre Hospitalier du Mans, Le Mans, [2]Centre Hospitalier de Périgueux, Périgueux, [3]Centre Hospitalier Dubois Brive, Brive La Gaillarde, [4]Centre Hospitalier Métropole Savoie, Chambéry, [5]Sandoz S.A.S., [6]AP-HP, Hôpitaux Universitaires Pitié-Salpêtrière, Paris, France

Background: Anemia is common in patients with cancer who are receiving treatment with chemotherapies. Symptoms of anemia, the most common of which is fatigue, can have a substantial negative impact on the quality of life (QoL) of affected patients.
Aims: To determine the correlation between fatigue and QoL in patients with hematological malignancies and treated with biosimilar epoetin alfa (Sandoz) for chemotherapy-induced anemia (CIA).
Methods: This was a non-interventional, prospective, multicenter study conducted in France (66 centers). Key inclusion criteria included: age >18 years; ≥2 cycles of chemotherapy planned after inclusion in the study; CIA and receiving treatment with biosimilar epoetin alfa. Data were collected on the first day of the chemotherapy cycle (inclusion, T0), after 2–3 chemotherapy cycles (follow-up; T1), and following 4–6 chemotherapy cycles (end of follow-up; T2). Fatigue (patient-reported outcome) was measured using a Visual Analog Scale (VAS; range 0–10) and QoL was evaluated using the EORTC QLQ C30 questionnaire.
Results: Data are reported for a subgroup of patients with hematological malignancies (FAS population; n=104): non-Hodgkin lymphoma, n=48; multiple myeloma, n=28; chronic lymphoid leukemia, n=18; Hodgkin lymphoma, n=7; other, n=3. At T0, the type of chemotherapy included: induction treatment, n=87; consolidation treatment, n=2; and salvage treatment after relapse, n=15. Mean (SD) hemoglobin level at baseline was 9.5 (±0.9) g/dL. Mean (SD) increase in hemoglobin between T0 and T1 was 1.3 (±1.8) g/dL, and 0.7 (±1.4) g/dL between T1 and T2. Improvements in fatigue and QoL were observed: mean (standard deviation [SD]) change in fatigue VAS score between T0 and T2 was –37.2 (±76.6)% and mean (SD) improvement in QoL between T0 and T2 was 58.6 (±88.4)%. The Pearson correlation co-efficient for fatigue and QoL was –0.255 at T0 (p=0.0001), –0.6459 at T1 (p<0.0001) and –0.7910 at T2 (p<0.0001). Assessment of fatigue by treating physicians was consistent with patients' perceptions of fatigue at T0 (5.1±1.7 *vs* 4.7±2.3), T1 (4.1±2.1 *vs* 4.3±2.2) and T2 (3.1±2.4 *vs* 2.7±2.3). Of the 108 patients in the safety population, 17 (15.7%) had adverse events (N=67 events) and of these, 49 AEs in 13 (12.0%) patients were serious. No AEs were considered related to the study treatment.
Summary and Conclusions: A correlation between reduced fatigue and improvement in QoL was observed. Treatment of CIA with biosimilar epoetin alfa in patients with hematological malignancies was well tolerated and effective, leading to improvements in fatigue and QoL.

PF706

DIAGNOSIS DISCLOSURE IN PATIENTS WITH HEMATOLOGICAL CANCERS IN INDIA

C. Cherian Philip[1,*], S. Singh[1], A. Mathew[1], A. Jindal[2], N. Kakkar[3], M.J. John[1]

[1]Clinical Haematology, [2]Immunohematology and Blood Transfusion, [3]Laboratory Haematology, Christian Medical College & Hospital, Ludhiana, India

Background: Disclosure of diagnosis is a challenge for health care providers in India(1). With family playing the significant role in healthcare decisions, there is often collusion between the doctor and the family to withhold information from the patient(2). This is however in good intent and stems from the societal belief that it benefits the patient (3). The fear of discrimination and stigma is further accentuated when the diagnosis is cancer, often perceived with a pervading sense of inevitability that relatives often request the doctor to withhold information(4). However, honest disclosure is reported to establish a satisfactory relationship between the doctor, patient and family members(5).
Aims: To estimate the percentage of patients with haematological cancers wanting complete diagnosis disclosure at our tertiary care centre and their attitude towards disclosure.
Methods: This prospective study involved a questionnaire based interview that assessed patient demographics, the information provided about cancer, patient preference about the diagnosis disclosure, and the roles of family and finances in treatment planning. Consecutive inpatients beginning August 2016 through June 2017 were interviewed in the absence of their relative with the intent to derive genuine patient response**.** The questionnaire was derived and modified from extensive literature search and its components had been validated elsewhere in the country. An informed consent was obtained prior to the interview.
Results: The patient demographics of the 100 patients who were interviewed are detailed in Table 1. The majority (80) had been to other treatment centres earlier for the current diagnosis. Ninety seven were satisfied with the diagnosis discussion and information they had been provided. Most (92 and 91) patients did not want to enquire further about their disease and treatment respectively. 72 preferred that the physician disclose their illness to relatives. 85 patients responded as being aware of the nature of their disease but only 35 were aware that they had cancer. The attitude of patients appears to approve of a paternalistic role for physicians in India. The patients who liked disclosure of diagnosis were younger [(36 (25-51) *vs* 55 (43-62)] and had a higher education (33 *vs* 13),P<0.05.

Table 1.

Patient demographics (N=100)

Variable	Patients (N=100) n or %/ Median (IQR)/Mean ±SD			
Age	46 (28-57)			
Gender (Male)	59			
Monthly family Income (€)	€375 (€187.5 – €500)			
Family history of Malignancy (Yes)	5			
Treatment decision maker (Spouse)	35			
Patient attitude towards diagnosis disclosure				
Question	Don't mind to know	Absolute need to know	Disclose to relative	Trust doctor's choice
What is your disease?	62	17	16	5
What is your specific medical illness?	40	19	14	27
What is your weekly progress?	43	20	29	8
What are the chances of cure?	46	23	24	7
What are the possible treatments?	32	13	19	36
What are the possible side effects of treatment?	48	18	14	20
How does the treatment work?	26	12	5	57
₹80 = 1€				

Summary and Conclusions: Our study though limited by patient numbers and being single centred, reveals some useful insights. In our analysis, most patients are neither aware nor in want of complete diagnosis disclosure. Despite only a minority of patients being aware of the exact nature of their illness, the majority of patients were satisfied with the information provided. There exist differences in regards to attitude and preference of patients towards diagnosis disclosure in cancer across societies. Personal beliefs and social practices possibly govern patient choice. Gaining an understanding of factors that influence decision making and diagnosis disclosure for each population should guide physicians on disclosure.

Sickle cell disease

PF707

NATIONAL EVALUATION OF QUALITY OF CARE DURING THE 5 FIRST YEARS OF LIFE FOR CHILDREN DIAGNOSED BY THE NEWBORN SICKLE CELL SCREENING PROGRAMME IN FRANCE: RESULTS OF EVADREP STUDY

I. Thuret[1,*], C. Arnaud[2], V. Brousse[3], E. Lesprit[4], B. Quinet [4], M.-H. Odièvre [5], M. Etienne-Julan[6], C. Guillaumat[7], G. Elana[8], M. Belloy [9], N. Garnier[10], A. Chamouine [11], C. Dumesnil[12], M. De Montalembert[3], C. Pondarre[13], N. Couque[14], J. Bardakjian[15], M. Benkerrou[16]

[1]Pediatric onco-hematology, thalassemia reference center,Timone Enfant hospital, APHM, Marseille, [2]pediatric, Sickle Cell disease reference center, CHIC hospital, Créteil, [3]pediatric, sickle cell disease reference center, Necker-Enfants Malades hospital, APHP, [4]pediatric, sickle cell disease reference center, Trousseau hospital, APHP, Paris, [5]pediatric, Louis Mourier hospital, APHP, Colombes, [6]Sickle cell disease reference center, University hospital of Pointe-a-Pitre, Guadeloupe, [7]pediatric, CHSF, APHP, Evry, [8]Sickle cell Unit, Martinique hospital, fort de France Martinique, [9]pediatric, Robert Ballanger hospital, Aulnay sous Bois, [10]Pediatric onco-hematology, IHOP, Lyon, [11]pediatric, Mamoudzou hospital, Mayotte, [12]Pediatric onco-hematology, Charles Nicolle hospital, Rouen, [13]Pediatric onco-hematology, Sickle Cell disease reference center, CHIC hospital, Créteil, [14]biochemistry and molecular biology laboratory, Robert Debré hospital, Paris, [15]Department of Biochemistry and Genetics, Henri Mondor hospital, Créteil, [16]Sickle Cell disease reference center, Robert Debré hospital, Paris, France

Background: The aims of newborn SCD screening programmes are to reduce early mortality from infection and acute splenic sequestration as well as morbidity especially stroke. Introduction of pneumococcal conjugate vaccines (PCV) and of Transcranial Doppler (TCD) are the main recent progress allowing to further improve outcome.
Aims: The goal of this national retrospective study was to evaluate at 5 years of age the performance of the French newborn SCD screening programme by describing the residual risks of death, stroke, and bacterial meningitis/septicaemia as well as TCD use and pneumococcal vaccines coverage.
Methods: Evadrep study (NCT 03119922) was approved by the French National Committee for Computerized Databases (CNIL-127020) and by EC/CCTIRS (12319). Data were collected from the patients' medical files up to the age of 5 years, including age first prescribed penicillin, occurrence of death/overt stroke/bacterial meningitis and septicaemia, use of pneumococcal vaccines/chronic TF programme / hydroxycarbamide/ HSCT and DTC results. Between 01/01/2006 and 31/12/2010, 1792 consecutive babies with SCD were identified through newborn screening programme by AFDPHE (Association Francaise de Depistage et de Prevention des Handicaps de l'Enfant). 152/1792 patients (8.5%) could not be located during the period of data collection (2014-2015), of whom 64 were known to be back to their country of origin. 1620 patients followed in 69 centres were included in EVADREP study, 71.8% with sickle-cell anaemia (SS) or sickle beta°-thalassemia (Sb°). 20.3% had SC disease. 59.6% were resident in Paris area, 9.4% in the French West Indies.
Results: Probability of survival at 5 years was 98.9% (95%CI: 98.2-99.3). 18 deaths occurred, 12 related to SCD (11 patients SS or SB0). Probability of overt stroke at 5 years for the whole study population was 0.8% (95%CI: 0.5-1.4%) and 1.1% (0.6%>1.9%) in SS/Sb° patients. All 12 patients with stroke had the SS genotype and 6 have not been investigated by DTC before. In the SS/Sb° group DTC was performed before 2 and 3 years of age in 56% and 81% of patients. The median number of annual TCD was 0.8. The probability of experiencing abnormal TCD (velocities ≥200cm/s) at 5 years was of 10.4% [8.6%>12.4%]. 26 patients underwent a severe infection (meningitis or septicaemia), lethal in 8 cases. Streptococcus pneumonia was identified in 8/26 cases. More than 99% of patients were prescribed prophylactic penicillin, started at a median age of 2.2 months of life. 87% of children received at least 3 injections of PCV during their first year of life. The 23-valent pneumococcal polysaccharide vaccine was less well administered (only 90% before 5 years). For patients SS and Sb°, probabilities at 5 years to be treated with hydroxycarbamide or to receive chronic transfusion programme were of 13.7% [11.7%>15.9%] and 18.4% [16.2%>20.8%]. Only 9 patients underwent a HSCT before 5 years of age. Vaccination and DTC coverage, use of TF programme were significantly higher in larger centers.
Summary and Conclusions: In this national newborn SCD screening cohort of children born between years 2006-2010 the residual risks under the age of 5 years of death, stroke and invasive infection were very low. Almost all infants benefited from PVC and a vast majority of children from TCD monitoring before 3 years reflecting an efficient access to French care system. These national results are near to those observed in SCD expert centres cohorts. Nonetheless national coverage with Pneumovax and TCD can be further improved.

PF708

VISUAL CORTEX CHANGES IN CHILDREN WITH SICKLE CELL DISEASE: A MULTIMODAL MRI STUDY

R. Manara[1], A. Dalla Torre[2], M. Lucchetta[2], M. Ermani[2], A. Favaro[2], C. Baracchini[2], S. Favaretto[2], F. Viaro[2], V. Munaretto[3], S. Sartori[2], S. Ponticorvo[4], A. G. Russo[4], G. Basso[3], L. Sainati[3], R. Colombatti[3,*]

[1]University of Salerno, Salerno, [2]Department of Neurosciences, [3]Clinic of Pediatric Hematology Oncology, Department of Child and Maternal Health, Azienda Ospedaliera-Università di Padova, Padova, Italy, Padova, [4]Department of Neurosciences, University of Salerno, Salerno, Italy

Background: The neural tissue is typically affected in the Sickle Cell Disease (SCD) SS-Sβ° genotype due to its vulnerability to micro and macrocirculation failure. Recently, advanced imaging analyses have revealed several structural and functional brain changes (white matter volume loss, cortical thinning, neural function impairment of cognitive- or pain-related networks) that go beyond silent infarcts and stroke (1-2). Among several neural systems, vision is known to be primarily affected by SCD and regular eye examination (visual acuity and dilated eye examination) since late childhood is recommended in SCD management guidelines. Retinal abnormalities include proliferative and non-proliferative retinopathy. As the retina is a visible and easily assessed part of brain tissue, retinal changes might represent a valuable "window" on the mechanisms of brain injury in SCD. On the other hand, neurons of the retina are subjected to the same interactivity that characterizes neurons in all parts of the brain. Subclinical retinal changes might have an effect on the entire neural visual system and reciprocally, brain changes in the visual network might affect the the retina. So far, all studies focused on the retina neglecting the changes that might be present in the cortical portion of the visual system
Aims: The aim of the present study was to investigate, with multimodal MRI analyses, structural and functional changes in the cerebral part of the visual system of children with SCD, using a control group for comparison and taking into account age-related changes
Methods: 25 SCD children and 31 healthy controls -mean age (yrs) 12.3±1.9 *vs* 12.7±1.6- underwent: MR-angiography and axial FLAIR for determining vascular and parenchymal lesion burden, 3D-T1-weighted scan and resting-state fMRI for cortical thickness and neural network connectivity analyses. SCD children had a routine ophthalmologic evaluation
Results: Ophthalmologic examination revealed: 3/25 SCD children with mild visual acuity deficits (8-9/10), none with biomicroscopy abnormalities, 2/25 with mild tortuosity of the retinal vessels (both children had normal acuity) at dilated eye examination. None had occipital infarcts at MRI nor abnormal Transcranial Doppler, while 6/25 disclosed posterior cerebral artery stenosis (5 mild, 1 severe) at MR-angiography. Compared to controls, SCD children had increased posterior pericalcarine cortical thickness (Table 1) with a different trajectory of cortical maturation and decreased connectivity within both medial and ventral visual neural networks (Figure 1).

Table 1. Cortical thickness whole brain analysis: showing clusters (>50 mm2) of different values in the primary visual cortex: increased thickness in SCD patients compared to controls.

Thickness	Hemisphere	Annotation	Size (mm^3)	TalX	TalY	TalZ
Increased	Left	Pericalcarine	714	-12.4	-88.0	6.2
Increased	Right	Pericalcarine	274	5.8	-79.6	11.3
Increased	Right	Lingual	134	4.6	-86.4	-6.5

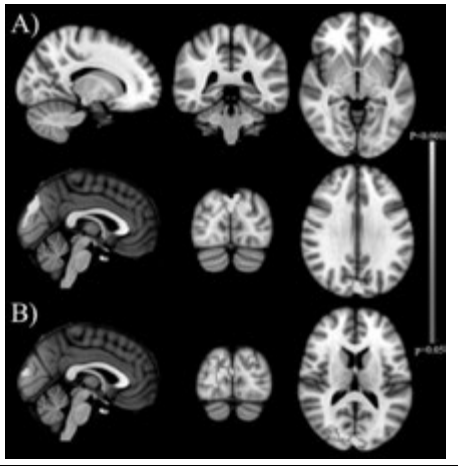


Figure 1.

Summary and Conclusions: Our findings provide novel evidence that SCD affects the development and tuning of the visual cortex leading to significant anatomical and functional changes in childhood, even without retinopathy at standard ophthalmologic examination. Longitudinal multimodal MRI (fMRI) and advanced ophthalmologic tools (i.e OCT) could determine if these changes can be predictors of visual impairment in adulthood, biomarkers of disease progression or treatment response

PF709

RESULTS FROM A PHASE 2A STUDY (GBT440-007) EVALUATING ADOLESCENTS WITH SICKLE CELL DISEASE TREATED WITH MULTIPLE DOSES OF VOXELOTOR (GBT440), A HBS POLYMERIZATION INHIBITOR

C. Brown[1,*], C. Hoppe[2], A. Inati[3], W. Wang[4], L.L. Hsu[5], V.R. Gordeuk[5], R. Liem[6], G. Woods[7], C. Piccone[8], E. Fong[9], G. Balaratnam[9], S. Dixon[9], M. Tonda[9], C. Washington[9], Y. Yaron[9], J. Lehrer-Graiwer[9]

[1]Children's Healthcare of Atlanta, Atlanta, GA, [2]UCSF Benioff Children's Hospital Oakland, Oakland, CA, United States, [3]Rafik Hariri University Hospital Beirut, Beirut, Lebanon, [4]St. Jude Children's Research Hospital, Memphis, TN, [5]University of Illinois at Chicago, Chicago, IL, [6]Ann and Robert Lurie Children's Hospital of Chicago, Chicago, IL, [7]Children's Mercy Hospital, Kansas City, MO, [8]UH Rainbow Babies and Children's Hospital, Cleveland, OH, [9]Global Blood Therapeutics, South San Francisco, CA, United States

Background: Sickle cell disease (SCD) is an autosomal recessive inherited disorder in which deoxygenated sickle hemoglobin (HbS) polymerizes and triggers downstream effects including red blood cell deformation (sickling), hemolysis, vaso-occlusion, and inflammation. Ischemic injury from SCD starts in infancy and accumulates over a lifetime, causing pain, fatigue, vaso-occlusive crisis (VOC), progressive end-organ damage, and other clinical complications that are associated with decreased quality of life and early death. Voxelotor (GBT440) is a first-in-class, oral, once-daily therapy that modulates hemoglobin (Hb) affinity for oxygen, thereby inhibiting HbS polymerization.
Aims: To assess the efficacy and safety of voxelotor at 900 mg in pediatric patients with SCD (HbSS or HbS ^{0} thalassemia) in the ongoing phase 2a study (GBT440-007).
Methods: This study is being conducted in 2 parts. Part A was single-dose voxelotor (600 mg) in pediatric (6-11 years) and adolescent (12-17 years) patients. PK data from part A have been completed and were reported previously. Part B includes treatment of adolescents for 24 weeks with multiple doses of voxelotor (900 and 1500 mg/d). The primary objective of part B is to assess the effect of voxelotor on anemia. Secondary objectives include measures of hemolysis, daily symptoms using an electronic patient-reported outcome (PRO) measure, and safety.
Results: Enrollment in part B of the 900 mg cohort is complete. As of 29Jan2018, 25 patients (11 females) have received voxelotor for up to 24 weeks (range, 3-24). Median age was 14 years (range, 12-17), median weight 50 kg (range, 31-93), and median baseline Hb value was 8.9 g/dL (range, 6.3-11.0). In this cohort, 88% of patients were receiving hydroxyurea (HU) and 44% had ≥2 VOC (range, 2-8) in the year prior to enrollment. Data for measures of hemolysis are available for 12 patients (92% on HU at study entry) who received voxelotor 900 mg for 24 weeks. Five of the 12 patients (42%) achieved Hb response of >1 g/dL increase (Figure 1), which was associated with improved measures of hemolysis. Median reduction in reticulocytes (32%) and indirect bilirubin (38%) was consistent with previous results in adults with SCD. Data collected from the PRO measure indicate total SCD symptom scores trended lower from screening to week 24. Among the 25 patients treated for up to 24 weeks, the majority of treatment-related adverse events (AEs) were grade 1 or 2, and there were no treatment-related serious AEs or drug discontinuations due to AEs. The most common treatment-related AEs included nausea (12%), vomiting (8%), headache (8%), and rash (8%). Data for all 25 patients treated with voxelotor 900 mg for 24 weeks will be presented. Statistical results will be available for presentation at the meeting.
Summary and Conclusions: Voxelotor at 900 mg was well tolerated in all 25 adolescents. Data from 12 adolescents at 24 weeks demonstrated sustained and durable improvement in Hb and reduction in clinical measures of hemolysis in patients already maximally managed with HU. PRO data suggest that clinical symptoms may improve with voxelotor. Overall, these results are consistent with *in vivo* inhibition of HbS polymerization by voxelotor and support the ongoing clinical evaluation of voxelotor as a potential disease-modifying therapy for adults and adolescents with SCD.

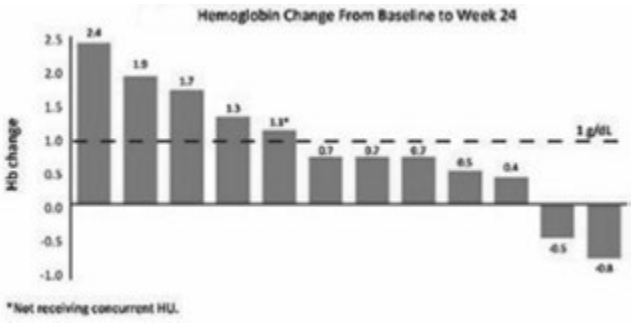


Figure 1.

PF710

EVALUATION OF CEREBRAL TISSUE OXYGENATION USING NIRS DURING TRANSFUSION EXCHANGES IN SICKLE CELL PATIENTS WITH CEREBRAL VASCULOPATHY.

F. Romain[1], Z. Fouzia[2], C. T. Rym[3], M. Dehbia [3], P. Sadaf [3], H. Anoosha [4], G. Frederic[4], P. France [3], R. Thomas [5], B. Pablo[4,*]

[1]Rhône, Service de Médecine Interne, Hôpital Edouard Herriot, HCL, Lyon, [2]Paris, Service d'Hématologie biologique, Hôpital Antoine Béclère, APHP, Clamart, [3]Paris, Etablissement Français du Sang, Hôpital Henri Mondor, APHP, Créteil, [4]Paris, Centre de Référence des Syndromes Drépanocytaires Majeurs, Hôpital Henri-Mondor, AP-HP, Créteil, Paris, [5]Savoie, Laboratoire Interuniversitaire de Biologie de la Motricité, Université Savoie Mont Blanc, EA 7424, Chambéry, Chambery, France

Background: Sickle cell disease is a genetic disorder caused by a mutation of beta globin, characterized by chronic hemolysis and vascular dysfunction. Stenosis of the large intracranial vessels represents one of the most serious complications, responsible for cerebrovascular accidents with a prevalence between 4 and 13% before the age of 20 years old, and a recurrence rate of approximately 46%. These lesions are preferentially localized to the internal carotid, middle and anterior cerebral arteries. Primary and secondary prevention of stroke is based on long-term transfusion exchange (TE) with an HbS goal <30%. However no evaluation was made on the benefit of cerebral tissue perfusion during TE.
Aims: The aim of this study was to evaluate the benefit of cerebral tissue perfusion during TE in sickle cell patients with cerebral vasculopathy.
Methods: We conducted a pilot, monocentric, observational, prospective study in adult sickle cell patients under TE protocol by erythrapheresis. Real-time evaluation of tissue oxygenation throughout the procedure was performed transcutaneously using a near-infrared spectroscopy (NIRS) system. Several parameters were analyzed at the muscular level and the two cerebral hemispheres: 1) quantification of tissue oxygen saturation (TSI%); 2) oxyhemoglobin (HbO2) with high level reflecting better cerebral perfusion; 3) deoxyhemoglobin (HHb); and 4) total hemoglobin (HbT).
Results: 21 patients were included, including 11 men and 10 women, with an average age of 36 years. 14 of them had cerebral vasculopathy (8 unilateral and 6 bilateral). The cerebral perfusion after TE was significantly improved, as shown by the increase of HbO2 (p=0.004) and HbT (p=0.003), without modification of the HHb. At the muscular level there was only a significant increase in HbO2 (p=0.03). Interestingly, the improvement in perfusion concerned only cerebral hemispheres with vasculopathy. Finally, TSI% did not differ during TE.
Summary and Conclusions: This is the first study to demonstrate the benefit of TE on cerebral perfusion in sickle cell patients with cerebral vasculopathy. These results confirm 1) the feasibility of evaluating real-time cerebral perfusion with NIRS; and 2) the need to maintain a transfusion therapy in patients with cerebral vasculopathy. It would be then interesting to perform respiratory maneuvers during the NIRS, in order to evaluate the vascular reserve which is an early marker of the risk of cerebral hypoperfusion.

PF711

COMPASSIONATE-USE VOXELOTOR (GBT440) FOR PATIENTS WITH SEVERE SICKLE CELL DISEASE (SCD) AND LIFE-THREATENING COMORBIDITIES

G. Blyden[1], K. Bridges[2,*], L. Bronte[1]

[1]Foundation for Sickle Cell Disease Research, Hollywood, FL, [2]Global Blood Therapeutics, South San Francisco, CA, United States

Background: Voxelotor (GBT440) is a first-in-class, oral, once-daily therapy designed to modulate the affinity of hemoglobin (Hb) for oxygen and prevent sickle hemoglobin polymerization, thereby blocking subsequent red blood cell damage and complications from SCD. Seven patients with severe SCD ineligible for ongoing clinical trials because of high mortality risk from comorbidities, including renal dysfunction, frequent hospitalizations for vaso-occlusive crisis (VOC), extreme anemia, and multiorgan failure, were provided compassionate-use access to voxelotor by the US Food and Drug Administration single-patient investigational new drug program.
Aims: To report the efficacy and safety of voxelotor administered to 7 patients with severe SCD and high mortality risk owing to multiple comorbidities.
Methods: Once-daily voxelotor began at 900 mg, with possible increase to 1500 mg. Baseline echocardiograms were performed. Initial assessments at 2 and 4 weeks were followed by routine evaluations at least monthly. Key data captured included Hb levels, reticulocyte count, indirect bilirubin, resting oxygen saturation (PO_2), pain level on 0 to 10 scale, transfusions, and hospitalizations for VOC. Patient Health Questionnaire-9 (PHQ-9) assessed depression.
Results: Four female and 3 male patients aged 22 to 67 years had severe comorbidities at baseline, including iron overload in all patients, frequent transfusions in 5/7, severe fatigue in 4/7, chronic oxygen supplementation in 2/7, progressive severe renal dysfunction (1/7), and multiorgan failure (1/7). In 5/7 patients, baseline Hb was ≤6.4 g/dL, with three <6 g/dL. Hb level increased in all 7 patients by 24 weeks of receiving voxelotor, with 5 reaching ≥1.0 g/dL. Hb increase from voxelotor was clearest in 3 patients, who had 1.0 to 5.4 g/dL rises without transfusion. The number of transfusions fell by 60% in all 7 patients. VOC hospitalizations fell by 67%. Baseline PO_2 improved to 98% to 99% from <95% in 4 patients following voxelotor treatment, permitting discontinuation of long-term supplemental oxygen in 2 patients. Improved overall well-being, typically 2 to 3 weeks after treatment initiation, occurred in all patients, and the PHQ-9 score registered reduced depression. Voxelotor was well-tolerated with no discontinuations during treatment periods of up to 17 months. There was 1 dose reduction because of grade 2 diarrhea. Two patients with advanced end-stage organ injury died of SCD complications unrelated to treatment.
Summary and Conclusions: Seven patients given compassionate-use voxelotor after exhausting all treatment options achieved substantial improvements in clinical, laboratory, and mental health parameters. Controlled clinical trials will be needed to confirm the benefit of voxelotor in patients with severe SCD and multiple comorbidities.

PF712

CRIZANLIZUMAB TREATMENT IS NOT ASSOCIATED WITH THE DEVELOPMENT OF PROTEINURIA AND HEMATURIA IN PATIENTS WITH SICKLE CELL DISEASE: A SAFETY ANALYSIS FROM THE SUSTAIN STUDY

K.I. Ataga[1,*], A. Kutlar[2], R. Cancado[3], D. Liles[4], L. Velez-Nandayapa[5], J. Lincy[5], A. Bruederle[5], J. Kanter[6]

[1]Division of Hematology–Oncology, University of North Carolina, Chapel Hill, NC, [2]Sickle Cell Center, Medical College of Georgia, August University, Augusta, GA, United States, [3]Department of Hematology–Oncology, Santa Casa Medical School of São Paulo, São Paulo, Brazil, [4]Division of Hematology–Oncology, East Carolina University, Greenville, NC, United States, [5]Novartis Pharma AG, Basel, Switzerland, [6]Division of Pediatrics, Medical University of South Carolina, Charleston, SC, United States

Background: Patients with sickle cell disease (SCD) are at risk of multiorgan complications, including renal disease. Sickle cell nephropathy is caused primarily by cortical hyperperfusion, medullary hypoperfusion and the renal vascular response to the stress of SCD, leading to renal vaso-constriction and vaso-occlusion. Renal complications of SCD include proteinuria, hematuria and renal failure. Crizanlizumab, a humanized anti-P-selectin monoclonal antibody, is under investigation for reducing the frequency of, or preventing, vaso-occlusive crises (VOCs) in patients with SCD. In the Phase II SUSTAIN study, crizanlizumab 5.0 mg/kg significantly reduced the median annual rate of VOCs by 45.3% *vs* placebo (median absolute difference of –1.01 *vs* placebo, 95% CI [–2.00, 0.00]; *P*=0.01). Crizanlizumab 5.0 mg/kg was also associated with longer Kaplan-Meier estimated median time to first VOC *vs* placebo (4.07 *vs* 1.38 months). The incidence of adverse events (AEs) and serious AEs was similar in patients receiving crizanlizumab and placebo (Ataga *et al. N Engl J Med* 2017). Although proteinuria (n=3) and hematuria (n=1) were rarely reported as AEs, and all were mild or moderate, the overall incidence across treatment groups (computed from the laboratory sample results) was 34.2% and 26.6%, respectively.

Aims: To compare the risk of developing proteinuria or hematuria during SUSTAIN for patients receiving crizanlizumab or placebo.
Methods: SUSTAIN was a Phase II, randomized, double-blind, placebo-controlled, 52-week study (NCT01895361). Patients (aged 16–65 years) with SCD were randomized 1:1:1 to receive intravenous crizanlizumab 5.0 mg/kg, 2.5 mg/kg or placebo. This safety subanalysis from SUSTAIN was performed with risk ratios (RR) as a measure of effect. The analysis compared two groups: patients receiving crizanlizumab (2.5 and 5.0 mg/kg combined) *vs* those receiving placebo, in patients without proteinuria or hematuria at baseline. Proteinuria and hematuria were each defined as a positive result for protein or red blood cells in dipstick urinalysis, respectively. Incidences of abnormal estimated glomerular filtration rate and increase in serum creatinine were also assessed.
Results: 192 patients received at least one dose of crizanlizumab 5.0 mg/kg (n=66), 2.5 mg/kg (n=64) or placebo (n=62). In total, 161 patients had no proteinuria at baseline (crizanlizumab, n=109; placebo, n=52) and 156/161 (n=106 and n=50) had available post-baseline protein data. During the study, proteinuria was reported in 35/106 (33.0%) patients receiving crizanlizumab *vs* 17/50 (34.0%) receiving placebo (RR=0.97, 95% CI [0.61–1.56]). At baseline, 109 patients did not have hematuria (crizanlizumab, n=77; placebo, n=32) and 100/109 (n=73 and n=27) had available post-baseline hematuria data. During the study, hematuria was reported in 18/73 (24.7%) patients receiving crizanlizumab *vs* 11/27 (40.7%) receiving placebo (RR=0.61, 95% CI [0.33–1.11]). The incidence of glomerular filtration abnormalities and increased serum creatinine was low.
Summary and Conclusions: This analysis suggests there is no association between the use of crizanlizumab and the risk of developing proteinuria or hematuria in patients with SCD in SUSTAIN. As such, the observed cases of proteinuria and hematuria in patients treated with crizanlizumab are likely associated with the underlying disease. These findings further support the use of crizanlizumab as a well-tolerated treatment for preventing VOCs in SCD. Further studies are needed to evaluate the effect of crizanlizumab in reducing proteinuria/hematuria in SCD.

PF713

PHARMACOKINETICS (PK) OF VOXELOTOR (GBT440) USING POPULATION PHARMACOKINETIC (PPK) AND PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING IN PEDIATRIC SUBJECTS WITH SICKLE CELL DISEASE (SCD)

C.B. Washington[1,*], M. Green[2], A.C. Inati[3], J. H. Estepp[4], C. Brown[5], M. Abboud[6], R. Drachtman[7], V. R. Gordeuk[8], C. C. Hoppe[9], L. Hsu[8], R. Liem[10], C. Piccone[11], W. Wang[4], G. Woods[12], B. Goldstein[1], S. Dixon[1], E. Fong[1], A. Hutchaleelaha[1], M.E. Tonda[1], J. Lehrer[1]

[1]Global Blood Therapeutics, South San Francisco, CA, [2]Certara Strategic Consulting, Menlo Park, CA, United States, [3]Rafik Hariri University Hospital, Beirut, Lebanon, [4]Department of Hematology, St. Jude Children's Research Hospital, Memphis, TN, [5]Department of Pediatrics, Emory University, Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta, Atlanta, GA, United States, [6]Department of Pediatrics, American University of Beirut, Beirut, Lebanon, [7]Pediatric Hematology Oncology, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, [8]University of Illinois at Chicago, Chicago, IL, [9]UCSF Benioff Children's Hospital Oakland, Oakland, CA, [10]Ann and Robert Lurie Children's Hospital of Chicago, Chicago, IL, [11]UH Rainbow Babies and Children's Hospital, Cleveland, OH, [12]Children's Mercy Hospital, Kansas City, MO, United States

Background: SCD is an autosomal recessive inherited disorder caused by a mutation in hemoglobin (Hb) that leads to the production of sickle hemoglobin (HbS). When deoxygenated, HbS polymerizes and deforms and damages red blood cells. These damaged red blood cells block capillaries and undergo hemolysis, which triggers downstream effects of anemia, fatigue, tissue ischemia, vaso-occlusive crisis, vascular injury, and organ damage leading to a decreased quality of life. Currently, pharmacologic treatments do not address the primary pathogenesis of SCD by direct inhibition of HbS polymerization. Voxelotor is a first-in-class, oral, once-daily therapy that is designed to modulate Hb's affinity for oxygen, thereby inhibiting HbS polymerization. GBT440-007 is a phase 2a study designed to assess the safety, PK, and efficacy of voxelotor in pediatric patients with SCD and consists of 2 parts: Part A (single-dose) and Part B (multiple-dose).
Aims: To report the single-dose PK of voxelotor in children (6 to <12 years; Part A) and multiple-dose PK of voxelotor in adolescents (12 to <18 years; Part B) with SCD.
Methods: Children and adolescents in Part A received a single oral dose (600 mg) of voxelotor. Adolescents in Part B received multiple once-daily oral doses (900 or 1500 mg) of voxelotor for up to 24 weeks. PK samples to measure whole blood and plasma voxelotor concentrations were collected up to 15 days following single-dose administration and up to 24 weeks following multiple-dose administration. Separate PPK models were developed to describe the concentration *versus* time profiles of voxelotor in whole blood and plasma using nonlinear mixed effects modeling (NONMEM, version 7.3). Additionally, PPK models and PBPK modeling were used to simulate voxelotor PK parameters and support dose selection for future evaluation in younger children (<12 years).
Results: Enrollment in Part A and Part B (900-mg cohort) is complete. Part A included 6 children (3 females; median age 8.5 years [range, 6-10]). Mean weight was 21.1 kg (range, 16-38 kg). Part B included 25 adolescents (11 females; median age 14 years [range, 12-17]. Mean weight was 49.9 kg (range, 31-93 kg). A 2-compartment model with first-order absorption best described the PK of voxelotor and is the same model structure previously used for adults with SCD. The half-life was similar in children, adolescents, and adults (Table 1). The% Hb occupancy following multiple 900-mg doses (~23%) was similar in adolescents and adults. Voxelotor PK exposures in adolescents were comparable to those observed in adults. However, as expected, higher PK exposures were observed in children supporting a weight-based dosing approach in this population. PPK and PBPK simulations resulted in similar dosing schemes for children <12 years.

Table 1.

Model PPK Parameter Estimates for Voxelotor in Children, Adolescents, and Adult Participants with SCD

PK Parameters/n	n = 6	n = 25	n = 42
	Children	Adolescents	Adults
Voxelotor Whole Blood PK Parameters			
CL/F, L/h	0.24	0.4	0.43
V/F, L	9.55	18.9	21.4
$T_{1/2}$, h	27.8	32.7	34.5
Voxelotor Plasma PK Parameters			
CL/F, L/h	3.67	5.48	7.63
V/F, L	140	267	373
$T_{1/2}$, h	26.4	33.8	33.9

CL/F, apparent clearance; h, hours; L, liters; $T_{1/2}$, half-life; V/F, apparent volume of distribution.

Summary and Conclusions: Voxelotor was well tolerated following single doses (600 mg) in children and multiple doses (900 mg) in adolescents. Multiple-dose PK were similar in adolescents and adults. PK exposures were higher in children compared with adolescents and adults, so lower doses are recommended in children (<12 years). The integrated PPK and PBPK strategy provides an innovative approach to minimize the need for clinical PK evaluation in children and to accelerate dose selection.

PF714

ACCESS TO EMERGENCY DEPARTMENT AND IDENTIFICATION OF SUBJECTS WITH SICKLE CELL DISEASE AND OTHER HEMOGLOBINOPATHIES IN REFUGEES

L. De Franceschi[1,*], C. Lux[2], F. Piel[3], B. Gianesin[4], F. Bonetti[5], M. Casale[6], G. Graziadei[7], R. Lisi[8], V. Pinto[4], M.C. Putti[9], P. Rigano[10], G. Ruffo[11], G. Russo[12], S. Spadola[13], A. Vianello[1], G. L. Forni[4]

[1]Department of Medicine, University of Verona AOUI Verona, Verona, [2]Department of Clinical and Biological Sciences, University of Turin, Turin, Italy, [3]Department of Epidemiology & Biostatistics, Imperial College, London, United Kingdom, [4]Centro della Microcitemia, Ospedale Galliera, Genoa, [5]Pediatric Haematology Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia, [6]Deparetment of Woman Child and General and Surgery, Università degli Studi della Campania L.Vanvitelli, Napoli, [7]UO Medicina Interna, Fondazione IRCCS Ca Granda Ospedale Maggiore Policlinico, Milano, [8]UOD Talassemia, Ospedale Garibaldi, Catania, [9]Clinica Emato-Oncologica Pediatrica, Università di Padova, Padova, [10]Campus of Hematogy, AOOR Villa Sofia-Cervello, [11]UOC Ematologia con Talassemia, ARNAS Ospedale Civico Di Cristina, Palermo, [12]Ematologia-Oncologia Pediatrica, Policlinico Vittorio Emanuele, Catania, [13]Centro per la Cura delle Talassemie, Ospedale di Ragusa, Ragusa, Italy

Background: The number of refugees coming to Europe has considerably increased since the beginning of the Syrian conflict which started in 2011. Hundreds of migrants still arrive on a daily basis along the Mediterranean coasts of southern European countries. The arrival of refugees in Italy represents a humanitarian emergency with needs which are still largely unmet. There are currently no signs of these fluxes slowing down or being interrupted. In hot-spot and in secondary level centers, refugees are routinely screened for communicable diseases and undergo vaccinations based on WHO recommendations. However, no screening for non-communicable disorders such as sickle cell disease (SCD) is currently in place. As indicated in a recent document from the US Department of Health and Human service

– division global migration and quarantine, screened in refugees coming from endemic areas for SCD is recommended.
SCD is a worldwide distributed red cell disorder defined as a global health priority issue by the African Union and WHO. Life-threatening complications of SCD include acute vaso-occlusive crisis (VOCs), which require early identification and intensive clinical management. Dehydration, psychologic stress, and exposition to high/low temperature can trigger VOCs and other severe complications in SCD patients.
Aims: To evaluate as hemoglobinopathies impact refugees
Methods: Here, we carried out a retrospective study on new diagnosis of SCD in refugees at their access to emergency departments for acute events in Italy. Data were collected from 2014-2017 by National reference centers for SCD and hemoglobinopathies, which are part of the SITE (Italian society for hemoglobinopathies studies) and ERN-Euroblood network (https://www.eurobloodnet.eu/).
Results: A total number of 67 patients were identified as first diagnosis of SCD related to acute event at their access to the emergency department (ED). SCD patients were 52% adults (86% male/14% female) and 48% children (81% male/19% female). Genotype distribution was as follow: SS (*n*=48), SC (*n*=9), bS (*n*=3), CC (*n*=2), AS (*n*=1). We also identified 4 subjects with non-transfusion dependent b-thalassemia. Patients identified came from a wide range of countries, including Nigeria (32%), Gambia (20.9%), Mali (7.5%), Senegal, Ghana (6%), Guinea, Syria (4.5%), Bangladesh (3%), Benin (1%), Burkina Faso (1%), Guinea Bissau (1%) and Kenya (1%). The main causes of access to the ED were: VOC (35.8%), anemia (19.4%), fever (7.5%), acute chest syndrome (4.5%), pneumonia (3%) and abdominal pain (3%). 60% of the identified SCD patients were then followed-up by the comprehensive Centers for hemoglobinopathies after their first access to ED.
Summary and Conclusions: Collectively, our data support the recommendation that SCD as a non-communicable disease should be screened in refugees coming from endemic areas for SCD. To address this health and humanitarian crisis, we propose (i) to develop flow-charts for the early and systematic identification of SCD patients at the arrival and second level refugee camps; (ii) to educate health operator in early identification and treatment of acute VOCs; (iii) to send SCD or symptomatic HbS-carrier refugees to the regional comprehensive SCD reference center for treatment and follow-up. Recent low-costs point-of-care screening devices could potentially play a more role in ensuring the implementation of such a program, which would allow to identify affected patients early and to prevent severe complications.

PF715

AN ANALYSIS OF PAIN CRISIS PATTERNS IN DIFFERENT GEOGRAPHIC REGIONS OF THE WORLD: ANALYSIS FROM THE CASIRE STUDY

R. Colombatti[1,*], B. Andemariam[2], I. Tartaglione[3], C. Antwi-Boasiako[4], C. Strunk[5], D. Manwani[6], C.M. Piccone[7], E.V. Asare[8], S. Perrotta[9], I. Ekem[8], L. Sainati[1], S. Rao[10], D. Boruchov[11], W. Zempsky[11], F. Sey[8], C. Segbefia[10], B. Inusa[12], A. Campbell[13]

[1]University of Padua, Department of Women's and Children's Health, Padova, Italy, [2]University of Connecticut Health Center, Farmington, United States, [3]Università della campania "Luigi Vanvitelli", Napoli, Italy, [4]Department of Physiology, University of Ghana Medical School, Accra, Ghana, [5]5ProMedica Toledo Children's Hospital, Toledo, [6]6Department of Pediatrics, Albert Einstein College of Medicine, Children's Hospital at Montefiore, Bronx, NY, [7]Pediatric Hematology/Oncology, Rainbow Babies and Children's Hospital, Cleveland, United States, [8]Ghana Institute of Clinical Genetics, Korle Bu Teaching Hospital, Accra, Ghana, [9]Università della Campania "Luigi Vanvitelli", Napoli, Italy, [10]Dept. of Child Health, University of Ghana Medical School, Accra, Ghana, [11]Connecticut Children's Medical Center, Hartford, United States, [12]Paediatric Haematology, Guys and St Thomas Hospital, London, United Kingdom, [13]Center for Cancer and Blood Disorders, Children's National Medical Center, Washington, United States

Background: Sickle cell disease (SCD) is global blood disorder affecting over 1 million people. It is characterized by chronic hemolysis, unpredictable pain crises(PCs), and chronic organ damage which leads to early death in patients affected by the disorder. Pain crisis frequency varies widely between patients and is directly related to disease severity and increased mortality (O Platt et al). Pain crises resulting in emergency room (ER) or day hospital visits and or hospitalizations have been used in many studies as an indicator of disease phenotype and inclusion criteria for eligibility for Phase II/III clinical drug trials in SCD.
Aims: To determine the pain phenotype across different geographic regions of the world.
Methods: The CASIRE group is an international multi-institutional collaboration of researchers studying the clinical severity of patients with sickle cell anemia on a global scale through a validated questionnaire, chart review and laboratory studies with 868 patients enrolled to date. Patients were enrolled into the CASIRE study after informed consent and assent was obtained from either the parent or patient when appropriate. The study was approved at each participating institution's IRB. A questionnaire was answered by the parents and/or patient, and baseline and current laboratory studies were collected. We investigated the sickle cell disease pain crisis patterns in three geographic regions of the world (USA, Europe [Italy, United Kingdom], and Africa (Ghana). Secondary data analyses of crises per year requiring ER/day hospital clinic visit (PC-ER/DH) or requiring hospitalization (PC-Hosp) were performed within each geographic region to assess PC frequency and care utilization variability.
Results: We analyzed 867 patients in this study for Pain crises per year requiring ER/day hospital clinic visit (PC-ER/DH) and requiring Hospitalization (PC-Hosp). We stratified patients based on age (pediatrics, n=443 *vs* adults, n=424) disease genotype: Severe (Hgb SS or Sickle Beta-Thal-Zero, n=678, 79%) *vs* Mild (Hgb SC /Hgb Sickle Beta thal-plus, n=189, 21%). Subjects included were receiving care either in Ghana (n=365), Italy/United Kingdom (n=258), or the United States (n=244). Fifty-two percent were female (n=446) while 49% were male (n=419). Overall, the Ghanaian pediatric patients had a significantly higher PC-ER/DH rate than the pediatric patients from the U.S. and U.K./Italy groups. Among patients with the severe disease genotypes, those receiving care in Europe experienced higher PC-Hosp rates than those who were receiving care in either the U.S. or in Ghana. Among pediatric patients with a mild SCD genotype, those from the U.S. experienced significantly lower rates of pain than the European and Ghanaian groups. When analyzing the adult SCD patients with severe genotype, Ghanaian patients experienced significantly lower PC-Hosp rates than their U.S./UK/Italy counterparts with a p<0.001. Conversely Adult American patients reported significantly higher PC-Hosp rates than their Ghana/European cohorts (Table 1).

Table 1.

Ghana : mean PC(sd)	n=57 1.51 (2.2)	**.028	N=57 1.27 (1.94)	0.114	N=204 2.15(3.08)	0.509	N=204 1.20(2.2)	** <0.001
UK/Italy: meanPC(sd)	n=200 0.81 (1.96)	0.153	n=200 2.35(4.3)	**.036	N=31 0.65(1.1)	**.017	N=31 1.97(4.0)	0.525
U.S. : mean PC(sd)	n=94 0.09(2.3)	0.303	n=94 1.65(2.9)	0.381	N=92 2.30(4.5)	0.403	N=92 2.54(3.5)	** <0.001
PEDIATRICS(<18Y/O)			TABLE 2: MILD GENOTYPE				ADULTS(>18 Y/O	
Country	PC-ER/DH	*p=	PC-Hosp	*p=	PC-ER/DH	*p=	PC-Hosp	*p=
Ghana: Mean PC (sd)	N=32 1.63(1.86)	<0.001	N=32 1.47(2.27)	0.534	N=72 2.15(3.01)	0.868	N=72 0.91(2.19)	0.064
UK/Italy: Mean PC (sd)	n=25 0.56(1.26)	0.239	n=25 1.68(3.35)	0.293	***N/A		***N/A	
U.S. : Mean PC (sd)	n=35 0.37(0.843)	0.153	n=35 0.77(1.19)	**.036	n=23 2.17(3.1)	0.913	n=23 2.04(2.2)	**.031

*One Way Anova: PC per year: Index Countries compared to all other countries combined;
p= <0.05; * N/A: number too small(N=2) for statistical analysis

Summary and Conclusions: A different pattern of admission to ER and inpatient services seem to be present in the various regions: Children in Europe presented more hospitalizations for SCD related painful crisis while adults in the USA seem to present more ER and hospital admissions compared to other areas. Whether this is related to disease phenotype, genetic modifying factors, environment characteristics or organization of health care needs to be investigated.

PF716

CHARACTERISTICS OF EPISODES OF ACUTE CHEST SYNDROME IN ADULTS PATIENTS WITH SICKLE CELL DISEASE IN GUADELOUPE AND PREDICTIVE FACTORS OF SEVERITY: A RETROSPECTIVE STUDY

M. Etienne-Julan[1,*], C. Mitaut[2], C. Lebouc[2], V. Tarer[3], O. Hippomene-Jerier[4], N. Lemonne[5], C. Charneau[6], M. Billaud[1], B. Pons[7], B. Tressieres[8]

[1]Unité transversale de la drépanocytose (Sickle cell Unit), Sickle cell reference centre,

[2]Unité transversale de la drépanocytose (Sickle cell Unit), [3]Reference centre for Sickle cell disease, [4]Service de médecine polyvalente, [5]Unité transversale de la drépanocytose (Sickle cell Unit), Sickle cell reference center, CHU de la Guadeloupe, Pointe-a-Pitre cedex, [6]Unité de la drépanocytose (Sickle cell Unit), Sickle cell competence center, Centre Hospitalier de la Basse-Terre, Basse-Terre, [7]Service de réanimation (Intensive Care Unit), [8]Centre d'Investigation Clinique Antilles-Guyane CIC 1424, CHU de la Guadeloupe, Pointe-a-Pitre cedex, France

Background: Acute chest syndrome (ACS) is the second leading cause of hospitalization and the most common cause for death among adult patients with sickle cell disease (SCD). It is a lung injury syndrome. Its pathophysiology is complex and associates, at different levels, vaso-occlusion, fat embolism, infection, *in situ* thromboembolism, hemolysis related vasoconstriction, hypoventilation. The evolution can be severe and drive to acute respiratory distress syndrome and/or acute pulmonary hypertension with acute cor pulmonale. Treatment is symptomatic. It is still difficult to predict which patients will have severe disease or develop life-threatening hypoxemia.
Aims: The goals of this non-interventional retrospective study were to describe the characteristics of ACS in an SCD affected adult population attending the referral centre for sickle cell disease in Guadeloupe and to identify predictive factors of severity
Methods: The CHU de Guadeloupe has the agreement (CNIL-2006302 v) from the French national Committee for computerized Databases for MR3 non interventional Studies. All patients with SCD, who were hospitalized at the University Hospital of Guadeloupe or at the Hospital of la Basse-Terre from January 1st 2011 to december 31st 2014 because of the occurrence of ACS, were included. The data were collected from the patients'medical files and included demographic data, sickle cell and non-sickle cell related medical antecedents, current treatment, the more recent biological steady state values, clinical symptoms before admission and at admission, radiological signs at admission, clinical evolution after admission, biological parameters evolution during hospitalization, transfer or not to Intensive Care Unit (ICU), transfusion therapy and the issue of the episode. Two groups were compared, according to the occurrence of a severe ACS, defined by clinical worsening, and/or an ICU stay of more than 48h, and/or the need for ventilatory support.
Results: During the study period, 71 episodes of ACS with 41 (58%) severe episodes were recorded. To be a younger adult (p=0.01), to have lower steady hematocrit level (p=0.013), long term treatment for SCD-related nephropathy by angiotensin-converting enzyme (ACE)-inhibitors and/or angiotensin receptor blockers (p=0.035), pregnancy for women patients (p=0.016), higher heart rate at admission (p=0.004), higher temperature at admission (p=0.01), lower limb located pain (p=0.035), higher leukocytes count (p=0.042), higher serum bilirubin, lacticodehydrogenase and C reactive protein levels at admission (p=0.002, 0.046 and 0.013 respectively) were associated with severe episodes. The multivariate analysis showed that to be younger than 28 years old, to have steady state hematocrit level lower than 25% and long term treatment for SCD-related nephropathy by angiotensin-converting enzyme (ACE)-inhibitors and/or angiotensin receptor blockers are independent factors associated with the severity of this complication in our population
Summary and Conclusions: Younger adults, pregnancy in SCD women, SCD nephropathy, and higher hemolysis level were associated with more severe ACS in adults with SCD in our study. A large-scale prospective study is needed to investigate the influence of these factors on the development of severe ACS in order to identify the patients who should benefit from early aggressive treatment in order to reduce mortality associated with this severe complication.

PF717

COULD LIVER INVOLVEMENT BE EARLY DETECTED IN SICKLE CELL DISEASE?

M. Bortolotti[1,*], R. D'Ambrosio[2], M. Fraquelli[3], P. Pedrotti[4], D. Cattaneo[5], M. D. Cappellini[6], G. Graziadei[7]

[1]Rare Diseases Center, General Medicine Unit, Department of Clinical Sciences and Community Health, University of Milan, [2]Gastroenterology and Hepatology Department, [3]Division of Gastroenterology and Endoscopy, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, [4]Cardiac Magnetic Resonance Laboratory, Cardiology 4, ASST GOM Niguarda, [5]Department of Clinical Sciences and Community Health, University of Milan, [6]Rare Diseases Center, General Medicine Unit, Department of Medicine and Medical Specialties - Department of Clinical Sciences and Community Health, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico - University of Milan, [7]Rare Disease Center, General Medicine Unit, Department of Medicine and Medical Specialties, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy

Background: Liver damage is a severe and frequent complication in Sickle Cell Disease (SCD), mainly characterized by intra-hepatic cholestasis. So far, no effective approaches to prevent or treat this condition are established.
Aims: Clinical, laboratory and imaging findings are evaluated longitudinally in SCD patients, comparing different sickle-genotypes, in order to identify possible early predictors of liver involvement.
Methods: Sixty-eight SCD patients were studied: 17 Sickle Cell Anemia (SCA, median age 42.8±10.3yrs, M:F 4:13), 38 Sickle Cell Thalassemia (HbS-βThal, 45.2±9.4yrs, M:F 14:24) and 13 HbS/HbC (HbSC, 35.6±8.7yrs, M:F 5:8). Patients with at least two Stiffness data (Transient Elastography TE) (T0 and T1), measured out of sickle crisis, were retrospectively evaluated (2007-2016). Liver function tests, HBV, HCV, iron status, and hemolytic indices, were recorded. Abdominal ultrasound (US) and Magnetic Resonance Imaging (MRI) T2* were also collected.
Results: In SCA pts Hb were 9±0.92 g/dL, HbS% 67.9±18.2% and HbF% 8.08±5.36; in HbS-βThal Hb 10±1.59, HbS 63.4±14.2% and HbF% 12.1±9.03; in HbSC Hb 11.9±1.1 (25.9-83.1), HbS 46.4±1% and HbF% 1.53±1.3 (SCA *vs* HbSC and HbS-βThal *vs* HbSC <0.0001).
Considering clinical manifestations, 76.5% of SCA, 60.5% of HbS-βThal and 30.8% of HbSC pts had >1 vaso-occlusive crisis during the decade (VOCs)/yr (SCA *vs* HbSC p=0.02; SCA *vs* HbS-βThal p=0.36; HbS-βThal *vs* HbSC p=00.1). Occasional transfusions (<4 RBCs Units/yr) occurred in 88.2% of SCA, 84.2% of HbS-βThal and 61.5% of HbSC pts. Hydroxy-Carbamide was prescribed to 58.8% of SCA, 65.8% of HbS-βThal and 15.4% of HbSC pts and iron-chelators to 23.5% of SCA, 23.7% of HbS-βThal and none of HbSC pts. At T0 AST, ALT, LDH were statistically higher in SCA pts than in HbS-βThal and HbSC (ALT p<0.0001) and in HbS-βThal compared to HbSC (ALT p=0.01). GGT, ALP were higher in SCA than in HbS-βThal and HbSC (GGT p=0.013; ALP p=0.006), but without statistical significance in HbS-βThal compared to HbSC (GGT p=0.23; ALP p=0.44). Liver synthesis indices were similar in the three subgroups; none was neither HbsAg nor HCV-RNA positive. No differences were found comparing laboratory indices at T0 and T1. TE Stiffness was statistically higher in SCA (KPa 8.3±6.86) than in HbSC pts (KPa 5.33±2.15; p=0.014). In HbS-βThal (KPa 6.17±2.58) was increased but not statistically significant compared to either SCA and HbSC pts (p=0.2). Liver Iron Concentration (LIC) (derived from MRI T2*) was higher in HbS-βThal than in SCA and HbSC pts (HbS-βThal *vs* HbSC p=0.0145) and in SCA comparing HbSC (p=0.018). Univariate analysis was performed to correlate GGT with ferritin (p=0.02), TE (p=0.002), US (p=0.107) and LIC (p=0.511) in all SCD pts. A good correlation between GGT and US liver echogenicity was present in SCA and HbS-βThal pts, with GGT values respectively 20% and 160% higher than normal. TE and US (p=0.045) in all SCD pts correlated positively. No differences were found in TE and MRI T2* at T0 and T1. US showed significant differences at T0 compared with T1 in HbS-βThal (p=0.04) and in HbSC pts (p=0.001), but not in SCA pts (p=0.46) probably because of higher Stiffness since T0. Multivariate analysis showed as independent risk factors: sex (male), low HbF values, high ferritin values, more severe sickle genotype as predictors of liver involvement.
Summary and Conclusions: Function liver tests associated with US, TE and when possible to MRI T2* taking into account sex, percentage of HbF, and SCD genotypes, are important to early detect and follow the sickle hepatopathy.

Stem cell transplantation – Clinical

PF718

SAFETY AND IMPROVED SUSTAINED RESPONSE RATES IN PATIENTS WITH CHRONIC GRAFT *VERSUS* HOST DISEASE TREATED WITH IBRUTINIB: 1-YEAR UPDATE OF A PHASE 1B/2 STUDY

E. Waller[1,*], D. Miklos[2], C. Cutler[3], M. Arora[4], M. Jagasia[5], I. Pusic[6], M. Flowers[7], A. Logan[8], R. Nakamura[9], J. Dubovsky[10], S. Chang[10], F. Clow[10], I. Lal[10], L. Styles[10], S. Jaglowski[11]

[1]Winship Cancer Institute of Emory University, Atlanta, GA, United States, [2]Stanford University, Stanford, CA, United States, [3]Dana Farber Cancer Institute, Boston, MA, United States, [4]University of Minnesota, Minneapolis, MN, United States, [5]Vanderbilt-Ingram Cancer Center, Nashville, TN, United States, [6]Washington University School of Medicine, St. Louis, MO, United States, [7]Fred Hutchinson Cancer Research Center, Seattle, WA, United States, [8]University of California San Francisco, San Francisco, CA, United States, [9]City of Hope, Duarte, CA, United States, [10]Pharmacyclics LLC, an AbbVie Company, Sunnyvale, CA, United States, [11]The Ohio State University Comprehensive Cancer Center, Columbus, OH, United States

Background: Ibrutinib, a once-daily inhibitor of Bruton's tyrosine kinase, demonstrated clinically meaningful sustained responses and an acceptable safety profile in a phase 1b/2 study involving patients with active chronic graft *versus* host disease (cGVHD) and inadequate response to corticosteroid-containing therapies (median follow-up, 1.2 years; Miklos *et al.*, *Blood* 2017). Observations from this study led to the approval of ibrutinib in US patients who failed ≥1 lines of systemic therapy.
Aims: To report updated data from the phase 1b/2 clinical study of ibrutinib in patients with inadequate response to corticosteroid-containing therapies.
Methods: Phase 1b/2, open-label, single-arm, multicenter study (PCYC-1129-CA; NCT02195869). Patients with steroid-dependent or -refractory cGVHD who received ≤3 prior therapies for cGVHD and had either a National Institutes of Health (NIH)-defined 25% body surface area erythematous rash or 4 total mouth score received ibrutinib 420 mg/day until progression or unacceptable toxicity. The primary endpoint was best overall cGVHD response by 2005 NIH Consensus Panel Response Criteria with modification based on the 2014 NIH Consensus Panel Response Criteria. Secondary endpoints included sustained response rate, changes in Lee cGVHD symptom Total Summary Score, steroid doses over time, and safety. All patients provided written informed consent.
Results: After a median follow-up of 2.1 years, the best overall cGVHD response rate was 69% (n=29/42; complete response [CR], 31% [n=13]; partial response [PR], 38% [n=16]). Among the 29 patients who responded, 20 (69%), 18 (62%), and 16 (55%) had sustained responses ≥20, ≥32, and ≥44 weeks, respectively. A total of 19/26 (73%) patients who responded and had ≥2 organs involved at baseline had a response in ≥2 organs and 6/10 (60%) patients who responded and had ≥3 organs involved at baseline had a response in ≥3 organs. The cGVHD response rate in patients with sclerosis at baseline was 61% (n=11/18; CR, 39%; PR, 22%). By week 52, 26/42 (62%) patients had a reduced steroid dose to <0.15 mg/kg/d; 8 patients discontinued steroids. A clinically meaningful (≥7 point) decrease in the Lee cGVHD Symptom Total Summary Score was observed in 12 (29%) patients on ≥2 consecutive visits. At 1 year, 16/29 patients who responded (55%) compared with 1/13 patients who did not respond (8%) showed an improvement in Lee cGVHD Symptom Total Summary scores. Common grade ≥3 adverse events (AEs) were pneumonia (n=6), fatigue (n=5), and diarrhea (n=4). Serious AEs (SAEs) were reported in 22 patients (52%); grade ≥3 SAEs occurred in 19 patients (45%) and included 6 patients with pneumonia. One patient with pneumonia died. The onset of new grade ≥3 AEs decreased from 71% in the first year of treatment to 25% in the second year of treatment (n=12). A total of 15 patients discontinued treatment because of AEs and 5 discontinued because of progressive cGVHD; 4 patients discontinued ibrutinib after cGVHD resolution.
Summary and Conclusions: An additional 1 year of follow-up of ibrutinib's activity and safety in adult patients with cGVHD that failed ≥1 lines of systemic therapy demonstrated durable responses and continued improvements from the previously reported 1-year results: CR rates increased from 21% to 31%, sustained response rates for ≥32 weeks increased from 48% to 62%, and patients with improvement in Lee cGVHD Symptom Total Summary Score on ≥2 consecutive visits increased from 24% to 29%. These results support ibrutinib's recent approval in the United States in this pretreated, high-risk population.

PF719

SINGLE CORD BLOOD UNIT PLUS THIRD PARTY DONOR CELLS (HAPLO-CORD) TRANSPLANTATION COMPARED TO ADULT UNRELATED DONORS IN PATIENTS WITH ACUTE LEUKEMIA: A RETROSPECTIVE CASE-CONTROL STUDY

G. Bautista Carrascosa[1,*], C. Canals[2], M. Kwon[3], I. Sanchez-Ortega[4], C. Regidor[1], P. Balsalobre[3], A. Bermúdez[5], M. Rovira[6], J.L. Díez-Martin[3], A. de Laiglesia[1], J.A. Pérez-Simón[7], L. Yañez[5], C. Ferrá[8], J. Sierra[9], J.L. Bello[10], I. Herrera-Arroyo[11], C. Solano[12], M.J. Pascual[13], T. Zudaire[14], L. López-Corral[15], A. Figuera[16], I. Heras[17], A. Sureda[4], R. Cabrera[1], R.F. Duarte[1]

[1]Hospital Universitario Puerta de Hierro Majadahonda Madrid, Madrid, [2]Banc de Sang i Teixits, Barcelona, [3]Hospital General Universitario Gregorio Marañón, Madrid, [4]Institut Català d'Oncologia Duran i Reynals, Barcelona, [5]Hospital Universitario Marqués de Valdecilla, Santander, [6]Hospital Universitari Clínic, Barcelona, [7]Hospital Universitario Virgen del Rocío, Sevilla, [8]Institut Català d'Oncologia Trias i Pujol, Badalona, [9]Hospital Santa Creu i Sant Pau, Barcelona, [10]H Clínico Universitario, Santiago de Compostela, Santiago de Compostela, [11]Hospital Universitario Reina Sofía, Córdoba, [12]Hospital Universitario Clínico, Valencia, Valencia, [13]Hospital Universitario Carlos Haya, Málaga, [14]Complejo Hospitalario de Navarra, Pamplona, [15]Hospital Clínico Universitario, Salamanca, Salamanca, [16]Hospital Universitario de La Princesa, Madrid, [17]Hospital General Universitario Morales Meseguer, Murcia, Spain

Background: The best alternative donor for allogeneic HCT (alloHCT) candidates without a matched sibling remains to be defined. In the absence of prospective randomized trials, additional data are needed to inform donor choice in these patients. Recently, Milano et al (N Engl J Med 2016;375:944-53) showed that cord blood HCT may improve relapse rates and overall survival compared to unrelated donors (UD) in patients with high-risk acute leukemia (AL) and residual disease prior to HCT.
Aims: We present a retrospective case-controlled study by the Grupo Español de Trasplante Hematopoyético y Terapia Celular (GETH) of first alloHCT for high-risk AL including 94 alloHCT recipients of single cord-blood units plus third-party donor CD34+ cells (haplo-cord) compared (1:2) with 188 recipients of UD alloHCT.
Methods: Case and controls were matched for age, gender, WHO diagnosis, disease status at HCT, time from diagnosis to HCT, prior auto-HCT, TBI use in conditioning and year of HCT. Haplo-cord cases included 57 men (61%) and 37 women (39%), median age 34 years (range 16-64), median weight 70 kg (42-111), 51 AML and 43 ALL, 49 in first CR, 16 in CR2 and 29 more advanced, including 23 with detectable disease. Six had a prior autologous HCT. Median time from diagnosis to alloHCT was 8.3 months (range 2-66).
Results: Overall outcomes for the whole series at 6 years are comparable between haplo-cord and UD-controls for non-relapse mortality (33.0%, 95CI: 24.7-44.0 *versus* 34.4%, 95CI: 28.1-42.0, respectively; n.s.), and show a statistical trend in favour of haplo-cord in relapse rate (24.5%, 95CI:17.2-34.9 in haplo-cord *versus* 30.7%, 95CI:24.7-38.2 in UD-controls; p=0.135) and overall survival (47.7%, 95CI: 37.6-57.8 in haplo-cord *versus* 37.0%, 95CI: 29.9-44.0 in UD-controls; p=0.079). In addition, grade II-IV acute GvHD was significantly lower in the haplo-cord group (12.1% *vs* 40.7%, p=0.001; 35.8% in matched *vs* 45.6% in mismatched controls) as well as chronic GvHD (29.9% *vs* 50.0%, p=0.02; 43.6% in matched *vs* 55.8% in mismatched controls).
Of note, high-risk AL patients transplanted with advanced disease (CR3 or later, partial remission or refractory disease), had significantly better 6-year outcomes following haplo-cord alloHCT than their UD-controls: relapse rate was 37.9% (95CI: 23.8-60.4) *versus* 47.8% (95CI: 35.4-64.7), respectively (p=0.069), progression-free survival was 24.1% (95CI: 8.6-39.7) *versus* 13.0% (95CI: 3.3-22.8), respectively (p=0.046), and overall survival was 31% (95CI: 14.2-47.9) *versus* 13.0% (95CI: 3.3-22.8), respectively (p=0.046). Patients with ALL had a particular benefit from the use of haplo-cord HCT. Their progression-free survival with haplo-cord at 6 years significantly improved compared with UD (43.6% (95CI: 28.0-59.2) *vs* 24.4% (95CI: 14.6-34.3); p=0.05) (Figure 1).
Summary and Conclusions: With the increase availability and use of alternative donors for alloHCT, data to inform donor choice are needed. It is unlikely that any single type of alternative donor will be the best choice for all patients lacking a matched related donor. Our data with haplo-cord HCT, in line with recent findings by Milano *et al.*, contribute to the evidence to suggest that unrelated CB reduces the incidence of GvHD while controlling the underlying AL, and might be a preferable donor choice with improved overall survival for patients with AL and a high-risk of relapse, especially in ALL patients.

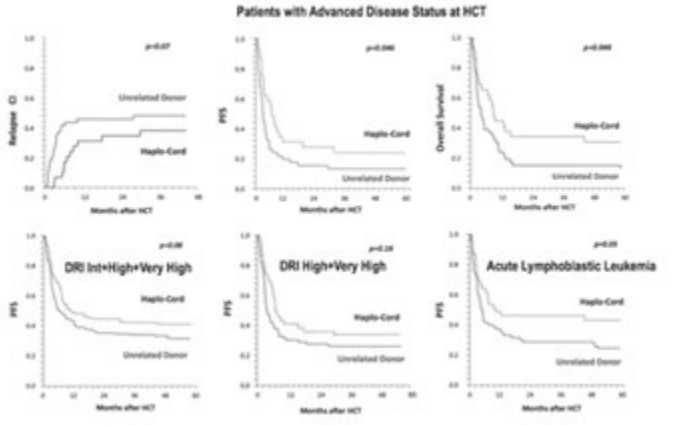


Figure 1.

PF720

CARFILZOMIB-CYCLOPHOSPHAMIDE-DEXAMETHASONE INDUCTION AND SALVAGE ASCT IN TRANSPLANT-ELIGIBLE MULTIPLE MYELOMA WITH FIRST RELAPSE AFTER UPFRONT ASCT

H. Gregersen[1,*], V. Peceliunas[2], K. Remes[3], F. Schjesvold[4], N. Abildgaard[5], H. Nahi[6], N. Frost Andersen[7], A. Juul Vangsted[8], T. Wirenfeldt Klausen[9], C. Helleberg[9], U. C. Frølund[10], P. Axelsson[11], O. Stromberg[6], C. Blimark[12], K. Carlson[13], A. Waage[14], M. Hansson[15], N. Gulbrandsen[4]

[1]Hematology, Aalborg Universitetshospital, Aalborg, Denmark, [2]Hematology, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania, [3]Hematology, Turku University Hospital, Turku, Finland, [4]Hematology, Oslo University Hospital, Oslo, Norway, [5]Hematology, Odense University Hospital, Odense, Denmark, [6]Hematology, Karolinska University Hospital, Stockholm, Sweden, [7]Hematology, Aarhus University Hospital, Aarhus, [8]Hematology, Rigshospitalet, Copenhagen, [9]Hematology, Herlev Hospital, Herlev, [10]Hematology, Zealand University Hospital, Roskilde, Denmark, [11]Hematology, Helsingborg Hospital, Helsingborg, [12]Hematology, Sahlgrenska University Hospital, Göteborg, [13]Hematology, Uppsala University Hospita, Uppsala, Sweden, [14]Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway, [15]Hematology, Skåne University Hospital, Lund, Sweden

Background: Salvage autologous stem cell transplantation (ASCT) is used in selected patients with relapsed multiple myeloma (MM) after up-front ASCT. However, there are limited data on the optimal induction therapy before salvage ASCT. There is strong support for the use of maintenance therapy after upfront ASCT in newly diagnosed MM whereas data on maintenance therapy after salvage ASCT are sparse.

The Nordic Myeloma Study Group (NMSG) initiated the CARFI trial (NCT02572492), an open randomized phase II study, to investigate the efficacy and safety of carfilzomib as part of induction and conditioning in salvage ASCT and to evaluate the role of carfilzomib/dexamethasone maintenance after salvage ASCT. Here we report safety and response data before start of maintenance on the first 118 included patients that have undergone induction and salvage ASCT.

Aims: To report safety and response data on the first 118 included patients that have undergone carfilzomib-cyclophosphamide-dexamethasone (CAR-CY-DEX) induction and salvage ASCT.

Methods: Patients with first relapse more than one year after up-front ASCT can be included. A total enrollment of 200 patients is planned. Induction therapy is four cycles of CAR-CY-DEX (iv carfilzomib 20 mg/sqm 36 mg/sqm on days 1, 2, 8, 9, 15 and 16, tablet cyclophosphamide 300 mg/sqm on days 1, 8 and 15 and tablet dexamethasone 20 mg on days 1, 2, 8, 9, 15 and 16 in each 28-days cycle). The conditioning regimen consists of iv carfilzomib 27 mg/sqm on day –2 and –1, and iv melphalan 200 mg/sqm on day –2. Two months after ASCT patients are randomized (1:1) to either observation or maintenance therapy with iv carfilzomib 27 mg/sqm 56 mg/sqm every second week and tablet dexamethasone 20 mg every second week.

Results: By January 15, 2018, 173 out of 200 planned patients have been enrolled in the study. 129 patients have completed CAR-CY-DEX induction and 118 patients have completed salvage ASCT. Twelve patients went off study during the induction phase (four discontinued due to progression, five due to serious adverse events (two infections, acute myocardial infarction, intracerebral hemorrhage and pulmonary insufficiency), two due to withdrawal of consent and one due to sub-optimal response (SD)). Two patients died due to serious infections during induction. Five patients went off study during the ASCT phase or within the following two months (two patients due to progression, one due to fatal Candida septicemia, one due to withdrawal of consent and one due to small cell lung cancer) (Table 1). A total of 53 Grade 3/4 serious adverse events were reported in 40 patients (31.0%) during the CAR-CY-DEX induction therapy (Table 1).

Responses after CAR-CY-DEX induction and after salvage HDT are summarized in Table 1. After CAR-CY-DEX induction ≥VGPR and ≥CR were observed in 46.5% and 9.3%, respectively, and at disease evaluation 2 months after salvage ASCT ≥VGPR and ≥CR were achieved in 64.4% and 16.9%, respectively. The corresponding figures for the same patients after upfront induction therapy were ≥VGPR in 46.6% and ≥CR in 10.9%, and after up-front ASCT ≥VGPR in 78.0% and ≥CR in 31.4%.

Summary and Conclusions: At first relapse after upfront ASCT CAR-CY-DEX induction therapy was feasible, tolerable and equally effective as induction at front-line.

Table 1.

Patient characteristics

N	129	
Age, median [IQR] [range]	62 [56;66] [41;72]	
Male, N (%)	71 (55.0%)	
Type of MM		
IgA	27 (20.9%)	
Other MM types	102 (79.1%)	
Up-front induction treatment		
VAD	1 (0.8%)	
CY-DEX	2 (1.6%)	
VELCY-DEX	90 (69.8%)	
CY-THALDEX	8 (6.2%)	
VELDEX	15 (11.6%)	
Other / mixed / multiple	10 (7.8%)	
ISS at enrollment		
I	66 (51.2%)	
II	48 (37.2%)	
III	10 (7.8%)	
FISH (high risk) at enrollment*	22 (17.1%)	
Response after induction therapy	Up-Front (N=129)	CAR-CY-DEX (N=129)
Stringent complete response (sCR)	2 (1.6%)	4 (3.1%)
Complete response (CR)	12 (9.3%)	8 (6.2%)
Very good partial response (VGPR)	46 (35.7%)	48 (37.2%)
Partial response (PR)	58 (45.0%)	52 (40.3%)
Stable disease (SD)	5 (3.9%)	8 (6.2%)
Progressive disease (PD)	1 (0.8%)	4 (3.1%)
Missing/not reported data	5 (3%)	4 (3.1%)
Response to treatments after ASCT	Up-Front (N=118)	Salvage ASCT (N=118)
Stringent complete response (sCR)	6 (5.1%)	17 (14.4%)
Complete response (CR)	11 (9.3%)	8 (6.8%)
Very good partial response (VGPR)	75 (63.6%)	51 (43.2%)
Partial response (PR)	25 (21.2%)	22 (18.6%)
Stable disease (SD)	0 (0.0%)	0 (0.0%)
Progressive disease (PD)	0 (0%)	2 (1.7%)
Missing/not reported data	1 (0.1%)	8 (6.8%)
Serious adverse events		
Grade 3/grade 4 during CAR-CY-DEX (N)	NA	53
Infection	NA	25 (46.0%)
Thrombosis	NA	11 (20.8%)
Septicemia	NA	3 (5.7%)
Pulmonary embolism	NA	1 (1.9%)
Other	NA	20 (37.7%)
Bleeding	NA	1 (1.9%)
Cardiac	NA	5 (9.4%)
Myocardial infarction	NA	2 (3.8%)
Atrial fibrillation	NA	1 (1.9%)
Other cardiac complication	NA	1 (1.9%)
Pulmonary edema	NA	1 (1.9%)
Other	NA	12 (22.6%)

Categorial variables described in N (%) and continuous in median [IQR] [range]. All values at enrollment if not.
* Defined by cytogenetics (del(17p), t(4;14) or t(14;16)).

PF721

THE ROLE OF COLLATERAL RELATED DONORS IN HAPLOIDENTICAL HEMATOPOIETIC TRANSPLANTATION: A COMPARATIVE STUDY BETWEEN COLLATERAL RELATED DONORS AND PARENTAL DONORS

X.-D. Mo[1,*], Y.-Y. Zhang[1], X.-H. Zhang[1], L.-P. Xu[1], Y. Wang[1], C.-H. Yan[1], H. Chen[1], Y.-H. Chen[1], K.-Y. Liu[1], X.-J. Huang[1]

[1]Peking University People's Hospital, Institute of Hematology, Beijing, China

Background: Human leukocyte antigen (HLA)-haploidentical related donor HSCT (haplo-HSCT) has become a valuable option in transplant procedures.Immediate family donor and the collateral relative donor (CRD) could both be a potential haploidnetical donor. But the role of CRDs in the donor selection for haplo-HSCT is unclear
Aims: We aimed to analyze the clinical outcomes and prognostic factors for patients receiving haplo-HSCT from CRDs. We also wanted to compare the clinical outcomes among patients receiving haplo-HSCT from CRDs, maternal donors (MDs), and paternal donors (PDs).
Methods: A total of 929 consecutive subjects with hematological malignances receiving haplo-HSCT January 2005 through December 2014 were considered; including CRDs (n=60), PDs (n=573), or MDs (n=296).
Results: Female donor/male recipient (FDMR) combination was significantly associated with higher non-relapse mortality (NRM) and poorer survival rates of CRDs. The 5-year cumulative incidence of relapse was comparable among the PD, MD, and CRD groups. The 5-year cumulative incidence of NRM was higher in CRD (25.0%) and MD (23.1%) groups compared with that of PD (12.5%) group (P<0.001). The 5-year probabilities of DFS (66.1%) and OS (72.2%) of PD group were both better than those of CRD (DFS: 53.2%; OS, 56.6%) and MD group (DFS: 59.5%; OS, 61.6%). All of the clinical outcomes were comparable between CRD and MD groups. FDMR CRD was associated higher platelet engraftment failure (HR=3.58, P=0.028), higher extensive chronic graft-*versus*-host disease (cGVHD) (HR=6.33, P=0.010), higher NRM (HR=13.20, P<0.001), lower DFS (HR=6.79, P<0.001) and lower OS (HR=8.89, P<0.001) compared with MD. The clinical outcomes of non-FDMR CRD group were comparable to those of MD. PD showed the best clincial outcomes.
Summary and Conclusions: Our results showed that for the patient without sibling or offspring donor, choosing PD was the most optimal selections. For those who were not available to choose PD, choosing MD or non-FDMR CRD is reasonable. Transplants from FDMR CRD should probably be avoided considering the low platelet engraftment, high GVHD, high NRM, and low survival rates.

PF722

EFFICACY OF BONE MARROW DERIVED MSC FOR STEROID REFRACTORY ACUTE GVHD ASSOCIATES WITH AGE AND A DEFINED MOLECULAR PROFILE OF MSC DONORS

L. Van Der Wagen[1,*], F. Fernando[2], N. Appukudige[2], A. Janssen[2], K. Westinga[3], R. Admiraal[4], M. Lorenowicz[5], P. Coffer[5], G. Huls[6], S. Zeerleder[7], J. Janssen[8], A. Broers[9], W. van der Velden[10], R. van Marwijk Kooy[11], J.J. Boelens[12], J. Kuball[1]

[1]Hematology, [2]Laboratory of Translational Immunology, [3]Cell Therapy Facility, [4]BMT Unit Pediatric Department, UMC UTRECHT, [5]Center for Molecular Medicine & Regenerative Medicine Center, UMC Utrecht and Utrecht University, Utrecht, [6]Hematology, UMC Groningen, Groningen, [7]Hematology, AMC Amsterdam, [8]VU MC, Amsterdam, [9]Erasmus MC, Rotterdam, [10]Radboud MC, Nijmegen, [11]Internal Medicine, Isala klinieken, Zwolle, [12]UMC UTRECHT, Utrecht, Netherlands

Background: Acute graft *versus* host disease (aGVHD) remains a life-threatening complication and substantially reduces efficacy of allo-HSCT. The outcome of patients with severe steroid-refractory aGVHD continues to be poor. Administration of Mesenchymal Stromal Cells (MSC) has been reported by others and us as an interesting treatment option (Le Blanc, Frassoni *et al.* 2008, von, Stolzel *et al.* 2009, Lucchini, Introna *et al.* 2010, Te Boome, Mansilla *et al.* 2015). Though insurance and fast track approval has recently been granted to MSC products in Japan and USA, variability arising from donor variability and the inability to link surrogate markers of the product to clinical efficacy could prove to be a major obstacle in daily clinical practice.
Aims: To evaluate the impact of individual MSC donors as well as MSC donor properties such as age on clinical response in a cohort of 102 patients with grade II-IV steroid refractory aGVHD treated within a prospective study as well as an extended hospital exemption program (Te Boome, Mansilla *et al.* 2015).
Methods: Bone marrow derived MSC from third party non-HLA matched donors were administered at day 1, 8, and 21 as reported (Te Boome, Mansilla *et al.* 2015). Primary outcome measures were 1-year overall survival (OS) and GVHD response. Cox proportional hazards models, competing risk analyses (Gray's test) and Kaplan Meier estimates were used for analyzing response and OS respectively. Two R statistical packages for RNA-seq analysis were used for calculating differentially expressed genes (DEGs) (DeSeq & EdgeR). DEGs were considered significant based on the overlap across the two methods and a False Discovery Rate/adjusted p-value<0.05.
Results: 102 patients received in total 299 MSC infusions derived from 10 different BM donors. Median number of infusions was 3 (range 1-4). 75,5% of patients received all MSC infusions from the same donor, 20,6% MSC from 2 donors and 3,9% MSC from 3 different donors. Two donors were used to treat 28,4% and 43,1% of patients respectively. When testing retrospectively impact on 1-year OS of an individual product no differences between patients treated with either the 2 main contributing donors or the patients treated with the 'other' MSC donors could be observed. However, a survival benefit was observed for patients treated with young donors (MSC donor age <10 years, Figure 1). Competing risk analysis also revealed a significant benefit for patients only treated with young donors. Furthermore, in multivariate analysis, MSC donor age remained predictive for OS (HR 2,00, p-value 0,025). RNA sequencing analysis was used to further dissect molecular differences between individual donors and allowed to identify a distinctive molecular profile in MSC which associated with improved clinical outcome.

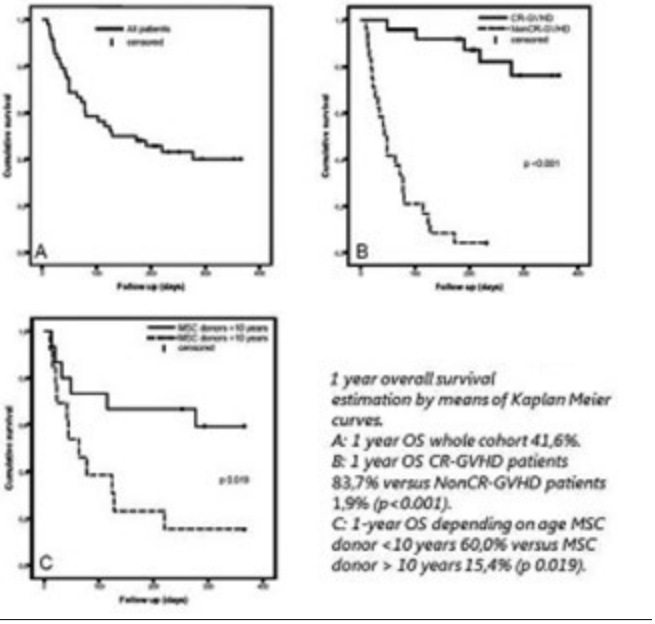


Figure 1.

Summary and Conclusions: In our cohort of 102 patients with steroid refractory acute GVHD that were treated with MSC we observed in this retrospective study differences in achieving both complete resolution of GVHD symptoms and 1 year OS. Patients only treated with MSC derived from young bone marrow donors (<10 years old) had significantly better resolution of GVHD symptoms as well as an improved OS. Furthermore we identified a distinctive molecular profile by means of RNA sequencing which associates with improved clinical outcome. These findings may have implications for approval of current products entering the market as well as on future and currently ongoing trials with MSC.

PF723

PROGNOSTIC INDEX FOR PATIENTS WITH RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA WHO UNDERWENT HEMATOPOIETIC CELL TRANSPLANTATION: A KSGCT MULTICENTER ANALYSIS

T. Tachibana[1,*], J. Kanda[2,3], T. Ishizaki[4], Y. Najima[5], M. Tanaka[1], N. Doki[5], S. Fujiwara[6], S.-I. Kimura[2], M. Onizuka[7], S. Takahashi[8], T. Mori[9], T. Saito[10], S. Fujisawa[11], E. Sakaida[12], K. Matsumoto[13], N. Aotsuka[14], M. Goto[15],

R. Watanabe[16], K. Shono[17], K. Usuki[18], N. Tsukada[19], H. Kanamori[1], Y. Kanda[2,20], S. Okamoto[9]

[1]Kanagawa Cancer Center, Yokohama, [2]Saitama Medical Center, Jichi Medical University, Saitama, [3]Graduate School of Medicine, Kyoto University, Kyoto, [4]Gunma University Graduate School of Medicine, Mabashi, [5]Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, [6]Jichi Medical University, Shimotsuke, [7]Tokai University School of Medicine, Isehara, [8]The Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo, [9]Keio University School of Medicine, [10]Jikei University School of Medicine, Tokyo, [11]Yokohama City University Medical Center, Yokohama, [12]Chiba University Hospital, Chiba, [13]Yokohama City University School of Medicine, Yokohama, [14]Japanese Red Cross Society Narita Hospital, Narita, [15]Tokyo Medical University, Tokyo, [16]Saitama Medical Center, Kawagoe, [17]Chiba Aoba Municipal Hospital, Chiba, [18]NTT Medical Center Tokyo, [19]Japanese Red Cross Medical Center, Tokyo, [20]Jikei University School of Medicine, Shimotsuke, Japan

Background: Generally, the outcomes of hematopoietic cell transplantation (HCT) in patients with non-remission acute myeloid leukemia (AML) have been poor. Particularly, the detection of cohorts with extremely poor prognosis is important, because medicosociological issues, including escalation of medical costs or donor burden, remain unresolved.
Aims: A multicenter retrospective study was performed to design a prognostic scoring index and to detect a population with lowest survival in patients with relapsed or refractory AML, who underwent HCT.
Methods: Patients with non-remission AML who received HCT between 2005 and 2015 at attending institutions were eligible. Using a clinical research form, further clinical information, such as the reason of non-remission or biomarkers prior to HCT, was collected. Inclusion criteria were the blast fraction in bone marrow ≥5% or detection of blast cell in peripheral blood prior to HCT. Extramedullary sarcoma without bone marrow involvement and untreated leukemia were excluded.
Results: A total of 639 patients with non-remission AML were found in the registry data. After excluding 120 patients, 519 patients were included in the analysis. The whole cohort was randomized for training (n=256) and validation (n=263). The median age of a training cohort was 50 years (range: 16–70). Disease status included primary induction failure in 145 patients and relapse after remission in 111 patients. The median blast fractions of peripheral blood and bone marrow were 3% (range: 0–99) and 20% (range: 0-99), respectively. The median duration from diagnosis to HCT was 7 months (range: 0–78). Donors came from related bone marrow in 26 patients, related peripheral blood in 50, an unrelated donor in 115, and a single unit of umbilical cord in 65, respectively. An HLA antigen-matched donor was used for 150 patients. Myeloablative and reduced intensity conditioning regimen was used for 150 and 106 patients, respectively. Cumulative incidence of engraftment at 50 days, grade to of acute graft-*versus*-host disease (GVHD) at 100 days, and chronic GVHD at 1 year was 85.5%, 48.3%, and 34.9%, respectively. With a median follow-up period of 36.7 months (range: 2.5–129), overall survival (OS), non-relapse mortality, and relapse mortality at 1 years was 42% and 36%, and 41%, respectively. Multivariate analysis demonstrated independent predictors for OS included C-reactive protein ≥1 mg/dL (hazard ratio [HR]=1.84, 95% confidence interval [CI]: 1.35–2.50, P <0.001), peripheral blood blast fraction ≥20% (HR=1.99, 95%CI: 1.48–2.69, P <0.001), poor-risk karyotype (HR=1.75, 95%CI: 1.30–2.35, P <0.001), performance status ≥2 (HR=1.74, 95%CI: 1.17–2.57, P=0.006), and bone marrow from unrelated donor (HR=1.56, 95%CI: 1.16–2.09, P=0.003). Scoring one point for each factor, a prognostic scoring index was designed. At 2 years, OS was 46%, 24%, 8%, and 0% for Good (Score 0, 1: n=118), Intermediate-1 (Score 2: n=75), Intermediate-2 (Score 3: n=39), and Poor (Score 4: n=24), respectively (P <0.001)(Figure 1A). Applying the index to the validation cohort, OS at 2years was 40%, 18%, 12%, and 0% for Good (n=133), Intermediate-1 (n=61), Intermediate-2 (n=49), and Poor (n=20), respectively (P <0.001). The index was reconfirmed in a validation cohort (Figure 1B).

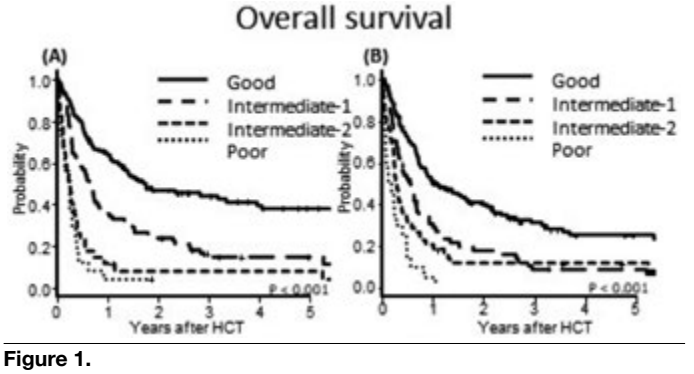


Figure 1.

Summary and Conclusions: Although further validation study is warrant, the scoring index may be useful to predict survival and to detect the population with the lowest survival prior to HCT in patients with relapsed or refractory AML.

PF724

IMPROVED SURVIVAL FOLLOWING OMS721 TREATMENT OF HEMATOPOIETIC STEM CELL TRANSPLANT-ASSOCIATED THROMBOTIC MICROANGIOPATHY (HCT-TMA)

A. Rambaldi[1,*], S. Khaled[2], M. Smith[3], M. Zecca[4], Y. L. Kwong[5], K. Claes[6], N. Leung[7], S. Whitaker[8]

[1]Bone Marrow Transplant Unit ASST Papa Giovanni XXIII, Bergamo, Italy, [2]City of Hope, Duarte, United States, [3]Canterbury District Health Board - Te Poari Hauora o Waitaha, Christchurch, New Zealand, [4]Fondazione IRCCS Policlinico San Matteo, Pavia, Italy, [5]University of Hong Kong, Hong Kong, Hong Kong, [6]UZ Leuven, Leuven, Belgium, [7]Mayo Clinic, Rochester, [8]Omeros Corporation, Seattle, United States

Background: Thrombotic microangiopathy is a potentially fatal complication of HCT. Mortality greater than 90% has been reported in high-risk cases. HCT-TMA is an endothelial injury syndrome associated with complement pathway activation. Other endothelial injury syndromes include graft *versus* host disease (GvHD) and diffuse alveolar hemorrhage (DAH). OMS721 is a human monoclonal antibody that inhibits mannan-binding lectin-associated serine protease-2 (MASP-2), the effector enzyme of the lectin pathway. OMS721 may improve patient outcomes by inhibiting complement-mediated injury during TMA.
Aims: This study's aims were to evaluate the efficacy and safety of OMS721 in patients with HCT-TMA.
Methods: OMS721 was evaluated in a 3-stage, uncontrolled Phase 2 study of patients with TMA, including HCT-TMA. Stage 1 evaluated 3 dose levels. One dose level was selected for cohort-expansion Stages 2 and 3. The protocol allowed 4 or 8 once-weekly IV doses of OMS721. Additional ½ doses could be administered to patients who received plasma therapy. HCT-TMA patients had persistent TMA defined as TMA that did not resolve following immunosuppression modification. One patient received OMS721 under compassionate use and received thrice-weekly dosing. A best-matched historical control from the literature was identified for comparison to the outcomes of HCT-TMA patients treated with OMS721. References were chosen to match OMS721-treated patients based on age, allogeneic transplantation, and reports that TMA did not resolve following immunosuppression modification. The references included individual patient survival data and were published in 2000 or later. Kaplan-Meier estimated mean survival was compared between OMS721-treated patients and historical control patients.
Results: A total of 19 patients were included in the analysis, 18 from the study and 1 from compassionate use. Nine OMS721-treated patients died, 4 during study participation. Figure 1 demonstrates a significant increase in median overall survival between OMS721-treated patients and historical controls (347 days *vs* 21 days from TMA diagnosis, respectively; Log-Rank p <0.0001).

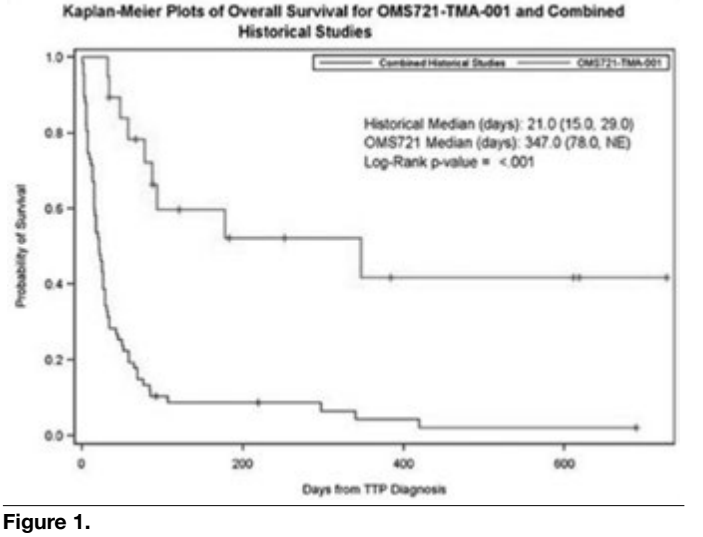


Figure 1.

Two patients from this population were previously presented. The compassionate-use patient's course was complicated by DAH and she did not tol-

erate previous eculizumab treatment. She responded well to OMS721 and was able to discontinue oxygen therapy, hemodialysis, and platelet transfusions. Another patient had steroid-refractory GvHD post-transplant. He developed TMA with co-existing GvHD and multiple disabling neurological complications. Following OMS721 treatment, his TMA and GvHD resolved, his neurological complications improved, and he was discharged and returned to work. OMS721 was well tolerated. The most common adverse events were diarrhea and neutropenia. One fatal adverse event of infection-related acute respiratory and renal failure was considered possibly related to OMS721 treatment because the investigator could not definitively rule out possible causation.

Summary and Conclusions: In this study, patients with high-risk HCT-TMA had significantly improved overall survival compared to best-matched literature controls. The safety profile was acceptable. This study shows positive outcomes with lectin pathway blockade by MASP-2 inhibition to treat HCT-TMA and other HCT-related complications (*i.e.*, GvHD and DAH) linked to endothelial injury.

PF725

TRANSPLANT-ASSOCIATED THROMBOTIC MICROANGIOPATHY: INCIDENCE, PROGNOSTIC FACTORS, MORBIDITY AND MORTALITY IN ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

E. Gavriilaki[1,*], I. Sakellari[1], D. Mallouri[1], I. Batsis[1], Z. Bousiou[1], A. Vardi[1], E. Yannaki[1], V. Constantinou[1], A. Tsompanakou[1], C. Vadikoliou[1], P. Kaloyannnidis[1], G. Bamihas[2], A. Anagnostopoulos[1]

[1]Hematology Department - BMT Unit, [2]Nephrology Department, G. Papanicolaou Hospital, Thessaloniki, Greece

Background: Transplant-associated thrombotic microangiopathy (TA-TMA) is a severe complication of allogeneic hematopoietic cell transplantation (HCT). Renewed interest has emerged in the field with the introduction of novel prognostic, diagnostic and treatment algorithms.

Aims: Therefore, we aimed to investigate the incidence, prognostic factors, morbidity and mortality of TA-TMA in allogeneic HCT recipients.

Methods: We enrolled consecutive patients who underwent HCT from 1990 to 2017 in our center. TA-TMA diagnosis was based on the International Working Group Criteria. Anti-thymocyte globulin (ATG, rabbit) was used as part of the conditioning in almost all non-sibling transplants. Graft-*versus*-host disease (GVHD) prophylaxis consisted of cyclosporine or tacrolimus plus methotrexate in myeloablative and mycophenolate mofetil plus cyclosporine in reduced intensity or toxicity conditioning transplants. Upon identification of TA-TMA, all possible causative factors were fully investigated. Management included withdrawal of calcineurin inhibitors, plasma infusion and plasma exchange combined with corticosteroid administration. Patients refractory to conventional management received humanized anti-CD20 monoclonal antibody (rituximab) until 2015 and since 2015, terminal complement inhibitor (eculizumab).

Results: Among 758 alloHCT recipients (transplanted from 451 sibling, 259 unrelated, 40 haploidentical, 2 twin donors and 6 cord blood), 116 (15.5%) patients were diagnosed with TA-TMA.

Regarding pre-transplant characteristics (age, gender, disease type and phase, lines of previous treatments, type of conditioning, ATG or TBI administration, type of donor, HLA matching), TA-TMA was associated only with TBI-based conditioning (34.4% *versus* 20.6%, p=0.004). TA-TMA patients also showed significantly increased rates of viral (p=0.001) and fungal (p=0.002) infections post-transplant, as well as severe acute (grade III-IV) and extensive chronic GVHD (p<0.001 for both). In the multivariate analysis, TBI-based conditioning (p=0.020), viral infections (p=0.024), acute (p=0.010) and chronic (p=0.004) GVHD remained independent predictors of TA-TMA. With a median follow-up of 23 (range 0.1-329) months, TA-TMA resulted in significantly lower overall survival (OS Figure 1, p=0.016). In the multivariate analysis, TA-TMA (p=0.029) remained an independent predictor of OS, along with relapse (p<0.001), acute (p<0.001) and chronic (p=0.002) GVHD. Among 116 TA-TMA patients, 70 developed renal (56) and/or neurologic (26) dysfunction that would be necessary for TA-TMA diagnosis according to the Bone Marrow Transplant Clinical Trials Network criteria. TA-TMA patients with renal dysfunction showed increased rates of acute GVHD (p=0.010), but no difference in OS compared to TA-TMA patients without renal dysfunction. However, neurologic dysfunction resulted in significantly lower OS (p=0.033) in TA-TMA patients.

Summary and Conclusions: Our results confirm that TA-TMA is associated with increased morbidity and mortality in allogeneic transplant recipients, irrespectively of pre-transplant characteristics (except for TBI). Successful prevention and treatment strategies of infections and GVHD need to be timely employed to improve survival in this complex setting. Novel diagnostic criteria are also warranted to early recognize patients that would benefit from novel treatment options.

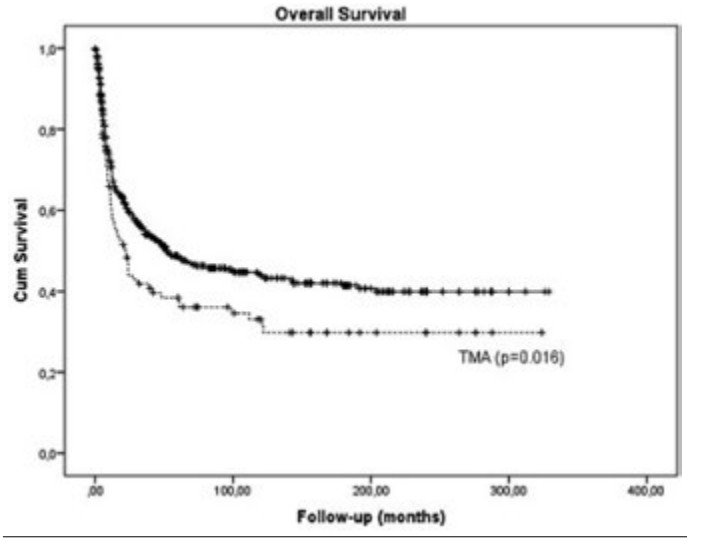


Figure 1.

PF726

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT IN THE OUTPATIENT SETTING: A SINGLE CENTER EXPERIENCE OF 924 CONSECUTIVE TRANSPLANTS

S. Saadeh[1,*], U. Durani[1], A. Kansagra[2], H. Alkhateeb[1], M. Shah[1], K. Ewert[3], S. Kendarian[1], S. Hashmi[1], M. Patnaik[1], M. Litzow[1], W. Hogan[1]

[1]Hematology, Mayo Clinic, Rochester, [2]Internal Medicine, UT Southwestern Medical Center, Dallas, [3]Information Technology, Mayo Clinic, Rochester, United States

Background: In most centers, allogeneic transplant patients are admitted for follow-up until engraftment and hematopoietic recovery a median length of stay of up to 31 days (Majhail *et al.*, 2013). Prolonged admissions can increase risk of nosocomial infections, deconditioning, in addition to associated financial toxicity. At our institution, patients are not routinely admitted and transplant followed outpatient with scheduled daily follow-up. Some require planned admissions for completion of conditioning or GVHD prophylaxis regimen among other reasons. During follow-up, patients are admitted for management of complications when deemed unsafe or high risk for management as outpatient

Aims: We aim to report on feasibility and outcomes of outpatient allogeneic stem cell transplant during the first 100 days

Methods: We retrospectively analyzed records of all adult allogeneic transplants performed from January 1989 to March 2017. Non-routine hospital admissions within 100 days after transplant were included. Descriptive statistics and multivariable logistic regression were used to characterize the cohort and determine predictors of admission

Results: A total of 924 transplants were identified. Males were 58%, median age was 51 (IQR 39-58), and 55.3% received myeloablative conditioning. There were 1,098 non-routine admissions with 32.7% of patients never requiring admission. Median number of admissions for the entire cohort was 1 (inter-quartile range (IQR) 0-2), and median time from transplant to admission was 23 days (IQR 9-54). Median length of stay was 6 days per admission (IQR 3-13) and in-hospital mortality was low at 5%. Direct admission to an intensive care unit occurred in 6.6% of admissions. In the cohort requiring admissions, median total number of days spent in the hospital was 17 (IQR 9-34). Most patients had an absolute neutrophil count greater than $0.5x10^9$/L and a platelet count greater than $20x10^9$/L prior to admission; 57% and 58%, respectively. Baseline characteristics and cause of admission are highlighted in Table 1 and Figure 1, respectively. patients with CLL were the least likely to require admission, and in a multivariable logistic regression (adjusted for age, sex, conditioning), patients with ALL (OR 2.66, p<0.01), AML (OR 2.44, p<0.01), benign disease (OR 3.25, p<0.02), lymphoma (OR 2.49, p<0.03), MPN (OR 2.93, p<0.02), and MM (OR 4.63, p<0.01) were more likely to be admitted compared to CLL. Bone marrow graft (*versus* peripheral blood) was a negative predictor of admission (OR 0.22, p<0.01) while unrelated donor was a positive predictor (OR 2.23, p<0.01).

Summary and Conclusions: Allogeneic stem cell transplant in the outpatient

setting is feasible with 32.7% of patients never requiring admission. Median number of in-hospital days was decreased to a median of 17 days compared to a reported median of 31 days. Interestingly, conditioning intensity was not a predictor of admission.

Table 1. Patient characteristics.

	Transplants (N=924)
Diagnosis	
ALL	124 (13%)
AML	352 (38%)
CLL	60 (4%)
CML	52 (6%)
Lymphoma	54 (6%)
MDS	140 (15%)
MPN	41 (4%)
MM	58 (6%)
Other leukemia	10 (1%)
Benign (AA, SCA, PNH)	33 (4%)
Graft source	
PBSC	707 (77%)
BM	172 (19%)
UCB	41 (4%)
PBSC + BM	4 (<1%)
Donor source	
Related	508 (58%)
Unrelated	362 (41%)
Haploidentical	13 (1%)
Myeloablative conditioning	494
Good performance status (ECOG<3 and/or KPS>60)	910 (98%)

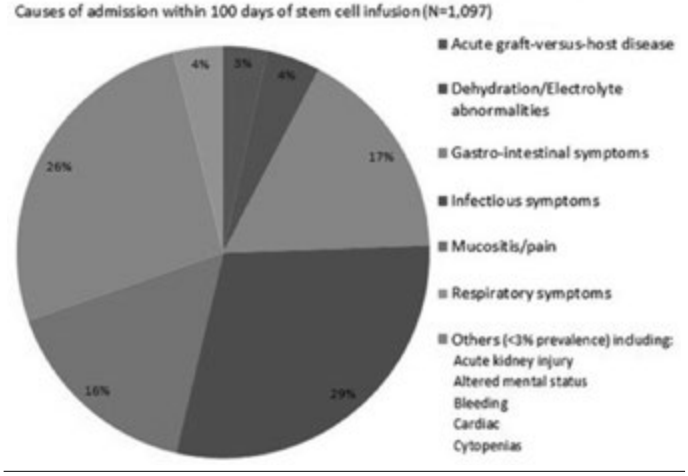


Figure 1.

PF727

PHASE II STUDY EVALUATING THE SAFETY AND EFFICACY OF BL-8040 FOR THE MOBILIZATION OF DONOR HEMATOPOIETIC STEM CELLS AND ALLOGENEIC TRANSPLANTATION IN PATIENTS WITH ADVANCED HEMATOLOGICAL MALIGNANCIES

M.P. Rettig[1,*], G.L. Uy[1], A. Bashey[2], S.M. Devine[3], S. Jaglowski[3], A. Vainstein-Haras[4], E. Sorani[4], H. Chen[4], O. Bohana-Kashtan[4], P. Westervelt[1], J.F. DiPersio[1]

[1]Washington University School of Medicine, St. Louis, [2]Blood and Marrow Transplant Group of Georgia, Northside Hospital Cancer Institute, Atlanta, [3]The Ohio State University Comprehensive Cancer Center, Columbus, United States, [4]BioLineRx Ltd., Modi'in, Israel

Background: Mobilization of hematopoietic stem and progenitor cells (HSPCs) with G-CSF requires 4-5 days of treatment prior to collection and is associated with bone pain and splenic rupture in donors, and higher rates of chronic GVHD in allogeneic hematopoietic cell transplant (alloHCT) recipients. Here, we sought to test BL-8040, a novel high affinity CXCR4 antagonist with rapid mobilizing kinetics and long receptor occupancy, in donors for alloHCT.
Aims: To assess the safety and tolerability of BL-8040 and the ability of a single BL-8040 injection to mobilize HSPCs for use in alloHCT.
Methods: This phase 2 study consists of 24 donor/recipient pairs. Part 1 enrolled HLA-identical pairs and donors were treated with a single SC dose of 1 mg/kg BL-8040. Part 2, following demonstration of safety, is enrolling both HLA-identical and haploidentical pairs and treating donors with 1.25 mg/kg BL-8040. Leukapheresis (LP) was performed 3 hours after injection and could be repeated the next day. When less than $5x10^6$ CD34 cells/kg recipient weight were collected, a second injection of BL-8040 and LP collection was performed the next day. The primary endpoint was collection of $\geq 2x10^6$ CD34 cells/kg recipient weight after up to 2 LP sessions.
Results: As of Feb. 2018, 22 healthy donors (16 HLA-identical and 6 haploidentical) with a median age of 54 (range 20-69) years have been enrolled in the study. One donor was not evaluable due to difficulties with vascular access and was replaced. 11 out of 13 donors (85%) treated at the 1 mg/kg dose and 8/8 donors (100%) treated at the 1.25 mg/kg dose of BL-8040 reached the primary goal of $\geq 2x10^6$ CD34 cells/kg recipient in up to 2 LP. 15 donors out of the 21 (71%) collected $\geq 2x10^6$ CD34 cells/kg in the primary LP and 13 (62%) collected $\geq 5x10^6$ CD34 cells/kg in up to 2 LP. The median CD34 cells/kg recipient in the primary LP collection was 2.3×10^6 (range 0.5×10^6 - 9.4×10^6; N=13) and 3.8×10^6 (range 1×10^6 - 8.7×10^6; N=8) in the 1 and 1.25 mg/kg BL-8040 group, respectively. The median CD34 cells/kg recipient in 2 LP collections was 5.2×10^6 (range 1.2×10^6 - 9.4×10^6; N=13) and 6.9×10^6 (range 2.3×10^6 – 13.8×10^6; N=8) in the 1 and 1.25 mg/kg BL-8040 group, respectively. Evaluation of CD34 kinetics following BL-8040 administration demonstrated stable CD34 cell concentration in the peripheral blood (15-21 cells/μL) throughout LP collection time. The most common BL-8040-related adverse events were injection site reactions and transient systemic reactions (mild to moderate hives). No serious adverse events were observed. 19 recipients, median age 51 years (range 26-69), with hematological malignancy (13 AML, 3 ALL, 1 MDS, 1 MPN and 1 HD), were transplanted with BL-8040-mobilized grafts. All transplanted recipients successfully engrafted with a median time to neutrophil engraftment (ANC≥500 cells/mm3) of 13 days (range 11-25 days) and to platelet engraftment (PLT≥20,000/mm3) 18 days (range 14-37 days). 13 out of 19 recipients passed day 100 post-transplant. Grade II acute GVHD was observed in 2 out of the 19 recipients (11%) and was resolved in one patient. Grade III-IV GVHD was observed in 3 out of 19 recipients (16%), of which 2 were resolved and 1 was downgraded to Grade 1.
Summary and Conclusions: BL-8040 as a single agent is a safe, rapid and effective HSPC mobilizing agent which results in prompt hematopoietic recovery after alloHCT with shortened collection time relative to G-CSF. Effects of BL-8040 on rates of chronic GVHD and relapse await longer follow up.

PF728

NON-HLA MATCHED, *EX-VIVO* EXPANDED CORD BLOOD PRODUCT SIGNIFICANTLY IMPROVES THE KINETICS OF HEMATOPOIETIC RECOVERY AND RESULTS IN EXCELLENT SURVIVAL IN PATIENTS UNDERGOING CORD BLOOD TRANSPLANTATION

F. Milano[1,*], E. Cox[1,2], S. Heimfeld[1,2], I. Bernstein[1], F. Appelbaum[1], C. Delaney[1,2]

[1]Clinical Research Division, Fred Hutchinson Cancer Research Center, [2]Nohla Therapeutics, Inc., Seattle, United States

Background: Allogeneic hematopoietic cell transplantation remains the only known curative approach for patients with high-risk leukemia; however, access is limited by donor availability. Umbilical cord blood (CB) has emerged as an important source of donor stem cells, particularly due to a decreased risk of graft-*versus*-host disease (GVHD) and potential improvement in graft *versus* leukemia effect. However, the low number of $CD34^+$ cells in CB grafts can lead to delayed hematopoietic recovery, resulting in increased risk of morbidity and mortality post-transplant.
Aims: With the goal of enhancing the kinetics of hematopoietic recovery and improving transplant outcomes, we developed methods to *ex-vivo* expand CB-derived hematopoietic stem/progenitor cells (HSPC) using an engineered Notch ligand to increase the number of rapidly repopulating HSPC for clinical applications. The final expanded product (NLA101) is a cryopreserved, non-HLA matched product for use as a T-cell depleted, universal donor graft source.
Methods: Between 2010 and 2012, 15 patients with hematologic malignancies were enrolled in a single center, Phase 2 trial to assess safety and feasibility of infusing NLA101 to augment myeloablative CB transplant (CBT). All patients received conditioning of fludarabine 75 mg/m^2; cyclophosphamide 120 mg/kg; and 13.2 Gy TBI. On transplant day, the unmanipulated CB unit(s) was infused first followed 4 hours later by NLA101. GVHD prophylaxis consisted of Cyclosporine/MMF. Hematopoietic recovery was analyzed using cumulative incidence (CI) rates to accommodate competing risks and was compared to a concurrent control cohort of 50 patients enrolled on a standard of care protocol who were treated identically. No significant differences between the two cohorts were found

with respect to age, sex, weight, disease, and MRD status. Disease-free survival (DFS) and overall survival (OS) were analyzed using Kaplan-Meier estimates. We herein report long-term follow-up data from this study.
Results: Fifteen patients, median age 21 years (range 5-45), being treated for ALL (n=8), AML (n=6) and MDS (n=1) were enrolled and included in this analysis. The median $CD34^+$ and TNC cell doses of NLA101 were 5.3 (range 3.1-11.6) $x10^6$ cells/kg and 5.8 (range 2.2-10.9) $x10^7$ cells/kg, respectively. Median follow-up for this study is now 6.5 years (range 5.6-7.4). As reported previously, time to neutrophil and platelet recovery was significantly improved over the control. CI at day 100 of neutrophil recovery was 100% *vs* 94% (p=0.005), with a median time of 19 days (range 9-31) *vs* 25 days (range 14-45) in the control. CI at day 100 of platelet recovery was 93% *vs* 74% (p=0.02), with a median time of 35 days (range 21 to 86) *vs* 48 days (range 24-158) in the control (Figure 1). No patients experienced transplant related mortality (TRM) or severe grade 3-4 acute GVHD. Two of the 15 patients (13%) relapsed post-transplant and subsequently died. Of the 13 evaluable patients, 9 patients (69%) were off immunosuppression therapy at 2 years post-CBT. DFS and OS remained excellent at 5 years post-CBT at 86%.

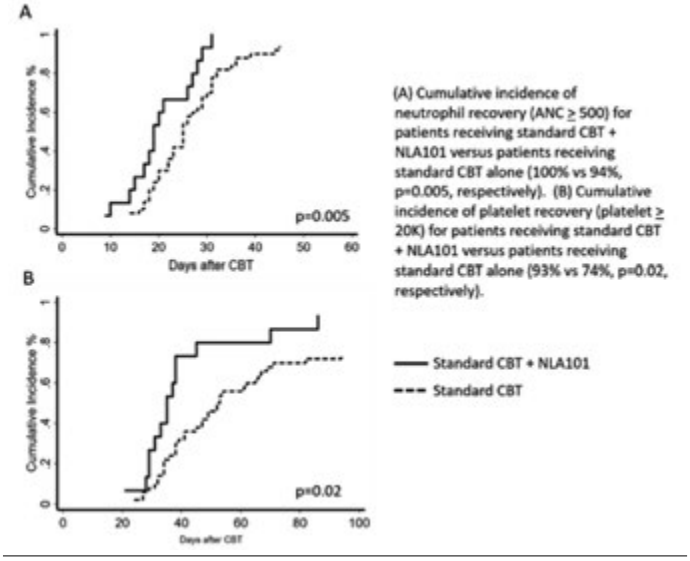


Figure 1.

Summary and Conclusions: These results demonstrate that infusion of NLA101 to augment myeloablative CBT was safe and led to faster neutrophil and platelet recovery, but more importantly showed excellent long-term outcomes of survival, with no TRM or severe grade 3-4 acute GVHD. The non-HLA matched NLA101 can be infused to enhance the kinetics of hematopoietic recovery, resulting in reduction of early transplant related mortality.

PF729

INCIDENCE AND RISK FACTORS OF ANTIBODIES TO HUMAN LEUKOCYTE ANTIGENS IN HAPLOIDENTICAL STEM CELL TRANSPLANTATION CANDIDATES: A MULTI-CENTER STUDY

M.-R. Huo[1], Y.-J. Xu[2], S.-Z. Zhai[1], M. Lv[1], Y. Wang[1], L.-Q. Cao[1], L.-P. Xu[1], X.-H. Zhang[1], H. Chen[1], Y.-H. Chen[1], F.-R. Wang[1], W. Han[1], Y.-Q. Sun[1], C.-H. Yan[1], F.-F. Tang[1], X.-D. Mo[1], M.-F. Zhao[3], K.-Y. Liu[1], X.-J. Huang[4], Y.-J. Chang[5,*]

[1]Peking University Institute of Hematology, Peking University People's Hospital, Beijing, [2]Department of Hematology, Xiangya Hospital, Changsha, [3]Department of Hematology, Tianjin First Central Hospital, Tianjin, [4]Peking University Institute of Hematology,Peking-Tsinghua Center for Life Sciences, Collabrative Innovation Center of Hematology, Peking University People's Hospital, [5]Peking University Institute of Hematology, Collabrative Innovation Center of Hematology, Peking University People's Hospital and Peking University Institute of Hematology, Beijing, China

Background: Currently, the incidence of and risk factors for antibodies to human leukocyte antigen (HLA) in haploidentical allograft candidates have not been thoroughly elucidated.
Aims: To investigate the incidence of and risk factors for antibodies to HLA
Methods: We investigated the incidence of and risk factors for antibodies to HLA in 1,663 haploidentical transplant candidates.
Results: Among these cases, 349 (21.0%) showed positive panel-reactive antibody (PRA) either for class I or class II HLA. Multivariate analysis showed the following: i) risk factors associated with the prevalence of PRA either for class I or class II HLA were female gender (P=0.018), prior transfusions (P<0.001) or pregnancy (P<0.001), and cases with MDS (P=0.018); compared to other patients, subjects with ALL had a lower incidence of class I antibodies (P=0.017); and ii) risk factors associated with the prevalence of PRA both for class I and class II HLA were female gender (P=0.014), prior transfusions (P=0.003), previous pregnancy (P<0.001), and diagnosis with MDS (P=0.035). The percentages of anti-HLA antibodies against a single locus, including HLA-A, -B, -C, -DP, -DQ, and -DR, among all cases were 15.6%, 17.3%, 10.5%, 5.6%, 8.5%, and 9.7%, respectively. Risk factors associated with specific antibodies against HLA-A, -B, -C, -DP, -DQ, and -DR were female gender, prior transfusion, previous pregnancy, and underlying disease. The median number of antibodies to a specific HLA locus was 28 (range, 1 to 148). Multivariate analysis showed a correlation between pregnancy and higher numbers of anti-HLA antibodies (P=0.004).
Summary and Conclusions: Our findings suggest that gender, prior pregnancy, previous transfusion and underlying diseases are risk factors for HLA sensitization. These findings could guide clinical monitoring for HLA antibodies and provide help with donor selection.

PF730

SAFETY AND EFFICACY OF VEDOLIZUMAB IN PATIENTS WITH STEROID REFRACTORY ACUTE GASTROINTESTINAL GRAFT-*VERSUS*-HOST DISEASE: A RETROSPECTIVE CHART REVIEW

Y. Fløisand [1], V. Lazarevic[2], J. Maertens[3], J. Mattsson[4], N. Shah[5], P. Zachée[6], A. Taylor[7,*], M. Akbari [8], S. Quadri[8], Y.-B. Chen[9]

[1]Department of Hematology, Oslo University Hospital, Oslo, Norway, [2]Department of Hematology, Oncology and Radiation Physics, Skåne University Hospital, Lund University, Lund, Sweden, [3]Department of Hematology, University Hospitals Leuven, Leuven, Belgium, [4]Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden, [5]Division of Hematology and Oncology, Medical College of Wisconsin, Milwaukee, United States, [6]Hematology Service, ZNA Stuivenberg, Antwerp, Belgium, [7]Takeda Development Centre Europe Ltd., London, United Kingdom, [8]Takeda Pharmaceuticals International Co, Cambridge, [9]Blood and Marrow Transplant Program, Massachusetts General Hospital, Boston, United States

Background: Allogeneic hematopoietic cell transplantation (allo-HCT) can be curative in patients with hematological malignancies but carries a significant risk of acute and chronic graft-*versus*-host disease (GvHD). Acute GvHD of grades II–IV and grades III–IV have been reported in 39–71% and 14–32% of allo-HCT recipients, respectively, despite standard prophylaxis. Corticosteroids are first-line treatment, but are effective in only approximately half of patients, with durable responses in one-third. Steroid refractory (SR) acute GvHD is associated with high rates of morbidity and mortality, especially when there is lower intestinal involvement. There are no approved treatments for acute gastrointestinal (GI) GvHD, but published case series suggest that vedolizumab, a gut-selective immunomodulator approved for treatment of inflammatory bowel diseases, may be effective in reducing ongoing tissue damage in patients with this disease.
Aims: The aims of this international retrospective cohort study are to evaluate the off-label use of vedolizumab for treatment of patients with SR acute GI GvHD, and to assess key clinical outcomes of these patients, including safety and effectiveness.
Methods: Data from medical charts of patients from seven sites in Belgium, Norway, Sweden and the USA were analyzed. To be eligible for inclusion, patients must have received only one allo-HCT and ≥1 dose of vedolizumab as treatment for SR acute GI GvHD, which was defined as active stage 1–4 GI GvHD following ≥1 previous treatment regimen containing ≥1 mg/kg methylprednisolone or equivalent for acute GvHD. Any donor source, conditioning regimen intensity and GvHD prophylaxis was allowed. Descriptive analyses were performed on collected data for treatment, acute GvHD response rate, overall survival (OS) and serious adverse events (SAEs).
Results: We identified 29 patients who met the inclusion criteria. Median age was 50 years (range 19–69). Median time from allo-HCT to diagnosis of acute GvHD was 36 days (range 20–131). Median duration of treatment with steroids before vedolizumab treatment was 15 days (range 1–236). Patients received 1–10 doses of intravenous vedolizumab 300 mg (median, 3 doses) as treatment for acute GI GvHD. The overall response rate (partial response or better), measured at 6–10 weeks after the first dose of vedolizumab, was 64%. The corresponding complete response rate was 28%. Three patients experienced relapse of their primary malignancy. OS at 6 and 12 months after the first dose of vedolizumab was 54% and 47%, respectively. After initiation of treatment with vedolizumab, there were 29

identified SAEs; 12 were infections (two of which were reported as possibly related to vedolizumab treatment), four were GI (one case of bleeding from the colon, with ileus and obstruction was possibly related to vedolizumab treatment), four were hematological, two were cardiovascular, two were in the central nervous system, two were respiratory, one was psychological, one was multiple organ failure and one was renal. Thirteen of these SAEs were fatal, of which seven were infections or sepsis (one of which was possibly related to vedolizumab treatment) (Figure 1).

Patient characteristics (N = 29)

Age, mean years (SD)		48.0 (15.3)
Age, median years (range, IQR)		50 (19–69, 37–59)
Female, n (%)		7 (24%)
Days from diagnosis of aGvHD to first dose of vedolizumab, median (range)		14 (2–147)
Primary disease indication for allo-HCT, n (%)	AML or related precursor neoplasm	8 (27%)
	Mature B-cell neoplasm	3 (10%)
	Myelodysplastic syndrome	3 (10%)
	Precursor lymphoid neoplasm	3 (10%)
	Myelodysplastic/myeloproliferative neoplasm	2 (7%)
	Mature T-cell and NK-cell neoplasm	1 (3%)
	Myeloproliferative neoplasm	1 (3%)
	Other[a]	8 (28%)
Conditioning regimen, n (%)	Myeloablative conditioning procedure	9 (31%)
	Non-myeloablative conditioning procedure	8 (28%)
	Reduced-intensity transplant conditioning procedure	12 (41%)
Donor relationship, n (%)	Related donor	7 (24%)
	Unrelated donor	22 (76%)
aGvHD grade, n (%)	II	0 (0%)
	III	26 (90%)
	IV	3 (10%)

[a]Aplastic anemia (1 patient), chronic lymphocytic leukemia (1), CMML converted to AML (1), CMML/MF (1), Hodgkin disease (2), multiple myeloma (1), very severe aplastic anemia (1).
aGvHD, acute graft-versus-host disease; allo-HCT, allogeneic hematopoietic cell transplantation; AML, acute myeloid leukemia; B-cell, B lymphocyte; CMML, chronic myelomonocytic leukemia; IQR, interquartile range; MF, myelofibrosis; NK-cell, natural killer cell; SD, standard deviation; T-cell, T lymphocyte.

Figure 1.

Summary and Conclusions: Evidence from this international retrospective cohort study suggests that treatment of patients with vedolizumab for SR acute GI GvHD leads to a 64% overall response rate at 6–10 weeks after the first dose and OS of 54% at 6 months after the first dose. These data support the ongoing prospective clinical trials designed to characterize fully the efficacy and safety of vedolizumab in this indication.

PF731

COMPARISON OF MOBILIZED PERIPHERAL BLOOD STEM CELLS *VERSUS* BONE MARROW HAPLOIDENTICAL TRANSPLANTATION USING POST-TRANSPLANT CYCLOPHOSPHAMIDE: A RETROSPECTIVE STUDY OF SFGM-TC IN 176 PATIENTS

N. Jacque[1,*], J. Lambert[2], I. Yakoub-Agha[3], R. Tabrizi[4], M. Michallet[5], E. Deconinck[6], S. Thepot[7], M. Meunier[8], F. Suarez[9], A. Charbonnier[10], P. Turlure[11], P. Ceballos[12], G. Guillerm[13], G. Michel[14], N. Contentin[15], T. Marchand[16], L. Manone[17], J. H. Dalle[18], J. V. Malfuson[19], B. Lioure[20], R. Peffault De Latour[21], M.-T. Rubio[22], S. NGuyen[23]

[1]Hematologie, Hopital Pitié Salpêtrière, [2]Biostatistic, Hopital Saint Louis, Paris, [3]CHRU Lille, Lille, [4]hematologie, Haut Leveque, Bordeaux, [5]5Centre Hospitalier Lyon-Sud, Pierre-Bénite, Lyon, [6]Hematologie, Hopital Jean minjoz, Besancon, [7]Hematologie, CH Angers, Angers, [8]Hematologie, CH Grenoble, Grenoble, [9]Hematologie, Hopital Ncker, Paris, [10]Hematologie, CH Amiens, Amiens, [11]Hematologie, CH Limoges, Limoges, [12]Hematologie, CHU Montpellier, Montpellier, [13]Hematologie, CH Brest, Brest, [14]Hematologie, Hopital de la Timone, Marseille, [15]Hematologie, Centre Henri Becquerel, Rouen, [16]Hematologie, CH Rennes, Rennes, [17]Hematologie, CH Nice, Nice, [18]Hematologie, Hopital Robert Debré, Paris, [19]Hematologie, Hopital Percy, Clamart, [20]CHU Haute Pierre, Strasbourg, [21]Hematologie, Hopital Saint Louis, Paris, [22]Hematologie, CH Nancy, Nancy, [23]Hematologie, Pitié Salpêtrière, Paris, France

Background: The administration of high dose cyclophosphamide post-transplant (HD-Cy) has improved the outcome of patients who receive an unmanipulated haploidentical graft. Mobilized peripheral blood (PB) is increasingly used as it is more convenient.

Aims: We report the French experience of haploidentical transplantation using HD-Cy and unmanipulated bone marrow (BM) or PB.

Methods: From 2012 to 2015, 176 patients with hematologic malignancy received a haploidentical transplant in 21 centers. We compared the outcome of patients who received a BM graft (n=89) with those receiving a PB graft (N=87). Median age at transplant was 44 year-old (2.5-72). One hundred thirteen (64%) patients had myeloid disease, 19 (11%) had acute lymphoblastic leukemia and 44 (25%) had lymphoid neoplasm. Prophylaxis of GVHD consisted in anticalcineurin and mycophenolate in 94% of the cases. All patients received HD-CY post-transplant (100 mg per kg total dose).

Results: The 2 groups were comparable for age, disease status at transplant, type of conditioning regimen (RIC 71% *vs* 80% for BM *vs* PB respectively; p=0.16), recipient cytomegalovirus (CMV) status, ABO mismatch. PB recipients were more likely to receive antithymocyte globulin (ATG) (N=19 *vs* 1) and to have a higher disease risk index (DRI). The number of CD34+ and CD3+ infused cells was higher in PB grafts (median 2.64x10^6 CD34/Kg and 26 x10^6 CD3/Kg for BM and 6.03x10^6 CD34/Kg and 191 x10^6 CD3/Kg for PB). Median follow-up was 525 days (94-1851). In univariate analysis, the rate of patients who recovered neutrophils and platelet (>50G/L) were similar in both groups. However, median time to neutrophil engraftment was significantly longer with BM than PB (20 days *vs* 18 days respectively, p=0.004). Median time to platelet recovery was not significantly different (30 days *vs* 24 days in BM and PB, p=0.14). At one year, OS, progression free survival (PFS) and relapse were similar in the 2 groups (OS=64% *vs* 53% (p=0.1), PFS= 53% *vs* 46% (p=0.17) and relapse rate 28% *vs* 34% (p=0.2) in BM and PB respectively). Acute GVHD II-IV or III-IV and chronic GVHD were similar in both groups (aGVHD II-IV= 18% *vs* 28% (p=0.09) and aGVHD III-IV=7% *vs* 9% (p=0.48), cGVHD=13% *vs* 15% (p=0.6) in BM and PB respectively), resulting in a similar GVHD-free relapse-free survival (GRFS) at 1 year (48% *vs* 37% (p=0.09)). High DRI and the lack of complete remission at transplant had a significant pejorative effect on OS, PFS, relapse, GRFS and platelet recovery. As ATG was mainly used with PB, patients who had received ATG were excluded and the same analysis was performed on the 156 remaining patients. One-year PFS was 52% for BM *vs* 42% for PB (p=0.06), aGVHd was 18% for BM *vs* 31% for PB (p=0.07) resulting in a better 1-year GRFS for BM (48% *vs* 33%; p=0.02). One year OS was 63% for BM *vs* 51% for PB (p=0.05).

Summary and Conclusions: In this retrospective study the use of PB instead of BM does not significantly impact the outcome of patients who received a haploidentical unmanipulated graft with HD-CY. However, GRFS and OS might be better with BM compared to PB without ATG. Prospective randomized studies are needed to evaluate the effect of PB *vs* BM in haploidentical settings.

PF732

G-CSF/ATG-BASED REGIMEN IS SUPERIOR TO PTCY-BASED REGIMEN IN TERMS OF ENGRAFTMENT AFTER MYELOABLATIVE HAPLOIDENTICAL TRANSPLANTATION FOR HEMATOLOGIC MALIGNANCIES

F.-F. Tang[1], H.-R. Chen[2], Y.-J. Xu[3], L.-P. Xu[1], X.-H. Zhang[1], Y. Wang[1], Q.-F. Liu[4], D.-P. Wu[5], X.-J. Huang[1,*]

[1]Peking University People's Hospital, Peking University Institute of Hematology, Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, Beijing, China, [2]PLA Army General Hospital, Beijing, China, Beijing, [3]Xiangya Hospital, Central South University, Changsha, Hunan, China, Changsha, [4]Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong, China, Guangzhou, [5]The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China, Suzhou, China

Background: The protocol in which granulocyte colony stimulating factor(G-CSF) mobilized marrow and blood grafts and anti-thymocyte globulin (ATG) in the conditioning is the most commonly used haploidentical hematopoietic stem cell transplantation (haplo-HSCT) regimen in China. The use of post-transplantation cyclophosphamide (PTCy) is a new emerging optional strategy for haplo-HSCT abord over the past decade. Data comparing the two regimens in myeloablative haplo-HSCT for hematologic malignancies are lacking.

Aims: We aim to compare these two approaches in patients with hematologic malignancies receiving haplo-HSCT.

Methods: We compared PTCy-based and G-CSF/ATG-based regimens after myeloablative haplo-HSCT for hematologic malignancies between January 2013 and March 2017 who were reported to the Chinese Bone Marrow Transplantation Registry. For each PTCy patient, G-CSF/ATG-based subjects (1:4) were randomly selected using the nested case-pair method.

Results: A total of 175 patients were analyzed: 35 in the PTCy group and 140 in the G-CSF/ATG group. Incidences of neutrophil engraftment at day

30(91.4% *vs* 96.4%, P=0.007) and platelet engraftment at day 180(85.7% *vs* 95%, P=0.04) were significantly lower in the PTCy group. Median times to neutrophil (17 days *vs* 12 days, P=0.000) and platelet (22 days *vs* 17 days, P=0.001) engraftment were prolonged in the PTCy group. In the PTCy and G-CSF/ATG groups, the cumulative incidences of grade 2-4 and grade 3-4 acute graft-*versus*-host disease(GVHD)at day 100 were 20% *vs* 27.2%(P=0.29) and 8.6% *vs* 9.4%(P=0.86), respectively; incidences of chronic and severe chronic GVHD at 3 years were 26.2% *vs* 42.3%(P=0.11) and 5.7% *vs* 9.5%(P=0.51), respectively. The 3-year cumulative incidences of non-relapse mortality(20.5% *vs* 10.2%,P=0.08), relapse (9.6% *vs* 15.7%, P=0.35),GVHD-free, relapse-free survival(58.7% *vs* 65.1%,P=0.46),progression-free survival(69.7% *vs* 73.3%, P=0.56), and overall survival(72.2% *vs* 78.4%, P=0.39) were comparable between two groups. In multivariate analysis, the incidences of neutrophil and platelet engraftment were lower in the PTCy group (Figure 1).

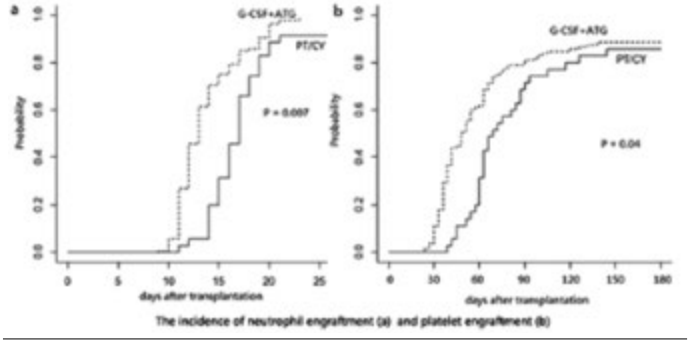


Figure 1.

Summary and Conclusions: G-CSF/ATG-based regimen is superior to PTCy-based regimen in terms of engraftment after myeloablative haplo-HSCT for hematologic malignancies.

PF733

BENDA-BEAM HIGH-DOSE THERAPY PRIOR TO AUTO-SCT IS EFFECTIVE IN RESISTANT/RELAPSED DLBCL: A PHASE II MULTICENTRIC STUDY

G. Visani[1,*], S. Guidi[2], P. Scalzulli[3], F. Loscocco[1], A. Olivieri[4], C. De Pau[5], S. Capria[6], F. Patriarca[7], B. Castagnari[8], F. Angrilli[9], D. Vallisa[10], G. Musuraca[11], A. Mengarelli[12], P. Galieni[13], F. Gaudio[14], P. Tosi[15], P. Zinzani[16], N. Cascavilla[3], A. Isidori[1]

[1]Hematology and Stem Cell Transplant Center, Pesaro, [2]Azienda Ospedaliera Universitaria Careggi, Hematology and Stem Cell Transplant Center, Firenze, [3]Hematology, IRCCS-Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, [4]Hematology, Ancona, [5]Armando Businco Hospital, Hematology, Cagliari, [6]Department of Biotechnologies and Hematology, Hematology, Rome, [7]Santa Maria della Misericordia Hospital, Hematology and Stem Cell Transplant Center, Udine, [8]Santa Maria delle Croci Hospital, Hematology, Ravenna, [9]Spirito Santo Hospital, Hematology and Stem Cell Transplant Center, Pescara, [10]Hematology, Piacenza, [11]IRST, Hematology, Meldola, [12]Regina Elena Institution, Hematology, Rome, [13]Hematology and Stem Cell Transplant Center, Ascoli Piceno, [14]Hematology and Stem Cell Transplant Center, Bari, [15]Hematology, Rimini, [16]Institute "Seragnoli", Bologna University, Bologna, Italy

Background: A major drawback affecting clinical trials of high-dose therapy (HDT) followed by autologous stem cell transplant (ASCT) in lymphomas is the high heterogeneity of histological entities. As a consequence, the statistical power is reduced when we focus on a specific histological subset, and data are often not conclusive.
Aims: We designed a phase II multicenter study to evaluate the efficacy of the BeEAM high-dose therapy regimen (bendamustine 200 mg/m2 on days -7,-6, cytarabine 400 mg/m2 days -5-4-3-2, etoposide 200 mg/m2 days -5-4-3-2, melphalan 140 mg/m2 day-1) followed by ASCT (day 0) in resistant/relapsed diffuse large B-cell (DLBC) non-Hodgkin lymphoma patients.
Methods: The study was registered at European Union Drug Regulating Authorities Clinical Trials (EudraCT) with the number 2011-001246-14. Until now, 76 out of 88 pre-planned patients (median age 55 years, range 19-69) with resistant/relapsed DLBCL were enrolled. The primary end-point of the study is to evaluate the 1-year complete remission rate.
Results: Briefly, 59/76 patients had advanced stage disease (III-IV); 25 were primary refractory and 51 had relapsed. 38/76 patients were in II or subsequent CR after salvage therapy, whereas 31 were in PR and 7 in progressive disease. A median number of 5.63x10^{6}CD34+/kg cells (range 2.21-11.40) collected from peripheral blood was reinfused to patients. All patients engrafted, with a median time to ANC>0.5x10^9/l of 10 days. Median times to achieve a platelet count >20x10^9/l and >50x10^9/l were 12 and 17 days respectively. Twenty-four out of 76 patients presented a FUO (32%), whereas 25 patients (34%) presented a clinically documented infection. All patients received G-CSF after transplant for a median time of 8 days (range: 8-13). One patient died due to an incomplete hematological recovery after transplant, for an overall transplant related mortality of 2.7%. Sixty-six patients are evaluable for response: 55/66 (72%) obtained a CR, 4/66 (5%) a PR, whereas 7/66 (9%) did not respond to therapy. The median follow-up after transplant was 20 months (range 0-69).
Summary and Conclusions: The stringent inclusion criteria at enrollment might allow to accurately evaluate the impact of BeEAM HDT regimenfollowed by ASCT in a highly selected population of DLBCL patients. Accordingly, our data provide the evidence that the Benda-BEAM regimen is highly effective in resistant-relapsed aggressive diffuse large B cell lymphoma, with a maneageble toxicity profile. **Acknowledgements:** The study was supported in part by AIL Pesaro Onlus. Mundipharma Italy provided Bendamustine free of charge.

PF734

A HIGH PRE-TREATMENT BONE MARROW CD34+/CD38- CELL BURDEN IN PATIENTS WITH MYELODYSPLASTIC SYNDROME IS A PROGNOSTIC FACTOR FOR DISEASE PROGRESSION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

M. Jentzsch[1,*], U. Geus[1], J. Grimm[1], S. Beinicke[1], J. Häntschel[1], V. Vucinic[1], W. Pönisch[1], G. Behre[1], G.-N. Franke[1], D. Niederwieser[1], S. Schwind[1]

[1]Hematology and Oncology, Universitätsklinikum Leipzig, Leipzig, Germany

Background: Myelodysplastic syndrome (MDS) is a highly heterogeneous clonal hematopoietic disorder. Allogeneic stem cell transplantation (HSCT) remains the only curative treatment, especially with respect to patients (pts) at high risk for progression to acute myeloid leukemia (AML). It has been shown that in MDS, CD34+/CD38- cells possess MDS stem cell potential & secondary AML (sAML) clones originate from MDS disease stage. To date no study evaluated the prognostic impact of a high MDS stem cell burden prior to therapy.
Aims: To analyze the impact of a high pre-treatment CD34+/CD38- cell burden in MDS pts on outcome after HSCT.
Methods: We retrospectively analyzed 124 MDS (n=105) or myelodysplastic/myeloproliferative neoplasm (n=19) pts receiving HSCT at our institution (median age at HSCT 61.3 [range 22.2-74.4] years [y]) after reduced-intensity (RIC, 44%; Fludarabine with Busulfan or Treosulfan) or non-myeloablative (NMA, 56%; Fludarabine with 2Gy or 3Gy total body irradiation)-conditioning. Prior to HSCT, 59% of pts received cytoreductive therapy with hypomethylating agents (25%), AML chemotherapy (24%) or both (10%). Median follow up after HSCT was 4.3 y. Karyotype analyses were performed centrally at our institution. IPSS-R was 0% very low, 7% low, 28% intermediate, 21% high, 36% very high & 7% unknown. CD34+/CD38- cell burden was evaluated by flow cytometry in untreated bone marrow (BM) material. A cut-off of 1% CD34+/CD38- cells was determined using R's OptimalCutpoint package & divided the cohort in pts with high (34%) or low (66%) CD34+/CD38- cell burden.
Results: A high pre-treatment BM CD34+/CD38- cell burden associated with higher expression of CD13 (P<.001), CD33 (P<.001) & CD117 (P<.001) in BM, an excess of blasts (P<.001) & worse IPSS-R (P=.03) risk group. Pts with a high CD34+/CD38- cell burden also had worse IPSS-R genetic risk (P=.02), were more likely to harbor an abnormal (P=.04), complex (P=.002) or monosomal (P=.004) karyotype & more often received cytoreductive treatment prior to HSCT (P=.008). Pts with a high CD34+/CD38- cell burden had a significantly higher cumulative incidence of relapse/progression (CIR, P<.001, Figure 1A), sAML (CIsAML, P<.001, Figure 1B) & shorter overall survival (OS, P=.12) by trend. In multivariate analyses, a high CD34+/CD38- cell burden was an independent factor for higher CIR (Hazard ratio [HR] 2.9, Confidence Interval [CI] 1.4-6.1, P=.005) after adjustment for IPSS-R genetic risk, for higher CIsAML (HR 4.6, CI 2.0-10.8, P<.001) after adjustment for age at HSCT & HLA match & for shorter OS (HR 2.1, CI 1.2-3.8, P=.01) after adjustment for HLA match & donor type. Analyzing IPSS-R low/intermediate & high/very high risk MDS pts separately, a high CD34+/CD38- cell burden indicated patients with higher CIR (P<.001, Figure 1C), higher CIsAML (P<.001, Figure 1D) & a trend for shorter OS (P=.07) irrespective of the IPSS-R risk group.
Summary and Conclusions: A high pre-treatment CD34+/CD38- cell burden associated with higher CIR, higher CIsAML & a trend for shorter OS after

HSCT. Despite the correlation with high risk disease a high CD34+/CD38- cell burden provided independent prognostic information for all endpoints in multivariable analyses & in separate analyses for IPSS-R low/intermediate & high/very risk pts. The observed prognostic impact is likely mediated by MDS stem cells within the CD34+/CD38- cell population initiating MDS relapse or progression to AML. New therapeutic strategies targeting MDS stem cells might improve outcomes.

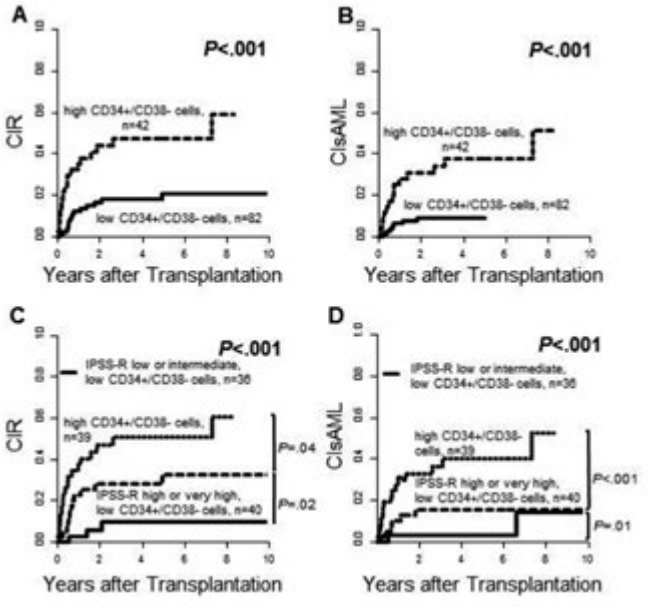


Figure 1.

PF735

HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH THERAPY-RELATED MYELODYSPLASTIC SYNDROME AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION FOR LYMPHOID NEOPLASMS: RETROSPECTIVE STUDY OF SFGM-TC

D. Jaimes Albornoz[1,*], L. Manonne[2], S. Nguyen[3], I. Yakoub-Agha[4], Y. Chalandon[5], C. Faucher[6], P. Chevallier[7], R. Tabrizi[8], M. Mohty[9], M. Meunier[10], M. Robin[11], L. Sutton[12], B. Lioure[13], G. Guillerm[14], P. Ceballos[15], J.-O. Bay[16], E. Van Den Neste[17], A. Huynh[18], N. Maillard[19], F. Suarez[20], S. Maury[21], E. Deconinck[22], Y. Beguin[23], M. T. Rubio[24], R. Peffault de la Tour[11], E. Gyan[1]

[1]Tours University Hospital, Tours, [2]Nice University Hospital, Nice, [3]Pitié Salpêtrière Hospital, Paris, [4]Lille University Hospital, Lille, France, [5]Geneva University Hospital, Geneva, Switzerland, [6]Paoli Calmette Institute, Marseille, [7]Nantes University Hospital, Nantes, [8]Haut-Lévêque University Hospital, Bordeaux, [9]Saint-Antoine Hospital, Paris, [10]Grenoble University Hospital, Grenoble, [11]Saint-Louis Hospital, Paris, [12]Argenteuil Hospital, Argenteuil, [13]Strasbourg University Hospital, Strasbourg, [14]Brest University Hospital, Brest, [15]Montpellier University Hospital, Montpellier, [16]Clermont-Ferrand University Hospital, Clermont Ferrand, France, [17]Saint-Luc University Hospital, Bruxelles, Belgium, [18]Toulouse University Hospital, Toulouse, [19]Poitiers University Hospital, Poitiers, [20]Necker Hospital, Paris, [21]Henri-Mordor Hospital, Créteil, [22]Besançon University Hospital, Besançon, France, [23]Liège University Hospital, Liège, Belgium, [24]Nancy University Hospital, Nancy, France

Background: Therapy-related myelodysplastic syndrome (t-MDS) after autologous stem-cell transplantation (ASCT) is uncommon and there is no curative treatment. Allogeneic hematopoietic stem cell transplantation (HSCT) may be considered in eligible patients.
Aims: The aim of the study was to evaluate the results of alloHSCT in patients with secondary MDS who have received an ASCT for lymphoid neoplasms.
Methods: Data were extracted from ProMISe for patients from French, Belgian, and Swiss centers, who received an alloHSCT between 2006 and 2016 for t-MDS after an ASCT for lymphoid neoplasms. Secondary acute myeloid leukemias were excluded. Overall survival (OS), relapse-free survival (RFS), and non-relapse mortality (NRM) were evaluated.
Results: We present 47 patients (37 males and 10 females), with a median age of 58 years (range: 30-71). Initial lymphoid neoplasm was Hodgkin's lymphoma in 8 patients (17%) and Non-Hodgkin Lymphoma in 36 (77%). 10 patients received more than two lines of chemotherapy before ASCT. Conditioning regimen was mostly BEAM (80.8%). CR was achieved in 37 patients (78.7%). The median time from ASCT to the diagnosis of t-MDS was 74.4 months (range 2.2 - 259). MDS type was MDS-EB-1 in 11 patients (23.5%) and MDS-EB-2 in 12 patients (25.6%), while 22 patients (46.7%) had other MDS. 2 patients (4.2%) had normal cytogenetics and 39 patients (82.9%) had at least one cytogenetic abnormality. Eleven patients (23.4%) were considered lower-risk (Low/Int-1 IPSS) and 28 (59.6%) higher-risk patients (Int-2 and High IPSS).32 patients (68%) received treatment before allo HSCT: 16 patients (34%) hypomethylating agents and 11 patients (23.4%) AML-like treatment. Ten patients (21.2%) achieved CR before alloHSCT. The median time interval from diagnosis of t-MDS to alloHSCT was 7.9 months (range 2.5-16.8). A myeloablative conditioning was used in 9 patients (19.1%) and a reduced intensity conditioning in 38 patients (80.9%). 19 patients (40.5%) had an HLA-identical donor, 17 patients (36.2%) had an matched unrelated donor and 10 patients (21.2%) an mismatched unrelated donor. The most frequent GvHD prophylaxis was cyclosporine (CsA) and mycophenolate mofetil (MMF) in 18 patients (38.3%) and 11 patients (23.4%) received cyclosporine and methotrexate (MTX). The Karnofsky score was equal or higher to 90 in 29 patients (61.8%) before alloHSCT. The median duration of post-transplant follow-up was 21.4 months (range 0 – 105.7) and median OS was 6.9 months (95% CI 0-14.6). OS rates was 45% (29–60%) and 30% (15-45%) in the first and third year after transplantation. In univariate analysis, prior therapy of MDS before alloHSCT (p=0.02) and mismatch HLA donors (p=0.004) were associated with poor OS. NRM and relapse rates were 44% (25-63%) and 41% (22-61%) at 3 years, respectively. The graft type (p=0.02) and mismatch HLA donors (p<0.001) were associated with higher NRM and MDS type at diagnosis (p=0.008) with higher relapse risk. On multivariable analysis, mismatch HLA was associated with higher NRM (HR 6.21; 95% CI 1.63- 23.62; p=0.007).
Summary and Conclusions: Although the number of patients is small, the results suggest that patients receiving an ASCT for a lymphoid neoplasm and developing a t-MDS have a short OS after alloHSCT, with few long-time survivors. The use of mismatched donors with standard GvHD prophylaxis should be avoided in such indication for alloHSCT. It will be worth to see if the implementation of CY post-transplantation will improve the outcome with mismatched donors.

PF736

A PHASE I/II STUDY OF PEGYLATED-INTERFERON-2ALPHA FOR RELAPSED HAEMATOLOGICAL MALIGNANCY AFTER ALLOGENEIC HAEMATOPOIETIC PROGENITOR CELL TRANSPLANTATION

A. Henden[1,2,*], J. Leach[2], E. Sturgeon[2], J. Avery[2], S. Olver[1], L. Samson[1], S. Durrant[2], J. Butler[2], J. Morton[2], A. Misra[2], S. Tey[1,2], E. Subramoniapillai[2], C. Curley[2], G. Kennedy[2], G. Hill[1,2]

[1]Bone Marrow Transplantation Laboratory, QIMR Berghofer Medical Research Institute, [2]Department of Haematology and Bone Marrow Transplantation, Royal Brisbane and Women's Hospital, Brisbane, Australia

Background: Relapse of hematologic malignancy after allogeneic hematopoietic progenitor cell transplantation (HPCT) reflects failure of immune-mediated graft-*versus*-malignancy (GVM) and is associated with very poor survival outcomes. In pre-clinical models of HPCT type I Interferons have capacity to regulate GVM and graft-*versus*-host disease (GVHD) by sensitizing residual malignancy to cytotoxicity and augmenting donor T and NK cell cytotoxic responses (Robb R *et al.*, Blood 2011, 118:3399 and Robb R *et al.*, Blood 2012, 119:5918). We sought to test the ability of pegylated-Interferon-2α (peg-IFN2a), a long acting type I Interferon, to improve outcomes in clinical transplantation.
Aims: To test safety and efficacy of peg-IFN2α when added to therapy for relapse of any haematological malignancy post HPCT.
Methods: Patients between 18 and 70 years with both haematological and minimal residual disease (MRD) relapse post HPCT were eligible. The study received approval from institutional HREC and informed consent was obtained for all participants. Treatment schema included cessation of immune suppression (where applicable) and those with haematological relapse received cytoreduction with Fludarabine and Cytarabine (FLAG). Upon count recovery peg-IFN2α was given in escalating weekly doses for up to 6 months, with stipulated dose adjustment based on cytopenias and toxicity. Those without grade II-IV acute GVHD or progressive chronic GVHD were eligible to receive DLI. 29 patients were planned for accrual. The primary endpoint is 2 year OS.
Results: Accrual is complete, the final patient having commenced therapy

on 31/03/2017. Current 2 year OS is 30% with median OS 330 days (median follow-up 1089 days). Median disease free survival (DFS) is 183 days (median follow-up 857 days). Peg-IFN2α was able to be given safely; 11 patients (38%) experienced grade 3 or greater adverse events however only 4 were thought probably or likely related to peg-IFN2α administration. 18 patients (62%) have had acute (11%) or chronic (89%) GVHD whilst on trial, 11 (61%) after administration of peg-IFN2α alone. 7 of 11 (64%) patients receiving DLI subsequently developed GVHD. GVHD was induced with peg-IFN2α in 7 of 12 (58%) patients who had not had any prior GVHD. 13 patients had measureable MRD or mixed chimerism after FLAG cytoreduction and prior to administration of peg-IFN2α and 8 of these (62%) demonstrated disease responses to peg-IFN2a, including 6 complete remissions (75%). Immune profiling demonstrates persistence of a high proportion of cytolytically active Tc1 cells (CD8+ T cells with Interferon γ secreting capacity and granzyme B positivity) in those patients who develop GVHD relative to those who do not.

Summary and Conclusions: Current 2 year OS of 30% compares favorably with an institutional control cohort who did not receive peg-IFN2α where 2 year OS was 9% (Curley C *et al.,* Int J Lab Haematol 2014, 36:197). Peg-IFN2α administration was associated with expected and manageable toxicity. Our data support the addition of peg-IFN2α to standard strategies of cytoreduction±DLI in patients that relapse after allo-HPCT. Patients who do not experience GVHD within 3 months of peg-IFN2α are candidates for alternative strategies. In the modern era, our cytokine-dependent approach to enhance GVL seems particularly well suited to patients with relapsed myeloid malignancies, where CAR based therapies are not available.

PF737

OUTCOME OF MESENCHYMAL STROMAL CELL TREATMENT IN CHRONIC GVHD PREDICTED BY THYMIC FUNCTION: A PHASE II CLINICAL STUDY

E. Boberg[1,2,*], L. von Bahr[1,2], N. Heldring[3], G. Afram[4,5], E. Iacobaeus[6,7], C. Lindström[1], K. Garming-Legert[8], E. Alici[5], P. Ljungman[4,5], R. Sugars[8], N. Kadri[2], K. Le Blanc[2,4]

[1]Department of Hematology, Karolinska University Hospital, [2]Department of Laboratory Medicine, [3]Center for Hematology and Regenerative Medicine, Karolinska Institutet, [4]Center for Allogeneic Stem Cell Transplantation (CAST), Karolinska University Hospital, [5]Department of Medicine, [6]Department of clinical neuroscience, Karolinska Institutet, [7]Department of Neurology, Karolinska University Hospital, [8]Department of Dental Medicine, Karolinska Institutet, Stockholm, Sweden

Background: No treatment for patients unresponsive to first line therapy for chronic graft *versus* host disease (cGvHD) has been established. Infusion of mesenchymal stromal cells (MSCs) is a promising alternative in this situation but treatment efficacy and mechanism of action have not yet been determined.

Aims: To evaluate the clinical and immunological effects of repeated MSC infusion treatment on steroid refractory (SR) cGvHD patients.

Methods: In this study, 11 patients with severe SR cGvHD were treated with repeated infusions of MSCs. The treatment consisted of 6 to 9 infusions (once every month) of $2x10^6$ allogeneic bone marrow MSCs/kg. Clinical response was evaluated using the National Institutes of Health (NIH) criteria. Immunophenotyping and cytokine analysis was conducted on blood samples taken before, and at several time points shortly after each infusion. Skin biopsies were also taken before and after treatment and blindly evaluated by an external pathologist.

Results: Nine patients completed the treatment protocol and were evaluated for response. Six patients responded (R) to MSC therapy according to NIH criteria. Five of the six showed long-lasting improvement 12 months after MSC treatment. Analysis of the immune cell distribution in R and the non-responders (NR) revealed significantly higher proportions and absolute numbers of naive CD4+ T-cells (CD3+ CD4+ CD45RA+ CD27+) and activated naïve B cells (CD19+ IgD+ CD38low) prior to MSC treatment in R compared to NR. A similar trend was observed for absolute numbers of immature NK-cells (CD56bright, p=0.066). These differences remained unchanged throughout the treatment. Further, in R, absolute numbers of these naïve lymphocyte subsets, as well as regulatory T-cells (Tregs, Figure 1A) increased 7 days after each MSC infusion, which returned to baseline by the next infusion. No such increases were seen in NR. Levels of four miRNAs increased one hour after MSC infusion in both R and NR, suggesting a MSC-specific effect. Multiplex analysis revealed long term improvements in the levels of the pro-inflammatory cytokines CXCL2 (Figure 1B) and CCL2 in R, while the levels of CXCL9, CXCL10 (Figure 1B), CXCL12, TNFa, IL-6 increased in NR. In skin biopsies, we observed resolution of epidermal inflammatory changes (keratinocyte apoptosis, vacuolization and mononuclear infiltration). Finally, we ascertained that differences in naïve T cell numbers and the short term increases after MSC infusion were due to mobilisation from the thymus, as the proportion of Ki67+ naïve CD4+ T-cells was similar in both R and NR. A significant difference in the percentage of recent thymic emigrants among naïve CD4+ T-cells suggested improved thymic function in the R (Figure 1C).

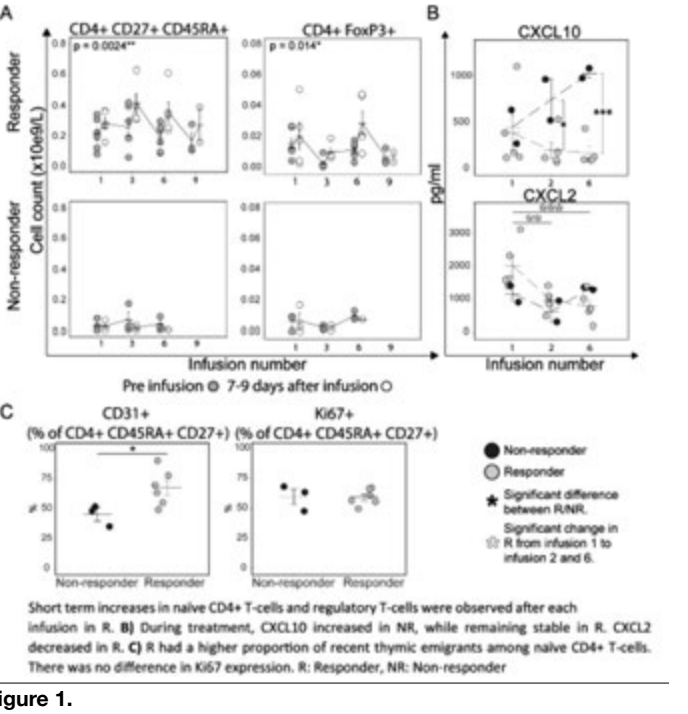


Figure 1.

Summary and Conclusions: In the current study, MSC treatment induced durable responses in patients with severe refractory cGvHD, as well as long term improvements in cytokine profile and skin pathology. Further, our data suggest that success of MSC treatment for patients with refractory cGvHD is dependent on the immunological characteristics of the individual patient. More specifically, we propose that sufficient thymic function, generating sufficient numbers of naïve T-cells and naïve Tregs, is required for responsiveness to MSC treatment in these patients.

PF738

IMPAIRED BONE MARROW ENDOTHELIAL PROGENITOR CELLS ARE INVOLVED IN THE PATHOGENESIS OF ACUTE GRAFT-*VERSUS*-HOST DISEASE AFTER ALLOTRANSPLANT

X.-N. Cao[1,*], Y. Kong[1], Y. Song[1,2], M.-M. Shi[1,2], H.-Y. Zhao[1], Q. Wen[1], Z.-S. Lyu[1,2], C.-W. Duan[3], Y. Wang[1], L.-P. Xu[1], X.-H. Zhang[1], X.-J. Huang[1,2]

[1]Peking University People's Hospital, Peking University Institute of Hematology, [2]Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Beijing, [3]Key Laboratory of Pediatric Hematology and Oncology Ministry of Health and Pediatric Translational Medicine Institute, Shanghai Children's Medical Center, Shanghai, China

Background: Graft-*versus*-host disease (GVHD) is a major complication that is frequently associated with bone marrow (BM) suppression after allogeneic hematopoietic stem cell transplantation (allo-HSCT), and the clinical management is challenging. While traditionally considered to be induced through an immune-mediated mechanism, GVHD is also associated with circulating endothelial cells (ECs) injury, which determines the severity of GVHD. BM endothelial progenitor cells (EPCs), the precursors of ECs, play crucial roles in the regulation of hematopoiesis and thrombopoiesis. We recently reported that impaired BM EPCs and hematopoietic stem cells (HSCs) contribute to the occurrence of prolonged isolated thrombocytopenia and poor graft function after allo-HSCT. However, little is known regarding the functional roles of BM EPCs in acute GVHD (aGVHD) patients.

Aims: To evaluate whether the number and function of BM EPCs and HSCs in allo-HSCT patients with aGVHD differed from those without aGVHD. Furthermore, to explore whether the severity of aGVHD and GVHD-mediated cytopenia was associated with impaired BM EPCs and HSCs in aGVHD patients.

Methods: In the current prospective case-pair study, we evaluated whether the percentages and functions of BM EPCs and HSCs in aGVHD patients differed from those in patients without aGVHD. In order to explore whether the severity of aGVHD was associated with impaired BM EPCs and HSCs in aGVHD patients, the percentages and functions of BM EPCs and HSCs were compared between patients with aGVHD >grade II and aGVHD ≤grade II. Moreover, to evaluate whether aGVHD-associated cytopenia was correlated with the impairment of primary BM EPCs and HSCs, the percentages and functions of BM EPCs and HSCs were compared between aGVHD patients with cytopenia and patients without cytopenia as well.
Results: Human BM EPCs were demonstrated as the spindle shape and the similar expression of CD34, VEGFR2 and CD133 at day 7 of cultivation among the enrolled subjects. Reduced and dysfunctional BM EPCs, characterized by decreased migration and angiogenesis capacities and higher levels of ROS and apoptosis, were found in aGVHD patients. Meanwhile, reduced and dysfunctional BM HSCs, characterized by higher levels of ROS and apoptosis, were identified in patients with aGVHD compared with those without aGVHD. In PB, increased frequency of ECs and decreased HSCs with higher levels of ROS and apoptosis were detected in patients with aGVHD compared to those without aGVHD. In addition, the frequencies of BM EPCs and HSCs in patients with the onset of aGVHD ≥grade II were significantly lower than those with aGVHD <grade II. By contrary, the levels of ROS and apoptosis in BM EPCs and HSCs in patients with the onset of aGVHD ≥grade II were remarkably higher than those with aGVHD <grade II. Moreover, the GVHD-mediated cytopenia was associated with BM EPCs impairment in aGVHD patients. And the EPCs impairment positively correlated with the ROS level, which may have led to the GVHD-associated cytopenia.
Summary and Conclusions: Taken together, our results suggest that reduced and dysfunctional BM EPCs may be involved in the pathogenesis of aGVHD. Although these findings require validation, our data indicate that improvement of BM EPCs represents a promising therapeutic approach for aGVHD patients.

PF739

PHASE II PROSPECTIVE STUDY OF DIFFERENT ATG DOSING IN ADDITION TO STANDARD HAPLOIDENTICAL TRANSPLANT CONDITIONING WITH POST-TRANSPLANT CYCLOPHOSPHAMIDE (PT-CY)

M. Hassanein[1,*], A. Alshomar[1], A. kotb[1], S. Al-Otaibi[1], S. Ahmed[1], F. Alfraih[1], F. Almohareb[1], H. Alzahrani[1], F. Alsharif[1], M. Shaheen[1], S. Alhayli[1], N. Chaudhri[1], A. Hanbali[1], S. Hashmi[1], G. Aldawsari[1], M. Aljurf[1], R. El Fakih[1]

[1]Adult Hematology/BMT, KFSHRC, Riyadh, Saudi Arabia

Background: Haploidentical stem cell transplant (Haplo-SCT) is accompanied by intense bidirectional alloreactivity due to the HLA mismatch between the donor and the recipient. Immunosuppression to control this alloreactivity is essential for the success of transplant. Currently 2 platforms for haploidentical transplant exist. The post-transplant cyclophosphamide (PT-CY) is the most frequently used regimen in the west (Europe and North America). The GIAC (Gcsf+Intense immunosuppression+ATG+Combined bone marrow and peripheral blood graft) is the most frequently used in China. We adopted the PT-CY after myeloablative conditioning as our standard haploidentical transplant platform.
Aims: To assess the risk of developing acute GVHD after the addition of ATG at different doses to standard Haplo-SCT conditioning with post-transplant PT-CY.
To study the impact of adding ATG on relapse risk, OS, CMV reactivation, and the risk of developing hemorrhagic cystitis (HCYST).
Methods: In this phase II prospective clinical trial we studied a total of 39 patients, who underwent Haplo-SCT at our institution. All studied patients (12 ALL, and 27 AML) had no active disease at time of transplant. Our first 12 patients received myeloablative conditioning (MA) with PT-CY without ATG. As we recognized the higher rates of aGVHD, we added ATG at a dose of (3mg/kg) (ATG-3) to our conditioning regimen. A total of 15 patients received ATG-3, and our first interim analysis revealed a marked decrease in the risk of aGVHD in the expense of a significant increase in post-transplant infections. We decided to cut down the ATG dose to be 2mg/kg (ATG-2), 12 of our studied patients received conditioning with this dose, and this is our second interim analysis.
Results: Our Haplo-SCT results showed that the probability of death without aGVHD is less than 11% at 100 days (Figure 1A), patients who received ATG-3 had a significantly lower risk of aGVHD 6.7%, compared to patients who received ATG-2 or no ATG (41.7% and 58.3%, respectively) p-value= 0.01 (Figure 1B). There was no significant difference in the cumulative incidence of relapse between the 3 groups of patients p-value= 0.4, and with longer follow it seems that the addition of ATG at a dose of 3mg/kg didn't affect the relapse risk 42% compared with 33.3% for no ATG (Figure 1C), and resulted in an even better median DFS when compared with no ATG (19 months and 9 months, respectively), but the difference was not statistically significant (p-value=0.7) (Figure 1D). Furthermore, the median OS was not reached for the patients who received ATG-3, but it was 18 months for patients who didn't receive ATG at 2 years follow up (Figure 1E). The rates of developing CMV reactivation and HCYST were not significantly different between the 3 patient groups (p-value= 0.2 and 0.4 respectively), with lower rates of non-relapse mortality (NRM) in patients who received ATG-3 (14%), compared to 25% for no ATG, and 32% for ATG-2 (Figure 1F).

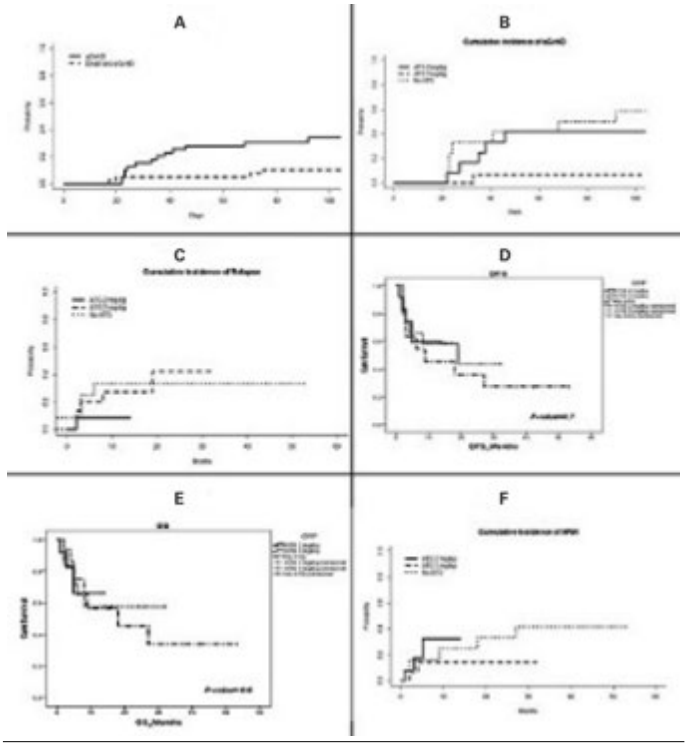


Figure 1.

Summary and Conclusions: ATG at a dose of 3mg/kg as a part of Haplo conditioning can significantly decrease the risk of aGVHD without increasing the risk of relapse or NRM, and with promising survival outcomes on longer follow up. However, decreasing the dose of ATG to 2mg/kg didn't seem to reduce the risk of aGVHD, but longer follow up is required to evaluate its long term effect on the relapse risk and overall survival.

PF740

EXTENSIVE TELOMERE SHORTENING IN MONONUCLEAR CELLS IS ASSOCIATED WITH INFERIOR OVERALL SURVIVAL AFTER NON-MYELOABLATIVE ALLO-HCT

J.H. Petersen[1,2,3,*], S.E. Bojesen[1,2,4], B. Kornblit[5], B.G. Nordestgaard[1,2,4], S.L. Petersen[5], H. Sengeløv[4,5]

[1]Department of Clinical Biochemistry, [2]The Copenhagen General Population Study, Herlev and Gentofte Hospital, Copenhagen University Hospital, Herlev, [3]Department of Hematology, Zealand University Hospital, Roskilde, [4]Faculty of Health and Medical Sciences, University of Copenhagen, [5]Department of Hematology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

Background: After allogeneic hematopoietic cell transplantation (allo-HCT), transplanted cells rapidly undergo multiple rounds of division. This may cause extensive telomere shortening, which could potentially prohibit further cell division and lead to increased mortality.
Aims: We characterized the development in mononuclear cell telomere length after non-myeloablative allo-HCT and tested the hypothesis that when compared to pre-transplant donor telomeres, extensive telomere shortening in recipients post-transplant is associated with low overall survival.
Methods: We studied 240 unselected consecutive patients undergoing non-myeloablative allo-HCT due to hematologic malignancies. Telomere length

was measured using quantitative polymerase chain reaction in mononuclear cells obtained from donors and recipients pre-transplant and in follow-up samples obtained from recipients post-transplant. Percentwise telomere length at 1 year post-transplant was calculated as the recipient telomere length at 1 year post-transplant in percent of donor pre-transplant telomere length.
Results: Although allo-HCT led to shorter mean telomere length in recipients when compared to donors, recipients had longer mean telomere length at 1 year post-transplant than they had pre-transplant. When compared to donor pre-transplant telomeres, recipients with extensive telomere shortening post-transplant had low overall survival (10-year survival from 1 year post-transplant an onwards 68% in the tertile with the longest percentwise telomere length at 1 year post-transplant, 57% in the middle tertile, and 39% in the shortest;p for trend=0.004). Similarly, when adjusting for potential confounders, recipients with extensive telomere shortening had high all-cause mortality (multivariable adjusted hazard ratio 1.84 per standard deviation shorter percentwise telomere length at 1 year post-transplant;95% confidence interval 1.25-2.72;p=0.002) and high relapse-related mortality (subhazard ratio 2.07;1.14-3.76;p=0.02). Neither donor nor patient pre-transplant telomere length were associated with overall survival.
Summary and Conclusions: Recipients with extensive telomere shortening post-transplant had low overall survival and high relapse-related mortality, indicating that telomere shortening may potentially be a clinically relevant marker for identifying patients at high risk of mortality.

PF741

G-CSF MOBILIZED MACROPHAGES POLARIZATION MAY PREVENT ACUTE GRAFT-*VERSUS*-HOST DISEASE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Q. Wen[1,2,*], Y. Kong[2], H.-Y. Zhao[2], Y.-Y. Zhang[2], T.-T. Han[2], L.-P. Xu[2], X.-H. Zhang[2], X.-J. Huang[1,2]

[1]Nanfang Hospital, Southern Medical University, Guangzhou, [2]Peking University People's Hospital, Peking University Institute of Hematology, Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, Collaborative Innovation Center of Hematology, Beijing, China

Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective treatment for malignant hematopoietic diseases. Granulocyte colony-stimulating factor (G-CSF) application in a healthy donor can modulate the balance between Th1/Th2/Th17 cells, promote regulatory T cell and tolerogenic dendritic cell differentiation, as well as affect myeloid-derived suppressor cells induction. Therefore G-CSF induced immune tolerance has been shown to play a critical role in allo-HSCT. Macrophage (MΦ) is an important population of immune cells. MΦs are now classified as M1 (classic) MΦs, which produce pro-inflammatory cytokines, and M2 (alternative) MΦs, which produce anti-inflammatory cytokines. The unbalanced polarization of MΦs has been shown to play a critical role in the occurrence and development of disease, such as cancer and diabetes. Therefore repolarization of MΦs may be a novel therapeutic option to treat diseases. The impact of endogenous G-CSF on MΦs induction has been described in mouse models, but no study focus on the function of G-CSF on MΦs in human. Moreover, little is known about the association of MΦs subgroups in allografts with the occurrence of acute graft-*versus*-host disease (aGvHD) in patients who underwent allo-HSCT.
Aims: To determine the effect of G-CSF on MΦs in healthy donors. Moreover, to investigate the association of MΦ subgroups in allografts with the occurrence of aGvHD in patients after allo-HSCT.
Methods: We investigated the effects of G-CSF on the percentages of M1(CD68+CCR2+), M2(CX3CR1+CD163+) MΦs both in bone marrow(BM) and peripheral blood(PB) of healthy donors by flow cytometry. Moreover, the effects of G-CSF on MΦs function, including DiI-Ac-LDL uptake, DAPI binding assay, migration assay and mixed-lymphocyte reaction were investigated *in vitro*. Subsequently, we evaluated the association of M1/M2 MΦs ratio with the occurrence of aGvHD in patients who underwent allo-HSCT.
Results: We found that G-CSF mobilized MΦs polarization in both PB and BM. A decrease in pro-inflammatory M1 MΦs were found in the G-CSF mobilized-BM (G-BM, 4 days after G-CSF) and G-CSF mobilized-PB (G-PB, 5 days after G-CSF) compared to the stable-BM (S-BM) and stable-PB (S-PB) pre-G-CSF administration. Moreover, a decrease in anti-inflammatory M2 MΦs were observed in the G-PB, whereas a reciprocal increase in anti-inflammatory M2 MΦs in the G-BM. As a result, the M1/M2 MΦs ratio was markedly decreased in G-PB and G-BM. After G-CSF mobilization *in vivo*, BM MΦs showed reduced migration and increased phagocytic activities. In the mixed lymphocyte reaction, G-CSF mobilized MΦs can promote the apoptosis of activated T cells, but inhibit the proliferation of T cells. Furthermore, after G-CSF mobilization, MΦs increased the percentage of Th2, Tc2 and regulatory T cell lineages, but depleted the percentage of Th1, Tc1 and Th17 lineages. Moreover, patients who received a higher ratio of M1/M2 MΦs infusion exhibited higher incidence of grade II–IV aGvHD.
Summary and Conclusions: These findings suggested that G-CSF induced immune tolerance may be mediated by MΦs polarization in allo-HSCT. The ratio of M1/M2 MΦs in bone marrow graft may be used to predict the occurrence of grade II–IV aGvHD. Moreover, G-CSF mobilized MΦs promise to be a novel immunotherapeutic strategy against aGvHD in the future.

PF742

INTERFERON-A IS EFFECTIVE FOR TREATMENT OF MINIMAL RESIDUAL DISEASE IN PATIENTS WITH T(8;21) ACUTE MYELOID LEUKEMIA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

X.-D. Mo[1,*], X.-H. Zhang[1], L.-P. Xu[1], Y. Wang[1], C.-H. Yan[1], H. Chen[1], K.-Y. Liu[1], X.-J. Huang[1]

[1]Peking University People's Hospital, Institute of Hematology, Beijing, China

Background: *RUNX1-RUNX1T1* transcript levels were established as a powerful marker for predicting relapse in patients with t(8;21) acute myeloid leukemia (AML). Monitoring of minimal residual disease (MRD) during chemotherapy can identify high-risk patients with t(8;21), and our prospective multicenter study demonstrated that allogeneic hematopoietic stem cell transplantation (allo-HSCT) significantly improved clinical outcomes of the high-risk patients.
Aims: We aimed to identify the efficacy of MRD-directed interferon-alpha (IFN-α) treatment in patients with t(8;21) AML who were positive for MRD after allogeneic hematopoietic stem cell transplantation (allo-HSCT) ($n = 42$).
Methods: MRD-positive status was defined as that a <4.5-log reduction from diagnosis in *RUNX1-RUNX1T1* transcripts and/or the loss of a ≥4.5-log reduction after 3 months post-HSCT. Patients with positive MRD received six cycles of IFN-α treatment (twice or thrice weekly of every 4 weeks cycle).
Results: The 1-year cumulative incidence of severe acute and chronic graft-*versus*-host disease after MRD-directed IFN-α treatment was 7.1% and 4.8%, respectively. After the treatment, 15 (35.7%), 5 (11.9%), 3 (7.1%), 3 (7.1%), and 6 (14.4%) patients achieved MRD negativity at 1, 2, 3, 4, and >4 months, respectively. Three patients relapsed after the IFN-α treatment, where the 1-year cumulative incidence of relapse was 7.2%. One patient died of severe infection at 460 days after treatment. The 1-year probabilities of event-free survival, disease-free survival, and overall survival post treatment were 76.0%, 92.4%, and 92.5%, respectively. The clinical outcomes in patients who received MRD-directed IFN-α treatment were significantly better than those of the MRD-positive patients without any interventions in the historical cohort.
Summary and Conclusions: MRD-directed IFN-α treatment is effective for patients with t(8;21) AML who were MRD-positive after allo-HSCT. The study was registered at http://clinicaltrials.gov as # NCT02027064.

PF743

EFFICACY AND SAFETY OF AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN ELDERLY MULTIPLE MYELOMA (MM) PATIENTS AGED ≥65 YEARS: A SINGLE CENTRE EXPERIENCE

J. Er[1], H. Rose[1], D. Routledge[1], D. Kipp[1], H. Fairweather[1], S. Ratnasingam[1], A. Friebe[1], J. Hempton[1], P. Campbell[1,*]

[1]Haematology, University Hospital Geelong, Geelong, Australia

Background: MM is a disease of the elderly, with a median age at diagnosis of 66 years. Induction chemotherapy followed by high-dose melphalan ASCT is considered the standard of care in patients aged <65 years without significant comorbidities. Transplant eligibility has historically been determined according to age, based on studies of young MM patients and the perceived risks associated with ASCT in the elderly. However, recent studies suggest that ASCT is both efficacious and safe in the latter group. Here, we report our single-centre experience of ASCT in elderly MM patients.
Aims: To evaluate the efficacy and safety of ASCT in 2 groups of patients aged ≥65 years and <65 years.
Methods: We identified 91 MM patients who underwent a single ASCT at our institution between 2008 and 2016. These patients were analysed based on age at ASCT. The "younger group" included patients aged <65 years, while the "elderly group" included patients aged ≥65 years. Patient charac-

teristics and comorbidity indices were collected including the haematopoietic cell transplantation-specific comorbidity index (HCT-CI). Patients were also retrospectively assessed using the recently developed revised myeloma comorbidity index (R-MCI). Response assessment included overall response rate (ORR), transplant-related mortality (TRM), progression-free survival (PFS) and overall survival (OS). The Kaplan-Meier method was used to estimate OS and PFS, and compared using the log-rank test. Multivariate analysis using the Cox proportional hazards model was used to identify factors independently associated with survival.

Results: Of 91 patients, 25 were in the elderly group (median age 66 years; range, 65-74) and 66 were in the younger group (median age 58 years; range, 38-64). Median follow-up time was 49 months. There were no differences in gender, ISS stage, cytogenetic risk status, or HCT-CI (p=0.359) between the 2 groups. More patients in the elderly group had R-MCI scores of 4-6 (intermediate fit) (44% *vs* 12%; p=0.011), while more patients in the younger group had R-MCI scores of 0-3 (fit) (83% *vs* 52%; p=0.004). 90% of patients in each group received the standard dose of melphalan (200 mg/m^2) as conditioning. Four patients received a dose of 140 mg/m^{2}due to elderly age, heavy pre-treatment, or reduced lung function, while 1 patient received a dose of 100 mg/m^{2}due to renal impairment. Overall response rates pre-ASCT were similar (elderly group, 96% *vs* younger group, 91%; p=0.4159) while the ORR post-ASCT also did not differ (88% *vs* 89%; p=0.8370). Toxicity, time to engraftment, and days of hospitalization were not significantly different between the 2 groups and TRM was low (3-4%) in both groups. The median PFS for the elderly group was non-inferior to the younger group (46 months *vs* 30 months; p=0.9390), while the median OS did not show any significant difference between the 2 groups (74 months *vs* not reached; p=0.2636) (Figure 1). In the uni- and multivariate analysis, higher ISS stage (hazard ratio [HR], 2.4; p=0.007) and high-risk cytogenetics (HR 7.8; p=0.005) were poor prognostic factors for OS. In the univariate analysis, higher R-MCI scores (HR 1.4; p=0.016) were also associated with inferior OS.

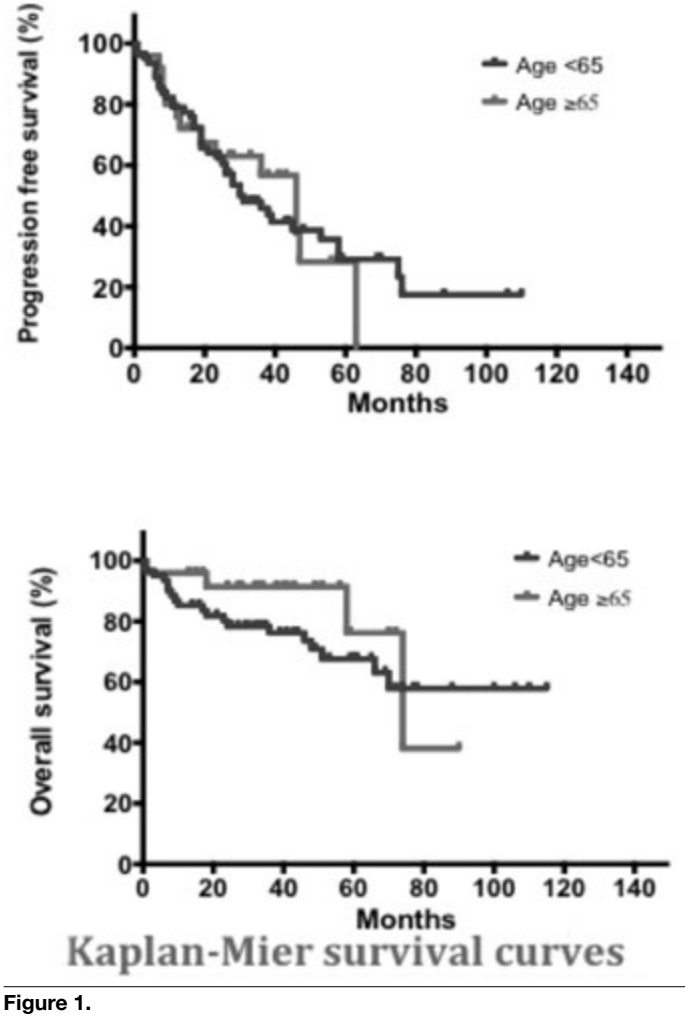


Figure 1.

Summary and Conclusions: This study suggest that ASCT is safe and effective in MM patients aged ≥65 years and should be considered based on performance status and comorbidities rather than age alone. In addition, this study suggests that the R-MCI could be useful in predicting outcomes for elderly MM patients undergoing ASCT.

PF744

PD-1 CONTRIBUTES TO THE DEVELOPMENT OF ACUTE GRAFT-*VERSUS*-HOST DISEASE IN PEDIATRIC PATIENTS RECEIVING HAPLOIDENTICAL STEM CELL TRANSPLANTATION

E. You[1], S. Jang[1], Y.-U. Cho[1], C.-J. Park[1], Y.J. Lee[2], N.-R. Park[2], E.S. Choi[3], H. Kim[3], K.N. Koh[3], H.J. Im[3], A. Ahn[4,*]

[1]Laboratory medicine, [2]Asan Clinical Research Center, [3]Department of Pediatrics, Asan medical center and University of Ulsan College, Seoul, Korea, Republic Of, [4]Department of Laboratory Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic Of

Background: In a tumor microenvironment, overexpression of PD-1, an inhibitory receptor on the surface of T cells, can lead to dysfunction of anti-tumor effector cells. Recent studies show PD-1 is associated with graft-*versus*-host disease (GVHD) and relapse after stem-cell transplantation (SCT). However, studies on PD-1 in patients received SCT are rare.

Aims: The aim of this study was to evaluate pediatric patients undergoing haploidentical hematopoietic stem-cell transplantation (HHSCT) to determine expression levels of PD-1 and to investigate the association between PD-1 and GVHD and relapse.

Methods: Between April 2017 and February 2018, a total of 153 peripheral blood samples with 34 patients with hematologic malignancies (n=18) and non-malignancies (n=16) were enrolled. Seventeen patients were experienced acute GVHD and 4 patients were experienced engraftment failure and 2 patients were expired. Flow cytometric analysis of PD-1 expression on T cells was performed by means of a Navios™ flow cytometer and Kaluza® Flow Analysis Software (Beckman Coulter, Miami, FL, USA).

Results: Patients within 14 days after graft infusion showed significantly lower level of PD-1 on CD8+ and CD4+ T cells than before HHSCT (P=0.000 and P=0.009). However, PD-1 expressions were not correlated with time after HHSCT. However, PD-1 expressions on CD8+ T cells and CD4+ T cells were significantly correlated quantitative chimerism results (P=0.000 and P=0.000). PD-1 expression on CD8+ T cells was higher in hematologic malignancies patients than non-malignancies patients (P=0.028). To investigate the relationship between PD-1 expression and acute GVHD, we analyzed the samples within 100 days after HHSCT. PD-1 expression in CD8+T cells and CD4+ T cells were significantly increased in the patients with acute GVHD (P=0.010 and P=0.034). However, no difference in PD-1 was observed between patients with engraftment and patients with engraftment failure.

Summary and Conclusions: In this study, the correlation between PD-1 expressions and quantitative chimerism results suggests that it might be associated with relapse after SCT. Blockade of PD-1 immune checkpoint may be an option of immunotherapeutic strategies for patients with increased quantitative chimerism results after HHSCT.

In particular, expression of PD-1 was found to be increased in patients with acute GVHD compared to non-GVHD patients and might be helpful in understanding the relationship between PD-1 and acute GVHD. However, the role of PD-1 in post-SCT patients should be evaluated in larger patient cohorts in future studies.

PF745

A PHASE 1 PILOT STUDY OF ULTRA-LOW DOSE IL-2 AS GVHD PROPHYLAXIS IN HAPLO-IDENTICAL STEM CELL TRANSPLANTATION

S. Ito[1,*], P. Jain [1], S. Cordes[1], X. Tian[2], C. Cantilena[1], C.P. Bradley[1], R. Panjwani[1], Q. Yu[1], S. Hauffe[1], J. Superata[1], D. Stroncek[3], J. Chen[4], M. Battiwalla[1], J. Barrett[1]

[1]Hematology Branch, [2]Office of Biostatistics Research, National Heart, Lung, and Blood Institute National Institutes of Health, [3]Department of Transfusion Medicine, Clinical Center, [4]Center for Human Immunology, Autoimmunity, and Inflammation, National Institutes of Health, Bethesda, United States

Background: Post-graft cyclophosphamide dramatically improved the outcomes of haplo-identical stem cell transplantation (haplo-SCT) by reducing the life-threatening graft *versus* host disease (GVHD). However, the optimal duration and choice of systemic immunosuppression in haplo-SCT remain undetermined especially for the subjects of high-risk hematologic malignancies requiring maximal benefit of graft *versus* leukemia (GVL) effects. Ultra-low dose interleukin 2 (ULD IL-2) can simultaneously prevent GVHD

and potentiate GVL through its unique biological properties to expand regulatory T cells (Tregs) and natural killer (NK) cells.
Aims: We updated our report of a phase 1 study evaluating the safety and feasibility of ULD IL-2 as GVHD prophylaxis in haploidentical allo-SCT (NCT02226861) with correlative biological data.
Methods: Twelve subjects (median age 35, range 22-66 years) with high-risk hematological malignancies were enrolled in the study receiving a myeloablative conditioning regimens of fludarabine 120mg/m^2 (day -10 to day -8) and total body irradiation (TBI; 600-1200 cGy, day -10 to day -6). Donor lymphocyte infusion (DLI) products (2x10^8 CD3+/kg) was infused on day -6, followed by post-DLI cyclophosphamide 120mg/kg on days -3 and -2. CD34$^+$ selected, peripheral blood stem cell product was infused on day 0. For GVHD prophylaxis, ULD-IL2 (aldesleukin, 100,000 IU/m^2) was given from day +1 subcutaneously daily for 12 weeks with a concurrent use of sirolimus for the first 60 days. The subsets of memory T cells, Tregs, NK cells were characterized by flow cytometry and plasma GVHD biomarkers (ST2, Reg3α, sTNFR1, ANG2) were measured by multiplex microfluidic channel assay. Virus and leukemia antigen specific T-cells were evaluated by both Elispot and flow cytometry. ULD IL-2-dependent transcriptome landscape was further analyzed at single cell level using 10x™ Chromium™ Single Cell Platform.
Results: Eight subjects were classified as high or very high risk by Disease Risk Index (DRI) and most subjects had high-risk EBMT score (median 5) and high HCT co-morbidity index (median 5). All subjects achieved successful engraftment and 100% donor myeloid and lymphoid chimerism by day 21. At the median follow up of 2 years, the overall survival and relapse-free survival was 56.2% and 37.5%, respectively. All evaluable subjects tolerated ULD-IL2 without significant toxicities. Grade II-IV and III-IV acute GVHD (aGVHD) were only observed in 25% and 8% respectively during ULD IL-2 treatment. However, five subjects experienced either *de-novo* or rapid exacerbation of aGVHD after discontinuation of ULD IL-2, resulting in the cumulative incidences of grade II-IV and III-IV aGVHD of 58% and 25% respectively (Figure 1A). ULD IL-2 expanded both Helios$^+$Tregs as well as CD56^{bright}NK cells. The onset of aGVHD was correlated to rapid decrease in Helios$^+$Tregs population with a sharp increase of ST2 (Figure 1B). Single cell RNAseq confirmed dynamic transcriptome changes in various cell populations induced by ULD IL-2 discontinuation, suggesting significant homeostatic roles of ULD IL-2 in GVHD prophylaxis. Both leukemia and virus antigen specific T cells were detectable as early as day 100 after haplo-SCT.

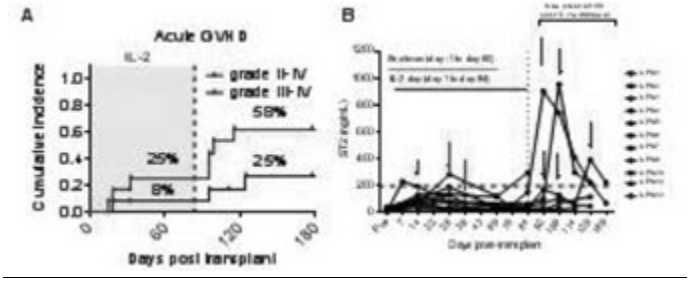


Figure 1.

Summary and Conclusions: This is the first study to demonstrate the safety and feasibility of ULD IL-2 as GVHD prophylaxis in haplo-SCT. Our study implies an acquired dependency of donor-derived Tregs to exogenous ULD IL-2 and the possible need of prolonged administration of ULD IL-2 after haplo-SCT.

PF746

THE IMPACT OF CMV REACTIVATION ON HSCT OUTCOMES IN CHILDREN WITH MALIGNANCIES: A 13 YEARS SINGLE CENTER EXPERIENCE

F. Baccelli[1,*], A. Di Gangi[2], S. Bernasconi[3], M. Nardi[3], L. Coccoli[3], G. Casazza[3], C. Favre[4], M. Menconi[3]

[1]University of Pisa, [2]Sant'Anna School of Advanced Studies, [3]Azienda Ospedaliera Universitaria Pisana (AOUP), Pisa, [4]Azienda Ospedaliera Universitaria Meyer, Firenze, Italy

Background: After the introduction of pre-emptive therapy, the incidence of CMV disease (CMVD) dramatically decreased in HSCT recipients, while indirect effects of CMV infection (CMVI) are still under investigation.
Aims: To critical evaluate indirect effects of CMVI and the incidence of CMVD in pediatric HSCT for malignancies.
Methods: We retrospectively analyzed children (aged<18 years) who underwent first allogeneic HSCT for malignancies at our institution between 01/2003 and 12/2016. CMVI was defined as virus isolation or detection of viral proteins (antigen pp65) or nucleic acid in any body fluid or tissue specimen while the definition of CMVD required specific organ signs/symptoms combined with CMV documented in the involved tissue by virus isolation, rapid culture, histopathology, immunohistochemistry or DNA hybridization techniques. Patients were classified according to the presence of CMVI. 5-years probabilities of OS, DFS, GVHD-free relapse-free survival (GRFS) and infection were calculated using the Kaplan-Meier method and the log-rank test. Cumulative incidence procedure and Gray's test were used to assess differences in relapse, NRM, aGVHD grades 2-4 and extensive cGVHD (e-cGVHD). Major endpoints are reported in Table 1 together with standard errors (SE).
Results: 92 patients were included (CMVI N=40). Groups were homogeneous for age, sex, diagnosis, donor (MSD N=13, MUD N=31, MMUD N=22, haploidentical (HAPLO) N=12, UCBU N=15), stem cell source and graft composition with the exception of lower median cellularity in UCBU. Eleven HAPLO were lymphocyte-depleted (6 α/β+ and 5 CD3+/CD19+) while one received post-transplantation cyclophosphamide. No significant differences were reported for OS, DFS, infections, GRFS, relapse and NRM (p>0.05). The mean day of CMVI diagnosis was 39.6 (28.2-51.0). Although statistically non-significant, aGVHD grade 2-4 was higher in CMVI+, suggesting a possible association. As reported in Figure 1, aGVHD has often preceded CMVI (N=7 *vs* N=1). In contrast, e-cGVHD was less frequent in CMVI+ patients. Remarkably, infections were slightly higher in the early period in CMVI+. Noticeable, only three patients have suffered of CMVD (all of them with gastrointestinal involvement) and two of them are alive at the time of the last follow-up.

Table 1.

Endpoints (SE)	Global	CMV infection	No CMV infection	p
OS	67.2 (0.05)	70.0 (0.07)	64.5 (0.07)	n
DFS	62.0 (0.05)	59.9 (0.08)	64.1 (0.07)	s
Relapse	24.6 (0.05)	25.1 (0.07)	24.2 (0.06)	
NRM	4.6 (0.03)	5.0 (0.04)	4.1 (0.03)	
Extensive cGVHD	9.7 (0.04)	6.3 (0.04)	13.1 (0.07)	
GRFS	41.4 (0.05)	35.0 (0.08)	46.4 (0.07)	
Infections	52.8 (0.06)	58.3 (0.09)	47.8 (0.08)	

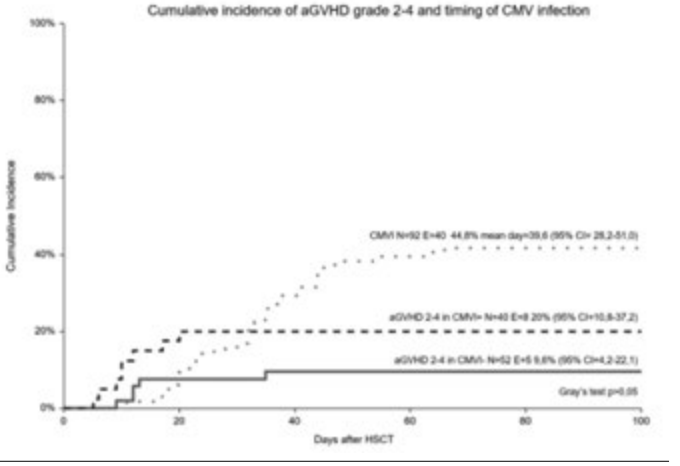


Figure 1.

Summary and Conclusions: Our data confirm low rates of CMVD in modern HSCT. Despite variations in HSCT procedures and the limited sample size, pre-emptive therapy determined a dramatical decrease in CMV indirect effects. Moreover, aGVHD is a possible risk factor for CMVI but not vice versa. However, further evidences are needed to enlighten the role of CMV in the modern era of pediatric HSCT.

PF747

A REAL-LIFE SINGLE CENTER RETROSPECTIVE ANALYSIS TO EVALUATE EBMT RISK INDEX AS A PROGNOSTIC FACTOR IN PEDIATRIC HSCT

A. Di Gangi[1,*], F. Baccelli[2], S. Bernasconi[3], L. Luti[3], E. De Marco[3], G. Casazza[3], C. Favre[4], M. Menconi[3]

[1]Sant'Anna School of Advanced Studies, [2]University of Pisa, [3]Azienda Ospedaliera Universitaria Pisana (AOUP), Pisa, [4]Azienda Ospedaliero Universitaria Meyer, Florence, Italy

Background: EBMT risk index (EBMT-RI) is a well-established tool for risk assessment in HSCT. Although it has been validated in children and adults as a predictor of OS and TRM, its association with other endpoints has not been extensively investigated in pediatric HSCT performed with more recent protocols.
Aims: To retrospectively investigate the role of EBMT-RI as a predictor of outcomes in modern pediatric HSCT.
Methods: We included patients <18 years who underwent first allogeneic HSCT between 01/2004 and 01/2017 for ALL, AML, MDS, CML and NHL. EBMT-RI was calculated following EBMT guidelines and patients were accordingly stratified: low risk (LR, score 0-3), intermediate risk (IR, score=4) and high risk (HR, score>4). Kaplan-Meier and log-rank tests were applied to estimate probabilities of 5-years OS, DFS and GRFS. Relapse, aGVHD grades 2-4, cGVHD, TRM were assessed by cumulative incidence accounting for competing risks and Gray's test. Donor types, donor and patient age, HSC source, AB0 compatibility, conditioning regimens, CMV infection and serostatus were included in univariate and multivariate Cox regression analyses.
Results: We analyzed data from 88 patients (LR N=62, IR N=19, HR N=7). Diseases were not uniformly represented within the sample (ALL N=51, AML N=24, MDS N=6, CML N=5, NHL N=2) and among groups. Matched sibling donors were present only in the LR group. 6 Haploidentical HSCT were lymphocyte-depleted (3 α/β+ and 3 CD3+/CD19+) and one received post-HSCT cyclophosphamide. TBI-based MA regimens and BM grafts were used in HSCT with the exception of haploidentical HSCT which were conducted with RIC regimens and PBSC grafts. Results with standard errors (SE) are reported in Table 1. Only 4 patients experienced TRM (LR N=3, IR N=1). OS and DFS were significantly associated with EBMT-RI (p<0.05). Relapse (Figure 1) and GRFS may show an association with EBMT-RI which is not statistically significant probably because of the nature of the study (p=0.06). Other endpoints did not differ between groups. Due to the small sample size, univariate Cox regression have demonstrated only weak correlations. Remarkably, a possible correlation was observed between DFS and EBMT-RI (p=0.08), donor types (p=0.02), HSC source (p=0.06) or AB0 compatibility (p=0.02). Similarly, correlation between OS and either donor types (p=0.03) or EBMT-RI (p=0.047) was observed. Multivariate analysis did not show any significant correlation.

Table 1.

Endpoints, % (SE)	All	LR	IR	HR	p
OS	65.7 (.05)	73.0 (.06)	57.4 (.12)	28.6 (.17)	<0.02
DFS	60.3(.05)	66.7 (.06)	51.5 (.12)	28.6 (.17)	0.05
GRFS	38.7 (.05)	44.2 (.07)	31.6 (.11)	14.3 (.13)	0.06
Relapse	25.7 (.05)	19.9 (.05)	32.8 (.11)	57.1 (.19)	0.06
aGVHD	14.8 (.04)	16.1 (.05)	10.5 (.04)	14.3 (.13)	>0.05
cGVHD	28.7 (.06)	28.0 (.07)	30.7 (.12)	20.0 (.18)	

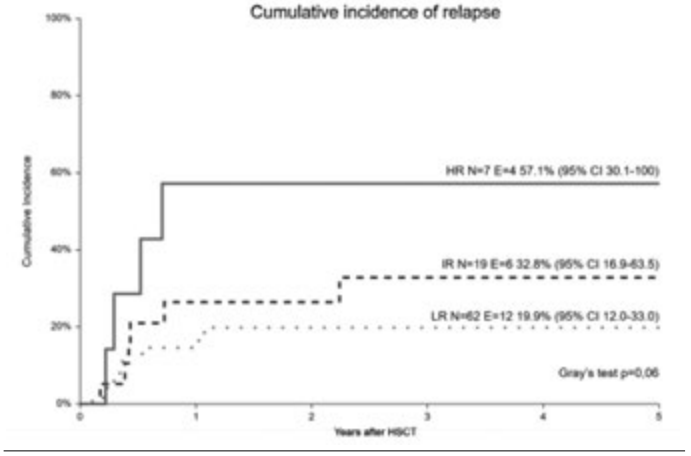


Figure 1.

Summary and Conclusions: EBMT-RI is a good predictor of OS and DFS. Furthermore, we found a putative association between the score and relapse and GRFS. Due to the low incidence of TRM, it was not possible to study correlation between TRM and EBMT-RI. Sample size and lack of homogeneous groups are important limiting factors of our study. Since EBMT-RI was not specifically developed for children, a reformulation of this score for pediatric HSCT may significantly improve risk stratification in this peculiar context. However, further studies are needed.

PF748

THE EXPERIENCE OF USING DEFIBROTIDE FOR THE PREVENTION AND TREATMENT OF HEPATIC VENO-OCCLUSIVE DISEASE AFTER PEDIATRIC HEMATOPOIETIC STEM CELL TRANSPLANTATION; A SINGLE CENTER EXPERIENCE

B. Antmen[1,*], H.I. Sasmaz[1,2], B. Karagun[1], M. Serbest[1]

[1]Pediatric Bone Marrow Transplantation Unit, Department of Pediatric Hematology, Adana Acibadem Hospital, [2]Pediatric Hematology, Cukurova University, Adana, Turkey

Background: Hepatic veno-occlusive disease (VOD) is a common and serious complication of hemotopoietic stem cell transplantation (HSCT) in children. Çorbacıo lu et al reported that, defibrotide prophylaxis seems to reduce incidence of VOD.
Aims: We aimed to assess prospectively the use of prophylactic defibrotide in pediatric patients undergoing HSCT and present of treatment results.
Methods: In this study, 157 patients who underwent HSCT were given defibrotide prophylaxis as 25 mg/kg per day in four divided intravenous infusions over 2h, starting on the same day as the pre-transplantation conditioning regimen. The mean duration of use of defibrotide is 25 days as a prophylaxis.
Results: In this study, 157 patients were recruited, 95 male patients and 62 female patients, with the average of 8.7 years, range 1-20; 9% infants, 58% children and 33% adolescent. There were 69 patients with thalassemia major, 58 patients with leukemia, 12 patients with aplastic anemia, one patient with Diamond Blackfan anemia, two patients with congenitale dyserythropoetic anemia, one patient with osteopetrosis, five patients with famial hemophagocytic lymphohistiocytosis, three patients with severe immune deficiency, threee patients with sickle cell disease and threee patients with Kostman syndrome. All transplants were allogeneic. No serious side effects were seen. In 13 patients developed clinical VOD (Seattle criteria). In these patients, defibrotide dose was increased to a treatment dose of 40-60 mg/kg per day. One infant patient with Kostman syndrome died due to hepatic and pulmonary veno-occlusive disease. After 48 months of follow up, 12 patients who developed VOD are being well and no patient have transplant related complications
Summary and Conclusions: Hepatic veno-occlusive disease, which is caused by hepatocyte and sinusoidal vessel endothelium damage, can ocur usually early phase after HSCT, and in its severe form, may lead to liver failure, hepatorenal syndrome, portal hypertension, and eventually death from multiorgan faillure. In this prospective study, we demonstrated that the use of defibrotide is safe and effective in preventing and treating VOD in pediatric patients at high risk group.

PF749

THE HEMATOPOIETIC CELL TRANSPLANTATION COMORBIDITY INDEX IN THE CONTEXT OF AUTOLOGOUS STEM CELL TRANSPLANTATION CAN PREDICTS CLINICAL OUTCOMES

A. Martínez-Roca[1,*], C. Talarn Forcadel[1], L. G. Rodríguez-Lobato[2], L. Escoda Teigell[1], R. Vallansot[1], M. Cervera Calvo[1], J. Do Nascimento[1], R. Aguinaco Culebras[1], T. Giménez Pérez[1], C. Araguás Arasanz[1], A. Vicen[1], M. Sola[1], J. Sarrà Escarre[1]

[1]Hematology, ICO-Hospital universitari Joan XXIII., Tarragona, [2]Hematology, Hospital Clinic, Barcelona, Spain

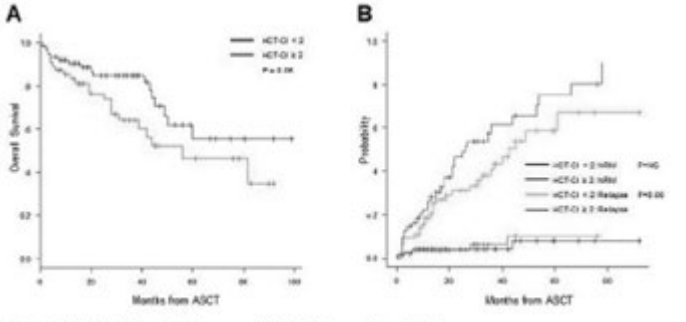
Background: Autologous stem cell transplantation (ASCT) is a standard of care in patients with multiple myeloma and lymphoma, nevertheless is associated with morbidity and mortality. The Hematopoietic cell transplant comorbidity index (HCT-CI) is a score used to evaluate the comorbidities for outcomes after allogenic hematopoietic cell transplant (HCT), proving to be a predictor of non-relapse mortality (NRM) after allogenic HCT. There are few publications that evaluate the use of HCT-CI in the setting of ASCT with heterogeneous results on the score ability to predict NRM and overall survivor (OS) in this group of patients.
Methods: We analyzed 130 ASCT performed in our center between November 2008 and April 2017. Individual comorbidities were prospectively collected at the time of ASCT. All patients were assigned an HCT-CI score. We tested the utility of HCT-CI as a predictor of NRM and survival in patients undergoing ASCT. Survival curves and probabilities were estimated using the Kaplan-Meier method and log-rank analysis. Multivariate analysis was performed using Cox proportional hazard regression model. Variables analyzed included HCT-CI, age, gender, type of disease (myeloma and lymphoma), disease stage and Karnofsky performance score

(KPS) (Table 1). NRM was defined as mortality after transplant in the absence of disease relapse/progression Thus, the cumulative incidence probabilities for NRM were calculated accounting for relapse as a competing risk.

Table 1. Patient Characteristics of the 2 Groups (HCT-CI Score <2 and ≥2).

Characteristic	TOTAL	HCT-CI <2	HCT-CI ≥2	P
Transplants	130	96	34	
Age at transplant (range) ≥ 50	94 (72.3)	47 (62.7)	47 (85.5)	0.004
Gender (%) Male	71 (54.6)	39 (52)	32 (58.8)	0.48
KPS (%) <90	74 (56.9)	40 (53.3)	34 (61.8)	0.33
Diagnose (%) Myeloma	70 (53.8)	36 (48)	34 (61.8)	0.12
Lymphoma	60 (46.2)	39 (52)	21 (38.2)	
Disease stage (%) CR	54 (41.5)	31 (41.3)	23 (41.8)	0.95

Results: Median follow-up was 29,5 months (CI, 11-44,25). Patients with a HCT-CI score ≥2 presented a median OS of 56 months (P=0.06) and trend towards to an inferior OS. In the multivariate analysis HCT-CI score ≥2 retained its independence adverse prognostic factor (HR=2.34; 95% CI, 1.166-4.71; P=0.016). NRM was 43 months in HCT-CI score <2, and 26 months in the HCT-CI score ≥2 group (P=0.083) (Figure 1).



OS and NRM of the 2 risk groups (HCT-CI Score <2 and ≥2).

Figure 1.

Summary and Conclusions: A higher HCT-CI score did not predict NRM but was associated with inferior survival in patients undergoing ASCT in our cohort.

PF750

INTRA-BONE CORD BLOOD TRANSPLANT WITHOUT *IN-VIVO* T-CELL DEPLETION: RESULTS IN 12 PATIENTS.

E. Xue[1,*], F. Giglio[1], R. Greco[1], D. Clerici[1], L. Lazzari[1], M. Bernardi[1], S. Piemontese[1], M.T. Lupo Stanghellini[1], J. Peccatori[1], F. Ciceri[1]

[1]Hematology and Bone Marrow Transplantation Department, IRCCS San Raffaele Scientific Institute, Milan, Italy

Background: Cord Blood Transplant (CBT) represents a valid option in patients affected by hematological malignances without HLA-matched donors. Anti-thymoglobulin (ATG) has been largely used as an *in vivo* T-cell depletion to reduce the risk of GVHD and prevent graft rejection, but its use is associated with a slow post-transplant immune recovery, which might lead to higher infection and relapse rate.
Aims: Aims of this study was to demonstrate the efficacy and safety of CBT without *in vivo* T-cell depletion in high-risk hematological disease.
Methods: Since 2015 our Center adopted a policy of GvHD prophylaxis without *in-vivo* T-cell depletion, based on sirolimus and mycophenolic acid. Twelve patients received a CBT: diagnosis were AML=8, T-ALL=2, NHL=2; median age was 45 years (range 22 - 65), and 5 pts were in active disease (AD) at transplant. None of them have received prior auto-SCT, whereas four have received prior allo-SCT (MUD=2, MRD =1, MMRD=1). In all but one case, CB was transplanted directly intra-bone to improve the speed and efficacy of engraftment. Conditioning regimens were based on full myeloablative doses of Treosulfan and fludarabine; Donor/recipient HLA matching were 5/8, 6/8, 7/8 in 4, 2, 5 cases respectively, and in one case 4/6. Antifungal prophylaxis included voriconazole (n=7), posaconazole (n=3), amphotericin (n=1) or micafungin (n=1), antimicrobial prophylaxis was based on quinolones (n=12), and antiviral prophylaxis included acyclovir (n=5) or acyclovir plus foscarnet (n=7).
Results: After thawing, median mono-nucleated CD45+ cells was 1.3 x 10e7/kg (range 0.69 – 2.9), median CD34+ cells infused was 0.08 x 10e6/kg (range 0.02 – 0.23), and median CD3+ cells was 2.75 x 10e6/kg (range 1.29 – 4-9). Neutrophils engraftment was documented in all but two cases (who died within one week after graft injection) with a median time of 25 days (range 16–59 days); platelets engraftment in seven out of 12 cases (median time=50 days, range 24 – 221 days). Median follow up is 206 days (range 9 – 1016 days). Three pts transplanted in active leukemia died from disease relapse, which occurred at 90, 180 and 305 days after CBT respectively; 3 pts died early from TRM (GvHD n=1, probable IFD n=3). In ten pts at least one viral reactivation was documented (CMV n=7, HHV6 n=10, others n=5), with a first reactivation median time of 25 days from CBT, in 7 cases with organ involvement (encephalitis n=2, gastrointestinal infection n=6). Two pts also had EBV reactivation, in one case associated to lymphoproliferative disorder. Three pts developed probable invasive fungal disease (IFD), treated with amphotericin (n=1) and voriconazole (n=2). One patient developed acute GvHD (overall grade 4; 59 days after CBT), whereas 4 pts developed a peri-engraftment syndrome (median time 13 days after CBT) which recovered after a short steroid course. Sirolimus and mycophenolic acid were withdrawn at a median time of 175 (range 64–353) and 27 days (range 27–31) respectively. No pts developed chronic GvHD. Interestingly, immune reconstitution of lymphocytes subsets was obtained at 3 months after CBT, showing a median of 157/μL CD3+ cells, 142/μL CD3+CD4+ cells, 38/μL CD3+CD8+ cells, 35/μL CD19+ cells, 129/μL NK cells at 3 months. Six pts are alive and in complete remission at last follow up (median FU 316 days, range 53-1016 days).
Summary and Conclusions: Intra-bone CBT without *in vivo* T-cell depletion is associated with fast hematopoietic engraftment and immune reconstitution, with low rate of GvHD.

PF751

HEMATOPOIETIC REGENERATION AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION WITH ACTUAL BODY WEIGHT BASED DOSING OF CONDITIONING CHEMOTHERAPY

D. Fuchs[1,*], R. Greul[1], K.J. Krenosz[1], M.A. Fridrik[1]

[1]Department of Internal Medicine 3 - Hematology and Oncology, Kepler University Hospital, Johannes Kepler University Linz, Linz, Austria

Background: The incidence of obesity is increasing, but it is unclear how to dose chemotherapy in these patients, especially in autologous stem cell transplantation (ASCT). Obese patients have more comorbidities and different pharmacokinetics than non-obese patients, which may put them at a higher risk for complications of ASCT. A majority of centers adjust dose for obese patients, but methods are heterogeneous and include using ideal body weight (IBW) or adjusted actual body weight (ABW) (*i.e.* IBW plus 25 or 40% of ABW minus IBW) and/or capping the calculated body surface area (BSA) at $2.0m^2$. In patients undergoing conventional chemotherapy, guidelines recommend full dosing based on ABW, because myelosuppression is not more pronounced in obese patients and adequate doses are need for optimal efficacy. In ASCT, there is little data and no consensus on how to dose conditioning chemotherapy in obese patients.
Aims: We aimed to examine whether ABW-based dosing of chemotherapy before ASCT leads to a prolongation in the time to hematopoietic reconstitution in patients with a BSA over $2.0m^2$.
Methods: We performed a retrospective analysis of patients with an ASCT from 2004 to2017 at our center, which uses full, ABW-based dosing of chemotherapy. In patients with a BSA of >=$2.0m^2$ those with a BSA <$2.0m^2$, reconstitution of leukocytes (days from ASCT to leukocytes >1.0 G/l) and thrombocytes (>20 and >100 G/l) were compared. We also examined overall survival (OS), 100-day OS and transplant associated mortality (TRM). Time to event analysis was summarized using Kaplan Meier curves and results were compared using the log rank test.
Results: We included 60 patients and 76 autologous transplants from 2004 to 2017. 69.7% were male, with a median age of 50.5±12.9 years (range 25.7-63.1, 95%CI 25.7-63.1). Median BMI was 25.3±4.4 (range 16.7-44.5, 95% CI 19.7-33.5). A majority was overweight (43.4%) or obese (10.5%; 40.3% normal weight, 5.3% underweight). 50.0% had a BSA over $2.0m^2$ (median 2.0±0.24, range 1.4-2.5, 95% CI 1.5-2.4). Chemotherapy protocols were BEAM (31.6%), carboplatin/etoposide (21.1%), melphalan (42.1%) and busulfan/cyclophosphamide (5.2%). Diagnoses were lymphoma (33.0%; Hodgkin's, 7.9%; follicular lymphoma, 13.2%, diffuse large B-

cell-lymphoma, 5.3%, other, 6.6%), testicular cancer (19.7%), multiple myeloma (42.1%) and acute myeloid leukemia (5.3%). Median time to reconstitution of leukocytes (>=1.0 G/l) was 10.0±0.354 days for BSA <2.0 and 10.0±0.233 days for BSA >=2.0 (p=0.643). There was no significant difference between reconstitution of thrombocytes >20 G/l (median 10.0±0.42 *vs* 10.0±0.328 days, p=0.481) or thrombocytes >100 G/l (median 18.0±0.581 *vs* 19.0±1.50 days, p=0.497). Similar results were seen when comparing BMI (underweight/normal weight *vs* overweight/obese; p=0.051 for leukocytes, p=0.624 and 0.729 for thrombocytes >20 and >100). Grade 3-4 infections were numerically higher in patients with BSA >=2.0, but this was not statistically significant (78.9% *vs* 92.0%, p=0.191). The same was true for other complications of grade 3 and higher (51.0 *vs* 65.8%, p=0.245). Therapy associated deaths were rare (n=1) and there was no difference regarding 100-day-OS (median N.R. *vs* N.R., p=0.558), but patients with a BSA >2.0 had a trend for a higher OS (N.R. *vs* 94.8 months, p=0.075) (Figure 1).

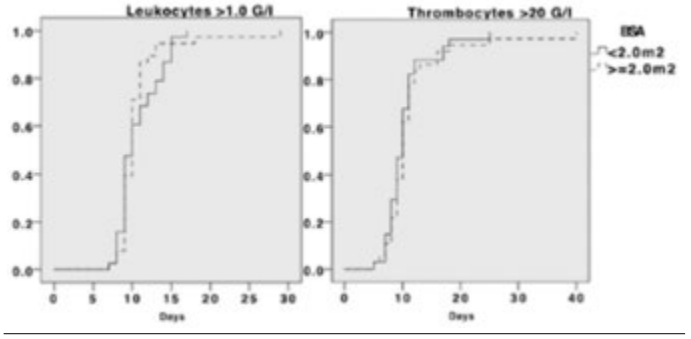


Figure 1.

Summary and Conclusions: Although this was a retrospective study and findings need to be validated, there was no difference in time to hematopoietic reconstitution, OS or TRM. ABW-based dosing of chemotherapy prior to ASCT was safe in patients with BSA >2.0m^2.

PF752

IMPACT OF MIXED CHIMERISM IN T REGULATORY CELLS ON RELAPSE RATE IN ACUTE LEUKEMIA PATIENTS AFTER ALLO-HSCT

D. Dubnyak[1,*], N. Risinskaya[2], M. Drokov[1], N. Kostritsa[3], J. Davydova[4], L. Kuzmina[1], N. Kapranov[4], V. Vasilyeva[1], N. Popova[1], E. Mikhaltsova[1], O. Koroleva[1], A. Sidorova[1], E. Usikova[1], Z. Konova[1], I. Galtseva[4], A. Sudarikov[2], E. Parovichnikova[1], V. Savchenko[1]

[1]Bone marrow transplant, [2]Molecular biology laboratory, National Research Center for Hematology, Moscow, Russian Federation, [3]School of Medicine, Lomonosov Moscow State University, [4]Immunophenotyping laboratory, National Research Center for Hematology, Moscow, Russian Federation, Moscow, Russian Federation

Background: It is known that T-regulatory cells (Treg) play an important role in maintaining tolerance after allo-HSCT. Tregs prevent the development of acute graft-*versus*-host disease (aGVHD) (Beres & Drobyski, 2013). At the same time these T-cell populations can limit the antitumor response (graft-*versus*-leukemia, GVL) and that limitation may cause relapses. Here we report the analysis of mixed chimerism in T-reg population and the frequency of relapse in patients with acute leukemia (ALL and AML) after allo-HSCT with comparable levels of donor chimerism.
Aims: To evaluate a possible relationship between the mixed chimerism in Treg cells and the rate of relapses in acute leukemia patients after allo-HSCT.
Methods: The study included 31 patients after allo-HSCT (ALL n=6, AML n=25). The median age was 38 years (19-66). Peripheral blood samples for analysis were taken on day 30 after transplantation. Immunomagnetic separation (Miltenyi Biotec, Germany) was used to isolate population with CD4+CD25+ phenotype which is predominantly associated with Treg cells. Extraction of DNA was performed from the obtained cells. Chimerism in DNA samples was determined using the STR-PCR method. The percentage of donor chimerism was calculated using standard procedures (Nollet *et al.*, 2001) Statistical analysis of the data was carried out using SPSS ver 23. (IBM, Chicago, Ill., USA). xact Fisher's test was used to analyze the 2 × 2 contingency tables.
Results: In the patients group with less than 11% of cells with host genotype (more than 89% of cells of donor origin) the relapse rate was significantly higher - 52.6% (10 of 19) than in the other group of patients with 11% and more cells with host genotype - 8.3% (1 of 12), (p=0.02). At the same time donor chimerism in the unselected bone marrow did not significantly differ between the groups (p=0.36) and amounted to 100% (75-100%) and 97.5% (90-100%). The study groups were balanced for all other factors that could affect the relapse rate: disease status, graft source, GVHD.
Summary and Conclusions: According to our data we proposed that host's Treg cells are not capable of suppressing GVL that explains significant differences in the frequency of relapses in patients with acute leukemia after allo-HSCT. Predominance of host's Treg cells may serve as a favorable prognostic sign but this hypothesis needs to be confirmed in the largest cohort of patients.

PF753

REDUCED-INTENSITY CONDITIONING WITH FLUDARABINE AND BUSULFAN *VERSUS* FLUDARABINE AND MELPHALAN FOR T DEPLETED ALLOGENEIC TRANSPLANT IN MYELOID MALIGNANCIES: AN EXPERIENCE OF A TERTIARY CENTRE

M.S. Lopes[1,*], P. Krishnamurthy[2], K. Tawana[2], J. Craig[2], B. Uttenthal[2], C. Crawley[2]

[1]Clinical Haematology, Centro Hospitalar Porto, Porto, Portugal, [2]Haematology, Cambridge University Hospitals, Cambridge, United Kingdom

Background: Fludarabine with busulfan (FB) and fludarabine with melphalan (FM) are two widely used reduced-intensity conditioning (RIC) regimens that are well tolerated in older patients and those with comorbidities. Until 2015, our centre has routinely used oral busulfan based regimens. Following data suggesting more favourable outcomes for acute myeloid leukaemia (AML) patients undergoing FM conditioning (Baron *et al.* 2015) we elected to switch to this regimen for all transplants for myeloid malignancies.
Aims: To review overall and progression free survival (OS and PFS) for patients undergoing T-cell depleted RIC FB or FM transplants for myeloid malignancies.
Methods: Data was collected retrospectively for myeloid patients who underwent allogeneic haematopoietic stem cell transplant (aHSCT) with FB or FM at our centre between February 2014 and December 2017. Busulfan 8mg/kg was given orally over 2 days and a single dose of melphalan (140 mg/m2) was given intravenously. All patients received aHSCT from a fully-matched sibling or an unrelated donor (VUD). All received alemtuzumab as graft-*versus*-host disease (GvHD) prophylaxis at 30-50mg total dose. Results were analysed using SPSS with Kaplan-Meier methodology for survival assessments.
Results: Of 78 patients observed, 46 (56%) were male, with a median age at transplantation of 59 years (range 35-71). The HCT comorbidity index (HCT-CI) score (Sorror 2005) was more than 3 in 25 patients (32%). Patients received transplants from a sibling (n=22, 28.2%) or VUD (n=56, 71.8%). The groups were matched for age, sex, HCT-CI, donor type, EBMT risk score (Gratwohl 2012) and months between diagnosis and transplantation. Patients were transplanted for AML (n=57, 73%) or MDS/MPN (n=21, 27%) and were equally distributed between groups. Fourteen patients (18%) had secondary AML (sAML). The median follow-up for FBC (FB+alemtuzumab) cohort was 39.1 months (range 26.7-48.6) and 11.5 months (range 1.7-47) for FMC (FM+alemtuzumab). Thirty-seven patients received FBC and 41 patients received FMC conditioning. Amongst the FBC group, there were 30 AML patients (*de novo*, n=20 (67%) and sAML, n=10 (33%)); amongst FMC patients, 27 were transplanted for AML (*de novo*, n=23 (85%), sAML n=4 (15%)) with a statistical difference (p=0.21). High risk AML patients by the ELN classification (Döhner *et al.* 2017) were equally distributed (p=0.14) between both conditioning groups (14/30 AML in FBC, 8/27 AML in FMC). Twenty-one MDS/MPN patients were equally distributed and classified in terms of IPSS-R as poor and very poor risk. Acute GvHD was observed in 23 (29.5%) patients and chronic GvHD in 14 (18%) patients, no significant differences between groups. Median OS was 19.7 months for FBC and not reached for FMC patients (p=0.005). Median PFS was 18.5 months for FBC and not reached for FMC (p=0.001). No other factors significantly influenced OS and PFS (Figure 1).
Summary and Conclusions: We demonstrate inferior OS and PFS in patients receiving FBC (oral Busulfan) when compared with patients who received FMC conditioning for transplantation in myeloid malignancies. Reasons for this could include the bioavailability and pharmacokinetics of oral busulfan. Whilst the data represents a small sample with short follow-up on FMC cohort, the earlier and greater number of relapses in the FBC cohort is significant. These results suggest that FBC (oral busulfan) is a suboptimal con-

ditioning for our data. Studies with longer follow-up and larger samples will be necessary to achieve major conclusions.

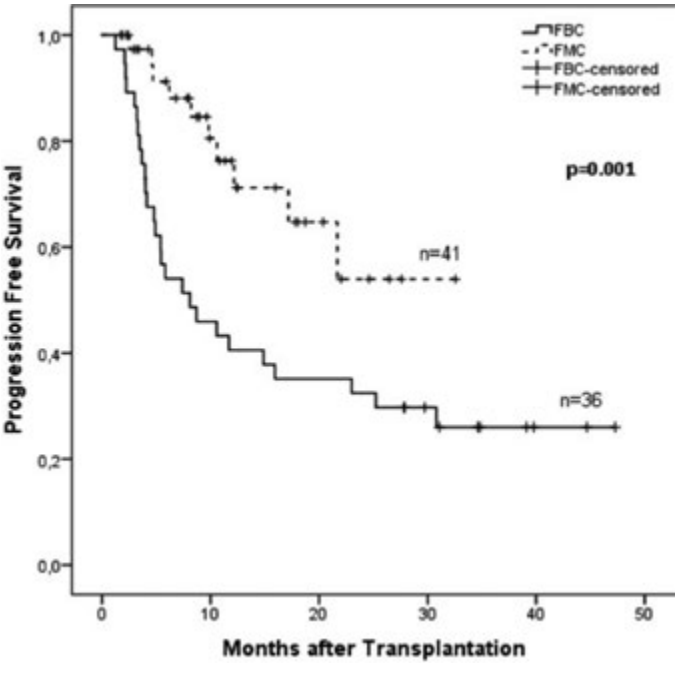


Figure 1.

Stem cell transplantation – Experimental

PF754

THYMIC FUNCTION AFTER HAPLOIDENTICAL ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

G. Lia[1,2], S. Butera[1], A. Evangelista[3], L. Tosti[2], L. Comba[2], S. Cena[1,2], R. Pulito[1], L. Brunello[1,2], M. Gilestro[4], M. Ruggeri[4], D. Oddolo[4], S.A. Caltagirone[4], V.E. Muccio[4], E. Saraci[4], M. Festuccia[1], G. Ciccone[3], E. Maffini[1,2], C. Dellacasa[1], M. Boccadoro[2,4], L. Giaccone[1,2], A. Busca[1], P. Omedè[4], B. Bruno[1,2,*]

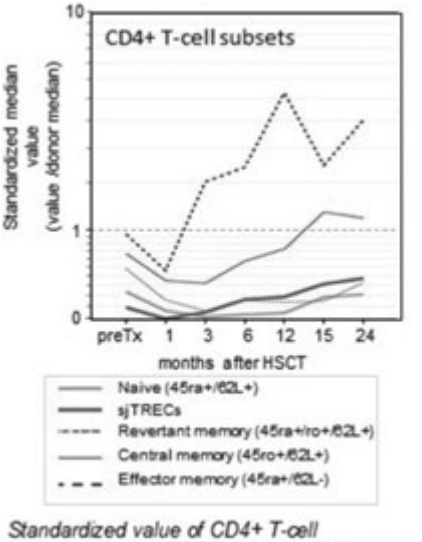
[1]Dipartimento di Oncologia e Ematologia, SSD Trapianto Allogenico di Cellule Staminali, A.O.U. Città della Salute e della Scienza di Torino, [2]Dipartimento di Biotecnologie Molecolari e Scienze per la Salute, Università degli Studi di Torino, [3]Clinical Epidemiology, [4]Dipartimento di Oncologia e Ematologia, A.O.U. Città della Salute e della Scienza di Torino, Torino, Italy

Background: The use of post-transplant cyclophosphamide (PTCY) has expanded the application of haploidentical haematopoietic stem cell transplantation (haplo-HSCT). Post-transplant immune-deficiency, especially in the T-cell compartment, may lead to increased risks of opportunistic infections and reduced graft-*vs.*-tumor effects. Adequate reconstitution of a near-normal/normal T-cell immunity may play a key role. The thymus is the primary source of naïve T-cells and highly contributes to maintain the peripheral T-cell pool.

Aims: To evaluate the kinetics of long-term thymus-dependent immune-reconstitution after PTCY haplo-HSCT.

Methods: The study population consisted of 29 consecutive haplo-HSCT patients of median age 53 (r 28-70) years. Donor median age was 39 (r 18-66). Blood samples were collected before conditioning and at 1, 3, 6, 12, 18, 24 months after transplant. Analysis of T-cell subsets (naïve, central, memory, revertant) by flow-cytometry was correlated with Real-Time PCR quantification of signal joint T-cell receptor excision DNA circles (sjTRECs), specific marker of naïve T-cells thymopoiesis. SjTRECs real-time PCR was performed on genomic DNA extracted from sorted CD4 and CD8 T-cells. Associations between sjTRECs and T-cell subsets and trends over time were evaluated by Generalized Linear Models.

Results: Following PTCY induced T-cell depletion, a constant gradual increase in absolute numbers of all T-cell subsets and of sjTRECs from the first month up to two years post-transplant was observed Patient median counts of T cell subsets at 1, 3, 6, 12, 15, 24 months were as follows: **naive CD4+CD45RA+CD62L+**: 1,9/ul; 2,3/ul; 5,5/ul; 9,3/ul; 31,9/ul, 40,2/ul; **central CD4+CD45RO+CD62L+**: 17,6/ul, 50,7/ul, 89,8/ul, 127,0/ul, 202,8/ul, 197,6/ul; **effector CD4+CD45RO+CD27-**: 5,2/ul, 41,37ul, 72,7/ul, 125,4/ul, 109,8/ul, 179,9/ul; **revertant CD4+CD45RA+/45RO+**: 3,9/ul, 11,1/ul, 36,6/ul, 62,2/ul, 42,2/ul, 55,4/ul. Median values of **sjTREC copies**/100 ng DNA from sorted CD4+ cells were: 2,8; 5,9; 36,2; 25,8; 22,4 at 3, 6, 12, 15, 24 months, and 84,6 in healthy donors respectively (Figure 1).



Standardized value of CD4+ T-cell subsets and sjTRECs reconstitution at different time-points after HSCT.

Figure 1.

Median values of healthy donor T cell counterparts were 284,3 /ul, 161,9/ul, 37,0/ul, and 152,6/ul respectively. Similar patterns of immune-recovery was observed in CD8+ T cell subsets. Overall, at two years, CD4 and CD8 T-cell levels and sjTRECs levels were lower than those observed in healthy donors. Molecular analysis of the sjTRECs kinetics was associated with the increase in CD4+ naïve T-cells (global p <0,008). This correlation clearly suggests that most of naïve T-cells derives from thymic re-education of donor precursor stem cells. Furthermore, an increase in revertant CD4 and CD8 memory T-cells was also significantly correlated with sjTRECs kinetic (p 0,041 and <0.001 respectively). By contrast, central and effector memory T-cells showed a faster thymic-independent expansion. Importantly, sjTRECs levels and overall thymic dependent immune-reconstitution were more robust in a cohort of 63 adult patients undergoing HSCT from HLA identical donors (data not shown).
Summary and Conclusions: Our results provide evidence of active thymic function despite age-dependent involution that substantially contributes to T-cell reconstitution after PTCY haplo-HSCT. Lower production of sjTRECs as compared after HLA identical sibling transplants may partly be due to a higher degree of "mismatching" of MHC molecules during thymic re-education after PTCY haplo-HSCT. Correlation between immune-reconstitution and clinical outcomes will be presented at the meeting.

PF755

A MESENCHYMAL STROMAL CELL (MSC)-DERIVED COMBINATION OF CXCL5 AND ANTI-CCL24 IS SYNERGISTIC AND SUPERIOR TO MSC AND CYCLOSPORINE FOR THE TREATMENT OF GRAFT-*VERSUS*-HOST DISEASE

X. Fan[1,*], D. Guo[1], A. Cheung[2], Z. Y. Poon[2], C. S. Yap[3], S. Goh[1], H. Li[4], S. Bari[2], S. Li[5], K.H. Lim[6], W. Hwang[7]

[1]Clinical Translational Research, [2]Hematology, Singapore General Hospital, [3]KK Research Centre, KK Women's and Children's Hospital, [4]Division of Medicine, Singapore General Hospital, [5]Cancer & Stem Cell Biology, Duke-NUS Medical School, [6]Department of Pathology, Singapore General Hospital, [7]National Cancer Centre Singapore, Singapore, Singapore

Background: The immunosuppressive properties of mesenchymal stromal cells (MSCs) have been clinically proven to be effective in treating graft-*versus*-host disease (GVHD). However, MSC therapy is limited by the need for laborious and expensive manufacturing processes that are fraught with some batch-to-batch variabilities.
Aims: Substitution of MSC therapy with key MSC-mediated immunomodulatory factors could be an option for GVHD treatment.
Methods: Cytokines presenting in the bone marrow-MSCs/mixed lymphocytes co-culture were identified with a cytokine antibody array. The key MSC-mediated immunosuppressive factors were screened out through serial factorial designs. The immunosuppressive efficacy of these key factors was validated in xenograft model of GVHD created by infusing human peripheral blood mononuclear cells to NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice. Human cell engraftment and cytokine/chemokine expression in mouse peripheral blood were measured by flow cytometry and Luminex assay.
Results: We identified a synergistic two-factor combination (2FC) of CXCL5 and anti-CCL24 from a panel of over 100 immunomodulatory factors as being equivalent to MSC in the modulation of mixed lymphocyte reactions. This 2FC was superior to cyclosporine in ameliorating both moderate and severe GVHD, while being equivalent to MSC in moderate GVHD and superior to MSC in severe GVHD. Its immunosuppressive efficacy could be further improved by extended treatment. Mechanistic studies revealed that, *in vitro*, the 2FC could only reduce the proliferation of T_H1 cells and T_H17 cells; while *in vivo*, CXCL5 acts in concert with anti-CCL24 antibody to reduce not only human T_H1 cells, T_H17 cells but also cytotoxic T lymphocytes and NK cells, increase mouse immunosuppressive neutrophils and without affecting human hematopoietic stem cell reconstitution. Concurrently, it reduced circulating human pro-inflammatory cytokines IFN-γ, IL-6, IL-17A, IL-8, MIP-1β and MCP-1.
Summary and Conclusions: Both *in vitro* and *in vivo* data suggest that CXCL5 and anti-CCL24 antibody act in concert to ameliorate GVHD via T_H1 and T_H17-triggered immunosuppression. We propose that this novel 2FC could substitute for MSC therapy in GVHD treatment.

PF756

GENERATION OF IMMORTAL MURINE HEMATOPOIETIC STEM/PROGENITOR AND LEUKEMIC STEM CELL LINES FROM TRANSGENIC MICE

E. Doma[1,*], F. Pfleger[1], A. Hoelbl-Kovacic[1], V. Sexl[1]

[1]Department of Biomedical Sciences, Pharmacology and Toxicology, University of Veterinary Medicine, Vienna, Austria

Background: Research on hematopoeitic and leukemic stem cells (LSCs) is currently limited as these cells are infrequent and their immortalization is hardly achieved.
Aims: We aimed to establish a long term *ex-vivo* culture system that allows maintenance and expansion of immortalized and functional LSK (lin$^-$, Sca-1$^+$, c-Kit$^+$) cells and LSCs, which trigger leukemia upon transplantation.
Methods: We adapted a technique described by the L. Carlsson lab and transduced high-purity sorted murine LSKs with *Lhx2*, a LIM-homeobox transcription factor, which has been reported to facilitate *ex vivo* expansion of immature hematopoietic cells.
Results: Lhx2 expressing- hematopoietc progenitor cell (HPC^{LSK}) lines require SCF (stem cell factor) and IL-6 and they can be maintained in a feeder-independent culture. They preserve LSK markers despite continuous proliferation and represent functional stem/progenitor cells. HPC^{LSK} cells differentiate *in vitro* upon stimulation with cytokines and repopulate lethally irradiated mice, where they reconstitute the entire hematopoietic cell compartment. HPC^{LSK} cell lines were established from a range of transgenic mice, underlining the overall applicability of this model. Using this system, we also established LSC lines that express BCR/ABL^{p210}, MLL-AF9, or Flt3-ITD; Nras^{G12D} and which do induce disease *in vivo*. These LSCs home to the bone marrow, differentiate and drive myeloid leukemia, when injected into NSG mice. To test the applicability of the model system for drug sensitivity tests we employed Imatinib mesylate (IM). As in people, HPC^{LSK}-BCR/ABL^{p210} cells show partial IM resistance and leads to the enrichment of undifferentiated LSKs.
Summary and Conclusions: We created a robust method of culturing functional hematopoietic stem/progenitor cells which are immortalized and can be expanded indefinitely. By oncogenic transformation we created leukemia triggering LSCs. Our model is a versatile tool allowing the investigation of hematopoietic and leukemic stem cells of any given genotype. Our system thereby represents a valuable tool for (cancer) stem cell biology and may assist in the development of new therapeutic avenues to combat LSCs.

PF757

GENOME WIDE METHYLATION IN HEMATOPOIETIC STEM CELLS AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

S. Trino[1,*], L. De Luca[1], A. Caivano[1], I. Laurenzana[1], P. Zoppoli[1], F. La Rocca[2], G. Calice[1], V. Simeon[3], D. Memoli[4], A. Weisz[4], D. Cilloni[5], G. Falco[6], A.M. Carella[7], L. Del Vecchio[8], P. Musto[9]

[1]Laboratory of Pre-clinical and Translational Research, [2]Laboratory of Clinical Research and Advanced Diagnostics, IRCCS - Referral Cancer Center of Basilicata (CROB), Rionero in Vulture (Pz), [3]Medical Statistics Unit, University of Campania "Luigi Vanvitelli", Naples, [4]Laboratory of Molecular Medicine and Genomics, Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Baronissi (SA), [5]Department of Clinical and Biological Sciences, University of Turin, Orbassano (TO), [6]Department of Biology, University of Naples Federico II, Naples, [7]Hematology and Bone Marrow Transplant Unit, IRCCS - Casa Sollievo della Sofferenza, San Giovanni Rotondo, [8]Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, Naples, [9]Scientific Direction, IRCCS - Referral Cancer Center of Basilicata (CROB), Rionero in Vulture (Pz), Italy

Background: Allogeneic hematopoietic stem cell transplantation (AHSCT) is a curative therapeutic approach for different hematological diseases. Currently, little is known about the biology of HSCs after transplant in terms of homing, self renewal and differentiation, as well as about the role of bone marrow (BM) microenvironment. Since DNA methylation is involved in the balance between HSC self-renewal and differentiation into lymphoid and myeloid lineages, this epigenetic modification may play a role in the reconstitution of the hematopoietic system after AHSCT.
Aims: We explored genome DNA methylation dynamics in BM HSCs aiming to describe the general pattern of methylation after AHSCT.
Methods: A total of 10 patients, affected by acute myeloid leukemia (n=6), acute lymphoblastic leukemia (n=3), Hodgkin lymphoma (n=1) and receiving BM AHSCT, were included in our study. CD34+ cells were sequentially collected from BM of donors (t0) and matched recipients at different time points, at day +30 (t1), +60 (t2), +120 (t3), +180 (t4), +360 (t5). DNA, extracted from CD34+ cells, was used to perform array-based methylation (Infinium Methylation EPIC, Illumina). M-values (log2 Methylated/Unmethylated signals) were used for analysis.
Results: We generated genome wide methylation profiles of donors and recipients interrogating about 850.000 CpG sites. We firstly analyzed global methylation level of all samples. Specifically, we applied principal component analysis to the most variable probes, observing that samples were uniformly distributed; moreover, unsupervised hierarchical clustering showed that, generally, each patient profiled into a specific methylation cluster; some donors clustered together, while others separated with their respective recipient. We

also observed that global methylation level was similar in all times (t0-t5), when considering all samples. Subsequently, to assess methylation changes after transplant, we compared methylation profiles of donor and recipient HSCs at different times. First, we evaluated differentially methylated probes (DMPs) observing the major differences in t1 *vs* t0 (n=12043 DMPs, 70% hyper- and 30% hypo-methylated); this data suggested that, after 30 days, CD34 recipient cells consistently modified their methylation pattern respect to donor cells. We also identified differential methylated regions (DMRs) observing a higher number of DMRs in t1 samples *vs* t0 (n=292). Genomic methylation distribution showed that percentage (%) of hyper-methylated DMPs resulted higher in CpG islands, shores and 3'UTR in all time points *vs* t0, while% of hypo-methylated DMPs was higher in open sea regions. To understand if DMPs maintained a long term modification (t5) or had a short term effect only present in t1, we generated two different signatures: (i) DMPs both in t1 and t5 against t0 (368 DMPs, 276 hyper- and 92 hypo-methylated) representing a long term signature (Figure 1), (ii) DMPs at t1 but not in t5 against t0 (2604 DMPs, 1987 hyper- and 617 hypo- methylated), defined as short term signature. Functional enrichment of both signatures revealed that differentially methylated genes were associated with hematopoiesis.

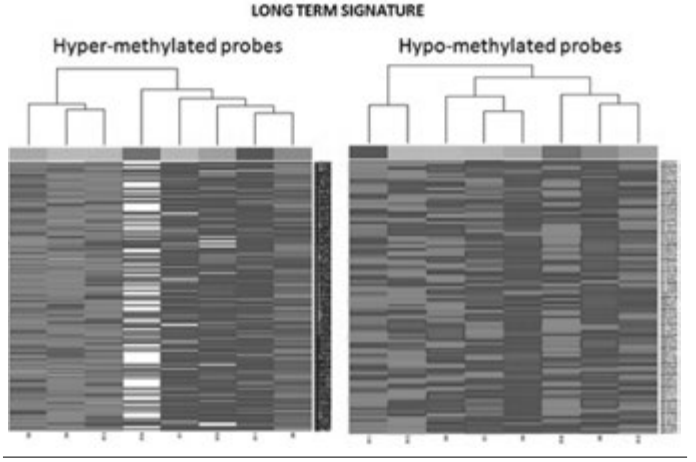


Figure 1.

Summary and Conclusions: Our study shows that global methylation pattern of HSCs prevalently changes in an early time after BM AHSCT, possibly, as a consequence of the adaptation of donor cells in recipient BM niche. Moreover, after 12 months, HSCs partially restored methylation pattern, similarly to donor cells at baseline. Ongoing studies on status and dynamic of HSC methylation could define methylation role in AHSCT outcome.

PF758

TYPE-I-INTERFERON INDUCING MICROBIAL METABOLITES PROTECT FROM INFLAMMATORY TISSUE DAMAGE

E. Thiele Orberg[1], H. Poeck[1,*]

[1]3. Medizinische Klinik; Klinikum Rechts der Isar, Technische Universität München, München, Germany

Background: Recent studies have demonstrated that signals of the innate immune system and the composition of local microbial communities (*i.e.*, the bacterial microbiota) in the gastrointestinal (GI) tract are linked to the outcome of graft-*versus*-host disease (GVHD) following allogeneic hematopoietic stem cell transplantation (allo-HSCT). Yet, the specific contribution of selected innate immune pathways and factors that mediate microbiota-dependent GVHD regulation remain poorly understood. Therefore, a better mechanistic understanding of microbiome-host interactions is needed for the development of clinical strategies based on targeted microbial interventions in patients. Recent work suggests a protective function of Type I Interferons (IFN-I) at epithelial surfaces and in the preventon of GVHD. Consistent with this, we have recently shown that activation of nucleic acid sensing pathways including RIG-I/MAVS and cGAS/STING during genotoxic stress and allo-HSCT results in protective type-I-interferon (IFN-I) signaling that maintains gut epithelial barrier integrity. Thus, we propose a key role for IFN-I signaling and nucleic acid sensor-mediated pathways for the maintenance and restoration of epithelial homeostasis during acute intestinal tissue damage. Notably, new research suggests naturally-occurring microbial-derived metabolites in the GI tract play an important role in protection against viruses in an IFN-I dependent manner.
Aims: We therefore aimed at characterizing the role of these microbial metabolites on intestinal homeostasis and self-renewal during genotoxic tissue damage and whether these metabolites can be harnessed for promotion of intestinal barrier function and prevention of GVHD.
Methods: We used intestinal organoid cultures derived from small intestine and colon to address tissue regeneration (proliferation assay, histology, FACS) and signaling (qPCR, immunoblot) in response to IFN-I-inducing metabolite treatment. We also studied treatment responses using a murine model of chemotherapy -induced gut epithelial damage.
Results: We found that treatment of intestinal organoid cultures with IFN-I-inducing metabolite (i) induced growth and proliferation, (ii) increased expression of interferon stimulated genes (ISG) and of antimicrobial peptides (RegIIIγ), (iii) enhanced NF-kB signaling (p65) and (iv) reduced apoptosis via inhibition of caspase-3 cleavage. In addition, mice receiving the respective metabolite were less sensitive to a chemotherapy (CTx)-mediated model of gut epithelial damage and showed improved recovery of intestinal stem cells (ISCs).
Summary and Conclusions: Our data suggest that prophylactic administration of IFN-I inducing metabolites coupled with maintenance of a permissive microbiome may improve the outcome of allo-HSCT recipients via maintenance of intestinal homeostasis.

PF759

EX-VIVO GENERATED HUMAN CORD BLOOD MYELOID-DERIVED SUPPRESSOR CELLS (HCB-MDSCS) ATTENUATE MURINE CHRONIC GRAFT-*VERSUS*-HOST DISEASES

J.-Y. Lim[1], D.-B. Ryu[1], M.-Y. Park[2], S.-E. Lee[1], G. Park[3], T.-G. Kim[2], C.-K. Min[1,*]

[1]Department of Internal Medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, [2]Catholic Hematopoietic Stem Cell Bank, College of Medicine, The Catholic University of Korea, [3]Department of Pathology, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea, Republic Of

Background: Myeloid derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells with anti-inflammatory activity. Although it has been shown that expanded murine MDSCs are capable of attenuating acute GVHD (AGVHD) severity, it is unclear whether *ex-vivo* cultured human cord blood-MDSCs (hCB-MDSCs) have therapeutic effects in preclinical chronic graft-*versus*-host disease (CGVHD) models.
Aims: We used two mouse CGVHD models to evaluate preventive and therapeutic effects of hCB-MDSCs.
Methods: First, GVHD recipients surviving in a classical C57BL/6 into MHC-mismatched BALB/c AGVHD model developed CGVHD. Second, donor pretreatment with G-CSF induced CGVHD. hCB-MDSCs ($1x10^6$) were intravenously injected to determine their preventive effects (on days 5, 7, 10 and 21) or therapeutic effects (on days 21, 28, and 35).
Results: In the first model, the onset of clinical cutaneous CGVHD was significantly delayed in preventive hCB-MDSCs-treated allogeneic recipients. Pathologic scoring of target organs confirmed these clinical results. Importantly, thymic tissues of GVHD mice treated with hCB-MDSCs were less severely damaged, showing higher number of double (CD4 and CD8) positive T cells with reduced expansion of donor-type CD4 and CD8 T cells. Moreover, late infusion of hCB-MDSCs controlled the severity of established CGVHD that had occurred in control recipients. In the second model, allogeneic recipients of G-CSF-mobilized stem cell graft had CGVHD with Th17 differentiation and due to promotion of Th2 differentiation, at least in part. hCB-MDSCs attenuated clinical and pathologic CGVHD severity. Increased production of IL-17 and more infiltration of T cells and macrophages in CGVHD mice were markedly reduced after hCB-MDSCs treatment. Importantly, $Foxp3^+$ regulatory T cells and IFN-γ-producing T cells were expanded while IL-17- and IL-4-producing T cells were decreased in allogeneic recipients of hCB-MDSCs.
Summary and Conclusions: Taken together, these results showed that hCB-MDSCs have preclinical capability of attenuating CGVHD by preserving thymus function and regulating Th17 signaling, suggesting a possible therapeutic strategy for clinical application.

PF760

EFFICACY OF DEFIBROTIDE FOR PREVENTION AND TREATMENT OF ACUTE GRAFT-*VERSUS* HOST DISEASE IN A FULLY MHC-MISMATCHED MURINE MODEL OF ALLOGENEIC BONE MARROW TRANSPLANTATION

D. García-Bernal[1,*], M. Blanquer[1], C.M. Martínez[2], A.I. García-Guillén[1], J.E. Millán-Rivero[1], R. Sackstein[3], J.M. Moraleda[1]

[1]Hematopoietic Transplant and Cellular Therapy Unit, [2]Experimental Pathology Unit,

Instituto Murciano de Investigación Biosanitaria, Murcia, Spain, [3]Departments of Dermatology and Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, United States

Background: Allogeneic bone marrow transplantation (allo-BMT) is an intensive therapy used for the treatment of high-risk hematological malignant disorders and other life-threatening hematological and genetic diseases. Acute graft *versus* host disease (aGvHD) remains the most frequent and adverse complication following allo-BMT and limits its extensive clinical application. Current pharmacologic agents used for prophylaxis and treatment of aGvHD have variable rates of success or provoke serious secondary side effects, arising the need for discovery and development of more effective and innocuous pharmacotherapies. Defibrotide is a drug clinically employed for the prophylaxis and treatment of veno-occlussive disease after allo-BMT. However, recent preliminary clinical evidences suggest that this drug could be also effective to prevent the occurrence and/or severity of aGvHD after allo-BMT.
Aims: Using a fully MHC-mismatched murine model of allo-BMT, we analyzed if defibrotide, either in prophylaxis or treatment, could reduce aGvHD incidence and severity in mice after allo-BMT.
Methods: On day +0 C57BL/6 recipient mice were transplanted with $1x10^7$ bone marrow cells from BALB/C donor mice (BM transplant), or with $1x10^7$ bone marrow cells and $1.5x10^7$ donor splenocytes to induce aGvHD. Control mice received syngeneic BMT by transplanting $1x10^7$ bone marrow cells and $1.5x10^7$ splenocytes, both from C57BL/6 mice. After aGvHD onset (from day +7 after allo-BMT), mice were treated with a daily infusion of 25 mg/kg of defibrotide for 20 days, or with 25 mg/kg of prophylactic defibrotide from 2 days before allo-BMT to 20 days after. Untreated mice received same volume of saline solution. Survival after allo-BMT was monitored daily and clinical aGvHD was graded using a previously described score. Histopathological aGvHD score was evaluated in distant areas of skin, liver, colon and oral mucosa (tongue) of mice by H&E staining. Also, T $CD3^+$ cell inflammatory infiltrate in same organs was detected by standard ABC anti-CD3 immunohistochemistry procedure.
Results: Mice with ongoing aGvHD and treated daily with defibrotide displayed a significantly lower aGvHD-related mortality and improved clinical aGvHD score than untreated mice group ($p<0.001$) (60% survival at day +60 post-allo-BMT). Control mice transplanted only with donor bone marrow cells w/o splenocytes (BM Transplant) or with syngeneic bone marrow plus splenocytes (Syngeneic Transplant) did not develop aGvHD. When defibrotide was used in prophylaxis, mice displayed a significant higher survival than untreated mice group ($p<0.001$) (87.5% survival at day +60 post-allo-BMT), also showing a significant reduced aGvHD clinical score from the aGvHD onset compared to untreated group of animals. Histopathologically, aGvHD-target organs revealed significant differences in the severity of aGvHD between the different experimental groups: liver, colon and tongue of untreated animals showed a higher degree of tissue damage than those receiving prophylactic defibrotide (liver: grade III *vs* grade I; colon: grade II/III *vs* grade I; tongue: grade I/II *vs* grade 0). Finally, we observed that liver, colon and tongue of allo-BMT recipient mice that received administration of prophylactic defibrotide displayed a substantial and significant decrease of $CD3^+$ T cells counts compared to untreated animals, mainly in the colon ($p<0.001$).
Summary and Conclusions: Defibrotide could represent a promising clinical alternative for both prophylaxis and treatment of aGvHD after allo-BMT, thereby opening new therapeutic strategies.

PF761

Abstract withdrawn.

Thalassemias

PF762

CONSTRUCTION OF A GFP KNOCK-IN CELL LINE FOR STUDYING GAMMA-GLOBIN CIS-ACTING ELEMENTS

M. Banan[1,*], S. Hesami[1], M. Safi[1], F. Ghasemi[1]

[1]Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran, Islamic Republic Of

Background: K562 is an erythroid cell line which expresses the γ-globin gene. In addition, its γ-globin expression is induced in response to several widely-studied chemicals. Therefore the K562 cell line is deemed to be a good model system for studying the γ-globin cis-acting regulatory elements (*i.e.* via CRISPR/Cas9 genome editing).
However, K562 cells have three normal copies and an abnormal copy of chromosome 11, where the β-locus is located (*i.e.* 4 Gγ and 4 Aγ copies). Thus in all likelihood, all eight putative cis-acting elements have to be edited in order to detect a robust effect on γ-globin expression. Given this limitation, K562 cells harboring a single copy of a reporter gene integrated into either γ-globin gene could be of great value.
Aims: The aim of the present study was to create K562 cells harboring a single copy of a GFP-(IRES)-Neo^R cassette integrated into either γ-globin gene by using CRISPR/Cas9 and HDR. Unlike previous studies (using TALENs), we wanted to avoid integrating an extra promoter (*i.e.* from PGK-Neo^R) into the locus.
Methods: Two sgRNAs binding upstream of the γ-globin start codon were cloned into the PX459 plasmid and screened in HEK 293T cells for functionality. The HDR construct was assembled in pBluescript by amplifying and cloning the GFP.Neo^R.pA cassette (from pSUPER.neo+GFP plasmid), and ~850 bp homology arms amplified from K562 cells. The homology arms precisely flanked the sgRNA-2 break site. K562 cells were co-transfected with the CRISPR/Cas9 and HDR constructs, selected, passaged, expanded, and characterized.
Results: Targeted integration of the cassette was verified by PCR. Subsequently, qPCR experiments showed the presence of ~2 GFP copies/cell, with a reciprocal decrease in the number of amplified γ-globin copies at the break junction. Expression of GFP was detected at both the RNA and protein (fluorescence) level. Of note, reporter gene expression was apparently higher than those detected in similarly created cell lines (by using TALENs and a td-Tomato/PGK-Neo^R cassette). Furthermore, reporter gene expression was induced upon both hydroxyurea and sodium butyrate treatment.
Summary and Conclusions: Our results suggest that the cell line hereby created could be useful for studying γ-globin cis-acting elements. However, further characterization of this cell line (*e.g.* interaction of the LCR with the γ-globin promoter adjacent to GFP) may be required for certain studies.

PF763

LONG-TERM LONGITUDINAL PROSPECTIVE CMR STUDY IN PATIENTS WITH THALASSEMIA MAJOR

A. Meloni[1,*], L. Pistoia[1], C. Gerardi[2], R. Mattei[3], M.G. Roberti[4], A. Carollo[5], M.F. Fiorenza[6], C. Cosmi[7], G. Palazzi[8], E. Facchini[9], G. Restaino[10], E. Grassedonio[11], V. Positano[1], A. Pepe[1]

[1]Fondazione G. Monasterio CNR-Regione Toscana, Pisa, [2]Presidio Ospedaliero "Giovanni Paolo II" - Distretto AG2 di Sciacca, Sciacca (AG), [3]U.L.S.S. 19, Adria (RO), [4]Azienda Ospedaliero-Universitaria OO.RR. Foggia, Foggia, [5]Azienda Ospedaliera "Sant'Antonio Abate", Trapani, [6]Ospedale "Sant'Elia", Caltanisetta, [7]zienda Ospedaliero-Universitaria di Sassari, Sassari, [8]Policlinico di Modena, Modena, [9]Azienda Ospedaliero-Universitaria di Bologna - Policlinico "S. Orsola-Malpighi", Bologna, [10]Fondazione di Ricerca e Cura "Giovanni Paolo II", Campobasso, [11]Policlinico "Paolo Giaccone", Palermo, Italy

Background: According to the International Guidelines, thalassemia major (TM) patients should perform a complete cardiac evaluation, including a CMR scan, every year. However, prospective CMR studies are limited beyond 3 years and longer-term studies are, therefore, important.
Aims: We aimed to determine longitudinal changes in cardiac iron and function assessed by CMR over 6 years in a large cohort of TM patients.
Methods: We considered 426 TM patients (205 males; 30.87±8.21 years) consecutively enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) Network with a CMR follow-up (FU) study at 72 months (6 years). The T2* in all 16 myocardial segments was quantified and 4 patterns of myocardial iron overload (MIO) were identified: no MIO (all segments with T2*≥20 ms), heterogeneous MIO (some segments with T2*≥20 ms and other segments with T2*<20 ms) and global heart T2*≥20 ms, heterogeneous MIO and global heart T2*<20 ms, and homogeneous MIO (all T2*<20 ms). Risk classes were defined on the basis of the patterns of MIO from worst to normal: homogeneous MIO heterogeneous MIO with global T2*<20 ms heterogeneous MIO with global T2*≥20 ms no MIO.
Biventricular function was quantified by cine images.
Results: In the 254 patients with baseline MIO (at least one segment with T2*<20 ms), the following changes were detected at the FU: - improvement, defined as a transition to a better risk class, in 182 (71.7%); - stabilization, defined as no change in the risk class, in 62 (24.4%); - worsening, defined as a transition to a worse risk class, in 10 (3.9%). Among the 172 patients without baseline MIO, 30 (17.4%) worsened, that is developed MIO at the FU. Biventricular end-diastolic volume indexes (EDVI) were significantly lower at the FU CMR. In patients with significant baseline MIO (global heart T2*<20 ms) a significant decrease in all biventricular volumes and a significant increase in left ventricular ejection fraction (EF) (mean difference: 3.83±8.48%, P<0.0001) as well as in right ventricular EF (mean difference: 1.79±9.04%, P=0.042) were detected with a concordant improvement of MIO status. The 50.7% of the patients changed the type of chelator during the FU based on CMR results. The percentage of patients who changed the chelation therapy was significantly higher in patients with significant MIO than in patients without MIO (60.2% *vs* 46.2%; P=0.008).
Summary and Conclusions: Over a period of 6 years, the continuous monitoring of cardiac iron levels and a tailored chelation therapy allowed an improvement in more than 70% of patients with baseline MIO and a consequent improvement of biventricular function.

PF764

CEREBROVASCULAR DISEASE IN BETA-THALASSEMIA: NOT A DISEASE RELATED COMPLICATION?

I. Tartaglione[1,*], S. Perrotta[1], M. Caiazza[1], M. Casale[1], R. Di Concilio[2], A. Ciancio[3], E. De Michele[4], G. Amendola[5], P. Gritti[6], P. A. Carafa[7], T. Ferrantino[8], A. Centanni[5], N. Ippolito[5], V. Caserta[9], T. Oliveto[5], I. Granato[5], G. Femina[10], F. Esposito[11], S. Ponticorvo[12], A. Russo[11], C. Russo[13], M. Cirillo[14], A. Elefante[13], R. Manara[10]

[1]Dipartimento della Donna, del Bambino e della Chirurgia generale e specialistica, Università della Campania "Luigi Vanvitelli", napoli, [2]Dipartimento di Pediatria, Ospedale "Umberto I", Nocera Inferiore, [3]Unità Operativa Ematologia, Ospedale "Madonna Delle Grazie", Matera, [4]Medicina Trasfusionale, AOU "San Gioanni di Dio e Ruggi D'Aragona", Salerno, [5]Ospedale "Umberto I", Nocera Inferiore, [6]Dipartimento di Salute Mentale e Fisica e Medicina Preventiva, Università della campania "Luigi Vanvitelli", Napoli, [7]Dipartimento di Salute Mentale, Servizio Psichiatrico di Diagnosi e Cura, ASL Salerno, salerno, [8]Università della Campania "Luigi Vanvitelli", [9]Università della Campania "L. Vanvitelli", Napoli, [10]Dipartimento di Medicina e Chirurgia, Scuola Medica Salernitana, Sezione di Neuroscienze, Università di Salerno, [11]Dipartimento di Medicina e Chirurgia, Scuola Medica Salernitana, Sezione di Neuroscienze, Università di Salerno Salerno, [12]Dipartimento di Medicina e Chirurgia, Scuola Medica Salernitana, Sezione di Neuroscienze, Università di Salerno Salerno, Salerno, [13]Neuroradiologia, Università degli Studi di Napoli "Federico II", [14]Dipartimento di Scienze Mediche, Chirurgiche, Neurologiche, Metaboliche e dell'Invecchiamento, Università della Campania "Luigi Vanvitelli, Napoli, Italy

Background: Little is known about brain involvement in beta-thalassemia: chronic hypoxia and thrombophilic state could be responsible of an increased risk of cerebrovascular disease (CVD). Silent cerebral infarcts and intracranial artery abnormalities have been recently studied in hemoglobinopathies, but their importance in beta-thalassemia has not been clarified, yet. Asymptomatic white matter lesions (WML) have been shown in both transfusion dependent thalassemia (TDT) and non transfusion dependent thalassemia (NTDT) while intracranial artery stenosis have been detected in NTDT (Karimi *et al.*, 2016; Karimi *et al.*, 2012; Pazgal *et al.*, 2016; Musallam *et al.*, 2011).
Aims: To define prevalence, severity and distribution of WML and intracranial artery stenosis, their impact on cognitive function, and to identify any possible risk factor for CVD in beta-thalassemia.
Methods: this cross-sectional study enrolled TDT and NTDT beta-thalassemic patients and healthy controls from 4 major Centers in the South of Italy. FLAIR and intracranial time-of-flight MR-angiography were performed with a 3T MR scanner. Three neuroradiologists blinded of clinical data evaluated the MR-exams. Both patients and controls were administered the Wechsler Adult Intelligence Scale-Fourth Edition (WAIS-IV) to detect cognitive ability, and Intelligence Quotient (IQ) was measured. Clinical and laboratory data were collected to correlate neuroradiologic and psychometric findings with disease history and severity.

Results: Seventy-four thalassemic patients (46/74 females, mean-age 35.2±11.4 years, range 16-66; 51 TDT and 23 NTDT; mean hemoglobin 9.3±1.4 g/dl, range 7.5-12; 41 splenectomized; 34 on prophylactic aspirin) and 56 controls (36/56 females, mean-age 34.2±11.7 years, range 17-66) were enrolled. No intracranial artery stenosis was detected; no difference between patients and controls with regards to aneurysms prevalence (9.46% *vs* 9.67%). WML were found both in patients and controls, and the prevalence (35/74; 47.30% *vs* 28/56; 50.0%), the mean-number (4.1 *vs* 4.6), the size and the Fazekas score did not differ between the groups. No differences in WML were found between TDT and NTDT, nor between patients on aspirin and others. Splenectomy was associated with an increased lesion burden only in TDT (mean 5.56 *versus* 1.53, p= 0.048); in splenectomized patients on aspirin the number and prevalence of WML was lower compared to those splenectomized not on prophylaxis, but the difference was not statistically significant. Patients with WML did not differ from others in terms of comorbidities, hemoglobin, platelets, erythroblasts, ferritin, hemolysis markers and chelation therapies. The presence of WML did not correlate with cognitive impairment as measured by IQ, both in patients and healthy controls. Patients' IQ was significantly lower than controls, and this finding remained significant after correction for education (82.75±11.11 *vs* 93.96±SD13.22, p=0.032).
Summary and Conclusions: beta-thalassemia patients present lower cognitive performances compared to healthy controls despite no difference in terms of WML and intracranial artery abnormalities. Cognitive impairment did not correlate with the presence of WML. Further analyses are warranted to understand the pathogenesis of cognitive impairment in beta-thalassemia.

PF765

CO-INHERITANCE OF SOUTHEAST ASIAN OVALOCYTOSIS WITH B-THALASSEMIA OR HEMOGLOBIN E HETEROZYGOTE LEAD TO ANEMIA IN CHILDREN

P. Banjerdlak[1,*], P. Sripornsawan[1], T. Chotsampancharoen[1], M. Wongchanchailert[1], N. Songthawee[1], S. Duangchu[1]

[1]Pediatric, Prince of Songkla University, Hat Yai, Songkhla, Thailand

Background: Southeast Asian Ovalocytosis (SAO) is an autosomal form of hereditary elliptocytosis wide spread in Southeast Asia, with an allele frequency of 0.01 in Southern Thailand. In this region Thalassemia is also prevalent, about 30% of Thai population being carriers of these abnormal genes. However, co-inheritance of SAO with β-thalassemia heterozygote has rarely been reported. It is still not known how SAO and β-thalassemia interact, nor its effect on clinical phenotype.
Aims: To determine the effects of co-inheritance of SAO with β-thalassemia, or Hb E heterozygote on clinical severity, hematological parameters compared to SAO patients without these mutations.
Methods: We conducted a cross-sectional analytic study on a total of 128 SAO patients during july - december 2017. Demographic data, clinical presentations, hematological parameters, peripheral blood smears and hemoglobin typing of patients were reviewed. The diagnosis of SAO was made based on peripheral blood smears that; had a presence of more than 25% of elliptocytes, macro-ovalocytes and stomatocytes(so called Theta cells " "). The molecular analysis confirmed that all patients were heterozygous 27bp-deletion of *AE1 gene.* β-thalassemia and Hb E heterozygote were diagnosed by use of a high performance liquid chromatography technique. Patients with other concomitant diseases such as; ABO incompatibility, G6PD deficiency, feto-maternal hemorrhage and thalassemia diseases were excluded. Student's t-test was used to compare continuous data, Chi-squared and Fisher exact test was used to compare categorical data.
Results: There were 77 patients who were diagnosed as SAO during the newborn period. Of these, 18 patients had co-inheritance of SAO with thalassemia (5 β-thalassemia trait & 13 Hb E trait), while the rest of these 59 patients were typical SAO cases. There was no significant difference in the incidence of neonatal anemia and jaundice between two groups. However, there was a higher degree of anisocytosis and poikilocytosis in the patients who had a co-inheritance of SAO and β-thalassemia (Figure 1). This finding correlated with a higher RDW in the co-inheritance group compared to the typical SAO (18.9±2.2 *VS* 18±1.3%, P= 0.03). Other hematological parameters (Hb, Hct, MCV, MCH) at birth were of no significant difference. At one year of age, there were 70 patients, whose hematological parameters were available for comparison. We found that all RBC parameters from 20 patients in the co-inheritance group (6 β-thalassemia trait & 14 Hb E trait), were significantly difference from those of the typical SAO group at: Hb (10.9±0.9 *VS* 11.9±0.8 g/dL, P<0.001), Hct (31.5±2.1 *VS* 33.5±2.1%, P<0.001), RBC count (4.8±0.4 x10^6 *VS* 4.5±0.3 x10^6/μL, P= 0.001), MCV (65.9±3.7 *VS* 74.7±2.8 fL, P<0.001), MCH (22.9±1.6 *VS* 26.5±1.2 pg, P<0.001), MCHC (34.7±1.1 *VS* 35.5±0.8 g/dL, P<0.001), respectively. More importantly, the co-inheritance group had a significant higher incidence of anemia at 1 year of age (60% *VS* 18%, P= 0.001). In addition there were more anisocytosis in the co-inheritance group as shown by a higher RDW (16.6±1.3 *VS* 15.3±1%, P<0.001).

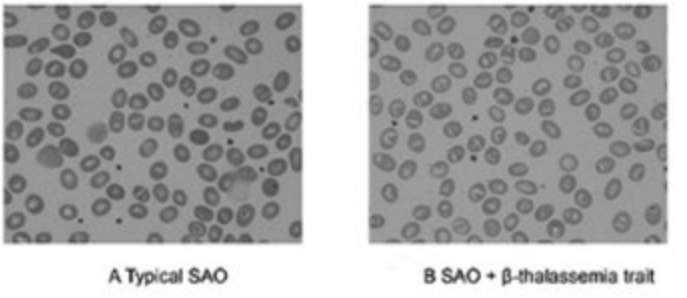


Figure 1.

Summary and Conclusions: Co-inheritance of SAO coupled with β-thalassemia did not affect the degree of neonatal anemia and jaundice at birth, however it lead to a higher incidence of anemia when patients reached one year of age. The presence of β-thalassemia mutation within the SAO population significantly increased the degree of anisocytosis.

PF766

DAILY ALTERNATING DEFERASIROX AND DEFERIPRONE AS NEW THERAPEUTIC SCHEME FOR IRON OVERLOAD IN UNTREATABLE TRANSFUSION DIPENDENT THALASSEMIA PATIENTS

V. Pinto[1,*], M. Balocco[1], S. Quintino[1], L. Bacigalupo[2], B. Gianesin[1], M. Rizzi[3], R. Malagò[4], L. De Franceschi[3], G. L. Forni[1]

[1]Centro della Microcitemia, [2]Radiology, Ospedale Galliera, Genoa, [3]Medicine, Policlinico GB Rossi, University of Verona and AOUI, [4]Radiology, University of Verona AOUI Verona, Verona, Italy

Background: Chelation therapy for treating iron overload has changed the prognosis for patients (pts) with Transfusion Dependent Thalassemia (TDT). Up to now, three drugs are available to reduce iron overload in these patients: deferoxamine (DFO), which is given subcutaneously, and the oral agents deferasirox (DFX), and deferiprone (DFP). The efficacy of combined chelation treatment has been shown to be additive and to increase the probability of success in patients, who previously fail on monotherapy. However, a small portion of patients do not tolerate mono or combined therapy, resulting in worsening iron over-load and in poor prognosis in term of life-expectancy.
Aims: To evaluate the impact of daily alternating regimen of DFP-DFX in otherwise untreatable TDT patients on iron-overload, determined by complementary approaches.
Methods: A total of eight TDT pts (4 males and 4 females) were placed on daily DFP (starting dose 75 mg/Kg/day)-DFX (starting dose 25 mg/Kg/day) alternating chelation therapy. Pts were defined as intolerant to monotherapy or classical combined therapy based on the following criteria: (i) significant proteinuria persistent after re-challenges; (ii) arthralgia with functional limitation; (iii) persistent neutropenia resolved after DFP discontinuation; (iv) gastrointestinal intolerance; (v) systemic reaction. Mean age was 28 years (ranging from 17 to 36). Medical records of TDT pts on daily alternating DFP-DFX were evaluated using serum ferritin and magnetic resonance imaging (MRI).
Results: Mean dose of DFP and DFX were 74 and 26 mg/kg/day, respectively and the median compliance was 90% (calculated as the percentage of drug taken in respect to that administered). Median iron intake from blood transfusions was 9.9 mg/year. Mean follow-up was 52 months (ranging from 12 to 104). The mean value of serum ferritin levels was 1632 at baseline and 1045 ng/ml at follow-up (p 0.2). The mean value of cardiac MRI-T2* was 27 and 37 millisecond (ms) at baseline and follow-up, respectively (p 0.21). Three patients showed at baseline cardiac iron overload (1 severe, 1 moderate and 1 mild) that was removed at follow-up. The mean value of the MRI-T2* of the liver was 5.8 and 12.8 ms at baseline and follow-up, respectively (p 0.19). Five patients showed at baseline liver iron overload (1 moderate-severe, 4 mild) that was removed at the follow-up. The mean value of the MRI-T2* of pancreas was 12.6 and 17 ms at baseline and follow-up, respectively (p 0.47). None of patients developed iron overload in the liver and heart during the treatment. No adverse events were reported. One patient was excluded from analysis due to lack of MRI follow-up (Table 1).

Table 1.

A. Demographic & clinical conditions of TDT patients on daily alternating chelation therapy

CASES	Gender	AGE (years)	Weight (Kg)	Splenectomy	Received Iron Via Blood Transfusion (g/year)	Chelation therapy (mg/kg/die)		Ferritin Levels (µg/dL)		Adverse events
						DFX	DFP	BASAL	FU	
1	F	17	51	Yes	9.9	28	79	4823	2564	No
2	F	36	63	Yes	8.7	18	68	298	407	No
3	F	24	60	Yes	8.5	34	94	2371	348	No
4	M	35	58	Yes	11.4	25	90	547	182	No
5	F	35	50	Yes	9.2	30	75	592	918	No
6	M	32	62	No	11.7	20	69	1500	1510	No
7	M	28	65	No	9.7	28	72	1300	1387	No

CASES	Follow up (months)	HEART			LIVER			PANCREAS		
		BASAL	FU	Difference	BASAL	FU	Difference	BASAL	FU	Difference
1	46	28	26	-2	8.7	6.1	2.6	11.5	16.3	4.8
2	104	28.2	34.3	6.1	13.8	6.8	-6.5	20.1	10.9	-9.2
3	88	8.3	35	26.8	3.4	30.8	27.4	4.9	36.2	31.3
4	70	11.6	48.5	36.9	4.1	23.7	19.6	6.5	7.2	0.7
5	32	19.1	36.4	17.3	10.5	7	-3.5	8.3	8.6	0.3
6	12	47.8	40.3	-7.5	3.3	10.3	9	-	16.7	-
7	12	48.2	37.3	-10.9	4.3	4.5	0.2	24.5	23.2	-1.3

Summary and Conclusions: In this case series, daily alternating regimen of DFP and DFX has been found effective in removing iron from heart and liver as well as maintaining the requested MRI-T2* in heart, liver and even pancreas during the follow-up period. None of the patients experienced moderate to severe adverse events with a safety profile. This new schedule of administration of oral iron chelators exploits the peculiarity of pharmacokinetic and pharmacodynamics of DFP and DFX, making these molecule synergizing and reducing their possible side effects. Our data generate the rationale to design larger studies to investigate this regimen as a potential treatment option for a broader spectrum of TDT patients.

PF767

DENOSUMAB EFFECTS ON SERUM LEVELS OF THE BONE MORPHOGENIC PROTEINS ANTAGONIST NOGGIN IN PATIENTS WITH BETA-THALASSEMIA MAJOR AND OSTEOPOROSIS

E. Voskaridou[1], J. Ntanasis-Stathopoulos[2], D. Christoulas[3], L. Sonnleitner[4], A. Papaefstathiou[5], M. Dimopoulou[1], A. Missbichler[4], N. Kanellias[2], K. Repa[1], A. Papatheodorou[6], M. Peppa[5], G. Hawa[4], E. Terpos[2,*]

[1]Thalassemia and Sickle Cell Disease Center, "Laiko" General Hospital, [2]Department of Clinical Therapeutics, National and Kapodistrian University of Athens, School of Medicine, [3]Department of Hematology, 251 General Air-Force Hospital, Athens, Greece, [4]FIANOSTICS GmbH, Vienna, Austria, [5]Endocrine Unit, Second Department of Internal Medicine-Propaedeutic, Research Institute and Diabetes Center, National and Kapodistrian University of Athens, School of Medicine, [6]Department of Biomedical Research, 251 General Air-Force Hospital, Athens, Greece

Background: Osteoporosis is a common complication of beta-thalassemia major (TM). Denosumab (DMB) is a fully human monoclonal antibody that binds RANKL, decreases osteoclast formation and function and is used for the treatment of osteoporosis. NOGGIN (NOG), a secreted homodimeric glycoprotein, is an antagonist of bone morphogenetic proteins (BMPs) that predominantly binds BMP-4 and BMP-2 and antagonizes their bioactivities by preventing their binding to the respective receptors. Thus, NOG seems to have a profound impact on osteogenesis and may be influenced by anti-resorptive therapy.
Aims: The aim of the study was to evaluate NOG in patients with TM and osteoporosis under DMB therapy.
Methods: NOG was measured in 63 TM patients with osteoporosis who participated in a randomized, placebo-control, phase 2b study. In that study, patients received either 60 mg DMB, sc, every 6 months for 12 months for a total of 2 doses (n=31) or placebo (group B, n=32). Measurement of bone mineral density (BMD) of three body sites (L1-L4, Femoral Neck, Wrist) was performed using DXA before treatment and after 12 months, along with a series of bone remodeling indices: sRANKL and OPG, Dkk-1 and SOST, CTX and RACP-5b, bALP and osteocalcin. NOG was measured, for the first time in TM patients, on days 0 and 180, using a recently developed high sensitivity fluorescent immunoassay based on plasmonic microtiter plates which increase the signal of fluorescent dyes several hundred-fold. Briefly the assay protocol includes: adsorptive coating of capture antibody in 50mM phosphate buffer (PBS)/150mM NaCl pH 7.4, overnight at 4°C followed by washing with PBS containing 0,1% Triton x100. Blocking of unspecific binding was achieved with a proprietary solution of FIANOSTICS containing synthetic polymers and mercapto-compounds. After another washing step, 20ul duplicates of standards/samples (serum) together with 25ul of anti-human NOG antibody labeled with AlexaFluor680 were incubated over night at RT temperature in the dark. Measurements were done using a standard fluorescence micro-plate reader. Samples reading above 100 pmol/l NOG were diluted with assay buffer and re-run to check for linearity of the signal.
Results: The effects of DMB on BMD were presented in EHA 2017 meeting. Regarding bone remodeling indices, patients of group A (DMB arm) achieved a dramatic decrease of sRANKL, sRANKL/OPG, CTX, TRACP-5b, bALP between baseline and after 12 months ($p<0.01$ for all comparisons), while there were no changes in Dkk-1, SOST and OC. On the contrary, patients of group B had an increase of sRANKL, OPG, Dkk-1, CTX, TRACP-5b, bALP ($p<0.01$ for all comparisons) and a slight increase of SOST and OC. Both groups showed an increase in Noggin serum levels after 12 months (mean±SD: 44.2±112.4 *vs* 19.4±49.1 for group A, $p<0.001$; 120.3±478.0 *vs* 12.2±22.1 for group B, $p<0.0001$). This increase was higher in the placebo group. In DMB group there was a strong correlation between NOG with WR T-score ($r=0.797$, $p<0.001$) and WR BMD ($r=-0.641$, $p=0.002$); this was not observed in the placebo group.
Summary and Conclusions: This is the first study where NOG is measured in TM. NOG elevation was lower in DMB than in placebo patients. Only in DMB group NOG changes correlated with WR-BMD. Since our assay detects free, bioactive NOG, higher NOG levels reflect more BMP inhibition, which in turn led to less bone formation of placebo group. Mechanistically these results suggest that denosumab possibly regulates NOG and leads to increased BMD in TM patients with osteoporosis via another mechanism of action.

PF768

FERRITN LEVELS BELOW 500 NG/ML IN TRANSFUSION DEPENDENT THALASSEMIA PATIENTS: HOW TO MODULATE CHELATION TREATMENT?

N. Scaramellini[1,*], E. Cassinerio[2], A. Marcon[3], N. Cieri[2], M. Bortolotti[2], G. Graziadei[2], M.D. Cappellini[2,3]

[1]Rare Disease Center, Department of Medicine and Medical Specialities, Ca' Granda Foundation IRCCS Ospedale Maggiore Policlinico, University of Milan, [2]Rare Disease Center, Department of Medicine and Medical Specialities, Ca' Granda Foundation IRCCS Ospedale Maggiore Policlinico, University of Milan, [3]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy

Background: Trasfusional iron overload is a major target of care in patients with transfusion dependent thalassemia (TDT) because accumulation of iron due to ineffective erythropoyesis and transfusions lead to multiorgans disfunctions; cardiac and hepatic iron overload is the preminent cause of morbidity and mortality in thalassemia patients. Serum ferritin (SF), iron intake, liver iron concentration (LIC) and cardiac T2* values are the parameters used to define when starting chelation: specific guidelines are available in literature for TDT. Three iron chelators are available for a tailored chelation therapy in clinical practice: Deferoxamine (DFO), administered intravenously or subcutaneously, and two oral iron chelators, Deferiprone (DFP) and Deferasirox (DFX). How to treat TDT with iron overload is clear, how to treat TDT with no signs of iron overload is blurred. For these reasons many specialists decide to stop chelation therapy when SF is below 500 ng/ml in absence of evident signs of iron overload thinking that side effects of chelation prevail on good effects.
Aims: We analysed patients with serum ferritin below 500 ng/ml of at least three years in whom we choose to modulate chelator's doses to prevent iron overload and adverse effects.
Methods: One-hundred thirty-two TDT patients were regularly followed at Rare Disease Center, Ca' Granda Foundation Ospedale Maggiore in Milan. All patients were transfusion-dependent and were treated with iron chelators. In a retrospective cohort study we analyzed data of 30 patients (10 males, 20 females, aged 40±6 years) who showed SF below 500 ng/ml from 2014 to 2016 and were treated with DFX (the most used chelator). All the participants underwent periodical evaluation of Hb, renal and hepatic function, SF, iron intake, and yearly T2* magnetic resonance imaging (T2*MRI) for regular follow-up of hepatic and cardiac iron overload. LIC (mg/g/dw) was calculated using the appropriate formulas. We registered the mean used doses of DFX, and their changes during time.
Results: We divided patients into 2 groups based on ferritin levels: group A (8 patients with SF <300 ng/ml) and group B (22 patients with SF between 300-500 ng/ml). Patients of both groups had a steady iron intake and a steady hemoglobin during all three years of observation. Moreover LIC and cardiac T2* values were normal at entering the study and they remained within the normal range over time. No major adverse effects related to DFX were documented during the observetional period. Overall 21 patients maintained a stable dose of DFX (mean dose of 20.6 +/- 6.93 mg/kg/day). In

group A, 3 patients had a reduction of dosage: one because of a slight increase of serum creatinine, one because of SF below 150 ng/ml. Only 1 patient had to increase the dosage because LIC had a mild increase (4.43 mg/g/dw) . In group B, 4 patients had a reduction of dosage for SF<150 ng/ml and only 1 patient had to increase the dosage due to a mild increase of serum ferritin without iron overload.
Summary and Conclusions: These data showed that iron chelation therapy is safe in patient with SF below 500 ng/ml. Chelators don't worsen renal and liver function if regularly monitored, and the reduction of ferritin levels below 300 ng/ml don't determine a reduction of Hb level during time or an increased in chelator's adverse events. More data are needed to support how to modulate chelation treatment in patients under transfusion but without iron overload and very low ferritin levels.

PF769

COMPARAISON OF MORBIDITIES BETWEEN NON TRANSFUSION AND TRANSFUSION DEPENDENT THALASSEMIA PATIENTS OLDER THAN 45 YEARS

A. Marcon[1,*], J. Sleiman[2], A. Tarhini[2], G. Graziadei[3], R. Bou-Fakhredin[2], E. Cassinerio[3], A. Taher[2], M.D. Cappellini[1]

[1]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy, [2]Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon, [3]Rare Disease Center, Department of Medicine and Medical Specialities, Foundation IRCCS "Ca' Granda" Ospedale Maggiore Policlinico, Milan, Italy

Background: The survival of Transfusion Dependent Thalassemia (TDT) patients over the last two decades dramatically improved, becoming similar to that of Non Transfusion Dependent Thalassemia (NTDT) patients.
Aims: To describe and compare clinical complications in TDT and NTDT patients older than 45 followed in two different Centers (Italy and Lebanon).
Methods: Clinical parameters and complications were evaluated in 90 (56 NTDT and 34 TDT) patients who are currently alive. All TDT patients aged >45 years were included in this study. For NTDT patients we included all patients >45 years except those with mild genotype (alpha gene triplication associated with one beta mutation).
Results: male to female ratio were similar in NTDT and TDT patients (33/23 and 18/16 respectively), mean age was higher in NTDT group (53.8±6 *vs* 47.9±3.6 years; p<0.05). The rate of splenectomized patients was higher in TDT group (85.3 *vs* 62.5%; p<0.05). Mean hemoglobin (Hb) was 8.9±1.5 in NTDT and 9.5±0.7 g/dl in TDT group. Mean ferritin levels were similar in both groups (807±567 *vs* 881±742 ng/ml) despite blood transfusion. Liver Iron Concentration (LIC) was higher in NTDT group (6.69±6.1 *vs* 3.52±4.9 mg Fe/g dw; p <0.05). Mean cardiac T2* was 42.05±9.76 ms in NTDT and 37.86±9.8 ms in TDT group; only 2 patients (both TDT) had myocardial iron overload (T2* <20 ms). All TDT and 40/56 (71.4%) NTDT patients were on Iron Chelation Therapy (ICT). Overall the most used drug was Deferasirox (55.5%) followed by Desferal (27%) and Deferiprone (13.5%); only 3 TDT patients (4%) were on combined ICT. The most commonly observed complications in both TDT and NTDT patients were bone disease and cholelithiasis (more than 50% of patients affected in both groups) followed by cardiac involvement, ocular disease and hypoacusia (more than 33% of patients affected in both groups). No statistically significant differences were observed between TDT and NTDT groups regarding fractures (44.1 and 35.7% respectively), in most cases due to traumatic events, cholecystectomy (32.3 and 39.2% respectively), and arterial hypertension (5.9 and 10.7% respectively). In TDT group we observed a higher prevalence of arrhythmic events (29.4 *vs* 10.7%) and nephrolithiasis (35.3 *vs* 14.3%). All endocrinopathies but adrenal insufficiency had higher prevalence in TDT group (p <0.05). Adrenal insufficiency was, so far, observed in 3/34 (8,8%) TDT and 3/56 (5.36%) NTDT patients. HCV infection had higher prevalence in TDT group (52.9% *vs* 8.9% p <0.05). Two TDT and 1 NTDT patients had hepatocellular carcinoma (HCC). Leg ulcers, pulmonary hypertension (PHT) extramedullary hematopoiesis (EMH) and venous thrombotic events were higher in NTDT group (p <0.05).
Summary and Conclusions: the comparison of morbidities in elderly TDT *vs* NTDT patients is still scanty so far. In TDT patients older than 45 years iron related comorbidities remain prevalent due to a long lasting transfusional iron accumulation not regularly counterbalanced by iron chelation. On the contrary NTDT patients showed higher rate of complications related to ineffective erythropoiesis, and anemia. The elderly NTDT patients also experience liver damage due to long lasting iron absorption. Based on these observations, preventive therapeutic strategies are mandatory in young patients.

PF770

A RANDOMIZED, CONTROLLED, PHASE III STUDY TO EVALUATE S-303/GLUTATHIONE PATHOGEN-INACTIVATED RED BLOOD CELLS IN THALASSEMIA MAJOR PATIENTS (SPARC)

Y. Aydinok[1,*], R. Origa[2], N. Huang[3], A. North[3], A. Erikson[3], N. Mufti[3], C. Ernst[3], J.-S. Lin[3], R. J. Benjamin[3], L. Corash[3], A. Piga[4]

[1]Ege University Hospital, Izmir, Turkey, [2]Ospedale Pediatrico Microcitemico, Calgieri, Italy, [3]Cerus Corporation, Concord, United States, [4]University of Torino, Torino, Italy

Background: Life expectancy of Thalassemia major patients is improved by chronic Red Blood Cell component (RBCC) transfusion support combined with iron chelation therapy, but longer duration transfusion exposure increases lifetime risk of acquiring transfusion transmitted infections, including HIV, hepatitis and emerging pathogens (*e.g.*, Zika, Chikungunya, Dengue). The INTERCEPT Blood System for Red Blood Cells (Cerus Corporation) is an investigational device that uses S-303 (amustaline) and glutathione (GSH) in an *ex vivo* treatment step to inactivate a broad spectrum of viruses, bacteria, protozoa and T-cells.
Aims: To evaluate the efficacy and safety of S-303/GSH RBCC in regularly transfused patients with Thalassemia.
Methods: A randomized, controlled, double-blind, non-inferiority, two-treatment period (6 transfusion episodes each), crossover study was conducted with informed consent to evaluate leukocyte-reduced (LR) S-303/GSH RBCC in comparison to conventional LR-RBCC, in >70 evaluable subjects ≥11 years old. Each patient maintained the same transfusion threshold as medically indicated before and during the study period (pre-transfusion hemoglobin (Hb) levels 9-10 g/dL). The primary efficacy endpoint was RBC Hb consumption during transfusion episodes #3-6 in each treatment period (the efficacy evaluation period) measured as the total Hb mass transfused per subject adjusted for body weight (kg) and time (Hb g/kg /day). Non inferiority was defined by a predetermined margin of 15% of the mean consumption of Hb in the Control efficacy evaluation period. The primary safety endpoint was treatment-emergent antibodies to S-303/GSH RBCC.
Results: 86 subjects were enrolled and 81 transfused with study RBCC at two sites in Italy (n=14) and one site in Turkey (n=67). Mean age was 26.1 ±8.1 years (range: 10-44), 45.7% were male, and 18.5% were aged ≤18 years. Eleven percent of patients had pre-existing RBC alloantibodies. In the intention to treat (ITT) population, mean RBCC exposure to S-303/GSH RBC was 12.5 components (range 3-18) and 12.5 (range 6 -18) with conventional RBCC, with a mean transfusion interval of 19.4 (Test) and 19.5 (Control) days. Total transfused Hb (per protocol (PP)+off-study RBCC) dose in the Test period was 687 ±97 grams (g) *versus* 700±116 g Hb (p =0.127) in the Control period. Total mean Hb consumption in the efficacy evaluation period was 0.113±0.04 g/kg/day for S-303/GSH RBCC, and 0.111±0.04 g/kg/day for conventional RBCC. Non inferiority was robustly achieved with a treatment difference of 0.002 g/kg/day, with upper bound of 95% 1-sided confidence interval (CI) for mean Test-Control difference in both ITT and PP populations of 0.005 g/kg/day (<0.017g/kg/day, the predetermined 15% non-inferiority margin). No treatment-emergent RBC alloantibodies or antibodies specific for S-303/GSH RBCC were detected. Adverse events occurred in 56/81 (69.1%) Test and 57/81 (71.3%) Control subjects, and were balanced between Test and Control periods. There were no deaths, grade 4 adverse events, or events deemed certain or likely related to S-303/GSH RBCC.
Summary and Conclusions: In transfusion-dependent patients with Thalassemia, S-303/GSH-RBCC are non-inferior to conventional RBCC based on Hb consumption and therefore transfusion iron burden. The safety profile was not different from conventional RBCC and no antibodies were detected to S-303/GSH-treated RBC. These results support the application of effective and safe pathogen inactivation in the clinical setting of Thalassemia major.

PF771

EVALUATION OF VASCULAR HEALTH OF E-BETA THALASSEMIA PATIENTS: EFFECT OF IRON OVERLOAD

D. De[1,*], U.K. Nath[2], P. Chakrabarti[3]

[1]Dept of Hematology, The Mission Hospital, Kolkata, [2]Aiims Rishikeh, Rishikeh, 3nrs Medical College, Kolkata, India

Background: Hb E-β thalassemia is the most common form of hemoglobinopathy in Southeast Asia and eastern India. Iron overload resulting from blood transfusion and increased intestinal iron absorption promotes the formation of reactive oxygen species (ROS), leading to oxidative stress, organ dysfunction, and tissue damage. Of these, cardiovascular complications are the leading cause of mortality. Impaired endothelial function is a biomarker of vascular

health in patients with cardiovascular risks. Therefore, assessment of endothelial function is a useful prognostic tool.
Aims: To determine the presence of vasomotor dysfunction and subclinical atherosclerosis in patients of E β thalassemia and its relationship with serum ferritin.
Methods: In the present study, 60 E- β thalassemia patients and 60 healthy, age, sex matched control subjects were taken. The mean hemoglobin and ferritin of thalassemic patients were 7.43gm/dl and 1032 mcg/dl respectively. The vascular health was compared by measuring flow-mediated vasodialation (FMD), arterial elastic parameters, and carotid intima-medial thickness (CIMT).
Results: There was lower FMD (7.49%) and higher CIMT (0.46mm) in thalassemic group than control (10.52% and 0.36mm respectively) (p value<0.05). Also arterial stiffness is elevated and arterial distensibility is lower in thalassemic patients than control. Among the thalassemic patients FMD or CIMT did not correlate with serum ferritin value.There was lower FMD (7.49%) and higher CIMT (0.46mm) in thalassemic group than control (10.52% and 0.36mm respectively) (p value<0.05). Also arterial stiffness is elevated and arterial distensibility is lower in thalassemic patients than control. Among the thalassemic patients FMD or CIMT did not correlate with serum ferritin value.
Summary and Conclusions: So, the E- β thalassemia patients had poor vascular health and are at a higher risk of developing atherosclerosis and cardio-vascular complication than normal population. The vascular dysfunction does not correlate with serum ferritin value, so regular monitoring with Doppler study is required for early diagnosis of subclinical atherosclerosis in this group of patients. However the effects of chelation therapy, Hydroxyurea, or other targeted therapies needs to be validated by further study.

PF772

CHRONIC HEPATITIS C VIRUS INFECTION IN THALASSEMIA MAJOR: A NEW CARDIOVASCULAR RISK FACTOR?

A. Meloni[1,*], L. Pistoia[1], R. Renni[2], S. Macchi[3], A. Quota[4], L. Tedesco[5], P. Giovangrossi[6], V. Carrai[7], S. Pulini[8], T. Grippo[9], M.P. Smacchia[10], G. Peritore[11], R. Righi[12], V. Positano[1], A. Pepe[1]

[1]Fondazione G. Monasterio CNR-Regione Toscana, Pisa, [2]Ospedale Civile "F. Ferrari", Casarano (LE), [3]Ospedale "Santa Maria delle Croci", Ravenna, [4]Ospedale "V. Emanuele III", Gela (CL), [5]Presidio Ospedaliero Locri - A.S.P di Reggio Calabria, Locri (RC), [6]Ospedale S. M. Goretti, Latina, [7]Azienda Ospedaliero - Universitaria Careggi, Firenze, [8]Ospedale Civile "Spirito Santo", Pescara, [9]Azienda Ospedaliera Regionale San Carlo di Potenza, Potenza, [10]Policlinico Umberto 1, Roma, [11]"ARNAS" Civico, Di Cristina Benfratelli, Palermo, [12]Ospedale del Delta, Lagosanto (FE), Italy

Background: Hepatitis C virus (HCV) infection is associated with a number of important extrahepatic manifestations.
Aims: The aim of this multicentric study was to assess the relationship between HCV infection and some traditional cardiovascular risk factors (CRF) and its predictive value and cardiovascular complications (CC) in thalassemia major (TM) patients.
Methods: We considered 827 TM patients (435 F) consecutively enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) network.
At the baseline assessment mean age of the patients, all free of CC, was 29.65±8.89 years and a categorization in 4 groups was performed: negative patients (group 0), patients who spontaneously cleared HCV (group 1), patients who eradicated the virus after treatment with antiviral therapy attaining a sustained virological response-SVR (group 2), and patients with chronic HCV infection (group 3).
Results: Patients in group 0 were significantly younger than patients in all the other three groups (P<0.0001) and had a significant lower frequency of diabetes than patients in group 3 (3.6% *vs* 11.0%; P=0.006).
Patients were followed-up for 79.53±28.71 months and 84 cardiovascular events (42 arrhythmias, 29 heart failure, and 13 vascular diseases) were registered. Table 1 shows the results of the univariate Cox regression analysis. Patients with chronic HCV infection had a significant higher risk of developing CC than negative patients.

Table 1.

	N(%) in Group	N(%) with CC	Univariate analysis HR(95%CI)	P
HCV group				
Group 0	290 (35.1)	19 (6.6)	Reference	0.258
Group 1	241 (29.1)	24 (10.0)	1.42 (0.78-2.59)	0.225
Group 2	43 (5.2)	5 (11.6)	1.84 (0.69-4.93)	0.006
Group 3	253 (30.6)	36 (14.2)	2.18 (1.25-3.79)	

Summary and Conclusions: HCV chronic infection is associated with a significant higher risk of CC in TM patients and, as a consequence, it should be analyzed as a systemic disease in which extrahepatic consequences increase the weight of its pathological burden.

PF773

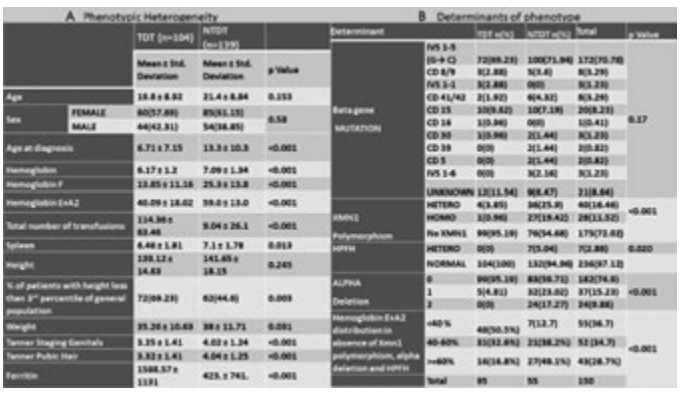
PHENOTYPIC HETEROGENEITY OF HAEMOGLOBIN E BETA THALASSEMIA AND ITS DETERMINANTS- IT'S NOT ONLY HAEMOGLOBIN F!

S. Kalantri[1,*], R. Ray[1], S. Choudhuri[1], S. Datta[1], M. Bhattacharyya[1]

[1]Clinical Hematology, Institute of Hematology and Transfusion Medicine, Medical College Kolkata., Kolkata, India

Background: HbE/ Beta Thalassemia is common problem(50 percent symptomatic thalassemias) in south-east asia and is phenotypically Highly diverse.Various factors influence the natural history of this disorder (example- XMN1 polymorphism, presence of HPFH, type of beta gene mutation, alpha deletion). Researchers have always focused on haemoglobin F and its induction in ameliorating severity of this disorder. Very few studies have focused on importance of haemoglobin E in modifying severity of this disease.
Aims: To document phenotypic heterogeneity and to investigate role of Haemoglobin E as determinant of severity.
Methods: This was comparative study. Diagnosed cases of HbE/ Beta Thalassemia, attending Thalassemia clinic and day care centre of IHTM between March 2015 and August 2016 were included in this study after obtaining informed consent. Patients were divided into two groups. 1) Requiring more than 8 transfusions/ year- **TDT (Transfusion dependent group).** 2) Requiring less than 8 transfusion/ year - NTDT **(Non- Transfusion dependent group).** Evaluation of patients comprised of a) Clinical Assessment B) Routine Laboratory evaluation & C) Molecular analaysis **(PCR) for** β-thal mutations, alpha deletion, HPFH gene persistence, XMN 1 polymorphism in both the arms.
Results: Two forty three HbE- Beta Thalassemia patients were included in the study. Various phenotypic parameters were compared in TDT and NTDT arm (Table 1A). Age at presentation was significantly lower in TDT arm (p value <0.001). TDT arm lagged in terms of linear and pubertal growth (Table 1A). Mean baseline haemoglobin, haemoglobin E percent, haemoglobin F percent were higher in NTDT arm (Table 1A).
It was observed that 7.91 percent (n=11) cases in NTDT arm had Haemoglobin E less than 40 percent and 50.36 percent (n=70) cases in this arm had greater than 60 percent of Haemoglobin E. As against this, only 16.35 percent (n=17) cases in TDT arm had haemoglobin E greater than 60 percent and 48.08 percent (n=50) cases had haemoglobin E less than 40 percent in this arm (p- value <0.001[u1]). In multivariate analysis higher haemoglobin E percentage was documented in NTDT arm; suggesting high haemoglobin E may be independently responsible for reducing severity (p value <0.001).
For beta mutation status no statistically, significant difference was found for frequency of various types of beta mutation (Table 1B). When subgroup analysis was done by excluding patients having xmn1 polymorphism, HPFH mutation and alpha deletion, higher prevelance of severe beta mutation IVS 1-5 (G C) mutation { 64(61.54 percent) *vs* 38(27.34) ; p value<0.001} was found in TDT arm.Xmn1 polymorphism, presence of HPFH mutation and alpha deletion were more prevalent in NTDT arm *vs* TDT arm (Table 1B).

Table 1.



Summary and Conclusions: We conclude that phenotype is not only modified by haemoglobin F percent but also by haemoglobin E percent. Further studies are warranted to detect the exact mechanism.

PF774

EFFICACY AND SAFETY OF RESVERATROL, AN ORAL HBF AUGMENTING AGENT, IN PATIENTS WITH BETA THALASSEMIA INTERMEDIA

M. Karimi[1,*], S. Haghpanah[1], T. Zarei[1], P. Eshgi[2], M. Bordbar[1], O. Zekavat[1], M. Hoormand[3], S. Parand[1]

[1]Hematology Research Center, Shiraz University of Medical Sciences,, Shiraz, [2]Pediatric Congenital Hematologic Disorders Research Center, Shahid Beheshti University of Medical Sciences,, [3]Department of Pharmacology, Iran University of Medical Sciences,, Tehran, Iran, Islamic Republic Of

Background: Recently, Resveratrol, (trans-3, 4, 5-trihydroxystilbene), showed induction of γ-globin mRNA synthesis in human erythroid precursors and reducing oxidative stress in red cells of thalassemia patients in many *in-vitro* studies.
Aims: We aimed to investigate the efficacy and safety of Resveratrol, for the first time, in non-transfusion dependent beta-thalassemia intermedia (B-TI) in Southern Iran.
Methods: In this double blind randomized clinical trial, 54 patients with B-TI were investigated from a total of 290 patients during 6 months between October 2016 and March 2017. Patients were randomly allocated into 3 groups by simple randomization method. Group 1 (hydroxyurea (HU) and placebo # 18 patients), group 2 (Resveratrol/Piperine and placebo #16 patients) and group 3(HU and Resveratrol/Piperine # 20 patients). HU was given with a dose of 8 15 mg per kg body weight per day and Resveratrol/Piperine, 2gram/10 mg per day orally. Primary end point was considered as change in hemoglobin levels and need for blood transfusion. Drug safety was considered as a secondary end point.
Results: Mean age of the patients was 28.2±5.6 (18-42) years. Response rate was not significantly different among the three groups (P>0.05). Higher percentages of adverse events were detected in groups 2 (31.3%) and 3 (25%) compared to group 1 (5.6%). However, the difference was not statistically significant (P>0.05). All reported adverse events were gastrointestinal symptoms.
Summary and Conclusions: Resveratrol showed a similar efficacy with HU in the small population of non-transfusion B-TI patients during a 6-month follow-up. Complications, mostly gastrointestinal, were observed more frequent in Resveratrol groups compared to the HU group. Although it was not statistically significant, more attention should be given to safety and efficacy of Resveratrol as an oral HbF augmenting agent.

PF775

HYPERURICEMIA, URINE URIC EXCRETION AND ITS COMPLICATIONS IN THALASSEMIA PATIENTS

J. Chaloemwong[1], A. Tantiworawit[1,*], T. Rattanathammethee[1], C. Chai-Adisaksopha[1], E. Rattarittamrong[1], L. Norasetthada[1], P. Charoenkwan[2], W. Louthrenoo[1]

[1]Internal Medicine, Division of Hematology, [2]Pediatric, Chiang Mai University, Maung, Thailand

Background: Thalassemia patients has high cell turnover rate due to its chronic hemolysis and ineffective erythropoiesis. Hyperuricemia is anticipated as complication.
Aims: To identify the prevalence of hyperuricemia, gout and nephrolithiasis, factor associated with high serum uric acid (SUA), urine uric acid excretion (UUA) and correlation to its complications in thalassemia patients.
Methods: This was a cross-sectional study in 15 years or older thalassemia patients at hematology clinic, Chiang Mai University. All patients had SUA and 24 hours UUA collection test. Renal ultrasound was performed in hematuria cases. Categorical variables were compared using the Chi-squared test or Fisher exact test. Continuous variables were compared by T-test or Mann-Whitney test.
Results: Of 112 thalassemia patients, there was female in 67.0%, beta thalassemia/Hb E in 64.3%, transfusion dependent in 76.8% and post splenectomy in 59.8%. The median age was 29 (range 16-58) years. Mean SUA was 6.7±2.0 mg/dl and hyperuricemia (SUA>6.8 mg/dl) was found in 47 cases (45.2%). The hyperuricemia was significantly identified in patients with intact spleen (51.1% *vs* 28.1%, p= 0.01), higher nRBC (222.7±155.8 *vs* 152.6±117.9 per 100 WBC, p=0.05), higher serum creatinine (0.6±0.2 *vs* 0.5±0.1 mg/dl., p=0.03) and lower fraction excretion of uric (FE uric) (7.9±2.6% *vs* 10.3±3.6%, p= 0.01) but reverse in deferasirox use (4.2% *vs* 19.3%, p= 0.019) compared with normal SUA. Seven (6.3%) had gouty arthritis and 9(8%) had microscopic hematuria which 1 case confirmed calyceal stone. The mean UUA excretion was 981.3± 335.0 mg/day which UUA hyperexcretion (>700 mg/24hours) was found in 83.3%. Renal hyperfiltration (eGFR>135 ml/min/1.73m^2) was documented in 46%, glomerular dysfunction (proteinuria and microalbuminuria) in 84% and renal tubular dysfunction (high urine neutrophil gelatinase-associated lipocalin (uNGAL)) in 7.7% from those who had UUA hyperexcretion. Mean uNGAL was 21.0± 8.2 mcg/L. The high uNGAL level that more than 37 mcg/L was significant correlated with higher serum creatinine (0.8± 0.4 *vs* 0.6± 0.1 mg/dl, p= 0.01), lower eGFR (89.9± 63.4 *vs* 127.9± 32.3 ml/min/1.73m^2, p= 0.04), higher FE phosphate (13.9± 4.9 *vs* 10.1± 3.3%, p= 0.04) and higher serum ferritin (2,659.1± 1,340.0 *vs* 1,434.2± 1,041.8 mcg/L, p= 0.03).
Summary and Conclusions: Hyperuricemia was found approximately forty percent but gouty arthritis was occurred only in six percent in thalassemia patients which may be explained by urinary hyperexcretion of uric acid which found in over eighty percent of thalassemia patients. The significant risk factor for hyperuricemia were intact spleen, higher serum creatinine and lower in fraction excretion of uric acid. The various renal abnormalities such as hyperfiltration, glomerular and tubular dysfunction were also detected in most cases.

PF776

THE VALUE OF MAGNESIUM LEVELS IN THE HAEMOSTASIS OF PATIENTS WITH B-THALASSAEMIA.

A. Kerimov[1], N. Aliyeva[1,*], T. Mamedova[1]

[1]Azerbaijan Scientific research hematology and transfusiology institute, Baku, Azerbaijan

Background: The study investigated the role of magnesium in hypercoagulation in different forms of β-thalassaemia patients.
Aims: The study was aimed to investigate the relationship between magnesium levels and hypercoagulation disorders of haemostasis in patients with different forms of β-thalassaemia.
Methods: The study involved 100 women aged 18-40 years with different clinical forms of β-thalassaemia (30 major, 25 intermedia and 45 minor). The diagnosis of β-thalassaemia was verified by haemoglobin fractions and characteristic clinical manifestation. Serum levels of magnesium and thrombinemia markers - D-dimer and soluble fibrin monomer complex (SFMC) have been identified. The patients had no clinical symptoms of hypercoagulation. The control group consisted of 30 healthy women of comparable age. Statistical analysis was done by variation statistics via standard software packages.
Results: β-thalassaemia major (β-TM) and intermedia (β-TI) patients exhibited decreased levels of magnesium, respectively, 0,73±0,04mmol/L (p<0,001) and 0,74±0,07mmol/L (p<0,001), ranging between 0,21 to 1,53 mmol/L. Magnesium levels of individuals with β-thalassemia trait (β-TT) were normal - 0,84±0,02mmol/L, ranging from 0,80 to 1,1mmol/L. Thrombinemia markers of β-TM and β-TI patients were increased: D-dimer - 496,0±5,1ng/mL (p <0,001) and 530,0±4,1ng/mL (p<0,001); SFMC - 5,83±0,2% (p<0,001) and 6,07±0,3% (p<0,001), respectively. 11 β-TM and 8 β-TI patients had hypercoagulation. Patients with latent hypercoagulation exhibited more expressed changes: magnesium levels of β-TM were 0,65±0,03mmol/L (p<0,001), D-dimer - 652,0±4,2ng/mL (p<0,001), SFMC - 6,81±0,18% (p<0,001); magnesium levels of β-TI were 0,63±0,05mmol/L (p<0,001), D-dimer - 690,0±7,1ng/mL (p <0,001), SFMC - 7,10±0,4% (p<0,001). No signs of latent hypercoagulation were detected in β-TT (no statistically significant difference with control values).
Summary and Conclusions: The plasma magnesium levels of β-TM patients was 1,6 times, and β-TI was 2,1 times less than control values. One-third of patients with β-TM and β-TI had latent hypercoagulation and lower magnesium levels. Based on our data, it can be concluded that, the low serum magnesium level of β-thalassemia patients is associated with an increased thrombosis risk.

PF777

ASYMPTOMATIC MALARIA IN CASES OF HBE BETA THALASSEMIA: A PILOT STUDY FROM EASTERN INDIA

S. Kalantri[1,*], R. Ray[1], S. Datta[1], S. Choudhuri[1], M. Bhattacharyya[1]

[1]Clinical Hematology, Institute of Hematology and Transfusion Medicine, Medical College Kolkata., Kolkata, India

Background: Haemoglobin E Beta thalassemia is the commonest symptomatic haemoglobinopathy in South East Asia & phenotype of this disease is heterogeneous. Many genetic and non genetic factors determine pheno-

type of this disease. Malaria is one such factor which was postulated to be important in affecting phenotypic severity of these patients. [O'Donnell *et al.* A 2009]The effect of thalassaemia on malaria parasite has been studied extensively but reverse is not true. Nancy F Olivieri opined in a review article that if studies from Asia and particularly from India also show higher frequency of asymptomatic malaria as shown in Sri Lanka it would be the basis for initiating anti malarial prophylaxis in these patients.[2]It has been observed that erythrocytes from subjects with homozygous and heterozygous haemoglobin E (HbE) infected with Plasmodium falciparum *in vitro* were phagocytosed to a greater extent by human monocytes than infected erythrocytes from normal subjects. [Bunyaratvej AH 1986] This gives protection against malaria but whether this excessive phagocytosis contributes to anemia and severity of thalassemia needs to be studied.

Aims: To document prevalence of asymptomatic malarial infection in patients with HbE Beta Thalassemia and compare the prevalence in transfusion dependent and non-transfusion dependent groups.

Methods: In a pilot study we evaluated 90 cases of HbE beta Thalassemia from Eastern India for detection of prevalence of asymptomatic malarial infection. Multiplex PCR was done using the primer set and protocols followed by Padley et al (2003). Both transfusion dependent (TDT) (those requiring more than 8 transfusions per year) and non-transfusion dependent (NTDT) (those requiring less than 8 transfusions per year)HbE – beta thalassaemia patients were included in the study. Result were compared in two arms.

Results: Prevalence of asymptomatic malarial infection in the total population was 35.5%. It was noted that out of 37 patients in the TDT arm 16 (43.24%) had asymptomatic malarial infection. In the NTDT arm of 53 patients 16 (30.19%) had asymptomatic malarial infection. When two arms were compared there was no statistically significant difference in prevalence of malarial infections (both Vivax and Falciparum). It is important to note that 43.24% and 30.19% cases in TDT and NTDT arm respectively had either falciparum or vivax infection. None of these patients gave past history of infection by malaria. Malarial infection was more prevalent in TDT arm though it was not statistically significant (Table 1).

Table 1.

Prevalence of Asymptomatic Malaria

		Group		Total N(%)= 90(100%)	p Value
		TDT N = 37	NTDT N=53		
Malaria PCR	FALCIPARUM	7(18.92%)	3(5.66%)	10(11.11%)	0.065
	VIVAX	9(24.32%)	13(24.53%)	22(24.44%)	0.982

TDT: transfusion dependent, NTDT: non- transfusion dependent

Summary and Conclusions: In a study from the same region where this study was also done, prevalence of asymptomatic falciparum malaria among general population using PCR was reported to be 8.4% (81/963) from Bandowan block of Purulia District. This was the block with highest prevalence of asymptomatic falciparum malaria of various others block included in that study. In the same block only 4 cases were detected to have asymptomatic vivax infection(0.41 percent). In the study from Sri Lanka authors reported that 28.6% of the children had DNA-based evidence of current infection with P. Vivax. Though this is a small pilot study it has clearly shown that prevalence of asymptomatic malaria is high in HbE- beta thalassaemia and its more in transfusion dependent group. More studies with larger sample size should be undertaken to understand the effect of malaria on phenotype of Hb E beta thalassemia particularly in South East Asian countries where both the conditions are common problem.

Thrombosis and vascular biology & translational research

PF778

ANTICOAGULATION IN THE OBESE - A PROSPECTIVE REGISTRY

G. McCaughan[1,2,*], H. Crowther[1,3], L. Pasalic[4,5,6,7], P. Rebeiro[8], J. Curnow[1,7]

[1]Department of Haematology, Westmead Hospital, [2]Sydney Medical School, [3]Department of Haematology, Blacktown Hospital, [4]Haematology, NSW Health Pathology, [5]Haematology, ICPMR, Westmead Hospital, Sydney, [6]Haematology, Westmead Hospital, Westmead, [7]Sydney Centres for Thrombosis and Haemostasis, [8]Haematology, Blacktown Hospital, Sydney, Australia

Background: The optimal anticoagulation strategy in the obese remains unclear. There is limited data to guide prescribing of low molecular weight heparin (LMWH) and direct oral anticoagulants (DOAC) in this population, particularly those with class III obesity (body mass index [BMI] ≥40kg/m^2).

Aims: To examine anticoagulant prescribing patterns in the obese in Australia and New Zealand; determine the most appropriate dosing strategy for LMWH; determine whether obese patients achieve appropriate peak and trough DOAC drug levels and evaluate clinical efficacy and safety of these agents in the obese.

Methods: We are prospectively registering all patients with a BMI ≥35kg/m^2 or weight >120kg receiving anticoagulants at 5 study sites in Australia and New Zealand (with 5 further sites in the process of submitting or awaiting ethics approval). Demographic data, weight, height, indication, anticoagulant choice, efficacy, adverse events and drug specific levels are being collected at registration and follow-up.

Results: As of abstract submission date we have recruited 21 patients at two sites with BMI ≥35kg/m^{2}with 3 sites commencing recruitmentthis month. Median BMI was 43kg/m^2 (range 39 - 80) and median weight was 132kg (range 108-229kg). 14/21 patients were female with a median age of 47 years (range 27-81). Indication for anticoagulation was deep vein thrombosis (11), pulmonary embolus (7), superior mesenteric vein thrombosis (1), extensive superficial vein thrombosis (1) and atrial fibrillation (1). Of those with venous thrombosis, 10/20 had a provoking factor and 7/20 had a prior history of DVT. Initial anticoagulation was: rivaroxaban (8), apixaban (6), dabigatran (1), enoxaparin (6) with transition to warfarin for 4/6. Of the four patients who received enoxaparin during the study period, 3 patients had a weight ≥200kg (BMI 70-80kg/m^2) and required 0.65-0.75mg/kg to achieve therapeutic Anti-Xa. The final patient had a weight of 108kg (BMI 43.2kg/m2) and required a dose of 0.92mg/kg. With regards to rivaroxaban 15mg twice daily, one peak level of 338ng/ml and three trough levels (<25, 37 and 55ng/ml) were available. There are no published 'on therapy' ranges for the rivaroxaban 15mg BD dose. For the 20mg dose, peak levels (n=4) ranged from 177-561 and median trough level (n=5) was 31ng/ml (range 28-122). 3/4 peak levels and 5/5 trough levels fell within the 5th to 95th percentile of published 'on therapy' ranges. For apixaban 5mg twice daily, median peak level (n=4) was 112ng/ml (range 97-156) and median trough level (n=5) was 40ng/ml (range 25-68). All peak levels and trough levels fell within the 5th to 95th percentile of published 'on therapy' ranges. 2 patients were changed from rivaroxaban to apixaban, due to menorrhagia in one patient and an undetectable trough rivaroxaban level in another. All patients that had progress imaging had radiological improvement and no patients had a symptomatic recurrent thrombotic event (median follow up 6 months).

Summary and Conclusions: There is limited data to guide prescribing of anticoagulation in the obese. Published 'on therapy' ranges have not been validated with clinical outcomes and there is no data to guide dose adjustment on the basis of drug levels. This makes interpreting the clinical significance of drug levels in the obese population difficult. The measured drug levels within this cohort appear appropriate relative to published 'on therapy ranges' and reassuringly no patients had a recurrent thrombotic event. Further data is required and we are now recruiting across multiple sites in Australia and New Zealand.

PF779

CHARACTERISTICS OF PLATELET COUNT AND SIZE AND DIAGNOSTIC ACCURACY OF MEAN PLATELET VOLUME IN PATIENTS WITH VENOUS THROMBOEMBOLISM. A SYSTEMATIC REVIEW AND META-ANALYSIS

S. Kovács[1], Z. Csiki[2], K. S. Zsóri[3], Z. Bereczky[4], H.A. Shemirani[5,6,*]

[1]Research Methodology and Statistics, Sectorial Economics and Methodology, [2]Medicine, Debrecen University, Debrecen, [3]Central Pharmacy, Erzsébet hospital, S.ujhely, [4]Laboratory Medicine, Debrecen University, [5]Laboratory Medicine, Dberecen

University, Debrecen, [6]Central Laboratory, Erzsébet hospital, S.ujhely, Hungary

Background: Venous thromboembolism (VTE) (pulmonary embolism and deep venous thrombosis) is the third most common cause of cardiovascular disease. Platelets play an active role in the thrombotic process and they contribute to the pathogenesis of atherothrombosis. There is strong evidence indicating that larger platelets are metabolically and enzymatically more active with higher thrombotic potential. Mean platelet volume (MPV) is potentially an important biomarker of platelet reactivity.
Aims: The purpose of this study was to evaluate the association between platelet (PLT) count and mean platelet volume (MPV) and venous thromboembolism (VTE). Thus, this study reviewed and perform a quantitative synthesis on data from the literature.
Methods: This meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. 18 studies were included in this paper. A random-effect meta-analysis was conducted for the assessment of heterogeneity using thrombosis place, type of analyzer, type of anticoagulant and incubation time of samples as covariates. A mixed-effect meta-regression was performed based on the subgroup for the whole samples using thrombosis place and method of measurement as moderators for MPV and PLT, respectively. The cumulative estimates and 95% confidence interval (95%CI) of specificity, sensitivity, area under the receiver operator characteristic curve (AUC) and diagnostic odds ratio (DOR) for MPV were calculated using a random effect model. The quality assessments were evaluated according to the quality assessment and diagnostic accuracy tool-2 (QUADAS-2). The primary outcome was the occurrence of VTE. Secondary outcomes included PLT and MPV.
Results: Patient with deep vein thrombosis is likely to have a higher value of MPV than control group (P <0.001). The presence of pulmonary embolism (PE) had no significant effect on the standardized mean difference of MPV between patients and controls. Patients are likely to have less PLT than the control group regarding all studies. However, subgroup analysis demonstrated that this effect was significant for patients with PE (P <0.05). The summary receiver operating characteristic (SROC) curve indicated that AUC was 0.745 (95% CI: 0.672-0.834). The DOR for MPV was 4.76 (95%CI: 2.3-9.85), with diagnostic accuracy of 0.66.
Summary and Conclusions: In our meta-analysis, significantly increased MPV and significantly decreased PLT were identified in patients with VTE. Additional studies are needed to investigate the clinical relevance of such relatively small but significant differences. Further studies are warranted in order to establish whether an increased MPV is either the cause or the consequence of venous occlusion in patients with VTE.

PF780

TISSUE PLASMINOGEN ACTIVATOR GENE POLYMORPHISM AND RECURRENCE OF VENOUS THROMBOEMBOLISM IN YOUNG PATIENTS FROM THE NORTH-WEST REGION OF RUSSIA

A. Chechulova[1,*], S. Kapustin[2], K. Svetlana[3], V. Shmeleva[3], V. Soldatenkov[3], L. Papayan[3], V. Soroka[1], P. Chechulov[4]

[1]Vascular surgery, Dzhanelidze Research Institute of Emergency Medicine, St. Petersburg, [2]laboratory of biochemistry, [3]Russian Research Institute of Hematology and Transfusiology, [4]Dzhanelidze Research Institute of Emergency Medicine, St. Petersburg, St. Petersburg, Russian Federation

Background: Tissue plasminogen activator (TPA) is an important component of fibrinolytic system. Inhibition of fibrinolysis can lead to venous thromboembolism (VTE). However, the role of TPA gene polymorphism in pathogenesis of VTE remains controversial.
Aims: To assess the role of TPA gene polymorphism in the risk of recurrence of VTE in young patients from the North-West region of Russia.
Methods: We analyzed 205 patients under 45 years old (101 men and 104 women, mean age – 37,4 years) with at least one confirmed episode of deep vein thrombosis (DVT) or/and pulmonary embolism (PE). The patients were genotyped for the 311 bp insertion/deletion (Ins/Del) TPA gene polymorphism by PCR. The differences in distribution of TPA genotypes were estimated by Fisher's exact method. Odds ratios (OR) together with their 95% confidence intervals (CI) and p-values were determined by using the SPSS software version 17,0 (SPSS Inc, Chicago, IL, USA).
Results: Recurrent VTE was diagnosed in 102 (49,7%) patients. The comparison group consisted of 103 patients without disease recurrence. The group with recurrent VTE included 71 (69,6%) patients with isolated DVT and 31 (30,4%) patients with the signs of PE. The frequency of the "normal" genotype TPA Del/Del was more than 2-fold decreased in patients with recurrent VTE when compared to those with single thrombotic episode (13,7% *vs* 29,1%, respectively; OR=0,4; 95% CI: 0,2-0,9; p=0,026). Moreover, in the group with non-recurrent isolated DVT, the frequency of the TPA Del/Del genotype was three times higher than in patients with recurrent DVT (28,5% *vs* 9,3%; OR=3,8; 95% CI: 1,6-9,1; p=0,005). At the same time, the TPA Del/Del genotype was almost equally represented in patients with or without recurrence of PE (20.8% *vs* 25.0%, p=0.68).
Summary and Conclusions: The presence of homozygous TPA Del/Del variant could have a significant protective effect against the risk of DVT recurrence and probably is not associated with the frequency of thromboembolic complications.

PF781

SEASONAL VARIATION IN PULMONARY EMBOLISM BUT NOT DEEP VEIN THROMBOSIS INCIDENCE: A KOREAN NATIONWIDE VENOUS THROMBOEMBOLISM EPIDEMIOLOGY STUDY

J. Hong[1,*], J. H. Lee[2], H. Park[3], J. Y. Lee[2], J.-O. Lee[2], W.-I. Choi[4], S. Ahn[5], Y.-H. Lim[6], S.-M. Bang[2], D. Oh[7]

[1]Department of Internal Medicine, Seoul National University Hospital, Seoul, [2]Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, [3]Department of Internal Medicine, Inha University Hospital, Incheon, [4]Department of Internal Medicine, Keimyung University Dongsan Medical Center, Daegu, [5]Medical Research Collaborating Center, Seoul National University Bundang Hospital, Seongnam, [6]Environmental Health Center, Seoul National University College of Medicine, Seoul, [7]Department of Internal Medicine, CHA University School of Medicine, Seongnam, Korea, Republic Of

Background: Seasonal variation is an environmental factor proposed to affect the incidence of venous thromboembolism (VTE). However, VTE seasonal variation is not well studied in Asian populations, which have different genetic determinants of VTE compared to Westerners.
Aims: The present study aimed at investigating seasonal variation of VTE occurrence and the effect of various demographic factors (*i.e.*, age, gender, and co-morbidities) on variation.
Methods: VTE seasonal variation was evaluated in 59,626 index cases (from January 2009-December 2013) in the Korean Health Insurance Review and Assessment Service (HIRA) database, a nationwide healthcare claims database basically covers near a whole Korean population (49,989,620 subjects registered in 2013). We quantified and compared VTE occurrence across four seasons, and additionally assessed monthly through a chronobiological analysis.
Results: VTE incidence varied both seasonally and monthly, with new cases peaking in the winter (January and February) and the lowest incidence in the summer (August and September). After adjusting for sex, age, type of VTE, and combined cancer diagnosis, winter remained a significant independent factor driving VTE incidence. Additionally, seasonal variation was prominent in patients aged 60 years or older and in patients with pulmonary embolism, but not in patients of aged less than 60 years and patients with deep vein thrombosis (Table 1).

Table 1.

Result of multivariate Poisson regression analysis of seasonality and VTE, DVT, and PE

	Venous thromboembolism			Deep vein thrombosis			Pulmonary embolism		
Subgroups	RR	95% CI	p	RR	95% CI	p	RR	95% CI	p
Male	1			1			1		
Female	1.20	1.18-1.22	<0.001	1.18	1.15-1.21	<0.001	1.22	1.20-1.25	<0.001
Age < 60	1			1			1		
Age ≥ 60	8.42	8.26-8.58	<0.001	6.07	5.90-6.26	<0.001	10.67	10.48-10.95	<0.001
Non-cancer	1			1			1		
Cancer	53.25	52.30-54.23	<0.001	58.20	56.53-59.91	<0.001	50.39	49.23-51.57	<0.001
Winter	1			1			1		
Spring	0.94	0.92-0.97	<0.001	0.97	0.93-1.00	0.0580	0.93	0.90-0.95	<0.001
Summer	0.91	0.89-0.93	<0.001	0.98	0.95-1.02	0.3743	0.86	0.84-0.89	<0.001
Autumn	0.94	0.92-0.96	<0.001	0.96	0.92-0.99	0.0143	0.93	0.90-0.96	<0.001

RR, relative risk; CI, confidence interval

Summary and Conclusions: Seasonal variation was a weak but independent contributor to VTE incidence in a Korean population diagnosed from 2009 to 2013, especially in those individuals suffering from a pulmonary embolism.

PF782

BLEEDING AND THROMBOTIC EVENTS RELATED TO PERIOPERATIVE CESSATION OF DIRECT ORAL ANTICOAGULANTS

S. Lam[1,*], D. Pepperell[1], S. P'ng[1]

[1]Haematology Department, Fiona Stanley Hospital, Perth, Australia

Background: Direct acting oral anticoagulants (DOACs) are increasingly prescribed long-term for atrial fibrillation or venous thromboembolism. These patients often require peri-procedural interruption of anticoagulation. The current management of DOACs perioperatively is based on expert consensus guidelines, with little published supporting evidence, particularly in the Australian population.
Aims: We aimed to assess the appropriateness of current perioperative DOAC cessation guidelines by measuring drug levels, physician compliance, and patient-centred outcomes.
Methods: Patients with planned invasive procedures were recruited to this single-centre observational study from haematology, cardiology and preoperative anaesthetic outpatient clinics. Perioperative anticoagulation management was at the discretion of the treating physicians, who were encouraged to utilise the guidelines by Tran (IMJ 2014).
Plasma DOAC levels were measured on the day of procedure and, when possible, within 24hrs after drug cessation. Peri-procedural bleeding events, and thrombotic events up to day 30 postoperatively were recorded.
Results: 36 patients with a mean age of 68 years (range 39-88) were recruited. The DOAC was apixaban in 21 patients (58%), rivaroxaban in 10 (28%) and dabigatran in 5 (14%). The indication for anticoagulation was atrial fibrillation in 92% of patients and venous thromboembolism in 8%. The timing of drug cessation preoperatively was consistent with guidelines in all patients undergoing high-risk procedures (n=13; mean 60hrs, range 40-107). However, 43% of patients undergoing a low-risk procedure had early cessation of anticoagulation (n=10; mean 59hrs, range 36-120).
Plasma DOAC levels were measured 24hrs following drug cessation in 12 patients, and were <50µg/mL in all but one (92%). On the day of procedure, plasma DOAC levels were <50µg/mL in 34 patients (94%). Both patients with plasma DOAC levels exceeding recommended limits underwent uncomplicated cardiac ablations; one patient had a rivaroxaban level of 72µg/mL (24hrs post-cessation, normal renal function), and the other did not cease rivaroxaban preoperatively and had a level of 192µg/mL 5hrs post-dose (at time of procedure).
There was one significant bleeding event in our cohort. The patient underwent cardiac ablation with a dabigatran level of 40 on the day of the procedure. This was complicated by cardiac tamponade with pericardial drain insertion required. There were no thrombotic complications.
Summary and Conclusions: In our observational study of perioperative DOAC cessation, plasma DOAC levels at the time of procedure were considered safe in almost all patients. The timing of DOAC cessation was consistent with guidelines in all patients undergoing high-risk procedures, however cessation was earlier than required in almost half of patients undergoing low-risk procedures. 2 patients had levels >50µg/mL at the time of procedure, but no immediate bleeding complications occurred in these patients. These findings are suggestive that the guidelines are overly cautious in DOAC cessation prior to high-risk procedures. Replication of these findings in a larger cohort may suggest that 50µg/mL is too conservative as a safety threshold. Perioperative interruption of anticoagulation did not result in any thrombotic events at 30-day follow-up.

PF783

TROMBOSIS IN LYMPHOMA, INCIDENCE, RISK FACTORS AND MANAGEMENT.

V. Pradillo[1,*], M. Bastos[1], E. Landete[1], D. Carbonell[1], J. Menarguez[1], P. Balsalobre[1], J.L. Diez-Martín[1], C. Pascual[1]

[1]Haematology, Hospital General Universitario Gregorio Marañón., Madrid, Spain

Background: Thrombosis is a frequent complication in patients with cancer. The incidence of thrombosis in patients diagnosed with lymphoma is estimated at 5%. These events increase the co-morbidity of these patients. New risk scores have been recently developed, and are being validated to predict thrombosis in this population.
Aims: The main objective of our study was to identify the risk factors associated with thrombosis disease in patients with lymphoma as well as to analyse the management in a single centre.
Methods: This is a retrospective unicentric study, all patients with diagnosis of non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL) were included consecutive since January 2014 to November 2017. A total of 250 patients were included. Basal characteristics of the patients are shown in Table 1A. Demographic, clinical and laboratory data and tumour characteristics were collected. Thrombotic events were assessed from diagnosis up to 6 months after. Likewise, thrombosis at the time of relapse was also assessed. In the incidence analysis, we defined as competitive event for thrombosis relapse, death and second neoplasm. We used R software for statistical analysis.

Results: For newly diagnosed patients we found 23 thrombotic events, with accumulative incidence of 8.7% at six months. At relapse, we found 5 events. The basis characteristics of the thrombosed patients are shown in Table 1B. Eight of the thrombosed patients (34%) presented the event at the moment of diagnosis, and in 15 patients (29%) thombotic event developed during chemotherapy. Among relapsed patients, we found 5 events in a total of 24 relapse patients (20%). In a univariate analysis, central venous catheter, ECOG and higher steroids dose were associated with thrombosis. In a multivariate, the variables independently associated with thrombosis risk were high aggressive lymphoma, previous thrombosis, platelet count at diagnosis, concomitant neoplasia and previous immobilization. Interestingly, the leucocytes or haemoglobin count were not associated with thrombosis; however we found increased risk with the highest platelet counts. Adenopathy location, bulky disease or extranodal involment were not associated with the event. No thrombosis was documented in patients under observation (16%) (p=0.034).Thirteen patients (5.2%) received antithrombotic prophylaxis, with LMWH. Thirty six patients were on antithrombotic treatment at diagnosis (14,4%): 10 anticoagulated and 26 anti-aggregated. None anticoagulated patients developed thrombosis, but six patients under anti-aggregant therapy did it. Rethrombosis was observed in two patients, both after treatment suspension (one with severe trombopenia in relapse and the other after complete anticoagulant treatment in complete remission). Regarding treatment, 27 of the thrombosed patients (75%) were treated with LMWH at full doses. Treatment adjustments were required in 10 patients due to thrombocytopenia (6), renal failure (1) or haemorrhagic events (3). Two patients under treatment had mild haemorrhagic complications (gluteal hematoma and fracture). No bleeding was observed in patients under prophylaxis.

Table 1.

A. CHARACTERISTICS OF PATIENTS

	n (250)	%
MALE/FEMALE	131/119	52/48
AGE (MEAN, RANGE)	62	(22-91)
HTA	91	36
DM	45	18
DL	68	27
BMI>30KG/M2	71	28
PREVIOUS TE	29	12
ARTERIAL/VENOUS	25/4	86/14
IMMOBILIZATION	61	24
OTHER NEOPLASIA	9	4
HISTOLOGY		
HODGKIN LYMPHOMA	41	16
AGRESSIVE LYMPHOMA	105	42
NON AGRESSIVE LYMPHOMA	97	39
T CELL LYMPHOMA	7	3
ECOG		
0-2	237	95
3-4	13	5
ANN ARBOR STAGE		
I-II	89	36
III-IV	161	64
B SINTOMS	94	38
BULKY DISEASE	50	20
EXTRANODAL LOCALIZATION	117	47
MEDIASTINUM MASS	20	8
CNS INVOLMENT	7	3
IDSI		
LOW	109	44
INTERMEDIATE	82	32
HIGH	59	24
BASELINE LABORTORY VALUES		
PLATELET COUNT X10^9/L (MEDIAN,RANGE)	262	(27-680)
HEMOGLOBINE G/L (MEDIAN,RANGE)	130	(61-185)
NEUTROPHILS X10^9/L (MEDIAN,RANGE)	4.9	(0.7-17)
CENTRAL VENOUS CATHETER	136	51
TREATMENT		
CHEMOTHERAPY (QT)	176	70
RADIOTHERAPY (RT)	12	5
QT+RT	18	7
SURGERY	3	1
NO TREATMENT	41	16
CHEMOTHERAPY		
R-CHOP	91	36
R-IDEGA	25	10
ABVD	35	14
EPOCH	4	2
HYPERCVAD	5	2
OTHERS	34	13

B. CHARACTERISTICS OF PATIENTS WITH THROMBOSIS

	n	%	Univariate analysis HR	p value	Multivariate analysis HR	p value
THROMBOSIS	23	100	HR	p value	HR	p value
MALE/FEMALE	12/11	52/48	0.968	NS		
AGE (MEDIAN, RANGE)	68	41-91				
HTA	10	43	1.39	NS		
DM	5	22	3.26	NS		
DL	10	43	2.036	NS		
BMI>30KG/M2	6	26	3.25	NS		
PREVIOUS TE	9	39	5.8	0.0000	5.22	0.0000
ARTERIAL/VENOUS	5/4	22/17				
IMMOBILIZATION	12	52	4.09	0.001	3.59	0.0048
OTHER NEOPLASIA	3	13	4.02	0.013	5.05	0.025
HISTOLOGY						
HODGKIN LYMPHOMA	4	17	1.25	NS		
AGGRESIVE LYMPHOMA	14	61	4.9	0.0011	5.023	0.0012
INDOLENT LYMPHOMA	5	22	0.39	NS		
ECOG			1.62	0.007	1.03	NS
0-2	19	82				
3-4	4	18				
ANN ARBOR STAGE						
I-II	9	40				
III-IV	14	60				
B SINTOMS	11	48				
BULKY DISEASE	6	26	1.46	NS		
EXTRANODAL LOCALIZATION	12	52	1.28	NS		
MEDIASTINUM INVOLMENT	3	13	1.76	NS		
CNS INVOLMENT	2	8				
BASELINE LABORTORY VALUES						
PLATELET COUNT MEDIA	241000		1.023	0	1.03	0.0000
HEMOGLOBINE LEVEL MEDIA	13		0.985	NS		
NEUTROPHILS MEDIA	5095		1	NS		
CENTRAL VENOUS CATHETER	17	74	2.87	0.026	1.85	NS
TREATMENT						
CHEMOTHERAPY (QT)	21	92				
QT+RT	2	8				

Summary and Conclusions: In our study, variables independently associated with thrombosis were: disease treatment, previous thrombosis, histology, immobilization, and a concomitant neoplasm. Also, a higher platelet count could confer an increased risk. Anti-aggregate therapy does not adequately protect patients. The adjustment of therapeutic doses has not been associated with rethrombosis. Relapse is a risk factor to consider.

PF784

PLATELET HYPERREACTIVITY AND POSTOPERATIVE VENOUS THROMBOEMBOLISM IN KNEE REPLACEMENT ARTHROPLASTY

J. Kim[1,*], Y. Kim[2]

[1]Laboratory Medicine, [2]Orthopaedics, Chungnam National University Hospital, Daejeon, Korea, Republic Of

Background: It is suggested that platelet hyperreactivity plays a role in the pathophysiology of arterial thrombi, such as myocardial infarction and

stroke, but the link between platelet hyperaggregability and venous thromboembolism is not well defined. The aggregometry using a submaximal concentration of epinephrine is proposed as a reliable method to detect hyperreactivity. Postoperative vein thromboembolism (VTE) in knee replacement arthroplasty (KRA) is a serious complication.
Aims: The aim of this study was to examine whether platelet hyperreactivity affects the development of VTE.
Methods: Seventy-six patients (71.2±5.4 years, 61 women and 15 men) without previous venous thrombotic history were enrolled. The unilateral KRA was performed by one surgeon. The complete blood count, coagulation assay and platelet function were checked in automated analyzers. We performed platelet aggregation using Chrono-log (Chrono-log Corporation, USA) in the presence of agonist epinephrine (0.4 mM) in duplicate and measured maximal aggregation (%) for 10 minutes.
Results: In aggregation response to 0.4 mM epinephrine, 53 (69.7%) exhibited relatively low platelet aggregation (<40%) and 11 (14.5%) demonstrated more than 60% aggregation, consistent with platelet hyperreactivity. The aggregation in 12 patients was distributed between 40% and 60%. Platelet hyperreactivity showed higher platelet count (P=0.040) and mean platelet volume (P=0.024) compared to group with low aggregation. The VTE was detected in 4 patients (5.3%) showing aggregation of 40% or more. The frequency of VTE was significantly lower in hyporeactivity phenotype group (P=0.008). The decrease of hemoglobin, RBC transfusion unit, CRP increase, and hospitalization time after surgery were similar among groups.
Summary and Conclusions: We conclude that platelet reactivity is a useful biological marker for the prediction of postoperative VTE. Postoperative transfusion requirement and the degree of inflammation are not related with platelet phenotype.

PF785

THROMBOEMBOLISM IN CHILDREN WITH CANCER; A RETROSPECTIVE MULTICENTER STUDY IN KOREA

H.S. Choi[1,*], H.J. Kim[1], C.R. Hong[2], H.J. Kang[2], J.W. Lee[2], H.Y. Shin[2], B.-K. Park[3], H.J. Park[3], Y.H. Lee[4], J.A. Lee[5], Y.-J. Lim[6], K.D. Park[2]

[1]Pediatrics, Seoul National University Bundang Hospital, Seongnam, [2]Pediatrics, Seoul National University Hospital, Seoul, [3]Pediatrics, National Cancer Center, Goyang, [4]Pediatrics, Hanyang University Hospital, [5]Pediatrics, Korea Cancer Center Hospital, Seoul, [6]Pediatrics, Chungnam National University Hospital, Daejeon, Korea, Republic Of

Background: Thromboembolism (TE) has been recognized as a significant cause of morbidity and mortality in adult cancer patients, however there is less knowledge about TE in pediatric cancer patients.
Aims: This study aims to determine the epidemiology of TE in Korean children with cancer.
Methods: During the period between January 2000 and July 2015, we retrospectively analyzed pediatric patients (0 to 18 years of age) newly diagnosed with cancer at six tertiary hospitals in Korea. Medical records were reviewed to evaluate the incidence, risk factors, treatment and outcome of TE.
Results: Among 3689 children with cancer, TE occurred in 33 patients (0.89%). Patients with acute lymphoblastic leukemia (n=12), brain tumors (n=5), lymphoma (n=4), and bone/soft tissue sarcomas (n=5) had a significantly higher incidence of developing TE. The male-female ratio was 17 to 16, and median age at diagnosis was 10 year 2 months (range 2 months-18 year 1 month). Symptoms including pain and swelling were present in 18 out of 33 patients (54.5%). Doppler ultrasonography was the most frequently used diagnostic method followed by computed tomography and magnetic resonance imaging. Locations of TE included three intracerebral, 23 upper venous, six lower venous and one combined upper and lower venous system. Additional risk factors for TE included central venous lines (CVL) in 11 patients, steroid and/or L-asparaginase in nine patients, and CVL and steroid and/or L-asparaginase in seven patients. Treatments included the use of unfractionated heparin, low-molecular-weight heparin, and warfarin according to the protocols of each institute. Of 33 patients, 23 exhibited complete resolution of TE, six partial resolution, two persistent disease and two recurrent disease. Aggravation of chronic subdural hemorrhage was occurred in one patient following warfarin treatment.
Summary and Conclusions: The incidence of TE is very low (0.89%) in Korean children with cancer but higher than general pediatric population and children hospitalized for diseases other than cancer. Further investigation of a larger pool of patients is warranted to determine the most effective strategies to prevent and treat TE in Korean children with cancer.

PF786

A RETROSPECTIVE ANALYSIS OF UTILISATION OF DIRECT ORAL ANTICOAGULANT LEVELS FROM A TERTIARY HAEMATOLOGY CENTRE

N. Denny[1,*], H. Belli[2], L. Keighley[2], M. Nash[1]

[1]Department of Haematology, [2]Department of Laboratory Haematology, Manchester University Foundation Trust, Manchester, United Kingdom

Background: Specialised laboratory tests for the measurement of direct oral anticoagulants (DOAC) are discussed in a number of international guidelines. Although DOAC may not require routine laboratory monitoring there are a number of situations in which measuring drug levels is useful. Our hospital is a tertiary centre for haematology and is the only hospital to process DOAC levels 24/7 in the area.
Aims: To assess the utilisation of these tests and to analyse the variation in DOAC levels in a real world setting.
Methods: We identified all DOAC levels requested at our institution over a 2 year period from 01/01/2016 to 31/12/2017 inclusive. Patient notes were reviewed and relevant blood results that were measured at the same time as DOAC levels were recorded. Levels were defined as peak if a DOAC had been taken within the last 12 hours. Dabigatran levels were measured using the Hemoclot® assay. Rivaroxaban and apixaban levels were measured using a chromogenic anti-Xa assay. Edoxaban levels were not available. Statistics were performed using SPSS and where appropriate the Mann Whitney U test was used.
Results: 220 levels were requested in 127 patients. 17 patients and 50 levels were excluded as they were from a different hospital and information was unavailable. 170 levels and 110 patients were included in the final analysis. The mean age was 67 years and DOAC use was as follows; apixaban 59 patients, rivaroxaban 38 and dabigatran 12. 1 patient had a DOAC level measured but was receiving LMWH. AF was the indication for DOAC use in 63 patients, VTE in 42 and in 5 use was off license. The reason for DOAC level requests is shown in Table 1. Bleeding and emergency surgery were the leading causes. A reversal agent was used in 27 patients; Praxbind in 6 and PCC in 21. Reversal agents were given prior to obtaining a result in all cases. The most common indication for reversal agent use was bleeding. 11 of the patients died during their admission and 7 received reversal agents. GI bleeding and polytrauma were the main reasons for death.
55% of DOAC levels were measured out of working hours. The mean turnaround time from receipt of sample to result was 108 minutes out of hours and 97 minutes in hours (no sig diff p=0.89). 84 apixaban levels were measured; 41 peak, 42 trough and 1 overdose. Mean peak apixaban level was 192ng/mL and mean trough level was 46ng/ml (sig diff p<0.00001). 61 rivaroxaban levels were measured; 19 peak and 42 trough. Mean peak rivaroxaban level was 290ng/ml and mean trough level was 43ng/ml (sig diff p<0.00001). 24 dabigatran levels were measured; 11 peak, 11 trough and 2 overdose. Mean peak dabigatran level was 86ng/ml (st dev 64ng/ml) and mean trough level was 55ng/ml (no sig diff p=0.178). There was minimal correlation between apixaban level and PT (r=0.11) or aPTT (r=0.39). There was moderate correlation between rivaroxaban level and PT (r=0.43) or aPTT (r=0.44). There was strong correlation between dabigatran level and both PT (r=0.98) and aPTT (r=0.94).

Table 1.

Reason for DOAC level request	Number of tests (n=170)
Bleeding	58
Pre-operative (emergency)	36
Monitoring efficacy	34
No clear reason	20
Change in renal function	13
Pre-operative (elective)	9

Summary and Conclusions: Our real world data show that DOAC levels are used clinically and provide helpful information about patients taking these drugs. Levels were predominantly requested in an emergency context and they are feasible in and out of hours with a turnaround time of under 2 hours. Interestingly, for direct Xa inhibitors, measured peak and trough levels correspond with expected levels in the BCSH guideline. There was minimal correlation between direct Xa inhibitor level and PT/aPTT highlighting the usefulness of measuring DOAC levels directly.

PF787

LONG- TERM MANAGEMENT OF RECURRENT VENOUS HROMBOEMBOLISM IN BLOOD CANCER PATIENTS AT HIGH BLEEDING RISK

M. Napolitano[1,*], S. Raso[2], S. mancuso[1], A. Passannanti[2], M. Santoro[2], L. LoCoco[3], S. Siragusa[1]

[1]Haematology Department, [2]Università di Palermo, [3]University Hospital "P.Giaccone", Palermo, Italy

Background: Recurrent venous thromboembolism (VTE), bleeding, and severe thrombocytopenia are frequently observed in haematological cancer patients. The optimal management of anticoagulation in such instances presents a major challenge to clinicians.
Aims: We here report efficacy and safety of anticoagulant treatment in patients with active haematological malignancies, a history of recurrent venous thromboembolism and stable thrombocytopenia
Methods: We here report results of a 12 months observational cohort study of patients with active haematological malignancies, a history of recurrent venous thromboembolism, at high bleeding risk for concomitant stable thrombocytopenia (Platelets count <50 x 10^9/L for at least three consecutive days), diagnosed between January 2014 and January 2017 at the Reference Regional Centre for Thrombosis and Haemostasis of University Hospital "P. Giaccone", Palermo. Haematological malignancies were either acute or chronic. Recurrent venous thromboembolism included two or more episodes of: deep vein thrombosis (DVT) of upper or lower extremities; atypical sites vein thrombosis; pulmonary embolism (PE) and catheter-related thrombosis. Long-term anticoagulant treatment was defined by continuous administration of anticoagulants.
Results: A total of 45 patients with a history of recurrent VTE were included (15 with chronic myeloproliferative neoplasms; 12 with acute leukemia; 8 with non-Hodgkin Lymphoma; 8 with multiple myeloma; 2 with Hodgkin Lymphoma). Twenty-five subjects had a history of recurrent DVT of the lower limbs, ten had sinus cerebral vein thrombosis and catheter-related thrombosis; five had PE and DVT of the lower limbs; 5 had mesenteric vein thrombosis and DVT of the lower limbs. All subjects received long-term anticoagulant treatment with Low Molecular Weight Heparin (LMWH) at reduced dose (50% of therapeutic dose),discontinued in case of severe thrombocytopenia (PLT<30 x10^9/L), active bleeding or new acute episodes of recurrent VTE. At 12 months follow-up, 27 (60%) subjects were still under treatment with a good compliance to daily LMWH. Among them, short-term discontinuation of LMWH was needed in 12 patients for severe thrombocytopenia secondary to chemotherapy and in 3 cases for active bleeding (2 recurrent epistaxis, 1 gum bleeding). At 12 months follow-up, seven subjects (15.5%) experienced recurrences while under treatment, in 4 (8.8%) patients a major bleeding occurred (2 gastro-intestinal bleeding, 1 genito-urinary bleeding, 1 intra-cerebral), six (13.3%) patients died of cancer.
Summary and Conclusions: Waiting for ad hoc studies, a patient oriented LMWH treatment tapering may be appropriate for the management of haematological cancer patients at high risk.

PF788

A MULTICENTER EXPERIENCE WITH IDARUCIZUMAB "IN REAL WORLD" AS REVERSAL ANTICOAGULATION OF DABIGATRÁN

M. Villalba Montaner[1,*], R. Martínez Fernández[2], O. Gavín Sebastián[2], I. Cuesta Gallardo[3], J.M. Real Campaña[4], L.I. Sancho Val[5], A.R. Millán Acero[6], I. Larrode Leciñena[4], E. Salvador Rupérez[7], S.F. Pinzón Mariño[7], G. Ferrer Garrido[7], B. Sánchez Quiñones[2], N. Fernández Mosteirín[7], J.M. Calvo Viñas[7]

[1]Hematology, Hospital Miguel Servet, Zaragoza, Spain, [2]Hematology, Hospital Clínico Universitario, Zaragoza, [3]Hematology, Hospital Obispo Polanco, Teruel, [4]Pharmacist, Hospital Miguel Servet, Zaragoza, Spain, Zaragoza, [5]Hematology, Hospital De Alcañiz, Teruel, [6]Hospital San Jorge, Huesca, [7]Hematology, Hospital Miguel Servet, Zaragoza, Spain

Background: Idarucizumab has been recently approved as reversal agent of anticoagulant effect of dabigatran in major bleeding and emergency surgeries however there is not patients reported outcomes of using idarucizumab "in real world.
Aims: Analyze the use of idarucizumab as reversal agent of dabigatran in current clinical practice in the hospitals of Aragón (Spain) from 2016 up to now.
Methods: From January-16 to date, 16 patients were registered in this multicenter prospective/retrospective study. We analyzed clinical variables and laboratory tests; efficacy, safety and thrombogenic capacity of the idarucizumab and related mortality with its use.
Results: The indications of dabigatran in patients were non-valvular auricular fibrillation in 15 patients and aortic valve prosthesis in 1 pt. The indications of idarucizumab were emergency surgeries in 11 pts, major bleeding in 4 pts and 1 pt with severe renal failure requiring dialysis received the drug off-label. Time from the last dose of dabigatran to the administration of idarucizumab was <12h in 15 patients.
Surgeries requiring reversal anticoagulation were 1 splenic rupture, 2 aortic dissections, 1 cardiac transplant, 1 cholecystectomy, 1 stroke fibrinolysis, 1 nephrectomy, 2 lower limb thrombectomies, 1 stroke thrombectomy, and 1 ankle osteosynthesis. One patient was at renal failure (clearance creatinine <30 ml/min). Activated partial thromboplastin time (aPTT) and prothrombin time (PT) were prolonged in 7 patients prior to idarucizumab infusion. Two hemorrhagic complications related with surgery: retrocardiac hematoma and cerebral hemorrhage, were registered.
The use of the drug in major bleeding was 2 pts with intracerebral hemorrhage, 1 with retroperitoneal hemorrhage and 1 lower gastrointestinal bleeding. aPTT and PT were prolonged, and one patient was at renal failure. Neither adverse effects nor thrombotic events were identified.
Four patients died in relation with intraventricular hemorrhage, heart transplant complications, septic shock and multi-organic failure respectively.
Summary and Conclusions: Our series includes 16 patients treated with idarucizumab to reverse the anticoagulation effect of dabigatran in hospitals of Aragon. In our experience "in real world" idarucizumab reversed immediately, completely, sustainably and safely the anticoagulation with dabigatran. The implementation of multidisciplinary protocols would optimize the cost/effectiveness ratio of idarucizumab in the approved clinical indications.

PF789

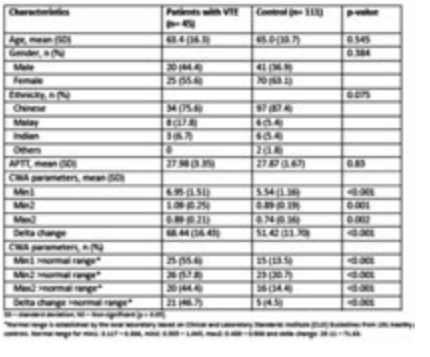
THE ASSOCIATION BETWEEN APTT-BASED CLOT WAVEFORM ANALYSIS AND ACUTE VENOUS THROMBOEMBOLISM

I.Q. Wu[1,*], C.W. Tan[2], H.H.M. Cheen[3], W.H. Wong[2], L.W.B. Chua[1], A.S. Hameed[4], C.K.L. Ng[1], M.H.Y. Chu[2], S.S. Lim[2], H.J. Ng[2]

[1]Singapore Health Services, [2]Haematology, [3]Pharmacy, Singapore General Hospital, [4]Ministry of Health Holdings, Singapore, Singapore

Background: Hypercoagulability contributes to the formation of venous thromboembolism (VTE). Increased levels of certain individual clotting factors and global hemostatic markers have been implicated as risk factors for VTE. Clot waveform analysis (CWA), as a global hemostatic assay, has been evaluated mainly in bleeding disorders, and its value in thrombotic conditions has not been explored.
Aims: We sought to evaluate the relationship between CWA parameters with acute VTE. We postulated that increased CWA parameters reflect a hypercoagulable state which will be present in patients with acute VTE compared with normal controls.
Methods: This project received institutional IRB approval prior to data collection. Inclusion criteria for this study included patients referred to the inpatient Hematology service at a tertiary hospital for objectively proven acute VTE with an activated partial thromboplastin time (aPTT) performed prior to initiation of anticoagulation. aPTT-based CWA was then performed on the CS2100i automated analyser using Dade Actin FSL reagent as per manufacturer's instructions. CWA parameters obtained were maximum velocity (min1), maximum acceleration (min2), maximum deceleration (max2) and delta change. Results from control patients were compared and retrospectively analysed. We also calculated the odds ratio for VTE based on comparison with the normal ranges for CWA parameters established by our laboratory.
Results: Whilst the baseline demographics and aPTT between VTE patients (n=45) and controls (n=111) were similar, all CWA parameters in the patients with VTE were significantly higher compared to the control groups (Table 1).

Table 1.



Similarly, there were significantly more cases within the VTE group exhibiting CWA values above the normal ranges of the respective CWA parameters than those within the control group (Table 1). In patients who had a CWA value above the normal range of that particular CWA parameter, the odds ratios for VTE were 8.0 (95% confidence interval [CI]: 3.6-17.8) for min1, 5.2 (95% CI: 2.8-11.1) for min2, 4.8 (95% CI: 2.2-10.5) for max2 and 18.6 (95% CI: 6.4-54.1) for delta change.
Summary and Conclusions: We have shown that increased aPTT-based CWA parameters were associated with hypercoagulability and were independent risk factors for acute VTE. aPTT-based CWA might be a potential diagnostic marker for acute VTE.

Transfusion medicine

PF790

A TERTIARY INSTITUTION BLOOD BANK EXPERIENCE AFTER THE IMPLEMENTATION OF A UNIFIED NATIONAL MASSIVE TRANSFUSION PROTOCOL (MTP) IN SINGAPORE.

C.-T. Lee[1,2,*], Y.L. Khng[3], S.T. Lim[3], E.S. Sumardi[3], K.P. Lim[3], S.Y. Lee[1,3], L.K. Tan[1,3]

[1]Department of Haematology-Oncology, NCIS, [2]Department of Medicine, Yong Loo Lin School of Medicine, National University Singapore, [3]Blood Transfusion Service, Department of Laboratory Medicine, National University Hospital, Singapore, Singapore

Background: Blood Services Group of the Singapore Health Sciences Authority had introduced a unified national massive transfusion protocol (MTP) to provide a standardized mechanism for prompt delivery of blood products to massively haemorrhaging patients in 6 acute general hospitals island-wide since 2012. Prior to this, within these hospitals, there was individual transfusion workflow in place for haemorrhaging patients.
Aims: To demonstrate the feasibility, usage, wastage, and patients' mortality after implementation of a nationwide MTP.
Methods: This is a retrospective analysis of blood bank data on blood product usage, returns and wastage of each MTP activation from 2012-2017. Clinical data of patients were analyzed to determine survival and Assessment of Blood Consumption (ABC) scores that was to used guide MTP activation in trauma. Our MTP consists of 3 packages. They are MTP1 and MTP3 which consist of 4 packed red cells (pRBC), 4 units of fresh frozen plasma (FFP) and 4 units of platelets pooled into 1 bag (pPLT). As for MTP2, additional 10 units of cryoprecipitate (CRYP) are added. After MTP1 is issued, the attending physicians will decide if MTP2 and MTP3 are required. Other components of the MTP included use of adjunctive anti-fibrinolytics to arrest bleeding.
Results: There were 401 MTPs activated, of which the median age was 52 years (13 - 93years). 185(46.1%) cases were trauma, 89(22.2%) gastrointestinal (GI) bleeding, 47(11.7%) peri-operative bleeding, 20(5.0%) ruptured aortic aneurysm (AAA), 11(2.7%) peripartum hemorrhage and 49 (12.3%) massive bleeding due to other causes. A total of 261(65.1%) MTP1, 87(21.7%) MTP2, 46(11.5%) MTP3 were activated, and 7(1.7%) cases required more than MTP3.This accounted for 5.8% of total blood products issued hospital-wide from 2012-2017. 5036(47.2%) of the blood units were issued to the EMD, 3394(31.8%) to ICUs, 1996(18.7%) to operating theatres, and 242(2.3%) to general wards with the median time of 9 minutes (interquartile range: 4-14 minutes). 1557(14.6%) units of blood products were returned unused including 568 units pRBC (5.3%), 121 units pPLT (1.1%), 693 units FFP (6.5%) and 175 units CRYP (1.6%). 72(17.9%) MTP activation had 528(4.9%) of the blood products issued returned due to patient's death. From 2014-2017, of the returned blood units, 9 out of 348 pRBC (2.6%), 67 out of 543 FFP (12.3%), 0 out of 89 pPLTs and 22 out of 155 CRYP (14.2%) were discarded after return, whereas the rest which met proper storage conditions were reissued. Overall calculated wastage was 1.8%. 300(74.8%) patients survived more than 1 day after MTP activation. The overall survivorship at 1 month was 139 (75.1%) for trauma cases, 48 (53.9%) GI bleeding, 31(66.0%) perioperative bleeding, 8(40.0%) ruptured AAA and 11 (100%) peri-partum bleeding. 37(9%) of all the patients who survived after activation expired within a month.
Summary and Conclusions: MTP packs are feasible and allow rapid and coordinated delivery of blood products to bleeding patients. Although return rates are moderately high at 14.6%, the overall wastage can be lower if proper storage conditions are maintained, thus, allowing product reuse. Our overall wastage was 1.8% which is lower than reported (*Dunbar et al.*) FFP and CRYP had the highest wastage, likely due to short expiry time after thawing. Development of better clinical predictive scoring to aid decision for MTP activation may also reduce return rates. MTP survivorship is acceptable and justifies the unified MTP as an adequate way for efficient blood product delivery in tertiary care hospitals.

PF791

DURATION OF RED BLOOD CELL STORAGE IS SIGNIFICANTLY ASSOCIATED WITH HEMOGLOBIN INCREASE AFTER BLOOD TRANSFUSION

J. Rydén[1,2,*], M. Clements[3], E. Hellström-Lindberg[1,2], P. Höglund[1,4], G. Edgren[3,5]

[1]Department of Medicine, Karolinska Institutet, Huddinge, Stockholm, [2]Department

of Hematology, Karolinska University Hospital, [3]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, [4]Department of Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, [5]Department of Cardiology, Stockholm South General Hospital, Stockholm, Sweden

Background: Red blood cells are commonly stored for up to 42 days. There has been conflicting evidence on the effect of red blood cell storage duration and clinical outcomes. Previous research has focused on determining the association between duration of storage time and risk for adverse outcomes including mortality, but most data has not supported such association. Surprisingly few studies have addressed the association between red blood cell storage and component efficacy.
Aims: The main aim of this study was to determine the effect of red blood cell storage duration with regard to hemoglobin increment in the transfusion recipient.
Methods: Transfusion data on a well-defined cohort of myelodysplastic syndromes were linked to hemoglobin measurements taken within 2 days before until 28 days following the transfusion. We applied a mixed effect regression model to study the impact of storage duration (categorized as <10, 10-19, 20-29 or ≥30 days) on the hemoglobin yield, per red blood cell unit.
Results: The study population consisted of 243 unique patients who were transfused at 4,419 transfusion occasions. Compared to patients who received units stored 1-9 days receipt of blood units stored 10-19, 20-29, or ≥30 days resulted in a hemoglobin increase that were 0.26 (95% confidence interval [CI], -0.11-0.63), 0.75 (95% CI, 0.26-1.23), and 1.12 (95% CI, 0.4-1.85) g/L lower, respectively, per red blood cell unit. Results were consistent in sensitivity analyzes.
Summary and Conclusions: We observed a gradual drop in the mean hemoglobin increase with prolonged red blood cell storage. Although the effect was statistically significant, the effect was modest and if this is clinically relevant in subgroups of patients must be investigated further.

PF792

RED CELL TRANSFUSION THRESHOLDS IN PATIENTS RECEIVING INDUCTION CHEMOTHERAPY FOR ACUTE MYELOID LEUKEMIA

A. Trottier[1,*], J. Tay[1]

[1]Department of Medicine, Division of Hematology, University of Calgary, Calgary, Canada

Background: Recent studies from a variety of clinical settings have provided evidence in favour of the use of a restrictive red cell transfusion strategy, however, data for patients with hematologic malignancies remains sparse. Patients undergoing chemotherapy for hematologic malignancies, such as induction chemotherapy for acute myeloid leukemia (AML), are high users of red cells and differ from other patient populations for numerous reasons such as, the presence of concurrent anemia and thrombocytopenia as well as a compromised immune system. The limited evidence available for the impact of red cell transfusion on clinical outcomes for these patients has been conflicting and unclear.
Aims: The aim of this study was to evaluate the impact of red cell transfusion threshold on clinically relevant outcomes for patients undergoing induction chemotherapy for AML.
Methods: This was a single-centre retrospective exploratory analysis of consecutive adult patients admitted between 2011 and 2016 for induction chemotherapy for newly diagnosed *de novo* or secondary AML. Baseline information including, age, gender, admission duration, induction regimen, and disease characteristics were collected. Transfusion data was compiled for each patient including the mean hemoglobin pre-transfusion (transfusion threshold) and the number of red cell units and platelet doses transfused during admission. Clinically relevant outcomes examined included remission status and 30-day mortality as well as WHO grade 3-4 bleeding events, myocardial infarction (MI), heart failure (HF), and venous thromboembolism (VTE) occurring during the admission period. Statistical analysis was performed using Stata 15.1 (StataCorp LLC). Comparison of the transfusion threshold for a given outcome was done using the two-sided two-sample unpaired t-test with a significance level of $p < 0.05$.
Results: 168 patients were included in the analysis. Baseline characteristics, transfusion data, and outcomes are shown in Table 1. The median transfusion threshold was 76 g/L (range: 64 – 86 g/L). The median number of red cell units and platelet doses transfused during admission were 10 and 7, respectively. The mean red cell transfusion threshold did not differ significantly between patients who suffered an MI, experienced HF, or had a VTE compared with those who did not (73 *vs* 75.3 g/L, p=0.1), (75.5 *vs* 75.2 g/L, p=0.8), (75.9 *vs* 75.2 g/L, p=0.3), respectively. There was no difference between patients who achieved a complete remission (CR) and those who did not (75.2 *vs* 76.1 g/L, p=0.1) or between patients who died within 30 days of starting induction and those alive (74 *vs* 75.3 g/L, p=0.2). There was no statistically significant difference in mean red cell transfusion threshold between patients who had a serious bleeding event and those who did not (74.3 *vs* 75.4 g/L, p=0.06). Younger patients (<56 years, the median cohort age) had a lower transfusion threshold than their older counterparts (≥56 years), however the magnitude of difference was small and unlikely to be clinically significant (74.8 *vs* 75.7 g/L, p=0.03).

Table 1.

Baseline characteristics, transfusion data, and outcomes

Characteristic (n=168)	Value
Median age, years (range)	56 (18 - 77)
Male sex (%)	95 (57%)
De novo AML (%)	150 (89%)
3+7 based induction	144 (86%)
Median admission duration, days (range)	32 (2 - 56)
Median Hgb threshold, g/L (range)	76 (64 – 86)
Median RBC units per pt (range)	10 (0 - 47)
Median Plt doses per pt (range)	7 (0 - 40)
CR* (%)	128 (76%)
30-day mortality (%)	13 (8%)
WHO grade 3-4 bleeding (%)	26 (15%)
Myocardial infarction (%)	3 (2%)
Heart Failure (%)	11 (7%)
VTE (%)	16 (10%)

*Complete remission (CR) = CR or Cri

Summary and Conclusions: In this single-centre retrospective analysis of patients with newly diagnosed AML admitted for induction chemotherapy, there were no significant differences in the mean red cell transfusion thresholds between patients who experienced severe bleeding, MI, HF, or VTE and those who did not. There was also no difference between patients who did and did not achieve CR or suffer 30-day mortality. Our results suggest that a restrictive red cell transfusion threshold is safe.

PF793

DEVELOPMENT OF HUMANIZED MOUSE MODELS TO EVALUATE AND PREVENT MOTHER-TO-CHILD HTLV-1 TRANSMISSIONS

T. Mizukami[1,*], K. Nojima[1], E. Sasaki[1], Y. Hiradate[1], K. Furuhata[1], Y. Sato[1], S. Matsuoka[1], K. Okuma[1], M. Satake[2], H. Akari[3], K. Uchimaru[4], I. Hamaguchi[1]

[1]Department of Safety Research on Blood and Biologicals, National Institute of Infectious Diseases, [2]Central Blood Institute, Japanese Red Cross, Tokyo, [3]Primate Research Institute, Institute for Virus Research, Kyoto University, Kyoto, [4]Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

Background: Human T-cell leukemia virus type 1 (HTLV-1) is a retrovirus that infects mature T cells and causes adult T-cell leukemia (ATL) and a chronic inflammatory central nervous system disease called HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). HTLV-1 transmissions in children mainly occur via the breast milk of HTLV-1(+) carrier mothers. Thus, avoiding long term breastfeeding (over 3 months) is recommended in the endemic areas of Japan. This political decision resulted in a drastic decrease in new HTLV-1 infections; however, new infections (5-8%) continued to occur even when HTLV-1(+) carrier mothers bottle fed infants. This suggested the possible involvement of alternative HTLV-1 transmission routes, such as placental or birth canal transmission. However, the transmission route most important to HTLV-1 vertical infection is still unknown.
Aims: In this study, we developed a humanized mouse model to evaluate HTLV-1 transmission mechanisms and to provide a platform that prevented mother-to-child transmissions.
Methods: NOD/SCID/gammac(null) (NOG) pregnant mice were intraperitoneally injected with human PBMCs before being transplanted into a mitomycin C-treated HTLV-1-infected cell line (MT-2).
Results: Although no HTLV-1-infected T cells were detected in the peripheral blood of newborn mice at day 30, HTLV-1-infected cells were exclusively observed in the liver tissues via FACS and PCR. To evaluate the importance of HTLV-1 infections via breastfeeding or before birth, we designed a study that switched HTLV-1-infected carrier mother (CM) mice with non-infected healthy mother (HM) mice. After switching, CM mice were maintained with HM mice pups (potentially HTLV-1(-)), and HM mice were maintained with CM mice pups (potentially HTLV-1(+)). This switching at birth resulted

in 30% of the potentially HTLV-1(-) pups becoming HTLV-1(+) since they were breastfed by an HTLV-1(+) mother. Similarly, 30% of the potentially HTLV-1(+) pups were HTLV-1(+) at 40 days after switching as they were breastfed by an HTLV-1(-) mother. These results suggested that both breast-feeding and canal or placental transmissions demonstrated equal risks with respect to HTLV-1 infections. Currently, we are trying to evaluate the efficacy of our anti-HTLV-1 immunoglobulin usage in this model.
Summary and Conclusions: In this study, we successfully developed the new humanized mouse models of mother-to-child HTLV-1 Transmissions. This model will provide platform to evaluate the mechanism of mother-to child HTLV-1 transmission and prevention.

PF794

A DESCRIPTIVE STUDY OF TRANSFUSION-RELATED ACUTE LUNG INJURY (TRALI) IN THE CANARY ISLANDS

C. Acosta Fleitas[1,*], L.M. Guerra Domínguez[1], L.M. González del Castillo[1], S. Fumero Guersi[2], R. López Hernández[3], A. Geijo Rodríguez[1], J. López Brito[1], P. Pecos Dorta[4], T. Marrero Quintana[5], E. Herrero[5], A. Oliva[6], T. Molero Labarta[1]

[1]Hematology, Hospital Universitario de Gran Canaria Doctor Negrín, [2]Hematology, Instituto Canario de Hemodonación y Hemoterapia, [3]Inmunology., Hospital Universitario de Gran Canaria Doctor Negrín, Las Palmas de Gran Canaria, [4]Hematology, Hospital Universitario de Canarias, Tenerife, [5]Hematology, Complejo Hospitalario Insular Materno Infantil, Las Palmas de Gran Canaria, [6]Hematology, Hospital Nuestra Señora de la Candelaria, Tenerife, Spain

Background: TRALI is a very relevant, but frequently infradiagnosed complication, that can result in potentially fatal consequences derived from blood-transfusion. It is associated with the presence of anti-leucocyte antibodies in the donor's plasma.
Aims: Hemovigilance programs have helped us establish more precisely its incidence, and whatsmore, they have opened the way to the development of preventive measures. The exclusive use of male plasma for transfusional purpose has shown its efficacy in reducing the incidence, even though it has not eliminated the diagnosed cases.
Methods: A review of the clinical history was performed. TRALI-cases taken place in Canarias between 2008-2016 were communicated. Luminex technology was used for the immunological study (Table 1).
Results: Demographic data were available for only 23 out of the 28 communicated cases. There were 12 male patients and 11 female. The age interval went from the neonatal period up to 97 years old, being 60 the median of the sample. The patients' diagnoses were heterogeneous, being mostly patients that had recently undergone surgery (10 cases), had an active infectious disease (7), or were suffering from a hematological condition (5). 89% cases presented a clinical and radiological presentation that was compatible with TRALI, with a severity of 2 and an imputability of 2-3 in 66% cases. 64% of the blood components that were related with TRALI were RBCs. When performed the determination of antiHLA and antiHNA antibodies in affected recipients, it was observed that only 6 of them had completed the study (31%), obtaining a positive result in at least one of them, being able to demonstrate a 100% "antigen-antibody" correlation with the involved donors. In Canarias, it has been possible to demonstrate the implication of 76 donors in various cases of TRALI, being able to complete the study in just 37 of them (49%), with a positive antiHLA and/or antiHNA result in 6 of them (25%) (Table 1).

Table 1.

Age	Gender	Diagnosis	Blood component	Severity / Imputability	Donor studied? (anti-HLA and/or HNA)	Recipient studied? (anti-HLA and/or HNA)
0	M	Respiratory distress	RBC	2/3	Negative	ND
69	F	MDS	RBC	2/3	Positive result	Anti-HLA I & II
ND	ND	Colitis	ND	2/3	Positive result	Anti-HLA II & Anti-HNA3
77	M	Tetraplegia	RBC	2/3	Anti-HNA3 & anti-HNA4	Negative
66	M	Renal cancer	RBC	2/2	Anti-HNA4 positive	Negative
80s	M	Cardiac surgery	RBC	2/3	Positive result	Anti-HLA II
30	M	Renal cancer	RBC	2/3	NO	NO
ND	ND	Cardiac surgery	RBC	2/3	Positive result	Anti-HNA3c
61	F	Cholecystectomy	RBC	2/3	NO	NO
67	M	Unstable angina	RBC	2/2	Anti-HLA II	No
ND	ND	Sepsis	ND	2/2	Negative	No
76	M	Burn oil	RBC	2/3	Negative	Negative
42	M	AML	RBC	2/3	Negative	Negative
75	F	Colon cancer	RBC	2/2	NO	No
ND	ND	Kidney donor	RBC	2/2	Negative	Negative
77	M	Aortic aneurysm	RBC	2/2	Anti-HNA3A & Anti-HNA3B	No
44	M	Abdominal sepsis	RBC	2/2	NO	Negative
60	M	Splenectomy	Platelets	2/2	Anti-HLA II & Anti-HNA3	Negative
ND	ND	Renal failure	ND	2/2	NO	No
47	F	Septic shock	FFP	2/3	Negative	Negative
57	F	Hip fracture	RBC	2/3	Positive result	Anti-HLA II
72	F	Peritonitis	RBC	2/2	Negative	Negative
56	F	MDS	Platelets	2/2	Negative	Negative
17	F	Tetraplegia	RBC	2/3	Negative	No
36	M	TTP	Plasma	2/3	Not considered	Negative
80	F	Colon cancer	RBC	2/3	Not considered	Negative
68	M	Brain aneurysm	RBC	2/3	Anti-HLA I & II	Negative
69	F	MDS	Platelets	2/3	Positive result	Anti-HLA I & II

Summary and Conclusions: TRALI's incidence in Canarias is 0.003%. The fact that most of the cases were communicated by HUDGCDN could be justified not only by the creation of a multidisciplinary formation program based on this matter, via the Hospital Transfusion Committee, but also due to the qualified nursing staff assigned to hemovigilance tasks. Independently of the severity of the case, 89% patients evolved favorably. 31% of the studied recipients presented antiHLA antibodies, with a 100% correlation with respect to donors-HLA-antigens. We could also establish, in case of donors carrying antiHLA/antiHNA antibodies, a 100% "antigen-antibody" correlation in relation to affected recipients. In spite of the study's limitation (as we could only analyze 49% implied donors), this study has led us identify the presence of anti-leucocyte antibodies in 6 frequent donors, finding that has led to their definite exclusion from the donation program as a preventive measure. Because of this, we have come to the conclusion that it is extremely important to establish training programs on this matter, as well as pursue an active formation in Hemovigilance and screen recipients at risk.

PF795

DYE-LIGANDS AFFINITY CHROMATOGRAPHY IN PROCESS OF FACTOR VIII PURIFICATION

N. Shurko[1,*], T. Danysh[1], V. Novak[1]

[1]State Institution «Institute of Blood Pathology and Transfusion Medicine NAMS of Ukraine», Lviv, Ukraine

Background: Industrial chromatographic fractionations have been used increasingly in the last few years for plasma fractionation. This has led to the emergence of a new generation of products derived of blood, especially factor coagulation. The commercial concentrates of factor VIII (FVIII) are used for the treatment of Hemophilia A. The main method of obtaining of plasma concentrates of FVIII remains the method of ion-exchange chromatography. The active dyes are important alternatives to natural ligands for specific affinity chromatography. We have developed a method for obtaining of FVIII by using dye-ligand affinity chromatography.
Aims: describe the main properties of FVIII concentrate obtained by a combination of different methods of purification.
Methods: Precipitation of proteins, ion-exchange on DEAE-Sepharose, dye-ligands affinity chromatography.
Results: To create a scheme for obtaining a highly purified preparation of the blood coagulation factor VIII from blood plasma, the research was conducted on combinations of the pre-fractionation method with the method of negative affinity sorption. It was determined that the pre-fractionation with barium citrate, aluminum hydroxide (III), and PEG-4000, provides removal of factors of prothrombin complex, fibrinogen, fibronectin, lipoproteins, denatured proteins, albumin etc. At this stage, we have reached hundredfold purification of the factor VIII preparation (for example, from 0.017±0.001 IU/mg protein to 1.88±0.11 IU/mg protein for sorbent Diasorb-Active purple 4GT). It has also been demonstrated that the use of ion-exchange chromatography on DEAE-Sepharose and affinity chromatography on selected sorbents (Diasorb-Active purple 4GT, Diasorb-Procion Gelb M4R, Diasorb-Procion Blue HB, Diasorb-Procion Blue MXR and Diasorb-Active bright blue KX) allows obtaining factor VIII from Cryoprecipitate with a degree of purification of 129 to 242 times and preservation of up to 73-76% of the initial procoagulant activity. The main losses from the initial activity of factor VIII and the von Willebrand's factor (up to 27%) occurred at the stage of ion-exchange chromatography.The phenomenon of negative affinity sorption provides almost 100.0% (96.34%) yield of the product. This is significant advantage of this method in the technology of obtaining a purified preparation of the coagulation factor VIII. It has been found that the combination of pre-fractionation, ion-exchange and affinity chromatography stages provides a purification rate of 239–700 times, depending on the type of selected sorbents. The best result was achieved when using Diasorb-Active purple 4GT.
Summary and Conclusions: The proposed method of purification of the blood coagulation FVIII, combining the stages of pre-fractionation, ion-exchange and affinity chromatography. A concentrate of FVIII with a specific activity of 50.58±1.68 IU/mg protein, vWF:R_{cof} – 3.07±0.18 IU/ml, FVIII/vWF:R_{cof} – 1.73±0.21 was obtained using the suggested method. The activity of thrombin and factor IX, as well as fibrinogen residues in the resulting preparation was not detected.

PF796

TEN YEAR EXPERIENCE OF RED BLOOD CELL TRANSFUSION ADEQUACY INDEX AS PART OF PATIENT BLOOD MANAGEMENT PROGRAM

G.D.C. Duarte[1,2,*], A. Celso Vigorito[1,2], F.J. P. Aranha[1,2], M. Veiga Rodrigues[1,2], J.F.C. Marques Jr[1,2]

[1]Hematology, Vera Cruz Hospital, [2]Hemotherapy, Cellular Hemotherapy Center in Medicine, Campinas, Brazil

Background: Transfusion is a vital and life-saving therapy. To achieve improved patient outcomes by avoiding unnecessary exposure to blood products through effective conservation and management of a patient's own blood, Patient Blood Management (PBM) program have been created. This program aims to promote medical education associated with blood transfusion alternatives in patient care and rationale blood utilization.
Aims: To optimize this task we have been studying the "Red Blood Cell Adequacy Index" since 2008, and this report demonstrate it's value in providing a useful tool for PBM program.
Methods: In our Institution, all transfusion requests are audited by staff physicians and classified as "proper" or "non-proper" based on internationally-adopted guidelines. "Non-proper" requests are then adapted to suit these protocols. From April to October 2008, requests for red blood cells (RBC), were identified that pre-transfusion hemoglobin higher than 9.0 g/dl, and absence of information on hemoglobin levels were associated with a higher likelihood of "non-proper" indication. These two characteristics were then used to calculate the so-called "RBC transfusion adequacy index" (RAI), which represents the proportion of RBC transfused in which any of these two characteristics was present. RAI was monitored prospectively is six different health care institutions of Campinas, Brazil, as a tool from the PBM program, providing information to promote interventions in the Institution practices.
Results: During the first part of the study, 1495 transfusions requests were analyzed, of which 12,9% were classified as "non-proper". In the second part of the study, from January 2008 until December 2017, 101.879 blood units were transfused and the RAI was monitored. The results are shown in Table 1. The data can show that the overall global RAI, assessing the whole six units, have a positive correlation regression value (R^2=0,49), but when each unit individually is evaluated, different realities are shown, with different patterns and tendencies (Table 1). For example, the Unit E shows an improvement in annual RAI and in the SD, unlike Unit A that has worse results in the last two years in both. The acceptable RAI can vary significantly from service to service according to Institutional focus and to local patterns of RBC transfusion. But the real value of the tool is to enable to follow the progress of each Institution during time and measure the efficacy of the PBM program. Another contribution of this tool, is that it makes possible to measure this index in any hierarchic level, *e.g.*, all the hospitals supplied by the blood bank, each hospital unit, a surgery specialty or even a specific physician. Therefore continuous monitoring of the RAI allows one to identify deviations from the established standards, identifying the focus of the deviation and providing information to more accurate interventions, ultimately improving transfusion safety.

Table 1.

Red Blood Cell Adequacy Index						
	Unit A Median ± SD (Monthly Range)	Unit B Median ± SD (Monthly Range)	Unit C Median ± SD (Monthly Range)	Unit D Median ± SD (Monthly Range)	Unit E Median ± SD (Monthly Range)	Unit F Median ± SD (Monthly Range)
2008	82 ± 6,9 (68,5-88,1)	85 ± 3,2 (81,5-93,4)	76,8 ± 6,2 (65,9-86,5)	*	85 ± 12,8 (66,67-100)	92 ± 12,3 (75-100)
2009	79,6 ± 5,9 (71,6-87,3)	91,7 ± 3,5 (85,4-96,7)	80,6 ± 6,2 (71,2-89,3)	*	84,9 ± 8,7 (70,6-100)	91,4 ± 8,6 (73,0-100)
2010	85,6 ± 5,6 (78,2-96,8)	89,6 ± 3,5 (82,6-93,8)	70,3 ± 7,4 (56,9-79,7)	*	90,7 ± 13,1 (60-100)	87,6± 6,9 (76-100)
2011	84,7 ± 5,2 (75,1-90,9)	90,5 ± 4,0 (84,3-94,7)	75,5 ± 5,0 (76,2-86,2)	*	85 ± 11 (53,8-100)	86,6 ± 3,9 (82,5-95,5)
2012	82,8 ± 5,7 (70,6-88,0)	87,0± 4,6 (77,9-95,2)	71,1 ± 6,9 (58,1-85,5)	*	69,6 ± 12,3 (56,2-93,1)	92,7 ± 4,6 (80,4-98,4)
2013	83,3 ± 7,1 (73,9-81,5)	92,0 ± 3,0 (85,0-92,1)	79,0 ± 5,0 (73,3-83,3)	*	91,6 ± 6,3 (78,1-100)	90,9 ± 6,2 (79,5-100)
2014	83,2 ± 5,5 (71,6-91,4)	92,2 ± 2,7 (89,7-98,1)	74,6 ± 1,3 (73,3-75,9)	*	89,4 ± 4,8 (79,7-96,3)	90,7 ± 9,4 (76,9-100)
2015	84,9 ± 7,8 (72,7-91,4)	88,3 ± 5,2 (78,9-97,0)	*	88,0 ± 5,2 (86,2-97,3)	87,9 ± 4,4 (81,1-94,3)	74,1 ± 12,9 (57,6-90)
2016	79,8 ± 6,3 (70,1-89,9)	92 ± 3,6 (83,9-95,1)	*	82,9 ± 10,2 (59,2-100)	89,9 ± 6,1 (75,7-95,4)	82,3 ± 4,5 (74-88,3)
2017	79,3 ± 7,6 (70,4-90,5)	85,7 ± 4,5 (76,2-94)	*	85,4 ± 8,2 (70,7-100)	85,1 ± 6,7 (71,7-92)	85,9 ± 5,7 (74-94,8)

Table 1. Red Blood Cell Adequacy Index per year, in each of the health units. *Indicates the period that the blood bank didn't supplied blood for that specific health unit. Median ± Standart deviation (Monthly RAI)

Summary and Conclusions: The optimal management of blood transfusion and patient related risks are an on course task. The RAI as part of a PBM programs can be a helpful tool in this process.

PF797

A 3-PART DIFF POCT CELL ANALYZER AT THE HEMATO-ONCOLOGY WARD FOR CLINICAL DECISION MAKING OF PLATELET TRANSFUSION AND CHEMOTHERAPY

N. Van den Eede[1,*], M.-B. Maes[1], R. Malfait[1], J. Van den Bossche[1], K. Deiteren[1]

[1]Laboratory of Hematology, UZA, Edegem, Belgium

Background: In our hospital a 3-part DIFF ABX Micros ES60 analyzer (Horiba, Kyoto, Japan) is used for screening of the patients' peripheral blood in follow up of their therapy at the hemato-oncology ward. The measurements are made by well-educated nurses. Clinical decisions such as platelet transfusion and start/stop of chemotherapy are based on the platelet count (PLT) and absolute neutrophil count (ABNEU) respectively of the POCT device.
Aims: In this study we compared the PLT and ABNEU POCT results with the 5-part DIFF ADVIA 2120 (Siemens, Munic, Germany) and Sysmex XN-1000 (Sysmex, Kobe, Japan) analyzers of the laboratory and we evaluated how many transfusions were not given or given erroneously based on a PLT threshold of 20x10E9/L or 10x10E9/L. At last we evaluated how many times the absolute neutrophil count was (in)correctly <0.5x10E9/L.
Methods: A 5 ml EDTA blood tube (BD) was analyzed for a complete blood count and differentiation on a POCT ABX Micros ES60 by a nurse at the hematology-oncology ward. Afterwards the tube was sent to the laboratory for reanalysis on the Advia 2120 and/or Sysmex XN-1000.
Results: A total of 20470 samples were analyzed using the POCT device during 2017. Investigation of unnecessary transfusion of platelet units showed that this occurred in ≤0,05% of samples (threshold ≤20 x10E9/L) or ≤0,02% (threshold ≤10 x10E9/L). In 0,6% or 0,4% of samples platelet transfusion was not given because PLT were >20 x10E9/L with POCT and ≤20 x10E9/L using Advia or Sysmex, respectively. When the threshold was lowered to 10 x10E9/L this number was reduced to 0,3%. A very small number of samples falsely indicated severe neutropenia (ABNEU <0.5x10E9/L) (0,03% and 0,06% in comparison with ADVIA and Sysmex, respectively). This observation may have resulted in unnecessary cessation of chemotherapy. In contrast, severe neutropenia was not detected using POCT in 0,6% and 0,3% of samples in comparison with ADVIA and Sysmex, respectively.
Summary and Conclusions: Only a minimum of cases were found discrepant for low platelet and absolute neutrophil count between POCT and high throughput hematoloy analysers Advia and Sysmex. We conclude that a small and easy-to-use 3-DIFF POCT device is a useful tool for obtaining fast and accurate results appropriate for clinical decision making of platelet transfusion or chemotherapy at the hemato-oncology ward.

PF798

EVALUATION OF OXYGEN TRANSPORT OF BLOOD IN PATIENTS WITH ANEMIA ON THE BACKGROUND OF THE RED BLOOD CELLS TRANSFUSIONS

N. Romanenko[1,*], A. Chechetkin[2], G. Grishina[3]

[1]Clinical Hematology, [2]Transfusiology, [3]Experemental Transfusiology, Russian Research Institute of Hematology and Transfusiology, Saint-Petersburg, Russian Federation

Background: Anemia frequently decreases survival rate and overall Quality-of- life in patients with hematological malignancies (HM). Red blood cell transfusions (RBCTs) can quickly correct severe symptoms of anemia, increase hemoglobin (Hb) and improve oxygen transport of blood.
Aims: To study efficacy of RBCTs in anemic patients with anemia before and after RBC transfusions.
Methods: The first group included patients (n=28) with hematological malignancies (acute myeloid leukemia (n=7), primary myelophibrosis (n=2), chronic myeloid leukemia (n=4), MDS (n=4), multiple myeloma in II and III st. (n=7), Non-Hodgkin's lymphoma in III-IV st. (n=4)), aged 65 years. The second group included patients with massive hemorrhage of traumatic genesis (n=12) at the age of 36 years. The indication for RBCTs was anemia with a hemoglobin level <8.0 g/dl, hematocrit <25%, target Hb was >8.0 g/dl in every groups. The efficiency of RBCTs was estimated as reduce clinical symptoms of anemia, increase concentration of hemoglobin and hematocrit, as well as to increase the saturation of venous blood (SvO_2).

Results: Mean baseline Hb concentration in our patients was <8.0 g/dl. During the hospitalization period were transfused 1-8 (Me=2 units) in both groups. There were revealed increasing of hemoglobin concentration and hematocrit: in the 1st group the Hb concentration increased from 6.41±0.27 g/dl to 9.02±0.17 g/dl, Ht – from 20.1±0.8% to 28.9±0.7%; in the second group the Hb concentration increased from 6.59±0.30 g/dl to 8.83±0.32 g/dl, Ht increased from 19.6±0.9% to 26.7±1.4%. Also observed an increasing of SvO_2: in the first group – from 42.0±3.3% to 57.6±4.1%; in the second group – from 51.3±1.9% to 69.0±1.3%. However, to compare of the frequency of achieving SvO_2 ≥60% in the 1st group was observed in 67.9% of patients after RBCTs, in the second group was in all (100%) patients. These data indicate that after RBCTs 32.1% of patients with hematological malignancies may persist tissue's hypoxia, despite the achievement of Hb >8.0 g/dl, Ht >25%.

Summary and Conclusions: In this study were shown the importance to access the saturation of venous blood in anemic patients, and in cases low SvO_2 (<60%) the patients should be continued RBCTs to increase of Hb concentrations to 9.5-10.0 g/dl, Ht to 33%.

SIMULTANEOUS SESSIONS II

Aggressive B-NHL: Immunotherapy

S799

AN UPDATED ANALYSIS OF JULIET, A GLOBAL PIVOTAL PHASE 2 TRIAL OF TISAGENLECLEUCEL IN ADULT PATIENTS WITH RELAPSED OR REFRACTORY (R/R) DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

P. Borchmann[1,*], C. S. Tam[2], U. Jäger[3], J. P. McGuirk[4], H. Holte[5], E.K. Waller[6], S.M. Jaglowski[7], M. R. Bishop[8], C. Andreadis[9], S.R. Foley[10], J. R. Westin[11], I. Fleury[12], P. J. Ho[13], S. Mielke[14], G. Salles[15], R. T. Maziarz[16], Ö. Anak[17], L. B. Pacaud[18], C. del Corral[18], R. Awasthi[19], S. Agoulnik[20], F. Tai[18], S.J. Schuster[21]

[1]Department of Haematology and Oncology, University Hospital of Cologne, Cologne, Germany, [2]Peter MacCallum Cancer Centre, St Vincent's Hospital and University of Melbourne, Melbourne, Australia, [3]Dept. of Medicine I, Div. of Hematology and Hemostaseology, Medical University of Vienna, Vienna, Austria, [4]Department of Blood and Bone Marrow Transplant, The University of Kansas Medical Center, Kansas City, United States, [5]Department of Oncology, Oslo University Hospital, Oslo, Norway, [6]Winship Cancer Institute of Emory University, Bone Marrow and Stem Cell Transplant Center, Atlanta, [7]The James Cancer Hospital and Solove Research Institute, The Ohio State University Comprehensive Cancer Center, Columbus, [8]University of Chicago, Hematopoietic Stem Cell Transplantation Program, Chicago, [9]Department of Hematology and Blood and Marrow Transplant, University of California San Francisco Helen Diller Family Comprehensive Cancer Center, San Francisco, United States, [10]Juravinski Hospital and Cancer Centre, McMaster University, Hamilton, Canada, [11]Department of Lymphoma and Myeloma, Division of Cancer Medicine, The University of Texas M.D. Anderson Cancer Center, Houston, United States, [12]Maisonneuve-Rosemont Hospital, University of Montreal, Montreal, Canada, [13]Royal Prince Alfred Hospital and Department of Medicine, The University of Sydney, Sydney, Australia, [14]Würzburg University Medical Center, Center for Allogeneic Stem Cell Transplantation, Würzburg, Germany, [15]Department of Hematology, Hospices Civils de Lyon, Université de Lyon, Lyon, France, [16]Oregon Health & Science Knight Cancer Institute, Center for Hematologic Malignancies, Portland, United States, [17]Novartis Pharma AG, Basel, Switzerland, [18]Novartis Pharmaceuticals Corporation, [19]Novartis Institutes for BioMedical Research, East Hanover, [20]Novartis Precision Medicine, Cambridge, [21]Lymphoma Program, Abramson Cancer Center University of Pennsylvania, Philadelphia, United States

Background: Tisagenlecleucel is a chimeric antigen receptor (CAR) T-cell therapy with a high rate of durable complete responses and a manageable safety profile in adult patients (pts) with R/R DLBCL.
Aims: To report long-term follow up of safety and efficacy in a single-arm, open-label, global phase 2 trial of tisagenlecleucel in pts ≥18 y with R/R DLBCL (JULIET; NCT02445248).
Methods: Eligible pts had r/r DLBCL, received ≥2 lines of therapy, including rituximab and anthracycline, and were ineligible for/failed autologous stem cell transplant (ASCT). Centrally manufactured CAR T cells were provided using cryopreserved apheresis and a global supply chain. The primary endpoint was best ORR (CR + PR) per independent review committee. Efficacy results are reported for pts in the main cohort with ≥3 mo follow-up or earlier discontinuation; safety is reported for all infused pts.
Results: At data cutoff (8 Dec 2017), 165 pts were enrolled and 111 infused (95 with US-manufactured [main cohort] and 16 with EU-manufactured [cohort A] tisagenlecleucel) with a single dose of tisagenlecleucel (median, 3.0×10^8 [range, $0.1\text{-}6.0\times10^8$] cells). 92% of pts received bridging therapy and 93% received lymphodepleting chemotherapy. Median time from infusion to data cutoff, 13.9 mo. Median age, 56 y (range, 22-76; 23% >65 y). At study entry, 76% of infused pts had stage III/IV disease, 17% had double/triple hits in MYC/BCL2/BCL6. 57% had germinal center B-cell; 41% had activated B-cell molecular subtypes. Median number of prior lines of antineoplastic therapy, 3 (range, 1-6; 95% had ≥2); 49% had prior ASCT. 93 pts had ≥3 mo and 81 pts had ≥12 mo of follow-up or discontinued earlier and were evaluable for efficacy. Best ORR was 52% (95% CI, 41-62) with 40% CR and 12% PR. Response rates were consistent across prognostic subgroups (including prior ASCT and double-hit lymphoma). Median duration of response was not reached; the 12-mo probability of being relapse-free was 65% (95% CI, 49-78; max follow-up, 17.3 mo). Median OS among all infused pts was 11.7 mo (95% CI, 6.6-NE); OS probability at mo 12, 49% (95% CI, 38.5-59). No pts proceeded to allo/ASCT while in remission. Tisagenlecleucel was detected in peripheral blood by qPCR for ≤693 days in responders. Grade (G)3 or 4 AEs of special interest ≤8 weeks of infusion included cytokine release syndrome (CRS; 14% G3 and 8% G4 by the Penn grading scale and managed by a protocol-specific algorithm), neurologic AEs (12%, managed with supportive care; no cases of cerebral edema were observed), cytopenias lasting >28 days (32%), infections (20%), and febrile neutropenia (14%). 15% received tocilizumab for CRS management. 3 pts died ≤30 days of infusion (all disease progression). No deaths were attributed to tisagenlecleucel or CRS. Since previous reports, no new deaths occurred due to causes other than disease progression. Analyses to better characterize and predict severe CRS, including relationships with baseline clinical/laboratory parameters, cytokines, dose, cellular kinetics and neurologic events will be presented.
Summary/Conclusion: Tisagenlecleucel produces high response rates in a cohort of highly pretreated adult pts with r/r DLBCL. With longer follow-up, these results confirm findings of an earlier primary analysis and show prolonged durable responses can be achieved. Centralized manufacturing was feasible in the first global study of CAR T cell therapy in DLBCL. CRS and other AEs could be effectively and reproducibly managed by appropriately trained investigators without treatment-related mortality.

S800

UPDATED SAFETY & LONG TERM CLINICAL OUTCOMES IN TRANSCEND NHL 001, PIVOTAL TRIAL OF LISOCABTAGENE MARALEUCEL (JCAR017) IN R/R AGGRESSIVE NHL

J. Abramson[1,*], L. Gordon[2], M. L. Palomba[3], M. Lunning[4], J. Arnason[5], A. Forero-Torres[6], M. Wang[7], D. Maloney[8], A. Sehgal[9], C. Andreadis[10], E. Purev[11], S. Solomon[12], N. Ghosh[13], T. Albertson[14], B. Xie[14], J. Garcia[14], T. Siddiqi[15]

[1]Massachusetts General Hospital, Boston, [2]Northwestern University Feinberg School of Medicine, Chicago, [3]Memorial Sloan-Kettering Cancer Center, New York, [4]University of Nebraska Medical Center, Omaha, [5]Beth Israel Deaconess Medical Center, Boston, [6]University of Alabama at Birmingham, Birmingham, [7]MD Anderson Cancer Center, Houston, [8]Fred Hutchinson Cancer Research Center, Seattle, [9]University of Pittsburgh Medical Center, Pittsburgh, [10]University of California San Francisco, San Francisco, [11]University of Colorado School of Medicine, Denver, [12]Blood and Marrow Transplant Group of Georgia, Atlanta, [13]Carolinas Healthcare Group Levine Cancer Institute, Charlotte, [14]Juno Therapeutics, Inc., Seattle, [15]City of Hope National Medical Center, Duarte, United States

Background: Lisocabtagene maraleucel (liso-cel; JCAR017) is a CD19-directed 4-1BB CAR T cell product administered in defined composition at a precise dose of CD8 and CD4 CAR T cells. A multicenter, seamless design pivotal phase 1 trial of liso-cel in R/R B-NHL (NCT02631044) has enrolled.
Aims: Long-term follow up of the initial cohort is presented herein.
Methods: Pts with R/R DLBCL, PMBCL, FL3B, or MCL and adequate organ function are eligible. Treatment includes lymphodepletion with fludarabine and cyclophosphamide, followed by liso-cel. Multiple dose levels (DLs) and administration schedules were evaluated; DL2 (10^8 CAR T cells) was chosen for the pivotal cohort. The FULL dataset includes all pts in the initial DLBCL cohort (DLBCL NOS, PMBCL, FL3B) treated with liso-cel at all DLs; CORE dataset includes only pts meeting inclusion criteria for the pivotal cohort, including DLBCL NOS (*de novo* or transformed from FL) and high grade lymphoma. Study objectives include safety, PK, and antitumor response.
Results: As of Oct 9, 2017, 91 pts were treated and evaluable for safety, 88 for efficacy. Pt characteristics were previously reported(Abramson ASH 2017). CRS was seen in 35% of pts; a single pt (1%) developed grade 3-4 CRS. Neurotoxicity (NT) developed in 19% of pts including 12% grade 3-4; all but one event resolved at time of data snapshot. Median onset of CRS and NT was 5 and 10 days respectively. Nineteen pts (21%) received tocilizumab and/or dexamethasone. Long term safety, including B cell aplasia, infections, and cytopenias, will be reported. Best ORR in FULL and CORE was 74% (65/88) and 80% (52/65), respectively; best CR was 52% (46/88) in FULL and 55% (36/65) in CORE. A higher rate of durable response at DL2 was observed in the CORE population, with 6-month ORR and CR of 50% and 50% (7/14), vs 40% (8/20) and 30% (6/20) at DL1. Long term efficacy, including 6-/12-month follow up from ~95/~55 pts, will be reported.
Summary/Conclusion: Liso-cel shows durable responses in pts with heavily pretreated R/R DLBCL and trends toward more durable responses at DL2. Observed acute toxicities have been manageable at all DLs tested and long-term safety from the initial cohort will be reported.

S801

AXICABTAGENE CILOLEUCEL (AXI-CEL) IN PATIENTS WITH REFRACTORY LARGE B CELL LYMPHOMA: OUTCOMES BY PRIOR LINES OF THERAPY IN ZUMA-1

F.L. Locke[1], A. Ghobadi[2], L.J. Lekakis[3], D. B. Miklos[4], C.A. Jacobson[5], E. Jacobsen[5], I. Braunschweig[6], O. O. Oluwole[7,*], T. Siddiqi[8], Y. Lin[9], P. Reagan[10], U. Farooq[11], A. Deol[12], A. Bot[13], J.M. Rossi[13], Y. Jiang[13], A. Xue[13], W.Y. Go[13], S.S. Neelapu[14]

[1]Moffitt Cancer Center, Tampa, [2]Washington University School of Medicine, St. Louis, [3]University of Miami Health System, Sylvester Comprehensive Care Center, Miami, [4]Stanford University, Stanford, [5]Dana-Farber Cancer Institute, Boston, [6]Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, [7]Vanderbilt-Ingram Cancer Center, Nashville, [8]City of Hope National Medical Center, Duarte, [9]Mayo Clinic, [10]University of Rochester School of Medicine, Rochester, [11]University of Iowa, Iowa City, [12]Karmanos Cancer Center, Wayne State University, Detroit, [13]Kite, a Gilead company, Santa Monica, [14]The University of Texas MD Anderson Cancer Center, Houston, United States

Background: In ZUMA-1 (NCT02348216), axi-cel, an anti-CD19 chimeric antigen receptor (CAR) T cell therapy, demonstrated significant benefit in patients with refractory large B cell lymphoma with an objective response rate (ORR) of 82% (complete response [CR] 58%; Neelapu & Locke et al. *N Engl J Med.* 2017). These results supported the recent approval of axi-cel by the US FDA for the treatment of adult patients with relapsed or refractory large B cell lymphoma after ≥ 2 prior lines of systemic therapy.
Aims: To assess outcomes of axi-cel by prior lines of therapy (LoT) in patients from Phases 1 and 2 of ZUMA-1.
Methods: All patients provided written informed consent. Patients with refractory large B cell lymphoma were leukapheresed and received 2×10^6 CAR T cells/kg after low-dose conditioning chemotherapy (Neelapu & Locke et al. *N Engl J Med.* 2017). Patients were evaluated by number of prior LoT: 2–3 vs ≥ 4. Autologous stem cell transplant (ASCT) was considered a prior LoT.
Results: As of 8/11/17, median follow-up was 15.4 months for the 108 patients treated with axi-cel. Sixty-two (57%) patients had 2–3 prior LoT and 43 (40%) had ≥ 4. Patients with 2–3 and ≥ 4 prior LoT had median ages of 60 and 55 y, 65% and 47% of patients had ECOG performance status 1, 18% and 42% had prior ASCT, and 76% and 93% had disease stage III/IV, respectively. ORRs were 94% and 67% for patients with 2–3 and ≥ 4 prior LoT, respectively, with CR rates of 65% and 53%; 44% and 42% of patients had ongoing responses as of the data cutoff (Table 1). Overall survival (OS) at 12 months was 65% and 51% for patients with 2–3 and ≥ 4 prior LoT, respectively. Grade ≥ 3 treatment-emergent adverse events (AEs) were reported for nearly all (100% and 93%) patients with 2–3 and ≥ 4 LoT, with similar rates of Grade ≥ 3 cytokine release syndrome (11% and 12%) and neurologic events (32% and 30%). There were 1 and 3 Grade 5 AEs unrelated to disease progression in the 2–3 and ≥ 4 LoT groups, respectively.

Table 1.

Response, % (95% CI)	LoT 2 – 3 (n = 62)	LoT ≥ 4 (n = 43)
ORR	94 (84 – 98)	67 (51 – 81)
CR rate	65 (51 – 76)	53 (38 – 69)
Ongoing ORR	44 (31 – 57)	42 (27 – 58)
6-month PFS[a]	49 (36 – 61)	51 (35 – 65)
12-month OS[a]	65 (51 – 75)	51 (35 – 65)

[a]Kaplan-Meier estimate.

Summary/Conclusion: Axi-cel demonstrated long-term clinical benefit for patients with refractory large B cell lymphoma, regardless of the number of prior LoT.

S802

ADDING POLATUZUMAB VEDOTIN (POLA) TO BENDAMUSTINE AND RITUXIMAB (BR) TREATMENT IMPROVES SURVIVAL IN PATIENTS WITH RELAPSED/REFRACTORY DLBCL: RESULTS OF A PHASE 2 CLINICAL TRIAL

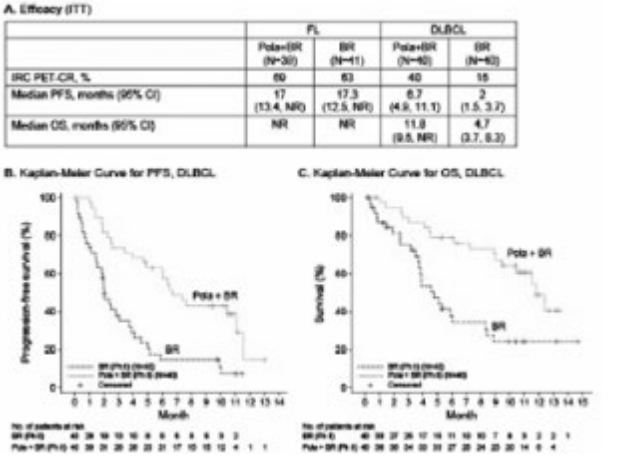
This abstract is embargoed until Saturday, June 16, 08:30 local time.

L.H. Sehn[1,*], M. Kamdar[2], A. F. Herrera[3], A. McMillan[4], C. Flowers[5], W.S. Kim[6], T.M. Kim[7], M. Özcan[8], M. Trněný[9], J. Demeter[10], M. Hertzberg[11], G. Salles[12], A. Davies[13], J. Hirata[14], J. Cheng[15], G. Ku[14], M.J. Matasar[16]

[1]BC Cancer Agency, Vancouver, Canada, [2]University of Colorado, Denver, [3]City of Hope, Duarte, United States, [4]Nottingham University Hospitals, Nottingham, United Kingdom, [5]Winship Cancer Institute of Emory University, Atlanta, United States, [6]Samsung Medical Center, [7]Seoul National University Hospital, Seoul, Korea, Republic Of, [8]Ankara University, Ankara, Turkey, [9]Charles University, Prague, Czech Republic, [10]Semmelweis University, Budapest, Hungary, [11]Prince of Wales Hospital, Sydney, Australia, [12]Hospices Civils de Lyon, Université Lyon-1, Lyon, France, [13]Cancer Research UK Centre, Cancer Sciences Division, University of Southampton, Southampton, United Kingdom, [14]Genentech, Inc., South San Francisco, United States, [15]Hoffmann-La Roche, Mississauga, Canada, [16]Memorial Sloan Kettering Cancer Center, New York, United States

Background: Pola is an antibody-drug conjugate targeting CD79b+ cells in B-NHL. Early results led to FDA breakthrough therapy status and EMA PRIME designation. We now report combined results for safety and efficacy from the randomized r/r FL and DLBCL cohorts of a phase 1b/2 study (ClinicalTrials.gov NCT02257567).
Aims: The primary objective is complete response rate by PET scan (PET-CR) 6-8 weeks after end of treatment by independent review committee (IRC) using modified Lugano criteria (PET-CR required PET score 1-3 and negative bone marrow if positive at screening).
Methods: After informed consent was obtained, 80 FL and 80 DLBCL transplant-ineligible patients (pts) were randomized 1:1 to pola 1.8 mg/kg + BR (B: 90mg/m^{2}x 2 days; R: 375mg/m^2) or BR for 6 cycles (q28 days FL, q21 days DLBCL). Pts were stratified by duration of response to last treatment ≤12 mo or >12 mo. FL pts were also stratified by high vs low disease burden.
Results: For FL pts (pola+BR *vs* BR), median age was 65 *vs* 63 years, both arms had median 2 prior therapies, 41% *vs* 42% were refractory to last therapy, and 64% *vs* 37% had FLIPI 3–5. As of 24 Oct 17, median follow up was 15 months. DLBCL characteristics and follow up were previously described (Sehn, ASH 2017). Safety: The most common grade 3–5 adverse events (AEs) that were higher in pola+BR *vs* BR in both FL and DLBCL were cytopenias, febrile neutropenia, and infections. Serious AEs higher in pola+BR *vs* BR were febrile neutropenia (FL, DLBCL) and infection (FL). Grade 5 AE rates were similar between treatment arms per histology: 5% (FL) and 18% (DLBCL). Efficacy: PET-CR by IRC and progression-free survival (PFS) by investigator (INV) were similar between FL arms (Figure 1 A). In DLBCL, pola+BR showed significantly higher IRC-PET-CR rates (p=0.012) and longer median (m) INV-PFS (p<0.0001) and overall survival (mOS; p=0.0008) (Figure 1 A,B,C). Also in DLBCL, longer PFS and OS were seen for pola+BR in 2nd-line (2L), 3rd-line plus (3L+), relapsed, and refractory pts. mPFS (pola+BR *vs* BR in months): 2L (11.1 *vs* 3.7), 3L+ (6.0 *vs* 2.0), relapsed (11.1 *vs* 5.1), refractory (6.0 *vs* 1.9). mOS: 2L (not reached [NR] *vs* 5.9), 3L+ (11.5 *vs* 3.8), relapsed (NR, NR), refractory (11.5 *vs* 3.8).

Figure 1.



Summary/Conclusion: The toxicity of pola+BR was manageable and as expected. In FL, pola+BR showed similar PET-CR rate compared to BR. Time-to-event endpoints are immature at this time due to low event rate. In contrast, in DLBCL, pola+BR led to significantly higher PET-CR rate and notably longer PFS and OS compared to BR regardless of prior treatment status.

S803

ATEZOLIZUMAB PLUS R-CHOP SHOWS ENCOURAGING ACTIVITY AND ACCEPTABLE TOXICITY IN PREVIOUSLY UNTREATED PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL): AN INTERIM ANALYSIS OF A PHASE I/II STUDY

A. Younes[1,*], J.M. Burke[2], C. Diefenbach[3], S. Ferrari[4], U. Hahn[5], E. Hawkes[6], C. Khan[7], I. S. Lossos[8], G. Musuraka[9], M. Tani[10], C. Ujjani[11], U. Vitolo[12], S. Yuen[13], S. Chitra[14], K. Krishnan[14], M.Z. Oestergaard[15], G. Sellam[15], R. Morariu-Zamfir[15], J. Sharman[2,16]

[1]Memorial Sloan Kettering Cancer Center, New York City, New York, [2]US Oncology Research, The Woodlands, Texas, [3]Perlmutter Cancer Center at NYU Langone Health, New York City, New York, United States, [4]Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy, [5]The Queen Elizabeth Hospital, Adelaide, South Australia, [6]Olivia Newton-John Cancer Centre, Austin Hospital, Melbourne, Victoria, Australia, [7]Allegheny Health Network Cancer Institute, Pittsburgh, Pennsylvania, [8]Sylvester Comprehensive Cancer Center, University of Miami, Miami, Florida, United States, [9]Istituto Scientifico Romagnolo Per Lo Studio e La Cura Dei Tumori, Meldola, [10]Ospedale Santa Maria delle Croci, Ravenna, Italy, [11]Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, District of Columbia, United States, [12]Azienda Ospedaliera Universitaria Città della Salute e della Scienza di Torino, Turin, Italy, [13]Calvary Mater Newcastle Hospital, Newcastle, New South Wales, [14]Genentech Inc, South San Francisco, California, United States, [15]F. Hoffmann-La Roche Ltd, Basel, Switzerland, [16]Willamette Valley Cancer Institute, Eugene, Oregon, United States

Background: Rituximab (R) plus CHOP (R-CHOP) is standard of care for pts with previously untreated DLBCL. Although most pts have long-term responses, up to 40% relapse or fail to achieve a remission. Atezolizumab (atezo) is a fully humanised anti-programmed death-ligand 1 (PD-L1) antibody with a complementary mechanism of action to R. An ongoing phase I/II study (NCT02596971) is evaluating the safety and efficacy of atezo in combination with R-CHOP (R-CHOP-atezo) in DLBCL pts. We report interim data.

Aims: To assess the safety and preliminary efficacy of R-CHOP-atezo in previously untreated pts with DLBCL.

Methods: Pts (aged ≥18 years, ECOG PS 0–2) with advanced DLBCL (Ann Arbor stage III/IV, International Prognostic Index [IPI] score ≥2 or stage II with bulky disease [at least one lesion ≥7cm]) received 8 cycles (each 21 days) of induction treatment with R-CHOP-atezo (R 375 mg/m^2 IV on Day 1 [Cycles 1–8], atezo 1200 mg IV on Day 1 [Cycles 2–8], CHOP [6 or 8 cycles as determined by the investigator (INV)]). Pts who achieved a CR at end of induction (EOI) received consolidation treatment with atezo 1200 mg IV on Day 1 of Cycles 9–25, every 21 days for 12 months. The primary endpoints were safety, and efficacy as determined by CR rate at EOI by independent review committee (IRC) using modified Lugano 2014 criteria. Secondary endpoints included CR rate at EOI assessed by INV using modified Lugano 2014, and by IRC and INV using Cheson 2007. An interim analysis was pre-planned after 15 consecutive pts had completed the EOI response assessment. The data cut-off was 20 November 2017.

Results: In total, 42 pts were enrolled and received treatment (safety population). At the time of data cut-off, 20 pts were receiving ongoing induction treatment, 5 had discontinued (3 AEs, 1 protocol violation, 1 withdrawal) and 17 had completed induction, of whom 14 had entered consolidation. Median (range) observation time was 5.3 (0.7–7.2) months for induction and only 1.6 (0.7–5.1) months for consolidation. Therefore, this report focuses on preliminary data from the safety population during induction only. Pt demographics and disease characteristics are shown in Table 1. Among 15 pts evaluable for response (efficacy population), 13 (87%) achieved a CR, and 2 (13%) had PD according to IRC and INV using modified Lugano 2014. Response assessment using Cheson 2007 showed 11 (73%) CRs, 2 (13%) PRs and 2 (13%) pts with PD, consistent between INV and IRC review. Preliminary safety data from induction therapy showed that all pts had ≥1 AE and 27/42 pts (64%) had a grade (gr) 3–4 AE. Regardless of causality, the most common gr 3–4 AEs were neutropenia (16 pts [38%], 6 received G-CSF prophylaxis) and febrile neutropenia (4 pts [9.5%], 3 received G-CSF prophylaxis). No deaths were reported. Serious AEs were reported in 13 pts (31%). AEs led to dose reduction in 8 pts (19%) (gr 4 neutropenia, gr 3 pancytopenia, gr 1–2 peripheral neuropathy) and withdrawal of any component in 3 pts (7%) (gr 3 neutropenia, gr 3 transaminase and asymptomatic lipase increases, gr 2 hyperthyroidism). Three pts had atezo-related AEs (gr 3 lipase and transaminase increases).

Table 1.

Pt demographics and disease characteristics for the safety population (n=42) and the efficacy population (n=15)

Characteristic, n (%)	Safety population (n=42)	Efficacy population (n=15)
Median age (range) at baseline, years	65 (22–84)	65 (31–78)
Age ≥65 years	22 (52)	9 (60)
Male	26 (62)	10 (67)
Ann Arbor stage III/ IV at diagnosis	39 (93)	15 (100)
IPI risk group at baseline		
Low (2)	13 (31)	3 (20)
High (≥3–5)	29 (69)	12 (80)
ECOG PS 0–1 at baseline	37 (88)	14 (93)
Bone marrow involvement at baseline	6 (14)	3 (20)
Extranodal involvement at baseline	30 (73)	11 (73)
LDH elevated at baseline	28 (67)	9 (60)
Bulky disease (≥7 cm) at baseline	26 (62)	7 (47)

Summary/Conclusion: Interim data demonstrate that first-line R-CHOP-atezo shows encouraging efficacy and acceptable toxicity in untreated pts with DLBCL. Updated results, including minimal residual disease and cell of origin data, will be presented.

Combination treatment with targeted agents in CLL

S804

HIGH RATE OF COMPLETE RESPONSE BUT MINIMAL RESIDUAL DISEASE STILL DETECTABLE AFTER FIRST-LINE TREATMENT COMBINING OBINUTUZUMAB AND IBRUTINIB IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): ICLL07 FILO TRIAL

A.-S. Michallet[1], M.-S. Dilhuydy[2], F. subtil[3], V. Rouille[4], B. Mahe[5], K. Laribi[6], B. Villemagne[7], G. Salles[8], O. Tournilhac[9], A. Delmer[10], C. Portois[11], B. Pegourie[12], V. Leblond[13], C. Tomowiak[14], S. de guibert[15], F. Orsini[16], A. banos[17], P. carassou[18], G. Cartron[4], L.M. Fornecker[19], L. Ysebaert[20], C. Dartigeas[21], M. Truchan[22], T. Aurran[23], F. Cymbalista[24], S. Iepretre[25], V. Levy[24], F. Nguyen Khac[13], M. Le garff tavernier[13], C. Aanei[11], M. Ticchioni[26], P. Feugier[27,*]

[1]Centre Léon Bérard, Lyon, [2]CHU de Bordeaux, Bordeaux, [3]Biostatistic, HCL, Lyon, [4]CHU Montpellier, Montpellier, [5]CHU Nantes, Nantes, [6]CH Le Mans, Le Mans, [7]CH departemental de vendee, La roche sur yon, [8]HCL, Lyon, [9]CHU Clermont ferrand, Clermont ferrand, [10]CHU Reims, Reims, [11]ICLOIRE, Saint Priest en jarez, [12]CHU Grenoble, Grenoble, [13]APHP La Pitié Salpétrière, Paris, [14]CHU Poitiers, Poitiers, [15]CHU Rennes, Rennes, [16]CH Annecy genevois, Annecy, [17]CH Côte basque, Bayonne, [18]CHR- METZ, Metz, [19]CHU Strasbourg, Strasbourg, [20]IUCT , Toulouse, [21]CHU Tours, Tours, [22]CHU Angers, Saumur, [23]IPC, Marseille, [24]APHP Avicennes, Bobigny, [25]Centre Henri Becquerel, Rouen, [26]CH Nice, Nice, [27]CHU Nancy, Nancy, France

Background: Achievement of CR with undetectable residual disease (uMRD) may be associated with a longer survival in CLL. New therapeutic agents have recently emerged, including new anti-CD20 antibodies and agents targeting BCR signaling.
Aims: We conducted a multicenter phase II trial aimed to explore the efficacy of an induction treatment associating obinutuzumab and ibrutinib, followed by immunochemotherapy only in case of PR or detectable MRD. The primary objective of this study was to obtain 30% of CR (according to IWCLL 2008 guidelines) with uMRD in BM at month 16.
Methods: FIT treatment-naïve patients with active Binet stage A to C CLL and no *TP53* mutation/deletion were eligible if CIRS score was < 7 and ECOG 0 or 1. Induction treatment consisted of 6 courses of obinutuzumab (1000 mg D1, D8, D15 for cycle 1 and D1 for cycles 2 to 6) along with ibrutinib 420 mg daily for 9 months. A first assessment of response was performed at month 9, including CT-scan, bone marrow (BM) biopsy and peripheral blood (PB) and BM MRD testing. Patients in CR with uMRD ($<10^{-4}$, by 8-color cytometry) received ibrutinib alone for 6 additional months whereas the others received 4 courses of fludarabine + cyclophosphamide and obinutuzumab while continuing ibrutinib. Patients with stable or progressive disease were taken off study. Final evaluation of response was performed at Day 1 Month 16.
Results: Between November 2015 and May 2017, 135 planned patients were enrolled including 89 males and 46 females; 7% were Binet stage A, 67% stage B and 26% stage C. The median age was 62 years (range, 35-80 years) and 57% have an unmutated mutational status. Patients with del11q, del13q and trisomy 12 were 20%, 51% and 22% respectively; and 13% had a complex karyotype (>3 abnormalities). A total of 37 serious AEs were observed with 24 related to the treatment. Two patients died during the study at the cut-off date, one sudden death and one of brain hemorrhage due to accidental fall not reliable to therapy. Among the other AE, 57% of the patients have presented, at least one G3-4 toxicity along the 6 cycles. Hematological toxicity was neutropenia (24% G3-4) and mainly during cycle 1 anemia and thrombocytopenia for respectively 6% and 31% of the patients. Infusion Related Reaction (IRR) only occurred during cycle 1 at day 1 for 69.5% of the patients (8% G3). Other significant toxicity was digestive (nausea, vomiting and diarrhea) occurring in 35% of the patients (grade 1 and 2) but only during cycle 1. At Month 9, 92% of the patients had received the 8 planned infusions of obinutuzumab; Ibrutinib dosage was reduced for 4 patients and definitively stopped in 3 out of them due to AE (atrial fibrillation, atrial flutter and neutropenia). One hundred twenty three are evaluable so far for the response at M9. The ORR was 100% with 41% in CR (IWCLL criteria) and 59% in PR. Only 16 patients (12.5%) had uMRD in PB and BM including 9 patients in CR and 7 in PR (2 bone marrow not evaluable but normal CT scan; 5 patients with lymph nodes > 15 mm respectively: 15, 17, 19, 20 and 21).
Summary/Conclusion: These preliminary results indicated that this 9 month « chemo-free » induction is associated with a high CR rate (41%) without excess of toxicity. However, the majority of the patients required subsequent immuno-chemotherapy because of detectable BM MRD.

S805

HIGH, DURABLE MINIMAL RESIDUAL DISEASE (MRD) NEGATIVITY WITH VENETOCLAX + RITUXIMAB IN RELAPSED/REFRACTORY CLL: MRD KINETICS AND RESPONSES IN CYTOGENETIC RISK GROUPS IN PTS FROM PHASE 3 MURANO STUDY

P. Hillmen[1,*], J. F. Seymour[2], A. W. Langerak[3], B. Eichhorst[4], C. Owen[5], S. Assouline[6], A. Janssens[7], P. Marlton[8], X. Badoux[9], R. Mous[10], B. Chyla[11], R. Humerickhouse[11], M. Boyer[12], K. Humphrey[12], E. Punnoose[13], J. Wang[13], J. Wu[13], Y. Jiang[13], M. Mobasher[13], A. P. Kater[14]

[1]St. James's University Hospital, Leeds, United Kingdom, [2]Peter MacCallum Cancer Centre, Royal Melbourne Hospital, and University of Melbourne, Melbourne, Australia, [3]Department of Immunology, Laboratory for Medical Immunology, Erasmus Medical Center, Rotterdam, Netherlands, [4]University of Cologne, Cologne, Germany, [5]Departments of Medicine and Oncology, University of Calgary, Calgary, [6]Segal Cancer Center, Lady Davis Institute, Jewish General Hospital, Montreal, Canada, [7]Universitaire Ziekenhuizen Leuven, Leuven, Belgium, [8]Princess Alexandra Hospital and University of Queensland, Brisbane, [9]St George Hospital Department of Haematology NSW Health Pathology, Kogarah, Australia, [10]UMC Utrecht Cancer Center, Utrecht, Netherlands, [11]Abbvie, North Chicago, United States, [12]F. Hoffmann–La Roche, Welwyn Garden City, United Kingdom, [13]Genentech, Inc., South San Francisco, United States, [14]Academic Medical Center Amsterdam, Amsterdam, Netherlands

Background: Improved survival outcomes with chemoimmunotherapy in patients with chronic lymphocytic leukemia (CLL) are associated with minimal residual disease negativity (MRD–; <1 CLL cell in 10,000 leukocytes [$<10^{-4}$]), but the importance of MRD with targeted agents and in the relapsed/refractory (R/R) setting remains unclear, mostly due to low MRD– rates with these agents. In the Phase 3 MURANO study, venetoclax + rituximab (VenR) showed superior PFS (hazard ratio 0.17) and higher peripheral blood (PB) and bone marrow (BM) MRD– vs bendamustine + R (BR) in R/R CLL patients.
Aims: To report MRD kinetics (percent of patients achieving MRD $<10^{-4}$, 10^{-4} to $<10^{-2}$, and $\geq 10^{-2}$, at various timepoints), and MRD response in CLL cytogenetic and molecular risk groups in patients from MURANO.
Methods: Patients were randomized to VenR for 6 months followed by single-agent Ven ≤1.5 years, or to BR for 6 months. PB samples were serially collected whereas BM samples were collected at the end of combination treatment assessment (EOCT; Month 9) or at best response; MRD was analyzed centrally by ASO-PCR and/or flow cytometry.

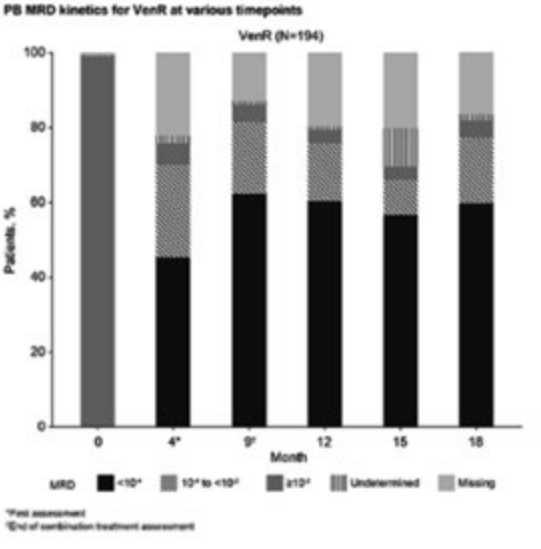


Figure 1.

Results: High concordance was observed between PB and BM MRD status (84%) in patients with paired samples. Higher agreement in MRD– between BM and PB was seen with VenR (45/50 [90%] PB MRD– were also BM MRD–) compared with BR (3/10 [30%]). Given this concordance and availability of serial PB sampling, we focus on PB MRD and outcome.
Best MRD– (at any time on study) rates were higher with VenR (84% *vs* 23% in BR), and were independent of high-risk cytogenetic and molecular factors only for VenR: del(17p) present vs absent: 83% vs 87%; *TP53* mutated vs unmutated: 73% vs 88%; IGVH unmutated vs mutated: 82% vs 89%.

PB MRD kinetics for VenR are shown in the Figure 1. Among 121/194 (62%) patients who were MRD– at EOCT with VenR: 100 (83%) maintained MRD– and were PFS free at a median follow-up of 13.8 (5.6–23.0) months; 2 patients developed progressive disease (PD); 2 died (unrelated); and 2 patients developed Richter's syndrome (with one MRD+ directly before). The remaining 15/121 (12%) patients converted to confirmed MRD+ (2 serial assay-positive assessments) at a median MRD+ follow-up of 5.6 (0.03–11.2) months; 1 patient had MRD ≥10^{-2} with PD, 14 patients had MRD 10^{-4} to <10^{-2}, 2 of which had PD, 1 patient died, and 11 patients remained PFS free. MRD kinetics by patient including treatment status will be presented.

Summary/Conclusion: The robust PB MRD and high concordance with BM MRD with VenR confirms the value of PB MRD for correlation with clinical outcome in patients with R/R CLL treated with this regimen. VenR achieves high, early, deep and durable PB MRD– regardless of risk features, unlike BR. Some reemergence of MRD+, mainly intermediate (10^{-4} to <10^{-2}) level, is seen only in a small number of patients, and may not lead to clinical PD, consistent with the PFS benefit observed in the MURANO study. *NCT02005471*

S806

IBRUTINIB LEAD-IN FOLLOWED BY VENETOCLAX IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA: PHASE 2 CAPTIVATE EARLY SAFETY AND EFFICACY RESULTS

P. Ghia[1,*], C. Tam[2], T. Siddiqi[3], I. Flinn[4], X. Badoux[5], T. Kipps[6], J. Allan[7], A. Tedeschi[8], J. Pagel[9], B. Kuss[10], E. Gonzalez-Barca[11], K. Eckert[12], C. Zhou[12], J. Ninomoto[12], J. Dean[12], D. James[12], W. Wierda[13]

[1]Department of OncoHematology, Università Vita-Salute San Raffaele, Milano, Italy, [2]Peter MacCallum Cancer Centre and St. Vincent's Hospital, Melbourne, Australia, [3]City of Hope National Medical Center, Duarte, CA, [4]Sarah Cannon Research Institute/Tennessee Oncology, Nashville, TN, United States, [5]St George Hospital, Sydney, Australia, [6]University of California San Diego, Moores Cancer Center, San Diego, CA, [7]Weill Cornell Medicine, New York, NY, United States, [8]ASST Grande Ospedale Metropolitano Niguarda, Milano, Italy, [9]Swedish Cancer Institute, Seattle, WA, United States, [10]Flinders Medical Centre, Adelaide, Australia, [11]Institut Català d'Oncologia L'Hospitalet, IDIBELL, Barcelona, Spain, [12]Pharmacyclics LLC, an AbbVie Company, Sunnyvale, CA, [13]University of Texas MD Anderson Cancer Center, Houston, CA, United States

Background: Ibrutinib (ibr), a first-in-class, once-daily BTK inhibitor, is approved in the US for CLL/SLL and in the EU for CLL treatment, including patients with del(17p). Single-agent ibr results in improved survival; however, rates of complete remission (CR) are low, and continuous therapy is required. Ibr and venetoclax (ven), a BCL-2 inhibitor approved by FDA, have complementary therapeutic activity, and synergistic anti-tumor activity has been shown in preclinical and clinical studies with these agents. Ven improves CR rates and can lead to minimal residual disease-negative (MRD(-)) responses in CLL, but increases tumor lysis syndrome (TLS) risk. Tumor debulking by single-agent ibr lead-in followed by combination ibr + ven (I+V) may improve clinical outcomes and lower TLS risk.

Aims: PCYC-1142 (CAPTIVATE) is a phase 2, multicenter study of I+V in first-line CLL (NCT02910583). The study is conducted in 2 phases. The first phase evaluates the MRD(-) clinical response rate of I+V, followed by MRD status-guided randomized treatment discontinuation. The overall objective is to evaluate whether achievement of MRD(-) remission after I+V allows for treatment holidays.

Methods: PCYC-1142 (CAPTIVATE) is a phase 2, multicenter study of I+V in first-line CLL (NCT02910583). The study is conducted in 2 phases. The first phase evaluates the MRD(-) clinical response rate of I+V, followed by MRD status-guided randomized treatment discontinuation. The overall objective is to evaluate whether achievement of MRD(-) remission after I+V allows for treatment holidays.

Results: At time of analysis, a total of 163 patients (median age, 58 years) were enrolled. The first 14 patients had completed the safety run-in of ibr lead-in and ≥6 cycles of I+V; 97 patients (including the safety run-in patients) had completed ibr lead-in and had initiated ven treatment (I+V Exposed). No dose-limiting toxicities occurred during safety run-in. At baseline, 14% of patients had del(17p), 15% had del(11q), and 33% had bulky disease with longest lymph node diameter (LDi) ≥5 cm. Of the 14 safety run-in patients, ORR was 100% (14/14; CR confirmed in 1/5 patients with early BM results at time of analysis and 13/14 confirmed PR); 9/11 assessed patients had MRD(-) status in PB. Common AEs (occurring in ≥20% of I+V Exposed patients) were diarrhea (39%), fatigue (23%), nausea (23%), and arthralgia (21%); grade ≥3 AEs in ≥3% were neutropenia (10%), hypertension (3%), and thrombocytopenia (3%). No patients met the clinical criteria for TLS; laboratory TLS was seen in 1/163. Of 30 I+V Exposed patients with baseline LDi ≥5 cm, 19 (63%) were reduced to LDi <5 cm after ibr lead-in. TLS risk was reduced to medium/low in 17/22 of high risk patients (77%). Overall, the proportion of patients with high-risk TLS decreased from a baseline of 23% to 3% after ibr lead-in.

Summary/Conclusion: These early study results support the safety, activity, and TLS risk reduction potential of ibr lead-in. The early data show promising activity of an I+V oral regimen with MRD(-) responses in 82% in first-line CLL. Safety profiles were consistent with AE profiles of single-agent ibr or ven. The protocol-specified efficacy analysis in the first 30 patients (including ORR) will be presented.

S807

A PHASE IB/II STUDY OF DUVELISIB IN COMBINATION WITH FCR (DFCR) FOR FRONTLINE THERAPY OF YOUNGER CLL PATIENTS

M. Davids[1,*], D. Fisher[1], S. Tyekucheva[1], H. Kim[1], M. McDonough[1], J. Hanna[1], K. Francoeur[1], J. Bazemore[1], J. Hellman[1], O. Odejide[1], P. Armand[1], J. Arnason[2], J. Brown[1]

[1]Medical Oncology, Dana-Farber Cancer Institute, [2]Medical Oncology, Beth Israel Deaconess Medical Center, Boston, United States

Background: FCR is a common initial therapy for younger CLL patients (pts); however, only about 20% will achieve CR/CRi with MRD negativity in the bone marrow (BM-MRD-). Duvelisib (formerly IPI-145) is a delta/gamma PI3K inhibitor with promising efficacy in CLL. We report on an investigator-initiated, phase Ib/II study of dFCR as initial treatment for younger CLL pts (NCT02158091).

Aims: The primary objectives in phase Ib were to determine duvelisib safety and the RP2D, and in phase II to assess the rate of CR/CRi with BM MRD- after dFCR. Secondary objectives included efficacy assessments.

Methods: A standard 3 + 3 phase I design included 2 dose levels of duvelisib (25 mg qd and 25 mg bid). Duvelisib was given for 1 week with FCR added on day 8. Up to 6 cycles of dFCR were given, followed by up to 2 years of duvelisib maintenance. Growth factor support, antimicrobial prophylaxis, and CMV monitoring were mandatory. Eligibility criteria included: age ≤ 65, requiring treatment by IW-CLL criteria, ECOG PS ≤1, and adequate organ function. Toxicity evaluations were performed by CTCAE v4.03/IW-CLL. Response evaluations by 2008 IW-CLL criteria occurred after 3 cycles, 2 months after final FCR, and q6 months thereafter. MRD was assessed by four-color flow cytometry (sensitivity of 10^{-4}).

Results: 32 pts were enrolled, including 6 pts treated with duvelisib 25 mg QD and 26 pts treated with duvelisib 25 mg bid. The median age at enrollment was 55 yrs (range 45-65). By FISH, 8 pts (25%) had del(11q), and 3 (9%) had del(17p). Unmutated *IGHV* was present in 18 pts (56%) and *TP53* mutation in 2 pts (6%). Gr3 febrile neutropenia at 25 mg QD (n=1) was the only DLT, and the RP2D of duvelisib was 25 mg bid. Heme toxicity included thrombocytopenia (69%; 34% gr 3-4), neutropenia (56%; 47% gr 3-4), and anemia (34%, 16% gr 3). Non-heme toxicities included nausea (72%, all gr 1/2), fatigue (69%, 3% gr 3), fever (53%, all gr 1/2), diarrhea (47%, 3% gr 3), transaminitis (34%, 28% gr 3/4), anorexia (34%, all gr 1/2), vomiting (28%, all gr 1/2), pruritus (16%, 3% gr 3), and inflammatory arthritis (9%, all gr 2). SAEs included transaminitis (n=5 gr 3, n=4 gr 4), febrile neutropenia (n=7, all gr 3), pneumonia (n=6, including 3 cases of PJP despite planned prophylaxis), colitis (n=1 gr 2, n=1 gr 3), gr 3 pruritus and gr 3 CMV infection (n=1 each). A median of 5.5 cycles of FCR were given, and 10 pts (31%) discontinued chemotherapy early due to toxicity. Nine pts (28%) required duvelisib dose-reduction. In the 29 pts evaluable for post-FCR response, the ORR was 97%, with 28% achieving CR (n=4) or CRi (n=4), and 69% achieving PR. The best rate of MRD- in the BM in pts with at least one evaluation was 21/26 (81%). The rate of CR/CRi with BM-MRD- (primary efficacy endpoint) was 28%. Two pts with del(17p) achieved MRD+ PR and one achieved MRD+ CR after 12 mo. of duvelisib maintenance. With a median follow-up among survivors of 21 mo. (range 6-42), 2 pts have progressed, including 1 with asymptomatic progression 6 mo. after maintenance ended and 1 with baseline del(17p) and complex karyotype who developed Richter's Syndrome and died 29 mo. after starting on study. Two other pts died, including 1 with metastatic melanoma (at 15 mo.) and 1 with glioblastoma (at 36 mo.). 2-year PFS and OS are both 97%. 8 pts have now completed 2 yrs of duvelisib maintenance.

Summary/Conclusion: dFCR is an effective regimen for the initial therapy of younger, fit CLL pts, leading to a high rate of BM-MRD negativity of 81%, although infectious and immune-mediated toxicities were observed.

S808

A PHASE 2 STUDY TO ASSESS THE SAFETY AND EFFICACY OF UMBRALISIB (TGR-1202) IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WHO ARE INTOLERANT TO PRIOR BTK OR PI3K DELTA INHIBITOR THERAPY

A.R. Mato[1,*], S.J. Schuster[2], N. Lamanna[3], I.W. Flinn[4], J. Barrientos[5], S. Kambhampati[6], B.D. Cheson[7], P. M. Barr[8], J.M. Pagel[9], J.A. Reeves[10], F. Lansigan[11], J.J. Pu[12], A. Skarbnik[13], G. Fonseca[14], C. Dorsey[1], E.T. Chatburn[2], H. Weissbrot[3], J. Svoboda[2], E.T. Luning[15], P. Tsao[15], A. Sitlinger[16], C.S. Ujjani[7], D. Paskalis[17], P. Sportelli[17], H.P. Miskin[17], M.S. Weiss[17], D.M. Brander[16]

[1]CLL Program, Leukemia Service, Memorial Sloan Kettering Cancer Center, New York, NY, [2]University of Pennsylvania, Abramson Cancer Center, Philadelphia, PA, [3]New York-Presbyterian Columbia University Medical Ctr, New York, NY, [4]Tennessee Oncology/Sarah Cannon Research Institute, Nashville TN, [5]Northwell Health/CLL Research and Treatment Program, New Hyde Park, NY, [6]Sarah Cannon Research Institute at Research Medical Center, Kansas City, KS, [7]Georgetown University Hospital Lombardi Comprehensive Cancer Center, Washington, DC, [8]Wilmot Cancer Institute, University of Rochester, Rochester, NY, [9]Swedish Cancer Institute, Seattle, WA, [10]Florida Cancer Specialists South/Sarah Cannon Research Institute, Fort Myers, FL, [11]Dartmouth-Hitchcock Medical Center, Lebanon, NH, [12]Penn State Health, Hershey, PA, [13]John Theurer Cancer Center, Hackensack, NJ, [14]Florida Cancer Specialists North/Sarah Cannon Research Institute, St. Petersburg, FL, [15]Clinical Immunology Laboratory at the Hospital of the University of Pennsylvania, Philadelphia, PA, [16]Duke University Medical Center, Durham, NC, [17]TG Therapeutics, Inc., New York, NY, United States

Background: Although kinase inhibitor (KI) therapies such as ibrutinib are generally well tolerated, intolerance is the most common reason for discontinuation (~50%, Mato et al, Blood 2016). Therefore, patients (pts) who respond to a KI but discontinue due to intolerance represent an unmet medical need. Umbralisib (TGR-1202) is a novel, highly-specific PI3K- inhibitor that also uniquely inhibits CK-1ε (Casein Kinase-1ε). Umbralisib is well-tolerated with a discontinuation rate due to Adverse Events (AEs) of < 10% as demonstrated in a safety analysis of 347 pts (Davids et al, ASH 2017).
Aims: This trial evaluates the safety/efficacy of umbralisib in CLL pts who are intolerant to prior BTK or PI3K- inhibitor therapy.
Methods: KI intolerant is defined as: ≥ 1 grade 3 or ≥ 2 grade 2 non-heme toxicities, ≥ 1 grade 3 neutropenia with infection or fever, and/or ≥ 1 grade 4 heme toxicity leading to KI (BTK and/or PI3K inhibitor) discontinuation. Toxicities must resolve to ≤ grade 1 prior to umbralisib. Prior KI must be discontinued for ≥ 14 days without CLL progression; however, pts could have progressed after 14 days of KI discontinuation. Pts must start umbralisib within 12 mos of discontinuing prior KI. All pts are treated with umbralisib (800 mg oral daily) until progression, toxicity, or study conclusion. The primary endpoint is progression-free survival (PFS). Secondary endpoints include duration of response, time to treatment failure, and umbralisib safety profile. Peripheral blood samples are being collected for correlative analyses to identify markers associated with KI intolerance.

Table 1.

Umbralisib AEs in > 15% pts (n=40)				
Adverse Events (All Causality)	All Grades	% All Grades	GR 3/4	% GR 3/4
Diarrhea	17	43%	3	8%
Nausea	17	43%		
Thrombocytopenia	11	28%	4	10%
Insomnia	9	23%		
Neutropenia	9	23%	7	18%
Dizziness	8	20%		
Fatigue	8	20%		
Rash	7	18%	1	3%

Results: 40 pts were treated as of 2/2018 (36 BTK & 4 PI3K intolerant). Baseline demographics include: median age 69 yrs (range 52-96), median prior therapies 2 (1-7), 55% were male, ECOG 0-1 (92%), del17p (20%), del11q (23%), IGHV unmutated (60%). 80% required treatment within 6 mos of prior KI discontinuation. Most AEs leading to prior KI discontinuation were: arthralgia, rash (9 events each), A-fib (6 events), diarrhea (4 events), bleeding, fatigue and weight loss (3 events each). AEs reported in 40 patients on umbralisib (regardless of causality) are listed in Table 1. Common GR ≥3 PI3K -associated AE's were limited: AST/ALT elevation (3%); diarrhea (7.5%); rash (3%). Four pts discontinued umbralisib due to intolerance (rash, pneumonia, pneumonitis, pancreatitis), and 1 patient due to study noncompliance. No patient has discontinued umbralisib as a result of a prior KI intolerant AE, and only 4 pts had recurrence of an AE that led to intolerance on their prior KI, however all recurrences were of lesser severity, and none led to discontinuation or dose-modification of umbralisib. Three pts (7.5%) had dose reductions (headache, hematologic, and colitis) and were successfully re-challenged (colitis patient on study in PR now 12 mos). Median PFS has not been reached with 90% of pts progression-free at a median follow up of 7 mos (range 1–16). No deaths were observed.
Summary/Conclusion: Umbralisib appears to be safe and effective in a KI intolerant CLL population. These are the first prospective data to confirm that switching from ibrutinib, acalabrutinib or idelalisib to an alternate PI3K- (umbralisib) can result in durable responses without recurrence of prior KI intolerance toxicities. Pre-umbralisib dosing samples are being analyzed for BTK resistance mutations and CYP-3A4 polymorphisms. Study enrollment is expected to be completed with 50 pts by the meeting.

S809

FINAL RESULTS OF THE DESTINY STUDY OF DE-ESCALATION AND STOPPING TREATMENT IN CHRONIC MYELOID LEUKAEMIA

R. Clark[1,*], F. Polydoros[2], J. Apperley[3], D. Milojkovic[3], K. Rothwell[4], C. Pocock[5], J. Byrne[6], H. de Lavallade[7], W. Osborne[8], G. Campbell[9], L. Robinson[10], J. Ewing[11], M. Raghavan[12], J. Tighe[13], J. Cullis[14], A. Whiteway[15], J. Coppell[16], A. Goringe[17], A. Mead[18], F. Dignan[19], B. Huntly[20], S. O'Brien[8], L. Read[2], L. Foroni[3], M. Copland[21]

[1]Institute of Translational Medicine, [2]Cancer Research UK Liverpool Cancer Trials Unit, University of Liverpool, Liverpool, [3]Centre for Haematology, Imperial College London at Hammersmith Hospital, London, [4]Haematology, St James's Hospital, Leeds, [5]Haematology, East Kent Hospitals, Canterbury, [6]Faculty of Medicine and Health Sciences, University of Nottingham, Nottingham, [7]Department of Haematological Medicine, Kings College Hospital, London, [8]Northern Institute for Cancer Research, Newcastle University, Newcastle, [9]Haematology, Colchester General Hospital, Colchester, [10]Haematology, Hereford County Hospital, Hereford, [11]Haematology, Heartlands Hospital, [12]Haematology, Queen Elizabeth Hospital, Birmingham, [13]Haematology, Aberdeen Royal Infirmary, Aberdeen, [14]Haematology, Salisbury District Hospital, Salisbury, [15]Blood Sciences, Southmead Hospital, Bristol, [16]Haematology, Royal Devon and Exeter Hospital, Exeter, [17]Haematology, University Hospital of Wales, Cardiff, [18]Institute of Molecular Medicine, University of Oxford, Oxford, [19]Haematology, Manchester Royal Infirmary, Manchester, [20]Haematology, Addenbrookes Hospital, Cambridge, [21]Paul O'Gorman Leukaemia Research Centre, University of Glasgow, Glasgow, United Kingdom

Background: The British study of De-Escalation and Stopping Treatment with Imatinib, Nilotinib or sprYcel (DESTINY) recruited from December 2013 until April 2015. Entrants were aged 18 or over, in first chronic phase, on the same tyrosine kinase inhibitor (TKI) for at least 3 years since original diagnosis (except that 1 change was permitted if intolerant to the initial TKI), and with all *BCR-ABL* qPCR transcript levels (minimum of 3) < 0.1% in the 12 months before entry. If all these results were also <0.01%, they were assigned to the 'MR4 group' (as studied in many other studies); patients with one or more result between 0.1% and 0.01% were allocated to a 'MMR but not MR4 group' (not previously studied in a stopping trial). Entry criteria were thus virtually identical to the EUROSKI study except that patients with MMR but not MR4 were also separately eligible.
Aims: TKI treatment was de-escalated to 50% of the standard dose (imatinib 200mg daily, dasatinib 50mg daily or nilotinib 200mg twice daily) for 12 months, then stopped altogether for a further 24 months. Centralised PCR monitoring was carried out 1-2 monthly, expressed according to International Scale. Molecular recurrence was defined as the first of 2 consecutive samples >0.1%; this required recommencement of the relevant TKI at full dose.
Methods: 174 patients (male 98; female 76) were recruited after giving informed consent from 20 UK centres. At entry, 148 patients were receiving imatinib, 16 nilotinib and 10 dasatinib, for a median duration of 6.8 years.
Results: We previously reported that after 24 months on study, molecular recurrence was lower in patients with stable MR4 at entry (29 of 125 patients; 23.2%) than in those in MMR but not MR4 (29 of 49 patients; 59.2%) (p<0.001). We now show in the Figure 1 that during the subsequent 12 months of complete treatment cessation (i.e. months 25-36 of study), only 5 further recurrences have occurred, all in patients with stable MR4 at entry, giving a recurrence free survival (RFS) of 72% (90% CI: 65-79%) at 36 months follow-up in this group of patients. The overall recurrence rate is higher in the MMR but not MR4 group (20 of 36 patients during cessation; 39% RFS overall (90% CI: 29-52%); p=< 0.001), though no new recurrences have been seen in months 25-36 in this group.
Multivariable Cox proportional hazards modelling revealed that the baseline entry PCR result did not add to the predictive effect on RFS of the PCR pattern in the 12 months prior to trial entry; however the duration of TKI treatment was an additional predictive factor (p=0.047; HR 0.93), as shown in other studies. The RFS probability remains unrelated to age, gender, performance status or prior TKI (imatinib vs second generation). No progression to advanced phase was seen; two deaths occurred due to unrelated causes, and one case lost haematological response. All relapsing patients have regained MMR within 4 months of resuming full dose TKI. No difference in RFS was seen between patients with a PCR level <0.0032% (MR4.5) at entry and those not.
Summary/Conclusion: The present finding of minimal (only 5) recurrences in the second stopping year, thus sustaining the excellent previously reported RFS out to 24 months of stopping, suggests that initial de-escalation is not simply delaying recurrence, though the mechanism of its benefit is not yet clear. Possibilities include gradual mobilisation of leukaemic stem cells into cycle and/or gradual improvement in the anti-leukaemic immune response at a time when TKI is still present. These require further study.

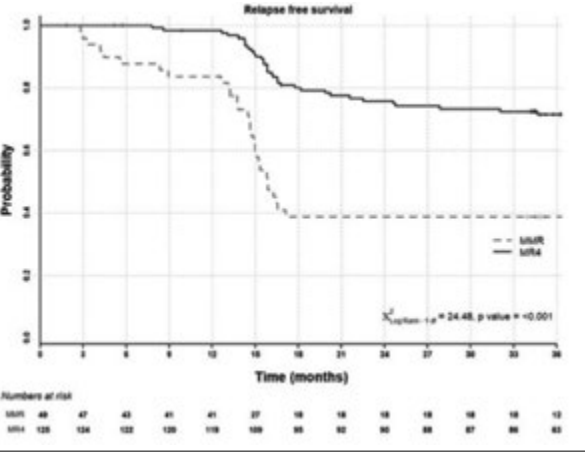


Figure 1.

S810

CHOICES: A RANDOMIZED PHASE II TRIAL FOR IMATINIB VS HYDROXYCHLOROQUINE PLUS IM FOR PATIENTS WITH CML IN MCYR WITH RESIDUAL DISEASE DETECTABLE BY Q-PCR

G. A. Horne[1,*], A. Mukhopadhyay[1], A.-L. Latif[1], J. Stobo[2], C. Kelly[2], J. Dixon-Hughes[2], L. McMahon[2], P. Cony-Makhoul[3], J. Byrne[4], G. V. Helgason[1], R. E. Clark[5], D. Milojkovic[6], L. Foroni[6], F. E. Nicolini[7], M. Copland[1], T.L. Holyoake[1]

[1]Paul O'Gorman Leukaemia Research Centre, University of Glasgow, [2]Cancer Research UK Clinical Trials Unit, Glasgow, United Kingdom, [3]Hematology Department, CH Annecy-Genevois, Pringy, France, [4]Faculty of Medicine & Health Sciences, The University of Nottingham, Nottingham, [5]Royal Liverpool University Hospital, Liverpool, [6]Imperial College London, London, United Kingdom, [7]Hematology department, Centre Léon Bérard, Lyon, France

Background: It is well documented that residual BCR-ABL positive leukemia stem cells are responsible for disease persistence in chronic phase CML (CP-CML), despite TKI therapy. Autophagy is rapidly induced following TKI treatment *in vitro* in CP-CML. Pharmacological autophagy inhibition, using lysosomotropic chloroquine (CQ), enhances the effect of TKIs in CP-CML, compared to Imatinib (IM) or CQ alone. Here, we report the results of the largest clinical trial of autophagy inhibition [CHOICES (CHlorOquine and Imatinib Combination to Eliminate Stem cells] trial. CP-CML patients in major cytogenetic response (MCyR) with residual disease detectable after at least one year of IM treatment were treated with IM and hydroxychloroquine (HCQ) or IM alone.
Aims: The primary study end-point was the proportion of treatment 'successes', defined as patients who had >0.5 log reduction in their 12-month (mth) qPCR level from baseline. The secondary study end-points were the proportion of treatment 'successes' at 24 mth, molecular response and progression at 12 and 24 mth, comparison of IM levels between study arms, and the proportion of patients who achieved whole blood HCQ levels >2000ng/ml.
Methods: CHOICES was an international multicentre, two-arm, parallel, randomised phase II trial with a safety run-in, designed to study the safety and efficacy of IM+HCQ. Patients were randomly assigned, using one-to-one allocation, to receive either IM alone, or IM+HCQ. IM was continued at the same dose that was taken prior to trial entry. At the end of 12 cycles, IM was continued. HCQ was initially started at 800mg/day.
Results: From 2010 to 2014, 62 patients were randomized to IM or IM+HCQ. Demographic data at study entry are listed in Table 1. The success rate in the IM+HCQ arm at 12 mnth was 1.2% lower than with IM alone (95% CI 21.1% lower to 18.4% higher; 1-sided p=0.58). MMR was achieved in 80% of IM alone vs 92% in IM+HCQ arm (p =0.21). At 24 mth, the 'success' rate in the IM+HCQ arm was 20.8% higher than the IM alone arm (p=0.059), and MMR was achieved in 79.2% IM and 88% in thr IM+HCQ (p=0.1) group. Within the IM+HCQ experimental arm, HCQ levels were determined over time and analysed by the 12 and 24 mth 'success' and 'failure' status. There was no correlation between achieving 'success' and achieving HCQ concentrations of >2000ng/ml at either time point.

During the trial period, 17 adverse events were reported; four were serious adverse reactions: 1 within the IM arm (dyspepsia), and 3 with IM+HCQ. Of these, 1 was unrelated, and 2 had a potential association, namely dyspnea and heart failure. An *in vitro* assessment of autophagy was performed within the CD34+ stem/progenitor and mononuclear populations from peripheral blood and bone marrow at each time point. Basal autophagy, assessed by LC3B-II levels, was higher in CD34+ stem/progenitor cells compared to mononuclear populations (p=0.002). Additionally, LC3B-II levels at 6 and 12 mth were higher in IM/HCQ arm than the IM arm, suggesting a block in autophagy flow (i.e. inhibition of autophagy-mediated degradation of LC3B-II), although this was not statistically significant.

Table 1.

Selected Demographics	IM	IM+HCQ
Age (years)	49.5 (21-79)	50 (20-78)
Male (%)	66.7	71.9
ECOG 0 (%)	93.1	87.5

Summary/Conclusion: We confirm that IM+HCQ is a tolerable combination in CP-CML, with significant improvement in overall qPCR levels and MMR at 24 mth. This suggests that there may be a potential long-term benefit of combined therapy on qPCR levels and achievement of deep molecular response. Longer follow-up will be required to confirm this.

S811

THE EUTOS LONG-TERM SURVIVAL (ELTS) SCORE IS SUPERIOR TO THE SOKAL SCORE FOR PROGNOSIS OF SURVIVAL PROBABILITIES OF PATIENTS WITH CHRONIC-PHASE CHRONIC MYELOID LEUKAEMIA

M. Pfirrmann[1,*], M. Baccarani[2], S. Saussele[3], A. Turkina[4], E. Faber[5], J.L. Steegmann[6], G. Ossenkoppele[7], M. Höglund[8], A. Zaritskey[9], A. Bogdanovic[10], R. Clark[11], L. Griskevicius[12], U. Olsson-Strömberg[13], S. Lejniece[14], S. Burgstaller[15], I. Zupan[16], A. Colita[17], H. Everaus[18], P. Koskenvesa[19], B. Labar[20], T. Sacha[21], F. Castagnetti[2], F. Cervantes[22], D. Zackova[23], W. Prejzner[24], J. Guilhot[25], R. Hehlmann[26], J. Hasford[1], V.S. Hoffmann[27]

[1]Institut für Medizinische Informationsverarbeitung, Biometrie und Epidemiologie (IBE), LMU München, München, Germany, [2]Clinical Department of Hematology and Oncology L. and A. Seragnoli, S Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy, [3]Medizinische Fakultät Mannheim, Universität Heidelberg, Mannheim, Germany, [4]National Research Center for Hematology, Moscow, Russian Federation, [5]Department of Hemato-oncology, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic, [6]Department of Hematology, Hospital Universitario de la Princesa, Madrid, Spain, [7]Department of Haematology, VU University Medical Center, Amsterdam, Netherlands, [8]Department of Medical Sciences, University of Uppsala, Uppsala, Sweden, [9]St Petersburg State Medical University, St Petersburg, Russian Federation, [10]Department of Hematology, Clinical Center of Serbia and Faculty of Medicine, University of Belgrade, Belgrade, Serbia, [11]Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom, [12]Faculty of Medicine, Vilnius University and Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania, [13]Department of Medical Sciences and Division of Hematology Uppsala University Hospital , University of Uppsala, Uppsala, Sweden, [14]National Haematology Centre, Clinic Linezers, Riga East Clinical University Hospital, Riga, Latvia, [15]Abteilung für Innere Medizin IV, Klinikum Wels-Grieskirchen, Wels, Austria, [16]University Medical Centre Ljubljana, Ljubljana, Slovenia, [17]Romanian Academy of Medical Sciences, Bucharest, Romania, [18]Tartu University Hospital, Tartu, Estonia, [19]Helsinki University Central Hospital and Hematology Research Unit, Biomedicum Helsinki, Helsinki, Finland, [20]Department of Hematology, University Hospital Center Zagreb, Zagreb, Croatia, [21]Department of Hematology, Jagiellonian University Hospital, Kraków, Poland, [22]Hospital Clinic, IDIBAPS, University of Barcelona, Barcelona, Spain, [23]Department of Internal Medicine, Hematology and Oncology, University Hospital Brno and Masaryk University, Brno, Czech Republic, [24]Department of Hematology, Medical University of Gdansk, Gdansk, Poland, [25]Clinical Investigation Center, INSERM CIC 1402, CHU de Poitiers, Poitiers, France, [26]Universität Heidelberg and ELN Foundation, Mannheim, [27]Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt, Neuherberg, Germany

Background: The European Treatment and Outcome Study (EUTOS) registry contains data on adult patients with chronic-phase (CP) chronic myeloid leukaemia (CML). In 2,205 imatinib-treated patients, the EUTOS long-term survival score (ELTS) was developed to discriminate three risk groups with different probabilities of dying from CML. Still, many investigators continue to apply the Sokal score for the prognostic discrimination of CML-patients treated with tyrosine kinase inhibitors (TKIs).
Aims: The Sokal score had allocated 23% of patients to the high-risk group, the ELTS score 12%. Long-term outcome of TKIs suggests that allocating >20% CP CML patients into a high-risk group is too pessimistic. The aim of this analysis was to compare risk group allocations and prognosis between the two scoring systems.
Methods: Due to the success of TKIs, the number of deaths from CML has distinctly declined. To optimise power, 2,949 patients from other registry sections were added. Survival was calculated from the date of start of treatment to death and censored at the latest follow-up. Cumulative incidence probabilities (CIPs) of dying of CML were compared with the Gray test and overall survival probabilities (OS) with the log-rank test. Only death after confirmed disease progression was regarded as "death due to CML". Progression was defined in accordance with the current ELN recommendations (Blood 2013). Level of significance was 0.05.
Results: The 5,154 patients in the combined registry sections had a median observation time of 5.3 years. Six-year OS probability was 90% (95% confidence interval (CI): 89-91%). Of 429 deceased patients, in 175 CML progression prior to death was confirmed (40%). The 6-year CIP of dying of CML was 4% (CI: 4-5%). From low to high risk groups, the Sokal score resulted in 6-year CIPs of 3% (n=1,982 (38% of 5,154), CI: 2-3%), 4% (n=1,975 (38%), CI: 3-5%), and 8% (n=1,197 (23%), CI: 6-10%) and the ELTS score in 6-year CIPs of 2% (n=3,037 (59%), CI: 2-3%), 5% (n=1,449 (28%), CI: 4-7%), and 12% (n=668 (13%), CI: 9-15%). Of the 1,197 patients allocated to high risk by the Sokal score, the ELTS score classified 671 (56%) as non-high-risk. Compared to the 526 high-risk patients according to both scores (6-year CIP of dying: 12%, CI: 9-16%), the CIPs of dying were lower for the non-high risk patients (p=0.0003, 6-year CIP: 5%, CI: 3-7%). The Sokal high but ELTS non-high-risk patients (6-year OS: 88%. CI: 85-91%) showed higher OS than the 526 common high-risk patients (p=0.0036, 6-year OS: 81%, CI: 76-85%). Of the 3,037 patients identified as low risk by the ELTS score, the Sokal score allocated 1,200 (40%) to non-low-risk groups. Without significant CIP differences to the latter group, at 6 years, the CIP of dying was 2% (CI: 1-3%) in the 1,837 low-risk and 2% in the 1,200 non-low-risk patients (CI: 2-4%). OS of the Sokal non-low-risk patients (6-year OS: 92%. CI: 90-94%) was lower than in the cases classified as low-risk by both scores (6-year OS: 95%. CI: 94-96%, p=0.0186).
Summary/Conclusion: The Sokal score allocated 13% (n=671) more patients to the high-risk group than the ELTS score. As these patients had significantly and clinically relevant lower CIPs of death and higher OS probabilities, the allocation of the Sokal score was not appropriate. OS probabilities but not the CIPs of 1,200 patients assessed as low-risk by the ELTS and non-low-risk by the Sokal score were different from the probabilities of 1,837 assessed as low-risk patients by both scores. For prediction of long-term survival, the use of the ELTS score is recommended.

S812

PHASE 2 STUDY OF RUXOLITINIB IN COMBINATION WITH 5-AZACITIDINE IN PATIENTS WITH MYELOFIBROSIS

L. Masarova[1,*], S. Verstovsek[1], J. Cortes[1], N. Pemmaraju[1], E. Jabbour[1], P. Bose[1], M. Ohanian[1], L. Zhou[1], S. Pierce[1], R. Gergis[1], G. Borthakur[1], Z. Estrov[1], G. Garcia-Manero[1], H. Kantarjian[1], N. Daver[1]

[1]Leukemia, UT MD Anderson Cancer Center, Houston, United States

Background: Ruxolitinib (RUX) is effective in controlling symptoms and organomegaly in patients with myelofibrosis (MF). Combination with azacitidine (AZA) may further improve its efficacy.
Aims: To evalute efficacy and safety of RUX and AZA combination.
Methods: This was a single insitutional, phase 2 study. RUX 15 or 20 mg orally twice daily was given continuously since cycle 1. AZA 25 to 75 mg/m^2 on days 1-5 of each 28-day cycle was added starting cycle 4. Responses were assessed per International Working Group for Myelofibrosis Research and Treatment 2013 criteria (IWG-MRT).
Results: Forty nine patients were enrolled on study between 03/2013 and 08/2017. After median follow-up of 18.5+ months (range, 1-56+); 14 patients (29%) remain on therapy with a median overall follow-up of 28+ months. 41 patients (84%) received AZA on study, with a median of 15 cycles (range, 1-52). Median age was 66 years (range, 48-87), 37 patients (76%) had int-2/high DIPSS score, 37 (76%) had spleen ≥5cm, and 26 (53%) were JAK2^{V617F} positive (Figure 1, Part I). Twenty six patients (53%) were previously treated. Thirty-six patients (73%) achieved an IWG-MRT 2013 objective response (Figure 1, Part II). Median time to response was 1.7 months (range, 0.7-19). Eight responses (22% of responders) occurred

after the addition of AZA with median time to response from start of AZA of 4 months (range, 1-16.5). In total, 25 (68%) and 22 (59%) patients had palpable spleen reduction by > 50% at any time on study, and at week 24, respectively. Spleen responses occurred after AZA introduction in 6 patients with a median time of 1.8 months from start of AZA. Mean percentage spleen reduction by palpation was 73% (95% CI 59-86%) and 63% (95% CI 25-100%) at any time on study and at week 24, respectively. $JAK2^{V617F}$ allele reduction was noted in 14 (82%) of 17 evaluable patients, including > 50% reduction in 3 patients (18%). Thirty-one patients (63%) had bone marrow biopsies available for sequential evaluation. Sixteen patients (52%) had a documented improvement in bone marrow fibrosis by at least one grade per European grading (EUMNET) including 4 (13%) by two grades or more, with median time to first documented fibrosis improvement of 12 months (range, 6-18). Additionally, 12 (48%) and 9 (41%) achieved improvement in initially abnormal collagen (n=25) or osteosclerosis (n=22), respectively (Figure 1, Part III).

Overall, 31 patients (63%) experienced grade 3/4 toxicity while on therapy. New onset of G3/4 anemia, thrombocytopenia and neutropenia occurred in 33%, 24% and 22% of patients, respectively. Only 4 (8%) discontinued therapy due to toxicities.

The most common reasons for therapy discontinuation were elective stem cell transplantation (n=12), and uncontrolled disease (n=8), including progression to AML in 4 patients.

PART I.

Patient clinical characteristics	N (%) or median [range]
White blood cells x 10^9 / L	12.1 [2.2 - 79.8]
Hemoglobin g / dL	10.4 [6.8 - 16.2]
Platelets x 10^9 / L	272 [125 – 1070]
Peripheral blood blast ≥ 1%	31 (63)
Spleen ≥ 5 cm below costal margin	37 (76)
Total symptom score (TSS)	32 [1 - 84]
Bone marrow fibrosis grade: Gr 1 vs 2 vs 3+	4 / 24 / 21
JAK2 V617F positive	26 (53)
MPL / CALR positive	3 / 5
Karyotype abnormal	21/49 (43)
Additional molecular mutations by 28-gene panel	15/33 (45)

PART II.

IWG-MRT 2013	N (%)
Overall response	36 (73)
Partial remission (PR)	2 (4)
Clinical improvement (CI) spleen + TSS	9 (18)
CI TSS + CI hemoglobin	2 (4)
CI TSS + CCyR	1 (2)
CI spleen + TSS + molecular PR (JAK2)	2 (4)
CI TSS only	11 (22)
CI spleen only	9 (18)

PART III.

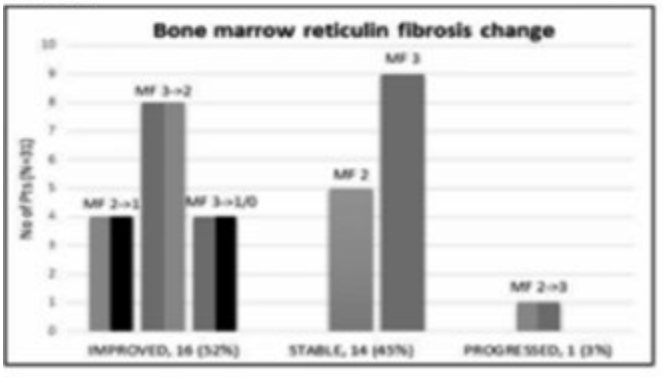


Figure 1.

Summary/Conclusion: Concomitant RUX with AZA was well tolerated with overall IWG-MRT response rate of 73%, including >50% spleen length reduction in 59% of patients at week 24. Moreover, 52% of patients achieved an independent pathologist committee reviewed, objective improvements in bone marrow fibrosis grade with a median time to improvement of 12 months, which compares favorably to single RUX. ClinicalTrials.gov Identifier: NCT01787487

S813

ESTABLISHMENT OF A SIMPLE RISK SCORE TO PREDICT SYSTEMIC MASTOCYTOSIS (SM) IN ADULT PATIENTS WITH MASTOCYTOSIS IN THE SKIN (MIS)

D. Fuchs[1,*], A. Kilbertus[2], P. Hockl[1], N. von Bubnoff[3,4,5], K. Shoumariyeh[3,4,5], R. Zanotti[6], P. Bonadonna[7], L. Scaffidi[6], M. Doubek[8], H.O. Elderink[9], L.F. Span[9], O. Hermine[10], C. Elena[11], P. Benvenuti[12], A.S. Yavuz[13], K. Brockow[14], A. Zink[14], E. Aberer[15], A. Gorska[16], J. Romantowski[16], E. Hadzijusufovic[17], A. Belloni Fortina[18], F. Caroppo[18], C. Perkins[19], A. Illerhaus[20], J. Panse[21], V. Vucinic[22], M. Jawhar [23], V. Sabato[24], M. Triggiani[25], R. Parente[25], H. Hagglund[26], C. Breynaert[27], J. Gotlib[19], A. Reiter[23], K. Hartmann[20,28], M. Niedoszytko[16], H. Kluin Nelemans[9], W. R. Sperr[17], P. Valent[17], R. Greul[1]

[1]Department of Internal Medicine 3 - Hematology and Oncology, [2]Department of Dermatology and Allergology, Kepler University Hospital, Johannes Kepler University, Linz, Austria, [3]Department of Hematology, Oncology and Stem Cell Transplantation, Medical Center, Faculty of Medicine, Freiburg, [4]German Cancer Consortium (DKTK), partner site Freiburg, Freiburg, [5]German Cancer Research Center (DKFZ), Heidelberg, Germany, [6]Section of Hematology, Department of Medicine, [7]Allergy Unit, Verona University Hospital, Verona, Italy, [8]University Hospital Brno, Brno, Czech Republic, [9]Department of Hematology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands, [10]Service Hèmatologie Adulte, Hôpital Necker-Enfants malades, Paris, France, [11]Department of Hematology Oncology, IRCCS Policlinico San Matteo Foundation , [12]Department of Hematology Oncology , School of Hematology, University of Pavia and IRCCS Policlinico San Matteo Foundation, Pavia, Italy, [13]Division of Hematology, Department of Internal Medicine, University of Istanbul, Istanbul, Turkey, [14]Department of Dermatology and Allergy Biederstein, Technische Universität München, Munich, Germany, [15]Department of Dermatology and Venereology, Medical University of Graz, Graz, Austria, [16]Department of Allergology, Medical University of Gdańsk, Gdańsk, Poland, [17]Department of Internal Medicine I, Division of Hematology and Hemostaseology, and Ludwig Boltzmann Cluster Oncology, Medical University of Vienna, Vienna, Austria, [18]Pediatric Dermatology Unit, Department of Medicine, University of Padua, Padua, Italy, [19]Stanford Cancer Institute/Stanford University School of Medicine, Stanford, CA, United States, [20]Department of Dermatology, University of Cologne, Cologne, [21]Department of Oncology, Hematology, Hemostaseology and Stem Cell Transplantation, Medical Faculty, University Hospital RWTH Aachen, Aachen, [22]University Hospital of Leipzig, Leipzig, [23]III. Medizinische Klinik, Universitätsmedizin Mannheim, Universität Heidelberg, Mannheim, Germany, [24]Faculty of Medicine and Health Sciences, Department of Immunology-Allergology-Rheumatology, University of Antwerp and Antwerp University Hospital, Antwerpen, Belgium, [25]Division of Allergy and Clinical Immunology, Department of Medicine, University of Salerno, Salerno, Italy, [26]Cancer Center, Uppsala University Hospital, Uppsala, Sweden, [27]University Hospitals Leuven and KU Leuven, Leuven, Belgium, [28]Department of Dermatology, University of Luebeck, Luebeck, Germany

Background: Mastocytosis is a clonal mast cell disease which, in adults, most commonly affects skin, bone marrow and other organs. Symptoms and serum tryptase levels vary, and there are patients with pure cutaneous mastocytosis (CM). It is generally appreciated that all adult patients with MIS require a bone marrow biopsy (BMB) in order to confirm the presence of SM. Although BMB is safe, it may be painful and cause complications. Existing risk scores are based on relatively few patients and a limited set of parameters.

Aims: We aimed at creating a simple and solid risk score to predict SM in adult patients with MIS. We hypothesized that there are patients at low risk of SM which may be relevant in clinical practice.

Methods: The European Competence Network on Mastocytosis (ECNM) registry is the largest collection of data on patients with mastocytosis. 1145 patients of at least 18 years with MIS who had a BMB within six months of diagnosis were included. Patients with advanced mastocytosis (ASM, MCL, SM-AHN) were excluded. We identified significant variables in univariate analysis and created a multivariate regression model using the whole population as a training and validation set (bootstrapping). A risk score was derived and the model and score were validated with receiver operating curve (ROC) area under the curve (AUC).

Results: Of 1145 patients, 944 had SM and 201 had CM. Among these 1145 patients, 63.7% were female, with a median age of 44±13.3 years (18-81 years) and good performance status (ECOG 0-1, 97.6%). They were highly symptomatic (skin symptoms, 81.4%; other, 65.5%). Almost all patients had typical skin lesions (>97%) and tryptase levels varied greatly (median 29.3±81.9 ng/ml, range: 1-885). In a univariate analysis to determine predictors of SM in patients with MIS, significant variables were typical MIS (p=0.001), a positive Darier's sign (p=0.020), constitutional/cardiovascular symptoms (p=0.002), bone symptoms/osteoporosis (p<0.001), gastrointestinal symptoms (p=0.004), serum tryptase (p<0.001), palpable spleen (p=0.003), age >65 years (p=0.009), lactate dehydrogenase (p=0.003),

monocytes (p=0.001) and beta2-microglobulin (p=0.047). In the multivariate regression model, tryptase level (p<0.001), constitutional/cardiovascular symptoms (p=0.014) and bone symptoms/osteoporosis (p<0.001) were identified as independent predictors of SM (p<0.001, Nagelkerke R2=0.462, Hosmer-Lemeshow p=0.177). The model correctly classified 88.1% of the cases as SM (sensitivity 90.7%, specifity 69.1%, positive predictive value 95.5%, negative predictive value 50.3%). A risk score was derived and points were assigned to all variables, creating a six-point scale with a risk of SM ranging from 10.7% to 98.0% (Figure 1). ROC AUC was 0.871 for the model and 0.867 for the risk score. Bootstrap validation (k=1000) led to an optimism-corrected AUC of 0.853 for the model and 0.849 for the score. Of all MIS patients, 17.4% (n=199) were at low risk (-1 and 0 points, 10.7 and 24.7% risk of SM), 14.5% (n=166) at medium risk (1 and 2 points, 47.1 and 70.7%) and 68.1% (n=779) at high risk (3-5 points, 86.8-98%).

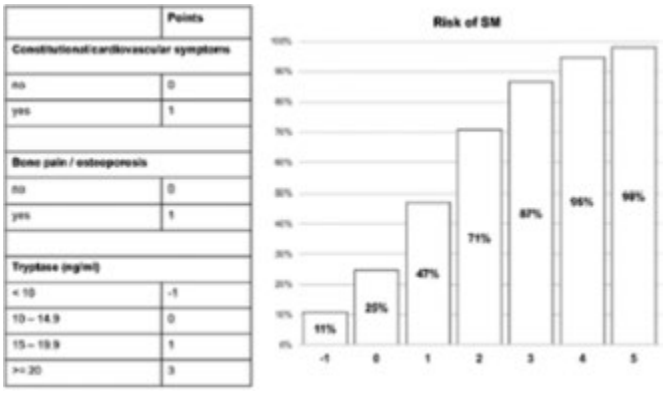


Figure 1.

Summary/Conclusion: Using a large data set of the ECNM registry, we created a risk score to better predict SM in patients with MIS. Although it will need validation in independent cohorts, our score seems to be valid and may discriminate between patients with SM and CM. Our new score may thus have clinical implications and may assist in the decision to recommend a BMB in adults with MIS.

AML biology & translational research II: Signalling

S814

IL1RAP POTENTIATES MULTIPLE ONCOGENIC SIGNALING PATHWAYS IN AML

K. Mitchell[1,*], L. Barreyro[1], T.I. Todorova[1], S. J. Taylor[1], I. Antony-Debré[1], S.-R. Narayanagari[1], L. A. Carvajal[1], J. Leite[1], Z. Piperdi[1], G. Pendurti[1], I. Mantzaris[1], E. Paietta[1], A. Verma[1], K. Gritsman[1], U. Steidl[1]

[1]Albert Einstein College of Medicine–Montefiore Medical Center, Bronx, United States

Background: Our group and others previously identified the surface molecule Interleukin 1 receptor accessory protein (*IL1RAP*) as one of the most significantly upregulated genes in hematopoietic stem and progenitor cells (HSPC) from AML patients and found that its overexpression is correlated with a poor prognosis. IL1RAP is emerging as a novel target for immunotherapy in AML and other myeloid malignancies, however, the cell-intrinsic functions and mechanism of action of IL1RAP in AML cells are largely unknown.
Aims: To investigate therapeutic targeting and the functional and mechanistic role of IL1RAP in AML.
Methods: We used complementary approaches to target IL1RAP (genetic knockout, RNA-interference, and antibody targeting), and measured the functional effects (cell growth, apoptosis, differentiation) of IL1RAP targeting *in vitro* in AML cell lines, AML patient samples (including leukemic stem cell-enriched populations), and healthy HSPC, and *in vivo* in AML xenograft models. We generated retrovirally induced leukemias in the *wt* vs. *Il1rap*$^{-/-}$ setting and performed competitive transplants of *wt* vs. *Il1rap*$^{-/-}$ normal bone marrow cells. Using western blotting, phospho-flow cytometry, EdU incorporation, co-immunoprecipitation and flow cytometric-based Förster resonance energy transfer (FRET), we assessed the molecular effects of IL1RAP targeting and the cellular role of IL1RAP.
Results: We found that IL1RAP targeting inhibited AML growth and viability and promoted differentiation in a cell-intrinsic manner and in the absence of immune effector cells, but did not have inhibitory effects on healthy HSPC. *in vivo*, IL1RAP targeting inhibited AML xenografts, and *Il1rap* knockout decreased leukemic stem cell function in an MLL-AF9 AML mouse model. Meanwhile, *Il1rap* knockout did not perturb healthy stem cell function in competitive transplantation experiments. In exploring the cell-intrinsic molecular bases for these effects, we unexpectedly found that IL1RAP is a promiscuous co-receptor in AML and its function is not restricted to its canonical role in the IL-1 receptor pathway. Specifically, studies by co-immunoprecipitation, FRET, and biochemical assays revealed that IL1RAP physically interacts with and mediates signaling and pro-proliferative effects through FLT3 and c-KIT, two receptor tyrosine kinases with known key roles in AML pathogenesis. Consistent with these mechanistic findings, we found differential sensitivity to IL1RAP targeting of FLT3-mutant and FLT3-wildtype AML cells, a finding with important implications for currently ongoing immunotherapeutic and precision oncology approaches targeting IL1RAP.
Summary/Conclusion: Our study reveals novel functional and mechanistic cell-intrinsic roles of IL1RAP in AML, and provides important insight for currently ongoing and future therapeutic strategies targeting IL1RAP.

S815

ARRAYED MOLECULAR BARCODING IDENTIFIES TNFSF13 AS A POSITIVE REGULATOR OF ACUTE MYELOID LEUKEMIA-INITIATING CELLS

M. Chapellier[1,*], P. Peña-Martínez[1], R. Ramakrishnan[1], M. Eriksson[1], M. Safaee Talkhoncheh[2], C. Orsmark-Pietras[1], H. Lilljebjörn[1], C. Högberg[1], A. Andersson Hagström[1], T. Fioretos[1], J. Larsson[2], M. Järås[1]

[1]Clinical Genetics, [2]Molecular Medicine and Gene Therapy, Lund Stem Cell Center, Lund University, Lund, Sweden

Background: Acute myeloid leukemia (AML) is characterized by an accumulation of immature myeloid blasts in the bone marrow. By providing cell-cell interactions and secreted factors, the bone marrow niche supports AML and normal hematopoietic stem and progenitor cells (HSPCs). A dysregulation of cytokines in the bone marrow microenvironment upon AML development contributes to the selective advantage of leukemia stem cells, a self-renewing population of leukemia cells that constitutes a chemo-resistant reservoir responsible for disease relapse.

Aims: To identify factors that regulate AML cells, we recently developed an *in vitro* cytokine screen using fluorescently labeled c-Kit$^+$ leukemia cells mixed with corresponding normal bone marrow cells, allowing us to successfully identify both negative and positive regulators of AML cells (Peña-Martínez *et al*., Leukemia 2017). However, to assess effects on leukemia stem cells, there is a strong demand to improve such screens to evaluate the impact of cytokines on the leukemia-initiating capacity of cells more directly using an *in vivo* readout. A major challenge for combining *ex vivo* screens with *in vivo* read-out of stem cell function is the large number of experimental animals needed to provide meaningful data. Hence, new methods that allow for a multiplexed *in vivo* read-out of leukemia-initiating activity are needed.
Methods: To identify cytokines that regulate AML stem cells using a competitive *in vivo* read-out of leukemia-initiating activity, we generated lentiviral vectors harboring 11 genetic barcodes in an arrayed setting. This approach allows for labeling of leukemia cell populations with distinct molecular barcodes followed by exposure to separate experimental conditions. Each labeled cell population was stimulated with one cytokine *ex vivo* and after culture, leukemia cells from up to 11 cytokine conditions were pooled prior to *in vivo* competition. To trace the effects of the cytokines to the leukemia-initiating capacity of barcoded cells, the representation of individual barcodes *in vivo* was assessed using next-generation sequencing (NGS).
Results: With this approach, we assessed the effect of 114 murine cytokines on *MLL-AF9* AML mouse cells and identified the tumor necrosis factor ligand superfamily member 13 (TNFSF13) as a positive regulator of leukemia-initiating cells. By using a *Tnfsf13*$^{-/-}$ mouse, we confirmed that TNFSF13 supports leukemia initiation and also normal myelopoiesis by regulating granulocyte and macrophage progenitor (GMP) cell levels in the bone marrow. TNFSF13 was secreted by normal myeloid cells but not by AML mouse cells, suggesting that mature myeloid bone marrow cells support AML cells by secreting TNFSF13. TNFSF13 supported leukemia cell proliferation in an NF-κB-dependent manner and suppressed apoptosis. Moreover, TNFSF13 supported the growth and survival of several human myeloid leukemia cell lines, demonstrating that our findings translate to human disease.
Summary/Conclusion: Taken together, using arrayed molecular barcoding, we identified a previously unrecognized role of TNFSF13 as a positive regulator of primitive AML cells. The arrayed barcoded screening methodology is not limited to cytokines and leukemia, but can be extended to other types of *ex vivo* screens, where a multiplexed *in vivo* read-out of stem cell functionality is needed.

S816

INVESTIGATING THE ROLE OF INPP4B AS A THERAPEUTIC TARGET IN AML

J. Woolley[1,2,3,*], M.H. Son[2], I. Dzneladze[2], R. He[2], A. Rashid[2], M. Minden[2], L. Salmena[2,3]

[1]Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, United Kingdom, [2]Ontario Cancer Institute, Princess Margaret Hospital, [3]Pharmacology & Toxicology, University of Toronto, Toronto, Canada

Background: Our studies have focused on characterizing a role for the lipid phosphatase Inositol polyphosphate 4-phosphate Type II (INPP4B) in Acute Myeloid Leukemia (AML). We have previously published data showing that elevated INPP4B expression in AML patients is predictive of lower response rates to induction chemotherapy and lower overall survival. We have since revealed that INPP4B protein plays a critical role in both human and murine HSC maintenance
Aims: To investigate the role of INPP4B in hematopoiesis and leukemia and how this contributes to leukemogenesis, both *in vitro* and *in vivo*.
Methods: We make use of *Inpp4b*$^{-/-}$ C57/BL6 mice to study the contribution of Inpp4b to the HSC niche. Functional studies of *Inpp4b*$^{-/-}$ HSCs were carried out *in vivo* (competitive transplant into CD45.1 B6.SJL mice) and *in vitro* (CFC assays). Leukemia models of Inpp4b-loss were generated by retroviral transduction of bone-marrow with MLL-AF9. A combination of *in vivo* transplants, *in vitro* CFC assays, mass cytometry and next-generation sequencing were utilized to study the contribution of *Inpp4b* to leukemia.
Results: We show compelling evidence that INPP4B expression is significantly enriched in human and mouse hematopoietic stem and progenitor cells (HSPC). *Inpp4b*$^{-/-}$ mice show a disrupted bone-marrow stem-niche with reduced long-term HSC, MPP and GMP compared to *wild-type* controls (49.1%, 32.4% and 19.7% respectively). Functional studies of *Inpp4b* in HSPC by competitive transplant of bone marrow demonstrated that *Inpp4b*$^{-/-}$ cells show a greatly diminished contribution to the myeloid compartment in secondary and tertiary transplants. Similarly, serial 5'-fluorouracil injections demonstrate a reduced overall survival of *Inpp4b*$^{-/-}$ mice compared to *wild-type* controls, indicating an impaired ability of *Inpp4b*$^{-/-}$ HSCs to repopulate the hematopoietic system post-insult.
Investigating *INPP4B* expression in a human primary AML patient sample grown in culture, we show its expression correlates with more primitive populations (CD34$^+$CD38$^-$). To further study the role of INPP4B in primitive leukemia populations we modeled Inpp4b-loss in an MLL-AF9 murine leukemia model. Transplanting MLL-AF9 leukemias into recipient mice in limiting dilution showed that loss of Inpp4b reduces the frequency of leukemia initiating cells (LICs), and increases disease latency. We also show in this model of Inpp4b-loss sensitizes the leukemia model to a 7-day regimen of cytarabine treatment *in vivo*. Mass-cytometry analysis showed that Inpp4b-loss leads to a more differentiated phenotype, with altered surface marker expression such as Gr-1, c-Kit, Flt3, CD117 and CD44. Also, next-generation sequencing demonstrated a transcriptional profile associated with loss of stem-related genes and upregulation of genes associated with differentiation in *Inpp4b*$^{-/-}$ MLL-AF9 leukemias.
Summary/Conclusion: Our results indicate that Inpp4b plays a role in maintaining stemness, and that Inpp4b loss leads to differentiation and chemosensitization of leukemia *in vivo*. We hypothesize that INPP4B plays a critical role in HSC maintenance and contributes to worse leukemia, and that its stem-related function is responsible for the poor therapy response observed in patients with elevated INPP4B.

S817

RAS MUTATIONS ARE THE DOMINANT MECHANISM OF SECONDARY RESISTANCE TO GILTERITINIB THERAPY FOR RELAPSED/REFRACTORY FLT3-MUTATED AML

C.M. McMahon[1,*], T.T. Ferng[2], J. Canaani[3], B. Rea[4], R.L. Sargent[4], J. Morrissette[4], D.B. Lieberman[4], C. D. Watt[4], R. Durruthy-Durruthy[5], M. Pellegrino[5], D.J. Eastburn[5], E.S. Wang[6], N.P. Shah[2], M. Carroll[1], C.C. Smith[2], A.E. Perl[1]

[1]Hematology-Oncology, University of Pennsylvania, Philadelphia, [2]Hematology-Oncology, University of California, San Francisco, San Francisco, United States, [3]Hematology, Sheba Medical Center, Givatayim, Israel, [4]Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, [5]Mission Bio, Inc, South San Francisco, [6]Medicine, Roswell Park Comprehensive Cancer Center, Buffalo, United States

Background: Gilteritinib is a potent, selective FLT3 inhibitor with substantial single-agent clinical activity in relapsed/refractory (R/R) AML patients with FLT3 internal tandem duplication (ITD) and/or FLT3-D835 tyrosine kinase domain mutations (Perl AE, et al *Lancet Oncol* 2017). However, gilteritinib monotherapy is not curative and, like other FLT3 inhibitors, our data suggest acquired sequence mutations eventually arise and contribute to drug resistance (McMahon CM, et al, ASH 2017 abstract #295). In some cases, FLT3-wild type leukemic populations are selected during gilteritinib therapy and establish clonal dominance at clinical progression; in others, the original FLT3 mutation persists but is accompanied at progression by new mutations, often N-RAS or FLT3-F691L. Higher gilteritinib doses might combat resistance from FLT3-F691L, but combinatorial strategies are likely needed to circumvent other mechanisms of resistance and optimize response quality and duration.
Aims: We sought to describe and clarify mechanisms of secondary resistance to gilteritinib therapy.
Methods: Adults with R/R FLT3-mutated AML who received at least 80 mg/day of gilteritinib on a phase 1/2 study (NCT02014558) at 3 institutions provided leukemia samples at baseline, scheduled study time points, and at study withdrawal; all clinical molecular and cytogenetic data were reviewed. Baseline and progression samples were tested by a 66-gene next generation sequencing (NGS) panel; a subset with treatment-emergent mutations at disease progression were analyzed by a 24-gene single-cell NGS technique (Tapestri). Dual FLT3-ITD+/NRAS+ cell lines (MOLM14-NRAS) were generated through long-term culture of MOLM14 cells in low concentrations of the FLT3 inhibitor quizartinib. Effects of gilteritinib and/or the MEK inhibitor trametinib on the *in vitro* growth and apoptosis of MOLM14-NRAS cells were studied.
Results: 29 subjects (median age 62) have complete, evaluable cytogenetic and/or NGS data; an additional 7 have data currently being analyzed. At study enrollment 26/29 (89.7%) had a FLT3-ITD mutation, including 5 (17.2%) with both FLT3-ITD and FLT3-D835 mutations. Three subjects (10.3%) had a FLT3-D835 mutation only. 23/29 progressed during gilteritinib therapy, 5 were withdrawn for transplant or toxicity, and one remains on gilteritinib in remission. 10/21 subjects (47.6%) with serial cytogenetic data developed new cytogenetic abnormalities during therapy, including a new BCR-ABL1 fusion at progression in 1 subject. Cytogenetic evolution

did not always correlate with disease progression. New gene mutations developed on gilteritinib in 12/27 subjects (44.4%) with available NGS data, including NRAS (n=7), KRAS (n=2), FLT3-F691L (n=3), IDH2 (n=1), PTPN11 (n=2), TBL1XR1 (n=1), and CBL (n=1). Single-cell NGS confirmed that treatment-emergent RAS mutations indeed occur in FLT3-mutated cells. MOLM14-NRAS cells were resistant *in vitro* to either gilteritinib or trametinib alone, but remained sensitive to the two drugs in combination. Both serial single cell genotyping to determine whether NRAS mutations precede gilteritinib therapy and whole exome sequencing of progression samples lacking new mutations by targeted NGS are ongoing and will be updated for presentation.
Summary/Conclusion: Ras mutations are the most common mechanism of acquired mutational resistance to gilteritinib and can occur through clonal selection for FLT3-WT or clonal evolution of FLT3-mutated cells. Combining gilteritinib and MEK inhibitors is a promising strategy to prevent or delay clinical resistance.

Stem cell transplantation – Experimental

S818

REDUCED FIBROBLASTIC RETICULAR CELL EXPRESSION OF TISSUE-RESTRICTED ANTIGENS IMPAIRS PERIPHERAL REGULATION OF SELF-REACTIVE T CELLS DURING ACUTE GRAFT-VERSUS-HOST DISEASE

S. Dertschnig[1,*], P. Evans[1], C. Bennett[1], R. Chakraverty[1]

[1]*Cancer Institute and Institute of Immunity and Transplantation, University College London, London, United Kingdom*

Background: Acute graft-versus-host disease (GVHD) is a known risk factor for the later development of chronic GVHD. Although thymic injury during acute GVHD leads to the *de novo* generation of self-reactive T cells, the "two hit" hypothesis proposes that the additional loss of peripheral mechanisms is required for autoimmunity. Non-hematopoietic lymph node stromal cells (LNSC) provide a platform for peripheral tolerance by expressing and directly presenting tissue-restricted antigens (TRAs) to T cells.
Aims: The aim of this work was to test the hypothesis that alloreactive T cells could directly kill LNSC in the GVHD recipient, thus disrupting a critical mechanism for preventing injury by auto-aggressive donor T cells.
Methods: To test this concept, we used a clinically relevant model of MHC-matched, B6 female → B6 male bone marrow transplantation (BMT) where GVHD is induced by the co-transfer of anti-HY TCR-transgenic MataHari $CD8^+$ T cells.
Results: We found that fibroblastic reticular cells (FRC), a LNSC subset identified as $CD45^-CD31^-gp38^+$, were reduced ~15-fold in mice with GVHD whereas other LNSC, including lymphatic or blood endothelial cells were less affected. FRC injury was associated with a profound loss of type 3 innate lymphoid cells (ILC3). Consistent with injury to FRC, transcription of both *Il-7* and *Ccl19* genes was significantly reduced in LN stroma of mice with GVHD. Furthermore, the repertoire of putative TRAs expressed by FRC was also substantially reduced. To test the hypothesis that acute GVHD interferes with FRC capacity to express TRAs and induce peripheral tolerance, we exploited the iFABPtOVA model where truncated ovalbumin (tOVA) antigen is expressed by intestinal epithelial cells and ectopically by FRC; in the steady state, direct presentation of tOVA by FRC induces clonal elimination of antigen-specific T cells. Transfer of HY-specific T cells into iFABPtOVA male BMT recipient reduced intranodal tOVA expression by 15-fold compared to no GVHD controls. To test whether acute GVHD-mediated FRC damage impairs tolerance induction, 10^6OT-I T cells (a surrogate for self-reactive T cells) were adoptively transferred to GVHD+ or GVHD- iFABPtOVA recipients at 6 weeks following BMT. Transferred OT-I T cells were efficiently tolerized and clonally deleted in GVHD- iFABPtOVA recipients. However, in GVHD+ iFABPtOVA, OT-I T cells were not eliminated, and a large number of $IFN\gamma^+$ effector cells expanded in LN and intestinal epithelium, leading to severe weight loss and intestinal injury.
Summary/Conclusion: Taken together, our data demonstrate that acute GVHD reduces TRA display by FRC and induces loss of a critical peripheral tolerance mechanism that prevents expansion and pathogenicity of autoreactive T cells. These findings provide a potential mechanism to explain the link between acute GVHD and the succeeding autoimmunity observed in chronic GVHD.

S819

MICRORNA-155 (MIR-155) INHIBIT T LYMPHOCYTE PRENYLATION TO PROTECT AGAINST ACUTE GRAFT VERSUS HOST DISEASE (AGVHD)

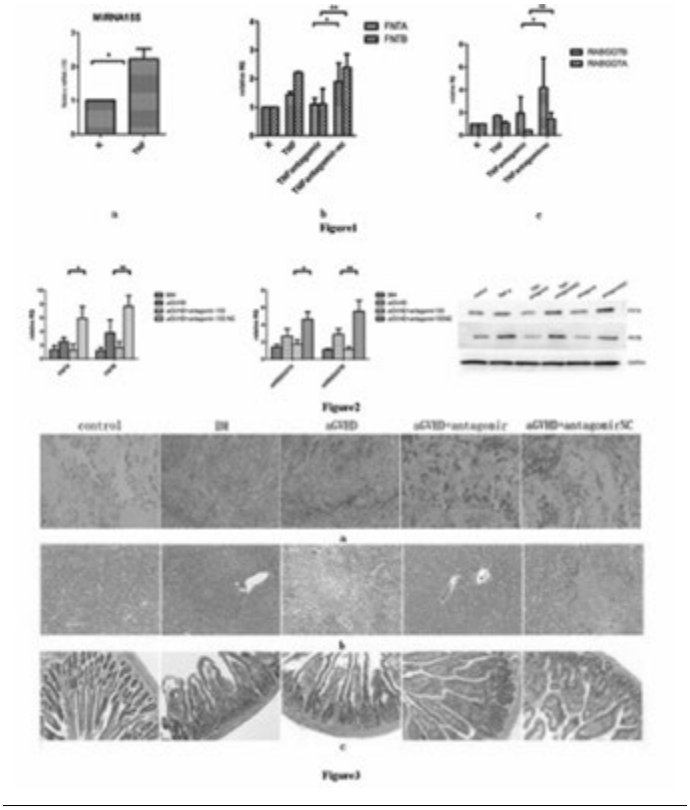
X. Wang[1,*], L. Xia[1]

[1]*Union Hospital, Tongji Medical College, Huazhong University of Science & Technology, WuHan, China*

Background: It is reported that farnesyl-transferase and geranylgeranyl-transferase inhibitors could help to reduce graft-versus-host (GVHD) disease significantly without having a negative impact on immune reconstitution. Previously, we have reported that miR-155 levels in T lymphocyte from peripheral blood of acut GVHD (aGVHD) patients and mice were significantly elevated and the inhibition of miR-155 can relieve the severe murine aGVHD.
Aims: we show a novel mechanism that inhibition of miR-155 protects against aGVHD via effects on prenylation of T cells.
Methods: TNF-α, which participates in the initiating events that culminate in aGVHD as well as amplifies the disease process once established. There-

fore, TNF-α induced T cell injury was used to simulate the course of aGVHD *in vitro*. To assess the contribution of miR-155 to prenylation of human T lymphocyte, T lymphocyte from human peripheral blood mononuclear cells (PBMCs) was selected through negative selection using magnetic bead, which was effected for 48h with antagomir-155 and antagomir-NC before stimulated with TNF-α (100 ng/ml) for 24h. To assess the contribution of miR-155 to prenylation *in vivo*, a well-established MHC mismatched bone marrow transplantation (BMT) model (C57BL/6 to BALB/c) was used to investigate the effect of deficiency of miR-155 on prenylation *in vivo*. 800 cGy X-rays irradiated BALB/c mice transplanted with 1×10^7 BM cells and 2×10^7 spleen cells from C57BL/6 mice, were treated with antagomir-155 and antagomir-NC at a loading dose of 25 mg/kg on +7d, 5 mg/kg intravenously twice weekly up to +21d post-transplant. Mice T lymphocyte magnetically isolated from PBMCs. we examined expression of prenyltransferase of both human T lymphocyte and mice T lymphocyte by Realtime quantitative-PCR and western blotting. We evaluated the severity of aGVHD using a histopathology scoring system and the farnesyltransferase activity by immunohistochemistry.

Results: The results indicated TNF-α stimulated T cells to imitate aGVHD *in vitro* induced a higher expression of miR-155 (Figure 1a, $p<0.05$), coincidentally, TNF-α stimulated T cells resulted a higher expression of farnesyltransferase(FT) subunits farnesyltransferase-α FNTA which is common to geranylgeranyltransferase -α and farnesyltransferase-β FNTB mRNA (Figure 1b) and protein levels(Figure 3), similarly increased geranylgeranyltransferase (GGT) subunits RABgeranylgeranyltransferase-α(RABGGTA) and RABgeranylgeranyltransferase-β(RABGGTB) mRNA expression (Figure 1c). Intriguingly, the suppression of miR-155 could reverse this phenomenon, which not only restrained FNTA, FNTB mRNA and protein expression (Figure 1b, $p<0.01$), but also decreased RABGGTA and RABGGTB (Figure 1c, $p<0.05$) mRNA levels. Simultaneously, in aGVHD mice models, compared with control groups, aGVHD group caused higher expression of FNTA, FNTB, REBGGTA and RABGGTB, however, which exacerbation can be reversed by inhibition of miR-155 (Figure 2a,b, $p<0.05$), all of this suggested that during the initiation and development of aGVHD, prenyltransferase and miR-155 levels were both increased, the suppression of miR-155 decreased th xpression of prenyltransferase *in vivo* and *in vitro*. Next, we consistently discovered that the miR-155 deficient aGVHD mice which had a lower histopathology score of live(Figure 3b) and small intestine(Figure 3c) possessed of decreased farnesylated proteins and farnesyltransferase activity in spleen (Figure3 a).



Figures 1,2,3.

Summary/Conclusion: this results confirmed that miR-155 simulate the role of farnesyl-transferase and geranylgeranyl-transferase inhibitors to relieve the effect of aGVHD.

S820

PIM1 INHIBITION EFFECTIVELY ENHANCES AMD3100-INDUCED HSC MOBILIZATION

T. Müller[1,*], S. Pennisi[1], A. Zwick[1], S. Decker[1], C. Klein[1], B. Rister[1], A. Rudorf[1,2], S. Kissel[1], M. Follo[1], R. Wäsch[1], A.L. Illert[1,2], C. Waskow[3], R. Oostendorp[4], D. Christine[1,5], J. Duyster[1,2]

[1]Department of Hematology and Oncology, University Medical Center Freiburg, Freiburg, [2]German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg, [3]Regeneration in Hematopoiesis and Animal Models in Hematopoiesis, Institute for Immunology, Medical Faculty Carl Gustav Carus, TU Dresden, Dresden, [4]Department of Internal Medicine III, Klinikum rechts der Isar, Technical University Munich, Munich, [5]Center for Biological Signaling studies, EXC294, BIOSS, Freiburg, Germany

Background: The interaction between hematopoietic stem cells (HSCs) and the bone marrow (BM) niche is best described for CXCR4 and its ligand, SDF-1/CXCL12. Therefore, CXCR4 antagonists like AMD3100 are used in the clinic for the collection of HSCs from patients who fail to mobilize HSCs in response to G-CSF. However, their effects are short-lived, limiting the collection time to only 4-6h and 30 % of the patients fail to collect the required amounts of CD34+ cells for autologous transplantation. We have previously demonstrated that the PIM1 kinase regulates CXCR4 receptor recycling and surface expression on HSCs. Consequently, deletion of *Pim1* results in reduced migration of HSCs towards a CXCL12 gradient and reduced homing to the BM.

Aims: We aimed to improve HSC mobilization by combining CXCR4 and PIM inhibition and demonstrate that the mobilized stem cells are capable of homing to the bone marrow niche.

Methods: In order to study the mobilization efficiency in the murine model, mice were treated with AMD3100 alone or in combination with LGB321, a novel pan-PIM inhibitor. The mice were sacrificed at various timepoints and peripheral blood (PB) was isolated. The percentage of HSCs was then determined by flow cytometry and colony-formation assays. To rule out long-term effects of PIM inhibition on stem cell engraftment, we transplanted mobilized cells into lethally irradiated recipient mice. The mechanism of HSC mobilization was studied in isolated HSC and stroma populations by analyzing mRNA levels of *Cxcl-12* and surface expression of CXCR4.

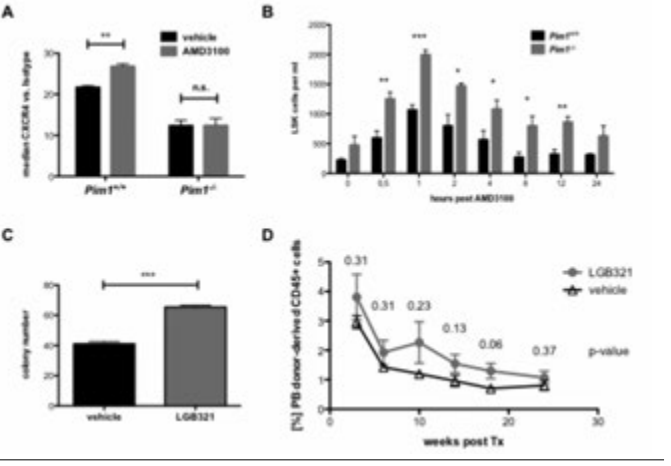


Figure 1.

Results: We found that CXCR4 inhibition using AMD3100 leads to a compensatory upregulation of CXCR4 surface expression on total BM cells as well as HSCs. This effect can be reverted by deficiency or inhibition of PIM1 (Figure 1A). As a consequence, HSC mobilization using AMD3100 is strongly enhanced and prolonged in *Pim1*-deficient mice compared to WT animals (Figure 1B). Likewise, treatment of WT animals with AMD3100 in combination with LGB321 leads to increased and prolonged HSC mobilization compared to animals treated only with AMD3100. Moreover, colony formation potential of LGB321/ AMD3100-mobilized LSKs was significantly elevated compared to AMD3100-only mobilized cells (Figure 1C). LSK cells mobilized with LGB321 and AMD3100 showed long-term engraftment (>1% donor-derived cells in 5/8 mice after week 18) after transplantation into lethally irradiated recipient mice. These cells even exhibited a strong

trend towards higher repopulating capacity compared to LSK cells mobilized only with AMD3100 (Figure 1D). Besides the downregulation of CXCR4 on HSCs, we found that the *Cxcl-12* expression as well as CXCR4 surface expression in CXCL12-abundant reticular (CAR) cells is dramatically decreased in *Pim1*-deficient mice, which even further increases the mobilization capacity of Pim1-deficient mice.
Summary/Conclusion: Our findings indicate, that PIM1 inhibition counteracts the compensatory upregulation of the CXCR4 receptor on HSCs after AMD3100 treatment and decreases CXCL12 levels within the bone marrow niche. Furthermore, PIM inhibition does not interfere with stem cell engraftment in the murine model. Therefore, targeting PIM kinases in combination with CXCR4 inhibition could improve the collection of stem cells in patients at risk for poor mobilization.

S821

PATIENTS' ABILITY TO INDUCE APOPTOSIS IN MSC PREDICTS CLINICAL RESPONSES IN GRAFT-VERSUS-HOST DISEASE

A. Galleu[1,*], C. Trento[1], C. Lomas[2], L. Dolcetti[1], D. Milojkovic[3], S. Ward[4], M. von Bonin[5], A. Bisquera[6], H. Hunter[7], N. Morley[8], E. Tholouli[9], K. Orchard[10], D.I. Marks[11], J. Apperley[3], V. Potter[12], K. Raj[12], M. Bornhauser[5,6], H. Walczak[4], C. Bennett[4], F. Dazzi[1,12]

[1]School of Cancer & Pharmaceutical Sciences, King's College London, [2]Institute of Immunity and Transplantation, University College London, [3]Imperial College Healthcare NHS Trust, [4]University College London, London, United Kingdom, [5]University Hospital Carl Gustav Carus, Dresden, Germany, [6]King's College London, London, [7]Plymouth Hospitals NHS Trust, Plymouth, [8]Royal Hallamshire Hospital, Sheffield, [9]Central Manchester University Hospital, Manchester, [10]University Hospital Southampton, Southampton, [11]University Hospitals Bristol, Bristol, [12]King's College Hospital NHS Trust, London, United Kingdom

Background: Mesenchymal stromal cells (MSCs) exhibit therapeutic activity in steroid-resistant acute Graft versus Host Disease (aGvHD). However, MSC efficacy is completely unpredictable.
Aims: We investigated the fate of infused MSCs and used our findings to design an assay to predict clinical responses
Methods: MSCs were used in a mouse model of GvHD where the disease was mediated by $CD8^+$ cells from Matahari mice (GvHD group) or defective cytotoxic $CD8^+$ cells from Matahari/perforin$^{-/-}$ mice (GvHD perf$^{-/-}$ group). Apoptosis in infused MSCs was evaluated 1 hour after infusion and infiltration of GvHD effector cells in spleen and lungs was assessed 4 days after. We then examined the cytotoxic activity against MSCs of Peripheral Blood Mononuclear Cells (PBMCs) obtained from 31 steroid-resistant aGvHD patients treated with MSCs. When performing the assay, the operator was blind to patients' clinical details. Informed consent was obtained from all patients in accordance with the local ethics committee requirements. Finally, MSCs made apoptotic *in vitro* (apoMSCs) before the infusion were tested for their ability to induce immunosuppression in our GvHD mouse model
Results: After infusion, we found that MSCs were induced to undergo apoptosis within the first hour but only in the presence of functional cytotoxic GvHD effector cells: mean caspase activation in MSCs was 26.1±7.4 and 6.2±5.6 in GvHD mice and GvHD perf$^{-/-}$ mice, respectively (p=0.0006). Notably, when we assessed the immunosuppressive activity of MSCs, we found that MSCs were able to reduce the infiltration of GvHD effector cells in lungs and spleen only in the GvHD group (Figure 1A-D). Taken together, our data demonstrate that cytotoxic cells are required to induce MSC apoptosis which is instrumental for the delivery of immunosuppression. Therefore, we inferred that the presence of cytotoxic cells in the recipient could be predictive of MSC therapeutic activity. PBMCs from 31 patients with steroid-resistant GvHD collected the day before MSC treatment were co-cultured with MSCs and MSC apoptosis was assessed using Annexin V and 7-AAD after 4 hours. The clinical overall response rate to MSCs was 37.5%. PBMCs from responders exhibited a significantly higher cytotoxicity against MSCs in comparison to non-responders (mean: 21.9%±15.4% versus 6.9%±4.7%, respectively (p<.0001) and a cut-off of 11.5% was predictive of response with 91.7% sensitivity and 90.0% specificity. The importance of the cytotoxic assay in predicting the response and its impact on the overall survival (OS) was then assessed. Higher cytotoxicity (>11.5%) was significantly associated with the response in multivariate logistic regression analysis (p<.001) and the median OS was longer in responders than in non-responders: median survival times were not reached and 60.5 days, respectively (log-rank test, p<.03). Finally, to further confirm the role played by MSC apoptosis, we tested the immunosuppressive activity of apoMSCs and we found that the infiltration of GvHD effector cells was significantly reduced both in lungs and spleen after apoMSC infusion (Figure 1E, F)
Summary/Conclusion: We propose the innovative concept that the presence of functional cytotoxic cells is required to induce MSC apoptosis which in turn is necessary to deliver immunosuppression. Therefore, patients should be stratified for MSC treatment according to their ability to kill MSCs. Alternatively, all patients may be treated with *ex vivo* apoptotic MSCs

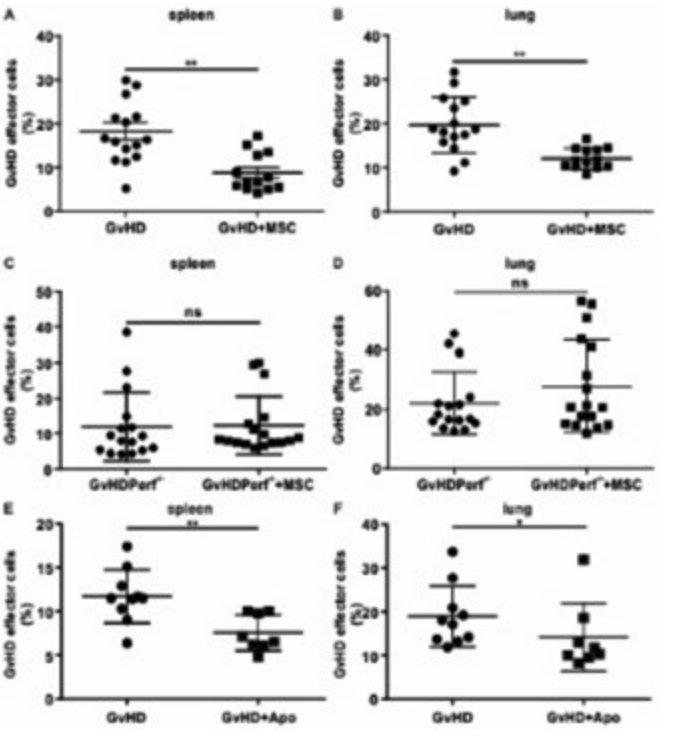


Figure 1.

S822

THE ROLE OF DONOR CLONAL HEMATOPOIESIS IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

M. Frick*[1,*], W. Chan*[1], R. Hablesreiter[1], A. Halik[1], C.M. Arends[1], J. Galan-Sousa[1], D. Noerenberg[1], O. Blau[1], M. Heuser[2], M. David[3], V. Wais[4], M. Stadler[2], K. Yoshida[5], J. Schetelig[6], E. Schuler[7], F. Thol[2], E. Clappier[8], M. Christopeit[9], F. Ayuk[9], M. Bornhäuser[6], I.-W. Blau[1], S. Ogawa[5], T. Zemojtel[10], A. Gerbitz[1], B. M. Spriewald[11], H. Schrezenmeier[12], F. Kuchenbauer[4], G. Kobbe[7], M. Wiesneth[12], M. Koldehoff[13], G. Socie[3], N. Kroeger[9], C. Thiede[6], L. Bullinger[1,14], F. Damm[1,14]

[1]Department of Hematology, Oncology, and Tumor Immunology, Charite, Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, [2]Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany, [3]Hematology and Transplantation Unit, Saint Louis Hospital, Paris / INSERM U1160, Institut Universitaire d'Hematologie, Paris / University Paris Diderot, Sorbonne Paris Cite, Paris, France, [4]Department of Internal Medicine III, University of Ulm, Ulm, Germany, [5]Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan, [6]Department of Internal Medicine I, Gustav Carus University, Dresden, [7]Department of Hematology, Oncology and Clinical Immunology, Medical Faculty, University of Duesseldorf, Düsseldorf, Germany, [8]Hematology Laboratory, Saint Louis Hospital, APHP, University Paris Diderot, Paris, France, [9]Department of Stem Cell Transplantation, University Hospital Eppendorf, Hamburg, [10]Berlin Institute of Health (BIH) Core Genomics Facility, Charité, University Medical Center, Berlin, [11]Department of Internal Medicine 5, Hematology and Oncology, University Hospital Erlangen, Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Erlangen, [12]Institute of Clinical Transfusion Medicine and Immunogenetics Ulm, German Red Cross Blood Transfusion Service Baden-Württemberg-Hessen and Institute of Transfusion Medicine, Ulm, [13]Department of Bone Marrow Transplantation, West German Cancer Centre, University Hospital of Essen, University of Duisburg-Essen, Duisburg, [14]German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg, Germany

Background: Clonal hematopoiesis of indeterminate potential (CHIP) occurs in the peripheral blood of approx. 20% of people > 60 years of age without history of hematologic disorders. CHIP associates with an increased risk of hematologic cancer, atherosclerotic disease and shorter overall survival. Case reports indicate that donor CHIP may lead to donor cell

leukemia in the recipient. Collectively, these data might raise worries about the eligibility of a stem cell donor with CHIP.
Aims: We performed a comprehensive investigation to elucidate how donor CHIP - transferred from the donor to the recipient - affects the outcome of allogeneic hematopoietic stem cell transplantation (HSCT).
Methods: We included PB or BM samples from 488 healthy HSCT donors aged ≥55 years (median 63y) collected prior to or at the time of donation. Whole blood DNA was subjected to targeted sequencing using a 60-gene panel. Clinical data from the 488 transplant recipients were collected using a standardized catalogue of HSCT outcome parameters. AML was the most frequent diagnosis leading to HSCT (n=239), followed by MPN (n=55), MDS (n=53), multiple myeloma (n=41), lymphoma (n=40), ALL (n=24), and CLL (n=18). Due to their elevated age, the majority of donors were related to the recipient (98.6 %).
Results: We identified 89 clonal mutations in 77/488 elderly donors (15.8%). The median VAF was 6.1% (range 2–43.0%) with 24 variants in 18 donors being present at a VAF ≥ 10%. 67 donors harbored one single CHIP mutation. Most frequently mutated genes were *DNMT3A* (37/488; 7.6%), *TET2* (2.3%), *ASXL1* (1.4%), *SF3B1* and *SRSF2* (0.8% each). Donor CHIP status was evenly distributed when looking at demographic, disease and transplantation characteristics of the recipient. Transplant failure, aGvHD and CMV reactivation were not affected by donor CHIP status, but cGvHD was significantly more often after HSCT with donor CHIP (54% vs. 39%; p=.018). Multivariate analysis confirmed donor CHIP as an independent risk factor for cGvHD (OR=1.8, 95% CI=1.05–2.95; p=.032). This was mainly attributed to *DNMT3A* mutations, which were associated with cGvHD in 64% of recipients (p=.004). Competing risk analysis identified donor CHIP to be associated with lower cumulative incidence of relapse/progression (CIR/P) in the recipient (p=.022) while non-relapse mortality was similar. Risk reduction was predominantly observed in recipients allografted in non-CR (n=297). Multivariate analysis confirmed donor CHIP as an independent risk factor for decreased CIR/P together with AML, cGvHD, CMV reactivation, donor age, ECOG performance and disease remission status (Fine and Gray test: HR=0.58; 95% CI=0.36–0.94; p=.029) in the entire cohort and in the subgroup of patients transplanted in non-CR (HR=0.36; 95%>CI = 0.151–0.842; p=.019). With respect to OS (median follow-up time for patients alive: 3.3y), neither donor CHIP status nor any single gene mutation affected survival, except for the MPN subgroup. MPN patients allografted with a clonal mutation (n=14) showed a reduced OS (p=.026). Serial quantification of 14 donor mutations in 50 follow-up samples suggests disproportionate expansion in most cases (Figure 1).

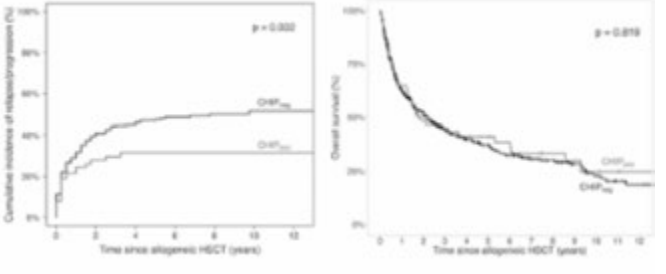


Figure 1.

Summary/Conclusion: Allogeneic HSCT from a donor with CHIP appears safe and results in similar OS in the setting of elderly and related donors. Surprisingly, donor CHIP may increase cGvHD rates and reduce relapse/progression risks. Future studies in younger and unrelated donors are warranted to confirm these results.

Acute lymphoblastic leukemia – Translational research

S823

SYNTHETIC LETHAL SCREENING IDENTIFIES CHK1 INHIBITION AS AN EXPLOITABLE VULNERABILITY IN EZH2 DEFICIENT T-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA

T.E. Leon[1,*], T. Rapoz-D'Silva[1], C. Bertoli[2], S. Rahman[1], M. Magnussen[1], N. Farah[1], J. Guerra-Assunção[3], R. Gupta[4], E. Nacheva[5], R. de Bruin[2], D.C. Linch[1], M.R. Mansour[1]

[1]Department of Haematology , UCL Cancer Institute, [2]MRC Laboratory for Molecular Cell Biology, [3]Bill Lyons Informatics Centre, UCL, [4]Stem Cell Laboratory, [5]Health Service Laboratories LLP, UCL Cancer Institute, London, United Kingdom

Background: Loss of function mutations of Enhancer of Zeste (Drosophila) homolog 2 (EZH2), the enzymatic component of Polycomb Repressive Complex 2 (PRC2), are recurrently found in T cell - Acute Lymphoblastic Leukaemia (T-ALL) and deregulation of PRC2 has been strongly associated with the pathogenesis of T-ALL. Moreover, PRC2 mutations are independent predictors of poor survival across all T-ALL subtypes and *EZH2* mutations alone are associated with a 60% risk of relapse in paediatric T-ALL, emphasizing the need for identification of drugs that selectively target PRC2 deficient leukemic cells.
Aims: Our aim was to identify specific synthetic lethal vulnerabilities induced by EZH2 deficiency, that can be exploited using drugs targeting other dependency pathways, potentially resulting in a clinically meaningful therapeutic strategy in T-ALL.
Methods: We used double-nicking CRISPR Cas9 to generate isogenic Jurkat T-ALL cells, with and without EZH2-inactivating mutations, and performed a cell-based drug screening using 220 well-characterized compounds, enriched for drugs targeting epigenetic, cell cycle and DNA repair machinery.
Results: Strikingly, we observed that *EZH2*-knock out (KO) cells are hypersensitive to checkpoint kinase 1 (CHK1) inhibition by the clinical-grade small molecule MK8776, showing an IC50 reduction of over 2-fold. To determine the specificity of this observation, we tested two different *EZH2*-KO cells against other CHK1 inhibitors (LY2603618 and CHIR-124) obtaining a similar sensitisation to CHK1 inhibition. Moreover, when we transduced the cells with shRNAs that specifically targeted CHK1 expression, we observed a significant reduction of cell growth in *EZH2*-KO cells compared to the parental control. Furthermore, pharmacological inhibition of EZH2 methyltransferase activity by its potent inhibitor GSK126 in other human T-ALL cell lines resulted in sensitization to CHK1 inhibition by MK8776. Notably, MK8776 resulted in apoptosis of EZH2-deficient cells as determined by Annexin V-PI staining. To investigate the mechanism, gene expression of two *EZH2*-KO Jurkat clones was compared to their isogenic parental control by RNA-seq. EZH2-KO cells showed a marked upregulation of genes associated with human ETP-ALL including *LYL1*, *MYCN*, *KIT* and *HHEX*, a finding that could be recapitulated with GSK126 treatment. Gene set enrichment analysis (GSEA) showed significant changes of expression in MYC target genes. Importantly, MYCN protein was markedly upregulated in both *EZH2*-KO and GSK126 treated cells. We speculated that increased MYCN expression induces high levels of replication stress and increased dependency on CHK1 for cell survival. In fact, EZH2 deficient cells showed a significant reduction in replication fork speed, compared with WT control, as determined by DNA fiber assay. In line with this observation, *EZH2*-KO cells exhibited markedly elevated levels of the ssDNA binding protein RPA and MK8776 treatment resulted in higher yH2AX expression in these cells.
Summary/Conclusion: Our findings suggest that specific inhibition of the CHK1 pathway can preferentially target PRC2-deficient leukemic cells, providing a potentially less toxic and more effective treatment for this high-risk subgroup of patients.

S824

PAX5-ELN ONCOPROTEIN PROMOTES MULTISTEP B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA IN MICE

L. Jamrog[1], G. Chemin[2], N. Prade[3], S. Lagarde[3], S. Hebrard[1], N. Rouquie[1], S. Mancini[4], E. Delabesse[3], A.A. Khamlichi[2], B. Gerby[1], C. Broccardo[1,*]

[1]CRCT, INSERM U1037, [2]IPBS, CNRS UMR5089, [3]IUCT, Toulouse, [4]CRCM, INSERM UM1068, Marseille, France

Background: *PAX5*, a gene encoding a key transcription factor in B cell differentiation, is altered in one third of B-cell acute lymphoblastic leukemia

(B-ALL). Its structural alterations (point mutations and translocations) are considered as primary oncogenic events of B-ALL while its deletion can be considered as an additional hit in leukemia process. Our previous work reported a new chromosomal t(7;9)(q11;p13) translocation in human B-ALL that juxtaposes *PAX5* and the sequence of elastin *ELN*. The resulting protein showed a dominant negative effect on the wild type PAX5 protein in patients cells.
Aims: Despite the well-known role of *PAX5* as haploinsufficient tumour suppressor gene in human B-ALL, the function of PAX5 fusion proteins in B-ALL initiation and transformation is less defined. The aim of this work is to study the impact of PAX5-ELN fusion protein in murine B cell development, decipher its molecular role and determine if the PAX5-ELN expression is a *princeps* event in the B-ALL process.
Methods: We have generated a transgenic mouse model in which PAX5-ELN is expressed from the *IgH* locus to ensure its early and restricted expression in B cell compartment.
Results: PAX5-ELN transgenic mice efficiently developed B-ALL with an incidence of 80%. Leukemic transformation systematically occurs with a minimal latency of 3 months suggesting requirement of additional oncogenic events. Indeed, exome sequencing revealed that mouse B-ALL induced by PAX5-ELN is associated with recurrent secondary mutations on *PTPN11*, *KRAS*, *PAX5* and *JAK3* genes affecting key signalling pathways required for cell proliferation. At the pre-leukemic stage, PAX5-ELN induces a partial blockade of B-cell differentiation *in vivo* characterized by an aberrant expansion of the pro-B cell compartment in steady state and in transplantation assay. At the molecular level, we showed that PAX5-ELN downregulates endogenous Pax5 expression and its canonical target genes in pro-B cells, consistent with a dominant-negative effect. At the functional level, PAX5-ELN is not able to restore B-cell differentiation of *Pax5*-deficient pro-B cells in an *ex vivo* complementation assay. Together, our functional studies demonstrate that PAX5-ELN perturbs PAX5 activity in pro-B cells to affect B-cell development before malignant transformation.
Summary/Conclusion: Our work strongly supports the role of PAX5-ELN as a potent oncoprotein in leukemia development and provides a valuable *in vivo* model recapitulating the multistep leukemogenesis process of human B-ALL.

S825

HIGH RECURRENCE OF FUSION GENES IN PHILADELPHIA-LIKE CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

G. Fazio[1,*], S. Bresolin[2], D. Silvestri[3], E. Vendramini[2], F. Locatelli[4], M.G. Valsecchi[3,5], G. Basso[2], A. Biondi[6], G. te Kronnie[2], G. Cazzaniga[1]

[1]Centro Ricerca Tettamanti, Fondazione Tettamanti, Monza, [2]Dept. Health of Woman and Child, Univ. of Padua, Padua, [3]CORS, Fondazione Tettamanti, Monza, [4]Dep. of Pediatric Hematology and Oncology , IRCCS Ospedale Pediatrico Bambino Gesù, Roma, [5]Medical Statistics, Medicine and Surgery, University of Milano-Bicocca, [6]Pediatric Dept., Univ. Milano Bicocca, Monza, Italy

Background: *Ph-like* (or *BCR/ABL-like*) ALL subtype encompasses 10-15% of BCP-ALL patients, predicts high incidence of relapses and defines a candidate subgroup for targeted treatment.
Aims: (i) To identify BCP-ALL Ph-like cases in patients treated in Study Protocols of the Italian Association of Pediatric Hematology and Oncology (AIEOP); (ii) to assess their prognosis; and (iii) to characterize their genetics basis, in terms of CNV and fusion genes.
Methods: Gene expression profiling was successfully performed on 400 Italian childhood BCP-ALL cases enrolled in AIEOP-BFM ALL2000/R2006 protocols. Of them, 142 negative for common fusion transcripts, non-high hyperdiploid and non-Down Syndrome, were defined as B-others. We implemented IKZF1-P335 MLPA kit (MRC Holland), in combination with ERG PCR to identify cases with an 'IKZF-plus' profile. RNA-target-NGS (panel with 1385 cancer-associated genes, Trusight RNA Pan Cancer, Illumina) has been setup to identify fusion genes involving recurrent genes with novel partners.
Results: Out of 142 B-other cases, 43 (30.3%, and 11% of the total BCP-ALL cohort) presented as a cluster with a gene expression signature close to the BCR/ABL signature, therefore referred to as Ph-like. Among B-others, Ph-like cases had a significantly increased proportion of males, age>10 years and WBC>20x10^9/L. The 5y event-free survival (EFS) of Ph-like patients was 54.8% (SE 8.2) vs 83.1% (3.9) in the remaining B-others patients (p<0.001), mostly due to an increased cumulative incidence of relapse (CIR: 33.9% (7.4) vs. 14.9% (3.7); p=0.009). Overall, 33 out of 142 cases experienced relapse, 14 Ph-like (33%) and 19 B-others not Ph-like (19%).
We analyzed 138/142 B-others by MLPA (in 59 cases also by DNA array), and detected 11/142 IKZF-plus patients (7/11 Ph-like and 6/7 experienced relapse). Regarding the *CLRF2* status, 15/142 cases resulted rearranged, 11/15 within Ph-like and 4/15 relapsed (exclusively in the Ph-like group). By NGS, we analyzed 61/142 patients, including all the Ph-like cases plus relapsed cases B-other not Ph-like. Among the 14 Ph-like relapsed cases, 11 were positive for a fusion gene, while the three cases negative were IKZF-plus. We detected four cases carrying *P2RY8/CLRF2*, 1 case with *TCF3/HLF*, 1 with *EBF1*-fusion, 1 with *IKZF1*-fusion, 1 with a *TTYH3*-fusion and 3 cases with *PAX5*-fusions. Considering the 28 Ph-like not relapsed patients, we detected 10 cases with various fusion genes and 4 additional cases with *PAX5*-fusion genes. Moreover, we detected two cases with *EBF1/PDGFRB*, 1 carrying *MEF2D/BCL9*, 1 positive for *RB1*-fusion, 1 with *DOT1L*-fusion and 1 patients with a noncanonical *MLL* fusion. Strikingly, the *PAX5* rearrangements are over-represented in the Ph-like subgroup (N=7), followed by *P2RY8/CRLF2* (N=6). We further identified IgH-CRLF2 (FISH) in two cases, one with a concomitant *PAX5*-fusion. Overall, 21/43 Ph-like cases were carrying a pathogenetic relevant fusion gene. Among relapsed B-others not Ph-like patients, only one carried a fusion gene, *ZNF384/CREBBP* originated from translocation t(12;16).
Summary/Conclusion: We dissected the Ph-like subgroup in the Italian cohort of children with BCP-ALL Ph-like patients, showing that, independently of other known risk features, these patients have a poor outcome and can be considered eligible for alternative treatments, in particular when they have high levels of MRD after induction. The genetic characterization of this subgroup revealed a high recurrence of fusion genes, this finding underlining the urgent need to define the pathogenetic mechanisms, with the aim to identify novel, maybe targeted, therapies.

S826

COMPREHENSIVE SCREENING OF B-OTHER ADULT ALL PATIENTS TREATED ON UKALL14 ESTIMATES FREQUENCY AND PROGNOSTIC IMPACT OF GENE ALTERATIONS AND IDENTIFIES JAK-STAT ABNORMALITIES AS MARKERS OF POOR OUTCOME

A. Moorman[1,*], E. Barretta[1], A. Kirkwood[2], C. Schwab[1], T. Creasey[1], P. Patrick[2], L. Clifton-Hadley[2], B. Patel[3], T. Menne[4], A. McMillan[5], C. Harrison[1], C. Rowntree[6], D. Marks[7], A. Fielding[8]

[1]Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, [2]Cancer Research UK & UCL Cancer Trials Centre, University College London, [3]Department of Haematology, Queen Mary University of London, London, [4]Department of Haematology, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, [5]Department of Haematology, Nottingham University Hospital NHS TRUST, Nottingham, [6]Department of Haematology, Cardiff And Vale University Health Board, Cardiff, [7]Department of Haematology, University Hospitals Bristol NHS Foundation Trust, Bristol, [8]UCL Cancer Institute, London, United Kingdom

Background: Chromosomal abnormalities are established prognostic markers in adult ALL. In the UKALL14 clinical trial patients with *BCR-ABL1*, *KMT2A-AFF1*, low hypodiploidy or a complex karyotype were classified as having high risk (HR) genetics. All remaining patients, including those lacking an established chromosomal abnormality (B-other ALL) were classified as having standard risk (HR) genetics. Recent genomic studies have identified a plethora of new gene fusions within B-other ALL.
Aims: The aim of this study was to screen UKALL14 patients with B-other ALL for ABL-class (*ABL1, ABL2, PDGFRB, CSF1R*), JAK-STAT (*CRLF2, JAK2*), *ZNF384*, and *MEF2D* rearrangements and determine their outcome to assess whether or not future such patients should be re-classified as HR.
Methods: Fixed cells left-over from diagnostic marrow samples were screened using commercially available and/or custom made dual colour break apart or dual fusion probes to detect gene rearrangements using standard methods. Endpoints were defined as per the trial protocol and analysed using standard methods. Patients were followed-up for a median of 2 years.
Results: Among 648 patients with B-cell precursor (BCP) ALL treated on UKALL14 (ISRCTN 66541317), 329 (51%) had HR genetics: *BCR-ABL1* (n=196), *KMT2A-AFF1* (n=47), low hypodiploidy (n=51) or a complex karyotype (n=35). The remaining 319 (49%) patients were classified as having SR genetics. Forty-seven patients had specific SR abnormalities: high hyperdiploidy (n=17), t(1;19) (n=15), other *KMT2A* (n=10), iAMP21 (n=4), *ETV6-RUNX1* (n=1)]. The remaining patients were classified as B-other (n=190) or had failed cytogenetics (n=82). Patients with B-other ALL, complex or failed cytogenetics were screened and rearrangements were detected at the following frequencies: *ABL1* (3/294, 1.0%), *ABL2* (0/185), *PDGFRB* (3/187, 1.6%), *CSF1R* (0/187), *CRLF2* (24/188, 13%), *JAK2* (3/190, 1.6%), *ZNF384* (11/145, 7.6%) and *MEF2D* (3/137, 2.2%). The following partners have been confirmed to date: *EBF1-PDGFRB* (n=1), *IGH-CRLF2* (n=19), *P2RY8-CRLF2* (n=5), *BCR-JAK2* (n=1), *PAX5-JAK2* (n=1) and *TCF3-ZNF384* (n=6). Among 19 cases with a complex karyotype, none

harboured an ABL-class or JAK-STAT abnormality but three had *ZNF384* (n=2) or *MEF2D* (n=1) rearrangements.
The Table 1 shows the demographic, clinical and outcome features of patients with ABL-class, JAK-STAT and *ZNF384* abnormalities. Patients with *ZNF384* or ABL-class abnormalities had a lower median age at diagnosis compared to other BCP-ALL cases (p=0.003 & p=0.06 respectively). Patients with JAK-STAT abnormalities had significantly inferior responses to initial treatment with higher rates of MRD positivity after phase 1 and phase 2 induction (p=0.015 & p<0.001, respectively). This poor response translated to a lower EFS and OS compared with other BCP-ALL patients (both p=0.01) due to higher rates of relapse/death and death: hazard ratio 1.74 (95% CI 1.10-2.73), p=0.017 and 1.81 (1.12-2.92), p=0.015, respectively. No patient with a *ZNF384* abnormality suffered a relapse or death after a median follow-up time of 2 years (10 months to 4.9 years). EFS and OS rates for the 11 patients identified were significantly higher than other BCP-ALL patients (p=0.005 & p=0.014).

Table 1.

	Total*	SR-Cyto	HR-Cyto	B-other**	ABL-class	JAK-STAT	*ZNF384*
Total	648	237	329	225	6	27	11
% Male	55%	58%	52%	59%	33%	56%	82%
Mean age (years)	45	43	46	43	37	43	35
Median WCC	8.0	6.1	13.6	5.9	9.3	12	4.6
% CR PP1	85%	87%	83%	84%	100%	77%	81%
% CR PP2	91%	94%	89%	92%	100%	85%	91%
% MRD positive PP1	62%	54%	68%	61%	50%	89%	64%
% MRD positive PP2	36%	35%	40%	38%	25%	81%	50%
EFS @ 3 y	42%	48%	36%	49%	50%	18%	100%
OS @ 3y	51%	56%	45%	55%	67%	27%	100%

* All patients with B-cell precursor ALL and available genetic data. ** Includes patients with complex karyotype. CR, complete remission PP1, post-phase 1 induction; PP2 post-phase 2 induction

Summary/Conclusion: The frequency of ABL-class abnormalities was <1% in BCP-ALL. Approximately 5% of patients harboured a *CRLF2* or *JAK2* abnormality and collectively had a significantly inferior outcome. We suggest that patients with a JAK-STAT abnormality should be classified as high-risk. Finally, 11 patients with a *ZNF384* fusion had 100% 3 years OS and EFS despite the lack of 100% MRD response.

S827

THE RIBOSOMAL RPL10 R98S MUTATION DRIVES IRES-DEPENDENT BCL-2 TRANSLATION IN T-ALL

K.R. Kampen[1,*], S. O. Sulima[2], B. Verbelen[2], T. Girardi[1], S. Vereecke[1], G. Rinaldi[3], J. Verbeeck[1], J. Verbeeck[1], J. Op de Beeck[1], A. Uyttebroeck[4], J. P. P. Meijerink[5], A.V. Moorman[6], C.J. Harrison[6], P. Spincemaille[7], J. Cools[8], D. Cassiman[7], S.-M. Fendt[3], P. Vermeersch[9], K. De Keersmaecker[1]

[1]Laboratoryfor Disease Mechanisms in Cancer, Oncology, [2]Laboratory for Disease Mechanisms in Cancer, Oncology, KU leuven, [3]Center for Cancer Biology, VIB-KU Leuven, [4]Department of Pediatric Oncology & Hematology, University Hospitals Leuven, Leuven, Belgium, [5]Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands, [6]Leukaemia Research Cytogenetics Group, Northern Institute for Cancer Research, Newcastle-upon-Tyne, United Kingdom, [7]Department of Gastroenterology-Hepatology and Metabolic Center, University Hospitals Leuven, [8]Laboratory of Molecular Biology of Leukemia, VIB-KU Leuven, [9]Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium

Background: While outcomes of pediatric T-ALL improved with the induction of multi-agent chemotherapy, event-free survival rates of 75% and long-term toxicity remain major problems of the current therapies. Thus, targeted treatments that specifically interfere with genetic subgroups are needed. As such, the R98S mutation in ribosomal protein L10 (RPL10 R98S) was identified in 8% of pediatric T-ALL cases.
Aims: Better delineation of the oncogenic role of RPL10 R98S in order to identify targeted therapies for RPL10 R98S positive T-ALL.
Methods: RPL10 R98S engineered Jurkat T-ALL cells, lineage negative (lin-) bone marrow (BM) cells from an RPL10 R98S knock-in mouse, and T-ALL PDX samples were used as models to study the effect of R98S on cell functions. Cell viability assays using PI exclusion or ATP measurements. Colony forming cell assays. Growth curves. Luciferase reporter assays. Treatment of T-ALL PDX models.
Results: A previously published quantitative proteomics screen showed that the peroxisome, the cellular organelle in which b-oxidation of very long-chain fatty acids occurs, as well as associated metabolic pathways were upregulated in RPL10 R98S Ba/F3 cells. The observed increase in peroxisomal protein expression was confirmed in RPL10 R98S JURKAT cells, in lin- RPL10 R98S mouse BM cells, and in T-ALL PDX samples. Peroxisomal β-oxidation produces high levels of the reactive oxygen species (ROS) hydrogen peroxide (H_2O_2). All RPL10 R98S mutant cell models presented a 20-40% increase in ROS expression as compared to RPL10 WT cells, causing mitochondrial dysfunction and the hypoproliferation defect of RPL10 R98S cells. Elevated ROS levels are a known source of DNA damage and may induce genomic instability in RPL10 R98S leukemia patients. Cells that encounter high ROS levels typically undergo programmed cell death, as observed in RPL10 WT cells. In contrast, RPL10 R98S cells showed enhanced survival phenotypes, especially under nutrient poor conditions. Proteomics data presented elevated levels of anti-apoptotic BCL-2 expression, which was confirmed by a 2-fold increased protein expression in all RPL10 R98S cell models, while other proteins regulating apoptosis were unchanged. BCL-2 has previously been shown to be regulated by stress-induced IRES-mediated translation. BCL-2 IRES reporter assays in Jurkat cells indicated that RPL10 R98S mutant ribosomes have an intrinsically higher capability to drive IRES-dependent BCL-2 translation, independent of oxidative stress. Elevated BCL-2 expression in RPL10 R98S cells may provide therapeutic opportunities, as BCL-2 inhibitors such as ABT-199/venetoclax are clinically used for other leukemias. We examined the efficacy of ABT-199 (50 mg/kg, once/week i.p.) on RPL10 WT and R98S mutant T-ALL PDX models. ABT-199 treatment completely suppressed appearance of human leukemia cells in the blood, prevented the occurrence of splenomegaly, and decreased the bone marrow engraftment by 30-50% in RPL10 R98S xenografted T-ALL animals, while RPL10 WT T-ALL PDX *in vivo* responses were undetectable at most leukemic infiltration sites (Figure 1).

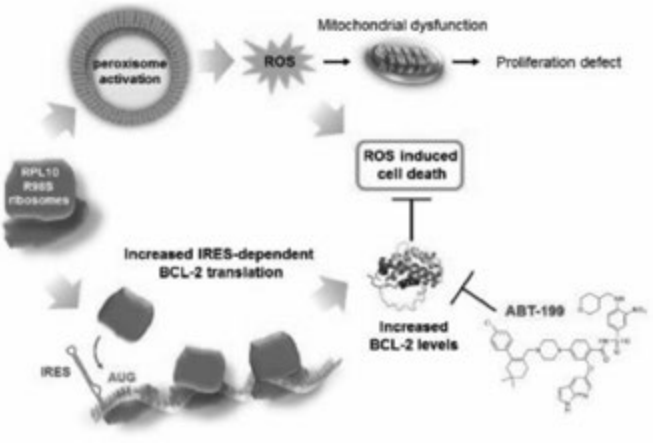


Figure 1.

Summary/Conclusion: The RPL10 R98S mutation enhances peroxisomal β-oxidation, resulting in accumulation of ROS, mitochondrial dysfunction and cellular hypoproliferation. Selective preference of RPL10 R98S mutant ribosomes for IRES-dependent BCL-2 translation allows R98S cells to survive under high oxidative stress. Finally, we demonstrate that RPL10 R98S positive T-ALL samples represent a subgroup that is highly sensitive to BCL-2 inhibitor ABT-199.

Lymphoma biology

S828

TH17 CELLS IN CLASSICAL HODGKIN LYMPHOMA ARE SUPPRESSED VIA THE PD1-PDL1 AXIS AND MAY PLAY AN IMPORTANT ROLE IN THE ACTION OF PD1 CHECKPOINT INHIBITORS

J. Taylor[1,2,*], A. Clear[1], P. Greaves[1], M. Poynton[1], M. Caporale[1], M. Calaminici[1], J. Gribben[1]

[1]Centre for Haemato-Oncology, Barts Cancer Institute, [2]Haematology , St Barts Hospital, London, United Kingdom

Background: Exciting responses are reported with Programmed Death 1 inhibitors (PD1i) in Classical Hodgkin Lymphoma (CHL). It is widely believed that PD1i act by reversing CD8 T cell exhaustion, however, predictors of PD1i response in CHL are elusive. Neither CD8 infiltration, PD1 expression nor lymphoma MHC1 expression are predictive as might be expected with a CD8-dependent mechanism of action (MOA). Instead, lymphoma MHC2 expression is associated with response and Programmed Death Ligand 1 (PDL1)-expressing tumor associated macrophages (TAM) interact primarily with PD1+CD4+ not CD8+ cells. These data suggest that CD4+T cells are central to the activity of PD1i in CHL and may be key to identifying better predictors of response.
Aims: To evaluate T cell subsets in CHL and their relationship to the PD1-PDL1 axis.
Methods: We assessed 146 patients with CHL and 24 reactive lymph node (RLN) controls. Immunohistochemistry (IHC) and novel phenotyping and spatial analysis methods were used to assess TAM, T subsets, lymphoma cells and the PD1 axis. Phenotyping was performed using IHC followed by stripping and re-staining of the same section, allowing accurate assessment of dual-positivity. Spatial analysis was performed using dual-staining on serial sections. CD4+T subset frequency by distance from the lymphoma cell was determined using HALO analysis software. To assess lymphoma influence on TAM, monocyte-derived macrophages (MdM) were differentiated from healthy PBMCs and treated with KMH2 CHL cell line supernatant.

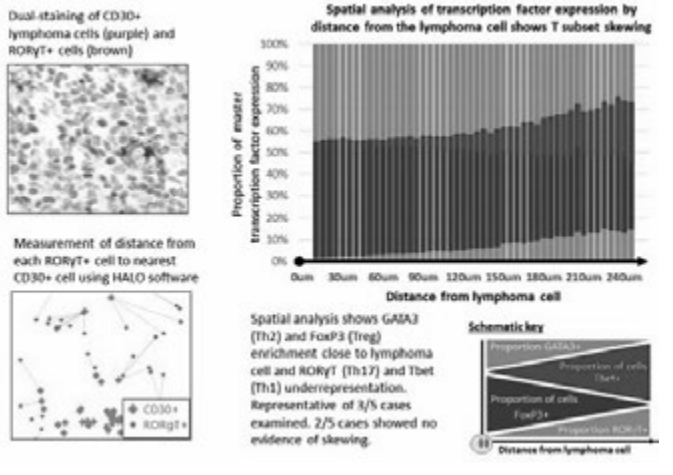


Figure 1.

Results: CD8+ lymphocytes were frequent, but PD1+ tumour-infiltrating lymphocytes (TIL) were absent or seen at low levels in CHL (median 33% vs 0.08% of nucleated cells). No correlation was seen between PD1+ TIL and PDL1. The absence of a significant PD1+ CD8 population or correlation to PDL1 is inconsistent with a central role for CD8 exhaustion. Expression of the Th17 transcription factor RORγT was reduced in CHL compared to RLN (p=0.001). Having identified that Th17 suppression was seen in CHL we assessed the role of Th17-suppressing factors; PDL1, Gal1 and IDO1 were upregulated on lymphoma cells and TAM. PDL1+TAM were enriched in areas proximal to lymphoma cells and MdM treatment with CHL cell line supernatant led to PDL1 upregulation (p=0.009) suggesting that TAM PDL1 expression is lymphoma induced. We observed an inverse correlation between RORγT+ TIL and PDL1 (p=<0.001) but no correlation between RORγT+ TIL and IDO1 or Gal1. No correlation was seen between PDL1 and other CD4+T-related transcription factors: Tbet (Th1), GATA3 (Th2) or FoxP3 (Treg). Spatial analysis revealed skewing of the T cell infiltrate by distance from the lymphoma cell. Skewed cases revealed enrichment of FoxP3+ and GATA3+ and underrepresentation of Tbet+ and RORγT+ TIL around lymphoma cells (Image, p=<0.0001). This powerful method corrects for variations in tumour content and highlights patterns that are hard to assess by eye. The finding of a distribution similar to Th1 suggests a Th17 anti-tumour role (Figure 1).
Summary/Conclusion: The data presented support a model of lymphoma suppression of Th17 cells via the PD1-PDL1 axis. This would explain PD1i activity despite a lack of observed PD1 expression. This is supported by: i) low Th17 numbers, ii) lymphoma-induced expression of Th17-suppressing factors, iii) an inverse correlation to PDL1 expression that is not seen with other CD4+ subsets and iv) a spatial distribution pattern similar to Tbet+ TIL. These data in conjunction with reports of an association between MHC2 with a response to PD1 inhibition highlight a central role for Th17 cells in the MOA of PD1i in CHL.

S829

EFFICACY OF VENETOCLAX AS A SINGLE AGENT AND IN COMBINATION WITH IBRUTINIB IN REFRACTORY OR RELAPSED T-PROLYMPHOCYTIC LEUKEMIA

C. Kornauth[1,*], B. Boidol[2], J. Koller[1], A. Ringler[2], E. van der Kouwe[1], L. Kazianka[1], G. Hörmann[3], I. Simonitsch-Klupp[4], U. Jäger[1], S. Kubicek[2], P. Staber[1]

[1]Clinical Department of Haematology, University Clinic for Internal Medicine I, Medical University of Vienna, [2]Center of Molecular Medicine (CeMM), Austrian Academy of Science, [3]Department of Laboratory Medicine, [4]Department of Pathology, Medical University of Vienna, Vienna, Austria

Background: T-cell prolymphocytic leukemia (T-PLL) is a rare and aggressive T-lymphoid malignancy usually refractory to current treatment strategies or complicated by relapse and associated with short overall survival.
Aims: We set out to identify novel effective treatments for T-PLL patients, especially new combinatorial treatment strategies.
Methods: We applied next-generation functional testing of primary patient-derived leukemia cells using a library 106 FDA approved anticancer drugs or compounds currently in clinical development. Combinatorial functional testing of T-PLL patient samples with venetoclax in combination with 14 other agents such as ibrutinib, idelalisib, 5-azacytidine, 6-mercaptopurin, alitretinoin, bendamustine, bortezomib, and cisplatin was performed. Bliss' independence was used to assess synergistic or antagonistic effects of the respective combination partners *ex vivo*.

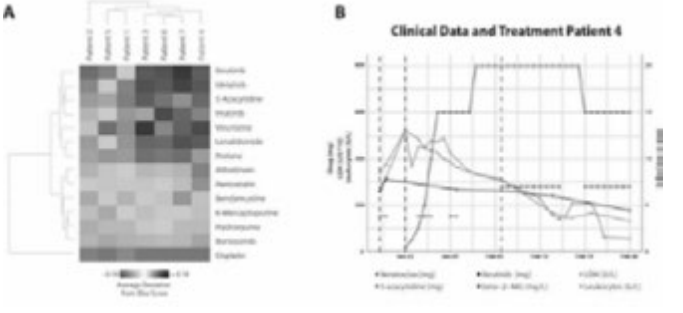


Figure 1.

Results: We found that the B-cell lymphoma 2 (BCL-2) inhibitor venetoclax (ABT-199) demonstrated the strongest T-PLL-specific response *ex vivo* when comparing individual drug response in 86 patients with refractory hematologic malignancies. Based on these results, off-label venetoclax treatment was commenced in 2 late-stage refractory T-PLL patients resulting in striking clinical responses (Boidol et al., Blood 2017). The most promising candidates for combination were the Bruton tyrosine kinase inhibitor ibrutinib, the PI3K-inhibitor idelalisib, and the DNA methyltransferase inhibitor 5-azacytidine, whereas cisplatin antagonized the effect of venetoclax across all patient samples tested (Figure 1A). Off-label co-treatment of venetoclax and 5-azacytidine was commenced in a T-PLL patient at third relapse after a failed re-treatment attempt with alemtuzumab presenting with dyspnea, leukocytosis, splenomegaly and reduced clinical condition. Combination of 5-azatiidine and venetoclax demonstrated modest clinical and laboratory improvement. In contrast, after initiating co-treatment with ibrutinib and venetoclax a dramatic response was observed as evidenced in significant clinical improvement, significant decrease in spleen size and disease related laboratory values such as leukocytosis, LDH, and B2-MiG (Figure 1B). At time of report the response is ongoing.

Summary/Conclusion: Our findings demonstrate first evidence of single-agent activity of venetoclax. Furthermore, we identify ibrutinib as an effective combination partner both *ex vivo* and in patients, offering a novel treatment concept in T-PLL.

S830

REFINING DIFFUSE LARGE B CELL LYMPHOMA SUBGROUPS USING INTEGRATED ANALYSIS OF MULTI-LEVEL MOLECULAR PROFILES IN A PROSPECTIVE COHORT: A LYSA STUDY

S. Dubois[1,2,*], B. Tesson[3], P.-J. Viailly[2], S. Mareschal[2], E. Bohers[2], P. Ruminy[2], P. Peyrouze[4], C. Copie-Bergman[5], J.-P. Jais[6], C. Haioun[7], G. Salles[8], R. Delarue[9], T.J. Molina[10], H. Tilly[2], F. Jardin[2]

[1]CHU de Rouen, [2]Centre Henri Becquerel, Rouen, [3]Institut Carnot CALYM, Paris, [4]Université de Lille 2, Lille, [5]IMRB – Inserm U955, AP-HP Hôpital Henri Mondor, Créteil, [6]Inserm UMRS 872, AP-HP Hôpital Necker, Paris, [7]Unité Hémopathies Lymphoïdes, AP-HP Hôpital Henri Mondor, Créteil, [8]CNRS UMR5239, Hospices Civils de Lyon, Lyon, [9]Department of Hematology, [10]Pathology Department, AP-HP Hôpital Necker, Paris, France

Background: Gene expression profiling has enabled the identification of three distinct DLBCL subtypes: ABC, GCB and PMBL. More recently, techniques such as Next Generation Sequencing (NGS) and Copy Number Variation (CNV) analysis have led to an increasingly detailed characterization of the genomic profiles of DLBCL.
Aims: The aim of this study was to perform a fully integrated analysis of mutational, genomic, and expression profiles of DLBCL to refine disease subtypes using molecular tools.
Methods: 215 patients with *de novo* DLBCL from the prospective, multicenter and randomized LNH-03B LYSA clinical trials were included. Gene expression profiling (GEP) was obtained with HGU133+2.0 Affymetrix GeneChip arrays. Mutational profiles were established by Lymphopanel NGS, targeting 34 key genes (Dubois *et al*., CCR 2016). CNV were analyzed by array Comparative Genomic Hybridization. FISH was performed to identify BCL2, BCL6 and MYC rearrangements. IHC was performed on CD10, BCL6, MUM1, BCL2, FOXP1, IgM and MYC. We applied unsupervised independent component analysis (ICA) to GEP data and identified 38 gene signatures (components) reflecting transcriptomic variability across our DLBCL cohort. SignatureDB and MSigDB databases were used to interpret the biological function of each component.

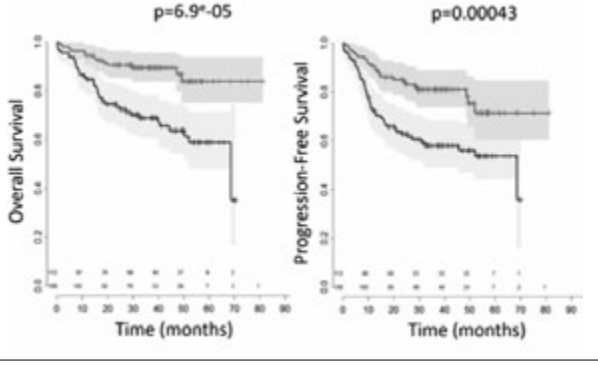


Figure 1.

Results: Many of the components were closely related to well-known DLBCL features such as cell of origin, stromal and MYC signatures, as evidenced by gene set overrepresentation analyses. Correlation of the components' expression with gene mutational status, CNV data, IHC and FISH identified interesting associations. Stromal components correlated positively with *B2M* and *TNFAIP3* mutations (FDR≈0.02) and negatively with *CD79B* mutations (FDR≈0.008). The GCB/ABC signature correlated positively with *EZH2*, *BCL2*, *STAT6* and *GNA13* mutations (FDR <10^{-3}-0.06) and negatively with *CD79B* and *PIM1* mutations (FDR 0.005 - 0.06). PMBL and interferon components shared numerous correlations with mutations including *SOCS1*, *B2M*, *ITPKB*, *STAT6*, *CD58*, *XPO1* and *TNFAIP3* (FDR <10^{-3}- 0.04). Both components were also linked to gain/amplification of the 9p24 locus containing *PDL1*/*PDL2*. Interestingly, PMBL with high interferon component expression also presented low T cell component expression, in keeping with the negative regulation of T lymphocytes by PDL1/2 during the immune response. ICA also identified variability among the GCB subgroup, delineating proliferation-high / T cell-low GCB patients versus GCB patients with the opposite phenotype. The Myc component was strongly linked to FOXP1, MUM1, IgM and MYC IHC expression, as well as to MYC rearrangement, showcasing a poor prognosis non-GCB population. Stromal components were strongly correlated with better OS ($p<10^{-4}$- $p<0.05$) and PFS ($p<10^{-3}$) (Figure 1). Another component correlated with *MYD88* and *CD79B* mutations as well as gain of 19q13 locus was found to be correlated with poor OS and PFS (p=0.01): gain of 19q13 locus has previously been shown to be linked to reduced event-free survival.
Summary/Conclusion: ICA is an unsupervised approach, which is sensitive and efficient for the analysis of DLBCL expression profiles. We characterized components linked to gene mutational status, to the presence of CNV, to IHC and FISH results and, notably, to patient outcome. The results of this type of integrated analysis should enable a global and multi-level view of DLBCL, as well as improve our understanding of DLBCL subgroups.

S831

EVIDENCE FOR INTERCLONAL TUMOR COOPERATIVITY IN MEDIATING IBRUTINIB RESISTANCE BY BTK-C481SER EXPRESSING MYD88 MUTATED LYMPHOMA CELLS

G. Yang[1,*], J. Chen[1], X. Liu[1], M. Munshi[1], L. Xu[1], N. Tsakmaklis[1], M. Demos[1], A. Kofides[1], M. L. Guerrera[1], G. Chan[1], C. Patterson[1], K. Meid[1], J. Gustine[1], T. Dubeau[1], P. Severns[1], J. Castillo[1], Z. Hunter[1], J. Wang[1], S. Buhrlage[1], N. Gray[1], S. Treon[1]

[1]Dana-Farber Cancer Institute, Boston, United States

Background: Acquired ibrutinib resistance due to BTKCys481 mutations occurs in B-cell malignancies, including those with MYD88 mutations. BTKCys481 mutations are usually sub-clonal, and their relevance to clinical progression remains unclear.
Aims: We sought to define the signaling pathways that promote ibrutinib resistance in MYD88 mutated WM and ABC DLBCL cells. Furthermore, we sought to delineate mechanisms responsible for promoting ibrutinib resistance in neighboring BTK wild-type tumor cells.
Methods: We engineered BTKCys481Ser and BTKWT expressing MYD88 mutated WM and ABC DLBCL cells, and performed signaling studies that included pathways responsible for aberrant MYD88 signaling, and downstream BTK signaling. Furthermore, cytokine release was evaluated by multiplex assay and correlated to findings in patients who progressed on ibrutinib after acquisition of BTK Cys 481 mutations. Lastly, co-culture experiments with BTK Cys 481 mutated and unmutated cells were performed and viability assessed.
Results: Comparing to BTK^{WT}, $BTK^{Cys481Ser}$ expressing cells re-activates BTK–PLCγ2–ERK1/2 signaling in the presence of ibrutinib. Use of ERK1/2 inhibitors triggered apoptosis in $BTK^{Cys481Ser}$ expressing cells, and showed synergistic cytotoxicity with ibrutinib. ERK1/2 re-activation in ibrutinib treated $BTK^{Cys481Ser}$ cells was accompanied by release of many pro-survival and inflammatory cytokines, including IL-6 and IL-10 that were also blocked by ERK1/2 inhibition. To clarify if cytokine release by ibrutinib treated $BTK^{Cys481Ser}$ cells could protect non-transduced BTK^{WT} MYD88 mutated malignant cells, we used a Transwell™ co-culture system, and showed that non-transduced BTK^{WT} MYD88 mutated WM or ABC DLBCL cells were rescued from ibrutinib induced cell death when co-cultured with $BTK^{Cys481Ser}$ but not their BTK^{WT} expressing counterparts (Figure 1). Use of IL-6 and/or IL-10 blocking antibodies abolished the protective effect conferred on non-transduced BTK^{WT} by co-culture with $BTK^{Cys481Ser}$ expressing WM or ABC DLBCL cell counterparts. Rebound of IL-6 and IL-10 serum levels also accompanied disease progression in WM patients with acquired BTK^{Cys481} mutations.

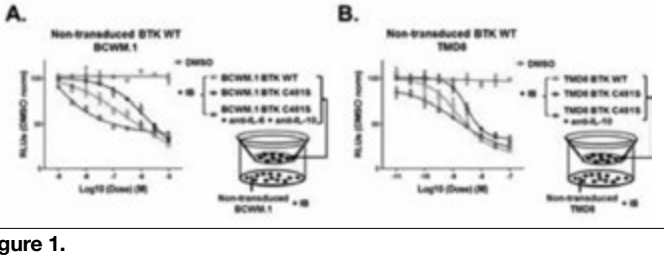


Figure 1.

Summary/Conclusion: Our findings show that the $BTK^{Cys481Ser}$ mutation drives ibrutinib resistance in MYD88 mutated WM and ABC DLBCL cells through re-activation of ERK1/2 activation, and can confer a protective effect on BTK^{WT} cells through a paracrine mechanism. The findings provide evidence for interclonal tumor cooperativity in mediating ibrutinib resistance.

Cell and gene therapy: Clinical results

S832

EFFICACY AND SAFETY OF CD22-DIRECTED CAR-T CELL THERAPY IN 15 PEDIATRIC REFRACTORY OR RELAPSED B ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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J. Pan[1,*], B. Deng[2], S. Liu[1], J. Wang[1], T. Wu[3], Z. Gao[3], Y. Lin[1], Y. Zhao[3], Y. Zhang[3], X. Tan[3], A. H. Chang[4], C. Tong[1]

[1]Department of Hematology, [2]Department of Immunotherapy, [3]Department of bone marrow transplantation, Beijing Boren Hospital, Beijing, [4]Clinical Translational Research Center, Shanghai Pulmonary Hospital, Shanghai, China

Background: Many patients with refractory or relapsed B cell acute lymphoblastic leukemia (rrB-ALL) have disease progressed again in one year after initial successful CD19-directed chimeric antigen receptors T (19-CAR-T) cell therapy. Most of them are probably refractory to secondary 19-CAR-T therapy. The relapse rate in rrB-ALL is still high even after allogeneic hematopoietic stem cell transplantation (allo-HCT). Therefore, new modalities are needed.
Aims: The efficacy and safety of a novel CD22-directed CAR-T (22-CAR-T) cell therapy in pediatric patients with rrB-ALL were evaluated.
Methods: Between July 6, 2017 and January 8, 2018, consecutive 15 pediatric patients (pts) with rrB-ALL who received 22-CAR-T cell therapy were enrolled in Beijing Boren Hospital. The median age was 8 (2-18) years old. The median disease course was 21 (5-84) months. 4/15 pts relapsed post-allo-HCT and 11/15 pts relapsed after chemotherapy. 14/15 pts received 19-CAR-T therapy before. One patient had weak CD19 expression on leukemia cells when relapsed. 11/15 pts had hematologic relapse with 42 (5-95.5) % blasts in bone marrow (BM), 2 pts remained minimal residual disease (MRD) positive by flow cytometry (FCM-MRD), and 2 pts developed extramedullary diseases (EMDs) only. All pts had CD22 expression on leukemia cells. A lentiviral vector was used to carry a second-generation CAR including anti-CD22 scFV derived from a humanized CD22 antibody, 4-1BB co-stimulatory and CD3z signaling domains. Manufacture of CAR-T cells from peripheral blood mononuclear cells commenced on day of leukapheresis and was completed in 7-8 days. The median doses of 22-CAR-T cells infused were 8.2 (0.5-34.7) × 10^5/kg in non-transplant pts, and 0.9 (0.7-5.0) × 10^5/kg in transplant pts. The 22-CAR-T cell expansion and cytokine releasing syndrome (CRS) were monitored after infusion. The efficacy in BM was evaluated on day 30, and EMDs were examined by imagine tests (ultrasonography, MRI and PET-CT) on day 30 as well. Long term follow-up was carried out and the outcome depended on several factors.
Results: Peak expansion of CAR-T cells (3.2%-71.7% of $CD3^+$ cells) was detected at day 11±1.8. On day 30, 13/15 (86.7%) pts had response. 10/11 hematologic relapsed pts achieved CR or CR with incomplete count recovery (CRi), and 9 of them became FCM-MRD negative. 1/2 $FCM\text{-}MRD^+$ case turned to $FCM\text{-}MRD^-$. 1/ 2 EMDs pts achieved CR, but the other patient had only partial response (PR) (mass size: from 10.0×5.3×5.9 cm to 6.2×2.3×3.1 cm). Both 2 pts with no response to 22-CAR-T therapy remained strong CD22 expression on leukemia cells. All pts experienced mild to moderate (grade 0-2) CRS only and 2 pts occurred grade 1 neurotoxicity. There was no significant difference in CRS between pts with and without allo-HCT (p=0.41). With a median follow-up time of 108 (46-199) days, 6 pts had been bridged to allo-HCT after 22-CAR-T cell therapy.11/12 CR/CRi pts achieved progression-free survival (PFS), and 1 of 12 CR/CRi pts had relapsed on day 50. The 6-month PFS rate was 91.7 (91.5-91.9) %.
Summary/Conclusion: Our results indicate that CD22-directed CAR-T cell therapy is quite effective and safe for treating pediatric rrB-ALL, and could be valuable especially for those who have failed to previous 19-CAR-T therapy. Extended observation period will be required to determine the long-term outcome and whether subsequent allo-HCT could further reduce the relapse rate after CAR-T cell therapy.

S833

LENTIGLOBIN GENE THERAPY FOR TRANSFUSION-DEPENDENT β-THALASSEMIA (TDT) IN PATIENTS WITH NON-$β^0/β^0$ GENOTYPES: UPDATED RESULTS FROM NORTHSTAR-2

F. Locatelli[1,*], M. Walters[2], J. Kwiatkowski[3], J. Rasko[4,5], S. Hongeng[6], G. Schiller[7], J. Porter[8], M. Sauer[9], A. Thrasher[10], I. Thuret[11], E. Yannaki[12], H. Elliot[13], B. Deary[13], M. Gayron[13], A. Ramirez-Santiago[13], A. Thompson[14]

[1]Department of Pediatric Hematology and Oncology, IRCCS Ospedale Pediatrico Bambino Gesù, Rome, Italy, [2]Blood and Marrow Transplant Program, Division of Hematology/Oncology, UCSF Benioff Children's Hospital Oakland, Oakland, [3]Division of Hematology, Children's Hospital of Philadelphia, Philadelphia, United States, [4]Institute of Haematology, Royal Prince Alfred Hospital, [5]Sydney Medical School, University of Sydney, Camperdown, Australia, [6]Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, [7]David Geffen School of Medicine, University of California Los Angeles, Los Angeles, United States, [8]Department of Haematology, University College London Hospitals, London, United Kingdom, [9]Medizinische Hochschule Hannover, Hannover, Germany, [10]Molecular Immunology Unit, UCL Great Ormond Street Hospital, London, United Kingdom, [11]Hématologie pédiatrique, Hôpital de la Timone, Marseille, France, [12]Gene and Cell Therapy Center, George Papanikolaou General Hospital of Thessaloniki, Thessaloniki, Greece, [13]bluebird bio, Inc., Cambridge, [14]Division of Hematology, Oncology & Stem Cell Transplant, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, United States

Background: LentiGlobin Drug Product (DP) contains autologous CD34+ hematopoietic stem cells (HSCs) transduced *ex vivo* with the BB305 lentiviral vector (LVV) encoding β-globin with the T87Q point mutation which permits quantification of transgenic hemoglobin (Hb) in transduced cells. The safety and efficacy of LentiGlobin in patients with transfusion dependent β-thalassemia (TDT) was evaluated in the phase 1/2 Northstar (HGB-204; NCT01745120) study where 9/10 patients with non-$β^0/β^0$ genotypes were free from chronic packed red blood cell (pRBC) transfusions for a median of 29 months with a median Hb of 10.2 g/dL (Kwiatkowski, ASH 2017). To further improve clinical results, the LentiGlobin DP manufacturing process was modified to increase transduction rates.
Aims: To evaluate the efficacy and safety of LentiGlobin DP with these refinements in patients with TDT and non-$β^0/β^0$ genotypes in the ongoing phase 3 Northstar-2 study (HGB-207; NCT02906202).
Methods: Patients with TDT (≥100 mL/kg/yr of pRBCs or ≥8 pRBC transfusions/yr) underwent HSC mobilization with G-CSF and plerixafor. Collected HSCs were transduced with the BB305 LVV. Patients underwent myeloablative conditioning with pharmacokinetic-adjusted busulfan dosage, were infused with transduced cells, and were followed for engraftment, pRBC transfusion independence, VCN, HbA^{T87Q}, adverse events (AEs), vector integration profile, and evidence of replication competent lentivirus (RCL). Summary statistics are presented as median (min – max).
Results: As of 13 October 2017, 7 patients with TDT and non-$β^0/β^0$ genotypes (age 20 [15 – 24] yrs) have been treated in Northstar-2 with a median follow-up of 3 (1 – 9) months. Data are reported for 6 treated patients with > 1 month follow-up. The time to neutrophil and platelet engraftment was 21.5 (17 – 26) and 44 (35 – 46) days, respectively. All patients successfully engrafted with no graft failure or death. AEs were consistent with myeloablative conditioning and no DP-related AEs were observed. Serious AEs after DP infusion included one AE each of transfusion reaction (grade 2) and hypotension (grade 3). No evidence of clonal dominance or RCL has been observed. As of 1 December 2017, 11 DP lots had been manufactured for 10 patients in Northstar-2 of which 9 could be assessed for % transduced CD34+ cells. In Northstar, 22 DP lots were manufactured for 18 patients. The refined manufacturing process used in Northstar-2 yielded higher DP VCNs (median 3.3 vs 0.7) and higher % transduced cells (median 82% vs 32%). In Northstar-2, peripheral blood VCN was 0.25 – 2.3 in 6 treated patients with 3 – 9 months of follow-up. The 3 patients with ≥ 6 months of follow-up have been transfusion-free for 7.5, 4.2, and 5.3 months with total Hb levels of 12.5, 8.4, and 11.5 g/dL at last visit. The first patient treated began phlebotomy 6 months post-infusion. By month 3, 5/6 patients had HbA^{T87Q} levels of ≥ 6.5 g/dL, and total Hb of 10.4 – 12.5 g/dL with HbA^{T87Q} contributing 58 – 91% of total Hb.
Summary/Conclusion: The refined manufacturing process for LentiGlobin in Northstar-2 has yielded higher DP VCNs and % transduced CD34+ cells. Early data suggest that these improved DP parameters translate into clinically significant levels of HbA^{T87Q} that can result in near-normal or normal total Hb production allowing patients to be free of transfusions. The safety profile to date is similar to that observed in Northstar. Longer follow-up and results observed in additional patients in Northstar-2 will be presented.

S834

LONG-TERM OUTCOME OF A COHORT OF ELDERLY ACUTE MYELOID LEUKEMIA PATIENTS, NOT ELIGIBLE FOR ALLOGENEIC STEM CELL TRANSPLANTATION, INFUSED WITH ALLOREACTIVE NATURAL-KILLER CELLS AS CONSOLIDATION THERAPY

S. Parisi[1,*], L. Ruggeri[2], A. Bontadini[3], E. Dan[1], M.R. Motta[1], S. Rizzi[1], S. Trabanelli[1], D. Ocadlikova[1], M. Lecciso[1], V. Giudice[3], F. Fruet[3], E. Urbani[2], C. Papayannidis[1], G. Martinelli[14], F. Bonifazi[1], R.E. Lewis[5], M. Cavo[1], A. Velardi[2], R. M. Lemoli[6], A. Curti[1]

[1]Department of Experimental, Diagnostic and Specialty Medicine, Institute of Hematology L. and A. Seràgnoli, S. Orsola-Malpighi Hospital, University of Bologna, Bologna, [2]Department of Medicine, Division of Hematology and Clinical Immunology, University of Perugia, Ospedale Santa Maria della Misericordia, Perugia, [3]Immunohematology Service and Blood Bank, S.Orsola-Malpighi Hospital, Bologna, [4]Department of Hematology, Istituto Scientifico Romagnolo per Studio e Cura dei Tumori, Meldola, Forlì-Cesena, [5]Department of Medical and Surgical Sciences, University of Bologna, Bologna, [6]Clinic of Hematology, Department of Internal Medicine (DiMI), University of Genoa, IRCCS Azienda Ospedaliera Universitaria S. Martino-IST, Genova, Italy

Background: Several pre-clinical and clinical studies revealed the potential of adoptive immunotherapy with alloreactive, natural-Killer (NK) cells in acute myeloid leukemia (AML) patients. Indeed, adoptively transferred NK cells can be successfully expanded *in vivo* and significantly impact on leukemic cells killing.
Aims: Aim of this work is to establish the impact on long-term survival and relapse rate of adoptive immunotherapy with alloreactive NK cells in a cohort of AML patients, who had been infused with alloreactive NK cells in CR, as post-consolidation therapy.
Methods: Seventeen AML patients, with a median age of 64 years (range 53-73), were enrolled in our study. Patients were in first complete remission (CR) after standard chemotherapy regimens and were unfit for allogeneic stem cell transplantation (ASCT). Patients in morphologic or better CR with an haploidentical KIR-L–mismatched donor, received NK cells after an immunosuppressive chemotherapy regimen based on fludarabine 25 mg/mq from day 7 to 3 and cyclophosphamide 4 g/mq on day 2. Two days after cyclophosphamide administration, patients received the NK cell infusion (day 0), which was followed by subcutaneous administration of IL-2 ($10x10^6$ IU/day, 3 times weekly) for 2 weeks (6 doses total). To correlate donor NK cell activity with clinical response, donor NK cells were assessed before and after infusion.
Results: As previously reported, NK cell infusion was well-tolerated and no signs of GVHD were described. The median follow-up is now extended to 55.5 months (range 6-125 months) *vs* 22.5 (range 6-68 months) in the original publication. Eight out of 16 evaluable patients (50%) are alive disease-free. Among relapsing patients (8 of 16), median time to relapse was 9 months (range 5-51 months). Three of the relapsed patients maintained a prolonged CR for 15, 24 and 51 months, respectively. Among them, the patient relapsing after 51 months received a second NK infusion, thus obtaining a second CR. All patients treated with molecular disease achieved molecular CR. Based on these data, 11 of 16 (69%) patients were considered as responders, whereas 5 of 16 (31%) were non-responders. These long-term clinical results were compared with the outcome of patients from a historical control cohort, treated with standard chemotherapy regimen and who did not receive NK immunotherapy. In the latter group, 14 out of 15 patients (93%) relapsed, with a median time to relapse of 11 months (range 3-79). Due to the low numbers of evaluable patients, the difference in terms of DFS between the two groups of patients is not statistically significant, although a trend toward an increase of DFS for patients who received NK infusion may be observed. In agreement with our previous work, the predictive impact of higher alloreactive NK donor repertoire on clinical outcome was confirmed also when a longer-follow up was evaluated.
Summary/Conclusion: In conclusion, adoptively transferred alloreactive NK cells have the potential to induce prolonged control of AML in the non transplant setting. Moreover, the composition of NK graft in terms of frequency of alloreactive NK cells is likely to influence the long-term clinical response.

S835

CART19-BE-01: A EUROPEAN ACADEMIC TRIAL ON THE USE OF ARI-0001 CELLS (A3B1:CD8:4-1BB:CD3Z CAR19 CELLS) IN PATIENTS WITH CD19+ RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA

J. Delgado[1,*], S. Rives[2], M. Castella[3], M. Caballero-Baños[3], V. Ortiz-Maldonado[1], A. Boronat[3], E. Garcia-Rey[4], T. Baumann[1], M. Torrebadell[2], J. Cid[5], M. Lozano[5], C. Llanos[6], B. Marzal[3], J. Esteve[1], J.M. Canals[7], J. Tabera[4], A. Vilarrodona[4], E. Trias[4], J. Yagüe[3], A. Urbano-Ispizua[1], M. Juan[3]

[1]Hematology, Hospital Clinic, [2]Hematology, Hospital Sant Joan de Deu, [3]Immunology, Hospital Clinic, [4]Banc de Sang i Teixits, [5]Apheresis Unit, Hospital Clinic, [6]Clinical Trials, Hospital Sant Joan de Deu, [7]University of Barcelona, Barcelona, Spain

Background: The prognosis of relapsed/refractory acute lymphoid leukemia (R/R ALL) is very poor, particularly in patients relapsing after allogeneic hematopoietic cell transplantation (alloHCT). New agents, such as inotuzumab or blinatumomab, have improved the complete response rate (CRR) in R/R ALL, but with a progression-free survival (PFS) shorter than 6 months. In the last decade, several chimeric antigen receptor anti-CD19 (CAR19) constructs have been developed. One of them (tisagenlecleucel) was approved by the FDA for pediatric or young adults with R/R ALL. The approval was based on a CRR around 80% with a 6-month PFS around 70%.
Aims: To develop our own academic CAR19 construct for clinical use.
Methods: We selected the anti-CD19 A3B1 hybridoma licensed by our institution, identified the scFv sequence and incorporated the CD8, 4-1BB and CD3z modules next to it. We cloned it into a 3rd generation lentiviral vector and transduced PBMCs from buffy coats after activation with CD3 and CD28 TransACT polymeric nanomatrix (ARI-0001 cells). Once cytotoxicity and specificity were confirmed *in vitro* and *in vivo* (in NALM6-xenograft NSG murine models), we scaled-up both lentiviral and cell production, the latter using the CliniMACS Prodigy System (Miltenyi). After reaching all pre-specified acceptance criteria in lymphoapheresis from 3 healthy donors, the Spanish Agency of Medicines approved our IND and also our first pilot clinical trial (clinicaltrials.gov NCT03144583) on May/2017. Eligibility criteria included R/R ALL (adult and pediatric), NHL and CLL who had failed all standard available therapy, but in this abstract we will only report the outcome of patients with R/R ALL.
Results: As of February 2018, we have recruited 8 patients with R/R ALL, all but one relapsing after alloHCT. Median age was 19.5 years (range 3-34) and 50% were female. Patients were included in the trial in first (1), second (5), and third (2) relapse. Two patients were in CR with negative minimal residual disease (MRD) at study inclusion. The median percentage of blasts in bone marrow was 92% (range 73-96%) for the remaining 6 patients. We successfully prepared ARI-0001 cells in all patients, although two patients required two procedures (2/10 [20%] production failure rate globally). After fludarabine (90 mg/m^2) and cyclophosphamide (900 mg/m^2) chemotherapy, we infused $0.5\text{-}5x10^6$ ARI-0001 cells/kg to 7 patients. In one patient the infusion was delayed due to pneumonitis. Only 4 patients are evaluable at this time (two were in MRD negative CR upon recruitment, one has not been evaluated yet), and all achieved CR with negative MRD (100% CRR) at day +28. Median follow-up is 114 days (range 13-219 days). All 6 patients have developed absolute B-cell aplasia, and none has relapsed so far. Cytokine release syndrome (CRS) has been observed in all 7 patients, but it was of grade III-IV in only one of them (14%). This patient's grade IV CRS was fully reversible with tocilizumab. Grade I neurotoxicity (confusion and tremor) has been observed in 2 (29%) patients but resolved spontaneously.
Summary/Conclusion: It is feasible to prepare CART19 (ARI-0001) cells in a purely academic setting using the automated CliniMACS Prodigy System and home-made lentiviral vectors. The treatment was safe, with only one case (14%) of severe but reversible CRS. The treatment was also efficacious, with a 100% CRR and no relapses so far, although with very limited median follow-up.

S836

RECENT PROGRESS IN GENE THERAPY FOR SEVERE SICKLE CELL DISEASE: UPDATED INTERIM RESULTS FROM A PHASE 1 CLINICAL STUDY OF LENTIGLOBIN GENE THERAPY

J. Kanter[1,*], A. Thompson[2], M. Mapara[3], M. Walters[4], J. Kwiatkowski[5], L. Krishnamurti[6], M. Schmidt[7], A. Miller[8], F. Pierciey[8], W. Shi[8], M. Asmal[8], J. Tisdale[9]

[1]Division of Pediatrics, Medical University of South Carolina, Charleston, [2]Division of Hematology, Oncology & Stem Cell Transplant, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, [3]Division of Hematology/Oncology, Columbia University Medical Center, New York, [4]Blood and Marrow Transplant Program, Division of Hematology/Oncology, UCSF Benioff Children's Hospital Oakland, Oakland, [5]Division of Hematology, Children's Hospital of Philadelphia, Philadelphia, [6]Department of Pediatrics, Division of Hematology/Oncology/BMT, Emory University School of Medicine, Atlanta, United States, [7]German Cancer Research Center (DKFZ), National Center for Tumor Diseases (NCT), Heidelberg, Germany, [8]bluebird bio, Inc. , Cambridge, [9]Sickle Cell Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, United States

Background: The purpose of β-globin gene transfer into hematopoietic stem progenitor cells (HSPCs) is to express a functional adult β-globin that reduces or eliminates sickle cell disease (SCD) symptoms. The investigational gene therapy LentiGlobin Drug Product (DP) contains autologous CD34+ HSPCs transduced with the BB305 lentiviral vector, which encodes β-globin with an anti-sickling mutation (HbA^{T87Q}). Initial results from a Phase 1 HGB-206 study (NCT02140554) of LentiGlobin in patients with severe SCD treated with CD34+ HSPCs from bone marrow harvesting (BMH) showed acceptable safety, but suboptimal HbA^{T87Q} production. The revised protocol instituted pre-harvest RBC transfusions, increased target busulfan levels, increased the DP VCN after manufacturing, and investigated plerixafor-mediated mobilization/apheresis for DP manufacturing.
Aims: To investigate the safety and efficacy of a revised LentiGlobin gene therapy protocol with plerixafor-mobilized HSPCs in severe SCD.
Methods: Adult patients with severe SCD (history of recurrent vaso-occlusive crisis, acute chest syndrome, stroke, or tricuspid regurgitant jet velocity of >2.5 m/s) were enrolled. In lieu of BMH-derived HSPCs for back-up and DP manufacturing (Group [Grp] A), the revised protocol instituted ≥2 months of RBC transfusions before plerixafor mobilization/apheresis for back-up HSPC collection but retained BMH for DP manufacturing (GrpB). A GrpC evaluated DP and back-up prepared with plerixafor-mobilized HSPCs. Following HSPC collection, patients received myeloablative conditioning, followed by DP infusion. Adverse events (AEs), engraftment, vector copy number (VCN) and HbA^{T87Q} production were monitored.
Results: As of Nov 30, 2017, 10 patients (18-42 yrs) received LentiGlobin DP. In 7 GrpA patients, the median DP VCN and cell dose were 0.6 (0.3-1.3) copies/diploid genome and 2.1 (1.6-5.1)×10^6CD34+ cells/kg, with 8% - 42% CD34+ cells transduced. Median follow-up was 21.6 (17.8-26.7) months, as of Oct 26, 2017. At last visit, the median peripheral blood (PB) VCN, total Hb and HbA^{T87Q} levels were: 0.1 (0.1-0.2), 9.7 (7.5-10.9) g/dL and 0.7 (0.5-2.0) g/dL. In 2 GrpB patients, DP VCN and cell dose improved: DP VCNs were 1.4/3.3 and 2.9/5.0 (2 DP lots per patient) and total cell doses were 2.2 and 3.2×10^6 CD34+ cells/kg, with 46% - 95% cells transduced. At 9 (pt 1313; GrpB) and 6 months (pt 1312; GrpB) follow-up, PB VCNs were 0.5 and 2.5, total Hb levels were 10.4 and 12.6 g/dL, and HbA^{T87Q} levels were 3.0 and 6.4 g/dL. Of 11 patients enrolled in GrpC, 6 completed plerixafor mobilization/apheresis. DPs in 4 GrpC patients had median VCN and cell dose of 3.3 (2.8-4.6) and 6.9 (3-8)×10^6 CD34+ cells/kg, with 78% - 88% cells transduced. One GrpC patient received DP and at 1-month follow-up (as of Dec 6, 2017) had a PB VCN of 2.5. Additional follow-up results will be presented. There were 17 Grade 3-4 AEs reported in 5 patients after BMH (N=9). There were 5 Grade 3-4 AEs reported in 3 patients after plerixafor mobilization/apheresis (N=7). The AEs that were Grade 3-4 after DP infusion were consistent with myeloablative busulfan. To date, there was no graft failure and no Grade 3-4 DP-related infusional toxicity/AEs.
Summary/Conclusion: Patients enrolled and treated in a revised protocol had improvements in DP VCN and HbA^{T87Q} levels with no new significant safety events to date. Plerixafor mobilization in patients with severe SCD appears safe and can result in DPs with VCNs comparable to, and cell doses higher than, BMH. Longer follow-up and additional experience will clarify the effect of protocol changes.

Thrombosis – Risk factors and treatment

S837

PLATELET ADHESION UNDER FLOW CONDITION IS SIGNIFICANTLY AFFECTED BY HEMATOCRIT LEVELS IN POLYCYTHEMIA VERA PATIENTS

S. Gamba[1,*], A. Vignoli[1], M. Marchetti[1], P.E. van der Meijden[2], C. Giaccherini[1], S. Tessarolo[1], F. Swieringa [2], H. ten Cate[3], G. Finazzi[4], A. Rambaldi[4], J.W. Heemskerk[2], A. Falanga[1]

[1]Division of Immunohematology and Transfusion Medicine, ASST Papa Giovanni XXIII, Bergamo, Italy, [2]Department of Biochemistry, [3]Department of Internal Medicine, CARIM, Maastricht University, Maastricht , Netherlands, [4]Division of Hematology, ASST Papa Giovanni XXIII, Bergamo, Italy

Background: Polycythemia Vera (PV) is complicated by a high rate of thromboembolic events. Aspirin therapy (81–100 mg once-daily) + phlebotomy with a target hematocrit (HCT) of 45% is currently recommended in all PV patients regardless of risk status. Recently, a novel mechanism by which elevated HCT can lead to an increased risk of thrombosis has been described. The red blood cells push the platelets closer to the vessel wall, increasing the probability of their adhesion and activation via von Willebrand factor and collagen (Blood 19: 2537, 2017). This may occur in PV, where multiple cellular events may be needed to trigger thrombosis.
Aims: In a cohort of PV patients, we aim to characterize the *in vitro* platelet thrombus formation capacity under flow conditions at arterial shear rate in relation to HCT levels.
Methods: Fifty-two PV patients (26 M/26F; median age 65 years, range: 38-87) were enrolled after giving informed consent. Measurement of whole blood thrombus formation was performed in a parallel plate flow-chamber for 4' at 1,000 s^{-1}shear-rate over a collagen-coated surface. Thrombi were then stained with an anti-P-selectin-FITC antibody to evaluate the platelet activation status, and annexinV-AlexaFluor647 to detect procoagulant phosphatidylserine (PS) exposure. Results are expressed as mean±SEM of the % of area covered by thrombi or the % of adherent platelets positive for either P-selectin or AnnexinV.
Results: Platelet adhesion was significantly increased in PV (48.9±1.6%) compared to healthy controls (37.5±1.7%, $p<0.01$). In both PV and controls, almost all platelets forming the inner thrombus core expressed P-selectin, while PS was expressed by some platelets at the external thrombus border. Patients' thrombi were usually larger and more often interconnected forming a network while thrombi from controls are smaller and well isolated from each other. Considering the total population analyzed, a significant positive correlation ($p<0.005$) was found between HCT levels and either platelet adhesion and P-selectin positive platelets. Differently, no significant correlation was found between platelet adhesion and platelet count in either PV or controls. On the basis of the median value of HCT of our patient population (43.7%), subjects with an HCT value above the median had a significantly higher adhesion than the subjects with an HCT below the median (42±1.9 vs 51±2.3%, $p<0.05$). After a multivariate analysis adjusted for sex, age, therapy and JAK2-V617F allele burden, the HCT value was still significantly associated with platelet coverage and p-selectin expression.
Summary/Conclusion: Our study demonstrates that an elevated HCT increases platelet adhesion to the vessel wall. This is likely favored by local rheological factors coming from the increased red blood cell count typical of this disease. These findings support the concept that an elevated HCT is a very relevant thrombotic mechanism in PV.

S838

COMPLEMENT C3 AND C4 ARE ASSOCIATED WITH POSTNATAL VENOUS THROMBOSIS

A.E.A. Dahm[1,2,*], A.F. Jacobsen[3], E.M. Jacobsen[4], H. Skuterud Wik[4], P.M. Sandset[2]

[1]Haematology, Akershus University Hospital, Lørenskog, [2]Institute of Clinical Medicine, University of Oslo, [3]Obstetrics and Gynaecology, [4]Haematology, Oslo University Hospital, Oslo, Norway

Background: Venous thrombosis (VT) is one of the most serious complications to pregnancy in developed countries. The complement system is part of the innate immune system. It has been suggested that the complement system and the coagulation system cross-talks and that high levels of C3 is associated with VT in the general population.

Aims: To investigate if high levels of complement C3 or C4 were associated with pregnancy related VT.
Methods: We investigated women in the Norwegian VIP study which is a population based case-control study of VT in pregnancy or within 3 months post partum (cases, n=313) and women without pregnancy related VT (controls, n=353). Blood samples were taken 3-16 years after the index pregnancy. We first investigated determinants of C3 and C4 in the control women with univariate linear regression. We then calculated the odds ratio (OR) for pregnancy related VT with multiple logistic regression including adjustments for other risk factors for pregnancy related VT associated with C3 or C4.
Results: In the control women C3 and C4 were associated with BMI. Control women pregnant at blood sampling had higher C3 than non-pregnant women. Both C3 and C4 were associated with CRP; the coagulation factors fibrinogen, factor VIII or factor IX; and with the coagulation inhibitors protein C, protein S, antithrombin, and free tissue factor pathway inhibitor. The strongest associations were seen between C3 and CRP (standardized regression coefficient beta 0.67), C3 and protein C (beta 0.49), C3 and free TFPI (beta 0.40), C3 and fibrinogen (beta 0.56), C3 and factor IX (beta 0.56). The crude OR for pregnancy-related VT was 1.8 (95% confidence interval (CI) 1.1-3.0) for C3 above the 90th percentile and 2.0 (95% CI 1.2-3.2) for C4 above the 90th percentile. Further stratification in antenatal and postnatal VT showed that the OR for antenatal VT for C3 above the 90th percentile was 0.87 (95% CI 0.43 to 1.7), and for C4 above the 90th percentile it was 1.4 (95% CI 0.73-2.6). For postnatal VT, the OR for C3 above the 90th percentile was 3.0 (95% CI 1.8-5.0), and for C4 above the 90th percentile 2.6 (95% CI 1.5-4.6). Adjustment for CRP, BMI, protein S, fibrinogen, factor VIII or factor IX only changed the odds ratios marginally.
Summary/Conclusion: Complement C3 and C4 were associated with several coagulation factors and coagulation inhibitors. High levels of C3 and C4 were associated with postnatal, but not antenatal VT.

S839

EFFECT OF COVARIATES IN THE PHARMACOKINETIC/PHARMACODYNAMIC MODEL OF ANDEXANET ALFA USED TO PREDICT THE REGIMEN FOR REVERSAL OF ANTICOAGULATION BY FXA INHIBITORS IN PATIENTS WITH ACUTE MAJOR BLEEDING

J.M. Leeds[1,*], Y. Xiong[2], M. Lovern[2], K. Der[1], J.W. Mandema[2], G. Lu[1], J.T. Curnutte[1], T.J. Milling[3], M. Crowther[4], S.J. Connolly[4], P. Yue[1], P.B. Conley[1]

[1]Portola Pharmaceuticals, Inc., South San Francisco, CA, [2]Certara, Menlo Park, CA, [3]Seton Dell Medical School Stroke Institute, Austin, TX, United States, [4]McMaster University, Hamilton, Canada

Background: Andexanet alfa (andexanet) is a recombinant protein that acts as a factor Xa (FXa) decoy to bind and sequester FXa inhibitors (apixaban, rivaroxaban, edoxaban, and betrixaban). A naïve-pooled pharmacokinetic (PK)/pharmacodynamics (PD) model, developed in Phase 2 studies in healthy subjects, predicted the andexanet regimen required to reverse anticoagulation by FXa inhibitors. Preliminary analysis of the ongoing Phase 3b/4 study (ANNEXA-4) demonstrated that the naïve-pooled model was predictive of the anti-FXa activity reversal in patients with acute major bleeding who were anticoagulated with FXa inhibitors. It is not known whether patient characteristics (e.g., impaired renal function, advanced age) may alter PK and affect the accuracy of the model.
Aims: The first interim data from the ANNEXA-4 study in patients with acute major bleeding was compared to predictions from the naïve-pooled PK/PD model. Additionally, enhanced analyses of the model that include evaluation of intrinsic factors that might affect both FXa inhibitor and andexanet levels in this patient population are ongoing.
Methods: In ANNEXA-4, an ongoing prospective, open-label study, bleeding patients anticoagulated with a FXa inhibitor received IV andexanet bolus (400 or 800 mg) followed by 120-min infusion (4 or 8 mg/min). Anti-FXa activity was measured before andexanet administration (baseline), at end of bolus (EOB), end of infusion, and 4, 8, and 12 h after infusion. The relationship between baseline anti-FXa activity and reversal in healthy subjects was used to develop the naïve-pooled PK/PD model and then to predict the percent reversal of anti-FXa activity for patients with acute major bleeding. Refinement of the PK/PD model includes assessment of intrinsic factors such as renal function, age and body weight on both FXa and andexanet exposure.
Results: In the first interim analysis of ANNEXA-4, 73 patients (apixaban, 39; rivaroxaban, 34) had plasma levels available for model qualification. The mean observed percent reversal of anti-FXa activity for rivaroxaban and apixaban was well predicted by the healthy subject PK/PD model; the point estimates fell within the 90% confidence intervals of predicted values (Table 1). The predicted reversal fit closely the observed confidence intervals through the first 4 h for rivaroxaban and apixaban; it extended through all evaluated time points for rivaroxaban but only through the post 4-h time points for apixaban, possibly due to higher baseline anti-FXa activity levels seen in some apixaban patients. The revised PK/PD model identified body weight as a significant covariate for andexanet exposure, and incorporated published covariates for rivaroxaban and apixaban, including renal function, age and lean body mass. This revised model is being used in all subsequent PK/PD analysis in samples from bleeding patients.

Table 1.

Comparison of mean predicted percentage of anti-FXa reversal vs. observed reversal (with 95% with 95% CI) for rivaroxaban and apixaban at different time points

Time	N	Predicted % reversal	Observed % reversal [95% CI]
Rivaroxaban			
EOB	32	76.4	74.4 [58.3 to 90.4]
EI	33	78.3	77.4 [67.6 to 87.2]
4 hrs	32	31.8	32.0 [18.0 to 45.9]
8 hrs	33	40.7	43.6 [35.0 to 52.2]
12 hrs	32	51.0	56.9 [50.2 to 63.7]
Apixaban			
EOB	34	84.1	83.9 [75.3 to 92.5]
EI	36	81.4	84.2 [77.3 to 91.1]
4 hrs	33	34.4	27.6 [10.2 to 45.0]
8 hrs	32	40.2	30.6 [25.1 to 36.1]
12 hrs	36	48.6	32.8 [26.1 to 39.5]

Summary/Conclusion: The naïve-pooled PK/PD model in healthy subjects closely predicted the percent reversal of anti-FXa activity by andexanet in patients receiving apixaban or rivaroxaban who presented with acute major bleeding. At later time points, the observed apixaban anti-FXa activity reversal did not overlap with that predicted by the naïve-pooled model. Incorporation of intrinsic factors (renal function and age) into the PK/PD model may provide more robust prediction of anti-FXa activity reversal in patients initially seen as outliers.

S840

A SYSTEMATIC REVIEW OF PREDICTORS OF VENOUS THROMBOEMBOLISM IN ACUTE LEUKEMIA

F. Al-Ani[1], A. Ahrari[1], P. Wang[2], A. Lazo-Langner[1,*]

[1]Medicine, [2]Western University, London, Canada

Background: The impact of venous thromboembolism (VTE) on morbidity and mortality is significant in hematological cancer patients, including acute leukemia (AL). Identifying risk factors for the development of VTE among patients with AL will enable clinicians to stratify patients according to their VTE risk and consider individualized approaches for anticoagulant prophylaxis.
Aims: We aimed to identify potential predictors of VTE in AL patients in previoslu published studies.
Methods: We conducted a systematic review of randomized trials and observational studies assessing VTE in the setting of AL including acute myeloid leukemia (AML), acute promyelocytic leukemia (APL) or acute lymphoid leukemia (ALL). Studies were included if they contained information on total VTE events, catheter related thrombosis (CRT) and cerebral vein thrombosis (CVT). Relative Risks (RR) for all VTE events, CRT and CVT were calculated among AL subgroups.
Results: A total of 30 studies were included (29 cohort and 1 case control) with 15,491 AL participants. Of the total participants, the number of evaluable patients was 10,788 for AML, 3,242 for ALL, and 1,031 for APL. The median time for VTE occurrence from AL diagnosis was reported in 22 studies, ranging between 5 and 92 days. The median time of VTE occurrence from AL diagnosis was <1 month in 14 studies (64% of evaluable studies); 1-2 months in 7 studies (32%); and only 1 study reported a median time of 3 months (~4%). The risk of VTE was higher in ALL patients compared to AML patients (excluding APL) [RR: 1.8; 95%CI: 1.58-2.10; p<0.00001]. When comparing APL patients to other AML subtypes, the risk was 1.5-fold significantly higher in APL [RR: 1.56; 95%CI: 1.24-1.97; p=0.0001]. The risk of thrombosis was higher with the presence of catheter [RR: 1.2; 95%CI: 1.04-1.36; p=0.0074]. A subgroup analysis of CRT comparing AL subgroups showed that the risk of CRT is similar between AML vs. ALL [RR: 0.9; 95%CI: 0.72-1.13; p=0.405]. The risk was also similar between peripherally inserted central line vs. central venous catheter [RR: 1.08; 95%CI: 0.81-1.45; p=0.557]. When comparing APL with ALL patients, the risk of CRT was higher among APL [RR: 1.54; 95%CI: 1.10-2.18;

p=0.0122]. Finally, the risk of CVT was 4-fold higher with ALL patients receiving l-asparaginase compared to AML patients [RR: 4.1; 95%CI: 1.75-9.73; p=0.0012].
Summary/Conclusion: Factors that seem to influence VTE risk in AL patients include leukemia phenotype, the presence of catheters, and probably the use of l-asparignase. The risk of thrombosis seems to be higher in the initial period following the diagnosis. Due to the lack of available data in published studies, other potential predictors could not be identified. A thrombosis risk prediction model study is warranted to develop risk stratification criteria for AL patients.

S841

PULMONARY EMBOLISM SEVERITY INDEX (PESI) SCORE AS A PREDICTOR FOR READMISSION IN ACUTE PULMONARY EMBOLISM IN EMERGENCY DEPARTMENT?

D. Prajapati[1,*], D. Suryanarayan[1], E. Lang[2]

[1]Department of Medicine, Devision of Hematology, [2]Department of Emergency Medicine, University of Calgary, Calgary, Canada

Background: Pulmonary Embolism (PE) management in Emergency Department (ED) confers a substantial cost burden representing opportunities for improvements in decision-making. The "Pulmonary Embolism Severity Index (PESI)" is a validated tool to prognosticate patients with PE supporting admit versus (vs.) discharge decisions from the ED. Despite existing evidence, PESI is under-used in patients with PE.
Aims: Our aim is to to evaluate PESI scores and patient disposition from 4 EDs within Calgary to determine discordance between them and the effect of the discordance on readmission and mortality.
Methods: Retrospective review of adult patients ≥18 years, diagnosed with PE between January-June 2016 at 4 EDs in Calgary Health Region. Patients were divided into high-risk PESI (score>85) and low-risk PESI (score 0-85). Chi-Square (χ^2) test was used for comparison between the groups. Primary outcome measure was rate of discordance between PESI risk and disposition decision and identify factors driving the discordance. Secondary outcome measures included comparing 30-day readmission rate, 30-day and 90-day mortality between the discordant PESI groups.
Results: 365 patients were diagnosed with PE in the study period with 60% being admitted and 40% discharged. The median PESI score in admitted patients was 85 (26-172) vs. 68 (20-163) in discharged patients. 51% of admitted patients had a low-risk PESI score and 24% of the discharged patients were high-risk PESI. 30-day readmission rate was 22.9% vs 5.3% (p=0.002) in discharged patients with high-risk PESI vs. discharged patients with low-risk PESI. Hypoxemia was the most common (62%) justification for admission in low-risk PESI groups. Among discharged patients we noted an 8.6% 90-day mortality in the high-risk vs. 0% in the low-risk PESI groups.
Summary/Conclusion: Discharging a PE patient from the ED with a high PESI score carries a significant risk of ED revisit and readmission. Hypoxia was the reason for admission in majority of low risk PE patients.

Thalassemia

S842

LUSPATERCEPT INHIBITS PSMAD2/3 SIGNALING AND PROMOTES ERYTHROID MATURATION THROUGH A GATA1 DEPENDENT MECHANISM

P. Martinez[1,*], M. Bhasin[2], R. Li[1], S. Pearsall[1], R. Kumar[1], R. Suragani[1]

[1]Preclinical, Acceleron Pharma, [2]Genomics and Proteomics Center, Beth Israel Deaconess Medical Center, Harvard Medical School, Cambridge, United States

Background: Previously, we reported elevated Smad2/3 signaling in diseases characterized by erythroid maturation defects (Ineffective Erythropoiesis; IE) such as MDS and β-thalassemia. Luspatercept (an erythroid-maturation agent [EMA]) binds and inhibits signaling by certain Smad2/3 ligands such as GDF11, activin B, and corrects IE/anemia in a murine model of thalassemia and MDS (Suragani et. al, 2014). However, the molecular mechanism through which Smad2/3 ligands negatively regulate erythroid maturation is not completely understood.
Aims: We investigated the molecular mechanism of action through which Smad2/3 ligands such as GDF11 inhibit erythroid maturation *in vitro*, *in vivo*, and how luspatercept prevents this inhibition.
Methods: β-thalassemic mice (*Hbb*$^{th3/+}$) were used to obtain splenic basophilic erythroblasts to be used for RNA-seq. MEL cells were treated with GDF11 in the presence or absence of luspatercept. *In vivo* studies were conducted using transgenic mice with inducible GDF11 overexpression under a ROSA26Cre promoter. CBC and erythroid differentiation analysis were carried out following 3-weeks of inducing GO (N= 5).
Results: To investigate the effect of GDF11 *in vivo*, we used transgenic mice with inducible GDF11 overexpression (GO). RBC and hemoglobin (Hgb) levels were significantly decreased in GO mice. RBC levels were 6.83×10^6/ l in GO mice vs. 8.7×10^6/ l in WT ($p<0.05$). Hgb levels were 9.92 g/dl in GO mice vs. 12.96 g/dl in WT ($p<0.05$). Additionally, bone marrow TER119+ cells were significantly reduced in GO mice (5.294%) compared to WT (29.16%, $p<0.001$) suggesting IE. Furthermore, we found that GATA1+ cells in splenic TER119+ erythroid cells was significantly reduced in GO mice (2.876%) compared to WT (7.342%; $p<0.05$).
Transcriptome analysis of sorted β-thalassemic erythroblasts identified 74 genes that were differentially expressed in RAP-536 (murine version of luspatercept) treated samples vs. VEH. Analysis of the significantly upregulated genes by RAP-536 revealed increased activity of GATA1. We investigated whether elevated pSmad2/3 levels by GDF11 affects GATA1 expression using MEL cells and found that GATA1 was decreased in both mRNA and protein levels, and importantly luspatercept treatment prevented the decrease. We also found increased nuclear accumulation of GATA1 in these cells consistent with increased transcriptional activity in β-thalassemic mice *in vivo*. Furthermore, we found that the expression of Transcription Intermediary Factor (TIF) 1γ in β-thalassemic erythroid tissues was significantly lower in VEH vs. RAP-536 treated mice. TIF1γ has been reported to compete with Smad4, binding pSmad2/3 and leading to erythroid differentiation (Wei *et. al*, 2006), and may act as a molecular link between pSmad2/3 and GATA1.
Summary/Conclusion: In this study, we showed that pSmad2/3 negatively regulates the levels of GATA1 protein, and prevents RBC maturation. We have emerging data to show TIF1γ as a link between pSmad2/3 and GATA1. Thus by preventing elevated pSmad2/3, and restoring GATA1 availability through TIF1γ, luspatercept treatment causes upregulation of genes involved in promoting terminal erythroid maturation, and consequently corrects anemia in β-thalassemia. Luspatercept is currently in phase 3 studies in patients with MDS and β-thalassemia.

S843

HEPCIDIN MIMETIC PTG-300 FOR TREATMENT OF INEFFECTIVE ERYTHROPOIESIS AND CHRONIC ANEMIA IN HEMOGLOBINOPATHY DISEASES

G. Bourne[1,*], L. Zhao[2], A. Bhandari[2], B. Frederick[2], J. McMahon[1], V. Tran[2], J. Zhang[1], A. Stephenson[1], R. Taranath[2], J. Tovera[2], T. Annamalai[2], L. Mattheakis[2], D. Patel[2], M. Smythe[1], D. Liu[2]

[1]Protagonist Therapeutics Pty Ltd., Brisbane, Australia, [2]Protagonist Therapeutics Inc., Newark, CA, United States

Background: Hepcidin is a 25–amino acid peptide synthesized by hepatocytes and is involved in iron homeostasis. The known molecular-target of hepcidin is the ferroportin receptor, which functions as the major transmembrane channel for the export of cellular iron. Hepcidin induces endo-

cytosis and proteolysis of ferroportin and thereby decreases the export of iron to plasma. Hepcidin is prone to aggregation and is difficult to synthesize, characteristics that hinder its future development. The hormone is a complex 4-disulfide bonded beta-hairpin structure that is highly conserved. Protagonist used a proprietary 'scaffold hopping' approach (Vectrix™) to identify a novel peptidic scaffold that matches key pharmacophores of hepcidin. After further optimization PTG-300 was selected as a clinical development candidate, based on its simpler structure and drug-like characteristics, such as human plasma stability $t_{1/2}$>24h and aqueous solubility >100 mg/mL. It reduces cell surface expression of human ferroportin with an EC_{50} of 5 nM and in healthy cynomolgus monkeys causes >80% sustained reduction of serum iron over 48 hr after a single subcutaneous dose of 1 mg/kg.
Aims: To evaluate the impact of the hepcidin mimetic PTG-300 on anemia in a mouse model of β-thalassemia
Methods: Wild-type control (WT) C57B6 and b-thalassemia intermedia (*Hbb$^{th3/+}$*) mice aged between 4 to 6 weeks were treated with PTG-300 by sc injection at various frequencies and doses. Hematological and iron parameters were examined, including analysis of erythropoiesis, molecular studies of sorted erythroid precursors, indices of hemolysis, various biomarkers (including EPO and liver expression) were carried out.
Results: PTG-300 was injected subcutaneously at several dosing frequencies (Q2D to Q5D) for 4 weeks in six week old *Hbb$^{th3/+}$*mice. This resulted in significant erythropoiesis as evidenced by a hemoglobin increase > 1.5 g/d, after Q5D dosing, reticulocyte count and spleen size reductions, and an increase in peripheral red cell number. The minimum effective dose (MED) was determined by comparing various doses levels between the acute mouse model (pharmacodynamic reduction in serum iron) and the Hbb$^{th3/+}$ mouse chronic model (erythropoietic increase in hemoglobin, peripheral red cell number and the decrease in reticulocytes). Using conditions of optimal dosing frequency and MED in *Hbb$^{th3/+}$* mice, flow cytometry studies of bone marrow and spleen erythroid populations from PTG-300 treated animals demonstrated an increase in the relative proportion of mature erythroid cells. Accumulation of tissue iron was also significantly reduced in the liver and spleen but not in the heart and duodenum. RBC survival increased in *Hbb$^{th3/+}$* mice to similar levels as in WT mice while EPO expression was decreased.
Summary/Conclusion: Our data suggest that PTG-300 ameliorates ineffective erythropoiesis as a treatment for chronic anemia in a mouse model of β-thalassemia. In conditions characterized by low endogenous hepcidin levels and high serum iron levels, such as β-thalassemia and myelodysplastic syndrome (MDS) patients, we believe that PTG-300 has the potential to treat the dysregulation of iron metabolism thereby reducing iron toxicity in the erythropoietic cascade and the chronic anemia. Ultimately PTG-300 has the potential to treat secondary iron overload by reducing the need for transfusions and by decreasing excessive dietary iron absorption.
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S844

IMPROVEMENTS IN HEMOGLOBIN, QUALITY OF LIFE, AND SIX-MINUTE-WALK DISTANCE IN ADULTS WITH β-THALASSEMIA TREATED WITH LUSPATERCEPT: LONG-TERM PHASE 2 STUDY

A. Piga[1,*], I. Tartaglione[2], M. R. Gamberini[3], E. Voskaridou[4], A. Melpignano[5], P. Ricchi[6], V. Caruso[7], A. Pietrangelo[8], J. Reynolds[9], C. Barron[9], X. Zhang[9], A. Laadem[10], P. G. Linde[9], M.L. Sherman[9]

[1]Turin University, Turin, [2]Università della Campania "L. Vanvitelli, Naples, [3]Arcispedale S. Anna, Cona, Ferrara, Italy, [4]Laiko General Hospital, Athens, Greece, [5]Ospedale "A. Perrino", Brindisi, [6]AORN "A. Cardarelli", Naples, [7]ARNAS Garibaldi, Catania, [8]CEMEF, Medicina 2 , Modena, Italy, [9]Acceleron Pharma Inc, Cambridge, [10]Celgene, Summit, United States

Background: Serious hematologic conditions such as β-thalassemia are associated with an erythroid maturation defect leading to anemia and other clinical sequelae. Luspatercept (ACE-536) is being developed as an erythroid-maturation agent (EMA) for treatment of β-thalassemia. Luspatercept binds to select TGF-β superfamily ligands, reducing aberrant Smad2/3 signaling and promoting late-stage erythroid maturation and increased hemoglobin (Hgb); it has corrected the effects of ineffective erythropoiesis in a mouse model of thalassemia (Suragani R, Blood, 2014), and increased Hgb and was well tolerated in a phase 1 study in healthy volunteers (Attie K, Am J Hematol, 2014).
Aims: This ongoing, phase 2, multicenter, open-label study followed by a long-term extension (ext) evaluates the effects of luspatercept in patients (pts) with either transfusion-dependent (TD) or non-transfusion dependent (NTD) β-thalassemia. Key endpoints include Hgb increase, pt-reported quality-of-life (QoL) and assessment of functional improvement in 6-minute walk distance (6MWD) in NTD pts, and reductions in RBC transfusion burden in TD pts.
Methods: Inclusion: age ≥18 yr and either NTD (<4 RBC U/8 weeks prior to first dose with baseline Hgb <10 g/dL) or TD (≥4 RBC U/8 weeks prior to first dose, confirmed over 6 months). Pts treated every 3 weeks subcutaneously for up to 5 doses (titration up to 1.25 mg/kg) in the base study were then eligible for treatment up to 5 additional years (base completed NCT01749540; ext ongoing NCT02268409).
Results: Data (as of 31Aug2017) were available for 63 pts treated at dose levels ≥0.6 mg/kg. 31 NTD, 32 TD; median (range) age (yr) was 38 (20-62); 67% had prior splenectomy. For NTD pts, at baseline, median (range) Hgb (g/dL) was 8.5 (6.5-9.8); mean (SD) liver iron concentration (LIC, mg/g dw) was 5.1 (3.6). For TD pts, at baseline, median (range) transfusion burden was 8 U/12 weeks (4-18 U); mean (SD) LIC (mg/g dw) was 4.7 (4.7). 22/31 (71%) NTD pts achieved mean Hgb increase of ≥1.0 g/dL and 17/31 (55%) achieved an even greater increase of ≥1.5 g/dL in mean Hgb over any 12-week period compared to baseline. Increases in mean Hgb over a 12-week period correlated positively with improvement in both a pt-reported QoL questionnaire (FACIT-F) and with improvements in 6MWD at weeks 16 and 48. At week 48, a statistically significant improvement from baseline in mean (SD) 6MWD was seen in NTD pts (n=9), 484 (121) vs 408 (68) meters, p=0.02. 22/32 (69%) TD pts achieved ≥33% reduction in transfusion burden over any 12-week interval compared to baseline. 12/29 (41%) achieved ≥33% reduction in transfusion burden over a fixed 12-week interval (weeks 13-24) compared to baseline, and 12/29 (41%) continued to have a response at a fixed interval of weeks 37-48 on study. Luspatercept was generally well tolerated, with no related serious adverse events and few grade 3 related AEs: bone pain (n=3), asthenia (n=2), and headache (n=1). The most frequent related AEs (≥10%) were bone pain, headache, myalgia, arthralgia, musculoskeletal pain, asthenia, injection site pain, and back pain.
Summary/Conclusion: In this phase 2 open-label study, long-term luspatercept treatment in pts with β-thalassemia was generally safe and well tolerated up to 2 years. Clinically relevant measures of luspatercept efficacy were observed in both NTD pts (increased Hgb levels and improved QoL) and TD pts (decreased transfusion burden).

S845

IMPAIRMENT OF THE MESENCHYMAL STROMAL NICHE OF BETA-THALASSEMIA PATIENTS: IMPLICATIONS OF PROLONGED IRON EXPOSURE

S. Crippa[1,*], V. Rossella[1], S. Rivis[1], L. Silvestri[2], A. Aprile[2], S. Scaramuzza[2], M.A. Avanzini[3], M. Zecca[4], S. Marktel[2], F. Ciceri[2], A. Aiuti[1], G. Ferrari[2], M.E. Bernardo[1]

[1]San Raffaele-Telethon Institute for Gene Therapy (SR-TIGET), Pediatric Immunohematology, [2]Ospedale San Raffaele, Milano, [3] Fondazione IRCCS Policlinico "San Matteo", [4]Fondazione IRCCS Policlinico "San Matteo", Pavia, Italy

Background: Bone marrow (BM) contains a population of mesenchymal stromal cells (MSCs) that, together with endothelial cells and osteoblasts, provide a specific microenvironment to support hematopoietic stem cell (HSC) homeostasis. Beta-thalassemia (BT) is a hereditary blood disorder characterized by reduced or absent synthesis of hemoglobin beta-chains amenable to allogeneic HSC transplantation and HSC-gene therapy (HSC-GT). Data on the mesenchymal compartment in BT patients are scarce.
Aims: Aim of this work is to characterize the mesenchymal compartment in BT patients before transplant procedures and to test whether these cells are functionally altered in their ability to support HSC.
Methods: We isolated MSCs from the BM of 12 BT patients (9 pediatric and 3 adults) and age-matched healthy donor (HD: 1:1). MSCs were characterized for their classical properties (clonogenic, proliferative and differentiation capacity, immunophenotype analyses) and for their functional properties in terms of BM niche supportive functions and response to iron overload.
Results: BT-MSCs showed a reduced clonogenic capacity (CFU-Fs), delay in colony formation and longer population doubling time. Similarly, we observed an altered differentiation capacity into adipocytes and osteoblasts both by immunohystochemical staining and RT-qPCR. Both HD- and BT-MSCs expressed canonical mesenchymal markers (positive for CD105, CD90, CD73; negative for hematopoietic markers). On the contrary, the expression of CD146 and CD271 was extremely reduced in BT-MSCs, indicating a pauperization of the most primitive stem cell pool. We measured a higher iron content in the BT BM microenvironment; moreover, we demonstrated that MSCs expressed iron transporters (TFR1, ZIP14, ZIP18, DMT1) and were able to uptake iron. Iron overload correlated with increased ROS level in BT-MSCs and robust decrease of primitive MSCs.

We showed that ROS levels were increased possibly due to altered anti-oxidant response caused by prolonged iron exposure associated with chronic blood transfusions. Indeed, at basal level BT-MSCs showed a reduced expression of anti-oxidant genes, which were not properly induced in response to iron. Moreover, we found that the expression of genes involved in the crosstalk between MSCs and HSCs (*Cxcl12*, *SCF* and *Angp1)* was reduced in BT-MSCs, leading to an altered capacity to attract CD34+ hematopoietic stem progenitors cells (HSPC) in transwell migration assays and impaired capability to maintain primitive HSPCs in *in vitro* 2D co-culture models. We demonstrated that iron dependent demethylases were more active in BT-MSCs, leading to an epigenetic remodeling of BT-MSCs associated with an altered MSC functionality.
Summary/Conclusion: In conclusion, we showed an impairment in the mesenchymal niche of BT-BM possibly associated with prolonged iron exposure and reduced anti-oxidative response. This underlines the importance of iron level for normal MSC function. Whether the ability of MSC to up-take iron represents a mechanism of protection for the BM niche and how the BT stromal niche impairment influences engraftment and HSC support after allogeneic HSC transplantation and HSC-GT is currently being investigated.

S846

A NOVEL BAEVRLESS-PSEUDOTYPED GLOBIN LENTIVIRAL VECTOR DRIVES HIGH AND STABLE FETAL HEMOGLOBIN EXPRESSION IMPROVING THALASSEMIC ERYTHROPOIESIS *IN VITRO*

E. Drakopoulou[1], M. Georgomanoli[1], C.W. Lederer[2], M. Kleanthous[3], C. Costa[4], O. Bernadin[4], F.-L. Cosset[4], E. Verhoeyen[4], E. Voskaridou[5], E. Papanikolaou[6], N.P. Anagnou[1,*]

[1]Cell and Gene Therapy, Biomedical Research Foundation of the Adademy of Athens, Athens, Greece, [2]Molecular Genetics Thalassemia Department , [3]The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, [4]International Centre for Infectiology Research, Inserm, Lyon, France, [5]Thalassemia Centre, Laikon General Hospital, [6]Laboratory of Biology, University of Athens, School of Medicine, Athens, Greece

Background: Ineffective erythropoiesis in β-thalassemia occurs primarily as a result of an early erythroid progenitor apoptosis, due to precipitation of the excess of α-chains at the polychromatic normoblast stage, becoming more profound upon enhanced activation of the autophagy pathway [Ann Hematol 90:7, 2011]. We have previously demonstrated that the self-inactivating γ-globin lentiviral vector GGHI, can significantly increase HbF ($\alpha_2\gamma_2$) in erythroid cells of thalassemia patients and improve the disease phenotype *in vitro*, by reducing apoptosis and restoring thalassemic erythropoiesis [Hum Gene Ther 23:1, 2012]. Furthermore, we have recently shown that the alternative baboon envelope glycoprotein BaEVRless specifically targets hCD34$^+$ cells and increases transduction at low multiplicity of infection (MOI) [Blood 119:5, 2012; Blood 124:8, 2014].
Aims: To this end, we generated a novel, improved, γ-globin vector designated as GGHI-mB-3D by 1) incorporating two regulatory novel elements, i.e the 3D HPFH-1 enhancer and the 3' β-globin UTR and 2) pseudotyping with the BaEVRless envelope glycoprotein, and assessed its potency to specifically target hCD34$^+$ cells from normal and thalassemic donors and its ability for stable and high transgene expression at low MOIs.
Methods: Both VSVG and BaEVRless envelope glycoproteins were used for pseudotyping γ-globin and GFP lentiviral vectors, while titration was carried out in 293T cells, using FACS and/or qPCR. Peripheral blood CD34$^+$cells from normal and thalassemic donors were isolated, transduced at low MOIs and cultured for up to 14 days in liquid and methylcellulose cultures. Assessment of HbF expression at both protein and mRNA levels was carried out at the terminal differentiation stage. Improvement of erythropoiesis was evaluated by both Annexin/7AAD staining and orthochromatic erythroblast determination, while *ATG5* expression at the mRNA level was determined using qPCR.
Results: We documented high and stable HbF expression in thalassemic cells for the novel GGHI-mB-3D/BaEVRless vector, exhibiting increased transduction efficiency, compared to the conventional GGHI-mB-3D/VSVG, with a concomitant 119% mean HbF increase (p=0.029, n=9), a mean VCN/cell of 0.86 and a mean transduction efficiency of 56.4%. Transduced populations also exhibited a trend towards late-erythroid, orthochromatic differentiation and reduced apoptosis, a further indication of a successful gene therapy treatment. Monitoring expression of *ATG5*, a key link between autophagy and apoptosis, showed that gene correction correlated with reduction of enhanced autophagy activation, a typical feature of thalassemic polychromatophilic normoblasts.
Summary/Conclusion: The novel GGHI-mB-3D regulatory elements, coupled with BaEVRless pseudotyping, can lead to efficient transduction and potentially therapeutic HbF levels, which can have significant implications for the clinical efficiency of the currently ongoing clinical trials.

SIMULTANEOUS SESSIONS III

New therapeutic strategies to improve the outcome of relapse/refractory plasma cell disorders

S847

OPTIMISMM: PHASE 3 TRIAL OF POMALIDOMIDE, BORTEZOMIB, AND LOW-DOSE DEXAMETHASONE VS BORTEZOMIB AND LOW-DOSE DEXAMETHASONE IN LENALIDOMIDE-EXPOSED PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

P. Richardson[1,*], A.O. Rocafiguera[2], M. Beksac[3], A.M. Liberati[4], M. Galli[5], F. Schjesvold[6], J. Lindsay[7], K. Weisel[8], D. White[9], T. Facon[10], J. San Miguel[11], K. Sunami[12], P. O'Gorman[13], P. Sonneveld[14], P. Robak[15], S. Semochkin[16], S. Schey[17], X. Yu[18], T. Doerr[18], A. Bensmaine[18], T. Biyukov[19], T. Peluso[19], M. Zaki[18], K. Anderson[1], M. Dimopoulos[20]

[1]Jerome Lipper Multiple Myeloma Center, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, United States, [2]Institut Català d'Oncologia and Institut Josep Carreras, Hospital Germans Trias i Pujol, Badalona, Spain, [3]Ankara University, Cebeci Yerleskesi, Dikimevi, Ankara, Turkey, [4]University of Perugia, Terni, Perugia, [5]A.O. Papa Giovanni XXIII, U.O. di Ematologia, Bergamo, Italy, [6]Oslo University Hospital, Oslo, Norway, [7]East Kent Hospitals University NHS Foundation Trust, Canterbury, United Kingdom, [8]University Hospital of Tuebingen, Tuebingen, Germany, [9]Dalhousie University and Queen Elizabeth II Health Sciences Centre, Halifax, Canada, [10]Service des Maladies du Sang, Hôpital Claude Huriez, Lille, France, [11]Clinica Universidad de Navarra, CIMA, IDISNA, Pamplona, Spain, [12]National Hospital Organization Okayama Medical Center, Okayama, Japan, [13]Mater Misericordiae University Hospital, University College Dublin, Dublin, Ireland, [14]Erasmus MC Cancer Institute, Rotterdam, Netherlands, [15]Medical University of Lodz, Lodz, Poland, [16]Pirogov Russian National Research Medical University, Moscow, Russian Federation, [17]Department of Hematology, King's College London, London, United Kingdom, [18]Celgene Corporation, Summit, United States, [19]Celgene International Sàrl, Boudry, Switzerland, [20]National and Kapodistrian University of Athens, Athens, Greece

Background: Despite recent therapeutic advances, multiple myeloma (MM) remains an incurable plasma cell malignancy. Pomalidomide (POM) is a standard-of-care treatment in relapsed or refractory MM (RRMM) and has demonstrated synergistic anti-myeloma activity with dexamethasone (DEX) and proteasome inhibitors, as well as cytotoxic effects in lenalidomide (LEN)-resistant cells. POM + DEX is approved in the European Union for patients with RRMM who received ≥ 2 prior therapies, including LEN and bortezomib (BORT), and has been investigated after LEN-based treatment. As LEN becomes increasingly established in up-front treatment of MM, patients who have exhausted the benefit of LEN represent a clinically relevant unmet medical need. Here we report final progression-free survival (PFS) and safety data from a phase 3 triplet trial in an entirely post–LEN-treated population, comparing POM, BORT, and low-dose DEX (PVd) vs BORT and low-dose DEX (Vd) in patients who received 1 to 3 prior therapies.
Aims: To compare the efficacy and safety of PVd vs Vd in LEN-exposed patients with RRMM.
Methods: Key eligibility criteria included 1 to 3 prior regimens and ≥ 2 cycles of prior LEN therapy. Patients were randomized 1:1 to receive PVd or Vd treatment in 21-day cycles: POM 4 mg/day on days 1-14 (PVd arm only); BORT 1.3 mg/m^2 on days 1, 4, 8, and 11 of cycles 1-8 and on days 1 and 8 of cycle 9 and beyond; and DEX 20 mg/day (10 mg/day if aged > 75 years) on the days of and after BORT. The primary endpoint was PFS. All pts provided informed consent.
Results: A total of 559 patients were enrolled: 281 patients in the PVd arm and 278 in the Vd arm. Demographic, baseline, and prior disease characteristics were generally well balanced between the 2 treatment arms. Median age was 67 and 68 years, respectively. All patients had prior LEN (71% vs 69% were LEN refractory), 72% vs 73% had prior BORT, and 70% vs 66% were refractory to their last treatment. Median number of prior lines of therapy was 2; 40% and 41% had 1 prior line of treatment in the PVd and Vd arms, respectively. Median follow-up was 16 months. Compared with Vd, PVd treatment significantly reduced the risk of progression or death by 39% and resulted in deeper responses in the intention-to-treat (ITT) population (Table 1). Median PFS was 11.20 vs 7.10 months in the ITT population and 20.73 vs 11.63 months in patients with only 1 prior treatment including LEN. Data for the overall survival analysis are not yet mature. Neutropenia (42% vs 9%), infections (31% vs 18%), and throm-

bocytopenia (27% vs 29%) were among the most frequently reported grade 3/4 treatment-emergent adverse events.

Table 1.

Efficacy	ITT		1 Prior Treatment Line	
	PVd n = 281	Vd n = 278	PVd n = 111	Vd n = 115
PFS, mos				
Median	11.20	7.10	20.73	11.63
HR (95% CI)	0.61 (0.49-0.77)		0.54 (0.36-0.82)	
P	< .0001		NA	
ORR (≥ PR), %	82.2	50.0	90.1	54.8
≥ VGPR, %	52.7	18.3	61.3	22.6

HR, hazard ratio; ITT, intention-to-treat; NA, not applicable; ORR, overall response rate; PFS, progression-free survival; PR, partial response; PVd, pomalidomide, bortezomib, and low-dose dexamethasone; Vd, bortezomib and low-dose dexamethasone; VGPR, very good partial response.

Summary/Conclusion: The OPTIMISMM phase 3 study in early RRMM reported a significant and clinically meaningful PFS improvement in patients who were entirely LEN exposed, including 70% of patients who were refractory to LEN. Of note, the PFS and overall response rate results showed improved benefit with PVd over Vd in patients who had only 1 prior line of treatment. Follow-up for long-term survival is ongoing. The safety of POM-based treatment was manageable and consistent with its well-established profile.

S848

MOR202 WITH LOW-DOSE DEXAMETHASONE (DEX) OR POMALIDOMIDE/DEX OR LENALIDOMIDE/DEX IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA (RRMM): A PHASE I/IIA, MULTICENTER, DOSE-ESCALATION STUDY

M. Raab[1,*], M. Chatterjee[2], H. Goldschmidt[3], H. Agis[4], I. W. Blau[5], H. Einsele[2], M. Engelhardt[6], B. Ferstl[7], M. Gramatzki[8], C. Röllig[9], K. Weisel[10], T. Jarutat[11], D. Weinelt[11], M. Winderlich[11], R. Boxhammer[11], C. Peschel[12]

[1]Department of Medicine V, University Hospital Heidelberg, Heidelberg, [2]Department of Internal Medicine II, University Hospital of Wuerzburg, Wuerzburg, [3]Department of Medicine V, University Hospital Heidelberg and the National Center for Tumor Diseases, Heidelberg, Germany, [4]Department of Medicine I, University Hospital of Internal Medicine – AKH Wien, Vienna, Austria, [5]Department of Internal Medicine III, Charité Campus Benjamin Franklin, Berlin, [6]Hematology & Oncology Department, Medical University Hospital, Freiburg, [7]Department of Internal Medicine 5 - Hematology and Oncology, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, [8]Department of Medicine, University Hospital Schleswig-Holstein Division of Stem Cell Transplantation and Immunotherapy, Kiel, [9]Department of Medicine I, University Hospital Carl Gustav Carus, Dresden, [10]Department of Hematology, Oncology, Immunology, Rheumatology and Pulmonology, University Hospital of Tuebingen, Tuebingen, [11]Morphosys AG, Planegg, [12]Department of Internal Medicine III, Technical University of Munich, Munich, Germany

Background: CD38 is a type II transmembrane glycoprotein expressed by MM cells. MOR202, a human IgG1 CD38 monoclonal antibody, has shown high single-agent activity in preclinical models of MM and synergy in combination with immunomodulatory drugs (IMiDs®), lenalidomide (LEN) and pomalidomide (POM).
Aims: The primary objectives of the study were to evaluate the safety, maximum tolerated dose (MTD)/recommended phase II dose of MOR202 in patients with relapsed or refractory multiple myeloma.
Methods: This is an analysis of a multicenter, dose-escalation phase I/IIa study of MOR202 with data presentation from patient (pt) cohorts treated with clinically relevant doses of MOR202 (2-hour IV infusion; 4, 8 and 16 mg/kg q1w) + Dex (≤40 mg), or at 8 or 16 mg/kg q1w with an IMiD+Dex combination.
Results: As of September 2017, 91 pts had been treated, including 56 in clinically relevant cohorts: 18 received MOR202 + Dex, 17 MOR202 + LEN/Dex and 21 MOR202 + POM/Dex. Pts received a median of 3, 2 and 3 prior treatment lines, respectively. The MTD of MOR202 was not reached. Combinations were generally well tolerated, with grade ≥3 adverse events (AEs) of mainly hematological nature. 2 pts discontinued due to a MOR202-related AE (one patient with a grade 4 thrombocytopenia and one patient with a grade 3 bacterial infection complicated by acute kidney failure). Infusion-related reactions (all grade 1 or 2) were observed in 4/56 (7%) pts. These mainly occurred during the first infusion. In the MOR202 + Dex cohort, 5/18 (28%) pts showed a response: 3 partial responses (PRs) and 2 very good PRs (VGPRs). Responses were also observed in 11/17 (65%, 7 PRs, 3 VGPRs, 1 complete response [CR]) pts in the MOR202 + LEN/Dex cohort and 9/21 (43%, 3 PRs, 4 VGPRs and 2 CRs) in the MOR202 + POM/Dex cohort. Longest response duration was 25 months (MOR202/Dex). Preliminary results indicate preservation of high CD38 levels on MM cells under MOR202 therapy.
Summary/Conclusion: 2-hour infusions of MOR202 administered at up to 16 mg/kg with Dex or in combination with an IMiD/Dex in heavily pretreated pts with RRMM showed a favorable safety profile, including excellent infusion tolerability. Promising preliminary efficacy and long-lasting tumor control were observed.

S849

ONCE-WEEKLY VS TWICE-WEEKLY CARFILZOMIB DOSING PLUS DEXAMETHASONE IN PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA (RRMM): RESULTS OF THE RANDOMIZED PHASE 3 STUDY A.R.R.O.W.

M.-V. Mateos[1,*], P. Moreau[2], J.R. Berenson[3], K. Weisel[4], A. Lazzaro[5], K. Song[6], M. A. Dimopoulos[7], M. Huang[8], A. Zahlten-Kumeli[8], A.K. Stewart[9]

[1]Hematology, Hospital Clinico Universitario de Salamanca-IBSAL, Salamanca, Spain, [2]Hematology Department, University of Nantes, Nantes, France, [3]Institute for Myeloma and Bone Cancer Research, West Hollywood, United States, [4]Universitatsklinikum Tubingen, Tubingen, Germany, [5]Department of Clinical Oncology and Hematology, Division of Hematology and Bone Marrow Transplant Center, Hospital Gulielmo da Saliceto, Piacenza, Italy, [6]Leukemia/Bone Marrow Transplant Program of British Columbia, Vancouver, Canada, [7]National and Kapodistrian University of Athens, Athens, Greece, [8]Amgen Inc., Thousand Oaks, [9]Division of Hematology/Oncology, Mayo Clinic, Scottsdale, United States

Background: Twice-weekly carfilzomib (K) at 20/27 mg/m^2 is approved for the treatment of RRMM. To develop a more convenient K regimen, once-weekly K plus dexamethasone (d) was assessed in the phase 1/2 CHAMPION-1 study, establishing a maximum tolerated dose of K 20/70 mg/m^2 for RRMM pts.
Aims: To present results from the pre-planned interim analysis of the phase 3 study A.R.R.O.W. comparing Kd once-weekly at 20/70 mg/m^2 (once-weekly group) vs twice-weekly at 20/27 mg/m^2(twice-weekly group).
Methods: Pts with 2–3 prior therapies and prior exposure to proteasome inhibitor and immunomodulatory agent were eligible. Pts were randomized 1:1 to receive either once- or twice-weekly K plus d. The once-weekly group received K (30-min IV) on days (D) 1, 8, and 15 of all cycles (20 mg/m^2 on D1 [cycle 1]; 70 mg/m^{2}thereafter). The twice-weekly group received K (10-min IV) on D1, 2, 8, 9, 15, and 16 (20 mg/m^2 on D1 and 2 during cycle 1 and 27 mg/m^2 thereafter). All pts received d at 40 mg on D1, 8, 15 (all cycles), and 22 (cycle 1–9 only). Treatment was given in 28-day cycles until disease progression or unacceptable toxicity. The primary endpoint was progression-free survival (PFS). Secondary endpoints were overall response rate (ORR), overall survival, safety, and pharmacokinetics.
Results: Baseline characteristics were generally balanced. Median PFS (once- vs twice-weekly) was 11.2 mo vs 7.6 mo (hazard ratio = 0.69; 1-sided p=0.0014). ORR (once- vs twice-weekly) was 62.9% vs 40.8% (p<0.0001); 7.1% vs 1.7% had a complete response or better. Grade ≥3 adverse events (AEs) occurred in 67.6% (once-weekly) and 61.7% (twice-weekly). Treatment-related grade 5 AEs occurred in 5 pts (2.1%) (once-weekly) and 2 pts (0.9%) (twice-weekly). The incidence of grade ≥3 hypertension and cardiac failure (once- vs twice-weekly) was 5.9% vs 5.5% and 2.9% vs 4.3%, respectively.
Summary/Conclusion: Once-weekly Kd at 20/70 mg/m^2 significantly improved PFS and ORR vs twice-weekly Kd at 20/27 mg/m^2. The incidence of AEs was comparable between groups. No new safety risks were found in the once-weekly group. Overall, once-weekly Kd showed favorable benefit-risk profile with a convenient dosing regimen vs twice-weekly Kd.

S850

FINAL RESULTS OF A PHASE IB STUDY OF ISATUXIMAB PLUS POMALIDOMIDE AND DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

J. Mikhael[1,2,*], P. Richardson[3], S. Usmani[4], N. Raje[5], W. Bensinger[6], C. Karanes[7], F. Dubin[8], Q. Liu[9], O. Vitse[10], K. Anderson[3]

[1]Mayo Clinic, Phoenix, [2]International Myeloma Foundation, Los Angeles, [3]Dana-Farber Cancer Institute, Harvard Medical School, Boston, [4]Levine Cancer Institute/Carolinas Healthcare System, Charlotte, [5]Massachusetts General Hospital, Boston, [6]Swedish Cancer Institute, Seattle, [7]City of Hope National Medical Center, Duarte, United States, [8]Sanofi, Vitry-sur-Seine, France, [9]Sanofi Oncology, Cambridge, United States, [10]Sanofi, Montpellier, France

Background: Isatuximab (ISA) is a monoclonal antibody targeting CD38-expressing tumor cells via several modes of action. We report final data from a Phase Ib dose-escalation/expansion study of ISA in combination with pomalidomide (Pom)/dexamethasone (dex) in patients (pts) with relapsed/refractory multiple myeloma (RRMM) (NCT02283775).
Aims: To evaluate combination therapy with ISA plus Pom/dex in pts with RRMM.
Methods: Pts with RRMM (≥2 prior MM therapies; includes lenalidomide and a proteasome inhibitor [PI]) received 5, 10, or 20 mg/kg ISA (4 weekly [QW] doses, then every 2 weeks until disease progression/intolerable toxicity), Pom 4 mg (Days 1–21), and dex 40 mg (QW; 20 mg if ≥75 yrs old) in 28-day cycles. All pts provided written informed consent to participate in the study. The primary objective was to determine the recommended dose of ISA plus Pom/dex. Secondary objectives included efficacy (International Myeloma Working Group criteria), safety, and pharmacokinetics (PK).
Results: Forty-five pts received ISA at 5 (n=8), 10 (n=31), or 20 (n=6) mg/kg. Median age was 67 (42–82) yrs. Median 3 (2–10) prior lines; 41 (91%), 37 (82%), and 38 (84%) pts were refractory to their last regimen, immunomodulatory drugs (IMiDs), or PIs, respectively. Six pts had high-risk (HR) cytogenetics. Median time on treatment was 9.6 mos; 19 (42%) pts remain on treatment. Two pts (10 mg/kg) discontinued due to an adverse event (AE) (grade [Gr] 5 intestinal perforation [due to underlying MM]; Gr 3 infusion-associated reaction [IAR]). One pt at each dose reported a dose-limiting toxicity; the maximum tolerated dose was not reached. The expansion cohort was initiated at 10 mg/kg based on efficacy, safety, and PK data. The most common treatment-emergent AEs besides IARs/hematologic abnormalities were fatigue (62%), upper respiratory tract infection (42%), and dyspnea (40%). Gr ≥3 neutropenia was observed in 83% of pts (Gr 4, 56%); all cases proved manageable with dose modification (dose delay in 16 [35.6%] pts and dose reduction in 22 [48.9%] pts) and/or granulocyte colony stimulating factor support. IARs occurred in 19 (42%) pts (Gr ≥3, 1 pt): 18 (40%) pts during the first infusion, 3 (7%) pts at later infusions. Overall response rate (ORR) was 62%; ORR in HR cytogenetics: 33%; IMiD refractory: 57%; PI refractory: 63%. Twelve (27%) pts achieved ≥ very good partial response (1 complete response; 1 stringent complete response). Median time to first response was 0.95 mos and median duration of response was 18.7 mos (95% confidence interval [CI] 12.5–not calculable). Median progression-free survival was 17.6 mos (95% CI 6.8–20.5). ISA PK is unaffected by co-administration with Pom/dex.
Summary/Conclusion: These final results confirm the promising clinical activity and manageable safety profile of ISA in combination with Pom/dex in heavily pretreated RRMM. A Phase III confirmatory trial is ongoing with results expected later in 2018.
Funding: Sanofi

S851

A PROSPECTIVE PHASE II STUDY OF DARATUMUMAB IN PREVIOUSLY-TREATED SYSTEMIC LIGHT- CHAIN (AL) AMYLOIDOSIS

A. Jaccard[1,*], B. Arnulf[2], A. M. Stoppa[3], L. Karlin[4], A. Perrot[5], M. Macro[6], A. Huart[7], L. Frenzel[8], P. Morel[9], V. Dorvaux[10], G. Merlini[11], G. Palladini[11], D. Lavergne[1], F. Bridoux[12], M. Rousselle[13]

[1]Hematology, CHU Limoges, Limoges, [2]Hematology, APHP, Saint Louis, Paris, [3]Hematology, IPC Marseille, Marseille, [4]Hematology, CHU Lyon, Lyon, [5]Hematology, CHU Nancy, Nancy, [6]Hematology, CHU Caen, Caen, [7]Nephrology, CHU Toulouse, Toulouse, [8]Hematology, APHP Necker, Paris, [9]Hematology, CHU Amiens, Amiens, [10]Hematology, CHU Metz-Thionville, Metz, France, [11]Hematology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy, [12]Nephrology, CHU Poitiers, Poitiers, [13]Hematology, CHU Toulouse, Toulouse, France

Background: Daratumumab (DARA), is a novel, high-affinity, IgG1κ human monoclonal antibody that specifically recognizes CD38. It has emerged as a breakthrough targeted therapy for patients with multiple myeloma. Monoclonal plasma cells in AL amyloidosis (AL) are similar to plasma cells in myeloma and express CD38.
Aims: We report here the preliminary results of a prospective multi-center, phase II study of DARA in AL (NCT02816476).
Methods: Forty patients will be recruited in this trial. Patients aged ≥18 years with evaluable AL amyloidosis, who have received ≥1 prior therapy and are not in very good partial response (VGPR) or better with a measurable plasma cell dyscrasia with dFLC >50 mg/L (difference between involved and uninvolved free light chain levels), with at least one major vital organ involvement, with ECOG performance status 0, 1 or 2, no chronic atrial fibrillation, a supine blood pressure >100 mmHg and NT-proBNp<8500 ng/L are eligible. They receive DARA intravenously in a standard schedule and dose: 16 mg/kg weekly during the first two 28-day cycles and every other week during cycles 3 through 6 for a total of six 28-day cycles. Hematologic responses are measured after 1 injection of DARA, at day 1 of each cycle and at the end of treatment visit. The objectives are to assess hematologic responses, organ responses and safety.
Results: To date 38 of the 40 planned patients have been enrolled in 11 French and 1 Italian centers. Here are the characteristics of the 36 patients included at data cut-off (Nov 13th, 2017). The median age is 69 years (range, 45-83). The median number of organ system involvement is 2 (range, 1-5). Twenty-three patients (64%) have cardiac and 21 patients (58%) renal involvement. The median time from diagnosis to enrolment is 24 months (range, 3.5-122). Median number of prior therapies is 3 (range, 1-5), 20 patients (56%) have received Melphalan and Dexamethasone, 34 patients (94%) bortezomib, and 19 patients (53%) lenalidomide. Nineteen patients (53%) have received 3 or more lines of treatment. There were two on-study deaths due to cardiac progression and lung cancer. Three other patients have discontinued study treatment before 6 cycles due to disease progression. Nine patients (25%) experienced at least one grade ≥3 AE (any cause) and only one was considered as drug related (lymphopenia). The most common drug-related AEs were infusion reaction seen in 11 patients (30%), all grade I or II. Very good partial response or better (VGPR) was observed in 14 of 32 evaluable (completing at least 1 cycle) patients (44%), and partial response in 5 patients (16%). The overall response rate is 59%. Responses were usually very rapid, after a single DARA injection, all 17 patients, with available dFLC measurement, who finally reached partial response or better had a dFLC decrease of more than 35 % with a median dFLC decrease after only 1 injection in these 17 responding patients of 70% (range 35-96).
Summary/Conclusion: Monotherapy with DARA demonstrates encouraging efficacy in previously-treated patients with AL amyloidosis with deep and rapid hematological responses. The administration of DARA in these patients is associated with a good safety profile and non-severe adverse events occurring mostly after the first infusion. The data, in particular hematological and organ responses, will be updated at the meeting. A prospective randomized international phase III study (AMY3001) in naive patients with DARA in combination with bortezomib, cyclophosphamide and dexamethasone is ongoing.

Waldenström's macroglobulinemia, mantle-cell lymphoma and hairy cell leukemia – Clinical

S852

MULTINATIONAL, RANDOMIZED PHASE 3 TRIAL OF IBRUTINIB-RITUXIMAB VS PLACEBO-RITUXIMAB IN PATIENTS WITH WALDENSTRÖM'S MACROGLOBULINEMIA

This abstract is embargoed until Friday, June 15, 08:30 local time.

M. Dimopoulos[1,*], A. Tedeschi[2], J. Trotman[3], R. García-Sanz[4], D. MacDonald[5], V. Leblond[6], B. Mahe[7], C. Herbaux[8], C. Tam[9], M.L. Palomba[10], J. Matous[11], C. Shustik[12], E. Kastritis[1], S. Treon[13], J. Li[14], Z. Salman[14], T. Graef[14], C. Buske[15]

[1]National and Kapodistrian University of Athens School of Medicine, Athens, Greece, [2]ASST Grande Ospedale Metropolitano Niguarda, Milano, Italy, [3]Concord Hospital, University of Sydney, Concord, Australia, [4]Hospital Universitario de Salamanca, Salamanca, Spain, [5]The Ottawa Hospital, University of Ottawa, Ottawa, Canada, [6]Département d' Hématologie Hôpital Pitié-Salpêtrière APHP, UPMC Université Paris, Paris, [7]Centre Hospitalier Universitaire de Nantes, Nantes, [8]Centre Hospitalier Régional Universitaire de Lille, Institute of Hematolog-Tranfusion, Lille, France, [9]Peter MacCallum Cancer Centre & St. Vincent's Hospital, Melbourne, Australia, [10]Memorial Sloan Kettering Cancer Center, New York City, [11]Colorado Blood Cancer Institute, Denver, United States, [12]Royal Victoria Hospital at McGill University Health Centre, Montreal, Canada, [13]Dana-Farber Cancer Institute, Boston, [14]Pharmacyclics LLC, an AbbVie Company, Sunnyvale, United States, [15]Comprehensive Cancer Center Ulm, Institute of Experimental Cancer Research, University Hospital of Ulm, Ulm, Germany

Background: Waldenström's macroglobulinemia (WM) is an incurable B-cell lymphoma with several treatment choices, however, there have been few randomized trials to help identify treatment standards. Single-agent ibrutinib is highly active in relapsed WM with reported PFS rates of 86% (at 18 mo) and 69% (at 24 mo) (Dimopoulos, Lancet Oncol. 2017; Treon, NEJM 2015) and is approved in the United States and Europe for WM.
Aims: To report the safety and efficacy of ibrutinib-rituximab (IR) vs placebo-rituximab (R) at a preplanned interim analysis in treatment naïve and relapsed patients who were not refractory to prior rituximab-containing therapies.
Methods: Patients with confirmed WM and symptomatic disease requiring treatment were randomized to daily ibrutinib (420 mg) or placebo, both with rituximab (375 mg/m^2/wk IV for infusions at wks 1-4 and 17-20). Patients previously treated with a rituximab-based regimen were required to have a response (≥MR) to the last rituximab therapy. The primary endpoint was progression-free survival (PFS) as assessed by an independent review committee (IRC). We also report response rates, sustained improvement in hemoglobin levels, time to next treatment (TTnT), overall survival (OS), and safety. All patients provided written informed consent.

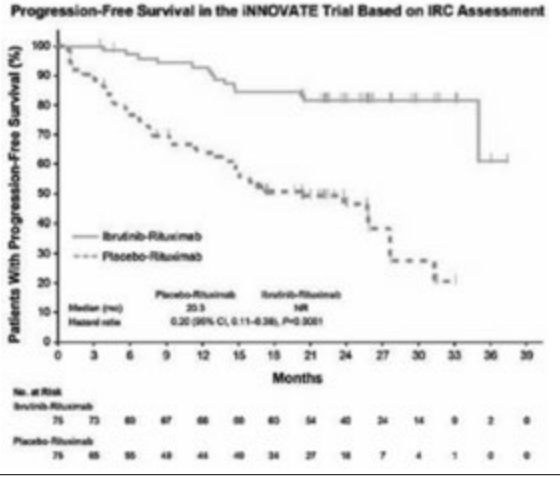


Figure 1.

Results: For 150 randomized patients, median age was 69 y, 38% had a high IPSSWM risk score, and 45% were treatment-naïve. Of 136 patients with available mutation data, MYD88^{L265P} and CXCR4^{WHIM} mutations were identified by target exome sequencing assay in 85% and 36%, respectively. At a median follow-up of 26.5 mo, IR significantly prolonged PFS compared with R representing a five-fold reduction in the risk of progression or death (median PFS, not reached vs 20 mo; hazard ratio [HR], 0.20; 95% CI: 0.11-0.38, p<0.0001; Figure 1). The 30-mo PFS rates were 82% vs 28% in each arm. Improvements in PFS were consistently reported in all relevant subgroups, including treatment-naïve (HR, 0.34; 95% CI: 0.12-0.95), relapsed (HR, 0.17; 95% CI: 0.08-0.36), MYD88^{L265P}/CXCR4^{WT} (HR, 0.17; 95% CI: 0.06-0.49), MYD88^{L265P}/CXCR4^{WHIM} (HR, 0.24; 95% CI: 0.09-0.66), and MYD88^{WT}/CXCR4^{WT}(HR, 0.21; 95% CI: 0.04-1.1). Overall (≥MR) and major (≥PR) response rates were significantly higher for IR vs R, 92% vs 47% (p<0.0001) and 72% vs 32% (p<0.0001), respectively. Improvements in hemoglobin were observed in 73% vs 41% of IR and R patients (p<0.0001). At the time of this analysis, 75% of IR patients continued on treatment. Median TTnT was not reached for IR and 18 mo for R (HR, 0.096; *P* <0.0001). The 30-mo OS rates were 94% vs 92% in the 2 arms. With a median time on treatment of 25.8 mo for IR, grade ≥3 treatment-emergent adverse events (AEs) occurred in 60% vs 61% of patients on on IR vs R. Serious AEs occurred in 43% vs 33% of patients on each arm. No fatal AEs occurred with IR vs 3 with R. Meaningful reductions in any grade IgM flare (8% vs 47%) and grade ≥3 infusion reactions (1% vs 16%) were observed with IR vs R.
Summary/Conclusion: The efficacy of the ibrutinib-rituximab combination was superior to that of placebo-rituximab, producing improvements in PFS for all WM patients regardless of prognostic or genotypic factors. The IR combination had a manageable toxicity profile and no new or unexpected AEs were observed. Based on these results, IR should be considered a standard therapeutic option for patients with WM.

S853

ACALABRUTINIB IN PATIENTS WITH WALDENSTRÖM MACROGLOBULINEMIA

R. Owen[1,*], H. McCarthy[2], S. Rule[3], S. D'sa[4], S. Thomas[5], F. Forconi[6], T. Anderson[7], M.J. Kersten[8], P.L. Zinzani[9], S. Iyengar[10], J. Kothari[11], M. Minnema[12], E. Kastritis[13], R. Izumi[14], J.G. Slatter[14], D. Mittag[15], H. Wei[14], D.-Y. Chen[14], P. Patel[14], R.R. Furman[16]

[1]St James's University Hospital, Leeds, [2]Royal Bournemouth Hospital, Bournemouth, [3]Plymouth University Medical School, Plymouth, [4]University College London Hospitals NHS Trust, London, United Kingdom, [5]The University of Texas MD Anderson Cancer Center, Houston, United States, [6]University of Southampton Hospital Trust, Southampton, United Kingdom, [7]Texas Oncology-Bedford, Bedford, United States, [8]Academic Medical Center, University of Amsterdam, on behalf of the Lunenburg Lymphoma Phase I/II Consortium - HOVON /LLPC, Amsterdam, Netherlands, [9]Institute of Hematology University of Bologna, Bologna, Italy, [10]Royal Marsden Hospital, London, [11]Churchill Hospital, Oxford, United Kingdom, [12]University Medical Center Utrecht Cancer Center, on behalf of the Lunenburg Lymphoma Phase I/II Consortium - HOVON /LLPC, Utrecht, Netherlands, [13]National and Kapodistrian University of Athens, Athens, Greece, [14]Acerta Pharma, Redwood City, United States, [15]Acerta Pharma, Oss, Netherlands, [16]Weill Cornell Medical College, New York Presbyterian Hospital, New York, United States

Background: Bruton tyrosine kinase (BTK) is a clinically validated target in Waldenström Macroglobulinemia (WM). Acalabrutinib is a highly selective, potent, covalent BTK inhibitor.
Aims: The efficacy and safety of acalabrutinib was evaluated in a Phase 2 study of patients with treatment-naive (TN) or relapsed/refractory (R/R) WM.
Methods: Patients with TN or R/R WM received 100 mg acalabrutinib BID (or 200 mg QD [n=6], later switched to 100 mg BID) in 28-day cycles until progressive disease (PD) or intolerance. The primary endpoint was investigator-assessed overall response rate (ORR). Secondary endpoints included duration of response (DOR), progression-free survival (PFS), overall survival (OS), safety and pharmacokinetics (PK).
Results: One hundred six patients (14 TN and 92 R/R) were treated. In all patients, the median age was 69 years (range 39-90), 94% had ECOG PS ≤1, and median serum IgM level was 3615 mg/dL (range 291-9740). R/R patients had a median of 2 prior therapies (range 1-7). At a 25-mo median follow-up, 7 (50%) TN patients and 70 (76%) R/R patients remain on treatment. Discontinuations were primarily due to PD (TN: 0 patients; R/R: 9 patients), adverse events (AEs; TN: 3 patients; R/R: 3 patients), and investigator decision (TN: 2 patients; R/R: 4 patients). BTK occupancy and PK parameters were consistent with previous acalabrutinib studies. Efficacy outcomes are listed in Table 1. The most common AEs of any grade were headache (39%), diarrhea (31%), contusion (29%), and dizziness (25%). The most common Grade 3/4 AEs were neutropenia (16%), pneumonia (7%), anemia, increased alanine aminotransferase, and hyponatremia (each 5%). Atrial fibrillation occurred in 3 patients; 1 case was Grade 3. Bleeding events occurred in 57% of patients, the most common of which were contusion (29%) and epistaxis (13%). Four bleeding events were Grade 3/4:

epistaxis, hematuria, dysfunctional uterine bleeding, and retinal hemorrhage. There were 5 Grade 5 events: pneumonia, glioblastoma multiforme, esophageal carcinoma, myocardial ischemia, and intracranial hematoma.

Table 1.

	Modified 3rd International Workshop on WM Criteria (Kimby 2006)	
	TN (n=14)	R/R (n=92)
ORR (≥ minor response [MR]), n (%)	13 (93)	86 (94)
95% CI	66, 100	86, 98
Major response rate (≥ partial response [PR])	11 (79)	72 (78)
95% CI	49, 95	68, 86
Complete response	0	0
Very good PR	1 (7)	29 (32)
PR	10 (71)	43 (47)
MR	2 (14)	14 (15)
24-mo rate, % (95% CI)		
DOR	90 (47, 99)	84 (73, 90)
PFS	90 (47, 99)	82 (72, 88)
OS	92 (54, 99)	89 (80, 94)

Summary/Conclusion: Acalabrutinib is a highly effective treatment for WM with durable responses and limited toxicity.

S854

PROSPECTIVE PHASE II STUDY OF VENETOCLAX (VEN) IN PATIENTS (PTS) WITH PREVIOUSLY TREATED WALDENSTROM MACROGLOBULINEMIA (WM)

J. Castillo[1,*], J. Gustine[1], K. Meid[1], T. Dubeau[1], J. Allan[2], R. Furman[2], T. Siddiqi[3], R. Advani[4], J. Lam[4], Z. Hunter[1], G. Yang[1], L. Xu[1], M. Davids[1], S. Treon[1]

[1]Dana-Farber Cancer Institute, Boston, [2]Weill Cornell Medicine, New York, [3]City of Hope, Duarte, [4]Stanford Cancer Institute, Stanford, United States

Background: Ven is an oral BCL2 antagonist approved for the treatment of chronic lymphocytic leukemia. However, the role of venetoclax in WM is unknown. Immunophenotypic and genomic sequencing studies have shown that BCL2 is highly expressed and activated in WM cells (Chng Blood 2006; Hunter Blood 2016). In a study of pts with non-Hodgkin lymphoma, ven showed efficacy in 4 pts with WM (Davids J Clin Oncol 2017).
Aims: We initiated a phase II study to evaluate the safety and efficacy of ven monotherapy in previously treated pts with WM (NCT02677324).
Methods: Treatment was given in the outpatient setting and followed a ramp-up of 200 mg daily days 1-7, 400 mg daily days 8-14, then 800 mg daily thereafter, for maximum of 2 years. Pts were closely monitored for tumor lysis syndrome (TLS) during the first 24 hours of each dose escalation. Toxicity was graded per CTCAE v.4.03. Response was assessed based on IWWM6 criteria.
Results: 30 pts with symptomatic WM were enrolled. Median follow-up time is 8 months (range 0.2-18 months). Median age was 66 years (range 39-80 years) and 17 pts (57%) were men. Indications to treat were constitutional symptoms (52%), anemia (44%), neuropathy (15%), extramedullary disease (11%) and thrombocytopenia (7%). Median number of previous lines of therapy was 2 (range 1-10), and 15 pts (50%) were previously exposed to BTK inhibitors (BTKi). The $MYD88^{L265P}$ mutation was detected in all pts, and CXCR4 mutations in 16 (53%). At baseline, median serum IgM was 3,543 mg/dl (range 642-7,970 mg/dl), median bone marrow involvement was 35% (range 4-95%) and median hemoglobin was 10.6 g/dl (range 6.4-13.5 mg/dl). All pts were successfully escalated to target dose of 800 mg. At 6 months, median serum IgM declined to 1,640 mg/dl (range 49-5,220 mg/dl), median bone marrow involvement declined to 5% (range 0-20%) and median hemoglobin increased to 12.6 g/dl (range 10-15.1 g/dl) ($p<0.001$ for all variables against baseline). At best response, very good partial response (VGPR) was attained in 5 pts (17%), partial response in 11 (37%), minor response in 8 (27%) and stable disease in 6 (20%), for overall response rate of 80% and major response rate of 53%. Major response rate was not statistically different based on relapsed or refractory disease, prior BTKi exposure or CXCR4 mutation status. However, VGPR rate at best response was lower in pts with previous BTKi exposure (7% vs. 27%), and those with CXCR4 mutations (6% vs. 29%). Median time to response (TTR) was 9 weeks and was slower in pts with prior BTKi exposure than in pts without (19 vs. 6 weeks; $p=0.02$). TTR was not impacted by relapsed vs. refractory disease or CXCR4 mutations. Only one instance of disease progression has been observed thus far, in a patient with MYD88, CXCR4 and TP53 mutations. Grade 4 neutropenia ocurred in 4 pts, who received G-CSG support; ven was dose reduced to 600 mg in 2 pts. Grade 3 adverse events included neutropenia (n=7), anemia (n=2), back pain (n=1), constipation (n=1), diarrhea (n=1), headache (n=1), upper respiratory infection (n=1). One instance of laboratory TLS occurred in a patient with significant extramedullary disease. No clinical TLS was seen. Most common grade 2 adverse events included anemia (n=5), nausea (n=4), neutropenia (n=3). Most common grade 1 adverse events included nausea (n=9), diarrhea (n=6), rash (n=5). No instances of IgM flare were observed, and there have been no deaths.
Summary/Conclusion: Our interim results show that venetoclax provides a safe and effective treatment option for pts with symptomatic, previously treated WM, including those previously exposed to BTKi.

S855

EFFICACY OF VENETOCLAX MONOTHERAPY IN PATIENTS WITH RELAPSED, REFRACTORY MANTLE CELL LYMPHOMA POST BTK INHIBITION THERAPY

T. Eyre[1,*], H. Walter[2], S. Iyengar[3], G. Follows[4], C. Fox[5], M. Dyer[2,6], N. Morley[7], G. Collins[1]

[1]Haematology, Oxford University Hospitals NHS Foundation Trust, Oxford, [2]Haematology, Leicester Royal Infirmary, Leicester, [3]Haematology, The Royal Marsden Hospital, London, [4]Haematology, Addenbrooke's Hospital NHS Trust, Cambridge, [5]Haematology, Nottingham University Hospitals NHS Trust, Nottingham, [6]The Ernest and Helen Scott Haematological Research Institute, Leicester, [7]Royal Hallamshire Hospital, Sheffield, United Kingdom

Background: Patients (pts) with relapsed, refractory (R/R) mantle cell lymphoma (MCL) have a typical survival of 2 years despite the recent approval of active novel agents including lenalidomide, ibrutinib and acalabrutinib. Although ibrutinib monotherapy provides impressive efficacy (overall response rate (ORR) 68% (complete response (CR) 21%)) and tolerability in R/R MCL, primary resistance (i.e. lack of response) occurs in 30% and most pts ultimately relapse, representing a cohort with clear unmet need. Documented responses post-ibrutinib are seen in <1/3 of patients and median overall survival (OS) following cessation of ibrutinib 4 months. Venetoclax is a potent, selective and orally bioavailable BCL2 inhibitor. A phase 1 first-in-human trial of venetoclax (n=106) included 28 Bruton tyrosine kinase inhibitor (BTKi)-naïve MCL pts; toxicity was minimal and an impressive ORR 75% (21% CR) was demonstrated.
Aims: There are no data regarding venetoclax efficacy in R/R MCL outside these initial data and none available in the post-BTKi setting. We provide data from the UK compassionate use programme.
Methods: 20 R/R MCL pts who had failed BTKi received venetoclax monotherapy in UK and Ireland between 03/2016-02/2018. Data at diagnosis and relapse were collected from hospital records by the treating physician. Response to and duration of BTKi and reasons for stopping BTKi were collected. Baseline data collected prior to venetoclax included LDH, performance status, extranodal disease/sites, stage, histological subtype, and KI67%.
Results: The median age was 69 years (range 43-84) with 86% male gender. Pts received a median of 3 prior lines (range 2-5). 38% received a Rituximab(R)-Maxi-CHOP/Ara-C-based induction, and 29% were consolidated with ASCT in 1st remission. Others pts received chemotherapy (fludarabine or CHOP-based) +/- R 1st line. At relapse (2nd or subsequent line), all but 1 pt received BTKi (ibrutinib (n=17), ibrutinib with DLI (n=1), tirabrutinib (n=2)). The ORR to BTKi was 55% (CR 15%), with a median progression free survival (PFS) of 4.8 months (95% CI 3.1-29.3 months). 9/20 pts did not respond to BKTi. 18 pts stopped BTKi due to disease progression and 2 for toxicity. Following BTKi, 4 pts relapsed with documented blastoid MCL and the median Ki67% was 45% (n=11). ECOG PS 0-1 (n=11) and PS 2-3 (n=9) were noted. LDH was raised in 75%. ORR to venetoclax monotherapy in the 20 BKTi exposure pts was 60% (20% CR). The median PFS was 2.6 months (95% confidence interval (CI) 1.5-9.9 months) and the median OS was 4.3 months (95% CI 2.1-16.0 months). The median duration of response was not reached (Figure 1). Although the ORR to venetoclax varied according to prior BTKi response (primary resistance to BKTi (n=9): ORR 44.4% *vs* response to prior BTKi (n=11): ORR 72.7%) differed ($p=0.198$)), this did not translate to an improved PFS (Figure 1). There were no cases of clinical tumour lysis syndrome (TLS) and 5 asymptomatic biochemical TLS. Venetoclax was otherwise well tolerated. 3 pts required dose reductions (800 mg to 600 mg od) due to adverse events (AEs) (Grade (G) 2 fatigue (n=1), G2 diarrhoea (n=2)). There were 13 AEs

reported including pneumonia (G3; n=3), sepsis (G4; n=2), fatigue (G2; n=2) and diarrhoea (G2; n=2). 11 pts have died; 9 from PD and 2 from the combination of PD and infection.

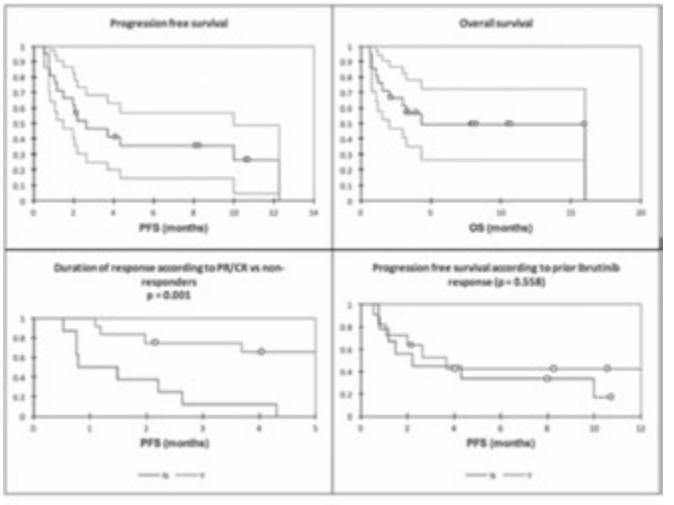


Figure 1.

Summary/Conclusion: In summary, venetoclax monotherapy provided an ORR of 60% with 20% CR in a poor-risk R/R BTKi-resistant MCL. Venetoclax was relatively non-toxic and provides scope for rational novel combination therapies in this setting.

S856

MOXETUMOMAB PASUDOTOX IN HEAVILY PRETREATED PATIENTS WITH RELAPSED/REFRACTORY HAIRY CELL LEUKEMIA: RESULTS OF A PIVOTAL INTERNATIONAL STUDY

F. Giles[1,*], C. Dearden[2], P. L. Zinzani[3], J. Delgado[4], L. Karlin[5], T. Robak[6], D. Gladstone[7], P. le Coutre[8], S. Dietrich[9], M. Gotic[10], L. Larratt[11], S. Lee[12], F. Offner[13], G. Schiller[14], R. Swords[15], L. Bacon[16], M. Bocchia[17], K. Bouabdallah[18], D.A. Breems[19], A. Cortelezzi[20], S. Dinner[21], M. Doubek[22], B.T. Gjertsen[23], M. Gobbi[24], A. Hellmann[25], S. Lepretre[26], F. Maloisel[27], F. Ravandi[28], P. Rousselot[29], M. Rummel[30], T. Siddiqi[31], T. Tadmor[32], X. Troussard[33], C. Arana Yi[34], P. He[35], S. Marshall[35], W. Wilson[36], I. Pastan[36], N.S. Yao[35], R.J. Kreitman[36]

[1]Developmental Therapeutics Consortium, Chicago, IL, United States, [2]Royal Marsden NHS Foundation, Sutton, Surry, United Kingdom, [3]Institute of Hematology, Seràgnoli University of Bologna, Bologna, Italy, [4]Hospital Clinic, Barcelona, Spain, [5]Centre Hospitalier Lyon Sud, Pierre-bénite, France, [6]Medical University of Lodz, Copernicus Memorial Hospital, Lodz, Poland, [7]Johns Hopkins Kimmel Cancer Center, Baltimore, MD, United States, [8]Charité Universitätsmedizin, Berlin, [9]Universitätsklinikum Heidelberg, Heidelberg, Baden-Württemberg, Germany, [10]Clinical Center of Serbia, Belgrade, Serbia, [11]University of Alberta, Edmonton, Alberta, Canada, [12]New York-Presbyterian Weill Cornell Medical Center, New York, NY, United States, [13]Ghent University Hospital, Ghent, Belgium, [14]David Geffen School of Medicine, UCLA, Los Angeles, CA, [15]Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL, United States, [16]St. James's Hospital, Dublin, Ireland, [17]Azienda Ospedaliera Universitaria, University of Siena, Siena, Italy, [18]Hôpital du Haut Lévêque, Pessac, France, [19]Ziekenhuis Netwerk Antwerpe, Antwerp, Belgium, [20]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and University of Milan, Milan, Italy, [21]Northwestern Medicine Feinberg School of Medicine, Chicago, IL, United States, [22]Masaryk University, Brno, Czech Republic, [23]Helse Bergen HF Haukeland University Hospital, Bergen, Norway, [24]Ospedale S. Martino, Genoa, Italy, [25]Department of Hematology and Transplantation, Medical University of Gdańsk, Gdańsk, Poland, [26]Inserm U1245 and Department of Hematology, Centre Henri Becquerel and Normandie Univ UNIROUEN, Rouen, [27]Clinique Sainte-Anne, Strasbourg, France, [28]MD Anderson Cancer Center, Houston, TX, United States, [29]Hôpital André Mignot, Le Chesnay, Cedex, France, [30]Justus-Leibig University, Giessen, Germany, [31]City of Hope National Medical Center, Duarte, CA, United States, [32]Bnai Zion Medical Center, Haifa, Israel, [33]L'hôpital Côte de Nacre, Caen Cedex 9, France, [34]University of New Mexico, Albuquerque, NM, [35]MedImmune, Gaithersburg, MD, [36]National Cancer Institute, National Institutes of Health, Bethesda, MD, United States

Background: Moxetumomab pasudotox, a first-in-class recombinant immunotoxin targeting CD22, is composed of an immunoglobulin light chain variable domain and a heavy chain variable domain genetically fused to a truncated form of *Pseudomonas* exotoxin PE38.
Aims: This pivotal multicenter, single-arm study evaluated the rate of durable complete response with moxetumomab pasudotox in patients with relapsed/refractory hairy cell leukemia (HCL).
Methods: Eligible patients had ≥2 prior systemic therapies, including ≥1 purine nucleoside analog. Patients received moxetumomab pasudotox 40 µg/kg intravenously on days 1, 3, and 5 of 28-d cycles, up to 6 cycles. Disease response and immunohistochemistry (IHC) minimal residual disease (MRD) status were determined by blinded independent central review. The primary end point was durable complete response (CR), defined as CR with hematologic remission (HR; blood count normalization) for >180 days.
Results: Eighty patients (63 male; median age 60 years) received moxetumomab pasudotox. The median number of prior systemic therapies was 3 (2–11); 39 patients (49%) had >3 prior lines of therapy and 60 (75%) had prior rituximab. At 16.7 months median follow-up, objective response (OR) rate was 75% (60/80), HR rate was 80% (64/80), CR rate was 41% (33/80), and durable CR rate was 30% (24/80). Of 33 patients achieving CR, 27 (82%) had IHC MRD negative status. Median time to HR was 1 month. Median duration of OR and median PFS were not reached. Most frequent treatment-related adverse events (AEs) were nausea (28%), peripheral edema (26%), headache (21%), and pyrexia (20%); 8% had infections and 3% had neutropenia deemed treatment related. Three deaths occurred; none were treatment related. Treatment-related AEs leading to discontinuation were hemolytic uremic syndrome (HUS; n=4 [5%]), capillary leak syndrome (CLS; n=2 [3%]), and increased blood creatinine (n=2 [3%]). Seven patients (9%) had CLS (grade 2: n=5; grade 4: n=2), 7 (9%) had HUS (grade 2: n=2; grade 3: n=3; grade 4: n=2), and 4 (5%) had both. Both CLS and HUS were manageable and reversible with dose interruption and discontinuation in severe cases (no plasma exchange in HUS). Median immunoglobulin levels remained unchanged after treatment. Median CD4 cell counts were stable or improved after the first week of treatment.
Summary/Conclusion: Moxetumomab pasudotox achieved a high rate of independently assessed durable CR, with the ability to eradicate MRD in heavily pretreated HCL patients, and showed a favorable safety profile without immuno/myelosuppression.

Intensive and immune therapy in AML

S857

PROSPECTIVE STUDY OF HLA-MATCHED DONOR AVAILABILITY AND SURVIVAL IN REMISSION AFTER AML IN OLDER ADULTS: 1ST PLANNED ANALYSIS FROM ECOG-ACRIN E2906 PHASE III RANDOMIZED TRIAL IN PATIENTS AGE ≥60 YEARS

J. Foran[1,*], Z. Sun[2], D. Claxton[3], H. Lazarus[4], E. Paietta[5], J. Godwin[6], S. Luger[7], J. Rowe[8], J. Altman[9], K. Pratz[10], M. Litzow[11], M. Tallman[12]

[1]Mayo Clinic Cancer Center, Mayo Clinic Florida, Jacksonville, [2]ECOG-ACRIN Biostatistics Center, Dana-Farber Cancer Institute, Boston, [3]Penn State Hershey Cancer Institute, Hershey, [4]University Hospitals Cleveland Medical Center, Cleveland, [5]Montefiore Medical Center, Bronx, [6]Providence Portland Medical Center, Portland, [7]Abramson Cancer Center, University of Pennsylvania, Philadelphia, United States, [8]Hematology, Shaare Zedek Medical Center, Jerusalem, Israel, [9]Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, [10]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, [11]Hematology, Mayo Clinic, Rochester, [12]Leukemia, Memorial Sloan-Kettering Cancer Center, New York, United States

Background: This is the 1st report of the planned analysis of donor availability and outcome in the E2906 phase III trial of intensive therapy in patients (pts) age ≥60 years with newly-diagnosed AML.
Aims: Determine availability of HLA-matched donor and its impact on relapse & survival from 1st remission in the E2906 prospective study of 'curative' therapy with integrated allogeneic transplantation (AlloBMT).
Methods: 727 pts were registered (2011-2015) to receive induction therapy on E2906, with results previously presented (Foran, ASH 2015, #217). All pts in 1st 'remission' [complete remission (CR), CRi (platelets (plts) <100K), or leukemia-free state] with 10/10 HLA-matched donor were eligible for protocol AlloBMT using a busulfan-fludarabine reduced intensity conditioning (RIC) regimen. Living siblings, intent to initiate donor search, & comorbidity (HCT-CI) score at AML diagnosis was recorded for all pts. Survival from remission was determined by Kaplan-Meier method, and we performed a multivariate analysis using Cox models to determine impact of matched donor availability on Overall (OS) & Leukemia-Free survival (LFS; relapse or death from remission) with established AML risk factors.
Results: 360 patients in 'remission' (centrally reviewed) were included in this pre-specified analysis; 135 (37.5%) had a donor identified (sibling 42%, unrelated 58%), & 225 did not. Patients with donor were more likely baseline age<70yrs (p<0.01), performance status (PS)=0 (p=0.03), & plts≥50K (p<0.01). 61/135 (45%) underwent protocol-specified RIC AlloBMT in 1st remission, and 55 additional pts underwent *non*-protocol AlloBMT, including 25 in the No Donor group. The median follow-up is 37.7 months, and OS by Donor status is noted in Figure 1 (p=0.013). On multivariate analysis, favorable risk cytogen., PS, & plts≥50K were significantly associated with OS (Table 1). After stratification by age, secondary (vs. *de novo*) AML & induction treatment, and adjusted for cytogenetics, plts, PS & HCT-CI, availability of matched Donor was associated with 24% reduction OS risk, however did not meet statistical significance on multivariate analysis (HR: 0.76, 95% CI [0.56, 1.04], p=0.09). Similar results were observed for LFS in multivariate model (HR: 0.76, 95% CI [0.56, 1.03], p=0.07).

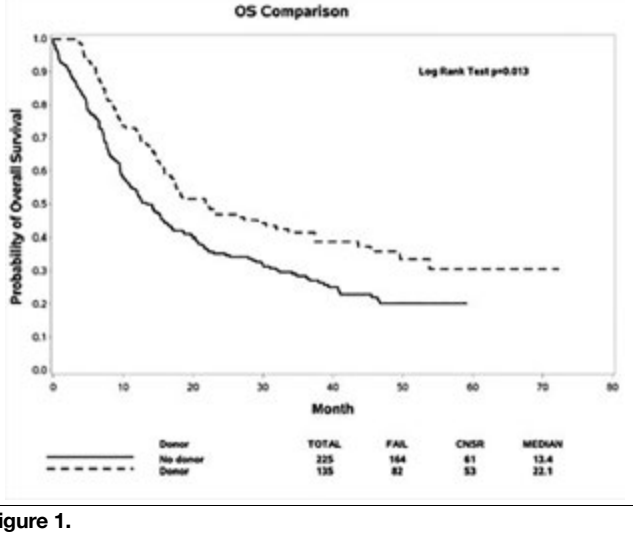


Figure 1.

Table 1.

Multivariate Cox Model – OS	P Value	Hazard Ratio (HR)	95% HR CI	
Donor vs. No donor	0.09	0.76	0.56	1.04
Baseline HCT-CI 0 vs. >2	0.33	0.82	0.56	1.22
Favorable vs. Unfavorable Risk Cytogenetics	0.002	0.25	0.11	0.59
Platelet <50K vs. ≥50K	<.0001	1.89	1.39	2.55
Performance status 2 vs. 0	0.02	1.68	1.07	2.63
Performance status 3 vs. 0	0.004	5.32	1.70	16.65
Unfavorable Risk Cytogen., Donor vs. No Donor	0.13	0.65	0.38	1.13

Summary/Conclusion: An OS advantage was observed in this 1st prospective Donor vs. No Donor analysis from E2906 in older adults, although matched donor status was not significant in the multivariate model. Further analysis is being performed to identify barriers to AlloBMT in this population, and its impact in CR1 vs. conventional therapy.

S858

PANOBINOSTAT, DECITABINE, AND DONOR LYMPHOCYTE INFUSION POST ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: THE HOVON 116 STUDY IN NEWLY DIAGNOSED POOR-RISK AML PATIENTS

J. Cornelissen[1,*], Y. van Norden[1], B. Kalin[1], M. van Gelder[2], D. Breems[3], J. Maertens[4], M. Jongen-Lavrencic[5], A. Broers[5], T. Grob[5], W. Zeijlemaker[6], G. Ossenkoppele[6], E. Meijer[6]

[1]Erasmus MC, Rotterdam, [2]Maastricht University Medical Center, Maastricht, Netherlands, [3]Ziekenhuis Netwerk Antwerpen, Antwerp, [4]University Hospital Leuven, Leuven, Belgium, [5]ErasmusMC, Rotterdam, [6]VUMC, Amsterdam, Netherlands

Background: Although the allogeneic graft versus leukemia (GVL) effect is operational in poor-risk AML, the relapse rate remains high. In order to exploit GVL more effectively, we explored the early initiation of epigenetic therapy after alloHSCT, interspersed with successive, low dosage donor lymphocyte infusion (DLI).
Aims: 1. First, feasibility of epigenetic therapy, consisting of either panobinostat (PNB) alone or PNB combined with decitabine, was evaluated with toxicities and DLT as endpoints. 2. Following the feasibility phase, the study continued as phase II focusing on outcome after transplantation.
Methods: Pts were registered early after diagnosis. Patients qualifying for alloHSCT all received standard reduced intensity conditioning (Flu/TBI). GVHD prophylaxis consisted of post-transplant (PT) cyclophosphamide and a short course of ciclosporine. First, feasibility of epigenetic therapy, consisting of either panobinostat (PNB) alone (20 mg orally at days 1, 4, 8, 11 of a 4 wk-cycle) or PNB combined with decitabine (DCB, either 10 or 20 mg/m^2 i.v. at days 1-3 of every 4 wk-cycle) was evaluated. After the feasibility phase, the study continued as phase II focusing on toxicities, GVHD, non-relapse mortality (NRM), relapse, residual disease (MRD), overall survival (OS), and relapse free survival (RFS) as from alloHCT.
Results: 140 Pts were registered early after diagnosis and 110 actually proceeded to alloHSCT at a median number of 110 days (range: 56-205) after diagnosis. 67 pts were in hematological CR, 38 in CR without complete blood recovery, and 5 in PR. Median age was 59 years (18-71). Donors included 41 sib and 69 MUD. Combining PNB with DCB at a dose of 20 mg/m^2 proved not feasible due to prolonged cytopenia, which was a dose limiting toxicity (DLT), but either PNB alone or the combination of PNB/DCB 10 mg/m^2 did prove feasible. Altogether, 87 out of 110 transplanted pts received PT epigenetic therapy, including 39 PNB alone, 13 PNB/DCB (20 mg/m^2), and 35 PNB/DCB (10 mg/m^2). Pts started at a median time point of 33 days (range: 27-90) after transplantation. CTC grade 3 and 4 side-effects after the first cycle of PNB/DCB included nausea in 7 pts (5 grade 3; 2 grade 4)), febrile neutropenia in 1 pt and general fatigue in 1 pt. No CTC grade 3 or 4 opportunistic infections were observed after the first 2 cycles of PNB/DCB. Outcome of 110 transplanted pts showed OS at 12 and 24 months from transplantation of 71% (se 5%) and 49% (se 6%), respectively. 44 Pts died, including 17 due to NRM and 27 due to relapse. RFS at 12 and 24 months was 64% (se 5%) and 46% (se 6%), comparing favourably to historical matched HOVON-pts with RFS of 43% and 39% at 12 and 24 m, respectively. So far, DLI could be administered in 62 pts, including 38 pts receiving 2 DLI's, and 20 pts a third DLI. None of the pts developed grade 3 or 4 acute GVHD before DLI. Out of 62 recipients of DLI, severe chronic GVHD occurred in 15 (24%) pts.

Summary/Conclusion: Collectively, these results suggest that: 1. alloHSCT with GVHD-prophylaxis by cyclophosphamide PT allows for early initiation of epigenetic therapy and DLI, and 2. as compared to a matched historical HOVON-cohort, encouraging results with respect to relapse, RFS, and OS were observed. 3. Limited side effects were observed in recipients of PNB alone or the combination of PNB and DCB at a dose of 10 mg/m^2; the incidence GVHD also appeared limited. Altogether these results might suggest enhanced GVL and, therefore, will be followed by an international prospective randomized study in (very) poor-risk AML patients.

S859

PHASE 1 FIRST-IN-HUMAN TRIAL OF AMV564, A BIVALENT BISPECIFIC (2×2) CD33/CD3 T-CELL ENGAGER, IN PATIENTS WITH RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA (AML)

P. Westervelt[1,*], G.J. Roboz[2], J.E. Cortes[3], H. M. Kantarjian[3], S. Lee[2], M.P. Rettig[1], T.H. Han[4], J. Guenot[4], E.J. Feldman[4], J.F. DiPersio[1]

[1]Washington University, St. Louis, MO, [2]Weill-Cornell Medical College, New York, NY, [3]MD Anderson Cancer Center, Houston, TX, [4]Amphivena Therapeutics Inc, South San Francisco, CA, United States

Background: AMV564 is a novel bivalent, bispecific (2x2) CD33/CD3 targeted immunotherapy that binds both CD33 and the invariant CD3ε on T-cell receptors with strong avidity, thus creating an immune synapse between CD33-expressing cells and T cells, initiating T-cell directed lysis of CD33 expressing cells, and inducing expansion, differentiation and proliferation of T cells. By design, AMV564 has reduced renal clearance and therefore has a longer half-life ($t_{1/2}$) than monovalent, bispecific T-cell engagers. In preclinical investigations using both leukemic cell lines and primary cells from AML patients, AMV564 eliminated myeloid blasts with picomolar potency and broad activity independent of cytogenetic risk, CD33 expression level, and disease stage, with no nonspecific activation of T cells (Reusch U et al. *Clin Cancer Res* 2016;22:5829-38). AMV564 also selectively eliminated myeloid-derived suppressor cells (MDSC) in myelodysplastic bone marrows at low doses with restoration of immune homeostasis and hematopoiesis (Cheng P *et al. Blood* 2017;130:51).
Aims: The primary objectives of this study are to characterize the safety, tolerability, and preliminary anti-leukemic activity of AMV564. Evaluation of AMV564 pharmacokinetics (PK) is a secondary objective.
Methods: This is an ongoing Phase 1 study with a 3+3 dose-escalation design (NCT03144245). Key inclusion/exclusion criteria are: adults with relapsed and/or refractory AML after 1-2 prior induction regimens (with a standard anthracycline-based regimen or hypomethylating agent) and no more than 2 prior salvage regimens. AMV564 is administered by continuous intravenous infusion (CIV) for 14 consecutive days for up to 2 induction cycles.
Results: Twelve patients (7M/5F) with a median age of 71 years (range 24-84) have been enrolled in 1 of 4 dosing cohorts: (0.5, 1.5, 5.0, and 15 mcg/day×14 days). Overall, 83% (10/12) had secondary AML and/or adverse cytogenetics, 75% (9/12) had received at least one prior salvage regimen, and 58% (7/12) had received one or more prior intensive chemotherapy-based regimens. No dose-limiting toxicity, cytokine release syndrome, or treatment-related grade ≥3 adverse events (AE) have been reported. The most common treatment-emergent grade ≥3 AE has been febrile neutropenia in 25% (3/12) of patients, but each case was considered unrelated to study drug. The 30-day mortality rate is 0% (0/12). AMV564 PK were linear with a terminal $t_{1/2}$ of 2-3 days. Plasma concentrations increased gradually, with time to steady-state concentrations of 3-7 days. Reductions in bone marrow blasts ranging from 13% to 38% were observed in 6 of 9 evaluable patients. Two of 3 patients with progression after cycle 1 had a reduction in bone marrow blasts after cycle 2. One patient with prior myelodysplastic syndrome had stable disease with a significant rapid rise in hemoglobin (>4 g/dL), achievement of transfusion-independence, and a rise in the absolute neutrophil count to >2,400/mm^3. Preliminary data demonstrated clearing of MDSC from the peripheral blood.
Summary/Conclusion: AMV564 is well-tolerated and can be administered without the need for incremental dosing and/or pre-medication. AMV564 has a unique PK profile compared to monovalent T-cell engagers, demonstrating a gradual increase in drug concentration and thus the potential for controlled T-cell activation. In a limited number of patients at the lowest dose levels, AMV564 demonstrated anti-leukemic activity. Dose escalation continues and correlation with T-cell expansion, T-cell activation, cytokine release, and other pharmacodynamic endpoints is ongoing.

S860

DECREASED INCIDENCE OF INFECTION, USE OF ANTIBACTERIALS AND DAYS IN HOSPITAL AFTER ADMINISTRATION OF CLT-008 MYELOID PROGENITOR CELLS TO SUBJECTS RECEIVING AML INDUCTION THERAPY: PHASE 2 STUDY RESULTS

P. Desai[1,*], L. Akard[2], C. Andreadis[3], J. Foran[4], O. Frankfurt[5], J. Hsu[6], S. Gill[7], J. Lister[8], J. Pagel[9], G. Schiller[10], M. Solh[11], C. Ustun[12], M. Wieduwilt[13], J. Brown[14], A. Wong[15], L. Kellerman[16], R. Van Syoc[16], D. Zimmerman[16], S. Panuganti[16], S. Solish[16], R. Mandalam[16], R. Mamelok[16], C. Abboud[17]

[1]Weill Cornell Medicine, New York, [2]Indiana Blood and Marrow Transplantation, Indianapolis, [3]University of California, San Francisco, San Francisco, [4]Mayo Clinic, Jacksonville, [5]Northwestern University, Chicago, [6]University of Florida, Gainesville, [7]University of Pennsylvania, Philadelphia, [8]Western Pennsylvania Hospital, Pittsburgh, [9]Swedish Cancer Institute, Seattle, [10]University of California, Los Angeles, Los Angeles, [11]Northside Hospital - The Blood & Marrow Transplant Group of Georgia, Atlanta, [12]University of Minnesota, Minneapolis, [13]Moores University of California, San Diego Cancer Center, La Jolla, [14]Stanford University Medical Center, Palo Alto, [15]Celllerant Therapeutics, Inc, [16]Cellerant Therapeutics, Inc, San Carlos, [17]Washington University, St. Louis, United States

Background: Standard induction chemotherapy (7+3) for AML results in prolonged neutropenia with a high risk of infection. CLT-008 is an off-the-shelf human allogeneic myeloid progenitor cell (MPC) preparation manufactured by ex vivo culture expansion of CD34+ cells. Following infusion, MPCs are expected to home to bone marrow (BM) and produce neutrophils.
Aims: Study the effect of CLT-008 on the reduction of infections during AML induction therapy.
Methods: 94 *de novo* AML subjects (age ≥55) receiving 7+3 induction therapy were randomized on Day 0 (first day of induction) to receive a regimen of CLT-008 ($7.5x10^6$ cells/Kg) on Day 9 + GCSF daily starting on Day 14 (intervention) or GCSF alone daily starting on Day 14 (control) until ANC recovery to ≥500/μL. The primary endpoint was days in a febrile episode (DFE) and secondary endpoints included clinically diagnosed (CDI) and microbiologically diagnosed (MDI) bacterial or fungal infection (adjudicated by a blinded independent committee), use of antimicrobials, and days in hospital (to Day 42). Data are reported for 2 periods: From infusion of CLT-008 on Day 9-28 and from 6 days after infusion of CLT-008 (1 day after start of GCSF) – Day 28 (denoted as Day 15-28). This is the period when CLT-008 derived neutrophils are likely to be circulating. Intervention and control subjects were evaluable if they received respectively CLT-008 or GCSF alone, were on study ≥28 days, and did not receive additional chemotherapy before Day 28. One-sided p-values are reported.
Results: The baseline characteristics, including mean age, mean WBC, and ECOG status were balanced between the intervention and control groups. The mean DFE during Day 9-28 in the intervention and control was 7.37 and 9.11 days respectively (p=0.099). The mean DFE for Day 15-28 was 3.24 and 4.77 days in the intervention vs. control respectively (p=0.057). The incidence of MDI or CDI during Day 9-28 was 33% vs 51% in the intervention vs. control, a decrease of 35% (p=0.058) and during Day 15-28 was 7.6% vs 28% in the intervention vs. control, a decrease of 73% (p=0.010). Mean number of days in hospital were 27.1 vs 30.6 days in intervention vs. control groups (p=0.003). Remission rates and days to ANC recovery were similar in the two groups. Eleven subjects assessed for chimerism had CLT-008 cells detected in peripheral blood (PB) on the day of infusion. Post infusion, chimerism was observed on day 13 or 14 in bone marrow in 5/6 subjects and in PB in 5/11 subjects between days 13 and 21 prior to ANC recovery. No infectious deaths were observed in the intervention group; there were two deaths attributed to pneumonia in the control group. CLT-008 was generally tolerated well (Table 1).

Table 1.

	Incidence of Subjects Receiving Antibacterial Agents	
	Empiric therapy with IDSA Group 2[a] Intervention (N = 39) vs Controls (N = 39)	Antibacterial treatment of MDI/CDI Intervention (N=39) vs Control (N=39)
Days 9-28	38% vs 54% p=0.084	38% vs 67% p=0.007
Days 15-28	31% vs 54% p=0.021	36% vs 64% p=0.007

[a]IDSA2: antimicrobials added to initial treatment of neutropenic fever due to suboptimal response to initial treatment.

Summary/Conclusion: The incidence of infections, use of antibacterial agents and days in hospital were decreased in subjects receiving CLT-008. Myeloid progenitors may provide a new option to reduce infections in AML patients undergoing induction therapy.

S861

RANDOMIZED COMPARISON OF 90 MG VERSUS 60 MG DAUNORUBICIN IN 7+3 STANDARD INDUCTION FOR NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA: RESULTS FROM THE SAL-DAUNODOUBLE TRIAL

C. Röllig[1,2,*], B. Steffen[3], R. Herbst[4], R. Noppeney[5], Z. Racil[6,7], K. Schäfer-Eckart[8], A. Krämer[9], A. Neubauer[10], C. Baldus[11], C. Schliemann[12], M. Kaufmann[13], J. Mertova[14], E. Jost[15], D. Niemann[16], J. Novak[17], S. Krause[18], S. Scholl[19], G. Held[20], S. Parmentier[21], T. Szotkowski[22], P. Zak[23], A. Rank[24], M. Wass[25], S. Buske[26], U. Platzbecker[27], M. Kramer[27], J. Schetelig[27], C. Müller-Tidow[28], H. Serve[3], M. Bornhäuser[27], W. Berdel[12], J. Mayer[6,7], G. Ehninger[1,27]

[1]Study Alliance Leukemie (SAL), [2]Medizinische Klinik und Poliklinik I, Universitätsklinikum Dresden, Dresden, [3]Universtitätsklinikum Frankfurt, Frankfurt, [4]Klinikum Chemnitz, Chemnitz, [5]Universitätsklinikum Esssen, Essen, Germany, [6]University Hospital Brno, [7]CELL Study Group, Brno, Czech Republic, [8]Klinikum Nord, Nürnberg, [9]Universitätsklinikum Heidelberg, DKFZ, Heidelberg, [10]Universitätsklinikum Marburg, Marburg, [11]Univeritätsklinikum Charite , Berlin, [12]Universitätsklinikum Münster, Münster, [13]Robert-Bosch-Krankenhaus, Stuttgart, Germany, [14]UHKT, Praha, Czech Republic, [15]Universitätsklinikum Aachen, Aachen, [16]Gemeinschaftsklinikum Mittelrhein, Koblenz, Germany, [17]Fakultni Nemocnice Kralovske Vinohradi, Praha, Czech Republic, [18]Universitätsklinikum Erlangen, Erlangen, [19]Universitätsklinikum Jena, Jena, [20]Westpfalzklinikum, Kaiserslautern, [21]Rems-Murr-Klinikum, Winnenden, Germany, [22]Fakultni Nemocnice Olomouc, Olomouc, [23]Fakultni Nemocnice Hradec Kralove, Hradec Kralove, Czech Republic, [24]Klinikum Augsburg, Augsburg, [25]Universitätsklinikum Halle, Halle, [26]Städtisches Krankenhaus, Kiel, [27]Universitätsklinikum Dresden, Dresden, [28]Universitätsklinikum Heidelberg, Heidelberg, Germany

Background: Seven days of cytarabine plus three days of anthracycline, most commonly daunorubicin, remains the standard approach for curative treatment of acute myeloid leukemia (AML). Two randomized trials have shown a survival benefit for a daunorubicin dose of 90 mg/m^2 versus 45 mg/m^2 as part of a standard 7+3 schedule using seven days of continuous cytarabine (c.i.) and three subsequent days of daunorubicin. However, a daunorubicin dose of 60 mg/m^2 rather than 45 mg/m^2 has been an established standard in many countries, posing the question if 90 mg/m^{2}would still be a superior approach. The NRCI-AML 17 study explored 90 mg/m^2 versus 60 mg/m^2 in a 10+3 induction using cytarabine bolus infusion twice daily plus daunorubicin on days 1, 3, 5 followed by diverse induction and consolidation options, but showing no differences in complete remission (CR) rates, early death or survival.
Aims: In order to compare 90 mg/m^2 versus 60 mg/m^2 in a standard 7+3 induction regimen, we set up the randomized multicenter DaunoDouble trial.
Methods: Patients with newly diagnosed AML without previous anthracycline treatment, normal cardiac and organ function and 18-60 years of age were randomly assigned to receive induction treatment with seven days of cytarabine 100 mg/m^2 c.i. plus daunorubicin infusion on days 3-5, either with 90 mg/m^2 (Dauno90) or with 60 mg/m^2 (Dauno60). Response assessment in bone marrow was done 15 days after commencement of chemotherapy. A blast count <5% was defined as good response. Responses were compared using Chi squared test and multivariable logistic regression analyses accounting for established prognostic factors. Incidence and grade of adverse events and early mortality were compared between Dauno90 and Dauno60. Patients with good response were eligible for a second randomization to proceed to a routinely administered second induction with 7+3 or to no further induction. This second part of the DaunoDouble trial is ongoing and results will be presented in the future.
Results: Between April 2014 and August 2017, 314 patients were randomized, 157 in each arm. The median age was 48 years, 87% had *de novo* AML, NPM1 mutation was present in 41% of patients, FLT3-ITD in 20%, CEBPA double mutation in 5%; favorable, intermediate and adverse risk (ELN 2017) was present in 7%, 73% and 20% of patients, respectively. No significant imbalances were observed between the two treatment arms. Response rates after 7+3 induction with 90 mg/m^2 daunorubicin were 47.8% (95%>CI, 39.7-55.9) versus 42,7% (95%>CI, 34.8-50.8) with 60 mg/m^2 (p=0.29). Adverse events (AE) were documented in 89.8% of patients in Dauno90 and 86.6% in Dauno60. AEs ≥ grade 3 were registered in 20.3% and 16.2%, respectively. The incidence of early deaths within 14 days after start of induction was 1.3% and 0.6%, whereas 28-day early mortality was 3.2% and 1.3% in Dauno90 and Dauno60, respectively (p=0.251).
Summary/Conclusion: Our results provide randomized evidence that in a standard 7+3 induction, a dose escalation of daunorubicin from 60 to 90 mg/m^2 does not result in a significant increase in response rates. The tolerability was similar in both dose levels with no obvious signs of excess toxicity. Survival follow-up is ongoing. Our preliminary results support the use of 60 mg/m^2 daunorubicin in 7+3 induction.

Acute lymphoblastic leukemia – Biology

S862

PIGGYBAC TRANSPOSON SCREENING IDENTIFIES NOVEL CANCER GENES AND REGULATORY ELEMENTS IN T CELL LEUKEMIA

A. Pfaus[1,2,3,4,*], R. Lersch[1,2,4], J. Weber[1,2,3], J. de la Rosa[5,6], A. Strong[5], L. Rad[5], U. Geumann[2], R. Öllinger[1,2], T. Engleitner[1,2,3], M. Friedrich[5], N. de Andrade Krätzig[1,2], K. Steiger[7], U. Kohlhofer[8], I. Gonzalez Menendez[8], L. Quintanilla de Fend[8], M. Heikenwälder[3,9], P. Liu[5], J. Cadiñanos[5,6], A. Bradley[5,10], G.S. Vassiliou[5,10], N. Conte[5,10], R. Rad[1,2,3,10]

[1]Center for Translational Cancer Research (TranslaTUM), Technische Universität München, [2]Department of Medicine II, Klinikum rechts der Isar, Munich, [3]German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, [4]Equal, contribution, Germany, [5]Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom, [6]Instituto de Medicina Oncólogica y Molecular de Asturias (IMOMA), Oviedo, Spain, [7]Institute of Pathology, Technische Universität München, Munich, [8]Institute of Pathology, University of Tuebingen, Tuebingen, Germany, [9]Equal senior, contribution, United Kingdom, [10]Institute of Virology, Technische Universität München, Munich, Germany

Background: Insertional mutagenesis by the *PiggyBac* transposon can be used for cancer gene discovery in mice. Previously, we have shown that transposon based genetic screening can overcome the challenge of pinpointing cancer drivers that are not mutated, but are among the thousands of transcriptionally, epigenetically, or posttranslationally dysregulated genes in cancer. Although the majority of transposon insertions are detected in intergenic regions, the consequence of these insertions for tumor development has not yet been investigated systematically.
Aims: We used *PiggyBac* for the identification of novel cancer genes, but also to decipher the role of non-coding regulatory elements in leukemogenesis and lymphomagenesis.
Methods: Using *PiggyBac* transposon mutagenesis in mice, we performed genome-wide surveys for cancer drivers and regulatory elements across distinct hematologic malignancies (n=256 cancers), including T cell, B cell, and myeloid neoplasms.
In addition, using intergenic knockout mouse models, we validated these newly identified regulatory elements *in vivo*.
Results: In line with previous studies more than 90% of *RosaPB;ATP2* mice developed hematopoietic malignancies. The subgroup of T cell lymphoblastic lymphomas/ T cell acute lymphoblastic leukemia (T-LBL/T-ALL, n=53) demonstrated a significantly reduced survival compared to other subgroups or single-transgenic control mice. Using semi-quantitative transposon insertion site sequencing (Friedrich *et al.*, *Nature Protocols* 2017) we found genes already known to be important in T cell malignancies (e.g., *Notch1*, *Ikzf1*, *Pten*, *Myb*) as well as potential novel candidate genes. Moreover, systematic genome-wide analyses of intergenic insertions identified cancer-relevant regulatory regions, including high-coverage insertions on chromosome 12 downstream of *Bcl11b* in the T-LBL/T-ALL subgroup. Two of these intergenic common insertion sites show an overlap with markers for open chromatin such as H3K27ac, H3K4me1, and DNase hypersensitivity sites confirming their potential role as regulatory elements. Knockout of two such intergenic regions (105 kb and 1 Mb) in novel mouse models resulted in lymphoma and solid cancer development in 30-40% of cases. Whereas the 105 kb knockout mice (n=148) showed a diverse tumor spectrum, the knockout of the 1 Mb region (n=47 mice) mainly led to the development of lymphomas (70% T-LBL/T-ALL). Consistent with this observation, the mRNA expression of *Bcl11b* was more prominently reduced in mice with the knockout of the 1 Mb region, thereby indicating a role of this region in *Bcl11b* regulation and T-LBL/T-ALL development. Importantly, the common insertion sites found downstream of *Bcl11b* share over 80% sequence similarity to the concordant human region and might therefore also play a role in human *BCL11B* regulation.
Summary/Conclusion: Taken together, our studies give comprehensive novel insights into the coding and regulatory landscapes in malignant hematopoiesis and show that an intergenic knockout model can recapitulate the phenotype of the transposon mice.

S863

MODELING HIGH RISK ACUTE LEUKEMIA WITH COMBINED T-CELL AND MYELOID PHENOTYPE CAUSED BY THE ETV6-NCOA2 FUSION GENE

H. Fishman[1,*], B. Vase[2], W. Van Loocke[3], Y. Kirschenbaum[1], I. Ganmore [1], P. Van Vlierberghe[3], J. Mulloy[2], S. Izraeli[1]

[1]Dept. of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel, [2]Cincinnati Children's hospital, Cincinnati , United States, [3]Ghent University, Ghent , Belgium

Background: Mixed-phenotype acute leukemia (MPAL) is a rare subtype of leukemia in which there is co-expression of myeloid and lymphoid markers on the same malignant cells. The pathogenesis of these leukemias is unknown and their treatment is challenging. This research is based on our discovery of the recurrent t(8;12)(q13;p13) chromosomal translocation in children with acute leukemia. The translocation generates a transcript in which the PNT domain of the transcriptional repressor ETV6 fuses with the epigenetic-modification domains of the co-activator NCOA2. Remarkably, this genetic event is always associated with acute leukemia co-expressing T-lymphoid and myeloid (T/M) markers and often presents with activating mutations of Notch1.
Aims: We hypothesize that ETV6-NCOA2 (EN2) fusion protein is a major driver of MPAL and of Early T-cell leukemia (ETP) with a T/M phenotype through transcriptional dysregulation.
Methods: To examine whether EN2 directly contributes to leukemogenesis we transduced lineage-negative BM progenitors with EN2 and performed *in vitro* and *in vivo* assays: methylcellulose replating assay to assess self-renewal; co-cultures on OP9DL4 to examine T-cell differentiation ability and transplantation into C57B/6 mice to examine leukemia development. Cord blood derived CD34+ hematopoietic stem and progenitor cells (HSPCs) were co-transduced with EN2 and a Notch1 allele with a weak activating mutation (L1601PdP) and transplanted into immunodeficient mice. To understand the initiating changes in gene expression we conducted RNA-sequencing of HSPCs transduced with EN2. To reveal the effect of EN2 fusion protein on wt-ETV6 we preformed CO-IP experiments to explore this interaction and luciferase reporter assay to examine changes in transcription.
Results: *In vitro* experiments demonstrated enhanced self-renewal ability and T-cell differentiation arrest at the DN1/DN2 stage. C57B/6 mice transplanted with lin- transduced with EN2 cells developed a lymphoma/leukemia malignancy characterized by extra medullary infiltration including spleen, liver, lungs and lymph nodes. Immunophenotypic analysis displayed a mixed T/M phenotype. Several leukemic/lymphoma cells developed spontaneous Notch1 mutations similarly to those found in human EN2 patients. The tumors were propagated in secondary transplantations. Immunodeficient mice transplanted with HSPCs co-expressing EN2 and Notch1-L1601PdP developed leukemia with co-expression of T-cell and myeloid markers. RNA-seq of CD34+ cells transduced with EN2 after five days in culture with myeloid and stem cell cytokines revealed that EN2 induces an early T-cell precursor's transcriptional program. We detected a significant up-regulation of ETV6 target genes. We hypothesize that EN2 has a dominant negative effect on wt-ETV6 through dimerization with its PNT domain and recruitment of histone acetylase EP300 to ETV6 targets. COIP experiments confirmed that EN2 and ETV6 interacts and Luciferase assay showed that EN2 expression can abolish ETV6's repression.
Summary/Conclusion: ETV6-NCOA2 is a driver of T/M MPAL by inducing a T-cell program in early hematopoietic progenitors, possibly by de-repression of ETV6 genes. These findings are consistent with an earlier report on dominant negative mutations in ETV6 in early T-cell ALL, characterized by a T/M phenotype. The development of the first preclinical model of T/M acute leukemia will contribute to the understanding of this disease and may serve as a future model for therapeutics. Furthermore, this research could shed more light on the lineage choice decisions of early hematopoietic progenitors.

S864

SYK TARGETING AS A POTENTIAL THERAPEUTIC STRATEGY FOR HIGH RISK TEL-AML1 PATIENTS

V. Serafin[1,*], E. Porcù[1], G. Cortese[1], E. Mariotto[1], S. Bresolin[1], G. Basso[2], B. Accordi[1]

[1]University of Padova, Padova, Italy, [2]Dipartimento della Salute della Donna e del Bambino, University of Padova, Padova, Italy

Background: The most frequent chromosomal rearrangement in childhood B acute lymphoblastic leukemia (B-ALL) is the reciprocal translocation t(12;21)(p13;q22) (ETV6-RUNX1). Although the presence of ETV6-RUNX1 appears to be a strong independent predictor factor of a favorable prognosis, relapses still occur in the 10% of patients, usually near the end of treatment and occasionally many years later. A distinctive marker able to distinguish, already at diagnosis, ETV6-RUNX1 patients at risk of relapse is still missing.
Aims: To identify new potential biomarker and/or therapeutic targets that could predict and/or prevent already at the moment of diagnosis ETV6-RUNX1 patients' relapse.
Methods: By Reverse Phase Protein Arrays (RPPA) we performed the phosphoproteomic profiling of pediatric ETV6-RUNX1 B-ALL patients. We evaluated by MTT assay the effects of treatment with selected inhibitors in cell lines and primary samples. We assessed by phosphoflow the phosphorylation of SYK.
Results: We performed a phosphoproteomic profiling at diagnosis of 62 pediatric ETV6-RUNX1 B-ALL patients. We analysed the activation status of several signaling pathways and we identified SYK as hyperactivated (SYK Y525) in patients who will experience relapse (n=11) compared to non-relapsed ones (n=51, p=0.02). Total SYK and *SYK* RNA were not differentially expressed in the two subgroups. We also evaluated the activation of SYK at relapse occurrence and we observed an increase of SYK Y525 compared to diagnosis (n=8, p=0.009). SYK is a non-receptor tyrosine kinase which mediates signal transduction downstream of a variety of transmembrane receptors including classical immunoreceptors such as the B-cell receptor. Thus, we first functionally validated SYK as a new therapeutic target by treatment of cell lines and primary cells from pediatric ETV6-RUNX1 patients with SYK inhibitors. We treated t(12;21) REH, AT-1 and AT-2 cell lines, that display SYK hyperphosphorylation, with Entospletinib, Fostamatinib and PRT-060318. After 48hours of treatment, all the compounds were able to decrease both cell proliferation and SYK Y525 phosphorylation. We also tested inhibitors in combination with conventional chemotherapeutic compounds (Vincristine, Dexamethasone and Cytarabine-Ara-C), and Entospletinib resulted the one with the best synergistic effect. This result was also confirmed combining all the three chemotherapeutic compounds (VDA) together with Entospletinib. We also validated SYK as a therapeutic target by treating primary cells from t(12;21) patients at diagnosis with Entospletinib and VDA alone or in combination. At diagnosis we observed an augmented response to Entospletinib in samples from patients who will experience relapse (n=3) compared to non relapsed ones (n=7, p=0.03), and more importantly an increased cell death with Entospletinib+VDA compared to VDA alone (p= 0.1) in patients who will relapse. Finally, we are now setting up the analysis of activated SYK by phosphoflow to validate it as a prognostic marker. We will screen an independent cohort of t(12;21) patients, and preliminary results seem to confirm what previously observed by RPPA.
Summary/Conclusion: SYK is hyperactivated in t(12;21) B-ALL patients who will experience relapse and also at relapse occurrence. Its inhibition using Entospletinib, a phase 2 inhibitor for Chronic Lymphocytic Leukemia treatment, is able to potentiate the effects of conventional chemotherapeutics. Thus, SYK could be considered as a new potential marker of relapse occurrence and as a new therapeutic target for t(12;21) pediatric patients.

S865

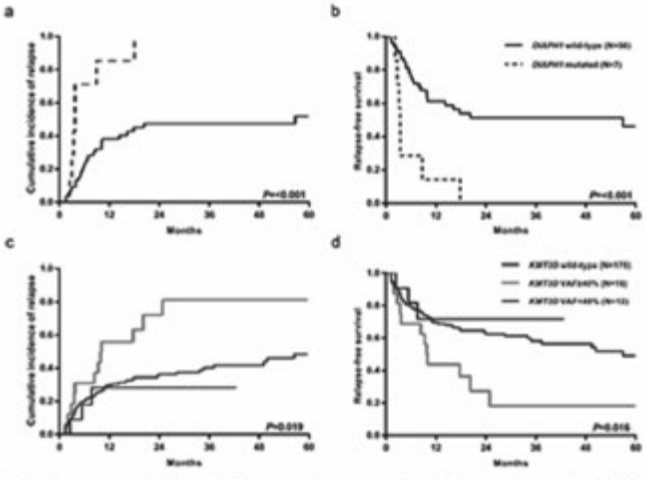
GENOMIC LANDSCAPE OF ADULT B-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA

L.-X. Wu[1,*], R.-Q. Lu[1], K.-Y. Liu[1], R. Peter Gale[2], Q. Zheng[3], J. Zhou[1], J.-L. Li[1], S.-J. Wang[1], H. Jiang[1], Q. Jiang[1], B. Jiang[1], X.-H. Zhang[1], L.-P. Xu[1], Y. Wang[3], C.-L. Song[3], Y.-R. Liu[1], X.-J. Huang[1#4], G.-R. Ruan[#1]

[1]Peking University People's Hospital, Beijing, China, [2]Haematology Research Center, Division of Experimental Medicine, Department of Medicine, Imperial College London, London, United Kingdom, [3]Novogene Bioinformatics Institute, [4]Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Beijing, China

Background: The overall prognosis of adults with B-cell acute lymphoblastic leukaemia (ALL) remains worse than that of children. One of the bottlenecks leading to the poor outcome of adults is that molecular analyses of risk cohorts in adult B-cell ALL has lagged behind progress in pediatric B-cell ALL.
Aims: Interrogate the genomic landscape of adult B-cell ALL and variants associating with leukaemogenesis and prognosis.
Methods: 226 consecutive, newly-diagnosed adults with B-cell ALL seen in Peking University People's Hospital between April, 2007 and December, 2015 were enrolled in the study. Whole-genome sequencing was performed for 8 paired samples (at diagnosis and at complete remission), whole-exome sequencing for 13 paired samples and target DNA sequencing for 210 samples including 88 paired samples. For validation, 5 paired samples were sequenced both by whole-exome and target DNA sequencing. Clonal evolution of 7 paired samples (at diagnosis and at relapse) was detected by whole-exome sequencing.
Results: We identified 2285 mutations in 791 genes (median, 11 mutations *per* subject) with ≥2 mutations identified in 190 subjects (84%; 95% confidence interval [CI], 79, 89%). *KMT2D*, also termed *MLL2*, represented

the most frequent significantly mutated gene in our cohort (N=31; 14% [9, 19%]). We also found 4 new mutated genes, *PRB2* (N=13; 6% [3, 9%]), *NBPF10* (N=12; 5% [2, 8%]), *TSFM* (N=8; 4% [1, 7%]) and *DIAPH1* (N=8; 4% [1, 7%]). 7 *DIAPH1* mutated subjects with remission were matched by 56 controls according to age (<35y *vs.* ≥35y), t(9;22)/*BCR-ABL* (+ *vs.* -) and time to CR1 (≤33 d *vs.* >33d) to compare CIRs and RFSs. *DIAPH1* mutation had a significantly higher CIR *vs.* those without a mutation (p=<0.001; Figure 1a) and worse RFS (p=<0.001; Figure 1b). In multivariate analyses *DIAPH1* mutation was independently-associated with CIR (hazard ratio [HR]=4.6 [1.7, 12.7], p=0.004) and RFS (HR=4.5 [1.6, 12.6], p=0.004). Besides, 90% of the samples showed 14q32.33 loss, validating by a long non-coding RNA, *KIAA0125* in this area. Mutation burden (40% VAF as a watershed) showed more importance in B-ALL development and patient survival than mutation itself. *KMT2D* mutation with VAF≥40% indicated higher relapse risk compared with the wild-type ones(Figure 1 c,d). Multivariate analyses also revealed that *KMT2D* mutation with VAF≥40% was an independent risk factor for CIR (HR=2.4 [1.3, 4.3], p=0.006) and RFS (HR=2.3 [1.2, 4.4], p=0.013). Moreover, genes with VAF ≥40% predispose them to persistence at relapse.



Univariate analyses. CIR (a) and RFS (b) analyses subjects with *DIAPH1* mutations and matched subjects without *DIAPH1* mutations; CIR (c) and RFS (d) analyses of subjects with *KMT2D* mutations VAF ≥40%, <40% and without *KMT2D* mutations.

Figure 1.

Summary/Conclusion: These data provide insights into the genomic landscape of adults with B-cell ALL, identify diverse mutations with 4 new mutated genes and frequent copy number loss in 14q32.33, which may highly correlate with leukaemogenesis. Besides, targeting mutations with VAF ≥40% may benefit patients as more clinically relevant. (Trial registered in the Beijing Municipal Health Bureau Registration N: 2007–1007 and the Chinese Clinical Trial Registry [ChiCTR-OCH-10000940 and ChiCTR-OPC-14005546]; http://www.chictr.org.cn)

S866

MESENCHYMAL STROMAL CELLS PROTECT ACUTE LYMPHOBLASTIC LEUKAEMIA CELLS FROM CYTARABINE INDUCED APOPTOSIS BY TRANSFER OF MITOCHONDRIA VIA TUNNELLING NANOTUBES IN A TREATMENT RESISTANT NICHE MODEL

R. Burt[1,*], A. Dey[1], K. Ng[1], C. Pospori[2,3], S. Hooper[4], C. Lo Celso[2,3], E. Sahai[4], A. Fielding[1]

[1]Cancer Institute, UCL, [2]Stem Cell Biology, The Francis Crick Institute, [3]Stem Cell Biology, Imperial College, [4]Cancer microenvironment, The Francis Crick Institute, London, United Kingdom

Background: In acute lymphoblastic leukaemia (ALL) models, mesenchymal stromal cells (MSC) can form a 'protective niche' critical for treatment resistance (Ebinger, 2016; Duan, 2014).
Aims: We set out to determine the existence of such a niche in primary human bone marrow from patients with ALL and to understand the mechanism by which MSC may support ALL cell survival in this niche, following standard chemotherapy drugs.
Methods: We used both primary MSC and blasts from bone marrow of 152 patients enrolled on the UKALL14 trial as well as B-ALL cell lines and HS27a stromal cell line. We employed immunocytochemistry, gene expression profiling, ELISA, cytokine bead assay, MTS assays, apoptosis assays, quantification of reactive oxygen species (ROS), assays of mitochondrial number and various live cell imaging techniques.
Results: Immunostaining for f-actin, a gene expression profile panel and ELISAs showed that a significant proportion of primary patient bone marrow specimens contained MSC with an irreversible, activated phenotype, analagous to cancer associated fibroblasts (ALL-CAF). We demonstrated that both primary ALL cells, ALL cell lines and cytarabine (AraC) were able to generate ALL-CAF *de novo* from both healthy donor MSC and HS27a cells. ALL-CAF formation was closely associated with an increase in reactive oxygen species (ROS). It was functionally significant; pre-treatment of HS27a with AraC before co-culture with ALL cells led to increased cell proliferation and chemotherapy resistance. This phenomenon was not observed in untreated, or VCR or DEX pre-treated HS27a. Given AraC induces apoptosis by increasing ROS, we postulated MSC may protect ALL cells by 'rescuing' them from oxidative stress. We exposed SEM cells to AraC for 48 hours and observed a 1.4 fold increase in intracellular ROS (p=0.01), a 2-fold increase in apoptosis (p=0.0009) and cell death (p=0.01) at 48 hours compared to an untreated control. When SEM cells (a B-ALL cell line that didn't induce ALL-CAF) were co-cultured with MSC in the presence of AraC, ROS reduced 4-fold (p = 0.0002) compared to SEM in mono-culture which correlated with a 2.5-fold reduction in apoptosis (p=0.02) and a 3.4-fold reduction in cell death (p=0.0001). The observations couldn't be replicated in a transwell system, suggesting a contact-dependent mechanism. Next, using a mitotracker flow cytometry assay, we showed transfer of mitochondrial from HS27a to ALL cell lines, proportionate to the ROS level of the ALL cell line. Mitochondrial transfer to healthy donor B-cell controls was minimal. Passive transfer was excluded in a transwell system. Treatment of the MSC and ALL cell co-cultures with AraC increased mitochondrial transfer to ALL cells 3-fold at 72 hours compared to an untreated control (p<0.0001) and was AraC dose-dependent. Treatment with DEX and VCR did not impact mitochondrial transfer. We confirmed mitochondria were transferred along tunnelling nanotubes (TNT) using time-lapse confocal microscopy. As further evidence for transfer via TNT, the actin inhibitor latrunculin B and the microtubule damaging agent nocodazole both significantly blocked the phenomenon (Figure 1).

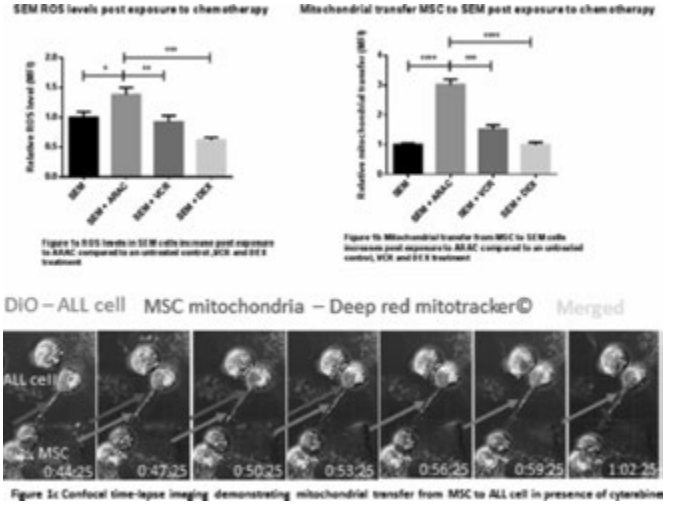


Figure 1.

Summary/Conclusion: We have shown that CAF-like MSC can provide support to ALL cells under oxidative stress by mitochondrial transfer via TNT. Our data may explain the ineffectiveness of ROS-inducing chemotherapy at eradicating residual disease at this niche and provide an explanation of why low dose non-ROS-inducing, microtubule damaging agents such as VCR used in maintenance therapy are effective in ALL.

Molecular mechanisms underlying CLL biology and response to treatment

S867

MULTIPLE MECHANISMS OF KRAS ACTIVATION IN TRISOMY 12 CHRONIC LYMPHOCYTIC LEUKEMIA

E. Vendramini[1,*], R. Bomben[1], F. Serra[1], F. Pozzo[1], D. Benedetti[1], F.M. Rossi[1], G. Del Poeta[2], F. Zaja[3], A. Chiarenza[4], G. Pozzato[5], V. Gattei[1], A. Zucchetto[1]

[1]Clinical and Experimental Onco-Hematology Unit, CRO Aviano National Cancer Institute, Aviano, [2]Division of Hematology, S. Eugenio Hospital and University of Tor Vergata, Rome, [3]Clinica Ematologica, Centro Trapianti e Terapie Cellulari "Carlo Melzi" DISM, Azienda Ospedaliera Universitaria S. Maria Misericordia, Udine, [4]Division of Hematology, Ferrarotto Hospital, Catania, [5]Department of Internal Medicine and Hematology, Maggiore General Hospital, University of Trieste, Trieste, Italy

Background: Trisomy 12 (Tri12) chronic lymphocytic leukemia (CLL) identifies a cytogenetic subset with a peculiar clinical behavior (Bulian *et al*, Haematologica, 2017) and specific biological features, including high frequency of stabilizing *NOTCH1* mutations (*NOTCH1-mut*) (Rossi et al, Blood, 2013). Moreover, *KRAS* and its transcriptional regulator *HNRNPA1*, key components of Ras-Raf-MEK-ERK pathway are both hosted in the trisomic chromosome 12.
Aims: To investigate the role of KRAS expression and/or activation in Tri12 and *NOTCH1-mut* CLL.
Methods: Tri12 was assessed by FISH. *NOTCH1* and *KRAS* mutations were assessed by either Sanger or NGS. QRT-PCR and western blot were employed to evaluate HNRNPA1 and KRAS expression and down-stream signaling in primary CLL or CLL cell line models. Active GTP-bound RAS pull-down was performed by Raf1 RBD agarose beads assay. CI and MEC-1 cell lines were used as human *in vitro* model of Tri12 *NOTCH1-mut* CLL and non-Tri12 *NOTCH1-wild type* (*wt*) CLL, respectively. Mann-Whitney test, unpaired t-test or Chi-Square test were used to compare differences between groups.

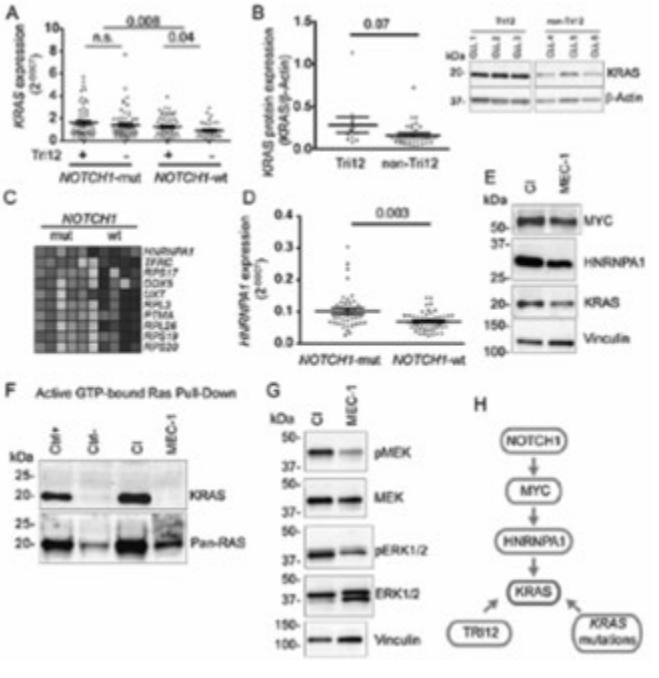


Figure 1.

Results: *KRAS* transcript was analyzed in 215 cases purposely enriched in Tri12 (118) and *NOTCH1-mut* (121). As shown in Figure 1 A, Tri12 were characterized by higher *KRAS* level in the context of *NOTCH1-wt* CLL (p=0.04); conversely, no differences in KRAS expression were found between Tri12 and non-Tri12 in the context of *NOTCH1-mut* CLL cases, which however expressed higher *KRAS* transcript compared to *NOTCH1-wt* CLL (p=0.008). Consistently, KRAS protein level was higher in Tri12 vs. non-Tri12 in the context of *NOTCH1-wt* CLL (Figure 1 B). A gene expression profiling identified *HNRNPA1* as the top ranked gene among MYC target genes upregulated in *NOTCH1-mut* vs. *NOTCH1-wt* CLL. Accordingly, higher *HNRNPA1* levels were found by QRT-PCR in additional 41 *NOTCH1-mut* vs. 47 *NOTCH1-wt* CLL samples (p=0.003, Figure 1 C,D) without differences between Tri12 (42) and non-Tri12 (46) CLL. In line with a KRAS transcriptional activation mediated by NOTCH1-MYC-HNRNPA1 axis, higher MYC, HNRNPA1 and KRAS protein levels were found in the Tri12 *NOTCH1-mut* CI cell line when compared to the non-Tri12 *NOTCH1-wt* MEC-1 cells. Furthermore, higher levels of active GTP-bound KRAS and higher phosphorylation levels of MEK and ERK were found in CI cells (Figure 1 E,F,G), supporting the hypothesis of sustained Ras-Raf-MEK-ERK signaling in Tri12 and *NOTCH1-mut* CLL. Finally, analysis of *KRAS* genomic aberrations revealed higher mutations incidence in Tri12 CLL (10/77, 13%) when compared to non-Tri12 CLL (2/68, 2.9%, p=0.03). Altogether, these data foster the hypothesis of multiple mechanisms of KRAS overexpression/activation occurring in Tri12 CLL via the NOTCH1-MYC-HNRNPA1 axis in *NOTCH1-mut* cases, or due to a higher incidence of KRAS mutations or an overexpression of KRAS by the supernumerary chromosome 12 (Figure 1 H).
Summary/Conclusion: Our data, by describing a synergism between Tri12, *NOTCH1-mut* and *KRAS-mut* in boosting KRAS expression and activity in CLL, indicate the Tri12 subset as particularly addicted to the Ras-Raf-MEK-ERK signaling pathway and likely to benefit of ERK/MEK inhibitors, as recently emphasized (Dietrich *et al*, J Clin Invest, 2018).

S868

REGULATION OF HIF-1A IN TP53 DISRUPTED CHRONIC LYMPHOCYTIC LEUKEMIA CELLS AND ITS POTENTIAL ROLE AS A THERAPEUTIC TARGET

V. Griggio[1], C. Vitale[1], M. Todaro[1], C. Riganti[2], J. Kopecka[2], C. Salvetti[1], R. Bomben[3], M. Dal Bo[3], D. Rossi[4], G. Pozzato[5], M. Marchetti[6], P. Omede'[1], L. Bonello[7], A.A. Kodipad[8], L. Laurenti[9], G. Del Poeta[10], F.R. Mauro[11], R. Bernardi[12], V. Gattei[3], G. Gaidano[8], R. Foa'[11], M. Massaia[13], M. Boccadoro[1], M. Coscia[1,*]

[1]Division of Hematology, University of Torino, A.O.U. Città della Salute e della Scienza di Torino, [2]Department of Oncology, University of Torino, Torino, [3]Clinical and Experimental Onco-Hematology Unit, CRO Aviano National Cancer Institute, Aviano, Italy, [4]Department of Hematology, Oncology Institute of Southern Switzerland and Institute of Oncology Research, Bellinzona, Switzerland, [5]Department of Internal Medicine and Hematology, Maggiore General Hospital, University of Trieste, Trieste, [6]Hematology Day Service, Oncology SOC, Hospital Cardinal Massaia, Asti, [7]Department of Molecular Biotechnology and Health Sciences, University of Torino, Torino, [8]Division of Hematology, Department of Translational Medicine, University of Eastern Piedmont, Novara, [9]Department of Hematology, Catholic University of Sacred Heart, [10]Division of Hematology, S. Eugenio Hospital and University of Tor Vergata, [11]Hematology, Department of Cellular Biotechnologies and Hematology, Sapienza University, Policlinico Umberto I, Roma, [12]Division of Experimental Oncology, IRCCS San Raffaele Scientific Institute, Milano, [13]Ematology Unit, ASO Santa Croce e Carle, Cuneo, Italy

Background: Treatment of high-risk chronic lymphocytic leukemia (CLL) patients remains an unmet clinical need. Disease aggressiveness can be ascribed to intrinsic features of the tumor cells (i.e. *TP53* disruption) and to the interaction of CLL cells with stromal cells (SC) of the microenvironment. HIF-1 is a transcription factor implicated in cell adaptation to hypoxia and is involved in the regulation of genes implicated in tumor progression. In CLL cells, the α subunit of HIF-1 (HIF-1α) is constitutively expressed even in normoxia and regulates the protective interactions that the leukemic cells establish with the microenvironment.
Aims: The aims of this study were to understand HIF-1α regulatory pathways in CLL cells from *TP53* disrupted ($TP53^{dis}$) and wild type ($TP53^{wt}$) patients, and to evaluate the ability of HIF-1α inhibition to exert synergistic cytotoxic effects in combination with fludarabine and ibrutinib.
Methods: Del(17p) in CLL cells was assessed by fluorescence in situ hybridization and the presence of *TP53* gene mutations was evaluated by Sanger sequencing. CLL patients with mutation of the *TP53* gene, or >40% del(17p) in the absence of *TP53* mutation, were included in the $TP53^{dis}$ subset. Patients with <10% del(17p) and without *TP53* mutation were considered $TP53^{wt}$. In selected experiments, CLL cells were cultured in the presence or absence of M2-10B4 SC, and exposed to PD98059, Y27632, LY249002, BAY87-2243, F-ara-A or ibrutinib. Culture conditions were 21% (normoxia) or 1% (hypoxia) O2, 5% CO2 at 37°C. Ras, ERK1-2, Akt, HIF-1α, Elk3 and pVHL expression was evaluated by Western Blot. RhoA and RhoA kinase acitivity was measured by specific immunoassays. *HIF-1A*, *p21* and *ENO1* gene expression was assessed by RT-PCR. Cell viability was analyzed by AnnexinV/propidium Iodide immunostaining.

Results: We found that primary CLL cells from patients carrying *TP53* abnormalities (*TP53*^{dis} CLL cells) had constitutively higher transcriptional activity and expression levels of the α subunit of HIF-1 compared to CLL cells isolated from *TP53*^{wt} samples (*TP53*^{wt} CLL cells). HIF-1α upregulation detected in the *TP53*^{dis}subset was due to a reduced expression of the HIF-1α ubiquitin ligase von Hippel-Lindau protein (pVHL) and more active PI3K/Akt and Ras/ERK1-2 signalling pathways. Hypoxia and SC further enhanced HIF-1α accumulation in both *TP53*^{dis} and TP53^{wt} CLL cells. Hypoxia-mediated HIF-1α upregulation was due to a decreased pVHL expression and to the activation of PI3K/Akt and Ras/ERK1-2 signalling pathways. SC did not affect pVHL expression, but induced an increased activity of Ras/ERK1-2, RhoA/RhoA kinase and PI3K/Akt pathways, leading to HIF-1α accumulation. Interestingly, *in vitro fludarabine-resistant* CLL cells were mostly *TP53*^{dis} and expressed significantly higher levels of *HIF-1A* mRNA compared to *fludarabine-sensitive* cells. The HIF-1α inhibitor BAY87-2243 reversed the constitutive fludarabine resistance of leukemic cells isolated from patients carrying *TP53* abnormalities, and counteracted the fludarabine resistance induced by SC. BAY87-2243 also elicited a strongly synergistic cytotoxic effect in combination with ibrutinib.
Summary/Conclusion: Overall, our data indicate that HIF-1α is overexpressed in CLL cells, especially in the presence of *TP53* abnormalities, and is susceptible of positive regulation by hypoxia and SC. From the translational standpoint, HIF-1α can be regarded as a crucial target whose inhibition warrants further evaluation, also in combination with currently available therapies.

S869

IBRUTINIB DOES NOT SUPPRESS CLONAL EVOLUTION IN HIGH RISK CHRONIC LYMPHOCYTIC LEUKEMIA

G. Forestieri[1], V. Spina[1], A. Bruscaggin[1], L. Terzi di Bergamo[1], L. De Paoli[2], A. Condoluci[13], C. Deambrogi[2], F. Guidetti[1], S. Favini[2], F. Diop[2], C. Cirillo Sanchez[1], G. Stüssi[3], B. Gerber[3], A. M. Frustaci[4], M. Mazzucchelli[4], F. Autore[5], M. Merli[6], L. Scarfo'[7], R. Lucchini[3], G. Del Poeta[8], R. Marasca[9], F. Zaja[10], A. Zucchetto[11], E. Zucca[3], M. Gregor[12], P. Ghia[7], F. Passamonti[13], L. Laurenti[14], J. Passweg[15], M. Montillo[4], A. Tedeschi[4], F. Cavalli[3], V. Gattei[11], G. Gaidano[2], D. Rossi[13*]

[1]Institute of Oncology Research, Bellinzona, Switzerland, [2]University of Eastern Piedmont, Novara, Italy, [3]Oncology Institute of Southern Switzerland, Bellinzona, Switzerland, [4]Niguarda Cancer Center, Niguarda Hospital, Milano, [5]Policlinico Gemelli Foundation, Catholic University of the Sacred Heart, Roma, [6]Policlinico Universitario Ospedale di Circolo e Fondazione Macchi, Varese, [7]IRCCS San Raffaele Scientific Institute and University Vita-Salute San Raffaele, Milano, [8]S. Eugenio Hospital and University of Tor Vergata, Roma, [9]Department of Medical and Surgical Sciences, University of Modena and Reggio Emilia, Modena, [10]University of Udine, Udine, [11]Centro di Riferimento Oncologico, IRCCS, Aviano, Aviano, Italy, [12]Division of Haematology and Central Haematology Laboratory, Cantonal Hospital Lucerne, Luzern, Switzerland, [13]Department of Medicine and Surgery, Hematology, University of Insubria, Varese, [14]Institue of Hematology, Università Cattolica del Sacro Cuore, Roma, Italy, [15]Department of Hematology, University Hospital Basel, Basel, Switzerland

Background: The B-cell receptor (BCR) is one of the most important surface molecules that CLL cells use to gain oncogenic signals from the microenvironment. The critical role of BCR signaling for the pathogenesis of CLL is supported by the therapeutic success of ibrutinib, a targeted agent that disrupts the BCR pathway. Beside microenvironment-promoted oncogenic signals, the biology of CLL is also driven by molecular lesions and clonal evolution, that mark CLL progression and treatment resistance. The interconnection between microenvironment-promoted oncogenic signals and clonal evolution has been postulated in CLL but never proven because of the lack of suitable *ex vivo* models.
Aims: Ibrutinib allows the unprecedented opportunity of assessing the contribution of BCR to cancer clonal evolution directly *in vivo* in patients.
Methods: The IOSI-EMA-001 study (NCT02827617) is an observational, non-interventional, multicenter study consisting in the prospective and longitudinal collection of peripheral blood samples and clinical data from high risk CLL patients treated with ibrutinib monotherapy. Tumor DNA derived from sorted CLL cells (purity >99%) and germline DNA derived from sorted T cells were used for somatic mutation identification by CAPP-seq targeted deep next generation. A gene panel including 133 genes recurrently mutated in mature B-cell tumors or targeted by the aberrant somatic hypermutation process, was used. The number of the libraries loaded in the NexSeq500 sequencer (Illumina) was tailored at obtaining at least a coverage >2000x in >80% of the region of interest. A stringent bioinformatic pipeline was applied to suppress the background noise allowing to call variants with a sensitivity of $3x10^{-3}$. To track clonal trajectories across serial samples, we first measured the variant allele fraction (VAF) of all mutations identified across the different timepoints per patient. VAFs were transformed to cancer cell fractions (CCFs) using the ABSOLUTE tool. For each patient, we compared the clonal composition of the baseline sample with all the available longitudinal samples (up to 72 weeks of therapy).
Results: The study cohort comprised 31 high risk CLL patients, including 15 treatment naïve, 16 relapsed, 80% IGHV unmutated, 42% 17p deleted and 55% TP53 mutated (Figure 1A). Median duration of ibrutinib treatment was 45 weeks (range 24-72 weeks). Overall, 285 individual mutations were longitudinally discovered and monitored across a total of 119 sequential timepoints collected during ibrutinib treatment. Significant changes in CCF over time, defined as a FDR adjusted p value of <0.1 for change in CCF >0.1 in the largest rising or falling clone was observed in 21/31 (67.7%) cases (Figure 1B), a proportion that is superimposable to the clonal evolution rate previously documented in CLL treated with chemoimmunotherapy. Clonal evolution appeared to be puzzled and involved different genes without a stereotypic targeting (Figure 1C). Consistently, none of the main driver gene mutations was homogeneously selected or suppressed by ibrutinib. Clonal evolution rate neither associated with IGHV or TP53 mutation status (Figure 1D), nor changed over time (Figure 1E).

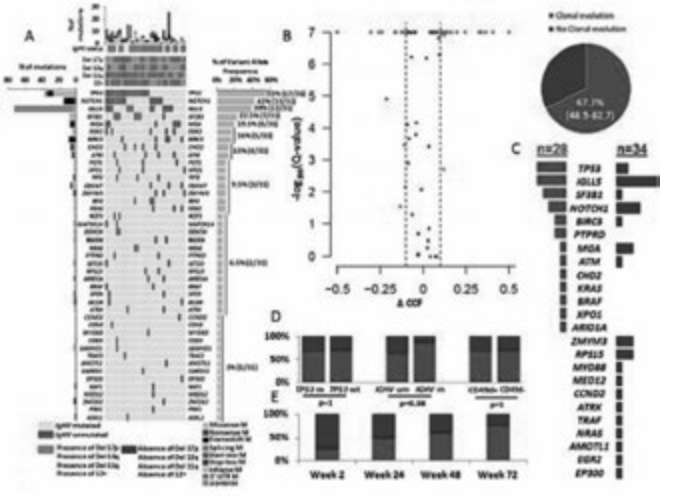


Figure 1.

Summary/Conclusion: Our results suggest that clonal evolution, a known pathogenic mechanism of progressive CLL: i) is not abrogated by ibrutinib; and ii) is quantitatively similar, but qualitatively different, than clonal evolution under chemoimmunotherapy, without specific pathways being targeted.

S870

RITUXIMAB INDUCES A PRO-INFLAMMATORY MICROENVIRONMENT THAT INCREASES NOTCH1 SIGNALLING IN CHRONIC LYMPHOCYTIC LEUKAEMIA CELLS

J. Edelmann[1,*], K. Holzmann[2], J. G. Gribben[1]

[1]Centre for Haemato-Oncology, Barts Cancer Institute, London, United Kingdom, [2]Genomics Core Facility, Ulm University, Ulm, Germany

Background: The addition of the anti-CD20 monoclonal antibody (mAb) rituximab to chemotherapy improves responses in almost all chronic lymphocytic leukaemia (CLL) patients, with the exception of those having *NOTCH1* mutations. NOTCH1 is a cell surface receptor releasing its intracellular domain (NICD1) after two ligand-induced cleavage steps performed by metalloproteases and γ-secretase. NICD1 acts as transcription factor and *NOTCH1* mutations in CLL lead to longer lasting transcription factor activity.
Aims: To understand the relationship between rituximab and NOTCH1, we studied the effects rituximab treatment has on NOTCH1 signalling.
Methods: Freshly isolated peripheral blood mononuclear cells (PBMCs) from CLL patients attending St. Bartholomew's Hospital, London were enriched for CD19+ cells and treated with rituximab. Whole protein lysates were obtained after 15, 30 and 60 min of mAb treatment; RNA was isolated after 150 min. NICD1 was semi-quantitatively assessed by western blot. Expression of *HES1*, the best established NICD1 target gene, and *CCL2* was quantified by TaqMan-probe-based quantitative PCR. Rituximab

F(ab')2 fragments and trastuzumab were used as controls.
Results: Peripheral blood CLL cells showed low NOTCH1 signalling activity. Following *in vitro* treatment with rituximab, we observed NOTCH1 receptor activation with NICD1 release in a time-dependent manner. In line with this, *HES1* gene expression was up-regulated after rituximab treatment. The extent to which *HES1* expression was up-regulated varied in between individual samples. Samples had variable amounts of residual non-tumour PBMCs and the sample with the highest up-regulation of *HES1* expression had the highest amount of residual non-tumour cells. We therefore reasoned that activation of immune effector cells via the Fc-fragment of rituximab might be involved in activating NOTCH1. Since relative effector cell numbers were low (≤4%), physical contact between CLL cells and activated ligand-expressing effector cells was unlikely to serve as only explanation. Effector cells such as monocytes secrete matrix metalloproteases (MMPs) upon activation. Therefore, we hypothesised that secreted metalloproteases might contribute to cleavage of the NOTCH1 receptor. Particularly MMP9 and MMP8 were found in the supernatant of healthy donor PBMCs treated with rituximab. Using this supernatant, NOTCH1 activation could be evoked in SU-DHL4 cells, which did not show NOTCH1 activation when adding rituximab to their standard culture medium. Also, the supernatant of trastuzumab treated PBMCs led to NOTCH1 activation in SU-DHL4 cells, whereas using rituximab F(ab')2 fragments had no impact. MMP9 and MMP8 are mainly secreted by monocytes. In line with this, up-regulation of *HES1* expression after rituximab treatment correlated well with up-regulation of *CCL2* expression. CCL2 is mainly produced by monocytes and secreted upon their activation.
Summary/Conclusion: Our data shows that rituximab treatment increases NOTCH1 signalling. In B-cells, only cell surface bound ADAM10 and ADAM17 were so far associated with first cleavage of NOTCH1. Here we show that proteins secreted by activated immune effector cells contribute to activate NOTCH1. MMP8 and MMP9 are the most likely candidates and active recombinant proteins will be used to prove their involvement. Activation of NOTCH1 in a pro-inflammatory environment occurs independent of the NOTCH1 mutation status, but abnormally long activity of mutant NICD1 probably has a different/stronger impact on the CLL transcriptome than wild-type NICD1.

Stem cell transplantation – Clinical II

S871

ADMINISTRATION OF BPX-501 CELLS FOLLOWING ALPHA/BETA T-CELL AND B-CELL-DEPLETED HLA HAPLOIDENTICAL HSCT (HAPLO-HSCT) IN CHILDREN WITH PRIMARY IMMUNODEFICIENCIES

D. Pagliara[1,*], A. Bertaina[1], M. Slatter[2], N. Kapoor[3], L. Krishnamurti[4], W. Qasim[5], S. Naik[6], V. Aquino[7], S. Baumeister[8], A. Woolfrey[9], P. Woodard[10], F. Locatelli[1]

[1]Department of Onco-Hematology and Cell and Gene Therapy, Ospedale Pediatrico Bambino Gesu, Rome, Italy, [2]Paediatric Immunology, Allergy, and Infectious Disease , Great North Children's Hospital, Newcastle upon Tyne, United Kingdom, [3]Hematology, Oncology, and Blood and Marrow Transplantation, Children's Hospital Los Angeles, Los Angeles, CA, [4]Blood and Marrow Transplant Program, Children's Healthcare of Atlanta, Atlanta, GA, United States, [5]Cell and Gene Therapy Program, Great Ormond Street Hospital, London, United Kingdom, [6]Bone Marrow Transplant/Stem Cell Transplant, Texas Children's Hospital, Houston, TX, [7]Pediatric Hematology and Oncology, University of Texas-Southwestern, Dallas, TX, [8]Hematology/Oncology, Boston Children's Hospital, Boston, MA, [9]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, [10]Clinical Development, Bellicum Pharmaceuticals, Inc., Houston, TX, United States

Background: Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) is a well-established treatment for children with a wide range of primary immunodeficiencies (PIDs). HLA partially-matched (haplo) donors are a suitable alternative option for children who lack a matched donor.
Aims: To evaluate the safety and efficacy of BPX-501 T cells administered after a T-cell receptor αβ and B-cell depleted haplo-HSCT in pediatric patients with PIDs. The purpose of this clinical trial is to determine whether BPX-501 infusion can enhance immune reconstitution and reduce the risk of life-threatening infections.
Methods: Fifty-nine (59) patients (EU:42, US:17; 34 males, 25 females; median age 4.6 years) with PIDs were enrolled in a multicenter, prospective trial utilizing αβ-T-cell and B-cell-depleted haplo-HSCT, followed by infusion of donor lymphocytes genetically modified to include the inducible Casp9 suicide gene (iCasp9; BPX-501 T cells). The study included, SCID: 19 patients, WAS: 9 patients, CGD: 7 patients, HLH: 6 patients, CID: 4 patients, and other immune deficiencies: 14 patients. In the EU, the conditioning regimen was primarily-treosulfan-based (used in 47.5% of all patients), whereas in the US conditioning was most frequently busulfan-based (used in 39% of all patients). All patients received rabbit anti-thymocyte globulin (Grafalon/Neovii® or Thymoglobulin/Sanofi® in the EU and US, respectively) as graft rejection prophylaxis. Patients received 0.25-1 x 10^6 BPX-501 cells/kg. The median time to BPX-501 infusion was 15 days (11.00 - 56.00).
Results: The median time for neutrophil and platelet engraftment was 16 and 11 days, respectively. Six children (10.1%) experienced primary graft failure (3 HLH, 1 CID, 2 CGD). Five patients engrafted after a successful second transplant (4 haplo, 1 unrelated cord blood). Twenty patients developed Grade I-IV aGVHD (cumulative incidence [CI] 35.1% [95% confidence interval (CoI): 22.6-47.6]). Ten patients developed Grade II-IV aGVHD (CI 17.0% [95% CoI: 7.4-26.6]). Three patients developed Grade III-IV aGVHD (CI 5.5% [95% CoI: 0-11.6]). Two patients developed mild to severe cGVHD (CI of 5.1% [95% CoI: 0-12.2]). The dimerizing agent AP1903 activating iCasp9 was administered in 6 patients (2 CRs, 2 PRs, 1 NE, 1 NR). Three of the 59 patients experienced transplant-related mortality (TRM CI 5.9%; 95% CoI 0-12.4%). Disease-free survival (DFS) was 88.1% (95% CI, 79.9-96.3%) after a median follow-up of 487 days (32-1147 days). The probability of overall survival was 88.6%. CD3+ and CD3+CD4+ T cells above 500 cells/ml were achieved by 90 and 180 days, respectively. IgA and IgM levels achieved normal values by 270 and 180 days, respectively. CD3+CD19+ cell counts above 100 cells/ml were achieved by day 90.
Summary/Conclusion: These data suggest that αβ-T-cell and B-cell depleted haplo-HSCT followed by infusion of BPX-501 cells represents a novel and highly effective transplantation strategy for many different PIDs, leading to a success rate comparable to that of matched-donor HSCT.

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GRAFT-VERSUS-LEUKEMIA EFFECTS IN SECONDARY ACUTE MYELOID LEUKEMIA: A RETROSPECTIVE STUDY FROM THE ACUTE LEUKEMIA WORKING PARTY OF THE EBMT

F. Baron[1,*], E. Beohou[2], M. Labopin[2], B. Savani[3], D. Niederwieser[4], A. Ganser[5], V. Potter[6], N. Kröger[7], D. Beelen[8], G. Socie[9], M. Itala-Remes[10], M. Bornhäuser[11], M. Mohty[12], A. Nagler[13]

[1]GIGA, ULiege, Liege, Belgium, [2]EBMT Paris Office, Paris, France, [3]Vanderbilt University Medical Center, Nashville, United States, [4]University Hospital Leipzig, Leipzig, [5]Hannover Medical School, Hannover, Germany, [6]GKT School of Medicine, London, United Kingdom, [7]University Hospital Eppendorf , Hamburg, [8]University Hospital Essen, Essen, Germany, [9]Hopital St. Louis, Paris, France, [10]HUCH Comprehensive Cancer Center, Helsinki, Finland, [11]Universitaetsklinikum Dresden, Dresden, Germany, [12]AP-HP, Hématologie Clinique et Thérapie Cellulaire, Hôpital Saint-Antoine, Paris, France, [13]The Chaim Sheba Medical Center, Tel-Hashomer, Israel

Background: The efficacy of allogeneic hematopoietic cell transplantation (HCT) in patients (pts) with acute myeloid leukemia (AML) depends both on chemo/radiotherapy given in the conditioning regimen and on immune-mediated graft-versus-leukemia (GVL) effects. Previous studies have observed an association between occurrence of acute (a) and chronic (c) graft-versus-host disease (GVHD) and a lower risk of AML relapse in pts with *de novo* AML (GvL effects).
Aims: It is well established that, in comparison to *de novo* AML, secondary AML (sAML) is less sensitive to chemotherapy. However, its susceptibility to GvL effects has not been studied in large cohorts of pts. This is the focus of the current study.
Methods: The study population included adult pts with sAML in first or second CR, bone marrow (BM) or peripheral blood stem cells (PBSC) as stem cell source, HLA-identical sibling or 10/ 10 (MUD) or 9/10 (MMUD) HLA-matched unrelated donor, no ex vivo T-cell depletion, and transplantation between 2005 and 2016. Previous studies might have overestimated the beneficial impact of GVHD on relapse prevention given the tight association between GVHD and non-relapse mortality (NRM) (since relapse and NRM are competing events). Thus, here we assessed the evolution of relapse rate over time according to GVHD condition by calculating the relapse rate per pt-year within sequential 90-day intervals, as previously reported (Baron *et al*., J Intern Med 2018, 283(2):178-18). The smoothed rates were plotted as curves for each GVHD condition. The impact of GVHD on relapse, NRM and overall survival (OS) was also assessed with classical multivariate Cox models modeling GVHD as time-dependent covariates.
Results: 3303 pts met the study inclusion criteria. Status at transplantation was CR1 in 2919 pts (88%) and CR2 in the remaining 384 ps. 1517 pts received grafts from MSD, 1427 from MUD and 359 from MMUD. 41% of the pts received a myeloablative conditioning (MAC), and 49% anti-thymocyte globulin (ATG). Stem cell source was PBSC in 90% of the patients. The proportion of patients with grade II and III-IV aGVHD was 15% and 10%, respectively. At 2 years, the cumulative incidence of cGVHD was 43% (18% extensive), the cumulative incidence of relapse 30% and OS 53%. Relapse rates declined gradually over time and were significantly lower in pts with cGVHD than in those without (p=0.009). In multivariate Cox models, grade III-IV aGVHD and cGVHD were each associated with a lower risk of relapse but not grade II aGVHD. Other factors associated with a lower risk of relapse included absence of poor-risk cytogenetic (p=0.008), MAC versus reduced-intensity (RIC) conditioning (p<0.001), and MUD (p=0.01). Interestingly, while there was a trend for better OS in pts with limited cGVHD (p=0.07), all other forms of GVHD were associated with lower OS. Other factors associated with increased mortality included high pt age (p<0.001), intermediate (p=0.01) and poor (p<0.001) risk cytogenetics, and MMUD (p=0.01).
Summary/Conclusion: Although this study showed that limited cGVHD was associated with lower risk of sAML relapse and a trend to a better OS, all other form of GVHD were associated with lower OS. In addition, while MUD recipients had a lower risk of relapse, they had higher NRM offsetting any potential OS benefit. Further studies should focus at optimizing the conditioning regimens and administering post-transplant maintenance therapies in an attempt to further reduce sAML relapse post HCT.

S873

KD025-208: A PHASE 2 OPEN-LABEL TRIAL OF KD025-208 FOR STEROID-DEPENDENT CHRONIC GRAFT-VERSUS-HOST DISEASE (CGVHD)

M. Jagasia[1,*], A. Lazaryan[2], A. Salhotra[3], B. Zoghi[4], J. Essell[5], C. Bachier[6], B. MacGillivray[7], O. Schueller[7], D. Eiznhamer[7], J. Ryan[7], S. Lee[8]

[1]Vanderbilt University, Nashville, TN, [2]University of Minnesota, MN, Minneapolis, [3]City of Hope, Duarte, CA, [4]Sarah Cannon Research Institute, San Antonio, TX, [5]Oncology/Hematology Care, Cincinnati, OH, [6]Sarah Cannon Research Institute, Nashville, TN, [7]Kadmon Corporation, LLC, New York, NY, [8]Fred Hutchinson Cancer Research Center , Seattle, WA, United States

Background: KD025 is a ROCK2-selective inhibitor in Phase 2 development for chronic graft-versus-host disease (cGVHD). By downregulating Th17 and Tfh cells while upregulating regulatory T cells and decreasing myofibroblast formation and proliferation, KD025 targets inflammatory and fibrotic components of cGVHD.
Aims: The aim is to evaluate the safety and activity of KD025 in subjects with steroid-dependent cGVHD.
Methods: KD025-208 is an open label, Phase 2 study in patients with steroid-dependent or refractory cGVHD. Three cohorts (200 mg QD, 200 mg BID, and 400 mg QD) of 16 patients each are planned, followed by an expansion cohort. The primary endpoint is the overall Complete and Partial Response rate, defined per the 2014 NIH Consensus criteria.
Results: Patients enrolled in Cohorts 1 (n=17) and 2 (n=16) had received a median of 3 and 2 prior lines of cGVHD therapy, respectively. The median corticosteroid dose (mg/kg/day) at baseline was 0.22 in Cohort 1 and 0.19 in Cohort 2. The most frequently involved organs in Cohorts 1 and 2, respectively, are eyes (82%, 69%), skin (76%, 75%), mouth (76%, 69%), joints (71%, 69%), and lung (24%, 38%). Forty-seven percent (47%) of patients in Cohort 1 and 69% in Cohort 2 had involvement of ≥ 4 organs. Sixteen patients (8 in each cohort) remain on treatment with KD025, with a median treatment duration of 37 and 28 weeks, respectively. As of a data cutoff date of January 3, 2018, the Overall Response Rate was 65% in Cohort 1 and 69% in Cohort 2. Responses were rapid, with 68% of responders achieving a response by the first assessment (at 8 weeks). Seven of 17 patients 41%) in Cohort 1 have sustained a response for ≥20 weeks. In responders with ≥4 organs involved, 75% and 33% in Cohorts 1 and 2, respectively, showed response in ≥4 organs. Responses were observed across all affected organ systems, including CRs in upper GI, lower GI, esophagus, mouth, skin, joints, eyes, and liver. The median corticosteroid dose reduced by 40% in Cohort 1 and 26% in Cohort 2 while on study. Four patients completely discontinued corticosteroid treatment while receiving KD025. Sixty-five percent (65%) and 38% of patients in Cohorts 1 and 2, respectively, achieved an improvement (≥7 point reduction) in the Lee cGVHD Symptom Scale Score. KD025 was well tolerated. Commonly reported AEs were AST/ALT elevations, anemia, nausea, diarrhea and URTI. Grade 3 or higher AEs were reported in 16 patients and SAEs in 9 patients. No apparent increase in incidence of infection was observed. Eleven of 33 patients discontinued treatment due to progression of cGVHD, 1 due to an adverse event, 2 due to recurrence of underlying malignancy, 2 due to voluntary withdrawal, and 1 due to investigator decision.
Summary/Conclusion: Treatment with KD025 has resulted in clinically meaningful and durable overall responses across all affected organ systems. Corticosteroid doses were reduced in both responders and non-responders. KD025 treatment was well tolerated with an AE profile consistent with that expected in cGVHD patients receiving corticosteroids. There was no apparent increased risk of infection observed with KD025. Enrollment targets in all three cohorts have been reached and treatment is ongoing. Updated follow-up data will be presented at the meeting.

S874

PREDICTION OF LEUKEMIA FREE SURVIVAL FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION IN AML: A RISK SCORE DEVELOPED BY THE ALWP OF THE EBMT

R. Shouval[1,*], M. Labopin[2], D. Bomze[3], M. Houhou[2], D. Blaise[4], T. Zuckerman[5], G. Baerlocher[6], R. Foa[7], E. Forcade[8], A. Huynh[9], R. Saccardi[10], M. Martino[11], N. Schaap[12], D. Wu[13], M. Mohty[2], N.C. Gorin[2], A. Nagler[1]

[1]Sheba Medical Center, Tel-HaShomer, Ramat-Gan, Israel, [2]Department of Haematology, Saint Antoine Hospital, EBMT Paris study office / CEREST-TC, Paris, France, [3]Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel, [4]Institut Paoli Calmettes, Marseille, France, [5]Rambam Medical Center, Haifa, Israel, [6]University Hospital Bern, Bern, Switzerland, [7]Univ. La Sapienza, Rome, Italy, [8]CHU Bordeaux Hôpital Haut-Leveque, Pessac, [9] Institut Universitaire du Cancer Toulouse, Oncopole, I.U.C.T-O, Toulouse, France, [10]Azienda Ospedaliera Universitaria Careggi, Firenze, [11] Grande Ospedale Metropolitano Bianchi Melacrino Morelli, Reggio Calabria, Italy, [12]Radboud University Medical Centre, Nijmegen, Netherlands, [13]First Affiliated Hospital of Soochow University, Suzhou, China

Background: Autologous stem cell transplantation (ASCT) has been proposed as consolidation therapy for patients with acute myeloid leukemia

(AML). Concern regarding the risk of relapse has led to decreasing use of this procedure. However, ASCT has been shown to be a putative strategy for reducing relapse and is associated with reduced non-relapse mortality (NRM) and a better quality of life compared to allogeneic SCT.
Aims: Develop a prediction model for Leukemia Free Survival (LFS) in AML patients treated with an ASCT.
Methods: This was a retrospective study of adult AML patients in complete remission (CR), treated with ASCT as consolidation therapy between 2000 and 2015 in European Society for Blood and Marrow Transplantation (EBMT) centers. The primary outcome was LFS, defined as death or relapse following ASCT. To develop a nomogram for prediction of 3-year and 5-year, we first performed a univariate analysis, introducing statistically significant ($p<0.1$) variables into a Cox regression multivariable model. The final model was chosen according to the Akaike information criterion. The performance was assessed using time-dependent c-statistic.
Results: A total of 2,298 AML pts, with a median age of 49 (IQR: 38-58) were included. The majority of pts were in CR1 (90%) and had intermediate risk cytogenetics (75%), followed by good (18.5%), and poor (6.5%). Peripheral blood was the cell source in 93% of the cases. TBI was used as the backbone of conditioning in 86% of the pts. The final variables included in the multivariable model were age, cytogenetic risk group (Medical Research Council classification), cell source, year of transplantation, and disease status and time from diagnosis to transplantation as an interaction term. The nomogram is present in Figure 1A. The cumulative points a patient receives corresponds with 3-year and 5-year LFS probability, described in the bottom rows. Prognostic discrimination was performed by dividing the predicted survival probabilities into quartiles that were then used to plot Kaplan-Meier curves (Figure 1B). Patients in the highest and lowest quartiles had a 60.8% and 27.7% probability of 5-year LFS. The c-statistic of the model for 5-year LFS was 0.64.

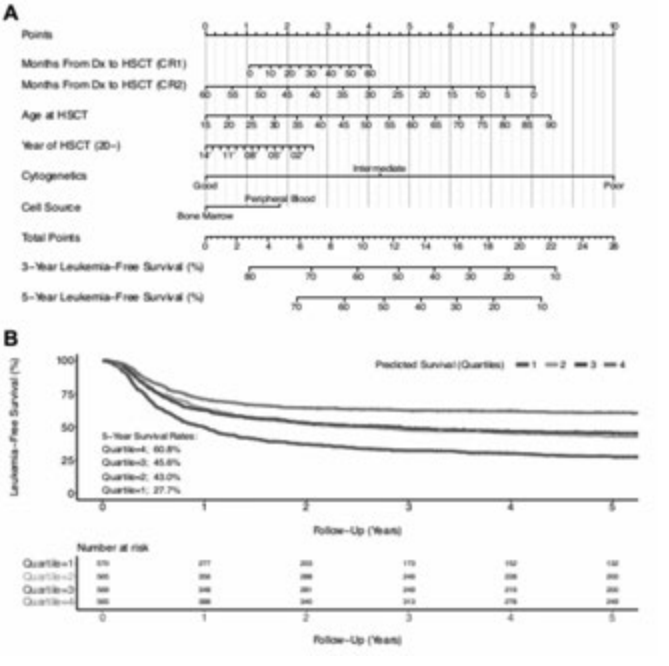

Figure 1.

Summary/Conclusion: We present the first predictive score for AML patients treated with ASCT. The score may help to better define patients who benefit from autologous transplantation as post-remission treatment.

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ALLOGENEIC STEM CELL TRANSPLANTATION FOR PERIPHERAL T-CELL LYMPHOMAS: A STUDY OF 284 PATIENTS FROM THE SOCIÉTÉ FRANCOPHONE DE GREFFE DE MOELLE ET DE THÉRAPIE CELLULAIRE

A.-C. Mamez[1], A. Dupont[2], D. Blaise[3], P. Chevallier[4], E. Forcade[5], P. Ceballos[6], M. Mohty[7], K. Bilger[8], F. Suarez[9], Y. Beguin[10], R. Peffault De La Tour[11], M.-T. Rubio[12], S. Nguyen Quoc[13,*]

[1]Département d'hématologie , Hôpitaux Universitaires de Genève, Genève, Switzerland, [2]service de biostatistique et information médicale, Hôpital Saint Louis, Paris, [3]Département d'hématologie, Institut Paoli Calmettes, Marseille, [4]Département d'hématologie, Hôtel Dieu, Nantes, [5]Département d'hématologie, Centre Hospitalier Universitaire de Bordeaux, Bordeaux, [6]Département d'hématologie , Centre Hospitalier de Montpellier, Montpellier, [7]Département d'hématologie, Hôpital Saint Antoine, Paris, [8]Département d'hématologie, Centre Hospitalier Universitaire de Strasbourg, Strasbourg, [9]Département d'hématologie, Hôpital Necker, Paris, France, [10]Département d'hématologie, CHU of Liège and University of Liège, Liège, Belgium, [11]Département d'hématologie, Hôpital Saint Louis, Paris, [12]département d'hématologie, Centre hospitalier Universitaire de Nancy, Nancy, [13]département d'hématologie, Hôpital La Pitié Salpêtrière, Paris, France

Background: Allogeneic stem cell transplantation for peripheral T-cell lymphomas: a study of 284 patients from the Société Francophone de Greffe de Moelle et de Thérapie Cellulaire.
Aims: The aim of the current study was to analyse the outcomes (overall survival, relpase/progression, non relapse mortality) in a cohort of patients who underwent an allo-HSCT for peripheral T cell lymphoma.
Methods: Based on the SFGM-TC database and on clinical files, we performed a retrospective multicentric analysis of adult patients who underwent an allo-SCT for non cutaneous PTCL between 2006 and 2014 in 34 centers. Primary cutaneous T cell lymphomas were excluded.
Results: A total of 284 patients with PTCL (NOS-T cell lymphomas: 39%, angioimmunoblastic T lymphomas: 29%, anaplastic T cell lymphomas 15%, others: 17%) were allo-transplanted in a median time of 12.6 months after diagnosis (3-322). Median age at transplant was 50 years (15 to 60) and 67% were males. At the time of transplant, 62% were in complete remission (CR), 27% in partial response (PR) and 11% in progressive disease (PD). Twenty-eight percent were transplanted in front line treatment, 36% after 2 lines of treatment and 35% after 3 or more lines of treatment; 23% had relapsed after a first autologous HSCT. Karnofsky index was up to 80% in 94% of the patients. Donors were matched related in 45%, matched unrelated in 36% and alternative in 19% (haplo-identical n=7, cord blood n=33, mismatched 9/10 n=13) and SC source was peripheral blood in 71% of the patients. Reduced intensity regiment was given in 147 patients (52%), myeloablative in 106 (38%) and non myeloablative (NMA) in 27 (10%). Fifty percent of the patient received an ex-vivo T cell depletion (ATG) whereas only 1% had an *ex vivo* T -depletion. Fourteen patients (14%) developed grade III-IV acute GvHd, and 34% developed chronic GvHd (extensive for 13%). Median follow up was 33 months. One and 2 year -OS were 68% (95% 0.62-0.73) and 64% (95% 0.58-07). Cumulative incidence (CI) of relapse was 18% at 1 year and 22% at 2 years. The median time from transplant to relapse was 94 days and only 10% of the relapse occurred after the first year post transplant. Non relapse mortality (NRM) was 22% at 1 year and 24% at 2 years. The main causes of death were relapse (35%), infection (27%) or GvHD (22%). In multivariate analysis, 5 year OS was significantly adversely influenced by the occurrence of grade III-IV aGvhd (HR: 2.52 (1.52-4.19), *p<0.01)*, low karnofsky score at the time of transplant (HR 2.22 (1.32-3.71), p:0.002), cord blood transplant compared to bone marrow (HR 2.01 (1.00-4.01), p:0.049). The main factor associated with NRM were patient's age (HR:1.02, p:0.084[MTR3]) a low Karnofsky score (HR 2.03 (1.08-3.83) p:0.029), female donor to male recipient (HR:1.87 (1.07-3.28), p: 0.027). The conditioning regimen intensity (RIC or MAC) was not found to have impact on OS. Among thirty patients transplanted in PD, 50% reached CR after allo-HSCT and 2 year-OS was 51% in this subgroup (Figure 1).

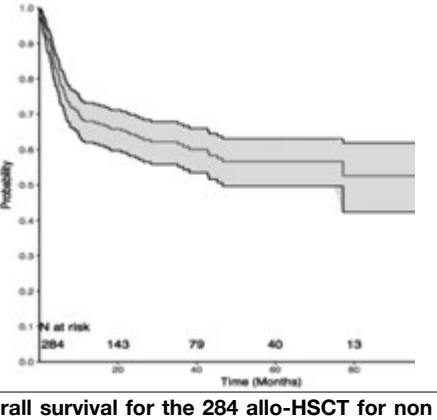

Figure 1. Overall survival for the 284 allo-HSCT for non cutaneous T-cell Lymphoma.

Summary/Conclusion: This is to our knowledge the largest cohort of allo-HSCT patients for T cell lymphoma, showing encouraging results in both MAC and RIC.

Myelodysplastic syndromes – Biology

S876

INTRINSIC DEFECTS CAUSED BY COMBINED CSNK1A1/RPS14 AND MIRNA145/146A DEFICIENCY DIRECTLY ALTER THE MICROENVIRONMENT IN A NOVEL MOUSE MODEL FOR DEL(5Q) MDS

F. Ribezzo[1,*], I. Snoeren [2], J. Stoelben [1], S. Ziegler[1], P. Olofsen[2], G. Buesche[3], U. Platzbecker[4], M. Raaijmakers[2], B.L. Ebert[5], R.K. Schneider[2]

[1]Department of Hematology, Oncology, Hemostaseology, and Stem Cell Transplantation, RWTH Aachen University , Aachen, Germany, [2]Department of Hematology, Erasmus MC Cancer Institute , Rotterdam, Netherlands, [3]Institute of Pathology, Hannover Medical School, Hannover, [4]Department of Hematology, University Hospital Carl Gustav Carus Technical University Dresden, Dresden, Germany, [5]Division of Hematology, Brigham and Women's Hospital, Department of Medicine, Harvard Medical School, Boston, United States

Background: Hemizygous, interstitial deletion of chromosome 5q is the most common cytogenetic abnormality in myelodysplastic syndrome (MDS) and has been the focus of functional analysis. Some patients with this molecular lesion have the 5q– syndrome, a disorder with a highly consistent clinical phenotype, characterized by a distinct clinical phenotype including severe macrocytic anemia, a high or elevated platelet count with hypolobulated megakaryocytes in the bone marrow and a low rate of progression to acute myelogenous leukemia. The full clinical phenotype is likely caused by the integration of effects from allelic insufficiency for multiple genes. *RPS14*, *CSNK1A1* and *mir-145* are universally co-deleted in 5q-syndrome but mouse models of each gene deficiency recapitulate only a subset of the composite clinical features of del(5q) MDS.
Aims: We aimed to dissect the combinatorial effect of haploinsufficiency for *Rps14*, *Csnk1a1* and *miRNA-145* on the hematopoietic stem cell function in a novel murine model for del (5q) MDS We hypothesized that compound loss of *Rps14*, *Csnk1a1* and *miRNA145/146a* has additive effects leading to recapitulation of the del(5q) MDS phenotype and an aggravated inflammatory response resulting in a more severe erythroid phenotype.
Methods: For *in vivo* studies, hematopoietic stem and progenitor cells (HSPCs) from compound haploinsufficient *Rps14*, *Csnk1a1* mice were retrovirally transduced and miR-145/146a were stably knocked down, followed by transplantation of the cells into lethally irradiated recipient mice. In order to further dissect the mechanisms, we generated granulocyte-macrophage colony-stimulating factor (GM-CSF) estrogen-receptor (ER)-Hoxb8 cells and Fms-related tyrosine kinase 3 ligand (FL) ER-Hoxb8 cells from the del (5q) murine models.
Results: We demonstrate that compound deficiency has high fidelity to human MDS and recapitulates the del(5q) MDS phenotype including 1) anemia, 2) thrombocytosis, 3) hypolobulated megakaryocytes and 4) clonal dominance. Macrophages, regulatory cells of erythropoiesis and the innate immune response, were significantly increased in *Rps14/Csnk1a1/miR-145/146a* deficient mice as well as in del(5q) MDS patient bone marrows and showed activation of the innate immune response, reflected by increased expression of S100A8, and decreased phagocytic function.
The bone marrow microenvironment is abnormal in MDS, but whether this is a direct consequence of changes in hematopoietic cells or instead independent of the disease has not been determined. Using 1) our new mouse model, 2) co-culture models with a defined mesenchymal stromal cell population, 3) immunohistochemistry and RNA-sequencing in del(5q) MDS patient samples, we demonstrate that increased expression of S100A8 in MDS-derived macrophages induces S100A8 expression in neighboring mesenchymal stromal cells. We show that S100A8-expression in stromal cells leads to loss-of their hematopoiesis-supporting capacity.
Summary/Conclusion: In conclusion, our data support the hypothesis that the combined haploinsufficiency of multiple genes on 5q has additive effects in the del(5q) phenotype and in the activation of the innate immune system. Our data indicate that intrinsic defects of the del(5q) MDS hematopoietic stem cell directly alter the surrounding microenvironment, which in turn negatively affects hematopoiesis as an extrinsic mechanism.

S877

EZH2 AND RUNX1 MUTATIONS COLLABORATE TO INITIATE DISTINCT HEMATOLOGICAL MALIGNANCIES DEPENDING ON THE TARGET CELL

C. Booth[1,*], W. H. Neo[1], N. Barkas[1], H. Boukarabila[1], E. Soilleux[2], N. Farnoud[3], A. Giustacchini[1,4], N. Ashley[1], J. Carrelha[1], L. Jamieson[1], D. Atkinson[1], T. Bouriez-Jones[1], E. Papaemmanuil[3], A. Mead[1], S.E. Jacobsen[1,5]

[1]Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, [2]Department of Pathology, University of Cambridge, Cambridge, United Kingdom, [3]Center for Molecular Oncology, Center for Heme Malignancies and Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, United States, [4]Department of Cell and Developmental Biology, University College London, London, United Kingdom, [5]Department of Cell and Molecular Biology and Center for Hematology and Regenerative Medicine, Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden
Adam Mead and Sten Eirik Jacobsen contributed equally to this work.

Background: How the identity of the cells targeted by oncogenic lesions impacts on the clinical picture of the resulting cancer has not been established. In myelodysplastic syndrome (MDS), all mutations in a malignant clone can be tracked back to the hematopoietic stem cells (HSCs) (Woll et al., Cancer Cell 2014). This suggests that MDS originates within and is propagated by the malignant counterparts of normal HSCs. However, the human phenotypically-defined HSC compartment also contains substantial numbers of non-HSCs, and it remains to be established to what extent also non-HSCs may be targeted and transformed by MDS-associated mutations. As well as in MDS (Papaemmanuil et al., Blood 2013), co-occurrence of inactivating mutations in EZH2 and RUNX1 is frequently observed in early thymic progenitor (ETP) leukemia, a clinically and molecularly distinct subtype of T cell acute lymphoblastic leukemia (Zhang et al., Nature 2012).
Aims: To determine the distinct impacts of inactivation of Ezh2 and Runx1 in different hematopoietic stem and progenitor populations in mice.
Methods: We used Mx1Cre, Flt3Cre and Rag1Cre to target conditional inactivation of Ezh2 and Runx1 to HSCs, multipotent progenitors (MPPs) or early lymphoid progenitors respectively.
Results: In agreement with previous studies using inactivation of Ezh2 combined with expression of a dominant-negative Runx1 mutant (Sashida *et al.*, Nat Commun 2014), we found that targeting of Ezh2 and Runx1 inactivation to HSCs using Mx1Cre induced an MDS phenotype. Competitive transplantation experiments demonstrated a clonal advantage of mutant cells over wild-type cells, combined with an extrinsic suppression of wild-type HSCs in recipient bone marrow.
Surprisingly, targeting of the same mutations to MPPs using Flt3Cre resulted in development of the same MDS phenotype with a similar latency to that induced by Mx1Cre. Absolute numbers of $Lin^-Kit^+Sca1^+CD48^-CD150^+$ HSCs were reduced in these mice, while $Lin^-Kit^+Sca1^+CD48^+CD150^-$ MPPs were expanded compared to wild-type controls, suggesting that MPPs rather than HSCs could represent the MDS-propagating malignant stem cells in this model. We confirmed this using transplantation of purified E13.5 fetal liver MPPs. Unlike wild-type MPPs, MPPs from Flt3Cre-induced Ezh2 and Runx1-inactivated embryos were able to engraft and reconstitute long-term hematopoiesis and an MDS phenotype in recipient mice, while transplanted MPPs from Flt3Cre-negative littermate controls showed no engraftment.
In striking contrast, when Ezh2 and Runx1 inactivation was targeted to early lymphoid progenitors using Rag1Cre, we did not observe development of an MDS phenotype. Rather, absolute numbers of ETPs within the thymuses of these mice were markedly expanded compared to wild-type controls. This expanded ETP population showed transcriptional signatures characteristic of human ETP leukemia. Addition of Flt3-ITD, a constitutively activating RAS signalling mutation recurrently found in ETP leukemia, induced an acute T cell/myeloid leukemia which could be propagated by the expanded ETP population.
Summary/Conclusion: Our findings reveal that targeting the same clinically relevant mutations to different hematopoietic stem and progenitor populations can induce distinct and clinically relevant malignant phenotypes. This provides experimental evidence that the identity of the target cell of specific oncogenic lesions might be a crucial factor for the phenotype, prognosis and therapeutic response of the resulting malignancy.

S878

CO-OCCURRENCE OF STAG2 AND RAS SIGNALING MUTATIONS DURING PROGRESSION FROM MDS TO SAML ANALYZED BY WHOLE-EXOME AND TARGETED-DEEP SEQUENCING

M. Martín-Izquierdo[1,*], M. Abáigar[1], J.M. Hernández-Sánchez[1], D. Tamborero[2], F. López-Cadenas[3], M. Hernández-Sánchez[1], E. Lumbreras[1], F. Ramos[4], A. Madinaveitia-Ochoa[5], M. Megido[6], J. Labrador[7], A. Redondo-Guijo[3], J. Sánchez del Real[4], C. Olivier[8], J. Dávila[9], C. Aguilar[10], J.N. Rodríguez[11], G. Martín-Núñez[12], R. Benito[1], N. López-Bigas[2], M. Díez-Campelo[3], J.M. Hernández-Rivas[13]

[1]Cancer Research Center-University of Salamanca (IBMCC, USAL-CIC), Hospital Clínico Universitario de Salamanca, Salamanca, [2]Research Program on Biomedical

Informatics, Hospital del Mar Medical Research Institute (IMIM), Universitat Pompeu Fabra, Barcelona, [3]IBSAL-Hematology, Hospital Clínico Universitario de Salamanca, Salamanca, [4]Hematology, Hospital Universitario de León, Institute of Biomedicine (IBIOMED)-University of León, León, [5]Hematology, Hospital Universitario Miguel Servet, Zaragoza, [6]Hematology, Hospital del Bierzo, Ponferrada, León, [7]Hematology, Hospital Universitario de Burgos, Burgos, [8]Hematology, Hospital General de Segovia, Segovia, [9]Hematology, Hospital Nuestra Señora de Sónsoles, Ávila, [10]Hematology, Hospital Santa Bárbara, Soria, [11]Hematology, Hospital Juan Ramón Jiménez, Huelva, [12]Hematology, Hospital Virgen del Puerto, Plasencia, Cáceres, Spain

Background: Myelodysplastic syndromes (MDS) are hematological disorders at high risk of progression to acute myeloid leukemia (sAML). Previous high-throughput sequencing studies have provided insight into the mutational dynamics and clonal evolution underlying disease progression. However, large longitudinal sequencing genomic studies are still required to define which mutations or combinations of them are important in disease progression.
Aims: To study the mutational profiles and mutational dynamics underlying progression from MDS to sAML.
Methods: A total of 846 samples of myeloid malignancies, divided in 3 cohorts, were examined: 1) a **discovery cohort** consisting of 94 serially collected samples from 47 MDS/CMML patients evolving to sAML that were studied two different time-points: at the time of diagnosis (disease presentation) and at sAML progression (disease evolution); 2) a control cohort consisting of 10 samples from 5 MDS/CMML patients who did not progress to sAML (median follow-up of 3 years); and 3) a validation cohort comprising 742 myeloid malignancies samples. Sequencing studies were performed by a combination of whole-exome sequencing (WES) and targeted-deep sequencing (TDS). WES was carried out, as first screening, on 40 diagnosis/progression-matched samples of the discovery cohort, and driver mutations were identified, after variant calling by a standardized bioinformatics pipeline, by using the novel tool "Cancer Genome Interpreter". Secondly, TDS was performed on these 40 samples of the initial discovery cohort, in order to validate WES results, and in additional 806 samples from all of the cohort (including 66 extra serial samples), using a custom MDS/AML-related capture enrichment panel (Illumina ®) of 117 genes.
Results: As a first result, all patients, progressing (discovery cohort) and not progressing (control cohort), presented similar number of mutations at diagnosis (p=0.15). However, patients who evolved to sAML displayed a statistically significant increase of mutations after progression (p=0.001) while control cohort did not during follow-up time (p=0.88). Thus, this greater number of mutations at second sampling may be indicative of a higher genomic instability during disease evolution. Then, to study the mutational dynamics during disease progression (discovery cohort), we compared the variant allele frequencies (VAFs) of mutations detected at both time-points in each patient. We identified 4 different clonal dynamics: mutations that were initially present but increased (type-1) such as *STAG2* mutations; decreased (type-2); were newly acquired (type-3) such as *NRAS/KRAS* and *FLT3* mutations; or persisted with similar allelic burden (type-4) at sAML stage, namely *SRSF2*, *TET2* and *DNMT3A* mutations. Interestingly, 13% of patients (6/47) included in this cohort showed co-occurrence of type-1 *STAG2* and type-3 Ras signaling mutations indicating this mutational combination could play an important role during disease progression. To confirm this hypothesis, we searched this combination in all patients of the validation cohort and we found that was present in 8 patients, and 7 of them were MDS patients who finally progressed to sAML.
Summary/Conclusion: Progression from MDS to sAML could be explained by different mutational processes, as well as by the occurrence of unique and complex changes in the clonal architecture of the disease during the evolution. Co-occurrence of *STAG2* and Ras signaling mutations could play an important role during disease progression.
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S879

COMPARED ANALYSIS OF MUTATIONAL LANDSCAPE IN PEDIATRIC AND ADULT PATIENT WITH MYELODYSPLASTIC SYNDROMES

W. An[1,*], B. Li[2], C. Liu[1], Y. Wan[1], Z. Xiao[2], X. Zhu[1]

[1]Pediatric blood diseases center, [2]MDS/MPN center, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, China

Background: Myelodysplastic syndromes (MDS) are uncommon in children. There are limited known on the gene mutations landscape in pediatric patients with MDS and the differences between pediatric and adult patients.
Aims: We aimed to elucidate the mutational landscape in pediatric MDS and compared with adult cases.
Methods: We performed both whole exome sequencing and targeted sequencing on 30 pediatric MDS patients and targeted sequencing on 57 pediatric cases and 510 adult patients. The diagnosis was according to the 2016 revised criteria of the WHO for childhood MDS. To be comparable with adult and on the basis of difficulty of precisely diagnosing of low-risk RCC, only pediatric patients who also meet the diagnostic criteria for adult MDS were included. DNA was obtained from bone marrow. Germline status was analyzed by CD3+ T-cell or oral epithelial cells.

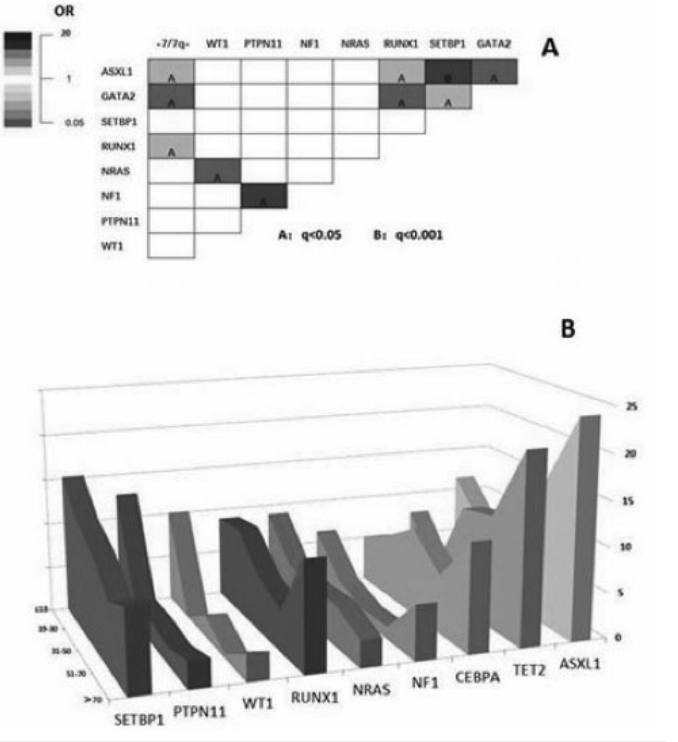


Figure 1.

Results: We found 208 mutations in 54 distinct genes with variant allelic frequency (VAF) from 2% to 100%. 83% patients had at least 1 gene mutation. 11 genes were mutated in more than 5% of patients, including *SETBP1* 15%, *PTPN11* 13%, *ASXL1* 10%, *WT1* 9%, *RUNX1* 8%, *NRAS* 8%, *TET2* 7%, *NF1* 7%, *CEBPA* 5%, *GATA1* 5%, *FLT3* 5%. Germline mutation of *GATA2* and *SAMD9/SAMD9L* were detected in 9% and 5% of the patients. Mutation of *DNMT3A*, *TP53*, *SF3B1*, *SRSF2* were rare (1%, 2%, 0%, 2%,). The most frequent pathways were the epigenetic modification (37%) and the RAS pathway (30%). In univariate analysis, somatic mutated *SETBP1*, *ASXL1*, *RUNX1* and deletion of chromosome 7 were significantly enriched in germline *GATA2* mutated subjects (p<0.05). And *NRAS* and *NF1* were significantly associated with *WT1* and *PTPN11* mutated subjects, respectively (Figure 1A). The multivariate analysis showed *ASXL1* was significantly enriched in *GATA2* mutated subjects, and *NRAS* and *NF1* were significantly associated with *WT1* and *PTPN11* subjects, respectively (p<0.05). According to the above results, the cohort could be divided into three subgroups, namely *GATA2/ASXL1/SETBP1/RUNX1* group, the *WT1/NRAS* group and the *PTPN11/NF1* group. Using copy number-adjusted VAF, we reconstructed the clonal architecture to explore ancestral and subclonal mutations. *ASXL1*, *NRAS*, and *PTPN11* were more likely to be an ancestral mutation. The 5-year overall survival (OS) was 57%. For patients with and without HSCT, the 5-year OS was 92% and 40% (p<0.001). Patients with deletion of chromosome 7, mutation of *PTPN11* and *SETBP1* tend to have worse OS. However, for patients accepted HSCT, there was no significant association between the genetic mutation and OS. In adult patients from our institution, the most common mutated genes were spliceosome and epigenetic associated genes. Pediatric patients had higher VAF than adult patients in *SETBP1*, *PTPN11*, *RUNX1*, *TET2*, *CEBPA* (the average VAF was 37.4% v.s. 20.2%; 38.3% v.s.18.6%; 38.3% v.s.18.6%; 44.0% v.s. 22.9%; 43.7% v.s. 17.0%; 36.2% v.s. 16.2%, p<0.001).We also found that *SETBP1*, *PTPN11*, *WT1*, *FLT3* more frequently occurred in pediatric and young adult patients, while, *TET2* and *ASXL1* were more likely existing in patients more than 70 years old (Figure 1B).
Summary/Conclusion: In this study, we found that mutations frequent encountered in adult MDS and in age-related clonal hematopoiesis were

rare in children. We also verified that germline mutations like *GATA2* and *SAMD9/SAMD9L* are common in pediatric patients. Somatic mutations in *SETBP1*, *ASXL1*, *RUNX1*, *WT1* and the RAS oncogenes define the genomic landscape of the pediatric MDS. Collectively, our results help define the mutational landscape in MDS in children.

S880

RAS PATHWAY HYPERACTIVATION IS ASSOCIATED WITH INFERIOR SURVIVAL IN PATIENTS WITH CHRONIC MYELOMONOCYTIC LEUKEMIA

K. Geissler[1,*], A. Barna[2], E. Jäger[3], M. Gurbisz[3], T. Alendar[1], E. Ljubuncic[1], B. Borjan[4], R. Jilch[5], T. Nösslinger[6], M. Pfeilstöcker[6], H. Tüchler[6], R. Reisner[6], T. Sliwa[6], F. Keil[6], C. Geissler[6], P. Bettelheim[7], S. Machherndl-Spandl[7], O. Zach[7], A. Weltermann[7], S. Burgstaller[8], J. Thaler[8], A. Zebisch[9], H. Sill[9], R. Stauder[10], R. Kusec[11], E. Ulsperger[12], B. Schneeweiss[13], J. Berger[14], L. Öhler[15], U. Germing[16], W. R. Sperr[17], P. Knöbl[17], I. Schwarzinger[3], G. Hörmann[3], U. Jäger[17], P. Valent[17]

[1]5th Medical Department with Oncology, Hospital Hietzing, Vienna, [2]Austrian Red Cross, Blood Transfusion Service for Upper Austria , Linz, [3]Department of Laboratory Medicine, Medical University of Vienna, Vienna, [4]Internal Medicine V, Hematology and Oncology, Labor für Tumorbiologie und Angiogenese, Medical University of Innsbruck, Innsbruck, [5]Department of Laboratory Medicine, Hospital Hietzing, [6]3rd Medical Department, Hospital Hanusch, Vienna, [7]1st Medical Department with Hematology, Stem Cell Transplantation, Hemostasis and Medical Oncology, Elisabethinen Hospital, Linz, [8]Department of Internal Medicine IV, Hospital Wels-Grieskirchen, Wels, [9]Department of Hematology, Medical University of Graz, Graz, [10]Internal Medicine V, Hematology and Oncology, Medical University of Innsbruck, Innsbruck, Austria, [11]University Hospital Dubrava, School of Medicine, University of Zagreb, Zagreb, Croatia, [12]Department of Internal Medicine, Country Hospital Horn, Horn, [13]Department of Internal Medicine, Hospital Kirchdorf, Kirchdorf, [14]Department of Internal Medicine, Hospital Schwarzach, Schwarzach, [15]Department of Internal Medicine/Oncology, Hospital St.Josef, Vienna, Austria, [16]Department of Hematology, Oncology, and Clinical Immunology, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany, [17]Division of Hematology and Hemostaseology, Department of Internal Medicine I, Medical University of Vienna, Vienna, Austria

Background: Although patients with chronic myelomonocytic leukemia (CMML) have surprisingly diverse genomic alterations, these events tend to occur in a limited number of pathways. The prognostic impact of pathway activation in CMML is poorly investigated. Recently we were able to show that the spontaneous *in vitro* growth of myeloid colonies (CFU-GM) may be a useful functional parameter of RAS pathway activation (Geissler K et al, Leukemia 2016).
Aims: To investigate the prognostic impact of RAS pathway activation in patients with CMML.
Methods: In this study we analyzed CMML patient samples which were collected in the "Austrian Biodatabase for CMML" (ABCMML) with regard to the presence of molecular aberrations in RASopathy genes by targeted next generation sequencing (NGS) and the presence of high spontaneous myeloid colony formation using semisolid *in vitro* cultures as described previously (Geissler K et al, J Exp Med 1996). From 225 CMML patients 288 samples (BMMNC or PBMNC) were analyzed by NGS and 207 samples (PBMNC) were used for *in vitro* cultures. Mutations with a VAF ≥5% were considered as positive in this study and autonomous CFU-GM formation ≥100/10^5 PBMNC was considered as high colony growth. Survival analysis was calculated from the sampling date. Molecular and functional data were correlated with patient survival using Cox regression analysis.
Results: Figure 1a shows the Kaplan-Meier plots, hazard ratios and p-values of the prognostic impact of RASopathy gene mutations including NRAS (23.3%), KRAS (11.6%), CBL (16.5%), NF1 (10.2%), and PTPN11 (8.0%), respectively. If the presence of a mutation in at least one of the RASopathy genes was used as a composite molecular parameter (55.5%) the prognostic impact was most significant with a p value = 0.00006. The prognostic impact of RAS pathway activation could be also demonstrated at a functional level. As shown in Figure 1b high autonomous colony formation (CFU-GM ≥100/10^5 PBMNC; 27.0%) was significantly associated with inferior survival (p <0.00001).
Summary/Conclusion: Our data show that RAS pathway hyperactivation, which was demonstrated at the molecular and at the functional level, is associated with inferior survival in patients with CMML. These data may be important for better understanding the pathogenesis of CMML, for improving risk stratification of individual patients and for the design of targeted treatment concepts.

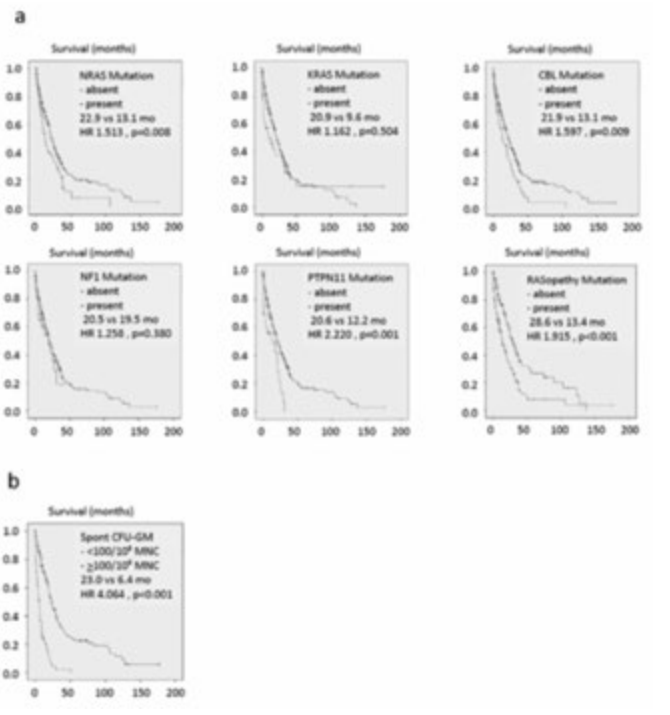


Figure 1.

Myeloproliferative neoplasms – Biology

S881

A NOVEL INHIBITORY MUTATION FOR JAK2 V617F TARGETING THE AROMATIC INTERACTION AROUND F617 RESTORES NORMAL FUNCTION FOR MOST OF JAK2-UTILIZING CYTOKINE RECEPTORS EXCEPT IFNGR

E. Leroy[1,2,*], A. Dusa[1,2], C. Pecquet[1,2], D. Colau[1,2], S.N. Constantinescu[1,2]

[1]Ludwig Cancer Research, [2]Université catholique de Louvain, Brussels, Belgium

Background: Dysfunction of JAK2 signaling is associated with hematopoietic malignancies. The JAK2 V617F mutation, which constitutively activates JAK2, is found in over 95% of Polycythemia Vera patients. JAK2 has a pivotal physiologic role in triggering several signaling pathways by binding to several cytokine receptors; therefore specific targeting of the mutant protein is absolutely required. Currently, no drug can discriminate between the mutant and the normal protein in myeloproliferative neoplasms. We have previously shown that an aromatic interaction between F617 and F594/F595 of pseudokinase domain helix C is required for constitutive signaling of JAK2 V617F, which constitutes the first molecular step in triggering hyperactivation in JAK2.
Aims: We aimed to find novel inhibitory strategies that directly destabilize the initial molecular step in V617F-mediated activation, namely the aromatic stacking between F617 and F594/F595 that only exist in the mutant conformation. We also aim to assess these approaches on the homologous JAK1 V658F, associated with ALL, and in the context of distinct cytokine receptors.
Methods: Via structure-guided mutagenesis, we assessed the role of key residues around the F617 aromatic interaction and used a combination of transcriptional activity luciferase-based assays, biochemical assays and cellular growth to measure the activity of JAKs in reconstituted JAK2 and JAK1 deficient cells.
Results: We created several aromatic mutations (S591F, A597F, A598F, S599F, V615F) strategically localizing around this aromatic interaction. We show that the specific mutation A598F preferentially inhibits JAK2 V617F over JAK2 WT, and without altering the normal function of the protein. Of importance, the inhibitory mutation A598F suppressed the hyperactivity of JAK2 V617F and other disease-associated JAK2 mutants such as JAK2 T875N and R683G, but not the JAK2 exon 12 mutant K539L. We found that the mutation A598F and the homologous A639F can inhibit basal JAK2 V617F and JAK1 V658F, respectively, in the context of distinct cytokine receptors, such as IL2-R, IL9-R, IFNGR and type I IFN receptor (IFNAR). Surprisingly, we found that the A598F mutation, as well as several other inhibitory mutations for JAK2 V617F, when in the background of the wild-type protein, drastically altered the cytokine responsiveness of the IFNGR, while it preserved normal cytokine response for EpoR, IL2-R, IL9R and IFNAR. This was even more compelling for JAK1 signaling with IFNGR; when these homologous inhibitory mutations were present in JAK1, they completely blocked the IFNγ signal transduction. Thus, it appears that the region of the JH2 helix C, which is required for JAK2 V617F and JAK1 V658F hyperactivation mechanism, is also crucial in specifically relaying the activation by cytokine in the heterodimeric IFNGR complex. Finally, we also show an unexpectedly low effect of JAK2 V617F on IFNGR complex compared to other cytokine receptors that physiologically utilize JAK2 (Figure 1).

Left, the pseudokinase domain (JH2) αC of JAK2 V617F is shown (PDB: 4FVR) with the three phenylalanine residues (pink) involved in the aromatic interaction. Right, modeling of substitution A598F (yellow) on the αC of JH2-V617F and the putative break of the aromatic interaction between F617-F595-F594.

Figure 1.

Summary/Conclusion: We identified a novel strategy to specifically inhibit JAK2 V617F and homologous JAK1 mutants, and we described an unexpected requirement for pseudokinase domain helix C specifically in the function of IFNGR. Inhibitory mutations localizing in the vicinity of JH2 helix aromatic interaction of JAK2 V617F restore normal function for most of JAK2-utilizing cytokine receptors except for the IFNGR.

S882

DELETION OF STAT2 INCREASES THROMBOCYTOSIS AND SURVIVAL IN A JAK2 V617F MOUSE MODEL OF MPN VIA A NON CELL-AUTONOMOUS MECHANISM

G. Vertenoeil[1,*], C. Pecquet[1], Y. Rahmani[1], J.-L. Villeval[2], W. Vainchenker[2], S.N. Constantinescu[1]

[1]Signal Transduction Unit, Ludwig Institute for Cancer Research Ltd Brussels Branch, Brussels, Belgium, [2]Hématopoïèse normale et pathologique (UMR 1170), Institut de cancérologie Gustave Roussy, Villejuif, France

Background: Myeloproliferative neoplasms are clonal hematopoietic disorders characterized by increased production of terminally differentiated myeloid cells. These diseases that encompass Polycythemia Vera (PV), Primary Myelofibrosis (PMF) and Essential Thrombocythemia (ET) are associated with drivers mutations inducing constitutive activation of JAK2/STAT signaling pathway.
The most prevalent driver mutation is the *JAK2*V617F mutation. During the last years, murine models of *JAK2*V617F-induced disease were developed and recapitulate the disease. These models have increased the understanding of the pathogenesis and have provided tools for preclinical testing of new therapies. Among those, interferon (IFN) alpha treatment is widely used in patients and induces significant hematologic and molecular responses. In mouse models, it was shown to target the mutated clone but the mechanism remains unknown.
Aims: STAT2 activation and formation of ISGF3 complex is the hallmark of IFN alpha signaling pathway, while STAT1 homodimer formation is shared by the signaling of many other cytokines and STATs. Thus, we wanted to assess the role of STAT2 in MPN phenotype induced by *JAK2* V617F knock-in and to evaluate the role of STAT2 in normal hematopoiesis.
Methods: We crossed our previously described *JAK2*V617F knock-in mice (based on cre-loxP system, with the use of the vav promoter) with STAT2 knock out mice to obtain $STAT2^{-/-}JAK2^{V617F}$ mice.
Results: The heterozygous KI JAK2V617F homozygous KO STAT2 mice exhibit a stronger myeloproliferative phenotype- regarding the blood counts, especially on the megakaryocyte lineage. However, mice exhibited a better survival compared to KI JAK2V617F mice, which was surprising, given the very high platelet and granulocyte levels. Myelofibrosis was apparently reduced in mice that do not express STAT2. The study of megakaryopoeisis in $STAT2^{-/-}JAK2^{V617F}$ mice compared to the $STAT2^{+/+}JAK2^{V617F}$ mice showed no significant increases in CFU-Mk and CD41+CD42+ cell numbers, suggesting that STAT2 deficiency targets the latter stages of megakaryopoeisis. To test whether this late effect on MK differentiation was due to an intrinsic or extrinsic effect, we performed bone marrow reconstitution assays. These experiments showed no differences in blood counts between mice reconstituted with $STAT2^{+/+}$ $JAK2^{V617F}$ and those reconstituted with $STAT2^{KO/KO}$ $JAK2^{V617F}$. Thus, the observed impact of STAT2 KO is due to the absence of STAT2 in non-hematopoietic cells. Survival also did not differ between the two types of reconstituted mice and was improved compared to the KI *JAK2*V617F mice resulting after the vav-cre crossing. The latter result suggests that mortality related to *JAK2*V617F in our knock-in model is probably related to its expression in endothelial cells (cre expression being under the control of vav promoter).
We will further use these murine models to assess the impact of STAT2 in interferon alpha response via competitive bone marrow reconstitution.
Summary/Conclusion: We report that in the vav-dependent *JAK2*V617F knock-in mouse model, the knock-out of STAT2 protects against myelofibrosis, increases survival, while leading to an increased thrombocytosis. These effects were extrinsic to the hematopoietic system. This raises the possibility that administration of type I IFN, in IFN-resistant patients, may enhance myelofibrosis by activation of STAT2 in non-hematopoietic cells and points to a potential extrinsic pro-inflammatory role of STAT2.

S883

A HISTIDINE PATCH IN THE N-DOMAIN OF MUTANT CALRETICULIN IS REQUIRED FOR MPN PATHOGENESIS

J. Rivera[1,*], A. Baral[1], R. Smyth[1], R. Mehta[1], E. Chen[1]

[1]Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom

Background: Recurrent somatic mutations in exon 9 of the calreticulin (CALR) gene are found in a majority of JAK2-unmutated myeloproliferative neoplasms (MPNs). All known mutations result in a +1-frameshift in the CALR reading frame that creates a novel C-terminal domain encoded by an alternative reading frame. We and others have previously demonstrated that mutant CALR binds to and activates the thrombopoietin receptor MPL and

confers cytokine-independent growth. Moreover, we showed that these oncogenic activities of mutant CALR are dependent on a threshold of basic amino acid residues in the mutant C-terminus. However, relatively less is known regarding the role of the N-domain of mutant CALR in oncogenic transformation.
Aims: We aim to identify key features of the N-domain of mutant CALR which are required for its oncogenic activity.
Methods: Mutations in the FLAG-tagged human CALR cDNA harbouring a "type I" del52 mutation ($CALR^{MUT}$) were introduced by site-directed mutagenesis. Transforming activity of $CALR^{MUT}$ variants was assessed based on their ability to confer cytokine-independence to Ba/F3 cell line expressing human MPL (Ba/F3-MPL). Interaction between $CALR^{MUT}$ variants and MPL was assessed by FLAG-pulldown experiments.
Results: We previously demonstrated that the lectin binding motif of $CALR^{MUT}$ was essential for oncogenic activity. The crystal structure of the CALR globular domain reveals that the carbohydrate binding pocket is proximal to a patch of histidine (His) residues which are required to coordinate Zn^{2+} and mediate regional domain structure of wild-type CALR. Here, we undertook a systematic mutagenesis screen to test the role of 3 His residues (His^{82}, His^{128} and His^{153}) and their effects on Zn^{2+} homeostasis in $CALR^{MUT}$ oncogenic activity. We observed that $CALR^{MUT}$ harbouring of any single His residue retained the ability to confer cytokine-independence, bind to MPL and activate Stat3/5. In contrast, $CALR^{MUT}$ harbouring loss of His^{82} in combination with either His^{128} or His^{153} (but not His^{25} which resides on the opposite face of the CALR N-domain) were compromised in their capacity to confer cytokine independence and bind MPL, suggesting that His^{82} needs to cooperate with other nearby Zn^{2+}-coordinating His residues on the carbohydrate binding face of $CALR^{MUT}$to facilitate oncogenic activity. The stability of all $CALR^{MUT}$ His variants exhibited comparable stability and folding *in vitro*, suggesting these changes in $CALR^{MUT}$ activity likely reflect a disruption of a critical domain within CALR rather than affecting protein stability. Finally, given the role of the His residues in wild-type CALR in regulating Zn^{2+} binding, we tested that $CALR^{MUT}$ activity may be affected by intracellular Zn^{2+} levels. We used CRISPRi to repress expression of metallothionein 1a (MT1a) which is required for Zn^{2+} uptake, and observed that MT1a depletion rendered CALR mutant cells more sensitive to the JAK inhibitor ruxolitinib. These data suggest that Zn^{2+} homeostasis is able to regulate MPN pathogenesis.
Summary/Conclusion: We show that Zn^{2+} binding by histidine residues are essential for $CALR^{MUT}$ to bind to MPL and engender transformation, and that disrupting intracellular Zn^{2+} levels has the potential to sensitise $CALR^{MUT}$-expressing cells to existing MPN therapies such as ruxolitinib.

S884

CALRETICULIN DEL52 KNOCK-IN MICE DEVELOP A DOSE-DEPENDENT THROMBOCYTOSIS AND PROGRESSION TO MYELOFIBROSIS

C. Marty[1,*], C. Benlabiod[1], L. Touchard[2], B. Panneau-Schmaltz[1], L. Lamrani[1], P. Gonin[2], J.-L. Villeval[1], H. Raslova[1], S. Constantinescu[3], P. Isabelle[1], W. Vainchenker[1]

[1]Inserm UMR1170, [2]Unité Mixte de Service 3655/US23, Gustave Roussy, Villejuif, France, [3]Ludwig Institute, de Duve Institute, Université catholique de Louvain, Brussels, Belgium

Background: The acquisition of somatic mutations in the hematopoietic stem cell (HSC) compartment leading to the constitutive activation of the JAK-STAT signaling pathway drives the development of myeloproliferative neoplasms (MPNs). Besides *JAK2*V617F, recurrent genetic abnormalities in the exon 9 of *calreticulin* (*CALR*) gene are the second most frequent driver mutations found in essential thrombocythemia (ET) and primary myelofibrosis (PMF). All CALR mutants induce a +1-bp frameshift of the same alternative reading frame and generate a common novel C-terminus tail. Retroviral models have shown that these mutants are driver of a MPN phenotype in mice dependent on the thrombopoietin receptor.
Aims: We investigated the consequences of physiological expression of one of the most frequent *CALR* mutations, the 52-bp deletion (*del52*) or type 1, in the pathophysiology of MPNs.
Methods: We generated a knock-in (KI) mouse model. As the analogous 52-bp deletion found in human patients would generate a mutated C-terminus tail with a different amino acid sequence in mice, our approach was to introduce fused mouse exons 8 and 9 framed with loxP sites and to humanized the C-terminal mutant tail in mouse exon 9. Floxed KI mice were crossed with tamoxifen-inducible Scl-$CreER^T$ (and Vav-Cre) mice to generate murine calr with the human mutated C-terminus sequence. Blood parameters and the different bone marrow and spleen hematopoietic compartments were analyzed over one year.
Results: Both heterozygous and homozygous KI *del52* mice were produced and developed a *del52* dose-dependent increase in both platelet and white blood cell counts. In contrast to heterozygous mice, ET-like phenotype in the homozygous *del52* mice progressed towards mild myelofibrosis in bone marrow (BM) but not in spleen after one year of induction. Myelofibrosis was accompanied with a decrease BM cellularity and splenomegaly. Both BM and spleen from heterozygous and homozygous mice presented megakaryocytic (MK) hyperplasia revealed using von willebrand factor staining and flow cytometry. *In vivo*, MKs achieved significantly higher ploidy in KI mice than in WT littermates (modal ploidy of 32N compared to 16N in WT). In BM, MK frequency increased mostly at the expense of erythroid ($CD71^+$Ter-119^+) cells. In spleen, increased MK frequency was associated with an increased frequency of erythroid cells resulting in a decreased frequency of B ($B220^+$) and T ($CD3^+$) cells. We observed a *del52* dose-dependent increase in the frequency of immature progenitors (SLAM cells) and MK progenitors (MkP) both in BM and spleen as well as a thrombopoietin-independent growth of the MK progenitors (CFU-MKs) in BM. Finally, using competitive BM transplantation with different ratios of homozygous *del52* KI cells and WT GFP^+ cells we found that the homozygous *del52* BM cells were able to outcompete WT BM cells and induce thrombocytosis from an initial 25% competitive engraftment.
Summary/Conclusion: Altogether, these results demonstrate that CALR del52 is sufficient to dose-dependently induce a thrombocytosis progressing to myelofibrosis in KI mice, thus mimicking the natural history of MPN patients. It will offer a good *in vivo* model to investigate therapeutic approaches for *CALR*-positive MPNs or cooperation with others mutated molecules.

S885

UNEXPECTED PREVALENCES OF THE JAK2V617F AND CALR MUTATIONS IN THE GENERAL POPULATION

S. Cordua[1,*], L. Kjaer[1], V. Skov[1], C. Ellervik[23], N. Pallisgaard[4], H.C. Hasselbalch[1]

[1]Department of Hematology, Zealand University Hospital, Roskilde, [2]Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, [3]Department of Research, Production, Innovation, Zealand Region, Soroe, [4]Department of Pathology, Zealand University Hospital, Roskilde/Naestvaed, Denmark

Background: The JAK2V617F mutation (JAK2) and calreticulin mutations type 1 and 2 (CALR) are predominantly found within the chronic Philadelphia-negative myeloproliferative neoplasms (MPN). Few studies have assessed the JAK2 prevalence in the general population with very limited reports on the association with MPN over time. To our knowledge, no corresponding studies of CALR exist.
Aims: 1) to determine the prevalence of JAK2 and CALR mutations in a Danish population study 2) to assess the kinetics of the allele burden and blood cell counts over time by inclusion of mutated citizens in a follow up program.
Methods: Inclusion of participants in the general population study in Region Zealand took place during 2010-2013 (N=20,000). When included, a broad range of blood samples were collected as well as DNA to be stored in a biobank, and all participants were asked for a written consent to allow the data and DNA to be used in future research. This DNA was in our study analyzed for JAK2 and CALR by digital droplet PCR. The assays used have high accuracy and sensitivity with a detection level of allele burden at 0.01%. Part of our ongoing follow up program during 2017-2018 on citizens with mutation includes blood samples and a current allele burden for JAK2/CALR, thereby obtaining a follow up time of 5-8 years.
Results: In a preliminary mutational analysis of 9,000 individuals, 285 (3.2%) were JAK2 positive and 18 (0.2%) were CALR positive corresponding to a JAK2:CALR ratio of 16. Among the citizens with mutation, median age was 63 years (range 23-93), and 82% presented low allele burden less than 1%. These proportions were similar when looking at the JAK2- and CALR-mutated groups separately. We noticed that 40% of the citizens concurrently with their mutation had an aberrant blood cell count – leukocytosis (defined as $>8.8\times10^9$/L) being the most frequent and present in 54% of the cases.
Summary/Conclusion: To our knowledge, we are the first to determine the prevalence of JAK2 and CALR in the general population with such a sensitive method, and our preliminary results indicate that the true prevalence of JAK2 in the general population is much higher than previously reported with dominance of low allele burdens. On the contrary, we find the CALR prevalence low compared to JAK2. The difference cannot entirely be explained by the distribution of the mutations within the MPN diagnoses. The long-term perspective of this study is to reduce thrombotic and hemorrhagic events in today's pre-MPN diagnosis phase by diagnosing MPN in earlier disease stages in the future. Thus, we find the high prevalence of low allele burden and mild cytosis intriguing, and we hope that the follow up program can elucidate whether we can discriminate between early signs of MPN and clonal hematopoiesis of indeterminate potential; though these two wordings in time might turn out to be two sides of the same coin.

Platelet and bleeding disorders II

S886

A STRATEGY WITH CHAPERONE-LIKE COMPOUNDS TO RESTORE EXPRESSION OF FACTOR IX VARIANTS AFFECTED BY FREQUENT MISSENSE MUTATIONS CAUSING HEMOPHILIA B

S. Pignani[1], A. Todaro[1], M. Ferrarese[1], S. Marchi[2], S. Lombardi[1], D. Balestra[1], P. Pinton[2], F. Bernardi[1], M. Pinotti[1], A. Branchini[1,*]

[1]Department of Life Sciences and Biotechnology, [2]Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Ferrara, Italy

Background: Missense mutations, representing the most frequent gene alteration associated with human disease including coagulation factor disorders, exert the most detrimental effect by impairing protein folding and intracellular trafficking. These effects at the protein level may be counteracted by small molecules with chaperone-like activity, with a concomitant improvement of the affected intracellular processes. Although some evidence exists for missense mutations associated with disorders involving intracellular proteins, only very few attempts have been made for secreted proteins, in particular in the coagulation field. Noticeably, a correction approach with chaperone-like compounds could be of relevance for coagulation factor disorders, where even modest increases of functional protein levels could have therapeutic implications.
Aims: The aims of our study were to i) detail the altered intracellular processing of a panel of factor IX (FIX) missense variants causing severe type I Hemophilia B (HB), and ii) to rescue their intracellular processing, secretion and activity through chaperone-like compounds.
Methods: Transient and stable expression of recombinant FIX (rFIX) variants in Human Embryonic Kidney (HEK) 293 cells, and evaluation of secreted/intracellular protein levels (ELISA, Western Blotting), intracellular trafficking (immunofluorescence) and activity (coagulant assays) before and after treatment of cells with chaperone-like compounds.
Results: As model mutation we selected the *F9* p.R294Q, representing the most frequent substitution (~100 patients) associated with severe/moderate type I HB. As comparison we chose other recurrent *F9* missense mutations (p.Y115C, n=9; p.Y161C, n=5; p.Y305C, n=9; p.F424L, n=3). Transient expression studies indicated that the selected mutations severely impair rFIX secretion (0.2-0.8% of wild-type rFIX), thus recapitulating findings in HB patients (antigen levels <1%). Immunofluorescence studies on stably expressing cells revealed that, at variance from wild-type rFIX, missense variants mainly co-localized in the ER and scarcely with Golgi, thus indicating impaired intracellular trafficking. This pattern was comparable to that of the rFIX-450C variant, previously demonstrated by us to cause severe type I HB and here chosen as additional control. These observations were in accordance with the very low secreted levels observed in expression studies. Screening of a panel of chaperone-like compounds identified sodium phenylbutyrate (NaPBA) as active in improving trafficking to Golgi and in appreciably promoting secretion (from 0.3±0.1% to 1.5±0.3%) of the rFIX-294Q variant in a dose-dependent manner. Noticeably, the rFIX-294Q variant revealed a remarkable specific coagulant activity that was higher (~2.0) than that of wild-type rFIX in all treatment conditions (0.5, 1.0 and 2.0 mM NaPBA). Importantly, activity after treatment was improved in terms of shortening of coagulation times (from 80±0.1 to 62±3 seconds at 2 mM NaPBA), with increased levels (~3%) that, if achieved in patients, would approach the therapeutic threshold.
Summary/Conclusion: Altogether our data contribute to detail molecular mechanisms underlying type I HB and candidate NaPBA as a potential "personalized" option for the high number of patients affected by the frequent p.R294Q mutation. In addition, our expression platform is proposed for other missense mutations leading to severe type I Hemophilia to select those being responsive to chaperone-like compounds.

S887

NLRP3 INFLAMMSOME ACTIVATION IN PBMCS CONTROLS THE T CELL RESPONSE IN ADULT PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA

M. Hua[1,*], X. Li[1], M. Hou[1], J. Peng[1]

[1]Department of Hematology, Qilu Hospital, Shandong University, Jinan, China

Background: Immune thrombocytopenia (ITP) is an acquired autoimmune disorder, which is characterized by imbalanced adaptive immunity of T cells. NLRP3 inflammasome has recently been reported to be involved in diverse inflammatory or immune diseases. However, NLRP3 inflammasome activation in the pathophysiology of ITP remains unclear.
Aims: To explore the effects of NLRP3 inflammsome activation on the T cells in adult patients with chronic ITP.
Methods: Peripheral blood was obtained from 28 adult patients with chronic ITP. PBMCs were isolated using Ficoll density-gradient centrifugation and the CD4+ cells were purified by positive immuno-magnetic microbeads selection. The PBMCs, CD4+ or CD8+ cells were primed with LPS, then stimulated with ATP and finally cultured in plate-bound anti-human CD3 and soluble anti-human CD28 plus IL-2. After four days, the percentage of CD4+ cells and CD8+ cells, the T helper cell subsets (Th cells) of Th1, Th2, Th17 and T-reg cells, the inhibitory molecules, PD1 and CTLA4, and the CFSE-labeled cells were analyzed by flow cytometry. IFN-γ, IL-4, IL-17 and TGF-β in cultured supernatants were assayed by ELISA. The mRNA expression level of cytokines associated with Th cells (*Ifn-γ, Il-6, IL-10, Il-17*), the key transcription factors of Th cells (*T-bet, Gata3, Foxp3, Ror-γt*) and the inhibitory molecules (*Ctla-4, Btla, Tim3, Lag3, Pd1 and Vista*) was determined by Real-Time PCR.
Results: We found that NLRP3 inflammasome activation in PBMCs initiated caspase-1–dependent IL-1β secretion, and thereby weakened the Th1 cell differentiation (unstimulated control 13.3% vs. stimulation 5.3%; p=0.015), which can be restored, at least in part, by caspase-1 inhibitor Z-YVAD-FMK (Figure 1). The production of IFN-γ and the expression of *Ifn-γ*, were significantly down-regulated after NLRP3 inflammsome activation. However, the percentage of Th2 or Th17 was not significantly different between the stimulated and unstimulated groups. Meanwhile, the percentage of T-reg cells was also decreased after stimulation by LPS plus ATP in PBMCs (unstimulated control 6.67% vs. stimulation 4.39%; p=0.033). More importantly, NLRP3 inflammasome activation significantly suppressed the proliferation but didn't induce the apoptosis of CD4+ cells and CD8+ cells (Figure 2). Accordingly, the mRNA expression of inhibitory molecules (*Ctla-4, Btla, Tim3, Pd1 and Vista*) of T cells and the PD1 or CTLA4 (Figure 3) on the membrane surface of CD4+ and CD8+ cells were up-regulated after the activation of NLRP3 inflammasome in PBMCs. Furthermore, the percentage of CD8+ cells significantly decreased after NLRP3 inflammasome activation in PBMCs. However, LPS plus ATP took no effect on purified CD4+ or CD8+ cells in contrast with PBMCs.

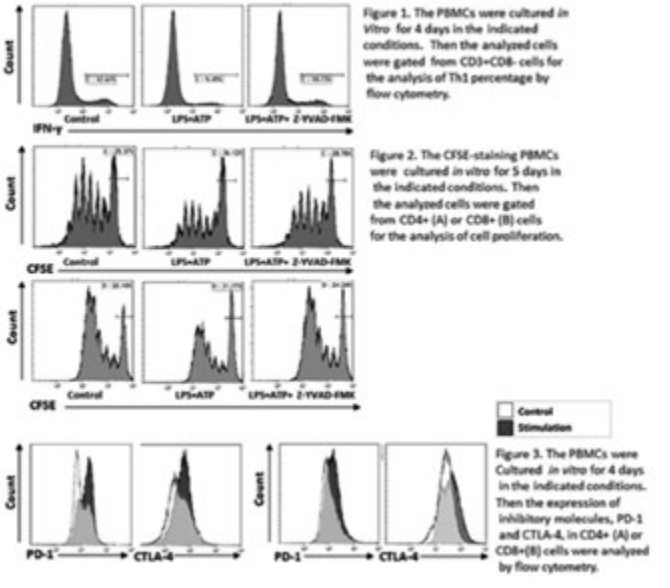


Figure 1.

Summary/Conclusion: NLRP3 inflammsome-mediated innate immune reaction may play an important role in controlling the T cell responses of adaptive immunity by the inhibitory molecules and might prevent disease progression in adult chronic ITP.

S888

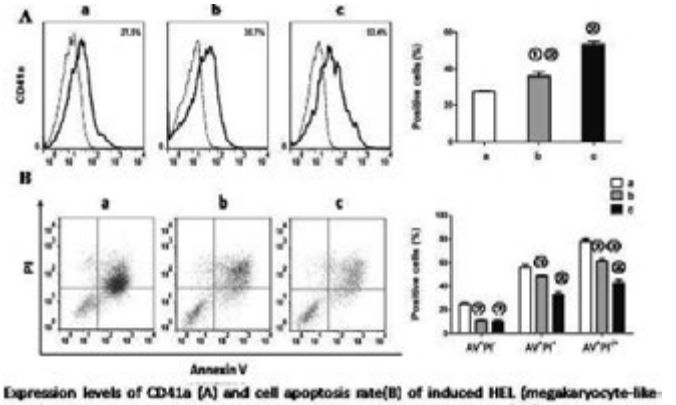
EFFECTS OF BONE MARROW MESENCHYMAL CELLS FROM IMMUNE THROMBOCYTOPENIA PATIENTS ON THE BIOLOGICAL BEHAVIORS OF MEGAKARYOCYTES

J. Wang[1], X. Li[1], T. Yin[2], J. Liu[2], X. Wang[3], L. Xu[1], A. Wang[1], H. Zhong[1,*]

[1]Department of Hematology, Ren Ji Hospital, South Campus,School of Medicine, Shanghai Jiao Tong University, [2]Department of Hematology, Ren Ji Hospital, School

of Medicine, Shanghai Jiao Tong University, [3]Department of Rheumatology, Ren Ji Hospital, South Campus,School of Medicine, Shanghai Jiao Tong University, Shanghai, China

Background: Immune thrombocytopenia(ITP) is an autoimmune disease characterized by increased platelet destruction as well as impaired production. The pathogenesis of impaired platelet production in ITP is still ill-defined. The bone marrow microenvironment plays the critical role in platelet generation. As the important component of the bone marrow microenvironment, bone marrow mesenchymal cells(BMCs) also play a critical role in regulating platelet production. BMCs can sustain MK differentiation and platelet formation through multiple mechanisms, including expressing many of the cytokines that are involved in megakaryocytic developments, as well as providing mechanical supports through adhesion molecules mediated direct contact. A series of studies have demonstrated the defective BMCs from ITP patients. However, whether the effects of BMCs in regulating megakaryopoiesis have changed in ITP is still not clear. In this study we focused on the interaction of BMCs and megakaryocytes, compared many aspects of BMCs from ITP and Normal controls, in order to deepen our understanding of the pathogenesis of ITP.
Aims: To investigate changes in the proliferation, survival and cytokines expression of BMCs derived from ITP patients, as well as to evaluate their influences on the biological behaviors of megakaryocytes.
Methods: Bone marrow samples were obtained from 7 ITP patients and 5 normal controls, and BMCs were cultivated by the whole marrow adherent method. The surface markers of BMCs and the basal cell apoptosis rate were analyzed by flow cytometry. Proliferation of BMCs were assessed by Cell Counting Kit-8 method. HEL cells were induced to undergo megakaryocytic differentiation by using nanomolar concentrations of Phorbol 12-myristate 13-acetate (PMA). The induced HEL cells were grouped and co-cultured with BMCs from different resources, followed by surface protein CD41a detection and apoptosis analysis through FCM. The expression levels of IL6 IL11 TPO SCF mRNA and protein in BMCs were detected by Real-time Fluorescent quantitative PCR and Enzyme Linked Immunosorbent Assay.
Results: BMCs from ITP patients grew progressively slowly, and cell basal apoptotic rates were higher than that of normal controls. BMCs from normal controls significantly promoted the CD41a expression as well as reduced the apoptosis rate of induced HEL(megakaryocyte-like cells) by co-culture *in vitro*. Whereas, this ability was much weaker in BMCs from ITP patients; The expression Levels of IL6, SCF mRNA and IL6 protein were significantly decreased in ITP BMCs (Figure 1).



Expression levels of CD41a (A) and cell apoptosis rate(B) of induced HEL (megakaryocyte-like cells) after co-cultured with BMCs from either ITP or normal controls(NC). a. Induced HEL cultured alone; b. Induced HEL co-cultured with ITP-BMCs; c: Induced HEL co-cultured with NC-BMCs. ①P=0.015, ②p=0.000, compared with group a; ③P=0.000, compared with group c

Figure 1. Effects of BMCs on the biological behaviours of megakaryocytes.

Summary/Conclusion: BMCs from ITP patients exhibited impaired proliferation and excessive apoptosis when cultured *in vitro*. BMCs from ITP also show defects in supporting megakaryocytic differentiation and survival under co-culture condition, which may be resulted from their deficiencies in expressing cytokines IL6 and SCF.

S889

ATRA COULD CORRECT THE IMPAIRED PROPLATELET FORMATION CAUSED BY THE TUBULIN β1 SINGLE NUCLEOTIDE POLYMORPHISM R307H IN CORTICOSTEROID-RESISTANT ITP PATIENTS

C.-C. Wang[1,2,3,*], Y.-N. Wang[1,2,3], Y. He[1,2,3], Q. Chen[1,2,3], X. Liu[1,2,3], J.-M. Zhang[1,2,3], J. Xue[1,2,3], H.-X. Fu[1,2,3], X.-S. Zhao[1,2,3], X.-Y. Zhao[1,2,3], Y. Kong[1,2,3], Y.-J. Chang[1,2,3], K.-Y. Liu[1,2,3], X.-J. Huang[1,2,3], X.-H. Zhang[1,2,3]

[1]Peking University Institute of Hematology, Peking University People's Hospital, [2]Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, [3]Collaborative Innovation Center of Hematology, Peking University, Beijing, China

Background: Primary immune thrombocytopenia (ITP) is an autoimmune disorder with reduced platelet production, and increased platelet destruction is considered to be the main pathogenic mechanism. However, the mechanism of corticosteroid-resistant ITP is still unclear. It is reported that single nucleotide polymorphisms (SNPs) in tubulin β1 in patients with ITP is related to the response of corticosteroid therapy (Paul A. Basciano et. al. 2013) and proplatelet formation (PPF) (Shinji Kunishima *et al.* 2013). Our study revealed that all-trans retinoic acid (ATRA), which has demonstrated to be a promising candidate for corticosteroid-resistant/relapsed ITP patients in our previous study (Feng FE *et al.* 2017), could correct the abnormal function of tubulin β1 in ITP patients with SNP and promote megakaryocyte maturation and PPF.
Aims: To explore the effect of ATRA on megakaryocyte maturation and PPF in ITP patients with tubulin β1 SNP R307H.
Methods: Thirty corticosteroid-resistant/relapse ITP patients and 10 healthy donors were enrolled in this study. MKs were isolated by cell sorting from bone marrow samples obtained by aspiration from the posterior iliac crest, and bone marrow samples were collected again after ITP patients received a 16-week oral ATRA (10 mg twice daily) therapy. The identification of R307H substitution in TUBB1 (the gene encoding tubulin β1) was performed by polymerase chain reaction (PCR) and Sanger sequencing. CD34+ cells were isolated from bone marrow samples by cell sorting for *in vitro* culture with TPO, and on day 8, differentiated MKs were enriched. Western blot (WB) was performed to verify the expression level of tubulin β1 in differentiated MKs. Immunofluorescence staining was performed for tubulin β1 and observed by inverted confocal fluorescence microscopy. In the ITP group, ATRA was added to the differentiating culture process. Proliferation, GPIIb/IIIa–GPIbIX expression, ploidy distribution and PPF were observed in the different groups.
Results: The allelic frequencies between a group of 30 ITP patients and the healthy control group showed no difference. The protein levels of tubulin β1 in cultured differentiated MKs from ITP patients with homozygote SNPs were significantly decreased compared with patients with wild type tubulin β1, and heterozygote patients also showed a similar trend, but no significant difference. The *in vitro* culture-differentiated MKs from ITP patients with homozygote SNPs displayed normal proliferation but decreased GPIIb/IIIa–GPIbIX expression and ploidy distribution, as well as impaired and decreased PPF compared with the control groups and patients with WT tubulin β1. Immunofluorescence results of the differentiated MKs showed that the expression of tubulin β1 and the distribution of microtubules were abnormal both in MKs and in pro-platelets from ITP patients with homozygote SNPs. In the homozygote SNP group, when ATRA was added to the culture, tubulin β1 expression was recovered, and as a result, the GPIIb/IIIa–GPIbIX expression, ploidy distribution and PPF was increased. Additionally, after receiving ATRA therapy, the expression and distribution of tubulin β1 in megakaryocytes and proplatelets returned to nearly normal compared with those before therapy.
Summary/Conclusion: The megakaryocytes of ITP patients with homozygote SNPs showed a decreased expression and abnormal distribution, which might lead to its dysmaturity and impaired PPF. ATRA might correct the function of impaired tubulin β1 and promote megakaryocyte maturity and PPF.

S890

VERY LOW DOSE PROPHYLAXIS IN CHILDREN WITH HAEMOPHILIA A: A RURAL INDIAN EXPERIENCE

D. De[1,*], S.S. Datta[1], B. Basak[2], S. Halder[2], P. Chatterjee[2]

[1]Department of Hematology, [2]Department of Pathology, The Mission Hospital, Kolkata, India

Background: Prophylactic therapy with factor concentrates administered 2 to 3 times a week on a regular basis is the gold standard treatment for haemophilia[1]. In countries with limited resources, initiating prophylaxis

with very low dose and at reduced frequency could be recommended.
Aims: We report our single centre experience from a Hemophilia Treatment Centre at rural eastern India with very low dose once weekly prophylaxis regimen in children with haemophilia A.
Methods: It was an open label prospective trial using historical data as control. Children with severe haemophilia A, below the age of 15 years, were included. The patients received very low dose prophylaxis with recombinant F-VIII, at the dose of 15 IU/kg once weekly. The dose was approximated to nearest 250 IU or 500 IU F-VIII, for optimal use of Factor vials. The Anti Hemophilic Factors (AHF) were provided by Hemophilia Federation of India on compassionate ground free of cost. The patients were followed up for 6 months (June 2017- November 2017) and clinical and joint evaluation parameters were compared with historical data in previous 6 month period. APTT-based Inhibitor screening was done at study entry and then at the end of 6 month.
Results: Altogether 18 patients were included in the study, but 3 patients were excluded from calculation, as they discontinued the prophylaxis because of logistics issues. The mean age of patients was 9.47 years (Range 3-15 years) and mean body weight were 28 Kg, (range 12-40 Kg). All the patients were previously exposed to AHF infusion as On Demand Therapy. There was significant reduction of ABR during prophylaxis therapy (23.73 before prophylaxis vs 1.87 after prophylaxis, P value <0.001). Similarly, no. of days of hospitalisation for rescue therapy was also reduced significantly. No patient developed inhibitor during the study period. The Social and functional parameters like PedQL score and days of school absenteeism also reduced significantly. The AHF use was about 1235 IU/Kg/year during on demand therapy, which came down to 821 IU/Kg/year during prophylaxis (Figure 1).

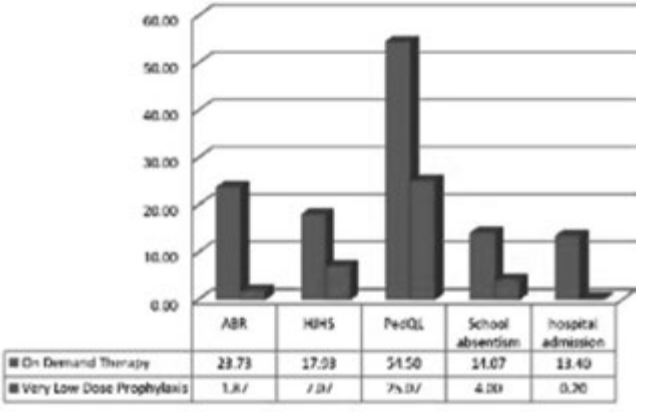


Figure 1.

Summary/Conclusion: Prophylaxis in haemophilia is a big challenge in resource constrained developing countries. Also the limited access to haemophilia treatment centre in rural India, difficulty in using public transport facility by haemophiliacs and lack of knowledge about self infusion and home based therapy makes prophylaxis program more difficult. The very low dose prophylaxis regimen of 15 units/kg once a week AHF was quite convenient and acceptable to the children with haemophilia. It is effective in preventing joint bleeds and overall bleeds and also is cost effective. Significantly less emergency visits, lesser days of school absenteeism and improved quality of life can be achieved in children with very low dose prophylaxis.

Iron: From basic science to clinical application

S891

INVESTIGATING THE ROLE OF NCOA4 IN ACUTE AND CHRONIC IRON RELEASE FROM STORES

A. Nai[1,2,*], M. R. Lidonnici[3], G. Federico[4], M. Pettinato[12], I. Artuso[15], G. Mandelli[3], G. Ferrari[23], C. Camaschella[1], F. Carlomagno[4], L. Silvestri[12]

[1]Division of Genetics and Cell Biology, San Raffaele Scientific Institute, [2]Vita-Salute San Raffaele University, [3]SR-TIGET – San Raffaele Telethon Institute for Gene Therapy, San Raffaele Scientific Institute, Milan, [4]CNR Endocrinology and Experimental Oncology Institute-Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, [5]Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy

Background: Nuclear Receptor Coactivator 4 (NCOA4) has been recently identified as the cargo receptor that promotes the autophagic ferritin degradation in conditions of iron deficiency (ID). Depletion of *NCOA4* increases ferritin aggregates and C57Bl/6 *Ncoa4-ko* mice show mild microcytic anemia and increased susceptibility to ID. Previously published *in vitro* and *ex vivo* data suggest a direct role of NCOA4 in erythroid differentiation and hemoglobinization.
Aims: We will address the role of NCOA4 in response to chronic and acute iron demand and we will identify the cell type mostly affected by *Ncoa4* deficiency *in vivo.*
Methods: The iron response has been studied in Sv129/J *Ncoa4-ko* and wt mice that were maintained in iron-balance (IB) or ID diet for 6 months. Complete blood count was periodically measured and the iron phenotype was evaluated at sacrifice. Ferritin degradation was also assessed *ex vivo* in hepatocytes and BM macrophages isolated from wt and *Ncoa4-ko* mice and treated with desferrioxamine. Moreover, in order to unambiguously elucidate the function of NCOA4, we lethally irradiated *Ncoa4-ko* mice for transplantation of bone marrow cells (BMT) from wt and *Ncoa4-ko* mice, and manipulated the dietary iron content of the transplanted animals. Two months after BMT, animals were fed an ID diet for 3 months and subsequently analyzed for hematological parameters and iron content.
Results: Sv129/J *Ncoa4-ko* mice, that are particularly enriched in iron content, showed microcytosis without anemia. The ID diet induced slight changes in hemoglobin (Hb) levels in wt mice, but severe microcytic anemia with very low transferrin saturation in *Ncoa4-ko* mice. Hepatic (LIC) and splenic (SIC) iron content were reduced by ID in both genotypes. On the other hand, acute iron chelation *ex vivo* induced progressive ferritin degradation in wt but not in *Ncoa4-ko* hepatocytes and macrophages.
In the transplantation model, red blood cells count and Hb levels were comparable between mice transplanted with wt and *Ncoa4-ko* BM at 2 months after BMT, while mean corpuscular volume was higher in the former. The ID diet caused a more severe anemia in mice transplanted with *Ncoa4-ko* cells than in animals transplanted with wt cells. High SIC and ferritin levels were observed in mice transplanted with *Ncoa4-ko* cells, while LIC was unchanged.
Summary/Conclusion: Our results demonstrate that Sv129/J *Ncoa4*-ko mice, although not anemic in basal conditions, are more susceptible to iron-deficiency anemia. Their hematological phenotype is due to a defect of BM-derived cells. *Ncoa4-ko* BM cells are able to reconstitute almost normal Hb levels in transplanted animals and this, accompanied by a normal erythropoiesis, indicates that *in vivo Ncoa4* deficiency has a limited, if any, effect on the erythroid lineage differentiation. On the contrary, the reduced iron recycling capacity of *Ncoa4-ko* macrophages *in vivo*, confirmed by *ex vivo* experiments, supports the role of NCOA4 in mobilizing the iron stores. Still, a prolonged ID diet leads to a substantial splenic and hepatic iron mobilization in *Ncoa4-ko* mice, and this suggests that other mechanisms of ferritin degradation are likely activated in chronic conditions. Therefore, to verify the role played by NCOA4 in response to acute increased iron demand *in vivo*, *Ncoa4-ko* mice are under study, exploiting an EPO based treatment protocol that induces rapid changes of iron bound transferrin, to analyse their ability to restore normal circulating iron levels after an acute erythropoietic expansion.

S892

LIVER IRON CONTENT DETERMINES HEPCIDIN LEVELS

S. Altamura[1,2,*], S. Colucci[1,2], J. Schmidt[1,2], K. Müdder[1], J. Neves[1], M. Hentze[2,3], M. Muckenthaler[1,2]

[1]Department of Pediatric Hematology, Oncology and Immunology, University of Hei-

delberg, [2]MMPU - Molecular Medicine Partnership Unit, [3]EMBL - European Molecular Biology Laboratory, Heidelberg, Germany

Background: The hepcidin-ferroportin regulatory system is crucial to maintain physiological systemic iron levels. Ferroportin is the only known iron exporter and mediates the release of iron from reticuloendothelial macrophages and from duodenal enterocytes. Mutations that affect ferroportin protein stability or its targeting to the cell surface cause iron retention in iron exporting cells and reduced systemic iron levels. Gain of function ferroportin mutations that cause resistance to hepcidin binding generate systemic iron overload. Hepcidin is a small hormone produced by the liver in response to iron levels. Hepcidin binds to and degrades ferroportin thereby reducing the amount of iron released into the blood stream. Recent evidences have shown that hepcidin is activated by the bone morphogenetic proteins BMP2 and BMP6, predominantly expressed by liver sinusoidal endothelial cells (LSECs). How iron levels are sensed by the liver to control BMP2 and BMP6 expression and the consequent hepcidin production is still unknown.
Aims: Study the impact of the haploinsufficiency of ferroportin on iron homeostasis and identify how iron levels are sensed by the liver to control BMP2 and BMP6 production and hepcidin synthesis.
Methods: Slc40a1(wt/trp) mice have been generated via homologous recombination by inserting into the 6th intron of the *Slc40a1* locus a promoterless bGEO selection marker cassette containing an upstream splicing acceptor. LSEC have been isolated from the liver via magnetic sorting using the CD146 magnetic beads. Gene expression analysis has been performed using SYBR-green qRT-PCR.
Results: We have generated and analysed mice with a heterozygous loss of the ferroportin allele (Slc40a1$^{wt/trp}$). In this mouse model we observed normal haematological parameters and plasma iron levels, while liver iron content is strongly decreased. In addition, plasma hepcidin levels are dramatically reduced suggesting a response to hepatic iron deficiency. Analysis of sinusoidal endothelial cells (LSECs) from the liver of Slc40a1$^{wt/trp}$ mice revealed a strong decrease of BMP6 levels compared to wild-type mice. Consistently, hepatic SMAD1/5/8 phosphorylation is decreased in Slc40a1$^{wt/trp}$ mice explaining low hepcidin expression. Reduced hepcidin in Slc40a1$^{wt/trp}$mice explains similar ferroportin protein expression in duodenal enterocytes and splenic macrophages compared to wild-type mice. As a consequence plasma iron is maintained within the physiological range to satisfy the demand for erythropoiesis.
Summary/Conclusion: Our results show that the hepatic iron content dominates over plasma iron levels in regulating BMP6 expression in LSECs and hepcidin expression in hepatocytes. Furthermore, low hepcidin expression can compensate for the lack of one ferroportin allele. This may explain why patients with heterozygous ferroportin null mutations have not been yet identified.

S893

DEVELOPMENT OF A GALNAC SIRNA CONJUGATE TARGETING TMPRSS6 FOR THE TREATMENT OF IRON OVERLOAD DISORDERS, SUCH AS B-THALASSEMIA

U. Schaeper[1,*], S. Altamura[2], S. Dames[1], S. Schubert[1], C. Frauendorf[3], M. Aleku[4], M. Muckenthaler[2], G. Grigoriadis[5], J. Vadolas[5], U. Zügel[1]

[1]Drug Discovery, Silence Therapeutics GmbH, Berlin, [2]Molecular Medicine, University of Heidelberg, Heidelberg, [3]Chemistry Manufacturing Controls, [4]Non Clinical Development, Silence Therapeutics GmbH, Berlin, Germany, [5]Centre for Cancer Research, Hudson Institute of Medical Research, Melbourne, Australia

Background: Hyperferremia is a major health problem in hemochromatosis, thalassemia and sickle cell anemia. Severe symptoms of excess iron can lead to liver cirrhosis, diabetes, and heart failure. In hereditary hemochromatosis, gene mutations in the hepcidin-ferroportin axis controlling iron homeostasis, lead to hepatic iron overload. In thalassemia major patients, iron overload develops as a consequence of frequent blood transfusions to ameliorate severe anemia. In addition, iron overload develops in patients with b-thalassemia intermedia, due to gastrointestinal iron hyperabsorption. Thus, in both indications iron overload is caused by dysregulation or dysfunction of the hepcidin-ferroportin axis. Importantly, expression of the peptide hormone hepcidin, which is the key player in iron homeostasis, is abnormally low and unable to block ferroportin-mediated intestinal iron absorption. Hepcidin is predominantly produced by the liver and is induced by activation of the BMP/SMAD signalling pathway. Hepcidin is furthermore under the negative control of the transmembrane protease matriptase 2, encoded by the TMPRSS6 gene. RNA interference is a powerful technology for inhibiting the expression of disease associated targets. Silence Therapeutics has developed siRNA conjugate technology for the selective inhibition of target gene expression in the liver. siRNAs conjugated to a GalNAc ligand bind efficiently to the asialoglycoprotein (ASGP) receptor expressed predominantly by hepatocytes. Thereby they provide a safe and efficient delivery technology for targeting disease modifying genes in the liver for therapeutic purposes. Here we report on the identification and pharmacological evaluation of SLN124, a GalNAc-siRNA conjugate targeting TMPRSS6 expression, for the treatment of iron overload disorders, such as b-thalassemia. Moreover, this study demonstrates that our GalNAc-conjugated siRNAs provide a safe and efficient delivery system and hold great promise for the targeted knock-down of modifier gene expression in the liver for therapy.
Aims: Identification and characterization of SLN124, a GalNAc-siRNA conjugate targeting TMPRSS6 expression, for treatment of b-thalassemia and iron overload.
Methods: Lead identification and pharmacological characterization of siRNA conjugates in cell based assays, in rodent disease models and in non-human primates.
Results: We will present the identification and pharmacological characterization of SLN124, the GalNAc-siRNA conjugate targeting TMPRSS6 expression. A single subcutaneous administration is sufficient to achieve significant modulation of target gene expression in mice and in non-human primates over several weeks. SLN124 reduces systemic iron levels, transferrin saturation and tissue iron levels in rodent models for hereditary hemochromatosis. In addition, we show the therapeutic efficacy of SLN124 in an animal model for b-thalassemia intermedia on erythropoiesis and anemia. Safety and tolerability are assessed in relevant preclinical models. SLN124 shows dose-dependent and long-lasting effects on target expression as well as on modulation of iron stores and normalization of erythropoiesis in b-thalassemia mice and was well tolerated.
Summary/Conclusion: GalNAc-siRNA conjugate SLN124 is a promising candidate for treatment of iron overload and anemia in b-thalassemia and related disorders. SLN124 is in preclinical development and we plan to enter clinical development by end of 2018.

S894

A PHASE 1, OPEN-LABEL STUDY TO DETERMINE THE SAFETY, TOLERABILITY, AND PHARMACOKINETICS OF ESCALATING DOSES OF LJPC-401 (SYNTHETIC HUMAN HEPCIDIN) IN PATIENTS WITH IRON OVERLOAD

A. Lal[1], A. Piga[2], V. Viprakasit[3,*], J. Maynard[4], A. Kattamis[5], D. Yaeger[6], B. Byrnes[6], L. Chawla[6], G. Tidmarsh[6]

[1]University of California San Francisco Benioff Children's Hospital, Oakland, United States, [2]Università degli Studi di Torino, Torino, Italy, [3]Siriraj-Thalassemia Center, Bangkok, Thailand, [4]CTI Clinical Research Center, Cincinnati, United States, [5]National and Kapodistrian University of Athens, Athens, Greece, [6]La Jolla Pharmaceutical Company, San Diego, United States

Background: Iron overload is a significant complication in patients with hereditary hemolytic anemia (esp. sickle cell disease and thalassemia) and hereditary hemochromatosis. LJPC-401, a synthetic form of endogenous human hepcidin, is being developed as a therapeutic intervention for iron overload in these conditions.
Aims: To determine the safety, tolerability, and pharmacokinetics (PK) and pharmacodynamics (PD) of escalating doses of LJPC-401 in patients at risk for iron overload.
Methods: This was a phase 1, multicenter, open-label, dose-escalation study. Patients (aged 18-85 years) were eligible for enrollment if they had one of the following: transfusion-dependent anemia (with blood transfusion [≥2 units in the past 2 months or 4 units in the past 6 months]; iron chelation therapy in the past 6 months; serum ferritin >1000 g/L, or hemochromatosis (requiring phlebotomy at least once every 2 months or iron chelation therapy in the past 6 months). Patients were assigned to receive LJPC-401 at doses of 1, 5, 10, 20, or 30 mg. A single subcutaneous injection was administered on day 1, and dose cohort escalations occurred only after the final patient at each dose level had been observed for ≥3 days with no evidence of study drug–related toxicity. Safety assessments included treatment-emergent adverse events (TEAEs), physical and laboratory evaluations, and immunogenicity. PK parameters of baseline corrected serum LJPC-401 were obtained by noncompartmental analysis, with blood samples collected at predose and at 0.5, 2, 4, 8, 24, 48, and 168 hours postdose. PD endpoints included effects on serum iron, ferritin, transferrin, iron-binding capacity, and transferrin saturation.
Results: Eighteen patients (55.6% female; 66.7% white) were enrolled and completed the study; 7 had hemoglobinopathies (sickle cell disease or thalassemia) and 11 had hemochromatosis. Sixteen patients (88.9%) reported TEAEs (34 mild and 4 moderate in severity). Rates of TEAEs were similar

across dose groups. The most frequently occurring AEs were injection site reactions (66.7%), nausea (11.1%), increased alanine aminotransferase (11.1%), decreased appetite (11.1%), and hypoesthesia (11.1%). There were no severe TEAEs, no TEAEs leading to early discontinuation, and no deaths during the study. One serious AE (sickle cell crisis) was reported but was not considered treatment related. Following LJPC-401 doses of 1 to 20 mg (but not 30 mg), the maximum serum concentration and area under the serum concentration-time curve from time 0 to 24 hours postdose increased with dose. Peak concentrations occurred at ~2 to 4 hours postdose for all doses. A larger reduction in serum iron was generally associated with increasing LJPC-401 doses up to 20 mg (Figure 1); at 30 mg, the magnitude of serum iron reduction was lower than that at 20 mg. Overall, there was a statistically significant dose response across the 5 dose groups (p=0.0478). Serum iron reductions were sustained up to day 8 in most patients. Data from other iron parameters will be presented.

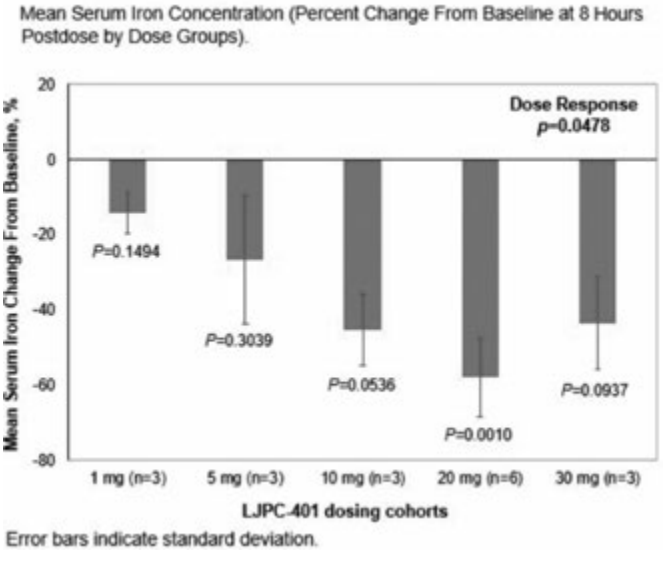


Figure 1.

Summary/Conclusion: LJPC-401 was well tolerated at doses between 1 and 30 mg in iron-overload patients, and showed significant decreases in serum iron levels compared with baseline, which were sustained in most patients up to 8 days. In healthy adults (abstract submission by Yaeger et al), LJPC-401 showed decreased serum iron levels that returned to baseline levels within 48 h. Additional studies are planned to further explore the iron-regulating effects of LJPC-401 in patients with iron-overload disorders.

S895

HEPCIDIN MIMETIC PTG-300 INDUCES DOSE-RELATED AND SUSTAINED REDUCTIONS IN SERUM IRON AND TRANSFERRIN SATURATION IN HEALTHY SUBJECTS

A. Nicholls[1], J. Lickliter[2], L. Tozzi[1], D. Liu[1], R. Shames[1,*]

[1]Protagonist Therapeutics. Inc., Newark, United States, [2]Nucleus Network Ltd., Melbourne, Australia

Background: PTG-300, an injectable peptide hepcidin mimetic, is being developed as a potential therapeutic agent for the treatment of hematologic diseases characterized by ineffective erythropoiesis, chronic anemia and iron overload including beta-thalassemia and myelodysplastic syndromes (MDS). PTG-300 improved anemia in a mouse model of beta-thalassemia and was well-tolerated in non-clinical studies.
Aims: A first-in-human (FIH) study of subcutaneous (sc) PTG-300 was conducted in normal healthy volunteers (NHV) to evaluate the safety/ tolerability, pharmacokinetics (PK) and the effect on serum iron parameters.
Methods: This was a randomized, double-blind, placebo-controlled single-ascending (SD) and repeat (RD) dose study of PTG-300 in 62 healthy male subjects. Eligible subjects had no recent history of oral iron, transfusion or blood donation and had normal baseline hematologic parameters. Informed consent was obtained and subjects randomized to cohorts of 10 subjects (single doses 1 to 40 mg, active: placebo 8:2) or 6 subjects (80 mg SD or 40 mg weekly×2; 5:1). Subjects consumed a standard diet throughout the study. PTG-300 was given as a single dose (1 mL sc) over 1-80 mg dose range or placebo. Subjects were followed for one week after dosing. In the RD cohort, two 40 mg doses were administered one week apart to further assess exposure-response. Blood sampling (iron studies, hematology and clinical chemistry), clinical observations and adverse event (AE) assessments were performed at frequent intervals after dosing.
Results: PTG-300 induced a dose-related reduction in serum iron with a maximum mean reduction of approximately 60% from baseline. The effect on iron appeared to plateau at a dose of 20 mg and was sustained for at least 72 h at higher doses. Recovery to pre-dose concentrations occurred at 144 h for the 20 mg dose while the 40 and 80 mg dose groups had not completely returned to baseline at this time point (Figure 1). Changes in serum iron were associated with a reduction in transferrin saturation; no changes in hematologic indices were observed in SD cohorts. In the RD cohort, the effects on iron were comparable for both administered doses. A small reduction in mean hemoglobin and a reticulocytosis were observed at 480 h, consistent with the return of available iron following PTG-300-induced iron restriction in subjects undergoing repeat blood sampling. PTG-300 was generally well-tolerated with no dose-limiting toxicities or serious AEs. The most frequent AEs were injection site reactions (ISRs), consisting mainly of transient erythema without systemic effects, headache and URIs. The maximum concentration in peripheral blood samples was observed up to 24 h after dosing. The decay in PTG-300 followed a simple exponential pattern, with AUC and Cmax increasing with dose; however, the increases were less than proportional to dose. Mean $t_{1/2}$ was 36.5 h (SD 17.6, n=24) for the 10-40 mg dose range.

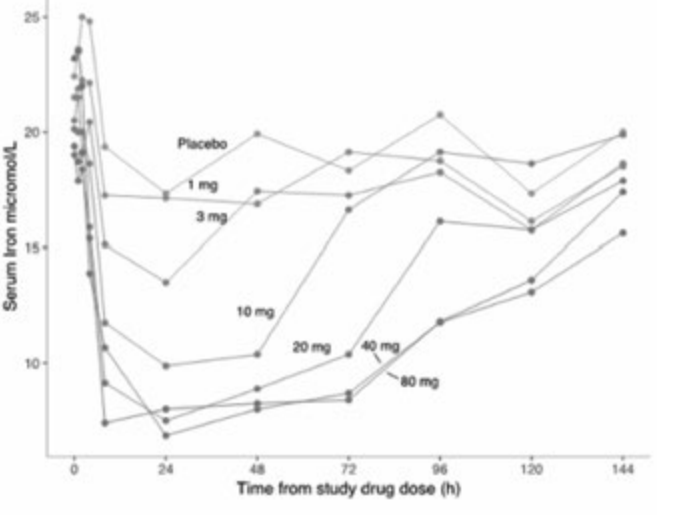


Figure 1.

Summary/Conclusion: PTG-300 was well-tolerated following single and repeat dose sc injection in NHV and demonstrated marked and sustained dose-related effects on iron distribution consistent with known activities of hepcidin and pre-clinical studies of PTG-300. This FIH study establishes PD-based proof of concept and provides a range of doses that could be evaluated in the treatment of iron-loading anemias, such as beta-thalassemia and MDS.

POSTER SESSION II

Acute lymphoblastic leukemia – Biology & Translational Research

PS896

INFLAMED BM-MSCS HIGHLY ATTRACT TEL-AML1+ PRE-LEUKEMIC CELLS AND PROVIDE A SUSTAINING NICHE FOR THEIR EMERGENCE

L. Beneforti[1,*], E. Dander[1], S. Bresolin[2], G. te Kronnie[2], T. Ford[3], G. D'Amico[1], A. Biondi[4], C. Palmi[1], G. Cazzaniga[1]

[1]Centro Ricerca Tettamanti, Fondazione Tettamanti, Monza, [2]Dept. Health of Woman and Child, Univ. of Padua, Padua, Italy, [3]Centre for Evolution and Cancer, Division of Molecular Pathology, The Institute of Cancer Research, Sutton, United Kingdom, [4]Pediatric Dept., Univ. Milano Bicocca, Monza, Italy

Background: Epidemiological and experimental data indicate that infections and inflammation play an important role in the conversion from TEL-AML1 positive pre-leukemia to leukemia. We showed that TGFβ, a pleiotropic cytokine produced after inflammation, limited the proliferation of normal B-precursors, favoring the TEL-AML1+ clone. Moreover, TEL-AML1+ Ba/F3 showed altered expression of adhesion molecules and an impaired migration towards SDF-1α, suggesting possible dysregulated interactions within the BM-niche, where MSCs are key regulators of HSPCs homeostasis. Moreover, MSCs posses pro- and anti-inflammatory properties representing a bridge between hemopoiesis and inflammation. In this complex equilibrium, pre-malignant cells are often characterized by oncogene induced senescence (OIS), which represents an anti-tumor barrier alternative to apoptosis. An important mediator of OIS is the CXCL1-CXCR2 axis; however, due to its link with inflammation, this pathway can also exert protumoral effects.
Aims: Aim of the study is to better elucidate intrinsic/extrinsic mechanisms favoring the TEL-AML1+ clone in the BM-niche, focusing on OIS, MSCs and inflammation.
Methods: Murine BM-derived MSCs were isolated, characterized and cultured for limited passages. Inflammatory conditions were reproduced by treating cells for 48-72-96h with IL6/IL1β/TNFα. Competitive growth assays were performed by mixing control and TEL-AML1+ Ba/F3 in presence of MSCs and/or cytokines.
Results: TEL-AML1 expression induces OIS in murine Ba/F3 proB cells, and accordingly, one of its mediator, the CXCL1-CXCR2 axis, is upregulated in pre-leukemic cells, as shown by GEP analysis and by the overexpression of CXCR2 receptor (MFI: TA=1378±807; ctr=284±167). Interestingly, preleukemic cells highly migrate toward recombinant CXCL1 respect to controls (% migrated cell/input: TA=21.5±6.7; ctr=2.2±1.7). Moreover, both murine and human BMMSCs basally produce CXCL1, but they dramatically increase its secretion after inflammatory stimulation (pg/mL: unstimulated=78±28; inflamed=30162±4760). Accordingly, TEL-AML1+ cells are more attracted by inflamed, but not unstimulated, BM-MSCs supernatants respect to control cells (% migrated cell/input: TA=30.2±9.1; ctr=14.3±9.6). Co-culturing control and TEL-AML1+ cells on unstimulated MSC doesn't provide any advantages to the latter; on the contrary, preleukemic cells increase if inflammatory cytokines are added to the MSC co-cultures (fold-increase=1.76±0.27). The advantaging effect depends on BM-MSCs, as inflammatory cytokines do not impact on Ba/F3 cells themselves, and it's mediated by soluble factors. Indeed, the inflammatory milieu in MSC co-cultures induces a strong anti-proliferative effect on normal B precursor (FI, CSFE-MFI: TA=1.18±0.49; ctr=2.34±0.58) and reduce their viability (FI,% ANN-V/7AAD: 0.76±0.18), while it exerts an anti-apoptotic effect on TEL-AML1+ cells (FI,% ANN-V/7AAD: 1.42±0.09). Blocking CXCL1 in inflammatory co-cultures doesn't affect results; TGFβ isn't likely implicated as well, as it doesn't increase in inflamed MSCs supernatants.
Summary/Conclusion: TEL-AML1+ murine B-progenitors are strongly attracted by inflamed BM-MSC, which provides at the same time an advantageous microenvironment for their emergence. While CXCL1 is fundamental for migration, it doesn't represent a pro-survival or pro-proliferative stimulus. However, blocking CXCL1/CXCR2 axis could avoid pre-leukemic cells to reach a sustaining niche, therefore representing a potential strategy to eradicate TEL/AML1+ pre-leukemic cells.

PS897

TRANSLATOME ANALYSIS REVEALS ALTERED SERINE METABOLISM IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

K. Kampen[1,*], L. Fancello[1], T. Girardi[1], S. O. Sulima[1], F. Loayza-Puch[2], B. Verbelen[1], S. Vereecke[1], G. Rinaldi[3], J. Verbeeck[1], J. Op de Beeck[1], J. Royaert[1], P. Vermeersch[4], D. Cassiman[5], S.-M. Fendt[3], R. Agami[2], M. Fiers[6], K. De Keersmaecker[1]

[1]Laboratoryfor Disease Mechanisms in Cancer, Oncology, KU leuven, Leuven, Belgium, [2]Division of Oncogenomics, The Netherlands Cancer Institute, Amsterdam, Netherlands, [3]Center for Cancer Biology, VIB-KU Leuven, [4]Department of Laboratory Medicine, [5]Department of Gastroenterology-Hepatology and Metabolic Center, University Hospitals Leuven, [6]Center for human Genetics, KU leuven, Leuven, Belgium

Background: Recurrent somatic mutations occur in ribosomal protein L10 (RPL10) at residue R98 (R98S missense mutation) in approximately 8% of pediatric T-ALL.
Aims: Integrate multi-omics approaches to define the overall changes in transcriptional and translational control of RPL10 R98S defective cells.
Methods: Merge multiple datasets to compare RPL10 WT and R98S cells; Quantitative proteomics by mass spectrometry analysis (published), RNA sequencing (3 datasets), Polysome profiling (published), and Ribosome footprinting. Immunoblot validation. $^{13}C_6$-Glucose metabolic tracing analysis. Genetic interference of T-ALL cells by shRNA
Results: RPL10 R98S was associated with 798 transcriptional gene changes. We consulted iRegulon to predict transcriptional regulators that may cause this R98S linked transcriptome. Ikzf2 (Helios) was predicted to control upregulated transcripts and was overexpressed at mRNA and protein level. The NKX2 family was a predicted mediator for downregulated mRNAs, which is of interest as RPL10 R98S mutant T-ALL cases are known to be enriched for NKX2 translocations. The RPL10 R98S associated translatome highlighted phosphoserine phosphatase (Psph), showing a significant increase in all datasets with enhanced translational efficiency (3-fold) on top of increased mRNA expression (6-fold), leading to a 5-fold increase in protein expression by mass spectrometry. PSPH is an enzyme catalyzing the third and last step in *de novo* serine synthesis. The transcriptional and translational upregulation of PSPH were confirmed by increased mRNA and protein expression in Ba/F3 and Jurkat T-ALL RPL10 R98S cell models. In line with these findings, $^{13}C_6$-Glucose tracing revealed enhanced incorporation of ^{13}C labeled carbons into serine and glycine in our RPL10 R98S cell models, supporting increased branching towards *de novo* serine/glycine synthesis. High PSPH protein expression was observed in all T-ALL cases and was not restricted to R98S cases, while low PSPH levels were observed in CD34+ normal bone marrow cells and other leukemia's, such as pediatric AML. Besides the involvement of RPL10 R98S mutations in controlling PSPH expression, preliminary data revealed an association between high PSPH expression and occurrence of PHF6 mutations in pediatric T-ALL. Knockdown (KD) of PSPH expression using two different shRNAs in three different T-ALL cell lines revealed that PSPH was required for T-ALL cell proliferation. PSPH KD T-ALL cells were blocked in their cell cycle progression potential by reduced phosphorylation of CDK2 (Thr160) and increased p21/p27 protein expression. This was not merely an *in vitro* effect, as PSPH KD reduced *in vivo* T-ALL xenograft growth by suppression of engraftment to the bone marrow and spleen. In addition, elevated PSPH levels in T-ALL PDX samples were associated with enhanced blood plasma serine/glycine levels in mice. Interestingly, supplementation of serine and/or glycine increased the survival of bone marrow niche cell types. These data indicate that not only T-ALL cells themselves, but also their surrounding niche may benefit from enhanced *de novo* serine synthesis in T-ALL (Figure 1).

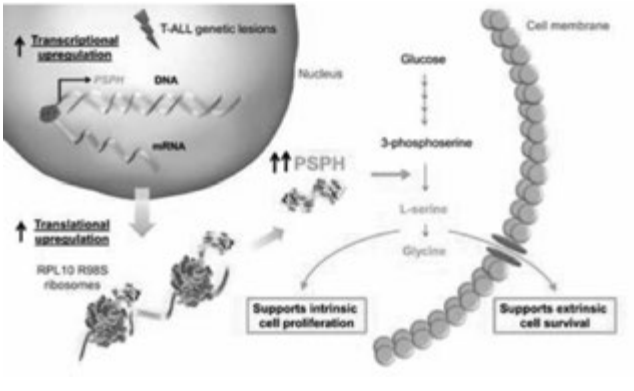


Figure 1.

Summary/Conclusion: Enhanced PSPH-driven serine/glycine synthesis is a common phenotype in T-ALL that can be mediated by specific mechanisms such as the RPL10 R98S mutant ribosome. Our results highlight the importance of PSPH for T-ALL proliferation and expansion capabilities, revealing for the first time a serine/glycine dependency of T-ALL cells.

PS898

MIR-497~195 CLUSTER IS ASSOCIATED WITH FAVORABLE OUTCOME AND HAS A TUMOR SUPPRESSIVE FUNCTION IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

E. Boldrin[1,*], E. Gaffo[2], S. Demir[1], K.-M. Debatin[1], G. te Kronnie[3], S. Bortoluzzi[2], L.-H. Meyer[1]

[1]Department of Pediatrics and Adolescent Medicine, Ulm University Medical Center, Ulm, Germany, [2]Department of Molecular Medicine, [3]Department of Women's and Children's Health, Padua University, Padua, Italy

Background: Despite high rates of successful treatment of B-cell precursor acute lymphoblastic leukemia (BCP-ALL), relapse is still associated with poor outcome. Previously, we found that rapid engraftment of BCP-ALL patient cells transplanted into NOD/SCID mice (short time to leukemia, TTL^{short}) is indicative of early relapse, while slow engraftment (TTL^{long}) is associated with favorable outcome. MicroRNAs (miRNAs) are short non-coding regulatory RNAs, which are deregulated in different cancers.
Aims: In this study, we analyzed miRNA expression profiles in BCP-ALL associated with distinct engraftment and outcome and addressed the functional impact of identified candidate miRNAs on leukemia development.
Methods: MiRNA expression was investigated in 13 patient-derived xenograft (PDX) BCP-ALL samples by small RNA sequencing, validated by qRT-PCR and associated with outcome data. Identified candidate miRNAs were lentivirally overexpressed in BCP-ALL cell lines and PDX samples. *in vitro* proliferation was analyzed by flow cytometry (eFluor and cell counting). *In vivo* leukemia development was assessed upon transplantation of transduced cells into NOD/SCID mice compared to empty vector (EV) control.
Results: We identified 18 differentially expressed miRNAs in ALL samples associated with distinct engraftment and patient outcome. Among the most significantly regulated miRNAs, the miRNA cluster miR-497~195 was overexpressed in TTL^{long}/good outcome ALL. This finding was validated in a large cohort of N=57 BCP-ALL xenograft samples, identifying significantly lower miR-497 levels in poor outcome ALL as compared to good outcome ALL (U-test; p=0.016). In addition, increasing miR-497 levels were significantly associated with longer remission duration (N=11; Spearman r=0.68; p=0.025). Interestingly, the miR-497~195 cluster has previously been reported to have tumor suppressive functions in solid cancers, in line with the high miR-497~195 expression we identified in good outcome ALL. Moreover, upon analysis of putative targets and biological functions associated with miR-497~195, we found cellular proliferation as the most enriched biological process (DAVID, p=0.004), further emphasizing a tumor-suppressor function in ALL. To address the functional impact of miR-497~195 on leukemia proliferation and growth, miR-497~195 were stably overexpressed in the BCP-ALL cell line EU-3. No differences in cell death and *in vitro* proliferation were observed as compared to control EV transduced cells. However, lower leukemia loads were observed in recipient animals transplanted with miR-497~195 overexpressing EU-3 cells as compared to EV control (N=5 per group, U-test p=0,008). We further addressed the functional role of miR-497~195 in leukemia growth of PDX-ALL *in vivo* and stably overexpressed miR-497~195 in an ALL with short engraftment, poor patient outcome and low miR-497~195 expression. Upon transplantation, high leukemia infiltration of EV control cells was observed (49% BM, 60% spleen, mean, N=4 in two independent experiments), whereas miR-497~195 overexpressing cells showed a clear delay in leukemia engraftment and lower ALL infiltration in both, spleen and BM (below 1%), indicating that miR-497~195 negatively regulated ALL growth *in vivo*.
Summary/Conclusion: We found that high miR-497~195 expression is significantly associated with good patient outcome in BCP-ALL. Overexpression of miR-497~195 in cell line and primary PDX BCP-ALL led to clearly prolonged ALL development and reduced ALL infiltration *in vivo*, indicating a tumor suppressor role of miR-497~195 in BCP-ALL.

PS899

MICRORNA-142 IS AN ESSENTIAL REGULATOR OF B CELL DEVELOPMENT AND MALIGNANT TRANSFORMATION

W.-L. Wang[1,*], N. Magilnick[2], E. Reyes[3], M. Boldin[4]

[1]Molecular and Cellular Biology,Irell & Manella Graduate School of Biological Sciences-City of Hope, Los Angeles, [2]Irell & Manella Graduate School of Biological Sciences-City of Hope, Los Angels, [3]Beckman Research Institute of the City of Hope, [4]Irell & Manella Graduate School of Biological Sciences-City of Hope, Los Angeles, United States

Background: MicroRNA-142 (*miR-142*) is predominantly expressed in cells of hematopoietic origin and plays a crucial role in the regulation of both innate and adaptive immune responses. Accumulating evidence suggests that *miR-142* dysregulation is associated with hematological malignancies. Acute myeloid leukemia (AML) patients were shown to carry somatic *miR-142* mutations that occur in the seed sequence of *miR-142* and therefore interfere with target repression. In addition, approximately 20% of patients with diffuse large B cell lymphoma (DLBCL) display *miR-142* lesions. Nevertheless, the role of *miR-142* in aberrant leukocyte proliferation is poorly understood.
Aims: Our previous findings indicated that abrogation of *miR-142* expression in mice results in a significant expansion of the immature and mature B cell compartments in part due to the upregulation of B-cell activating factor receptor (*BAFF-R*), a *bona fide* target of miR-142. We therefore hypothesize that *miR-142* deficiency confers developmental and proliferative advantages to B cell progenitors and promotes malignant transformation.
Methods: To better understand the role of *miR-142* in B cell ontogenesis, we examined early B cell development in *miR-142*$^{-/-}$ mice using Hardy fraction analysis. Since genetic lesions that disrupt control of B cell development frequently drive malignant transformation of B cells, we wondered whether *miR-142* dysregulation contributes to proliferative B cell disorders. To address this question, we bred *miR-142*$^{-/-}$ mice with Eμ-myc transgenic mice, which spontaneously develop a mix of precursor B cell lymphoblastic leukemia and mature B cell lymphoma.
Results: Here we show that *miR-142* deletion results in a significant increase in the frequency of pro-B, large pre-B and immature B cells (Fractions B, C and E), while the pre-pro-B cell population (Fraction A) decreased. In addition, *miR-142*-deficient pro-B cells displayed a significant proliferative advantage over the wild-type cells in *in vitro* differentiation assay in the presence of IL-7. Our data linked accumulation of pro-B and large pre-B cells in *miR-142*$^{-/-}$ bone marrow to an increase in their survival capacity. Furthermore, we found that *miR-142* haploinsufficiency dramatically accelerated development of leukemia/lymphoma and significantly shortened survival of Eμ-myc transgenic mice. We are currently testing a hypothesis that *miR-142* exerts its tumor suppressor activity in B cells by controlling BAFF-R and IL-7R signaling. This notion is supported by our *in vitro* observations that *miR-142*-deficient B cells display more robust proliferation than wild-type cells in response to BAFF or IL-7 treatments.
Summary/Conclusion: Collectively, our results establish *miR-142* as an essential molecular brake on B cell development and malignancy. Besides, it also demonstrates a great potential of using this miRNA as a biomarker to classify patients with dysregulated *miR-142* expression, and then corrects *miR-142*-directed target repression in malignant B cells e.g. BAFF-R signaling.

PS900

AN ESSENTIAL ROLE OF LCK IN T-ALL CELL PROLIFERATION AND SURVIVAL

Y. Shi[1,*], M. Beckett[1], O. Heidenreich[1], F. van Delft[1], A. Krippner-Heidenreich[1]

[1]Wolfson Childhood Cancer Research Centre, Northern Institute For Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom

Background: T-cell Acute Lymphoblastic Leukemia (T-ALL) is caused by malignant transformation of T cells showing differentiation arrest and uncontrolled proliferation. The checkpoints during T-cell development are dominated by pre-T-cell receptor (pTCR) for β-selection and T-cell receptor (TCR) for positive/negative selection. Gene lesions with proximity to these checkpoints are found in T-ALL such as *NOTCH, FBXW7, PTEN* and *NRAS*. As shown in B-cell Precursor Leukemia, kinases of the pre-B-cell receptor signaling complex (SYK and BTK) have been validated as drug targets, but whether critical components of the pTCR/TCR signaling such as LCK or ZAP70 play a similar role in T-ALL remains unclear. LCK (lymphocyte-specific kinase) is a central molecule in pTCR/TCR signaling transduction. The important role for LCK has been shown in Chronic Lymphoblastic Leukemia, however, the role of LCK in T-ALL has not been studied.
Aims: To investigate the importance of pTCR/TCR complex for T-ALL cell proliferation and survival.
Methods: We performed limited screens targeting six components of the pTCR/TCR signaling complex *in vitro* and *in vivo* in 4 T-ALL cell lines

and 2 T-ALL patient-derived xenografts (PDXs). To validate the screening results we conducted gene knockdown experiments in T-ALL cell lines using GFP-expressing lentiviral shRNA vectors and evaluated cell proliferation by competitive assays *in vitro* and *in vivo*. The activation and expression of LCK were determined in 11 T-ALL cell lines and 10 PDXs by qPCR, western blotting and Phosflow. The kinase activity of LCK was abrogated using Dasatinib. Synergism between Dexamethasone and Dasatinib was evaluated by drug matrix assays. The *ex-vivo* expansion of PDXs was achieved by co-culture with OP9-DL1 feeder cells and proliferation was assessed by Cell Trace Violet.

Results: A targeted *in vitro* and *in vivo* shRNA screen targeting central components of the pTCR/TCR signalling complex (*PTCRA*, *CD3ε*, *LCK*, *FYN, ZAP70* and *LAT*) in T-ALL cell lines and PDXs identified LCK to be crucial for T-ALL maintenance and engraftment. Knockdown of LCK in SUP-T1, CUTLL1 and MOLT4 cells revealed a significant loss of at least 50% of GFP+/*LCK* shRNA transduced cells over a time period of 2-3 weeks. This was also confirmed *in vivo* in competitive assays with MOLT4 cells showing a growth advantage of control shNTC cells over LCK knockdown cells in bone marrow, spleen and liver. Mechanistic analyses indicate that knockdown or inhibition of LCK by Dasatinib impairs cell proliferation by inducing G1/G0 arrest in both T-ALL cell lines and PDXs, but has little effect on the induction of cell death. The sensitivity of T-ALL cell lines towards Dasatinib seems to correlate positively with activated LCK (LCK p-Y394). Moreover, *LCK* knockdown significantly sensitizes T-ALL cells to Dexamethasone (Dex) and strong synergistic lethal effects between Dex and Dasatinib have been observed in various T-ALL cell lines and PDXs. Importantly, both Dex-sensitive and Dex-resistant T-ALLs are highly sensitive to Dex/Dasatinib combination treatment. To evaluate this further we currently perform a randomized phase- like mini-trial in NSG mice with 10 PDXs comparing four treatment arms including control, Dex, Dasatinib and combination.

Summary/Conclusion: Overall, our data demonstrate that LCK plays a critical role in T-ALL cell proliferation and engraftment. LCK inhibition with Dasatinib, in conjunction with Dex, reverses glucocorticoid resistance and induces cell death. The Dex/Dasatinib combination might provide a novel treatment strategy for refractory and relapsed T-ALL patients.

PS901

TITLE: PROGNOSTIC IMPACT OF THE BCR/ABL1-LIKE SIGNATURE IN ADULT B-LINEAGE ALL. FIRST ANALYSIS OF THE GIMEMA LAL1913 PROTOCOL

S. Chiaretti[1,*], M. Messina[1], A. Lauretti[1], A. Piciocchi[2], I. Della Starza[1], A. Laginestra[3], V. Apicella[1], A. Vitale[1], M. Vignetti[2], C. Frairia[4], L. Marbello[5], F. Di Raimondo[6], C. Cattaneo[7], B. Neri[8], A. Candoni[9], M. Bocchia[10], A. Guarini[11], R. Bassan[12], R. Foà[1]

[1]Hematology, Department of Cellular Biotechnology and Hematology, Sapienza University, [2]GIMEMA Data Center, GIMEMA Foundation, Rome, [3] Department of Experimental, Diagnostic and Specialty Medicine (DIMES), S. Orsola-Malpighi Hospital, Bologna, [4]Dipartimento di Oncologia ed Ematologia S.C. Ematologia [2] A.O., Citta' della Salute e della Scienza di Torino San Giovanni Battista, Turin, [5]SC Ematologia, Ospedale Niguarda Ca Granda, Milan, [6]Cattedra di Ematologia - Ospedale Ferrarotto, Universita' di Catania, Catania, [7]U.O. Ematologia, Spedali Civili - Brescia - Azienda Ospedaliera, Brescia, , Brescia, [8]Ud.O.C. Ematologia, Ospedale S.Eugenio, Rome, [9]Clinica Ematologica-Centro Trapianti e Terapie cellulari, Azienda Ospedaliero-Universitaria, Udine, [10]U.O.C. Ematologia e Trapianti - A.O. Senese -, Policlinico Le Scotte, Siena, [11]Department of Molecular Medicine, Sapienza University, Rome, [12]Hematology Unit, Ospedale dell'Angelo and Ospedale Ss Giovanni e Paolo, Mestre Venezia, Italy

Background: In B-lineage acute lymphoblastic leukemia (B-ALL) lacking the major fusion genes (i.e. *BCR-ABL1*, *ETV6-RUNX1*, *TCF3-PBX1* and r-*KTM2A)*, defined B-NEG, the outcome is negatively influenced by the *BCR-ABL1*-like profile, *CRLF2* overexpression, JAK/STAT and RAS pathway mutations. However, their prognostic value in the setting of a pediatric-oriented, Minimal Residual Disease (MRD)-driven trial for adult ALL has not been fully dissected.

Aims: To evaluate - within B-NEG ALL patients enrolled in the GIMEMA LAL1913 front-line protocol for adult Ph-negative ALL - the prognostic role of: i) *BCR-ABL1*-like profile, ii) *CRLF2* overexpression, iii) RAS and JAK/STAT pathway mutations.

Methods: Within a total of 120 B-NEG ALL patients enrolled in the GIMEMA LAL1913 protocol, 92 (median age 38.5 years, range 18.1-64.6) were screened for the *BCR/ABL1*-like profile (Chiaretti *et al*., BJH, in press), *CRLF2* expression levels (Chiaretti *et al*., Leuk Res, 2016), JAK/STAT (*JAK1*, *JAK2*, *JAK3*, *IL7R*, *CRLF2*) and RAS pathway (*FLT3*, *NRAS*, *KRAS*, *PTPN11*) mutations, the latter by Truseq custom amplicon kit and Illumina MiSeq (CA, USA). Molecular markers were correlated with clinical variables (age, gender, WBC count), complete remission (CR) achievement, disease-free and event-free survival (DFS, EFS) at 12 months.

Results: By applying the *BCR/ABL1*-like predictor, we identified 25 (27.2%) *BCR/ABL1*-like cases. *CRLF2* overexpression was found in 19 cases (20.6%) and was associated with a *BCR/ABL1*-like signature (40% *BCR/ABL1*-like *vs* 13.4% of non-*BCR/ABL1*-like ALL, p=0.008). Similarly, JAK/STAT pathway mutations, detected in 14 (15.9%) patients, were more frequent in *BCR/ABL1*-like (33.3%) than in non-*BCR/ABL1*-like cases (11.5%) (p=0.035). RAS pathway mutations were documented in 43 (48.8%) cases, with a higher incidence in non-*BCR/ABL1*-like cases (51.9%) than *BCR/ABL1*-like (33.3%) cases. No association was found between *BCR/ABL1*-like signature, *CRLF2* overexpression, JAK/STAT or RAS mutations and clinical correlates. Among the molecular markers evaluated, only the *BCR/ABL1*-like profile and RAS mutations impacted on outcome. Indeed, there was a trend towards an inferior CR rate in *BCR/ABL1*-like compared to non-*BCR/ABL1*-like cases (78.3% *vs* 90.6%). Consistently, survival analyses showed that *BCR/ABL1*-like cases had a significantly inferior EFS and DFS than non-*BCR/ABL1*-like (49.2% *vs* 74.4%, p=0.02 - Figure 1 - and 60.3% *vs* 82.6%, p=0.047, respectively). Clonal RAS pathway mutations did not impact on CR achievement, whereas they affected DFS: RAS-M cases had a significantly lower DFS than RAS-WT (58.6% *vs* 85%, p=0.04); subclonal (VAF <20%) RAS mutations did not influence patients' outcome. In a multivariate model for EFS, the only statistically significant variables were the *BCR/ABL1*-like profile (p=0.017, HR=0.4 CI 95% 0.2-0.85) and age (p=0.003, HR=1.03, CI 95% 1.01-1.06). The impact of the *BCR/ABL1*-like profile on MRD achievement will be presented.

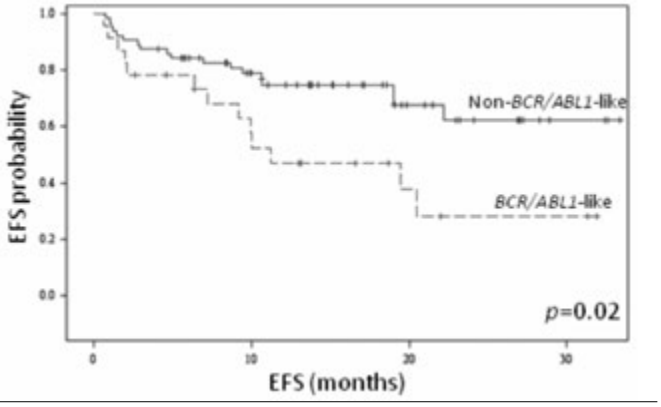


Figure 1.

Summary/Conclusion: In this first analysis of the GIMEMA LAL1913 protocol, our model identified as *BCR/ABL1*-like 27.2% of B-NEG ALL cases and showed that also in a pediatric-oriented and MRD-driven clinical trial adult *BCR/ABL1*-like ALL patients are characterized by a lower CR rate, EFS and DFS. RAS pathway mutations also negatively influenced DFS. In multivariate analysis, only the *BCR/ABL1*-like profile retained statistical significance. These findings further underline the need to identify *BCR/ABL1*-like ALL cases at presentation in order to allow a proper risk stratification and that treatment of this unfavorable subset of patients should include novel strategies upfront.

PS902

CHARACTERIZATION OF PEDIATRIC T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA WITH DNA METHYLATION STATUS

S. Kimura[1,2,*], M. Seki[1], T. Kawai[3], K. Yoshida[4], T. Isobe[1], H. Ueno[4], Y. Shiozawa[4], H. Suzuki[4], Y. Shiraishi[5], K. Chiba[5], K. Ohki[6], M. Kato[6], K. Koh[7], R. Hanada[7], H. Moritake[8], R. Kobayashi[9], T. Deguchi[10], Y. Hashii[11], T. Imamura[12], A. Sato[13], N. Kiyokawa[6], A. Manabe[14], K. Horibe[15], A. Ohara[16], M. Sanada[15], M. Kobayashi[2], A. Oka[1], Y. Hayashi[17], S. Miyano[5], S. Ogawa[4], K. Hata[3], J. Takita[1]

[1]Department of Pediatrics, The University of Tokyo, Tokyo, [2]Department of Pediatrics, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, [3]Department of Maternal-Fetal Biology, National Research Institute for Child Health and Development, Tokyo, [4]Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, [5]Human Genome Center Institute of Medical Science, The University of Tokyo, [6]Department of Pediatric Hematology and Oncology Research, National Research Institute for Child Health and Development, Tokyo, [7]Department of Hematology/Oncology, Saitama Children's Medical Center, Saitama,

[8]Division of Pediatrics, Faculty of Medicine, University of Miyazaki, Miyazaki, [9]Department of Pediatrics, Sapporo Hokuyu Hospital, Sapporo, [10]Department of Pediatrics, Mie University Hospital, Tsu, [11]Department of Pediatrics, Osaka University Graduate School of Medicine, Suita, [12]Department of Pediatrics, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, Kyoto, [13]Department of Hematology and Oncology, Miyagi Children's Hospital, Sendai, [14]Department of Pediatrics, St. Luke's International Hospital, Tokyo, [15]Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya, [16]Department of Pediatrics, Toho University, Tokyo, [17]Gunma Children's Medical Center, Maebashi, Japan

Background: Genetic profiles of pediatric T-cell acute lymphoblastic leukemia (T-ALL) have been well studied and we reported recurrent *SPI1* fusions associated with dismal prognosis. However, the epigenetic profiles and their potential contribution to clinicopathological features of T-ALL remain largely elusive.
Aims: To describe epigenetic landscape of T-ALL, we performed methylation array analysis.
Methods: Seventy-nine pediatric T-ALL samples mainly obtained from Tokyo Children's Cancer Study Group (TCCSG) and Japan Association of Childhood Leukemia Study (JACLS) including 7 cases with *SPI1* fusions were analyzed using Illumina HumanMethylation EPIC array, and conducted combined analysis whole transcriptome sequencing (WTS) and targeted-capture sequencing for 158 ALL-related genes.
Results: After normalization, 939 probes were selected to identify the most variable methylated probes. Unsupervised consensus clustering clearly identified 4 distinct sample clusters, characterized by *TAL1* fusions/high expression with intermediate methylation (M1 cluster; n=39), high TLX/HOX expression with intermediate methylation (M2 cluster; n=20), high TLX//HOX expression with high methylation (M3 cluster; n=11), and *SPI1* fusions/high expression with low methylation (M4 cluster; n=9), respectively. In M1 cluster, *PTEN* alterations were especially enriched, indicating a crucial role of PI3K-AKT pathway in this cluster. In M2 cluster, genes related to chromatin remodeling and JAK-STAT pathway were frequently mutated. *DEPTOR* related to activation of PI3K-AKT1 pathway was highly expressed. Mutation status of M3 cluster was similar to the M2 cluster, and frequent mutations of epigenetic regulators and JAK-STAT related genes were also observed in M3. Intriguingly, all *SPI1* fusion cases were classified into M4 cluster with 2 *SPI1* highly expressed cases without *SPI1* fusions. In M4 cluster, *SPI1* and *RASGRP4* were differentially expressed, and significant enrichment in RAS and NF-kB pathways was observed. Importantly, patients in M4 cluster showed significantly poor prognosis (Log-rank $p=4.4\times10^{-7}$). Next, we investigated cell surface markers of each methylation cluster. Cases in M1 cluster exhibited a late cortical thymocyte profile after T-cell receptor gene rearrangement (CD4+CD8+TCRαβ+), whereas most cases in M3 cluster did not show TCRαβ expression (CD4+CD8+TCRαβ–). In M2 and M4 clusters, most cases showed an uncommitted double negative T-cell profile (CD4–CD8–Myeloid+). Furthermore, most cases in M2 cluster represented more early stage of development (M2; CD1a–, M4; CD1a+). Finally, we analyzed CpG island methylator phenotype (CIMP) status of our cohort using previously reported 450K CIMP panel (1176 probes). As previously reported, two distinct CIMP groups were identified. Cases in M1 and M4 clusters were classified as CIMP–, exhibiting a similar methylation profile to normal CD3+ T-cells. In contrast, cases in M2 and M3 clusters were classified as CIMP+. Importantly, patients with a CIMP– profile had an significantly inferior prognosis (Log-rank $p=8.0\times10^{-4}$).
Summary/Conclusion: Based on DNA methylation profiles, pediatric T-ALL is clustered into 4 distinct subtypes, which exhibited remarkable correlation with genetic signatures, expression features, and clinical outcomes as well as profiles of T-cell development. Our results suggested that the biological phenotype of T-ALL is mediated by both genetic and epigenetic regulations, and explorations for aberrant DNA methylation along with genetic alterations might be helpful for a new therapeutic strategy for T-ALL.

PS903

THE PHILADELPHIA-LIKE SUBTYPE IN CHILDREN WITH FIRST RELAPSE OF B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA: A STUDY OF THE GERMAN ALL-REZ BFM 2002 TRIAL

M. Pogodzinski[1,*], J. Toedling[1], A. von Stackelberg[1], C. Eckert[1], R. Kirschner-Schwabe[1]

[1]Department of Pediatric Oncology and Hematology, Charite - Universitaetsmedizin, Berlin, Germany

Background: Philadelphia-like acute lymphoblastic leukemia (Ph-like ALL) is characterized by a gene expression profile similar to that of Philadelphia chromosome positive (*BCR/ABL* rearranged) ALL. Ph-like ALL lacks the *BCR/ABL* rearrangement, but frequently harbors other kinase activating gene fusions that can be targeted by kinase inhibitor treatment. Patients with Ph-like ALL have an elevated risk of relapse after primary treatment. However, the frequency of Ph-like ALL in children with relapsed ALL and the outcome of this subgroup after relapse treatment is currently unknown.
Aims: We investigated the frequency, clinical presentation and outcome of Ph-like ALL in relapsed patients of the German ALL-REZ BFM 2002 relapse trial to determine the eligibility of this subgroup for kinase inhibitor treatment during second-line therapy.
Methods: We performed gene expression profiling of 97 leukemic samples from children with first bone marrow relapse of B-cell precursor ALL by Affymetrix HG-U133 Plus 2.0 microarrays. For the identification of Ph-like cases, we used the previously established *Prediction Analysis of Microarrays* (PAM) method.
Results: Our study cohort comprised 11 *BCR/ABL*-positive and 86 *BCR/ABL*-negative samples. Within the BCR/ABL-negative group, 26 samples had defined cytogenetic aberrations (*ETV6-RUNX1, KDMT2A*-rearrangement, hyper- or hypodiploidy) that are in large parts mutually exclusive with the Ph-like subtype. Therefore, we used this subgroup (hereafter referred to as cytogenetic group) together with the *BCR/ABL*-positive group to train a *BCR/ABL* classifier. The classifier contained 351 genes and achieved a prediction accuracy of 100% for *BCR/ABL*-positive ALL. As a proof of concept, the same approach was applied to a published data set from primary ALL (GSE79533), where the classifier also reached a prediction accuracy of 100% and contained 732 genes. Next, we used the BCR/ABL classifier built on our ALL relapse training set to predict Ph-like ALL in the remaining 60 ALL relapse samples (test set). Using a PAM coefficient cut-off of ≥85%, the classifier predicted 31 of 60 relapse samples to be highly similar to *BCR/ABL* positive ALL, that is Ph-like ALL. In agreement with primary ALL, relapses of the Ph-like subtype were associated with an increased rate of *IKZF1* deletions (65%, p=0.004) and *JAK* mutations (*JAK2* and *JAK3*, 30%, p=0.006) and with an increased proportion of *CRLF2* high expressing samples (36%, p=0.004) compared to other cases of the study cohort. Interestingly, the rate of deletions in the PAR1 region leading to the kinase activating P2RY8-CRLF2 fusion was only slightly increased in our relapsed Ph-like ALL patients (13.3%, non-significant). A more in depth genetic analysis of the Ph-like relapses is currently ongoing. With respect to their clinical presentation, relapsed ALL patients with the Ph-like subtype comprised a heterogeneous group that did not significantly differ from the total ALL-REZ BFM 2002 trial cohort. Similarly, the probability of event-free survival (pEFS) of relapsed Ph-like patients was highly similar to the overall pEFS of the total ALL-REZ BFM 2002 trial cohort (pEFS 0.452±0.089 vs. 0.517±0.022; p=0.368).
Summary/Conclusion: In conclusion, we found that the Ph-like subtype is present in more than 30% (31/97) of children with relapsed B-cell precursor ALL. In contrast to primary ALL, the Ph-like subtype is not linked to inferior prognosis after relapse treatment. However, tyrosine kinase inhibitor treatment may still improve the currently intermediate outcome of relapsed Ph-like ALL patients.

PS904

HUMAN T CELL ACUTE LYMPHOBLASTIC LEUKEMIA DEVELOPS HYPOXIA-RELATED CHEMORESISTANCE THROUGH HIF1A AND MTOR PATHWAY INTERACTIONS

L. Fahy[1,*], J. Calvo[1], R. Kulkarni[1], I. Naguibneva[1], J. Rucci[1], S. Chabi[1], M.-L. Arcangeli[1], F. Pflumio[1], B. Uzan[1]

[1]CEA/UMR967, Fontenay-aux-Roses, France

Background: T cell acute lymphoblastic leukemia (T-ALL) is a malignant hematological disorder characterized by an increased proliferation of T lymphocytes precursors unable to complete their maturation. These leukemic cells accumulate in bone marrow, blood, thymus and/or lymph nodes and interfere with the production and function of normal hematopoietic cells. T-ALL treatment is based on intensive chemotherapy, which is effective in 80% of cases in children. However, children with relapse have a very poor prognosis as well as current treatments have significant side effects that should ideally be reduced. Besides the involvement of intracellular oncogenic pathways in leukemic development, extracellular signals from the microenvironment also participate in the development and progression of T-ALL. These data highlight the importance of targeting tumor cells as well as their environment to achieve sustainable results in patients. Bone marrow microenvironment, a crucial site of propagation and maintenance of leukemia, is hypoxic with oxygen levels varying from 0.1 to 5%.
Aims: We studied the consequences of a hypoxic environment on T-ALL growth and chemoresistance.
Methods: Growth, apoptosis, cell cycle, chemoresistance and graft capacity in NSG mice were quantified using human pediatric T-ALL cells cultured in

normoxia (21% O_2) or in hypoxia (3.5% or 1% O_2). Hypoxia target gene expressions were measured in T-ALL cells and HIF1α knock down (KD) was obtained using a lentiviral vector encoding shRNA specific to HIF1α (shHIF1α). Activation of mTOR and its direct targets (S6K and 4EBP1) was measured by flow cytometry. Efficiency of combining drugs with Rapamycine was tested on the growth and chemoresistance of T-ALL.
Results: We observed that hypoxia inhibits T-ALL cell growth by slowing down cell cycle progression, decreasing their metabolism and increasing apoptosis. Nevertheless leukemic cells recovered from hypoxia cultures keep their ability to propagate leukemia after transplantation into immune deficient NSG mice. In addition, T-ALL cells treated with vincristine, dexamethasone or cytarabine (drugs commonly used in T-ALL treatment) *in vitro* are better protected in hypoxia than in normoxia as they maintain their growth *in vitro* and are able to initiate leukemia *in vivo*. As expected HIF1α signaling pathway is activated in hypoxic T-ALL cells whereas HIF1α and hypoxia target gene expressions (*VEGF*, *Glut3* and *CXCR4*) are decreased in shHIF1α/T-ALL cells cultured in hypoxia. Functionally HIF1α KD in T-ALL reverses the drug resistance observed in hypoxia. Because mTOR is a potential therapeutic target in aggressive T-ALL, and as mTOR is indirectly regulated by HIF1α, we measured mTOR pathway activation. We observed that mTOR phosphorylation is decreased in T-ALL cells in hypoxia compared to normoxia. Importantly treatment of T-ALL with the mTOR inhibitor Rapamycine during cultures in normoxia mimicked the chemoresistance effects observed in hypoxia and drug treated T-ALL cells are more chemoresistant in hypoxia with Rapamycine than without. These results highlight a mechanistic loop between HIF1α and mTOR in hypoxia-related T-ALL chemoresistance.
Summary/Conclusion: Our results show that hypoxic niches may play a protective role during T-ALL treatment and induce chemoresistance. Inhibition of HIF1α and/or activation of the mTOR pathway would help abolishing the chemoresistance. A better understanding of the signaling pathways involved in T-ALL chemoresistance will undoubtedly allow more efficient therapeutic targeting of chemoresistant cells.

PS905

METABOLOMIC ANALYSIS REVEALS CEREBROSPINAL FLUID CREATINE AND XANTHINE CONCENTRATIONS AS POTENTIAL BIOMARKERS FOR CENTRAL NERVOUS SYSTEM INVOLVEMENT IN ACUTE LYMPHOBLASTIC LEUKAEMIA

A. Cousins[1,*], D. Sumpton[2], G. MacKay[2], J.H.M. Däbritz[2], A. Michie[1], E. Gottlieb[2], C. Halsey[1]

[1]Institute of Cancer Sciences, University of Glasgow, [2]Cancer Research UK Beatson Institute, Glasgow, United Kingdom

Background: Prevention and treatment of central nervous system (CNS) relapse remains a major challenge in childhood acute lymphoblastic leukaemia (ALL). The ability to colonise the leptomeninges (the site of CNS relapse) appears to be a common feature of pre-B ALL cells, and without CNS-directed therapy up to 80% of children will experience CNS relapse. The standard test for CNS involvement in ALL– cerebrospinal fluid (CSF) cytology – has poor sensitivity. Better tests for detecting and quantifying disease in the CNS are needed to assess individual risk of CNS relapse to enable accurate risk-adapted CNS-directed therapy. ALL cells in the CNS reside in a normally hypocellular environment, bathed in nutrient-poor CSF. We hypothesised that the presence of leukaemic cells in this environment would cause detectable alterations in the metabolome of CSF, creating opportunities for identification of metabolic biomarkers of CNS disease in ALL.
Aims: To detect metabolic signatures of leptomeningeal ALL using metabolomic analysis of CSF .
Methods: Metabolomics using high-performance liquid chromatography - mass spectrometry (LC-MS) was carried out on CSF of 20 children at pre-B ALL diagnosis (i.e. with high disease burden and prior to CNS-directed therapy) and while on maintenance chemotherapy (in complete remission, n=19), and of 17 unmatched control CSF samples (from patients investigated for possible neurological illnesses with negative test results). Promising compounds with differences in abundance between groups were confirmed with standards, and validated using LC-MS analysis of CSF and plasma from a human leukaemia xenotransplantation model in NSG mice, and of CSF from children at CNS relapse. LC-MS analysis was also performed on the intracellular matrix of human ALL cells retrieved from the CNS and spleen in the xenotransplantation model.
Results: Untargeted metabolomic analysis of CSF shows clear differences between children with ALL (either at diagnosis or on maintenance therapy) and non-ALL controls, and between children with ALL at diagnosis and the same children on maintenance therapy. Xanthine and creatine abundances were significantly different in children with ALL at diagnosis (xanthine 5-fold higher, creatine 1/3 lower at diagnosis than either patients on maintenance or non-ALL controls, $p<0.0001$). There was no correlation between allopurinol therapy or CSF allopurinol concentration and CSF xanthine abundance. In a murine xenotranplantation model of human leukaemia we detected a clear reduction in CSF creatine in the presence of CNS leukaemia (1/3 lower in leukaemic *vs.* control CSF, $p=0.0056$) but no change in xanthine concentration. Analysis of leukaemic cells retrieved from the CNS and spleens of these mice showed increased intracellular creatine in CNS ALL (9-fold higher, $p=0.02$). In our analysis of CSF from 4 children at CNS relapse, there was a reduction in creatine in the sample closest to relapse in 3 children, but a wide baseline creatine concentration. There was a clear increase in xanthine in the CSF in 3 children at the time of CNS relapse.
Summary/Conclusion: LC-MS metabolomic analysis is a powerful tool for investigating clinical samples. We identified clear changes in the CSF metabolome related to both ALL and ALL therapy, and present two new potential biomarkers for the presence of CNS ALL. Prospective analyses in independent cohorts are required to determine the clinical utility of these novel biomarkers for prediction of CNS relapse risk.

PS906

CLONAL EVOLUTION OF ACUTE LYMPHOBLASTIC LEUKEMIA CELLS FROM DIAGNOSIS TO RELAPSE

S. Sayyab[1,*], A. Lundmark[1], M. Larsson[2], M. Ringnér[3], J. Abrahamsson[4], L. Fogelstrand[5], K. Pokrovskaja[6], M. Heyman[7], E. Forestier[8], G. Lönnerholm[9], E.C. Berglund[10], A.-C. Syvänen[1]

[1]Dept. of Medical Sciences, Molecular Medicine and Science for Life Laboratory, Uppsala University, Uppsala, [2]Dept. of Physics, Chemistry and Biology, National Bioinformatics Infrastructure Sweden, Science for Life Laboratory, Linköping University, Linköping, [3]Dept. of Biology, National Bioinformatics Infrastructure Sweden, Science for Life Laboratory, Lund University, Lund, [4]Dept. of Pediatrics , Queen Silvia Children´s Hospital, [5]Sahlgrenska University hospital, Gothenburg University, Gothenburg, [6]Dept of Oncology-Pathology, Karolinska Institute, [7]Childhood Cancer Rsch. Unit, Karolinska University Hospital, Stockholm, [8]Dept. of Medical Biosciences, Umeå University, Umeå, [9]Dept. of Women's and Children's Health, Pediatric Oncology, Uppsala , [10]Dept. of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden

Background: Acute lymphoblastic leukemia (ALL) is the most frequent pediatric cancer. ALL originates from the malignant transformation of B- or T-cell lymphocyte progenitors into leukemic cells. ALL cells are characterized by large-scale chromosomal aberrations that define genetic subtypes of the ALL patients. Relapsed ALL presents a survival rate of ~45% and remains one of the leading causes of cancer-related death in children. Our previous results of whole genomes (WGS) and transcriptomes (RNA-seq) of diagnostic ALL samples (Lindqvist *et al*., 2015) and targeted sequencing of 872 cancer genes of diagnostic and relapse ALL samples (Lindqvist *et al*., 2016) revealed KMT2D and ATRX as novel putative driver genes in ALL, and EP300, ARID1A and SH2B3 as novel relapse-associated genes.
Aims: To determine clonal heterogeneity and clonal evolution patterns in ALL patients from diagnosis to relapse, using whole-genome sequencing, transcriptome sequencing and "linked-read" single-cell RNA sequencing and to increase the understanding of the molecular mechanisms that lead to relapse in pediatric ALL.
Methods: Here, we have performed WGS and RNA-seq of samples collected at diagnosis and relapse and at remission of ALL from 29 patients of six different BCP-ALL subtypes and T-ALL. For 9 of the patients, relapse samples at two time points were sequenced. RNA-seq will be used to determine the expression of somatic mutation in protein-coding regions and non-coding regions regulating gene expression. To resolve large, complex structural rearrangements in the ALL cells that remain undetected in the WGS data, we will use "linked-read" sequencing (Chromium, 10xGenomics) for genome-scale phasing (haplotyping).
Results: We have found 10 driver genes in our diagnostic cohort including 7 previously known in ALL, such as *NRAS, KRAS, ETV6, PTPN11, PTEN, FBXW7, SH2B3* and three that appear to be novel candidate driver genes in ALL. In the current study, we identified somatic driver mutations in coding and non-coding regions of the ALL genomes, aided by recently established genomic resources with data on functional genomic regions in relevant cell types, including ALL cells.
Summary/Conclusion: By combining the results from WGS, RNAseq and SNP-genotyping data for each individual, our results will provide novel insight into the mechanisms such as clonal competition and evolutionary adaptation that govern the treatment resistance in ALL.

PS907

REFINED DETECTION AND PHASING OF STRUCTURAL ABERRATIONS IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA WITH LINKED-READ WHOLE GENOME SEQUENCING

J. Nordlund[1], Y. Marincevic-Zuniga[1,*], L. Cavelier[2], A. Raine[1], T. Martin[1], A. Lundmark[1], J. Abrahamsson[3], E. Forestier[4], G. Lönnerholm[5], A.-C. Syvänen[1]

[1]Dept. of Medical Sciences, Molecular Medicine, [2]Dept. of Immunology, Genetics and Pathology, Uppsala University, Uppsala, [3]Dept. of Pediatrics, Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, [4]Dept. of Medical Biosciences, University of Umeå, Umeå, [5]Dept. of Women's and Children's Health, Pediatric Oncology, Uppsala, Sweden

Background: Structural chromosomal rearrangements that may lead to in-frame gene-fusions represent a leading source of information for diagnosis, risk stratification, and prognosis in pediatric acute lymphoblastic leukemia (ALL). The information on genetic variation from standard whole-genome sequencing (WGS) remains incomplete due to the inability of short sequence reads to accurately detect or phase large-scale chromosomal aberrations to the individual chromosome of each homologous pair in diploid ALL genomes.
Aims: To evalute "linked-read" WGS (lrWGS) for the detection of pathogenic structural aberrations in a set of well-characterized patients with pediatric ALL.
Methods: By sequencing library preparation from single DNA molecules (10X Genomics) in combination with short-read WGS (Illumina) we performed "linked-read" WGS (lrWGS) for unbiased detection of somatic chromosomal rearrangements in an ALL cell line (REH) and primary samples of varying DNA quality from 12 patients diagnosed with ALL. By analysis of the data from lrWGS we assessed the effect of input DNA quality on phased haplotype block size and the detectability of copy number aberrations (CNAs) and structural variants (SVs).
Results: We successfully detected all known pathogenic variants in the 13 analyzed DNA samples using lrWGS. By analysis of high-molecular weight DNA (~127 Kb average size) we detected phased haplotype blocks spanning over 18 megabases of genomic DNA with up to 99% of phased SNPs at a WGS coverage over 20x. Biobanked DNA isolated by standard column-based extraction methods (~56 Kb average size) was sufficient to detect chromosomal rearrangements associated with ALL pathogenesis even at as low as 10x sequencing coverage, as well as aneuploidy assessment comparable to that of microarrays. The genomic breakpoints of two balanced translocations involving the *ZNF384* gene were detected at single base-pair resolution. With use of haplotype information from the linked-reads, we also identified previously unknown structural variants, such as compound heterozygous deletions of *ERG* in a patient with the *DUX4-IGH* fusion gene.
Summary/Conclusion: Linked-read WGS allows detection of pathogenic variants in ALL genomes at a resolution beyond that of traditional karyotyping or short-read WGS. Refined accuracy at ALL diagnosis will allow more precise risk stratification of the ALL patients and will potentially translate into more effective therapy with less adverse drug effects in the future.

PS908

AMPLICON-BASED NGS AS A TOOL FOR THE IDENTIFICATION OF NEW MARKERS WITH POOR PROGNOSIS IN B-ALL

A. Montaño[1,*], M. Forero[2], J. Hernández-Sánchez[1], A. E. Rodríguez-Vicente[1], E. Lumbreras[1], M. Martín[1], J. L. Fuster[3], N. de Las Heras[4], A. García-de Coca[5], M. Sierra[6], J. Dávila[7], M. Vargas[8], J.A. Queizan[9], J. Labrador[10], J. Martínez[11], T. González[12], S. Riesco[13], J.M. Hernández-Rivas[12], R. Benito[1]

[1]Molecular genetics in onco-hematology Research Group, Institute for Biomedical Research of University of Salamanca (IBSAL) - University of Salamanca, Salamanca, Spain, [2]School of Biological Sciences (GICBUPTC research group), Pedagogical and Technological University of Colombia (UPTC), Colombia, Colombia, Colombia, [3]Department of Pediatric Oncohematology, Clinical University Hospital Virgen de la Arrixaca, Murcia, Spain, Murcia, [4]Department of Hematology, Virgen Blanca Hospital, León, Spain, León, [5]Department of Hematology, Clinical University Hospital of Valladolid, Valladolid, Spain, Valladolid, [6]Healthcare Complex of Zamora, Zamora, Spain., Zamora, [7]Department of Hematology - University Hospital of Salamanca, Salamanca, Spain, Salamanca, [8]Department of Hematology - Hospital Jarrio, Asturias, Spain, Asturias, [9]Hematology and Hemotherapy Service - Segovia Health-Care Complex, Segovia, Spain, Segovia, [10]Department of Hematology, University Hospital of Burgos, Burgos, Spain, Burgos, [11]Department of Hematology, [12]th October University Hospital, Madrid, Spain, Madrid, [12]Department of Hematology, University Hospital of Salamanca, Salamanca, Spain, [13]Department of Pediatrics, University Hospital of Salamanca, Salamanca, Spain, Salamanca, Spain

Background: B-cell precursor Acute Lymphoblastic Leukemia (B-ALL) is a neoplasm that can present both children and adults, being more frequent in childhood (80%). Despite the increase in the survival rate in patients, it is necessary to identify new markers that explain the cases with relapse or that do not respond to treatment.
Aims: Evaluation of an amplicon-based next-generation sequencing panel for detection of mutations in ALL patients.
Methods: A total of 116 samples with B-ALL were analyzed at the time of diagnosis or prior to treatment. All patients were treated according to PETHEMA and SEHOP protocols and minimal residual disease (MRD) data were available in more than 70% of cases. Sequencing was carried out by means of a panel of amplicons (Illumina) that allowed the analysis of 52 genes with importance in B-ALL.
Results: 1) A mutation rate of 84.4% was identified. The genes most frequently mutated were: *KRAS* (17.5%), *NRAS* (14.6%), *PTPN11* and *STAG2* (10.7%) and *JAK2* and *JAK3* (9.7%). There were differences between children and adults. In children, the most recurrent mutations were in *KRAS* (28.3%) and *NRAS* (19.6%), *JAK3* and *STAG2* (13%). While in adults the most frequently mutated genes were *TP53* (14%), *JAK2* (12.3%), *NRAS*, *PAX5* and *PTPN11* (10.5%). 2) Mutations in *RAS* pathway were frequently detected in *BCR-ABL1* negative cases (p=0.001). The univariate survival analysis in whole B-ALL cohort showed that mutations in *TP53* were associated with lower OS (p=0.001), EFS (p=0,005) and RFS (p=0.001), *JAK2* mutations with a decreased EFS (p=0.034) and RFS (p=0.017), NF1 mutations were associated with lower OS (p=0.006), EFS (p=0.017) and RFS (p=0.017), while mutations in epigenetic regulators and chromatin structure modifiers (*SETD2*, *PHF6*, *IDH2*, *EZH2* and *CREBBP*) showed a lower OS (p=0.05) (Figure 1). In the multivariate analysis of the whole B-ALL cohort, mutations in *NF1* were an independent risk factor associated with shorter OS (HR=11.583; p=0.015), EFS (HR=11.310; p=0.006) and RFS (HR=24.718; p=0.002). Mutations *TP53* and *JAK2* were an independent risk factor associated with shorter EFS (HR=10.439; p=0.047) and RFS (HR=4.598; p=0.018) and mutations in *TP53* were an independent risk factor associated with shorter RFS (HR=13.830; p=0.034).

Figure 1.

Summary/Conclusion: The mutations in *NF1*, *JAK2* and *TP53* could be considered as novel biomarkers associated with poor prognostic in B-ALL patients.

PS909

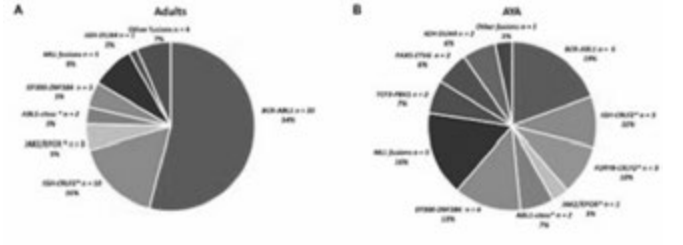
EXPLORING THE GENOMIC DIVERSITY OF AYA AND ADULT HIGH-RISK B-ALL CASES BY MRNA SEQUENCING

S.L. Heatley[1,2,3,4,*], B. Mayne[1], B.J. McClure[1,2], C. Kok[1,2], T. Sadras[1,2], P. Dang[1], M. Osborn[4,5,6], T. Revesz[4,5], N. Shanmuganathan[1,6], T.P. Hughes[1,2,6,7], D.T. Yeung[1,2,6,7], D.L. White[1,2,4,7,8]

[1]Cancer Theme, South Australian Health & Medical Research Institute, [2]Faculty of Health & Medical Science, University of Adelaide, Adelaide, [3]The Kids' Cancer Project, Sydney, [4]Australian Children's Haematology/Oncology Group , Melbourne, [5]Dept of Haematology, Women's & Children's Hospital, [6]Dept of Haematology, Royal Adelaide Hospital, Adelaide, [7]Australasian Leukaemia & Lymphoma Group, [8]Australian Genomics Health Alliance, Melbourne, Australia

Background: Acute lymphoblastic leukemia (ALL) remains one of the most challenging malignancies in adults, with more than 60% of patients suffering disease relapse. A comprehensive understanding of the genomic factors that influence leukaemogenesis and disease relapse is required to both improve outcomes in high-risk subtypes and guide the development of new therapeutic approaches.

Aims: The aim of this study was to identify the frequency of genomic alterations in adolescent/young adults (AYA) (16-39 years) and adults (>40 years) with B-ALL.
Methods: Samples from 63 AYA (ranged between 16-39 years, with a median age of 21) and 63 adult patients (40-88 years, median age of 59) underwent mRNA sequencing (mRNAseq). The cohort also contained Philadelphia (Ph)+ samples (n=33 adults, n = 6 AYA) but these were not sequenced. Fusions were identified using FusionCatcher, SOAPfuse and JAFFA. Variants were called using GATK HaplotypeCaller and underwent several filtering steps to eliminate possible germline alterations and common SNPs.
Results: Structural genomic abnormalities were identified in 112/126 (89%) samples by mRNAseq, including a number of clinically relevant, known oncogenic lesions associated with high-risk malignancy. Many of these lesions are considered to be Ph-like (15/63, 24% of adults and 9/63, 14% of AYA), with the majority of these being *CRLF2* rearrangements (*CRLF2*r) (n = 16). Together with Ph+ ALL, these two subsets define the bulk of the adult cohort. *MLL* rearrangements were also found in both AYA (n=5) and adults (n=5). Other recurrent high-risk lesions include variants identified in *NRAS, KRAS, PTPN11, NF1, TP53, JAK2, CSF1R, RB1, CREBBP, RUNX1, FLT3, NOTCH1* and *NOTCH2* with an average of 1-2 variants per patient and slightly more occurrences found in adults (n=90) than AYA (n=73). Broadly, many of these lesions inactivate tumor suppressor genes or activate kinase, cytokine or other pathways likely to be involved in leukemogenesis. Of significance, 21/63 (33%) AYA patients harbored *RAS* mutations compared to 10/63 (16%) adults (p=0.037). *TP53* mutations were found in only 2/63 (3%) AYA yet were present in 13/63 (21%) adults (p=0.004). Somatic *TP53* mutations have been reported in hypodiploid ALL patients and all *TP53* mutations reported here occurred within the DNA-binding domain, critical to biological activity. Interestingly, in patients with *TP53* mutations no concomitant gene fusions could be identified. While Ph+ and some Ph-like ALL patients may benefit from adding appropriate tyrosine kinase inhibitors to their chemotherapy, for many of the other gene fusions and alterations identified here, suitable targeted agents may not yet be clinically available (Figure 1).



Gene fusions identified in adults and AYA B-ALL patients. Fusions found in (A) adults and (B) AYA by mRNA seq and including Ph+ patients. Fusions that may be considered as Ph-like ALL are indicated by a *. Other fusions in adults included *TCF3-PBX1, IGH-MYC, TRIM24-FGFR1* and *DDX3-CXCR4* (one each) and in AYA 1 x *TCF3-MKNK2*.

Figure 1.

Summary/Conclusion: In this adult/AYA cohort we demonstrate that the majority of high-risk B-ALL cases harbor rearrangements or mutations in genes associated with malignant transformation. Importantly, Ph+ and Ph-like ALL, both high-risk subtypes, represent the majority of adult B-ALL cases. In the remaining patients, significant associations were found with *RAS* mutations in AYA and *TP53* mutations in adults. Furthermore, mRNAseq may eliminate discrepancies seen with different algorithms currently used to identify Ph-like ALL and may provide a deeper understanding of the genomic alterations associated with high-risk B-ALL. The increasing genomic complexity of high-risk B-ALL highlights the need for continued efforts to identify appropriate targeted therapies.

PS910

FUNCTIONAL CHARACTERIZATION OF ABERRANT FAT1 EXPRESSION IN T-ALL

S. Liebig[1,*], M. Neumann[1,2,3], J. Ortiz Tanchez[1], K. Isaakidis[1], C. Schlee[1], M.P. Schroeder[1], L. Bastian[1,2,3,4], L. Mochmann[1], C.D. Baldus[1,2,3,4]

[1]Department of Hematology and Oncology, Charité, University Hospital Berlin, Campus Benjamin Franklin, Berlin, [2]German Cancer Research Center (DKFZ), [3]German Cancer Consortium (DKTK), Heidelberg, [4]Berlin Institute of Health, Berlin, Germany

Background: T-cell acute lymphoblastic leukemia (T-ALL) has an unfavorable outcome in adult patients. The protocadherin FAT1 is frequently mutated and aberrantly expressed in many cancer types, including acute lymphoblastic leukemia (ALL). We have previously described FAT1 mutations in 16% of T-ALL patients. Aberrant expression occurred in 54% of T-ALL patients, in contrast to T-cells and progenitors from healthy donors lacking FAT1 expression. Moreover, it has been reported that FAT1 expression was associated with a shorter relapse-free and overall survival in ALL. Thus, FAT1 is considered a promising candidate for future targeted therapy, yet its functional consequences are unclear in T-ALL.
Aims: This study investigates the functional role of FAT1 in T-ALL.
Methods: FAT1 gene expression profiles were generated using a microarray dataset of adult T-ALL (Affymetrix U133 Plus 2.0; GSE78132, n=83) and an independent cohort of adult T-ALL samples analyzed by RNA-seq (n=84). Gene Set Enrichment Analyses (GSEA) and pathway enrichment analyses for genes coexpressed with FAT1 (ANOVA, p<0.01) were performed. The effect of FAT1 expression was studied by knockdown of FAT1 with siRNA and FAT1 overexpression of a functional truncated protein (kindly provided by Morris, L.G. *et al.*, 2013) in T-ALL cell lines. WNT pathway activity was modulated by treatment with 6-Bromoindirubin-3'-oxime (BIO), XAV-939 and CHIR00921 at 1μM, 5μM and 10μM for 48h in T-ALL cell lines. Proliferation was assessed by WST-1 assay. Downstream gene expression effects were analyzed by RT-PCR.
Results: RNA-seq based profiling revealed high expression of FAT1 in 53% of T-ALL patients (defined as FAT1$^+$, median TPM 155, range 36-1368) whereas 47% had negative FAT1 expression (FAT1$^-$, median TPM 0.5, range 0-27). GSEA using the Affymetrix data set and validated by the RNA-seq data, exhibited a positive enrichment for mature T-cell signatures (p=0.025) in FAT1$^+$ samples. In contrast, FAT1$^-$ samples correlated with early T-cell precursor ALL (p<0.001) and hematopoietic stem cell (p=0.064) signatures. FAT1$^+$ T-ALL samples had higher levels of immunophenotype maturation markers CD4 (p<0.001), CD8 (p=0.003) and CD1 (p=0.005) harboring a distinct expression pattern. Pathway enrichment analyses revealed coregulated genes with a strong enrichment for DNA replication (p<0.001), cell cycle (p<0.001), T cell receptor signaling (p<0.001), pathways in cancer (p=0.011) and WNT signaling pathway (p=0.036) compared to FAT1$^-$ samples. FAT1 was expressed in 6/7 tested T-ALL cell lines with significantly higher mRNA expression in the more mature T-ALL cell lines Jurkat, HPB-ALL and MOLT4 in contrast to immature cell lines RPMI-8402, BE13, Loucy and PER117. Functional analyses for FAT1 knockdown led to a FAT1 downregulation of 64% (p<0.01) compared to control siRNA in Jurkat, resulting in 14% reduction (p=0.02) of proliferation after 72h. FAT1 overexpression resulted in a proliferative advantage with a 42% increase (p=0.02) compared to WT controls in Jurkat. Furthermore, FAT1 knockdown mediated a downregulation of WNT target genes CCND1 (p=0.005), LEF1 (p=0.016), JUN (p=0.036) and IGF2 (p=0.02) compared to controls. Treatment of T-ALL cell lines with WNT pathway activating drugs BIO and CHIR00921 caused a dose-dependent upregulation of FAT1, yet WNT pathway inhibitor XAV-939 led to a FAT1 downregulation.
Summary/Conclusion: Aberrant FAT1 expression, associated with maturation, induces proliferation and WNT pathway interaction in T-ALL. This study contributes to understanding the role of FAT1 in T-ALL biology aiding in further therapeutic approaches.

PS911

"TRIPLE NEGATIVE" ACUTE GENE EXPRESSION CLUSTERING IDENTIFY NEW ADULT LYMPHOBLASTIC LEUKEMIA SUBGROUPS

A. Ferrari[1,*], S. Vitali[2], V. Robustelli[1], A. Ghelli Luserna di Rorà[1], S. Righi[3], G. Pasquini[2], C. Papayannidis[1], G. Marconi[1], G. Ferrari[1], E. Imbrogno[1], A. Santoro[4], J. Hernández-Rivas[5], C. Baldazzi[1], M. Abbenante[1], S. Paolini[1], N. Testoni[1], G. Castellani[2], E. Sabattini[3], M. Cavo[1], D. Remondini[2], G. Martinelli[6]

[1]DIMES, Institute of Hematology "L. e A. Seràgnoli", [2]Fisica e Astronomia, Bologna University, [3]DIMES, Hematopathology Unit, Bologna, [4]Hematology, A.O. Ospedali Riuniti Villa Sofia-Cervello, Palermo, Italy, [5]Fundación de Investigación del Cáncer , Universidad de Salamanca, Salamanca, Spain, [6]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori , IRST-IRCCS, Meldola (FC), Italy

Background: Although the remarkable progress, there is a need in improving the molecular dissection of subtypes, identifying genetic alterations that predict the risk of treatment failure and developing novel and targeted therapies. B-ALL patients (pts) that doesn't have the most recurrent adult rearrangements (*BCR-ABL1* t(9;22)*; TCF3-PBX1* t(1;19); *MLL-AF4* t(4;11)) are collectively referred to as "triple negative" (Ph-/-/-) ALL.
Aims: Biological characterization of Ph-/-/- ALL considering *CRLF2* overexpression event (that represents near to 57% of B-ALL; Roberts KG, J Clin Oncol. 2016), in in order to define and assess biomarkers in this subgroup to test new drugs.
Methods: Gene Expression Profiling (GEP; HTA 2.0 Affymetrix) were per-

formed on 60 Ph-/-/- ALL, 30 B-ALL Ph+ at different time point of the disease and on 7 mononuclear cell of healthy donors. Data were normalized and analyzed with the Expression Console and the Transcriptome Analysis Console (TAC) Software (Affymetrix). Successively we cluster triple negative GEP data with our validated pipeline, based in a top ten gene list. Ph-/-/- ALL samples were then characterized for the presence of gene fusions, Copy Number Alterations (CNAs) and mutations using different approaches (TruSight Pancancer-Illumina; MLPA and/or dMLPA-MRC-Holland; SNP Array-Affymetrix and PCR).

Results: Comparing all GEP of Ph-/-/- to donors we found some top upregulated genes to focus on (e.g. *EBF1, PDLIM1, PXDN, TCL1A, BLNK, CD19, RASD1, NAV1, DNTT, SOCS2, CTGF, CD200, CRLF2*). In triple negative ALL GEP top upregulated gene analysis we identify a defined 2-clusters-subdivision (Gr1 and Gr2; Figure 1) furthermore a third group, in the Gr1, can be identified by the algorithm without ambiguous assignments. The Gr2 is characterized by *CTGF, CRLF2* and *CD200* overexpression and it represents 12.2% of all B-ALL. Two groups t-test has been performed between Ph+ and the isolated subgroups of Ph- to determine the similarity of these two groups to Ph+. The Gr2 GEP is similar to Ph+ one. Fusion and mutational screening done, detected that Gr2 has a higher frequency of Ph-like associated lesions, that mainly affect JAK-STAT pathway. Also *IKZF1* deletion are significantly associated to Gr2 (p=0.0028).

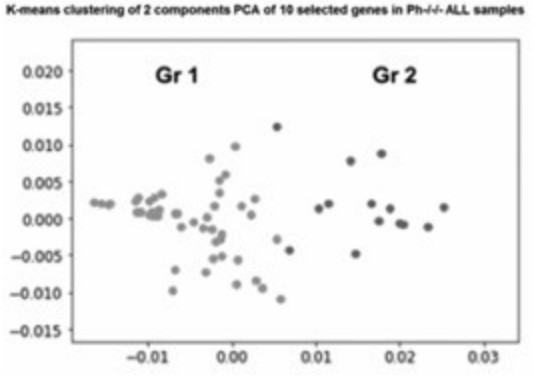


Figure 1.

Summary/Conclusion: We identified a new signature, related to *CRLF2* high expression, to classify Ph-/-/- ALL B-based on 10 genes. Gr2 represents 12.2% of all B-ALL and it is characterized by a) high co-expression of three main genes: *CRLF2*, *CTGF* and *CD200;* b) *IKZF1* deletion (CDK2 inhibitor downregulate *CRLF2* overexpression in *IKZF1* deleted ALL); c) JAK-STAT pathway mutations/fusions/deletions. Gr1 represents 45.4% of all B-ALL. Gr2 GEP similarity to Ph+ one, suggests that this Gr2 could contain Ph-like pts. This new Ph-/-/- subclassification identify new potential therapeutic targets with available drug (α-CTGF, α-CD200, CDK2 inhibitor; tyrosine kinase inhibitors already effective on Ph+ and Ph-like) to test as a single agent or in combination. Supported by: ELN, AIL, AIRC, project Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project, HARMONY project, Fondazione del Monte BO e RA project.

PS912

MOLECULAR PROFILE REFINES THE MRD-BASED PROGNOSTIC ASSESSMENT IN ADULTS WITH PHILADELPHIA NEGATIVE B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

J. Ribera[1,*], M. Morgades[1], L. Zamora[1], S. Vives[1], M. Batlle[1], M. Cabezón[1], S. Marcé[1], D. Domínguez[1], E. Genescà[1], P. Montesinos[2], I. Gómez-Seguí[2], S. Mercadal[3], R. Guàrdia[4], J. Nomdedeu[5], M. Pratcorona[5], M. Tormo[6], J. Martínez-Lopez[7], J.-M. Hernández-Rivas[8], J. Ciudad[8], J. González-Campos[9], P. Barba[10], J. Esteve[11], L. Escoda[12], F. Solé[1], E. Feliu[1], A. Orfao[8], J.-M. Ribera[1]

[1]Clinical Hematology Department, Catalan Institute of Oncology at Hospital Germans Trias i Pujol, Jose Carreras Leukaemia Research Institute, Badalona, [2]Clinical Hematology Department, Hospital La Fe, Valencia, [3]Clinical Hematology Department, Catalan Insitute of Oncology at Hospital Duran i Reynals, Hospitalet de Llobregat, [4]Clinical Hematology Department, Catalan Institute of Oncolgy at Hospital Josep Trueta, Girona, [5]Clinical Hematology Department, Hospital Sant Pau, Jose Carreras Leukaemia Research Institute, Barcelona, [6]Clinical Hematology Department, Hospital Clínico, Valencia, [7]Clinical Hematology Department, Hospital Doce de Octubre, Universidad Complutense, CNIO, Madrid, [8]Hospital Clínico Universitario de Salamanca, Institute of Biomedical Investigation of Salamanca, Institute of Molecular and Cellular Biology of Cancer, Center of Investigation of Cancer, Spanish National Research Council at University of Salamanca, Salamanca, [9]Clinical Hematology Department, Hospital Vírgen del Rocío, Sevilla, [10]Clinical Hematology Department, Hospital Vall d'Hebron, [11]Clinical Hematology Department, Hospital Clínic, IDIBAPS, Barcelona, [12]ClinicalHematology Department, Catalan Institute of Oncology at Hospital Joan XXIII, Tarragona, Spain

Background: Minimal residual disease (MRD) is the most important prognostic factor in acute lymphoblastic leukemia (ALL) at all ages. However, around 25% of MRD-negative adult patients ultimately relapse, suggesting that MRD has limitations for prognostic assessment. Molecular characterization may contribute to more precise risk stratification. Unfortunately, reliable molecular prognostic markers are still warranted, especially for Ph-negative B-cell precursor ALL (Ph-neg BCP-ALL).

Aims: To identify molecular abnormalities with prognostic significance in a series of homogeneously treated adult Ph-neg BCP-ALL patients.

Methods: Multiplex Ligation-dependent Probe Amplification (MLPA) was performed for the most recurrent copy number alterations in 128 adult Ph-neg BCP-ALL patients treated according to PETHEMA protocols. MRD was centrally evaluated by flow cytometry and good early response was considered when MRD was $<10^{-3}$ at the end of induction (End Ind). Outcome was also assessed in cases with MRD$<10^{-4}$ at End Ind.

Table 1.

Outcome variable	Variables	HR (IC95%)	p
Overall Survival including $<10^{-3}$ MRD at the end of induction	Age	**1,036** (1,013 ; 1,060)	0,002
	WBC	**1,007** (1,003 ; 1,011)	<0,001
	MRD≥10^{-3}	**2,223** (1,121 ; 4,410)	0,022
Overall Survival including $<10^{-4}$ MRD at the end of induction	Age	**1,048** (1,020 ; 1,076)	0,001
	WBC	**1,006** (1,003 ; 1,009)	0,001
	MRD≥10^{-4}	**2,605** (1,267 ; 5,355)	0,009
	CDKN2A/B	**2,003** (1,001 ; 4,006)	0,050
Disease Free Survival including $<10^{-3}$ MRD at the end of induction	Age	**1,026** (1,005 ; 1,047)	0,015
	WBC	**1,007** (1,003 ; 1,010)	<0,001
Disease Free Survival including $<10^{-4}$ MRD at the end of induction	Age	**1,022** (1,002 ; 1,043)	0,033
	WBC	**1,006** (1,003 ; 1,010)	<0,001
Cumulative Incidence of Relapse including $<10^{-3}$ MRD at the end of induction	WBC	**1,007** (1,004 ; 1,01)	<0,001
Cumulative Incidence of Relapse including $<10^{-4}$ MRD at the end of induction	WBC	**1,007** (1,004 ; 1,01)	<0,001

Results: At baseline, patients with intragenic IKZF1 deletion showed higher cumulative incidence of relapse (CIR) than patients without deletion (5-year CIR 83% [45%; 96%] vs. 43% [31%; 54%] p=0.005). Patients with CDKN2A/B deletion had poorer outcomes than those without deletion (5-year OS 34% [20%; 48%] vs. 57% [43%; 71%], p=0.042; 5-year DFS 25% [12%; 38%] vs. 47% [33%; 61%], p=0.029; 5-year CIR 56% [40%; 70%] vs. 41% [27%; 54%], p=0.090). Patients with both IKZF1 and CDKN2A/B deletions had an extremely high CIR (5-year CIR 91% [29%; 99%] vs. remaining, p=0.003).

MRD data at End Ind were available for 75/128 patients. Patients with MRD ≥10^{-3} (n= 19/75, 25%) showed a trend for poorer OS than those with good early response (5-year OS 26% [5%; 47%] vs. 56% [35%; 63%], p=0.175). Fewer patients with IKZF1 deletion achieved CR than patients without deletion (18/25 vs. 77/88, p=0.064) and more patients with IKZF1 deletion had MRD>10^{-3} at End Ind compared to patients without deletion (5/11 vs. 13/53, p=0.150). Among patients with good early response (MRD<10^{-3}) at End Ind, those with CDKN2A/B deletion had lower OS and DFS than those without CDKN2A/B loss (5-year OS 37% [19%; 55%] vs. 61% [42%; 80%], p=0.062 and 5-year DFS 24% [7%; 41%] vs. 51% [32%; 70%], p= 0.041). Patients with MRD<10^{-4} at End Ind, showed better outcome than those with MRD≥10^{-4} (5-year OS 48% [31%; 65%] vs. 29% [12%; 46%], p=0.136). CDKN2A/B deletion was also a significant marker of poor prognosis among patients with MRD<10^{-4} at End Ind (5-year OS DEL 32% [11%; 53%] vs WT 70% [45%; 95%], p= 0.018; 5-year DFS

DEL 9% [0%; 24%] vs. WT 66% [42%; 90%], p=0.006; 5-year CIR DEL 70% [36%; 89%] vs. WT 27% [(%; 52%], p=0.048) and showed independent prognostic significance in multivariate analysis (Table 1).
Summary/Conclusion: Molecular markers such as CDKN2A/B add prognostic information independent of MRD in adult patients with Ph-negative BCP-ALL. Combined MRD and molecular data should be used for risk stratification and treatment assignment.

PS913

TCR ALPHA GENE REARRANGEMENTS AS A NEW TYPE OF CLONAL MARKERS IN PEDIATRIC T-LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA

A. Komkov[1,2,*], A. Miroshnichenkova[1], G. Nugmanov[2], Y. Lebedev[2], Y. Olshanskaya[1], I. Mamedov[2], M. Maschan[1]

[1]National Research Center of Pediatric Hematology, Oncology and Immunology, [2]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russian Federation

Background: Relapse is still the major cause of poor outcome in therapy of T-lineage acute lymphoblastic leukemia (T-ALL) in children. According to the previously published data, up to 30% cases of T-ALL relapses in T-ALL are accompanied by changing of the major leukemic clones because of the "bottle-neck" caused by chemo-therapy. At the present time the most reliable markers for analysis of clonal architecture of T-ALL in debut as well as in relapse are rearrangements of immunoglobulin and T-cell receptors (TCR) genes: TCR beta, TCR gamma and TCR delta. However, involvement of rearrangements of fourth TCR genes family – TCR alpha genes – in clonal diversity formation in T-ALL remains unclear.
Aims: The main purposes of this study are identification and characterization of clonal TCR alpha locus rearrangements in pediatric T-ALL and analysis of possibilities to use them for precise reconstruction of clonal structure of T-ALL together with other TCR genes (first of all TCR beta) rearrangements.
Methods: Detection of TCR alpha and TCR beta genes rearrangements was based on targeted high-throughput sequencing (HTS). Amplicons for HTS was obtained in series of multiplex PCRs with genomic DNA isolated from bone marrow (BM) and degenerate primers covering the vast majority of all V- and J-genes combinations of both TCR alpha and TCR beta loci. Identification of rearrangements specific for leukemic clones was performed based on analysis of clone frequencies in obtained TCR repertoires using 5% cut-off.
Results: We analyzed a cohort consisted of 75 patients aged 2-18 years with T-ALL diagnosis. We identified over 90 clonal rearrangements of TCR alpha locus and over 120 clonal rearrangements of TCR beta locus in total. 15 samples contained TCR alpha rearrangements with clonal rate more than 40%, 37 samples contained 1-4 TCR alpha rearrangements with clonal rate 5-35%, 23 samples didn't contain any leukemia specific TCR alpha rearrangements. In the same time, 53 analyzed samples contained 1-2 TCR beta rearrangements with clonal rate above 40%, 10 samples contained TCR beta rearrangements with clonal rate 5-35% and the rest ones didn't contain leukemia specific TCR beta rearrangements. The vast majority of TCR alpha rearrangements was detected in samples with dominant TCR beta rearrangements. Samples without TCR beta rearrangements also didn't contain rearranged TCR alpha loci.
Summary/Conclusion: For the first time we evaluated frequency of occurrence of clonal rearrangements of TCR alpha locus in pediatric T-ALL. This type of clonal markers is presented in more than half of T-ALL cases what makes them fully applicable for analysis of clonal structure of T-ALL along with other TCR genes rearrangements. Detection of rearranged TCR alpha loci with rearranged TCR beta loci in the same samples allow to increase resolution of clonal structure reconstruction in T-ALL in both onset and relapse. This research was supported by Russian Science Foundation grant #17-75-10113, Russian Foundation for Basic Research grants #17-04-01280 and # Russian President's Fellowship SP-4059.2016.4.

PS914

Abstract withdrawn.

PS915

COHESIN GENES MUTATIONS IN ACUTE LYMPHOBLASTIC LEUKEMIA

G. Fazio[1,*], V. Massa[2], A. Grioni[13], V. Bystry[3], S. Rigamonti[1], C. Saitta[1], M. Galbiati[1], C. Rizzari[4], C. Consarino[5], A. Biondi[4], A. Selicorni[6], G. Cazzaniga[1]

[1]Centro Ricerca Tettamanti, Fondazione Tettamanti, Monza, [2]Department of Health Sciences, University of Milan, Milan, Italy, [3]Central European Institute of Technology, CEITEC , Masaryk University, Brno, Czech Republic, [4]Pediatric Dept., Univ. Milano Bicocca, Monza, [5]Ematologia ed Oncologia Pediatrica, Presidio Ospedaliero Ciaccio-De Lellis, Catanzaro, [6]Pediatric Dept., Presidio S. Fermo, ASST Lariana, Como, Italy

Background: Cornelia de Lange syndrome (CdLS) is a rare genetic disorder characterized by pre- and post-natal growth retardation, mental retardation, facial dysmorphism and upper limb abnormalities. The main causes of the disease are mutations in *NIPBL*, *SMC1A*, *SMC3*, *HDAC8* and *RAD21* genes, which encode proteins of cohesin complex or associated to it. *NIPBL* is involved in about 55% of CdLS cases, while the others account for 5%. Mutations in the cohesin genes have recently been identified in AML, CML and myelodysplastic syndromes.
Aims: The present study aims to describe the first case of a CdLS pediatric patient who developed precursors B-cell Acute Lymphoblastic Leukemia (BCP-ALL). Furthermore, we investigated the presence of cohesin variants in pediatric BCP-ALL patients not affected by CdLS.
Methods: RNA NGS-Targeted Capture strategy was setup, by implementing the TruSight Pan-Cancer (Illumina, 1385 genes), which included cohesin genes complex, such as *NIPBL*, *SMC1A*, *SMC3*, *RAD21*, *STAG2*.
Results: The patient was previously diagnosed as CdLS based on clinical features, but without genetic characterization. When he was 8-years old, he developed the B-cell precursor ALL and was enrolled in the AIEOP-BFM ALL 2009 study protocol. The biological investigations performed on bone marrow blasts at onset did not reveal any prognostically relevant cytogenetic abnormalities and was enrolled to high risk treatment group for MRD analysis. Through NGS Trusight Pan-Cancer analysis, we identified two mutations in heterozygosity of *JAK3* in exon 1 (shared only with the father) and in exon 16 (shared with whole family in heterozygosity), respectively noted as rs7254346 (benign) and rs3213409 (known as benign if germline and somatic in ALL and AML). A germline mutation of *TP53* exon4, rs1042522, known as benign and potentially involved in chemotherapeutic resistance mechanisms was identified. This mutation is present in homozygosity also in the mother. More importantly, we have identified variants in cohesin genes, in particular in exon 46 of *NIPBL*, in heterozygosity. This *NIPBL* variant is a new mutation that causes frameshift and a premature stop codon. This anomaly was confirmed on the patient's bone marrow DNA both at diagnosis and in remission of leukemia and in a buccal smear sample. Both parents and brother are negative, phenotypically normal and not affected by hematological diseases. We further analyzed 86 pediatric BCP-ALL cases after the first CdLS-ALL patient. We analyzed cohesin complex genes, including *NIPBL*, *RAD21*, *SMC1A*, *SMC3*, *STAG2*, and overall we detected 36 variants, including recurrent known variants in addition to 10 novel variants, mainly affecting the *NIPBL* gene. PCR/Sanger validation is ongoing and already confirmed three variants out of ten.
Summary/Conclusion: It has commonly accepted that cancer originates by two or more mutations accumulated in somatic cells, and there is growing evidence of the association of germline genetic aberrations and cancer. Some cancer predisposing syndromes have been associated with ALL in children as for example Down syndrome, Noonan syndrome and Li- Fraumeni syndrome.
Although mutations in cohesins have been found in many tumor cells, in particular in AML, only few cases of cancer have been reported in patients affected by cohesinopathies. Nevertheless, the present study reports a CdLS pediatric patient with concomitant ALL. The role in leukemogenesis of the new NIPBL gene mutation, identified in the first CdLS pediatric patient with BCP-ALL, and the biological role in leukemia of cohesin genes deserves further investigation.

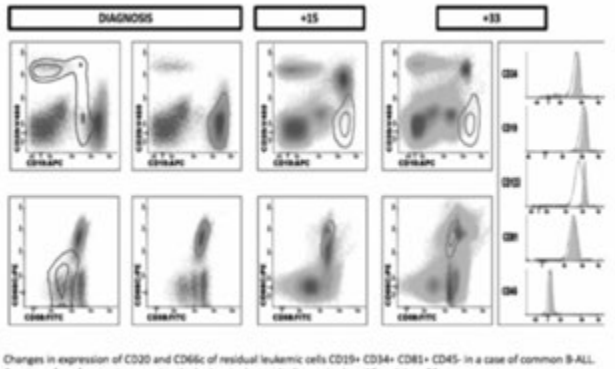
PS916

EVALUATION OF SAMPLE QUALITY AND CREATION OF DATABASES FOR FOLLOW-UP ASSESSMENT OF BLAST INFILTRATION AT DAY +15 IN CHILDHOOD B CELL ACUTE LYMPHOBLASTIC LEUKEMIA

P. Manresa[1,*], J. López[1], H. Sarmiento[1], F. López[1], O. Alda[1], E. Arranz[1], V. Rodríguez[1], I. Molina[1], M. Pérez[1], P. Ruiz-Sogorb[1], L. Blázquez[1], C. García[1], J.J. Verdú[1], F. De Paz[1], M. Del Cañizo[2], M. Tasso[2], C. Esquembre[2], P. Marco[1], F. Tarín[1]

[1]Department of Hematology, [2]Department of Pediatrics, Hospital General Universitario de Alicante, Alicante, Spain

Background: Evaluation of blast percentage on day +15 (BM+15) is critical in decision-making in patients with childhood B acute lymphoblastic leukemia (B-ALL). The current protocols establish risk criteria based on the optical count and minimal residual disease (MRD) evaluated by flow cytometry (FCM), both being critical in therapeutic decisions. However, different studies show the existence of relevant discordances in both optical and immunophenotypic count. These discrepancies are due to different aspects, highlighting sample contamination by peripheral blood (PB), lack of consensus in panels and strategies used, subjective interpretation of the results, and antigenic modulation produced by the active treatment, all of which can hinder the identification of residual population.
Aims: Investigate the significance of sample quality and immunophenotypic changes in residual/neoplastic populations, in data interpretation and final results in BM+14 samples.
Discuss the usefulness of the new multidimensional analysis tools applicable to the 8-color standardized FCM in the resolution of discrepancies.
Methods: Patients: 51 patients diagnosed of B-ALL (August 2013-February 2018) and treated according to the current SEOP-PETHEMA protocols. Estimation of contamination degree: evaluation of total cellularity (nucleated cells/µl) in bone marrow (BM) (ncBM) and PB (ncPB) measured the same day in order to estimate the BM contamination percentage by PB (% = ncPB /ncBM). Optical count: infiltration percentage evaluated by 3 independent observers. FCM panels: B-ALL panel proposed by Euroflow standards. Residual blasts count measured with 8 color FCM evaluated by 3 independent observers. FCM images are compared with normal sample databases (normal lymphoid maturation) and pathological (individual samples of each patient with the original pathological populations) (Figure 1).
Results: 1) The average percentage PB sample contamination was 26.03% (+/- 22.08%) almost 4 times higher than the day of control+33 (6.82 +/- 11.62%) reaching> 75% in 10% of the samples. The PB contamination degree correlated inversely with the erythroid precursors percentage (R2 = 0.613, p <0.05). 2) Optical count correlation was low among three different observers (R2 = 0.22 p= 0.06) and was not significant with FCM count (p> 0.1). 3) Relevant immunophenotypic changes were observed compared to the original leukemic population, highlighting an increased expression of CD20 (almost X10 more intense in 95% of cases) and CD45 (75%) as well as a decrease in CD10 (90%), CD66c (75%) and CD34 (60%). The antigenic expression of CD19, CD38, CD58 and CD123 remained stable. 4) Nonetheless, the FCM count correlation was very high among the different observers (R2 = 0.87 p=<0.01) using the tools previously described, as residual populations continued showing identifiable aberrant expression patterns when compared with databases of the different stages of normal lymphoid maturation.



Changes in expression of CD20 and CD66c of residual leukemic cells CD19+ CD34+ CD81+ CD45- in a case of common B-ALL. Creation of a reference image using the leukemic cluster. At diagnosis, day +15 and day +33

Figure 1.

Summary/Conclusion: 1) BM+15 samples present a relevant degree of PB contamination that can be evaluated by simple parameters such as PB/BM ratio and erythroid precursors percentage. 2) Identification of the residual population using "similarity criteria" to the original leukemic cells is exposed to relevant pitfalls that can be overcome using the new FCM interpretation tools. 3) The generalization of FCM standardized procedures and tools as well as the consensus of minimum sample quality criteria are necessary in order to reach diagnostic concordance and reproducibility.

PS917

GENETIC ALTERATIONS OF T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA IN TAIWAN: A COMPARISON BETWEEN PEDIATRIC AND ADULT COHORTS

Y.-J. Huang[1,*], T.-C. Yeh[2], H.-C. Liu[2], M.-C. Kou[1,3], T.-H. Jaing[3,4], S.-H. Chen[3,4], P.-N. Wang[1], T.-L. Lin[1], J.-Y. Hou[2], Y.-S. Hung[1], H. Chang[1,3], C.-P. Yang[4], L.-Y. Shih[1,3]

[1]Division of Hematology-Oncology, Chang Gung Memorial Hospital at Linkou, Taoyuan, [2]Division of Pediatric Hematology-Oncology, Mackay Memorial Hospital, Taipei, [3]College of Medicine, Chang Gung University, [4]Division of Pediatric Hematology-Oncology, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan, Republic of China

Background: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic disease and caused by multistep processes of genetic lesions. Recently, many recurrent genetic alterations have been identified by genome-wide approaches in children, however, those in adult T-ALL remain to be defined. There may be existence of significant differences between children and adults.
Aims: We aimed to analyze the frequencies of genetic alterations of different pathways involved in both children and adults with T-ALL in Taiwan and to determine whether different genetic lesions existed in the two cohorts.
Methods: Bone marrow samples obtained from 102 children (< 18 years) and 70 adult patients with T-ALL between 1994 and 2015 were examined. Fusion transcripts including *SIL-TAL1, CALM-AF10,* and *KMT2A*-rearranged (*KMT2A*-R) were detected by RT-PCR. RQ-PCR with TaqMan assay was used to measure the expression of transcription factor oncogenes, including *HOX11* (*TLX1*), *HOX11L2* (*TLX3*), *TAL1,* and *LYL1.* Detection of gene deletion or amplification was carried out with multiplex ligation dependent probe amplification kit (SALSA MLPA P383 T-ALL). The hotspot regions of *NOTCH1, FBXW7*, *JAK1*, *JAK2*, *PHF6*, *RUNX1*, *WT1*, *NRAS* and *KRAS* were amplified by PCR-based assays and followed by direct sequencing.
Results: Totally, 32 genetic alterations involving bHLH family, Homeobox family, LMO family, NOTCH1 pathway, cell cycle, signaling pathway, transcription factors, and epigenetic regulators were analyzed in Taiwanese children and adult patients with T-ALL. The frequency of genetic alterations involving NOTCH1 pathway (*NOTCH1* and *FBXW7* mutations) and transcription factors (*WT1* mutation, *RUNX1* mutation, *LEF1* deletion, *MLLT3* deletion, and *MYB* duplication) was similar in both cohorts. The four most common genetic abnormalities were *TAL1* over-expression, *P16* deletion, *P15* deletion, and *NOTCH1* mutation occurring in 74.7% and 64%, 63.3% and 35.6%, 50.0% and 35.3%, and 46.9% and 43.5%, respectively, of children and adults with T-ALL. Among bHLH, Homeobox and LMO family genes, *SIL-TAL1* fusion was more frequently detected in children (19.2% vs. 7.5%, p=0.043), whereas the frequencies of other genetic abnormalities including presence of *CALM-AF10, KMT2A-R,* or *RAG2-LMO2,* expression of *HOX11*, *LYL1*, and *TAL1* oncogenes, or *LMO1* amplification were not significantly different in both cohorts. A trend of *HOX11L2* over-expression in children compared with adults (17.2% vs. 6.0%, p=0.07) was observed. Of the cell cycle-related genes, *P16* deletion was more common in children than adults (p=0.001) and there was no difference in the frequency of *P15* or *MTAP deletion* between children and adults. Among the genetic alterations of signaling pathway (deletion of *NF1*, *CASP8AP2*, *PTEN*, or *PTPN2*, or mutations of *NRAS*, *KRAS*, *JAK1*, *JAK2*, or *NUP214-ABL1* fusion), only the frequency of *NF1* deletion was significantly different in the two cohorts (8.8% in adults vs. 0% in children, p=0.028). Of the three epigenetic regulator genes (*EZH2*, *PHF6*, and *SUZ12*), adult patients had a higher rate of *PHF6* mutation/deletion (34.1% vs. 16.5%, p=0.038) and *SUZ12* deletion (11.8% vs. 0%, p=0.008) than children, and a comparable frequency of *EZH2* deletion.
Summary/Conclusion: The present study showed that the genetic alterations in T-ALL were different between children and adults. *SIL-TAL1* fusion and *P16* deletion occurred more frequently in children whereas *PHF6* mutation and *NF1* or *SUZ12* deletion were more frequently present in adults.

Acute lymphoblastic leukemia – Clinical

PS918

PROGNOSTIC VALUE OF IKZF1 DELETIONS ON THE UKALL14 TRIAL; SIGNIFICANT ROLE OF BCR-ABL1 STATUS

R. Mitchell[1,*], A.A. Kirkwood[2], S. Lee[1], K. Zuborne Alapi[1], N. Zareian[3], P. Patrick[2], E. Lawrie[2], L. Clifton-Hadley[2], D.I. Marks[4], A. Douglas[2], C.J. Rowntree[5], T. Menne[6], A. K. McMillan[7], B. Wrench[8], A.V. Moorman[9], A.K. Fielding[1]

[1]UCL Cancer Institute, [2]CRUK and UCL Cancer Trial Centre, University College London, [3]Departmant of Haemato-Oncology, King's College, London, [4]Bristol Haematology and Oncology Centre, University Hospitals Bristol NHS Foundation Trust, Bristol, [5]Department of Haematology, University Hospital of Wales , Cardiff, [6]Department of Haematology, Newcastle Upon Tyne Hospitals NHS Foundation Trust, Newcastle Upon Tyne, [7]Centre for Clinical Haematology, Nottingham University Hospitals NHS Trust, Nottingham, [8]Barts Cancer Institute, QMUL, London, [9]Leukaemia Research Cytogenetics Group, Northern Institute for Cancer Research, Newcastle University, Newcastle Upon Tyne, United Kingdom

Background: *IKZF1* deletions *(ΔIKZF1)* are common in acute lymphoblastic leukaemia (ALL), with the most frequent, Δ4-7 exerting a dominant negative (DN) effect, with others causing loss of function (LOF). There are varying reports on the role of *ΔIKZF1* in prognosis.
Aims: We determined the prognostic impact of DN and LOF *ΔIKZF1* in 655 patients with B-precursor ALL aged 23-65 years UKALL14 (ISRCTN 66541317).
Methods: Samples were screened for Δ4-7, Δ2-7, Δ4-8, and Δ2-8 using a multiplex PCR assay with primer sites located in intron 1, intron 3, intron 7 and 3' UTR close to the breakpoints. All deletions were confirmed as unique by Sanger sequencing. At a median follow up of 29 months, we analysed the impact of any, LOF, DN or both *ΔIKZF1*, on CR, presence of minimal residual disease (MRD) at >1x10^{-4} (Ig/TCR or BCR-ABL1 quantitation, using EuroMRD criteria) after phases 1 and 2 of induction therapy, EFS, time to relapse (TTR) and OS.

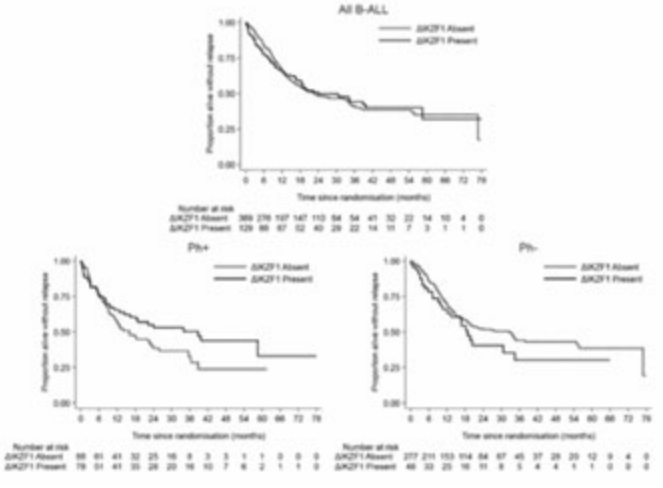


Figure 1.

Results: Diagnostic DNA was available from 498/655 (76%) patients enrolled with B-ALL. The median age of those screened was 46 years (range 23-65) and was not different from that of the trial population overall. The only difference between the screened and total population was presenting WCC (9.7 vs. 8.0 p<0.001) and a higher proportion of patients with Ph+ ALL (34% vs 31%, p=0.003). 142 *ΔIKZF1* lesions were detected in 129 (26%) patients. As expected, Δ4-7 was the most common (n=83, 59%) of deletions followed by Δ2-7 (n=31, 22%), Δ4-8 (n=22, 15%) with Δ2-8 (n=6, 4%) being the rarest deletion. Seventy-one (14%) patients harboured a DN deletion, 46 (9%) a LOF deletion, and 12 (2%) carried both DN and LOF. The frequency of *ΔIKZF1* was 78/166 (47%) of Ph+ cases and 48/325 (15%) of Ph- cases. Overall, patients with any *ΔIKZF1* were significantly less likely to reach CR (84.4% vs 93.4%, p=0.009) and significantly more likely to be MRD positive after both one (68.5% vs 53.5% p=0.016) and two (45.9% vs 32.2%, p=0.03) courses of therapy. However, when analysing these data by Ph status, this finding was strongly driven by Ph-data; as an example, 57.1% vs. 28.8% were MRD+ after 2 courses (p=0.004) in Ph- specimens as compared to 40.7 vs. 41 p=0.98 in Ph+ specimens. We did not find a significant impact of *ΔIKZF1* on EFS, OS and TTR. A subanalysis of *ΔIKZF1* impact by Ph status showed hazard ratios (HR) entirely >1 in the Ph- setting and largely <1 in the Ph+ setting, although none of the HR differences were statistically significant. For EFS: all patients *ΔIKZF1* HR 1.02, (0.76-1.36) p=0.92, Ph- only *ΔIKZF1* HR 1.34 (0.87–2.06), p= 0.18 and Ph+ only *ΔIKZF1* HR 0.7 (0.45 – 1.08) p=0.01. The Kaplan-Meier survival curves shown illustrate the differential relationship of *ΔIKZF1* to EFS by Ph status and show a significant interaction effect, p=0.038. A multivariable analysis (MVA) for EFS showed a negative prognostic impact of age in 10 year increments (HR 1.45, p<0.001) and high-risk cytogenetics (HR 1.91 p= 0.001). Among the Ph+ cohort the HR for EFS was 0.69 (0.39-1.24) for patients with a *ΔIKZF1* (p=0.21) compared to 1.67 (1.23-2.45) for Ph+ patients who did not have a *ΔIKZF1* (p=0.01). Conversely, when patients with *ΔIKZF1* were considered, the HR was 1.56 (0.92-2.62) p=0.096 among Ph- ALL and 0.65 (0.42-0.99) p=0.046 in Ph+ ALL. Thus, the MVA also implies a different prognostic impact of *ΔIKZF1* depending on Ph status (Figure 1).
Summary/Conclusion: *ΔIKZF1* are readily screened by multiplex PCR, a highly sensitive method. On the UKALL14 trial *ΔIKZF1* best predicts adverse early outcomes in patients with Ph- ALL. The impact of *ΔIKZF1* in Ph+ ALL is, at best, neutral and may be positive.

PS919

FIRST RESULTS OF NEW GIMEMA TRIAL LAL1913 FOR ADULT PATIENTS WITH PHILADELPHIA-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA (PH- ALL)

R. Bassan[1,*], S. Chiaretti[2], F. Paoloni[3], E. Audisio[4], L. Marbello[5], E. Borlenghi[6], P. Zappasodi[7], F. Di Raimondo[8], G. Martinelli[9], D. Mattei[10], A. Cortelezzi[11], M. Bocchia[12], P. De Fabritiis[13], M. Bonifacio[14], A. Candoni[15], V. Cassibba[16], P. Di Bartolomeo[17], G. Latte[18], M. Offidani[19], I. Della Starza[2], O. Spinelli[20], A. Santoro[21], L. Elia[2], M. S. De Propris[2], A. Guarini[22], A. Vitale[2], P. Fazi[3], M. Vignetti[3], A. Rambaldi[20], R. Foà[2]

[1]UOC Ematologia, Ospedale dell'Angelo, Mestre-Venezia, [2]Dip. Biotecnologie Cellulari e Ematologia, Università "La Sapienza", [3]Centro Raccolta Dati, GIMEMA, Roma, [4]SC Ematologia, AO Città della Salute e della Scienza, Torino, [5]SC Ematologia, Ospedale Niguarda Ca Granda, Milano, [6]UO Ematologia, AO Spedali Civili, Brescia, [7]SC Ematologia, Fondazione IRCCS policlinico S. Matteo, Pavia, [8]Cattedra di Ematologia, Università Ospedale Ferrarotto, Catania, [9]Istituto di Ematologia Seragnoli, Università Policlinico S.Orsola-Malpighi, Bologna, [10]SC Ematologia, ASO S. Croce e Carle, Cuneo, [11]UOC Oncoematologia, Fondazione IRCCS Ca Granda Ospedale Maggiore, Milano, [12]UOC Ematologia, AO Senese Policlinico Le Scotte, Siena, [13]UOC Ematologia, Ospedale S. Eugenio, Roma, [14]Div. Ematologia, AO Istituti Ospitalieri Policlinico Borgo Roma, Verona, [15]Clinica Ematologica, Azienda Ospedaliero-Universitaria, Udine, [16]Div. Ematologia, Ospedale S. Maurizio, Bolzano, [17]UO Ematologia Clinica, Azienda USL, Pescara, [18]Sez. Ematologia Clinica, Ospedale S. Francesco, Nuoro, Italy, [19]Div. Ematologia, Azienda Ospedaliero-Universitaria, Ancona, Israel, [20]SC Ematologia , AO Papa Giovanni XXIII, Bergamo, [21]Div. Ematologia, Ospedali Riuniti Villa Sofia-Cervello, Palermo, [22]Dip. Medicina Molecolare, Università "La Sapienza", Roma, Italy

Background: In adult ALL, pediatric-type treatment (PTT) in association with risk/MRD (minimal residual disease)-oriented strategy for allogeneic stem cell transplantation (SCT) can improve outcome, as documented by recent studies. One such pilot program yielded a 55% 5-year overall survival (OS) rate (Bassan R *et al.*, Blood 128:176;2016). This regimen was adopted and modified by the GIMEMA by adding pegylated-asparaginase (PegASP, 2000 IU/m^2×4 doses).
Aims: A first trial analysis was performed to assess the feasibility and reproducibility in a wider national context (vs NILG study), by examining the complete remission (CR) rate and early survival in different age and disease subsets.
Methods: The GIMEMA LAL1913 protocol (Clinical.Trials.gov NCT02067143) for adults aged 18-65 years with Ph- ALL combined PTT together with MRD evaluation for MRD/risk-guided SCT decision. This program consisted of a 5-drug remission induction phase including PegASP, followed by three modified BFM-like blocks (two with pegASP), three lineage-targeted MTX blocks (5 g/m^2 for T-ALL and 2.5 g/m^2 for B-ALL [1.5 g/m^2 if age >55 years]; two with high-dose Ara-C and one with PegASP) and reinduction, plus twelve triple prophylactic intrathecal injections. Bone marrow MRD monitoring was studied centrally by quantitative PCR at the end of courses 1 (TP1, week 4, w4), 3 (TP2, w10), 5 (TP3, w16) and 7 (TP4, w22). Patients achieving a CR were risk-stratified for SCT (high-risk, HR) or maintenance (standard-risk, SR). The HR group was defined by WBC >100x10^9/l (T-ALL), highly adverse cytogenetics or pre-T/mature T-

ALL; by late CR, WBC >30x10^9/l (B-ALL) or pro-B phenotype if MRD not available; by MRD ≥10^{-4} at TP2/TP3 or positive at TP4. The SR group was defined by MRD <10^{-4} at TP2/TP3 and negative at TP4, or by the absence of above risk factors in patients without MRD.

Results: Between 2014 and 2016, 203 patients were eligible for the study. Median age was 39.8 years (range 18-65, 19.2% ≥55 years), 58% were male, 64 had T-precursor lymphoblastic leukemia/lymphoma (32%) and 76 expressed HR clinical features (41.8%). The CR rate was 89.6% (n=182), correlating with T-cell phenotype (98.4% vs 88.6% in B-ALL, p=0.02) but not with the clinical risk profile (95% SR vs 87.8% HR, p=0.09) or age (p=0.80). After a median follow-up of 17.4 months (range 1.8-34.7), the 1-year OS and disease-free survival (DFS) rates are 80% and 77.9%, respectively. OS was slightly better in patients aged ≤40 years: 92.5% <25 years (n=44) and 86% 25-40 years (n=58) *vs* 72.3% 40-55 years (n=61) and 73.2% >55 years (n=39) (p=0.08, Figure 1). OS was significantly better in patients with T-cell disease: 89.3% *vs* 77.6% (p=0.04) and very similar among clinical risk groups: 83.9% SR *vs* 79.7% HR (p=0.19). The DFS rate was 77.9%, with better results in patients aged ≤40 years: 89.3% <25 years (n=42) and 87.4% 25-40 years (n=52) *vs* 69.6% 40-55 years (n=53) and 62.8% >55 years (n=34) (p=0.006); and no/minor differences between different diagnostic (B *vs* T, p=0.29) and clinical risk (SR *vs* HR, p=0.97) groups. Results of MRD evaluations and related treatment allocation will be analyzed and presented.

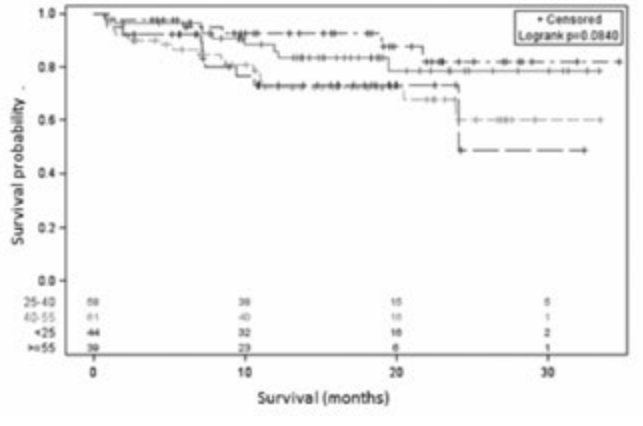


Figure 1.

Summary/Conclusion: The first analysis of the GIMEMA LAL1913 trial has documented a high CR rate with very favorable early OS and DFS, reproducing data from the interim analysis of the reference trial. The outcome seems excellent in T-ALL and younger adults, while HR patients fared no worse than clinical SR, to validate the adopted post-remission strategy. These results could be partly related to the use of PegASP in the present study.

PS920

LOW MIR-151 AND MIR-451 LEVELS IN BONE MARROW AT DIAGNOSIS IS CONFIRMED AS A PROGNOSTIC FACTOR FOR RELAPSE OF B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS FROM THE AIEOP 2000 CLINICAL TRIAL

J. Yarden[1,*], N. Dotan[1], S. Avigad[2], K. Shichrur[2], M.B. Youdim[3], I. Yaniv[4], G. Basso[5]

[1]Curewize Health Ltd., Yokneam, [2]Molecular Oncology, Felsenstein Medical Research Center, Petach-Tikva, [3]Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, [4]Pediatric Hematology Oncology, Schneider Children's Medical Center of Israel, Petach-Tikva, Israel, [5]Haematology-Oncology Division, Department of Women's and Children's Health, University of Padova, Padova, Italy

Background: Previous findings showed that the microRNA (miRNA) expression profile of miR-151-5p, miR-1290 and miR-451 measured at diagnosis in bone marrow (BM) aspirations can predict risk of relapse in pediatric precursor B-cell acute lymphoblastic leukemia (B-ALL) patients treated according to BFM-based protocols and DCOG protocols (Avigad *et al.*, Genes, Chromosomes & Cancer; 55:328–339, 2016).

Aims: Confirm miR-151-5p, miR-451 and miR-1290 expression profile measured in BM aspirations at diagnosis, as a prognostic factor for B-ALL and standardize the classifier on a new platform method for measuring miRNA.

Methods: The miRNA expression was measured in BM samples from a subcohort of B-ALL patients (n=272) from the Associazione Italiana di Ematologia Oncologia Pediatrica (AIEOP) enrolled in the AIEOP-BFM 2000 study under inform consent (Sep-1, 2000 to Jul-31, 2006). Patients' clinical status was unknown to the lab. A fixed amount of synthetic non-human miRNA (Ref-miR) was spiked in total miRNA extracted from BM samples before reverse transcription (RT) for quality control. RT was performed with stem-loop primers. Relative quantities (RQ) of the miRNA, measured by RT-qPCR, were calculated by the ddCt method using the Ref-miR as a reference, and a mix of synthetic miRNAs as a calibrator. Kaplan-Meier was performed for testing event and relapse-free-survival (EFS and RFS). Cox proportional-hazards regression was performed to investigate the relationship of the miRNA with other prognostic factors.

Results: Forty-nine of the 272 patients had an event (38 relapses, 2 second malignancies and 9 deaths). Optimal cutoffs for predicting an event (positive biomarker) was determined at < median level of miR-151-5p and < upper quartile level of miR-451 of whole population. Analysis of miR-1290 didn't continue due to nonsignificant association with EFS. Low levels (positive biomarker) of the miRNAs predicted worse EFS compared to high levels: miR-151-5p (75% vs. 89%, p=0.003) and miR-451 (79% vs. 92%, p=0.02). Combining miR-151-5p/miR-451 to a single classifier led to a linear decrease in EFS with increasing number of positive miRNA: 0 positive (96%), 1 positive (86%), 2 positive (74%) (p=0.0005, logrank trend). Combined miR-151-5p/miR-451 associated with PCR-MRD risk classification: 2 positive miRNA in 25/80 (31%) standard risk (SR), 50/123 (41%) medium risk (MR) and 11/13 (85%) high risk (HR), whereas <2 positive in 55/80 (69%) SR, 73/123 (59%) MR and 2/13 (15%) HR (p=0.002, chi^2 for trend). The combined miR-151-5p/miR-451 classifier (HR 2.0, 95%CI 1.3-3.3, p=0.004) was an independent predictor of EFS when testing in relation to white blood cell (WBC) count, age (1-6 yrs. vs. >6 yrs.) and AIEOP-BFM final risk stratification. An incremental classifier was created by the summation of miR-151-5p/miR-451 positive status (0, 1 or 2), WBC count 3 levels (1, 2 or 3), and age > 6 (0 or 1) leading to a score of 0 to 6. There was a linear decrease in EFS and RFS (p<0.0001) as the score increased. Subcohort analyses showed the predictive power of the classifier in AIEOP-BFM HR patients, PCR-MRD MR patients and patients negative for TEL/AML or t(4;11) mutations (Figure 1).

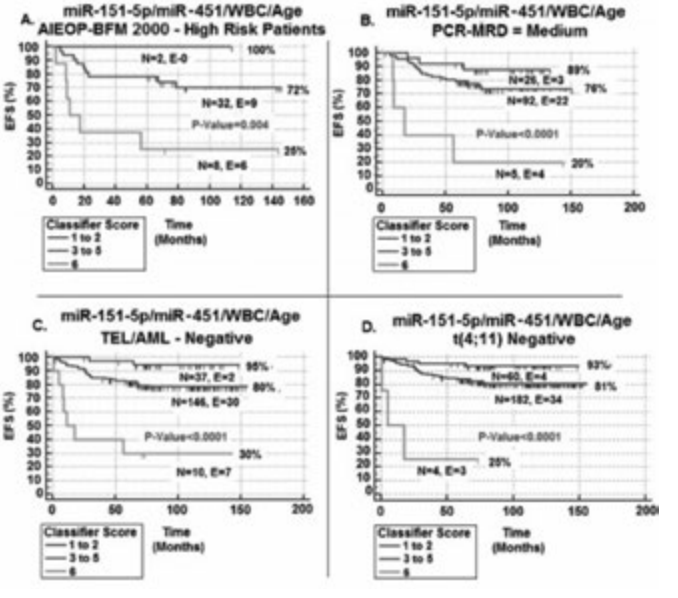


Figure 1.

Summary/Conclusion: The mir-151-5p/miR-451 classifier was confirmed as an independent prognostic factor for EFS and RFS of B-ALL patients from the AIEOP-2000 cohort. To confirm the prognostic independent value, these results should be validated in another independent cohort using this standardized method for the semi-quantification of miR-151-5p and miR-451.

PS921

THE CELL SURFACE PROTEIN VANIN-2 IDENTIFIES AGGRESSIVE SUBTYPES OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

V. Rodriguez Martinez[1,*], A. Rinaldi[1], M. Schuette[2], G. Cario[3], P. Mirkowska[1], C. Eckert[4], B. Marovca[1], M. Temperli[1], C. Möller[1], R. Siegenthaler[1], A. Mecklenbräuker[5], M. Dworzak[5], A. Schumich[5], R. Panzer-Grümayer[5], E. Mejstrikova[6], B. Buldini[7], P. Scarparo[7], G. Basso[7], M. Stanulla[8], M. Zimmermann[8], M.-L. Yaspo[9], B. Bornhauser[1], J.-P. Bourquin[1]

[1]Oncology, University Childrens Hospital Zurich, Zurich, Switzerland, [2]Alacris Theranostics , Berlin, [3]Department of Pediatrics, University Hospital Schleswig-Holstein, Kiel, [4]Pediatric Hematology and Oncology, Charité University Hospital, Berlin, Germany, [5]St. Anna Children's Hospital and Children's Cancer Research Institute, Vienna, Austria, [6]Department of Pediatric Hematology and Oncology, Charles University Hospital Motol, Prague, Czech Republic, [7]Department of Pediatrics, University of Padova, Padova, Italy, [8]Pediatric Hematology and Oncology, Hannover Medical School, Hannover, [9]Department of Vertebrate Genomics, Max Planck Institute for Molecular Genetics, Berlin, Germany

Background: The *in vivo* response to treatment assessed by minimal residual disease (MRD) during induction therapy is the most applicable predictor of the risk of relapse in acute lymphoblastic leukemia (ALL) of childhood. However, most relapses occur within an MRD-intermediate risk group, and better predictors are needed to stratify the patients at risk for targeted agents.
Aims: The aim of this project was to identify cell surface markers that are associated with MRD persistance and increased risk of relapse for possible inclusion in diagnostic immunophenotyping.
Methods: We have mapped the cell surface proteome of 11 good prognosis and 8 high risk ALL samples using a chemoproteomic cell surface capture technology. This revealed exclusive identification of the cell surface glycoprotein and fetal liver hematopoietic stem cell marker Vanin-2 (VNN2) in high risk ALL samples. In retrospective (by qPCR) and prospective (by flow cytometry) analyses performed in 5 different centres within the AIEOP-BFM ALL study group we validated this association of VNN2 positivity with high risk disease. We performed low coverage whole genome and whole exome sequencing in 26 VNN2-positive ALL cases and transcriptome sequencing in 16 VNN2-positive cases to identify lesions that associate with VNN2 positive ALL.
Results: Comparative analysis of the ALL high and low risk surfaceome revealed association of VNN2 with very high risk of relapse cases. Using mRNA analysis of VNN2 levels by qPCR, we evaluated the risk association of VNN2 high ALL in 776 cases retrospectively (ALL-BFM 2000 study). Very high levels of VNN2 transcripts were enriched in MRD intermediate and high risk ALL. Overall, the median event-free survival probability of VNN2-high ALL was significantly lower than in VNN2-low ALL, in particular in IR-ALL. To validate this association with risk, we have prospectively integrated VNN2 in the flow cytometry diagnostics of our international cooperative study group (AIEOP-ALL-BFM 2009) and have so far detected VNN2 positivity in about 10% of pediatric ALL patients (62/468), with an enrichment in MRD-high risk (20/35 = 57%) and MRD-intermediate risk (10/35 = 29%) cases. Immunophenotypic analysis of VNN2-positive cases did not show a pattern of myeloid co-expression, indicating that only a low percentage of cases were classified as biphenotypic (6/42 = 14%) or mixed-phenotype acute leukemia (3/42 = 7%). Genomic analysis of 26 VNN2-positive ALL cases revealed heterogeneous genotypes with frequent deletions of PAX5 or IKZF1, which were often combined with CDKN2A/B deletions and single nucleotide variants in RAS pathway genes. Indeed, around 25% of the cases were IKZF1+ (7/26), a recently described poor outcome subgroup. In a few cases known fusions such as JAK2-BCR (1/26 = 4%), TCF3-ZNF384 (2/26 = 8%) and PAX5-ESRRB (1/26 = 4%) were detected. The fatal ALL subtype positive for the translocation TCF3-HLF was always strongly positive for VNN2 (11 cases tested), providing a biomarker for rapid and early identification of such cases. Surprisingly, over 50% of all VNN2 positive cases had recurrent mutations in epigenetic regulators, such as EZH2, MLL2 and KDM6A.
Summary/Conclusion: VNN2 positive leukemia is a genetically heterogeneous group, appears associated with high risk of relapse, is always detected on poor risk TCF3-HLF ALL and has high frequency of mutations in epigenetic regulators. Prospective evaluation of VNN2 by flow cytometry in the upcoming AIEOP-BFM-ALL study will clarify the significance of this marker for risk stratification in ALL and will be useful for early detection of TCF3-HLF positive cases.

PS922

NILOTINIB COMBINED WITH LOWER-INTENSITY CHEMOTHERAPY FOR FRONT-LINE TREATMENT OF YOUNGER ADULTS WITH PH-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): INTERIM ANALYSIS OF THE GRAAPH-2014 TRIAL

Y. Chalandon[1,*], P. Rousselot[2,3], J.-M. Cayuela[4], X. Thomas[5], E. Clappier[4,6], V. Havelange[7], P. Chevallier[8], F. Huguet[9], N. Boissel[4,6], C. Berthon[10], G. Stüssi[11], S. Maury[12], S. Chantepie[13], F. Pasquier[14], I. Plantier[15], V. Lhéritier[16], N. Ifrah[17], S. Chevret[6,18], H. Dombret[4,6]

[1]HUG, Geneve, Switzerland, [2]CH, [3]UVSQ University, Versailles, [4]University Hospital Saint-Louis, AP-HP, Paris, [5]CHU Lyon Sud, Lyon, [6]University Paris Diderot, Paris, France, [7]UC, Louvain, Belgium, [8]CHU, Nantes, [9]IUCT-Oncopole, Toulouse, [10]CHRU, Lille, France, [11]Oncology Institute, Bellizona, Switzerland, [12]University Hospital Henri Mondor, AP-HP, Creteil, [13]CHU, Caen, [14]Institut Gustave Roussy, Villejuif, [15]CH, Roubaix, [16]GRAALL Coordination, Lyon, [17]CHU, Angers, [18]Biostatistics, University Hospital Saint-Louis, AP-HP, Paris, France

Background: The introduction of tyrosine kinase inhibitors (TKIs) to treat patients with Philadelphia chromosome-positive (Ph+) ALL allows reducing the intensity of associated chemotherapy. In our previous randomized GRAAPH-2005 trial with imatinib, we have reported better short-term results when patients received lower-intensity chemotherapy (vincristine, dexamethasone and intrathecal chemotherapy infusions [ITs] only) during the first treatment cycle (Y. Chalandon *et al.* Blood 2015).
Aims: In this ongoing GRAAPH-2014 trial with nilotinib, we are pursuing random evaluation of reducing chemotherapy intensity by omitting high-dose cytarabine during the second treatment cycle. We are also repeating cycles 1 and 2 for a total of 4 cycles in order to prolong TKI exposure and reach a deeper response prior to allogeneic or autologous stem cell transplantation (SCT) in these patients.
Methods: After a common steroid prephase, patients aged 18-59 years old with newly diagnosed previously untreated *de novo* Ph+ ALL and an ECOG-PS ≤3 are eligible for randomization. Treatments are detailed in Figure 1. Primary study endpoint is non-inferiority of major *BCR-ABL1* molecular response (MMR) in the no-cytarabine arm after cycle 4, defined as *BCR-ABL1/ABL1* ratio <0.1% in the bone marrow. Secondary endpoints are hematologic complete remission (CR), early mortality, progression-free survival (PFS), per protocol event-free survival (EFS, also including initiations of unplanned subsequent therapies as events) and overall survival (OS). An interim analysis was planned after the first 60 patients enrolled became evaluable to make sure that the overall MMR rate does not significantly differ from the anticipated 80% rate.

Figure 1.

Results: This analysis included thus 60 patients randomized between March 2016 and June 2017 (M/F, 29/31; median age, 47 years; minor *BCR-ABL1* breakpoint, 72%). The median follow-up was 14 months (IQR, 11.6-16.2) and the minimal follow-up of alive patients was 3.8 months. All patients but one who early died from a septic shock achieved hematologic CR after

cycle 1 (CR rate, 98%). During the initial 4-cycle period of time, 4 additional CR patients discontinued the planned therapy during cycle 1 (1 septic shock, 1 chest pain, 1 paraplegia, 1 cannabis arteritis) and 1 during cycle 3 (prolonged cytopenia). In intent to treat analysis, the MMR rate was 43/54 patients (80%) after cycle 2 and 38/41 patients (93%) after cycle 4. Based on these as good as expected MMR rates, the trial is continuing in order to randomize the 265 patients needed to answer the no-cytarabine question. At the time of analysis, 31 and 13 patients had received allogeneic and autologous SCT in first CR, respectively (transplant rate, 73%). Seven out of the 59 CR patients relapsed (including 4 post-SCT relapse) and 3 patients died (including 2 deaths in CR, 1 of whom after SCT). At one year, resulting PFS and OS were estimated at 84.5% (95% CI, 75.0-95.2) and 95.9% (95% CI, 90.3-100), respectively. When taking into account initiation of unplanned therapies prior to SCT as events (3 dasatinib, 1 imatinib), per protocol EFS was estimated at 79.7% (95% CI, 69.5-91.4) at one year.
Summary/Conclusion: Front-line combination of nilotinib and lower-intensity chemotherapy appears to be a promising option to treat adult patients with Ph+ ALL, associated with a high major molecular response rate and a good short-term outcome.

PS923

PEDIATRIC PATIENTS WITH ACUTE LEUKEMIA AND MLL REARRANGEMENT SHOW A DISTINCTIVE EXPRESSION PATTERN OF HISTONE DEACETYLASES

N. Vega-Garcia[1,2,*], R. Malatesta[1,2], C. Estella[1,2], S. Pérez-Jaume[3], E. Esperanza-Cebollada[1,2], M. Torrebadell[1,2,4], A. Català[2,4,5], S. Gassiot[1,2], S. Pont[1,2], S. Rives[2,4,5], M. Camós[1,2,4]

[1]Hematology Lab, Hospital Sant Joan de Deu, [2]Institut de Recerca Hospital Sant Joan Deu, [3]Developmental Tumor Biology Laboratory,, Barcelona, [4]CIBERER, Instituto de Salud Carlos III, Madrid, Madrid, [5]Pediatric Hematology Oncology Depart, Hospital Sant Joan de Deu, Barcelona, Spain

Background: Histone deacetylase inhibitors (HDACi) have emerged as potential drugs. However, the lack of target specificity makes it difficult to predict their toxicities, limiting their use. There is a need to elucidate the role of HDACs.The study of *HDAC* expression in pediatric leukemia has been scarcely addressed and could help to tailor treatment with HDACi in a more personalized approach.
Aims: To analyze the expression of *HDACs*, *MEF2C* and *MEF2D* in pediatric patients with acute leukemia.
Methods: We analyzed HDACs MEF2C and MEF2D mRNA expression in pediatric patients with acute leukemia from 2003 to 2017 uniformly treated according to the SEHOP consecutive protocols.
Results: We studied 211 patients (57% males, median age 5.8 years, range 0-17.4), including 134 B-cell precursor acute lymphoblastic leukemia (BCP-ALL) cases, 33 T-ALL and 44 patients with acute myeloid leukemia (AML). We observed a global overexpression of *HDACs* and nearly all patients expressed class I *HDACs* homogeneously. In T-ALL patients, high *HDAC4* expression correlated with a poor prednisone response at day +8 of induction therapy, although not significantly (p=0.053). Interestingly, *MEF2C* expression seemed to reflect the oncogenic pathway of leukemic blasts. Thus, all patients with absent expression of *MEF2C* were *NOTCH1/FBXW7* mutated, while all but one patients with high *MEF2C* expression were wild-type; the only *NOTCH1*-mutated patient who expressed *MEF2C* gene had an immature pre-T phenotype. Noticeably, we identified a distinctive signature for patients with *MLL* rearrangement, with high *HDAC9* and *MEF2D* expression, regardless of age, *MLL*partner and lineage (p=0.005 and p=0.034, respectively). Regarding outcome, in BCPALL patients, low *HDAC2* expression (cut-off 3.29, around percentile 50) and very high *HDAC9* (cut-off 3.62, around p65) impacted negatively on event-free survival (EFS) at 5 years (70.5±7% *vs.* 95.4±3%, p=0.002; 69±1% *vs.* 97.9±2%, p<0.001, respectively). *HDAC9* expression was an adverse prognostic factor both in *MLL*-rearranged and in *MLL* wild-type cases. AML patients with *HDAC8* expression above the median (cut-off 2.34, p55), had a significantly worse EFS at 5 years (30.1±12% *vs.* 61±11%, p=0.015).
Summary/Conclusion: We identified an HDAC signature for MLL-rearranged patients,with overexpression of HDAC9 and MEF2D.Our results provide useful knowledge on HDACs in childhood leukemia and support the use of more specific HDACi.

PS924

PROTEOLYTIC L-ASPARAGINASE DEGRADATION AS A MECHANISM FOR NON-ANTIBODY-RELATED DRUG INACTIVATION

J. Walenciak[1,*], E. Polakowska[2], K. Wyka[2], W. Mlynarski[1], B. Zalewska-Szewczyk[1]

[1]Department of Pediatrics, Oncology, Hematology and Diabetology, [2]Laboratory of Immunopathology and Genetics, Medical University of Lodz, Lodz, Poland

Background: Immune-mediated L-asparaginase (L-asp) inactivation is the major process limiting the efficacy of the drug. Apart from clinical allergy, in some proportion of patients occurs a phenomenon called 'silent inactivation', where L-asp activity is below its therapeutic level, while the recipient does not present symptoms of hypersensitivity. Interestingly, approximately 1/3 of patients with inadequate activity of the drug are negative for the presence of anti-L-asp antibodies (Willer *et al*., 2011). The mechanism responsible for L-asp inactivation in this case remains unknown. We hypothesized that proteolytic enzymes involved in *in vitro* L-asp degradation (Patel *et al*., 2009) may influence drug activity *in vivo*.
Aims: The aim of our study was to evaluate an effect of L-asparaginase-degrading enzymes on L-asp treatment efficacy. For that reason, we evaluated serum concentrations of AEP, CTSB and its secreted proenzyme, procathepsin B (proCTSB), as well as their inhibitor, cystatin C (CysC), in 44 L-asp recipients undergoing induction remission therapy for ALL.
Methods: Serum concentrations of L-asp degrading enzymes: asparaginyl endopeptidase (AEP) and cathepsin B (CTSB) and its proenzyme – procathepsin B (proCTSB) were assessed prior to L-asp therapy and at the end of induction remission using immunoenzymatic method. At the same time the level of the main serum protease inhibitor – cystatin C (CysC) was assessed using the same technique. Ratios of the evaluated proteases to CysC and to serum total protein were evaluated. L-asp activity was assessed prior each drug administration according to the treatment protocol using spectrophotometric method. Inadequate L-asp activity was defined as mean activity below 100 U/l from all measurements.

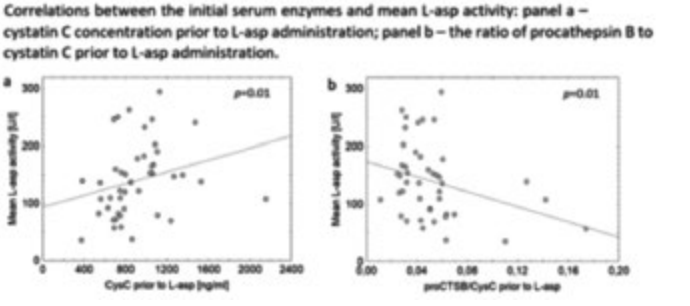


Figure 1.

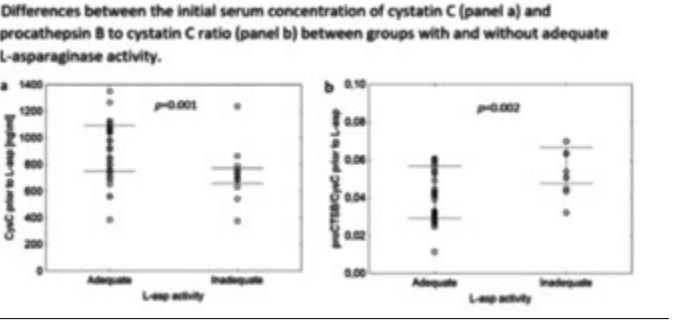


Figure 2.

Results: At the end of L-asp therapy we found an increase in the concentration of proteolytic enzymes (median of 1.29 vs. 1.41 ng/ml for AEP, 46.97 vs. 36.92 ng/ml for proCTSB and 0.51 vs. 0.30 ng/ml for CTSB) whereas their inhibitor level decreased (698.34 vs. 816.94 ng/ml for CysC). The proCTSB/CysC and CTSB/CysC ratio also increased significantly during the treatment. Mean L-asp activity correlated with initial CysC concentration (R=0.34, p=0.01) and initial proCTSB/CysC ratio (R=-0.35, p=0.01) (Figure 1). We did not observed any case of clinical hypersensitivity to L-asp, although inadequate activity of the drug was present in 13/44 patients (29%). In the group with inadequate L-asp activity we noted significantly lower CysC concentrations at the beginning of L-asp treatment (731.41 ng/ml vs. 977.34 ng/ml, p=0.001) and a higher initial proCTSB/CysC ratio (0.067 vs. 0.045, p=0.002, see also Figure 2).

Summary/Conclusion: Our results provide, for the first time *in vivo*, a possible explanation for the mechanism of L-asp inactivation apart from the development of anti-L-asparaginase neutralizing antibodies. To our knowledge, this is the first study demonstrating changes in the protease-antiprotease equilibrium upon L-asparaginase administration that may be involved in clinically significant blood L-asp clearance.

PS925

EVALUATION OF CORRELATION BETWEEN EARLY IMMATURE T-ALL IMMUNOPHENOTYPE, ABSENCE OF BIALLELIC DELETION OF TCR-Γ AND MEF2C EXPRESSION

S. Jay[1,*], N. Rajpoot[1], D. Verma[1], R. Kumar[1], S. Bakshi[2], R. Seth[3], J. Kumar[4], A. Sharma[2], A. Chopra[1]

[1]Laboratory Oncology, [2]Medical Oncology, [3]Pediatrics, [4]Biochemistry, all India Institute of Medical Sciences New Delhi, New Delhi, India

Background: T-ALL is an aggressive hematologic malignancy. Though it is generally associated with more unfavorable clinical features compared with B-lineage ALL, the prognostic markers in this disease are relatively inadequate for risk stratification and treatment planning. Because of this, there is interest in finding markers of poor risk disease that would indicate the likelihood of failure of remission induction on standard chemotherapy. Three potential markers of bad risk disease that show very poor treatment response, in the overall disease category of immature T-ALL (CD5+/-, CD1a-, CD8-; EGIL pro- and pre-T ALL), have emerged from recent work. The first is the presence of immunophenotypic characteristics that define the newly described entity of early thymic precursor T-ALL (ETP-ALL). The second is the absence of biallelic deletion of TCRγ chains (ABD) which also is a marker of immaturity. The third is overexpression of transcription factor, MEF2C, the driving oncogene for immature ETP T-ALL cases. In this study,we determined the correlation between T-ALL immunophenotype, ABD and MEF2Cexpression.
Aims: To determine how the three parameters of immaturity in T-ALL – immunophenotypically defined ETP-ALL, and molecularly determined absence of biallelic deletion of TCR y chains (ABD) and MEF2C, correlate with each other.
Methods: A total of 118 T-ALL cases, including 91 pediatrics and 27 adults, were included.Immunophenotyping was done on bone marrow/peripheral blood samples using CD3, CD7, CD45, CD5, CD8, CD1a, CD13, CD33, CD117, HLA-DR, CD34, CD65 and CD11b antibodies. ABD was done in 92 patients in which DNA was available by quantitative DNA PCR (Q-PCR) for TCR-γ rearrangements. *MEF2C* gene expression was quantified in patients (n=86) in which RNA was available by Q-PCR. Kruskal-Wallis test followed by Mann-Whitney test was performedto determine the correlation of *MEF2C* Expression with immunophenotypes and TCRγ chain status. Pearson's chi-squared test and Fisher exact test was performed to determine the significance of TCRγ chain status among various T-ALL immunophenotypes.
Results: Immunophenotypically, T-ALL cases were classified into mature(n=28), cortical(n=40) and immature(n=49) groups. Immature consists of ETP-ALL (n=17), Pro-TALL (n=9) and Pre-TALL(n=23) subgroups.As compared to mature and cortical, *MEF2C* expression was increased in immature group($p<0.05$).Within the immature group, *MEF2C*gene was found to be significantly over expressed in ETP-ALL subgroup as compared to mature and cortical ($p=0.004$ and 0.002; respectively). While comparing between ETP-ALL and non-ETP-ALL, *MEF2C* had high expression in ETP-ALL cases ($p=0.004$). Out of 92 patient analyzed for TCRγchains status, ABD was found in 29.3% (n=27) of patients. Also in comparison to mature ABD, it was significantly present in Pro-TALL and ETP-ALL groups ($p= 0.02$). Patients having ABD had higher expression of *MEF2C*($p<0.05$).
Summary/Conclusion: Taken together, ABD, MEF2C and immunophenotype can be used interchangeably to identify and risk stratify T-ALL cases.

PS926

EXTRAMEDULLARY LATE RELAPSES IN ATYPICAL SITES OF ACUTE LYMPHOBLASTIC LEUKEMIA: A RETROSPECTIVE STUDY OF THE ITALIAN ASSOCIATION OF PEDIATRIC HEMATOLOGY AND ONCOLOGY (AIEOP)

G. Giagnuolo[1,*], D. Silvestri[2], E. Barisone[3], O. Ziino[4], L. Lo Nigro[5], R.M. Mura[6], R. Parasole[1], F. Petruzziello[1], C. Rizzari[2], M. C. Putti[7], S. Ladogana[8], C. Micalizzi[9], L.D. Notarangelo[10], P. Pierani[11], V. Conter[2], F. Locatelli[12], A.M. Testi[13]

[1]Department of Pediatric Hemato-Oncology, Santobono-Pausilipon Children's Hospital, Napoli, [2]Department of Pediatrics, Ospedale San Gerardo, University of Milano-Bicocca, Monza, [3]Department of Pediatric Hemato-Oncology, Regina Margherita Children's Hospital, Torino, [4]4Department of Pediatric Hemato-Oncology, ARNAS Ospedali Civico, G Di Cristina, Palermo, [5]Department of Pediatric Hemato-Oncology, Azienda Policlinico-OVE, Catania, [6]Paediatric Haematology-Oncology, Ospedale Pediatrico Microcitemico, Cagliari, [7]Hemato-Oncology Division, University of Padova, Padova, [8]8Department of Pediatric Hemato-Oncology , Ospedale "Casa Solievo della Sofferenza", San Giovanni Rotondo, [9]Department of Pediatric Hemato-Oncology, IRCCS I G Gaslini, Genova, [10]Pediatric Onco-haematology and BMT Unit, Children's Hospital, Spedali Civili of Brescia, Brescia, [11]Division of Pediatric Hematology and Oncology, Ospedale G.Salesi, Ancona, [12]Department of Pediatric Hemato-Oncology, IRCCS Ospedale Bambino Gesù, [13]Department of Cellular Biotechnologies and Hematology, University La Sapienza, Roma, Italy

Background: Despite significant improvement of childhood acute lymphoblastic leukemia (ALL) outcome, disease recurrence remains the most important cause of treatment failure. ALL relapse occurs more frequently during treatment or within the first 2 years from primary diagnosis; following, the risk gradually reduces.
Aims: Herein, we report the AIEOP experience on late (>30 months from first diagnosis) ALL relapse with atypical extramedullary localizations.
Methods: We retrospectively analyzed late relapses with atypical extramedullary localizations among ALL patients enrolled in subsequent AIEOP study protocols (ALL91, ALL2000, ALL2006 and ALL2009). Clinical data about primary diagnosis, relapse, treatment and outcome, were recorded by filling a data collection form in each AIEOP centers. We considered atypical, both extramedullary isolated and combined relapses, excluding central nervous system (CNS) and testicular involvement.
Results: We collected 19 patients (11 female and 8 male), from 12 AIEOP centers, with late atypical extramedullary relapse diagnosed within 21 years. Mean age at relapse was 13 years (range 6-22) and mean time from primary diagnosis was 59 months (34-93). The most frequent involved sites were the ovary (n=5), pelvis (n=3), mediastinum (n=3), kidney (n=2) and lymph nodes (n=2). Other localizations were liver, abdomen, skin, bladder, maxillary sinus, nasopharynx, parotid and retina, each involved in 1 patient. Four patients had extramedullary localizations, already at the time of first diagnosis. Minimal residual disease (MRD) evaluation on bone marrow, at disease recurrence, was available only in 9 patients, and was positive in 5. Patients received different treatment protocols: 10 were enrolled in the AIEOP REC 2003, 6 in the IntReALL SR 2010, 2 in the AIEOP 9503 and 1 in the AIEOP LNH-97 protocols. Patients with ovary involvement were submitted to ovariectomy and local radiotherapy was performed in 2 other cases. Allogenic hematopoietic stem cell transplant (HSCT) was performed in 8 patients, while 2 received autologous HSCT. Second complete remission (CR) was obtained in 18 patients. After a median follow-up of 48 months (7-158), 14 patients (73,6%) are alive, in second CR (2 still on treatment, 2 completed follow-up after 10 and 13 years, and 12 continue controls). All but one patient (sepsis death), treated with allogenic HSCT are alive. Overall, 5 patients dead, 4 from progression disease and 1 from post-transplant complications. The estimated overall survival probability was 60%.
Summary/Conclusion: The Italian experience confirms that extramedullary ALL localizations, other than CNS and testicles, have a very low incidence (<1%); the most frequent involved sites are ovary, pelvis, kidney and lymphnodes, while mediastinum prevails in T-ALL, as expected. Patients have a good prognosis: 94,7% achieved a second CR, and 73,6% are in continuous CR after a median time of 48 months. Sixteen patients, at relapse, received a risk-adapted protocol with MRD monitoring, according to which treatment intensification with allogenic HSCT was established. Since most of the transplanted patients are alive, we underline the importance of MRD evaluation during salvage treatment in order to identify patients at high risk who can benefit from HSCT. Further study on specific genetic abnormality, microenvironment and adhesion molecules could clarify pathogenesis of these atypical relapse and the specific tropism for some tissues of leukemic cells.

PS927

INCIDENCE AND OUTCOME OF RELAPSES IN YOUNG ADULTS (18-60 YR) WITH PH—POSITIVE ALLTREATED WITH IMATINIB, CHEMOTHERAPY AND ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (PETHEMA ALLPH08 TRIAL)

J. M. Ribera[1,2,*], O. Garcia[1,2], M.J. Moreno[3], P. Barba[4], I. García-Cadenas[5], S. Mercadal[6], P. Montesinos[7], M. Barrios[8], T. Bernal[9], J. Ribera[2], S. Vives[12], M. Cervera[10], E. Lavilla[11], E. Abella[12], M. Tormo[13], E. Genescà[2], M. Calbacho[14], E. Feliu[12]

[1]ICO-Hospital Germans Trias i Pujol, [2]Josep Carreras Leukaemia Research Institute (IJC), Badalona, [3]Hospital Clínico de Málaga, Málaga, [4]Hospital Vall d'Hebron, [5]Hospital de Sant Pau, Barcelona, [6]ICO Duran i Reynals, Bellvitge, [7]Hospital Universitario y Politécnico de La Fe, Valencia, [8]Hospital Carlos Haya, Málaga, [9]Hospital Central de Asturias, Oviedo, [10]ICO-Hospital Joan XXIII, Tarragona, [11]Hospital Lucus Augusti, Lugo, [12]Hospital del Mar, Barcelona, [13]Hospital Clínico Universitario de Valencia, Valencia, [14]Hospital Ramón y Cajal, Madrid, Spain

Background: The concomitant use of tyrosine kinase inhibitors (TKI) and chemotherapy has improved the prognosis of patients with Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) allowing them to achieve a high rate (>95%) of complete remission (CR) and to perform allogeneic hematopoietic stem cell transplantation (HSCT) in a high proportion of patients (>70%), most of them in good molecular status. However, relapses occur in around 30% of young adults, their treatment and outcome has not been extensively analyzed.
Aims: The aim of this study was to analyze the incidence, characteristics and outcome of relapses in young adults with Ph+ ALL treated with imatinib, chemotherapy and allogeneic HSCT according to the PETHEMA ALLPh08 trial (NCT01491763)
Methods: The PETHEMA ALL Ph08 trial included 120 patients aged 15-60 yrs. CR was achieved in 111 (93%), and allogeneic HSCT was performed in 91 patients (82%) in first CR (Leuk Lymphoma 2018; 59:146-154). Twenty-six out of 111 patients (23%) experienced relapse. The following characteristics were analyzed: type of relapse (molecular/clinical), timing (before/after HSCT), location (BM/CNS/combined), presence of kinase domain mutations at relapse, type of treatment and outcomes (response, DFS and OS).
Results: 17/26 patients were male, with a median age of 42 years (range 17-56). At the time of relapse the median value of Hb was 116 g/L (85-134), WBC count 5.1 x10^9/L (0.97-111.4), platelet count 122x10^9/L (9-1125) and BM blast percentage of 4% (0%>90%). Four relapses (15%) occurred before HSCT and 22 (85%) after HSCT; 10 (39%) were molecular and 16 (61%) were systemic (BM [n=11], CNS [n=2], combined [n=3]). Mutations were studied in 12 patients (T315I [n=4], E225K [n=3], Y253H + E255V + E355G [n=1], no mutations [n=4]). Table 1 shows the results of treatment according to the type of relapse. Two patients did not receive treatment and one patient died during rescue therapy. At the time of analysis 12/20 patients who achieved a second CR relapsed and 16/26 patients died. The median of DFS was 8.5 months (95% CI: 2.1-14.9), and the median of OS was 15.3 months (95% CI: 6.7-23.9). DFS and OS probabilities were significantly higher for those patients with molecular relapse than for those with systemic relapse.

Table 1. Response to rescue therapy according to the type of relapse.

	Molecular relapse (n=10)	Systemic relapse (n=16)
Type of treatment	Increase imatinib dose (n=2) Change TKI (n=8)	TKI+Intensive chemotherapy (n=10) Intensive chemotherapy only (n=1) Change TKI (n=3) No treatment (n=2)
CR	-	13/13*
Molecular CR	7	9/10
Second HSCT after CR2	0	7
Relapse	3/7	9/13
DFS (median, 95%CI), months**	NA	6.3 (2.2-10.3)
Dead	4/10	12/16
OS (median, 95% CI), months***	NA	11.5 (8.4-14.6)

*early death in 1 patient; ** p=0.014; ***p=0.031

Summary/Conclusion: One fifth of young adults with Ph+ ALL treated in the ALLPh08 trial relapsed, most of them after allogeneic HSCT, and 40% of relapses were molecular. Mutations at relapse were detected in two thirds of patients. Although the response to rescue therapy was high, most patients showed a second relapse. The outcome of patients with molecular relapse was significantly better than that of patients with systemic relapse.

PS928

VENETOCLAX AND NAVITOCLAX WITH CHEMOTHERAPY IS EFFICACIOUS IN PATIENTS WITH RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA

T. Alexander[1,*], N. Lacayo[2], S. L. Khaw[3], J. Rubnitz[4], M. Schmidt[5], Y. Zhou[5], J. Ross[5], L. Rosenwinkel[5], S. Y. Kim[5], E. Jabbour[6], W. Stock[7]

[1]Department of Pediatrics, University of North Carolina School of Medicine, Chapel Hill, NC, [2]Lucile Packard Children's Hospital, Stanford University, Palo Alto, CA, United States, [3]Royal Children's Hospital, Melbourne, Australia, [4]Department of Oncology, St. Jude Children's Research Hospital, Memphis, TN, [5]AbbVie, Inc, North Chicago, IL, [6]Department of Leukemia, University of Texas MD Anderson Cancer Center, Houston, TX, [7]Section of Hematology/Oncology, Department of Medicine, University of Chicago and University of Chicago Cancer Research Center, Chicago, IL, United States

Background: Children and adults with relapsed or refractory (R/R) acute lymphoblastic leukemia (ALL) continue to have poor prognosis despite intensive chemotherapy regimens and novel therapeutic approaches. There remains significant unmet medical need for patients with ALL that are unsuitable for, or progress on, available therapies. In preclinical models of ALL, venetoclax (Ven; a highly selective BCL-2 inhibitor) has shown synergistic antitumor effects when combined with navitoclax (Nav; BCL-2, BCL-X_L and BCL-W inhibitor).
Aims: To present preliminary efficacy data, evaluating Ven and Nav in combination with chemotherapy in patients with R/R ALL.
Methods: This is an open-label, phase 1, multicenter dose escalation study (NCT03181126) in patients (4 45 years; ≥20 kg) who have R/R ALL. Patients receive the weight-adjusted equivalent of 200 mg daily Ven exposure on day 1, and 400 mg daily equivalent thereafter. Daily oral Nav administration is initiated on day 3 and given in three dose levels: 25, 50, or 100 mg weight-based equivalent for weight ≥45 kg. Patients may also receive chemotherapy: peg-asparaginase (1,250 IU/m^2 intravenous [IV] on days 9 and 22), vincristine (1.5 mg/m^2 IV on days 9, 15, 22 and 29), and dexamethasone (20 mg/m^2/day orally on days 9–13 and 22–26). Chemotherapy administration can be delayed, repeated for a second cycle, or not administered based on investigator discretion. Bone marrow aspirate and/or biopsy is required on days 8 and 36 for disease assessment and minimal residual disease (MRD; 10^{-4} cutoff) evaluation by flow cytometry before and after the first dose of chemotherapy, respectively. Absolute neutrophil counts (ANC) and platelet counts are reported 10^3 cells/mL.
Results: Baseline characteristics and prior therapies of the first three patients enrolled and treated are shown in the Table 1; all three patients had B-cell ALL. Patient A had a CRi with 1% MRD in the bone marrow on day 8; chemotherapy was not started to allow for count recovery. At day 36, this patient achieved a CR and was MRD-negative with an ANC of 2.74 and platelet count of 212. Patient B had stable disease (SD) with persistence of bone marrow blasts on day 8; by day 36 following one cycle of chemotherapy, this patient achieved CRp (platelet count of 62) and 0.7% MRD. Patient C had SD at treatment day 8 with persistence of bone marrow blasts. After one cycle of chemotherapy at day 36, this patient had achieved an MRD-negative CRi (ANC of 0.17).

Table 1. Baseline characteristics and disease response.

	Patient A	Patient B	Patient C
Age (years)	45	19	25
Sex	Male	Female	Male
Date of Diagnosis	Oct 2015	Jan 2013	Jun 2012
No. of prior therapies	6	7	3
Prior Therapy	1. intensive chemotherapy; 2. blinatumomab, cytarabine; 3. inotuzumab; 4. maintenance chemotherapy; 5. CAR-T; 6. SCT	1. intensive chemotherapy; 2. intensive chemotherapy; 3. blinatumomab, chemotherapy; 4. intensive chemotherapy, vorinostat, decitabine; 5. intensive chemotherapy, bortezomib, dasatinib; 6. fludarabine, cyclophosphamide; 7. CAR-T	1. intensive chemotherapy; 2. SCT; 3. SCT
Time on study	79 days	63 days	43 days
Day 8			
Response	CRi	SD	SD
MRD	1.0%	88%	90%
Day 36			
Response	CR	CRp	CRi
MRD	Negative	0.7%	Negative

CAR-T, chimeric antigen receptor therapy; SCT, stem cell transplant; CR, complete remission; CRi, CR with incomplete blood count recovery; CRp, CR with incomplete platelet recovery; SD, stable disease

Summary/Conclusion: Preliminary data suggest that the combination of

Ven and Nav is efficacious in patients with relapsed ALL that have failed multiple lines of therapy. The efficacy of Ven and Nav is potentiated by the addition of chemotherapy, and led to CR, CRi or CRp in all patients and MRD-negativity in 2 of the 3 patients. Long term follow-up to assess durability of response in these patients is ongoing. Results from this trial, along with data from an ongoing phase 1 study using Ven monotherapy followed by chemotherapy (NCT03236857) will help assess the overall contribution of Nav to the activity of Ven in this patient population.

PS929

IDENTIFICATION OF BCR-ABL-LIKE PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) PATIENTS BY REAL-TIME PCR

G. Tsaur[1,*], T. Mukhacheva[2], Y. Olshanskaya[3], S. Kovalev[2], O. Soldatkina[3], A. Druy[3], P. Sibiryakov[4], A. Vlasova[4], L. Vakhonina[1], O. Arakaev[1], L. Saveliev[5], L. Fechina[1]

[1]Regional Children's Hospital [1], Research institute of Medical Cell Technologies, [2]Ural Federal University named after the First president of Russia B.N. Yeltsin, Ekaterinburg, [3]National medical research centre of Pediatric Hematology, Oncology and Immunology named after D. Rogachev, Moscow, [4]Regional Children's Hospital [1], [5]Ural State Medical University, Ekaterinburg, Russian Federation

Background: Current strategy of *BCR-ABL*-like ALL identification is based on gene expression profile either by microarray, next-generation sequencing or TaqMan low-density array. All techniques are laborious and hardly transferrable. However easy and reliable characterization of *BCR-ABL*-like ALL is of high demand.
Aims: To work out the real-time PCR system for the identification of *BCR-ABL*-like ALL patients and to estimate clinical and genetic characteristics of pediatric *BCR-ABL*-like ALL cases
Methods: Based on previously published data (*R. Harvey et al. ASH 2013 abs #826.*) we estimated expression of *IGJ, SPATS2L, MUC4, CRLF2* and *CA6* genes in bone marrow samples of *BCR-ABL*-positive childhood ALL patients (n=9) obtained at the time of initial diagnosis by real-time PCR. Genes' expressions were estimated by ΔCt method in relation to *ABL* expression. Based on obtained results we made a classifier that was later applied on 97 pediatric 'B-others' ALL patients aged 1.2-17.0 years (median 4.0 years). 'B-others' group were formed after exclusion of translocations t(9;22)(q34;q11), t(12;21)(p13;q22), t(1;19)(q23;p13), 11q23/*MLL* rearrangements, high hyperdiploidy, hypodiploidy. In case of having expression results similar to *BCR-ABL*-positive cases, patients with *BCR-ABL*-negative ALL were attributed as *BCR-ABL*-like ALL. In 97 'B-others' ALL we evaluated *IKZF1* status by MLPA using P335 kit (MRC-Holland). We tested *BCR-ABL*-like ALL group for the presence of *ABL1, ABL2, CRLF2, IgH, JAK2, PDGFRb/CSFR1* gene rearrangements by FISH. Prognostic significance of *BCR-ABL*-like expression profile was estimated in 77 'B-others' patients treated according to the ALL-MB 2008 protocol. Informed consent was obtained in all cases.
Results: Hierarchical cluster analysis and principal component analysis showed that 15 cases out of 97 pediatric 'B-others' ALL patients were clustered together with 9 *BCR-ABL*-positive ones. In concordance with previously published data our *BCR-ABL*-like group identified by 5-genes approach was enriched by *IKZF1* deletions, *JAK2* and *CRLF2* rearrangements. *IKZF1* gene deletions were found more frequent in *BCR-ABL*-like ALL patients (67%) in comparison to non-*BCR-ABL*-like cases (11%) (p<0.001). Among 9 *BCR-ABL*-like cases *JAK2* rearrangements by FISH were found in 2, *CRLF2* in 4 (2 cases of *CRLF2-IgH* and 2 *CRLF2-P2RY8*). In 2 patients none of tested gene rearrangements were revealed. Moreover, in one case of *BCR-ABL*-like signature iAMP21 was identified, which is also in line with earlier published results (*J. M. Boer et al., Oncotarget 2017*). *BCR-ABL*-like profile in the observed cohort of patients was associated with female gender (80% vs. 57% p=0.011), high initial WBC (≥30*10^9/L) (67% vs. 18%, p=0.001), M3 bone marrow status on day 15 of induction remission (27% vs. 4%, p=0.010). As a results of high initial tumor load, *BCR-ABL*-like ALL patients more often were stratified to high-risk group (40% vs. 9%, p=0.001). *BCR-ABL*-like ALL patients had significantly worse outcome. Event-free survival in this cohort of patients was lower compared to non-*BCR-ABL*-like group (0.25±0.14 vs. 0.89±0.04, p<0.001) while cumulative incidence of relapse was remarkably higher (0.55±0.16 vs. 0.03±0.02, p<0.001).
Summary/Conclusion: Thus, we showed that real-time PCR technology based on expression data of 5 genes allowed us to detect the group of pediatric *BCR-ABL*-like ALL patients, enriched with *IKZF1* gene deletions, *JAK2, CRLF2* rearrangements. The observed group of patients had initial high-risk features, slower treatment response and as a consequence the unfavorable outcome within ALL-MB 2008 protocol.

PS930

DELIVERY OF INTRATHECAL METHOTREXATE CHEMOPROPHYLAXIS TO ADULTS AND TYA PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA – AN AUDIT OF FACTORS AFFECTING PROTOCOL ADHERENCE

S.A. Sommerfeld[1,*]

[1]Haematology, Christie Hospital, Manchester, United Kingdom

Background: ALL is a curable disease in 30-40% of adults up to 60 years and 60-70% of young adults aged 16-25 years. CNS relapse is associated with a poorer prognosis. Intrathecal chemoprophylaxis with methotrexate (IT MTX) is a standard of care and integral part of modern treatment protocols. In the UK, protocol schedules like UKALL14 and UKALL 2011 define total numbers of IT MTX. Keeping up with scheduled IT MTX prior to maintenance can be challenging and we recognize this in our clinical practice
Aims: Objective 1: Compare numbers of administered IT MTX with protocol scheduled doses to determine compliance. Objective 2: Examine factors leading to cancellation of IT MTX
Methods: Included were patients aged 16 to 60 years with a diagnosis of B or T – ALL or LBL who received IT MTX following either the UKALL 2011 or the UKALL 14 protocol. Excluded were patients with CSF disease at presentation, systemic or CSF relapse or cessation of protocol guided treatment for other reasons. Objective 1: EPR data from 31.8.2011 to 31.8.2017 were reviewed for IT MTX. Compliance with the IT MTX schedule as per current protocol phase was assessed by a compliance score (number of administered doses divided by protocol defined doses). Objective 2: Cancellations of protocol scheduled prescribed IT MTX between 1.1.2016 and 31.7.2017 were reviewed.
Results: Objective 1: 56 patients were identified. Of these, a total of 29 (51.8%) were TYA patients (16-25 years of age at time of commencing treatment) and 27 (48.2.7%) were adult patients (26 – 60 years). Of scheduled IT MTX doses, 135/176 (76.7%) were given in adults and 221/279 (79.8%) in TYA (78.6% in total). Compliance scores in adult and TYA did not differ significantly. Objective 2: 75 events leading to 70 cancellations were identified and separated in categories (Platelets, Coagulation, Rescheduled, Did not attend/communication issues, Infection, Toxicity, Vincristine administration, Anticoagulated, Failed lumbar access, Other). There were 56 events in 16 TYA and 19 in 10 adult patients. The 3 patients with the most events had overall compliance scores of 72.7%, 81.8% and 90.9%, respectively. Comparing TYA and adult events for "coagulation" by chi square test for independence, there was a statistically non-significant relationship that cancellation of an IT MTX due to abnormal coagulation parameters differed between the TYA group and the adult group (p-value 0.143538) (Figure 1).

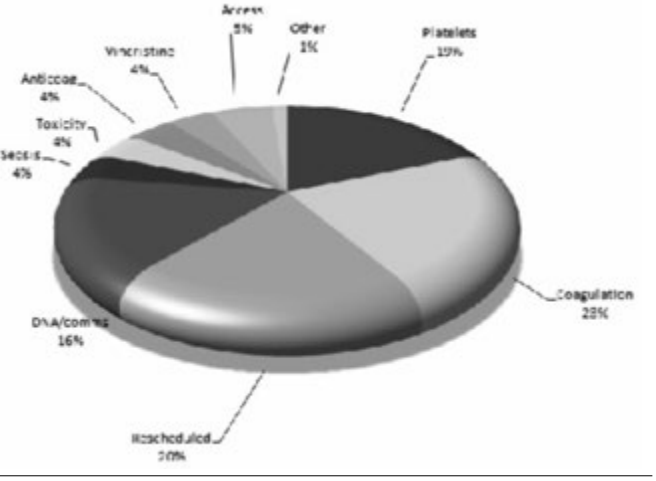


Figure 1. Causes for cancellation of IT MTX.

Summary/Conclusion: We believe this is the first audit examining IT MTX compliance in defined treatment schedules. The strength of this work lies in review of real life data. Limitations are a relatively small case numbers and bias. Data from objective 1 suggest IT MTX following a trial protocol is deliverable. The analysis of events in objective 2 shows compelling clinical reasons for most cancellations, but also scope for improvement. We suggest that targeting scheduling issues by integration of records into a single information system may improve overall efficacy of IT MTX. More data by other centres and re-audit are encouraged.

PS931

THE PROGNOSIS FOR PATIENTS WITH PHILADELPHIA CHROMOSOME POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA WHO WERE INELIGIBLE FOR ALLOGENEIC STEM CELL TRANSPLANTATION IN THE TKI ERA

A. Shigematsu[1,*], K. Minauchi[1], S. Takahashi[1], J. Iwasaki[1], T. Yamakawa[1], M. Ogasawara[1], M. Imamura[1], N. Kobayashi[1], S. Ota[1]

[1]Department of Hematology, Sappro Hokuyu Hospital, Sapporo, Japan

Background: The survival of the patients with Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ALL) has improved by the introduction of tyrosine kinase inhibitors (TKIs), especially for those who received allogeneic stem cell transplant (allo-HSCT). However, there has been a few data about the prognosis for patients with Ph+ALL who were ineligilble for allo-HSCT.
Aims: We conducted a retrospective analysis of prognosis for patients with Ph+ALL who were ineligible for allo-HSCT.
Methods: Clinical data of patients were retrospectively collected from medical records in Sapporo Hokuyu Hospital. The patients' eligibility criteria were as follows: diagnosed as Ph+ALL since 2003 and aged more than 25 years. Descriptive statistical analysis was performed using the Fisher's exact test for categorical variables and using the 2-sided Wilcoxon rank sum test for continuous variables. The probability of survival was estimated using the Kaplan-Meier method. All P-values were two-sided and a P-value of 0.05 was used as the cutoff for statistical significance.
Results: Sixty-four patients were eligible for the study, and median age of the patients was 41 years (range; 31-89). Median count of WBC at diagnosis was 11000/μL (860- 367300), and 31 of the patients had additional chromosomal abnormalities. Sixty-one patients received induction therapy including a TKI (Imatinib, n=44; Dasatinib, n=17), and 57 patients achieved complete remission. Thirty-five of the patients were received allo-HSCT (HSCT group) and the remaining 29 were did not receive allo-HSCT (non-HSCT group). Age was significantly higher in the non-HSCT group (non-HSCT, 65 years; HSCT, 45 years, p<0.001), and other characteristics of the patients; such as sex, WBC count at diagnosis and additional chromosome did not differ between the two groups. At the median follow-up days of 955 (range: 305-5077 day), overall survival was significantly worse in the non-HSCT group (p=0.009). The median day of death of the non-HSCT group was 451 (15-1284), and the major cause of death was relapse (81.0%). Only five of the fifteen relapsed patients achieved CR2, and all of the 5 patients relapsed early after achievement of CR2 (Figure 1).

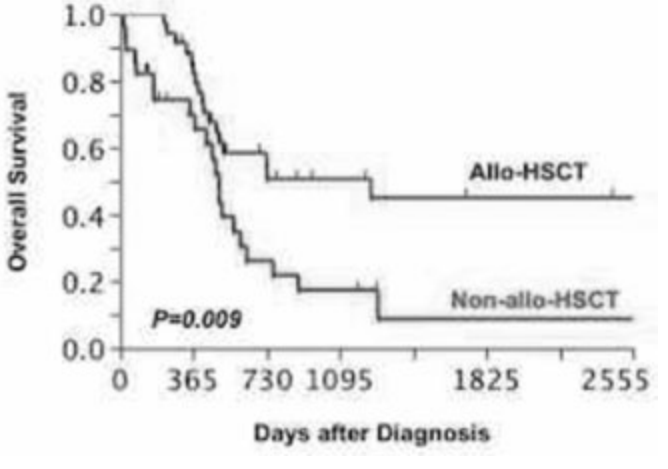


Figure 1. Overall survival according to allo-HSCT.

Summary/Conclusion: Our analysis suggests that overall survival was inferior for the Ph+ALL patients who did not received allo-HSCT, and the major cause of death was relapse. Few patients achieved CR2 after relapse, and more effective consolidation therapy is therefore needed for improvement of survival for patients who are ineligible for allo-HSCT.

PS932

COMPARISON OF NEXT GENERATION SEQUENCING AND RT-PCR APPROACHES FOR MINIMAL RESIDUAL DISEASE MONITORING IN ACUTE LYMPHOBLASTIC LEUKEMIA

A. Miroshnichenkova[1,*], A. Komkov[1,2], E. Zerkalenkova[1], I. Mamedov[2], Y. Olshanskaya[1]

[1]Laboratory of Cytogenetics and Molecular genetics, Dmitry Rogachev National Research Center of Pediatric Hematology, Oncology and Immunology, [2]Laboratory of Comparative and Functional Genomics, Shemyakin-Ovchinnikov Institute of bioorganic chemistry, Moscow, Russian Federation

Background: Being the major cause of relapse in leukemia, minimal residual disease (MRD) is considered to be the strongest prognostic factor, allowing to evaluate the efficiency of a treatment, and to make a decision on the subsequent therapy. Detection of MRD by Next generation sequencing (NGS) is proven to be one of the most efficient and sensitive techniques. Detection of clonal rearrangements of immunoglobulin (Ig) and T-cell receptor (TCR) genes is widely used for clonality assessment and for MRD monitoring along with RT-PCR of fusion transcripts, and unlike the latter one, is applicable in most cases of lymphoblastic malignancies.
Aims: The purpose of this study is to compare a new approach for monitoring MRD in acute lymphoblastic leukemia (ALL) by targeting massive sequencing of clonal rearrangements of immunoglobulin (IG) and T-cell receptor (TR) genes with MRD monitoring via RT-PCR of fusion transcripts.
Methods: A cohort of uniformely treated patients consisted of children aged 4 to 13 years diagnosed with ALL. DNA was extracted from patients' bone marrow (BM) samples on the 1st and 36th day of treatment. Initial detection of patient specific clonal rearrangements was carried out by 8 multiplex PCRs of Ig and TCR loci followed by NGS. MRD detection included targeted NGS of previously detected rearrangements in post-treatment follow-up samples in serial dilutions. The rearrangements chosen for future MRD diagnostics were chosen using 5% cut-off. Quantitative analysis was based on "Digital PCR"-like statistical approach.
Results: We identified over 500 leukemic rearrangements in 97 patients using NGS technique. All cases had detectable Ig and/or TCR rearrangements with a median number of 3 per sample. 65% of samples contained rearranged TCRG, and in 85% of samples IGH was rearranged. Complete VDJ rearrangements dominated over incomplete DJ rearrangements in both IGH and TRB. We compared the MRD levels of 21 follow-up samples with data obtained by RT-PCR of fusion transcripts including t(12;21)(p13;q22) ETV6/RUNX1, t(5;14)(q35;q32) BCL11B/TLX3, and t(4;11)(q21;q23) KMT2A/AFF1. Three KMT2A/AFF1 positive cases revealed a strong correlation between MRD level measured by RT-PCR and NGS. Among 17 RT-PCR negative results, 14 were confirmed negative by NGS, however 3 of those follow-up samples showed significant MRD level, verified by flow cytometry.
Summary/Conclusion: RT-PCR of fusion genes is a reliable and widely used MRD detection technique, however, it is only applicable for patients with known fusion transcripts. Moreover, our data showed that in 17% of cases MRD negativity by RT-PCR was not confirmed by NGS, clearly showing the presence of tumor clones in analysed samples, undetectable by RT-PCR. Therefore, NGS-based detection of rearrangements of Ig and TCR loci, being a sensitive, specific, and widely applicable method, allowing for clonality evaluation and MRD quantification, is likely to become a routine procedure for MRD diagnostics in the nearest future.
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PS933

ENHANCING MANAGEMENT OF PH+ ALL BY MONITORING WITH AN ANALYTICALLY VALIDATED MULTIPLEX ASSAY FOR BCR-ABL1 MINOR BREAKPOINT (E1A2) WITH HIGHLY SENSITIVE DETECTION OF 1:40,000 (0.0025% OR 4.6 LOGS)

J. Brown[1,*], M. Dodge[1], I. Beldorth[1], S. Filipovic-Sadic[1], K. Jefferson[1], M. Fahey[1], J. hedges[1]

[1]Asuragen, Austin, United States

Background: Management of ALL requires MRD quantification to assess response dynamics. For Philadelphia-chromosome-positive acute lymphoblastic leukemia (Ph+ ALL), such assessment of tumor burden can be achieved through flow cytometry and molecular monitoring (PCR-based) of the BCR-ABL1 fusion transcript of t(9;22), frequently e1a2 (minor break-

point, m-BCR). MRD is valuable post-induction and to assess eligibility for stem cell transplantation (SCT) after consolidation. For Ph+ ALL, prolonged monitoring of BCR-ABL1 MRD levels is recommended by the ESMO Clinical Practice Guidelines to guide changing TKI. As therapeutic progress results in deeper clinical responses, analytical sensitivity has become a critical topic for reliable measurement of complete molecular remission (MolCR), the most important prognostic factor for disease-free and overall survival. This level requires an MRD assay for minor breakpoint that confidently and consistently calls molecular responses of ≥1:10,000 (≥4 logs or ≤0.01%).

Aims: We describe the analytical validation and method comparison of a CE-marked IVD multiplex system reporting continuous BCR-ABL1:ABL1%ratio values via automated analysis.

Methods: Encapsidated RNA molecules form blends of nuclease-resistant BCR-ABL1 and ABL1 transcripts to calibrate and control the system. Multiplexed 4-point curves using such blends provide copy values and account for the relative run-specific efficiency of the RT step. Controls (high, low, negative) were also developed using encapsidated RNAs. cDNA generation and qPCR were optimized to allow high mass of nucleic acid without inhibition to drive analytical sensitivity. Cell line RNA was diluted into non-leukemic human RNA to create challenge panels for most validation studies. RNAs negative for e1a2 tested specificity. Peripheral blood from e1a2-positive ALL patients were collected (n=13) with informed consent from age range 33-73 (median 59) across the 4 groups: 15-35 (1), 36-55 (5), 56-70 (6), and >70 (1). They were tested against a second CE-marked IVD kit capable of e1a2 detection as a comparator. The software includes a logic algorithm that flags any specimen requiring further review. Statistical analyses were carried out after log transformation to achieve normal distributions. Specifically, we introduced "Log Reduction" (LR) values as the log reduction from theoretical totality of 100% (LR = log10(100%÷%ratio)), similar to Molecular Response (MR) values for Major breakpoints.

Results: Our gains in analytical sensitivity allowed detection of background e1a2 mRNA in non-leukemic specimens. Specifically, an LOB was determined both for BCR-ABL1 copy number of 1 copy/qPCR and for%ratio of 0.0010% (LR5.00, 1:100,000). The LOD (classical parametric) and LOQ were statistically distinct from LOB at 0.0025% (LR4.61, 1:40,000) and 0.0039% (LR4.45, 1:26,000), respectively. Despite deep analytical sensitivity, this system maintains analytical specificity ("below LOD" for BCR-ABL1-negative samples); however, it does demonstrate the expected low-level crosstalk from contrived clonal specimens of extremely high BCR-ABL1 Major breakpoint transcripts. Linearity was validated to encompass 4 logs, from 0.0025% to 25% (LR4.61 to LR0.61).

Summary/Conclusion: The newly developed test kit quantifies deep response dynamics to 1:40,000 (4.6 log) and improves workflow with streamlined reagent formulation, multiplex format, and automated software analysis.

PS934

THE RISK OF DEVELOPING HEPATIC TOXICITY FROM PEGYLATED-ASPARAGINASE IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS IS HIGHLY AFFECTED BY CONCOMITANT MEDICATIONS

P. Minetto[1,*], N. Bisso[1], F. Guolo[1], M. Clavio[1], R. Marcolin[1], M. Miglino[1], F. Ballerini[1], R.M. Lemoli[1], M. Gobbi[1]

[1]*Clinic of Hematology, Department of Internal Medicine (DiMI), University of Genoa, Policlinico San Martino, IRCCS per l'Oncologia, Genoa, Italy*

Background: The application of pediatric-inspired regimens for treating adult acute lymphoblastic leukemia (ALL) has led to a significant improvement in clinical outcome. However, concerns about the use of pegilated L-Asparaginase (PEG-ASP) have emerged. While patient-related risk factors as high BMI or hepatic steatosis have already been identified, few data are available on the role of concomitant drugs potentially contributing to PEG-ASP toxicity.

Aims: To identify patients and therapy-related factors related to PEG-ASP toxicity in a cohort of adult ALL patients.

Methods: Since 2013, 26 adult ALL patients received PEG-ASP in our Center. Median age was 47 years (range 19-76); 19 patients were treated in frontline setting (13 according to a full pediatric protocol) whereas 7 patients received PEG-ASP during salvage therapy for relapsed/refractory disease. We analyzed each course of therapy including PEG-ASP administration as an independent event, accounting 51 episodes. Patients' features, concomitant therapies were analyzed as factors potentially affecting PEG-ASP toxicity. The incidence of grade III-IV thrombotic/bleeding, hepatic or pancreatic toxicity was recorded.

Results: No grade III-IV pancreatic, thrombotic or hemorrhagic adverse event were observed. Grade III hepatotoxicity was observed in 5 patients; 3 patients had grade IV hepatotoxicity and experienced unexplained severe weight gain and painful hepatomegaly, a clinical picture resembling sinusoidal occlusive disease. Ultrasonography showed the onset of acute liver steatosis. All 3 patients had received concomitant therapy with Idarubicin (IDA), vincristine (VCR) and vancomycin. In univariate analysis age > 45 yrs, administration of PEG-ASP therapy with active leukemia or BMI >25 were not related with an increased incidence of grade III-IV hepatotoxicity which was instead significantly higher when a cumulative dose of IDA of at least 20 mg/sqm (p 0.047, HR 1.49) was administered. Cumulative dose of VCR of at least 2 mg/sqm determined a borderline increase in toxicity risk (p 0.055, HR 4.75). No increase was observed with any dose of steroids, daunorubicin, cyclophosphamide, cytarabine, methotrexate, and 6-mercaptopurine. Among antimicrobial therapies, vancomycin administration increased the risk of grade III-IV hepatotoxicity (p 0.009, HR 1.86). No significant increase in the risk of toxicity was observed with carbapenems and azoles. Notably, none of the patients undergoing full pediatric induction, which contains higher cumulative doses of PEG-ASP, experienced grade IV hepatotoxicity, regardless of age (range: 19- 55). A multivariate logistic regression analysis disclosed that administration of IDA or vancomycin were independent predictors of grade III/IV hepatotoxicity (p 0.004, 0.054; Table 1).

Table 1. Factors affecting Hepatic Toxicity.

	Grade III/IV hepatic toxicity (%)	HR	p (univ)	p (multiv)
Age >45	5/22 (23)	-	0.440	-
BMI >25	2/15 (13)	-	1.000	-
Full pediatric schedule (higher PEG-ASP dose)	3/27 (11)	-	0.440	-
Daunorubicin	4/23 (17)	-	1.000	-
Vincristine	7/26 (27)	4.75	0.055	0.137
Cyclophosphamide	5/23 (22)	-	0.698	-
Idarubicin	3/6 (50)	1.49	0.047	0.004
Cytarabine	1/15 (7)	-	0.406	-
Mercaptopurine	0/12 (0)	-	0.173	-
Methotrexate	0/9 (0)	-	0.322	-
Steroids	8/37 (22)	-	0.173	-
Vancomycin	4/7 (57)	1.86	0.009	0.054
Azoles	6/23 (26)	-	0.125	-
Carbapenems	5/15 (33)	-	0.086	0.543
Overall	8/41 (20)			

Summary/Conclusion: In our experience the toxicity profile of PEG-ASP in adult patients is overall manageable and even higher dose of PEG-ASP were administered without excess toxicities. However, our data suggest that concomitant drugs play a crucial role contributing to PEG-ASP hepatotoxicity.

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PS935

CELL OF ORIGIN INFLUENCES DISEASE AGGRESSIVENESS AND CHEMOTHERAPY RESISTANCE IN A MOUSE MODEL OF MLL-AF9 REARRANGED ACUTE MYELOID LEUKEMIA

C. H. Nguyen[1,*], K. Bauer[1], R. Wieser[1]

[1]Department of Medicine I, Medical University of Vienna, Vienna, Austria

Background: The cell from which a cancer originates has a profound impact on its clinical properties. This has also been suggested for acute myeloid leukemia (AML), where the presence of a stem-cell like gene expression signature in leukemic blasts was associated with a poor prognosis. Especially AML with rearrangements of the MLL locus in 11q23 is thought to be able to arise from either stem or progenitor cells, and the cell of origin appears to influence the course of disease
Aims: Using a mouse model of MLL-AF9 (MA9) driven AML, we further investigate the impact of the cell of origin on clinical properties of resultant AML.
Methods: A well-established and clinically relevant model of AML driven by expression of the fusion oncogene MA9 was used. The hematopoietic stem cell-enriched $Lin^-Sca\text{-}1^+c\text{-}kit^+$ (LSK) cell and the common myeloid progenitor (CMP) populations were retrovirally transduced with MA9 and transplanted into sub-lethally irradiated mice. Leukemic cells were harvested from terminal ill mice and subjected to flow cytometric analysis to determine the frequency of leukemic stem cells (LSCs) and of mature myeloid ($Mac\text{-}1^+$ and $Gr\text{-}1^+$) cells. Cell cycle analysis of LSCs was performed by intracellular staining with Ki-67 and DAPI. Furthermore, cell renewal capacity was determined using the serial replating assay, and sensitivity to drugs used in standard chemotherapy (cytarabine and daunorubicin) of AML was measured using a metabolic activity assay.
Results: LSK_MA9 cells were more potent in inducing AML in mice than CMP_MA9 cells, indicated by a shorter disease latency and a higher white blood cell count at the time of sacrifice. LSK_MA9 derived AML cells had a significantly higher frequency of LSCs and a lower frequency of mature myeloid cells as determined by cell surface marker phenotype. Also, LSCs derived from LSK_MA9 was more quiescent in G_0 phase of cell cycle. Accordingly, LSK_MA9 derived AML cells formed more colonies than CMP_MA9 derived AML cells through 4 serial replatings. Furthermore, high expression of *Evi1*, a stem-cell associated proto-oncogene linked to a poor prognosis in AML, was found in LSK_MA9 but not CMP_MA9 derived AML. Finally, LSK_MA9-derived AML cells were less responsive to cytarabine and daunorubicin than those derived from CMP_MA9.
Summary/Conclusion: Our data lend further support to the notion that the cell of origin influences the clinical outcome of AML. Specifically, expression of MA9 in LSK cells resulted in more aggressive and chemotherapy resistant AML as compared to AML derived from MA9 transduced CMPs.

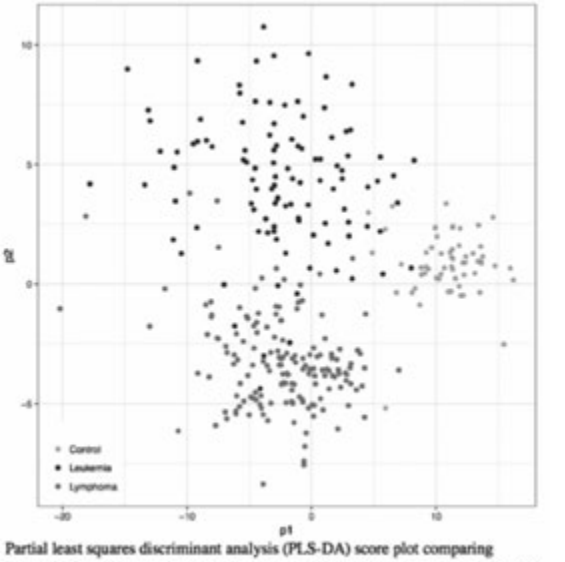
PS936

PLASMA PROTEOME IN PATIENTS WITH LEUKEMIA AND LYMPHOMA REVEALS MAJOR DIFFERENCES RELATED TO HEMOSTASIS, INFLAMMATION AND STROMA

A. Eriksson[1,*], A. Abu Sabaa[2], Q. Shen[2], E. Bergfelt Lennmyr[1], A. Enblad[1], G. Gammelgård[2], A. Hein[2], E. Freyhult[3], M. Kamali-Moghaddam[2], M. Höglund[1], G. Enblad[2]

[1]Department of Medical Sciences, [2]Department of Immunology, Genetics & Pathology, [3]Department of Medical Sciences, National Bioinformatics Infrastructure Sweden, Science for Life Laboratory, Uppsala University, Uppsala, Sweden

Background: Acute leukemias and lymphomas are highly malignant disorders associated with poor prognosis and high frequency of relapse.
Aims: In order to increase the knowledge of the disease biology, and to investigate the diagnostic and prognostic value of plasma proteins for these diseases, a large number of proteins were analyzed in plasma samples from consecutive acute leukemia and lymphoma patients and compared to healthy controls
Methods: Multiplex proximity extension assays (PEA) were utilized to assess plasma samples (1 µL), from leukemia and lymphoma patients included in the U-CAN biobank as well as age-matched healthy controls, using two Olink™ multiplex protein panels (ONCII and CVDIII). In multiplex PEA, each target protein is recognized by a pair of proximity probes that in proximity are hybridized to each other allowing enzymatic DNA polymerization and subsequent DNA amplification. In total, 183 proteins were measured in 265 plasma samples taken at diagnosis; 113 acute leukemias (Acute Myeloid Leukemia, AML: 69, Acute Lymphoblastic Leukemia, ALL: 35, Acute Promyelocytic Leukemia, APL: 9) 158 lymphomas (Diffuse Large B-Cell Lymphoma, DLBCL: 105, Hodgkin's Lymphoma, HL: 53) and 60 healthy controls. Results were obtained as Normalized Protein Expression (NPX) on a log2 scale where a high NPX value corresponds to a high protein concentration. For each protein, the difference in NPX between groups (leukemia vs controls, lymphoma vs controls and leukemia vs lymphoma) was assessed using a linear regression model, adjusting for age and gender. In addition, multivariate modeling (PLS-DA) was performed.
Results: Highly significant differences were observed for plasma protein levels between leukemia and lymphoma patients and healthy controls. Comparing the leukemia samples to controls, the 10 most significantly differentiating proteins were: vWF, SYND1, TNF-RSF6B, MPO, VIM, TNF-R1, IL-6, CTSD, ADAM-TS15 and FURIN; all with higher levels in leukemias except for ADAM-TS15. When lymphoma samples were compared to controls, the top 10 differentiating proteins were: PAI, MMP-9, VIM, AZU-1, MPO, HGF, PDGF subunit A, S1000A11, TGF-α and FURIN; all with higher levels in lymphomas. The analysis also allowed distinguishing between patients with leukemia and lymphoma, for which the top 10 identified proteins were MMP-9, PDGF subunit A, SPARC, PGLYRP1, vWF, TNFRSF10C, PAI, TR, TGF-α and GPNMB. The PLS-DA model distinguished between leukemia and lymphoma with very few patients being misclassified. Of the 5 ALL patients clustering with the lymphomas, 4 out of 5 samples had lymphoblastic lymphomas. For the 2 DLBCL cases misclassified as leukemias, both had bone marrow involvement by lymphoma. When the subgroups of leukemias and lymphomas were compared, major differences were observed between AML, APL and ALL as well as between HL and DLBCL (Figure 1).



Partial least squares discriminant analysis (PLS-DA) score plot comparing leukemia, lymphoma and controls. The cross-validated average error rate is 0.028.

Figure 1.

Summary/Conclusion: Using the highly specific and sensitive PEA technology, we identified sets of plasma proteins significantly distinguishing samples from patients with leukemia, lymphoma, and healthy controls. Samples from patients with acute leukemia had higher levels of proteins associated with hemostasis, cell to matrix integration and inflammation, whereas samples from lymphoma patients had higher levels of proteins associated with stroma and neutrophil activation. This technology appears to be a feasible screening technique for plasma protein biomarkers in minute sample volumes, and may be a valuable tool in the diagnostics and prognostics of various subgroups of acute leukemias and lymphomas.

PS937

IDENTIFICATION OF CALM/AF10 (PICALM/MLLT10) COOPERATING MUTATIONS IN A ZEBRAFISH LEUKAEMIA MODEL

O. Delfi[1,*], Y.J. Sung[1], P.J. Browett[1], P.M. Kakadia[1], S. Bohlander[1]

[1]Department of Molecular Medicine and Pathology, University of Auckland,

Leukaemia & Blood Cancer Research Unit, Auckland, New Zealand

Background: Acute Myeloid Leukaemia (AML) is one of the most frequent and aggressive types of blood cancer and can lead to the death of patients just a few weeks after diagnosis. AML is caused by somatic genetic aberrations, such as recurring chromosomal translocations and point mutations. The *PICALM/AF10* fusion gene results from the t(10;11)(p12;q14) that is found in about 1% of AML cases as well as in some acute lymphoblastic leukaemia cases.
Aims: Establish a CALM/AF10 transgenic zebrafish leukaemia model using the minimal fusion of CALM/AF10 (CA-MF), which has been shown to induce leukaemia in mice.
Methods: In our model, CA-MF is expressed under the control of the murine Runx1+23 promoter, which restricts CA-MF expression to zebrafish hematopoietic stem cells. Two transgenic constructs (pTol2-Runx1+23_CA-MF_IRES-EGFP and oTol2-Runx1+23_CA-MF_IRES-mCherry) were injected into fertilized zebrafish eggs at the one cell stage. Two days after injection, embryos were sorted based on the expression of the heart marker.
Results: More than 85% of the transgenic embryos died in the first 5 days after injection and only about 5% of F0 zebrafish reached four months of age. Between 3 to 8 months, 50% (5 out of 10 fish) fish developed signs of disease. They became less active, sat at the bottom of the tank and their abdomens became distended. At this stage, the fish were euthanized and dissected. The autopsies showed tissue overgrowth around the kidney and histological sections revealed increased cellularity of the kidney and liver with massive infiltration of hematopoietic cells in the tissue sections of these organs. In flow cytometry, the cells from the kidney marrow from the transgenic fish showed a different pattern in the forward scatter (FSC) and side scatter (SSC) profile compared to the FSC/SSC profile of normal zebrafish kidney marrow cells. The changed FSC/SSC profile was compatible with an expansion of the hematopoietic progenitor cell population in the transgenic F_0 zebrafish. These findings strongly suggest that the fish had developed a leukaemia-like disease. Currently, we are establishing tissue transplantation to determine whether the disease is transplantable.
To identify potentially cooperating mutations, we performed whole exome sequencing (WES) of the tumor cells and germline tissue (muscle) of six CA-MF zebrafish. We obtained about 10 gigabases of sequence per exome. Preliminary analysis of the WES data identified somatic mutations among others in genes whose human homologues are frequently mutated in patient samples, like *kras*, *dnmt3*, and *ezh1*. The identification of somatic mutations was complicated by the fact that the tumor and germline DNA samples that we used for our sequencing experiments only had a purity of between 70 to 80% (i.e. the germline DNA samples contained between 20 to 30% tumor DNA and the tumor DNA samples contained about 20 to 30% of germline DNA).
Summary/Conclusion: While the flow cytometry, histology and sequencing data from our transgenic zebrafish are strongly suggestive of leukaemia, additional experiments are required to further characterize our model and confirm the malignant nature of the observed tumors (e.g. by performing transplantatioan experiments).
To date only a handful of zebrafish leukaemia models have been established. Our new CA-MF zebrafish model together with the ease with which additional genetic manipulation can be performed in zebrafish will be a helpful tool to understand leukaemia biology.

PS938

FLT3-ITD ANALYSIS ON RNA CONFERS ENHANCED SENSITIVITY AND ALLOWS EARLIER RELAPSE PREDICTION

E. Oppliger Leibundgut[1,2,*], R. Joncourt[1], N.A. Porret[1], U. Bacher[1], M. Andres[1], G. Baerlocher[1,2]

[1]Hematology, University Hospital Bern, [2]Biomedical Research, University of Bern, Bern, Switzerland

Background: *FLT3* internal tandem duplications (ITD) are reported in ~30% of patients with acute myeloid leukemia (AML) and have been associated with a high relapse rate and poor outcomes. The FLT3 inhibitor midostaurin has been approved for targeted therapy of *FLT3* mutated AML, conferring a survival benefit. Thus, rapid molecular testing for *FLT3*-ITD is required as well as techniques for more sensitive molecular monitoring of response and minimal residual disease (MRD) assessment. *FLT3*-ITD analysis is currently performed by fragment length analysis (FA) that has a limited sensitivity of 2-5%. There is no consensus on whether DNA or RNA is the specimen of choice. Newer protocols are based on DNA including the FDA-approved companion test (Leukostrat *FLT3*). Due to *FLT3* overexpression on AML blasts, we hypothesized that use of RNA might increase the sensitivity of FA.
Aims: We compared the sensitivity of *FLT3*-ITD detection by FA on paired DNA and RNA samples.
Methods: Bone marrow (BM) or peripheral blood (PB) samples from 42 patients with AML were collected at diagnosis and follow-up. Samples were subjected to DNA and RNA extraction followed by PCR and FA using the Leukostrat Assay 2.0 (Murphy KM, *et al.* JMD 2003). ITD mutations and allelic ratios (ITD-mutated/wildtype) were determined. The assay was validated down to a limit of detection (LOD) of 2%.
Results: Eighty-two paired samples from 42 patients with AML (median age 62 y, 45% females, 33 ITD-mutated, 9 wildtype) were assessed including 31 BM and 51 PB samples. 30 were diagnostic and 52 follow-up samples from patients with a known ITD. 20 samples (24%) showed corresponding ITDs in RNA and DNA, and 52 (63%) were negative for ITD on RNA and DNA. In 12 samples, ITDs were only detected on RNA but not on DNA. McNemar's chi-squared test for symmetry was highly significant (p=0.00053), indicating that higher sensitivity with the RNA-based method outweighed potentially false negative cases. Of the 12 samples with ITD only on RNA, 4 had ITDs on DNA below the detection limit of 0.05 and therefore were reported as ITD negative. Allelic ratios across all samples ranged between 0.02 and 23.5. Differences of allelic ratios between RNA and DNA were 0.3-226-fold (mean 16-fold). Higher ratios on RNA were found in 75% of cases with positivity on both materials. Three patients with early hematological relapses were studied in detail. In two ITD-mutated patients, ITDs on DNA became undetectable during treatment, and relapses occurred at 5 and 7 months with reappearance of ITDs. On RNA, both patients had detectable ITDs at all time points under treatment and high levels at relapse. The 3rd patient had wildtype *FLT3* on DNA at diagnosis and a relapse after 7 months with 2 ITDs. On RNA, both ITDs were retrospectively detectable already at diagnosis at low levels of 0.02 and 0.04.
Summary/Conclusion: In the era of FLT3 inhibitors, sensitive detection of *FLT3*-ITD at diagnosis and monitoring of response to therapy and MRD are important prerequisites for therapy guidance. Systematic comparison of DNA and RNA analysis revealed positivity on RNA in 12 (15%) cases that were missed on DNA. Follow-up analyses showed that 1) persistence of ITD clones under treatment was relevant for prognosis and associated with earlier relapses, and 2) small initial ITD clones can evolve and require reevaluation of therapy. Higher sensitivity of *FLT3*-ITD detection by using RNA for FA will allow optimizing of therapeutic strategies and elucidate the dynamics of clonal evolution from diagnosis to relapse.

PS939

PHARMACOLOGIC INHIBITION OF CELLULAR METABOLIC PROCESSES IMPACTED BY AGING SENSITIZE ACUTE MYELOID LEUKEMIA (AML) CELLS TO THE NOVEL LIPOATE DERIVATIVE CPI-613

R. Anderson[1], K. Pladna[1], T. Pardee[1,2,*]

[1]Internal Medicine, Section on Hematology and Oncology, Wake Forest Baptist Health, Winston-Salem, [2]Rafael Pharmaceuticals , Cranbury, United States

Background: Altered metabolism is a hallmark of cancer cells, including AML. CPI-613 is a novel lipoate derivative in clinical development by Rafael Pharmaceuticals that inhibits the TCA cycle enzymes, pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase. In a phase one study, CPI-613 in combination with high dose cytarabine and mitoxantrone demonstrated encouraging efficacy especially in older AML patients. One possible explanation is that the cellular metabolic pathways affected by aging contribute to the increased response. Two well documented pathways are the decline in mitochondrial electron transport chain quality that occurs with aging and the decline in autophagy.
Aims: To determine if pharmacological inhibition of the electron transport chain and autophagy would increase the response to CPI-613.
Methods: To determine the effect of TCA cycle inhibition on autophagy and mitochondrial membrane potential a Pdha deleted murine AML cell line was created using CRISPER/Cas9. Human and murine AML cell lines were treated with CPI-613, the electron transport chain inhibitor metformin and the autophagy inhibitor chloroquine alone and in combination. The effects of PDH loss or CPI-613 on autophagy were assessed by Western blotting for p62 and LC3B. The effect of metformin on mitochondrial membrane potential was assessed by TMRM staining. In vivo effects of combination treatment were assessed in a syngeneic, orthotopic, therapy resistant murine AML model.
Results: The murine Pdha deleted cells were significantly more sensitive to autophagy inhibition with chloroquine. They also displayed a decrease in mitochondrial membrane potential and were more sensitive to CPI-613 treatment. In PDHA wild type cells CPI-613 treatment induced autophagy in all AML lines tested. Inhibition of autophagy with chloroquine significantly sensitized AML cells to CPI-613 *in vitro*. *In vivo*, animals treated

with the combination of chloroquine and CPI-613 had a significantly improved survival compared to controls and singly treated animals. In addition, metformin treatment decreased mitochondrial membrane potential and significantly sensitized all AML cells to CPI-613 cell killing *in vitro*. *In vivo*, treatment with CPI-613 and metformin conferred a significant survival benefit.

Summary/Conclusion: These data suggest that response to the novel lipoate analog CPI-613 is influenced by metabolic pathways that are affected by aging. This may help explain the increased benefit seen in older patients treated with CPI-613 in combination with high dose cytarabine and mitoxantrone in a phase I trial. These data further support that AML cells containing less functional mitochondria or those with reduced autophagy capacity may be particularly susceptible to this approach. Future studies will be needed to determine if pretreatment assessment of these functions can be used as a biomarker to predict which patients will most benefit from CPI-613.

PS940

GENOMIC CHARACTERIZATION OF DIAGNOSTIC AND REFRACTORY/RELAPSED ACUTE MYELOID LEUKEMIA SAMPLES THROUGH A NEXT GENERATION SEQUENCING (NGS) PANEL OF 62 GENES

D. Carbonell[1,2,*], C. Martínez-Laperche[1,2], J. Suárez-González[2,3], M. Chicano[1,2], M. Bastos[1,2], G. Rodríguez-Macías[1], M. Kwon[1,2], J.L. Díez-Martín[1,2], I. Buño[1,2,3]

[1]Department of Hematology, Gregorio Marañón General University Hospital (HGUGM), [2]Gregorio Marañón Health Research Institute (IiSGM), [3]Genomics Unit, Gregorio Marañón General University Hospital (HGUGM), Madrid, Spain

Background: Acute myeloid leukemia (AML) is an entity with a complex clonal structure, which presents from 3 to 5 driver mutations at diagnosis. These alterations may be useful as diagnostic and prognostic biomarkers; moreover, during follow-up, they may provide information about treatment response and clonal evolution within the disease. Thus, the study of these variants is becoming essential in a routine clinical practice. During the last years, advances in next generation sequencing (NGS) have revolutionized the world of genetics and cancers

Aims: The aim of this study is to characterize, using NGS, the genomics of AML at diagnosis and at refractoriness/relapse

Methods: Thirty-eight patients diagnosed of AML (non-promyelocytic) were consecutively included in a prospective observational study between November 2015 and February 2018. Samples were obtained from the entire cohort at diagnosis, at refractoriness in a subgroup of 12 of these patients and at relapse in another subgroup of seven patients. As a whole, 57 samples were analyzed. Therefore, two groups of paired samples were obtained: diagnosis-refractoriness and diagnosis-relapse. For NGS analysis, an enrichment-capture custom panel (LMA-GeneSGKit® 62 Genes, GeneSystems, Valencia, Spain) was used. This panel, designed with collaboration of our team, can detect SNP and short indels (<30 bp) in 62 AML-related genes. A mean depth of 300x was reached, obtaining a 10% sensitivity. The GeneSystems software was used for the bioinformatics analysis, and the variant classification was performed according to the American College of Medical Genomics (ACMG) 2015 score. All variants were confirmed by Sanger sequencing or GeneScan fragment analysis

Results: Non-benign variants were detected in 37 of the 38 diagnostic samples. A total of 194 variants in the 70.97% (44/62) of the genes analyzed were found; 99 of these variants were classified as unknown significance (VUS) and 95 as pathogenic or likely pathogenic variants (PV). The most frequently altered gene with VUS was *NF1* and with PV, *DNMT3A*, *FLT3* and *NPM1*. The median number of variants per sample was 5 (range 0-10). In refractoriness and relapse samples, 99 variants in the 53.23% (33/62) of the genes were found, 53 VUS and 46 PV. The main gene with VUS was, as in the case of diagnostic samples, *NF1*. In contrast to diagnostic samples, the most frequent genes with PV were DNMT3A and ASXL1. The median number of variants per sample, as in diagnosis, was 5 (range 2-10). Gain or loss of variants was observed in 73.68% of patients with paired samples (14/19), being *NF1* the gene with the most changing behavior in VUS, followed by *ASXL1*, *FLT3* and *IDH1*, which are affected by variants of pathogenic nature (Figure 1).

Summary/Conclusion: The use of NGS gene panels is useful at diagnosis of AML for an appropriate risk-group classification and at determining the heterogeneity of the diverse subpopulations taking into account the allele frequency of each variant. Moreover, the study of refractory or relapsed samples and its comparison with those at diagnosis, could add to the understanding of the clonal evolution of the disease. Thus, identifying leukemic cells that have a resistance-related variant, treatment selection could be more effective by targeting all potentially malignant clones. However, the lack of consensus in the interpretation of variants makes it a very subjective endeavor, being VUS a very frequently type of variant detected. Functional studies are therefore needed to test the actual cellular consequences of such variants which would lead to a better clinical management of AML patients.

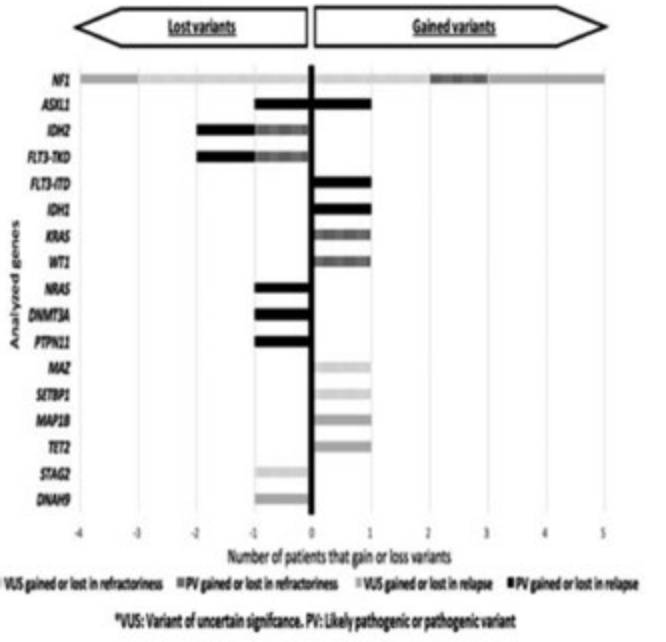


Figure 1.

PS941

HEMATOPOIETIC FUNCTIONS OF R396Q AND R204X GATA2 MUTANTS SUGGEST DISTINCT MECHANISMS LEADING TO THE GATA2 SYNDROME

M. Pasquet[1], S. Lagarde[2], N. Prade[2], L. Jamrog[3], A. Carrette[3], N. Rouquie[3], S. Hebrard[3], P.-Y. Juvin[3], P. Bories[3], C. Bellanne Chantelot[4], J. Donadieu[5], B. Gerby[3], C. Broccardo[3], E. Delabesse[2,*]

[1]Haematology, CHU Toulouse, [2]Haematology, Institut Universitaire de Cancérologie de Toulouse, site Oncopôle CHU de Toulouse, [3]Team [16], CRCT, Toulouse, [4]CHU Pitié, [5]CHU Trousseau, Paris, France

Background: Germline *GATA2* mutations have been identified in patients associated with predisposition to hematological, immunological and vascular disorders. Few data are available regarding the molecular pathogenesis of these mutations.

Aims: We analyze two representative germline GATA2 mutations, R204X leading to a premature stop codon before the N-terminal zinc finger and R396Q, a missense mutation located after the C-terminal zinc finger.

Methods: Human GATA2 coding region was cloned upstream of the IRES of the MSCV-IRES-EGFP retroviral vector. Retroviral particles were produced. Bone marrow cells from C57BL/6 8-week female mice were sorted and infected twice with retroviral vector. Infected lineage-negative cells were plated in triplicate in multipotential methylcellulose.

Results: The cellular location of these mutant proteins is distinct, nuclear for the R396Q mutant and restricted to the cytoplasm for the R204X mutant protein. *in vitro* differentiation of hematopoietic progenitors showed that the cells transduced with the GATA2 R396Q mutant remain blocked at an early stage of granulo-monocytic differentiation in contrast to the GATA2 R204X mutant which has no effect compared to cells transduced with wild type GATA2. The R396Q mutant, unlike R204X, specifically induces leukemic transformation of cells *in vitro* after serial replating of transduced progenitors leading to leukemia cell lines. The R396Q mutant although behaving as a null allele demonstrates a distinct and specific activation of *Runx1t1*.

Summary/Conclusion: Our *in vitro* experiments demonstrate that the heterogeneity of the GATA2 syndrome relies on biological distinct defects, the R204X mutant acting only as a complete loss of function mutant in contrast to the R396Q mutant combining to its loss of the normal GATA2 function a specific oncogenic activation of *Runx1t1* explaining the increased risk of myeloid transformation of this mutant In epidemiological studies.

PS942

SYNERGISTIC INHIBITORY EFFECT OF HYDROXYUREA AND DECITABINE ON THE PROLIFERATION OF AML CELLS

K. Byrgazov[1,*], P. Valent[2,3], T. Lion[1,4]

[1]Children's Cancer Research Institute, [2]Department of Internal Medicine I, [3]Ludwig Boltzmann Cluster Oncology, [4]Department of Pediatrics, Medical University of Vienna, Vienna, Austria

Background: Treatment of acute myeloid leukemia (AML) in patients who are unable to tolerate standard intensive chemotherapy remains a challenge. Hydroxyurea (HU) and decitabine (DCB) have been used in palliative treatment of AML, and several studies have examined drug efficacy in this setting, but the combination of both drugs has not been studied in detail.
Aims: We examined the individual and combined effects of HU and different small molecules, including DCB, on intracellular molecular pathways, implicated in differentiation and proliferation of AML cells, as a potential basis for the development of improved treatment strategies in AML patients who are unfit for intensive therapy.
Methods: Six different AML cell lines (HL-60, ML-2, THP-1, MV4-11, KASUMI-1, U937) were treated with HU *in vitro*, and alterations in various signalling pathways involving over 20 proteins and genes previously identified as hallmarks of cancer were assessed by Western blot analysis (Figure 1A) and qPCR. Several small molecules targeting oncogenic pathways and epigenetic drugs, including DCB, were selected for further testing of potential synergism with HU.
Results: In four of the cell lines tested (HL-60, ML-2, THP-1, MV4-11), HU down-regulated the Hedgehog transcription factor GLI-1 and its downstream target MYC (Figure 1A). By contrast, HU induced expression of the differentiation factor SPI-1/PU.1 which is putatively repressed by MYC. Among various small molecules tested, DCB showed the most prominent synergy with HU in all AML cell lines examined (Figure 1B), as calculated by the "bliss-over-excess" method. Combined exposure to HU and DCB was efficient in killing HL-60, ML-2, THP-1, and MV4-11 cells, whereas drug combination effects on U937 and KASUMI-1 cells were moderate.

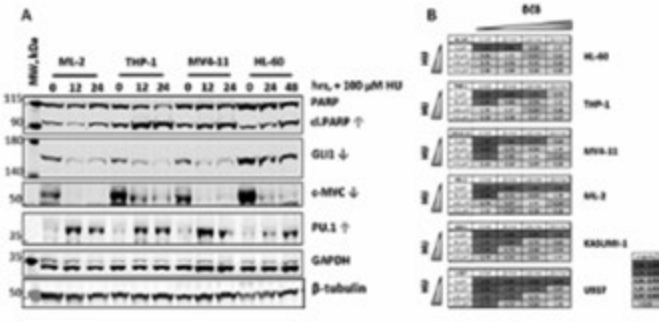


Figure 1.

Summary/Conclusion: HU and DCB are cytoreductive agents widely used in AML patients who cannot tolerate intensive therapy. Our data show that HU inhibits production of the MYC protein in AML cell lines. Moreover, we show that the combination of HU and DCB exerts synergistic anti-leukemic effects on AML cells which may pave the way for the development of new therapeutic strategies for AML patients, who are not amenable for intensive therapy, after tests on primary AML cells and *in vivo*.

PS943

MINIMAL RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIA USING MULTIPARAMETRIC FLOW CYTOMETRY IS HIGHLY PREDICTIVE OF OUTCOME

C. Kakirde[1,*], R. Kodgule[1], G. Raval[1], S. Kadechkar[1], Y. Badrinath[1], S. Ghoghale[1], N. Deshpande[1], D. Shetty[2], S. Chaudhary[1], S. Joshi[1], R. Mascarenhas[1], A. Gokarn[3], H. Jain[3], B. Bagal[3], H. Menon[4], M. Sengar[3], N. Khattry[3], P. Tembhare[1], P. Subramanian[1], S. Gujral[1], N. Patkar[1]

[1]Haematopathology, [2]Cytogenetics, [3]Adult Haematolymphoid Disease Management Group, Tata Memorial Center, Navi Mumbai, [4]Haemato-Oncology, Cytecare Cancer Hospitals, Bangalore, India

Background: One of the mainstays of chemotherapy in acute myeloid leukaemia (AML), other than acute promyelocytic leukaemia, is induction with a goal to achieve morphological complete remission (CR) as evident by less than 5% blasts. Post-remission strategies then focus on either consolidation chemotherapy or allogeneic bone marrow transplantation (aBMT). However, not all patients with this remission criterion achieve long-term remission and a subset of patients relapse. Detection of minimal residual disease (MRD) following chemotherapy, early in the course of treatment, is highly predictive of outcome and offers a window of opportunity to intensify treatment and prevent relapse.
Aims: Determining the prognostic outcome for Acute Myeloid Leukemia (AML) by Minimal Residual Disease (MRD) detection using multiparametric flow cytometry.
Methods: We accrued 311 consecutive patients of adult (>18 years) AML, other than with t(15;17), over a 32 month period (May 2015-Feb 2018) after obtaining consent. These patients were treated with standard 3+7 induction followed by high dose cytarabine MRD testing was done post induction and if feasible, post 1st Cycle HiDAC using a two tube 10 colour assay (CD15, CD13, CD19, CD34, CD56, CD7, CD45, CD11b, HLA-DR, CD117, CD14, CD123, CD64, CD33, CD36 & CD38). A minimum of 1.6 million events were acquired per tube on a Navios flow cytometer. An identical panel was used at MRD time points as well as on the diagnostic sample. Analysis of MRD was done using Kaluza 1.3. Conventional karyotyping and FISH was done as per standard recommendations and patients were classified into cytogenetic risk groups. The presence of *FLT3*-ITD, *NPM1* and *CEBPA* mutations was detected by a fragment length analysis based assay. Overall survival (OS) was calculated from the date of diagnosis to time of last follow up or death. Relapse-free survival (RFS) was calculated after achieving of 1st remission (CR) till relapse or death or last follow up if in CR. Results were analyzed for their impact on OS and RFS using Log Rank test of Kaplan–Meier survival analysis.
Results: Based on cytogenetics, 29.9% were classified as favourable risk whereas 59.4% and 10.6% were intermediate and poor risk respectively. FLT3-ITD, NPM1 and CEBPA mutations were harboured by 20.9%, 26.3% and 10.6% of patients respectively. The median follow-up was 8.2 months. Of these, 40 had induction death and 49 had refractory disease. Median OS was 5.1 months and RFS was 6.15 months. Post induction MRD was assessed in 264 patients of which 124 (46.9%) had a detectable residual disease (range 0.01-66.5%, median:0.35%). Post-consolidation MRD was assessed in 123 patients of which 28 (22.7%) were MRD positive (range 0.002-12.9%, median: 0.03%). Favorable risk and Intermediate risk cytogenetics were predictive of better OS than Poor risk patients (p=0.028) and (p=0.051) respectively and also provided a better outcome for RFS (p<0.001) and (p=0.001) respectively. The patients harbouring MRD at the end of induction were associated with inferior OS (p<0.001) and RFS (p=0.004), whereas post consolidation positive MRD status did not show a significant change in OS and RFS.
Summary/Conclusion: Our data is in agreement with other studies that determination of immunophenotypic MRD is extremely important in predicting an outcome. AML MRD is a very useful guide for guiding post remission strategies in AML and should be incorporated into routine treatment algorithms.

PS944

EXPRESSION OF CD25 FLUCTUATES IN THE LEUKEMIC STEM CELL POPULATION OF CD25-POSITIVE AML

Y. Kageyama[1,*], H. Miwa[1], R. Arakawa[2], K. Ohishi[3], M. Masuya[1], K. Nakase[4], F. Ishikawa[5], N. Katayama[1]

[1]Hematology and Oncology, Mie University Graduate School of Medicine, [2]School of Medicine, Mie University, [3]Blood Transfusion Service, [4]Cancer Center, Mie University Hospital, Tsu, [5]Laboratory for Human Disease Models, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan

Background: A small but distinct population of leukemic stem cells (LSCs) is responsible for the initiation and propagation of acute myeloid leukemia (AML) and its recurrence. IL-2 receptor α chain (CD25), which is expressed on leukemia cells of 10 to 20% cases of AML, has been reported to be associated with poor prognosis of AML (Nakase *et al*., PLoS One, 2015). CD25 is also demonstrated to be one of the LSC-specific surface antigens that are not expressed on normal hematopoietic stem cells (Saito *et al*., Sci Transl Med, 2010). It is possible that CD25-targeting may be a promising novel therapy against AML.
Aims: We elucidated the relationship between CD25 expression and stem cell property using patient-derived xenograft model.
Methods: We examined nine CD25-positive AML samples with the detectable CD34 expression. CD25-positive or -negative cells in $CD34^{+}CD38^{-}$ population when this population was detected or otherwise

$CD34^+$ population were transplanted into 120 to 150 cGy irradiated newborn NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/Sz (NSG) mice *via* facial vein within 48 hours of birth. Then engraft rate and phenotype of the engrafted cells were analyzed. Engrafted leukemic cells were subjected to secondary transplantation.
Results: Leukemic engraftment was observed with both $CD34^+CD25^+$ and $CD34^+CD25^-$ populations in three of nine CD25-positive AML samples. Phenotypical analyses of bone marrow cells from mice with leukemic engraftment revealed that those engrafted cells had both $CD34^+CD25^+$ and $CD34^+CD25^-$ populations. Engrafted cells of these cases were again divided into CD25-positive and -negative $CD34^+$ populations, each of which reconstituted both fractions again in the secondary recipient mice.
Summary/Conclusion: LSC has been defined as having the abilities to initiate leukemia in a recipient mouse and to grow out after re-transplantation into secondary recipients and preferably in tertiary recipients (Lapidot *et al.*, Nature, 1994). LSC-specific markers should satisfy the following conditions: 1) low or undetectable expression in normal tissues, especially normal HSCs, which ensures no or negligible adverse effects upon target therapy, 2) universally expressed in LSCs, which leads to eradication of LSCs by target therapy. Our results demonstrate that LSCs of CD25-positive AML reside in both CD25-positive and CD25-negative populations and that CD25 is not a *bona fide* LSC-specific marker of AML.

PS945

INTEGRIN ALPHA 7 EXPRESSION IS ASSOCIATED WITH PHENOTYPE AND GROWTH OF ACUTE MYELOGENOUS LEUKEMIA

N. Kobayashi[1,*], M. Takizawa[2], T. Ishizaki[1], K. Honma[3], H. Shimizu[1], A. Yokohama[4], T. Saitoh[3], N. Tsukamoto[5], H. Handa[1]

[1]Department of Hematology, Gunma University Graduate School of Medicine, [2]Medical Security and Safety Management Center, Gunma University Hospital, [3]Gunma University Graduate School of Health Science, [4]Blood Transfusion Service, [5]Oncology Center, Gunma University Hospital, Maebashi, Japan

Background: Acute myelogenous leukemia (AML) with granulocytic sarcoma (GS) at diagnosis is related to poor prognosis. A study using an established GS cell line demonstrated that a 92 kDa type IV collagenase enhances tissue invasiveness. However, other factors that promote the formation of extramedullary lesions are unknown.
Aims: To clarify the mechanism of tissue invasion and proliferation in local lesions of AML cells and its clinical prognosis.
Methods: A total of 77 AML patients participated in this study after informed consent. RNA was extracted from the bone marrow mononuclear cells of seven AML patients, with clinical complications of GS and seven AML-M2 patients (French-American-British classification; FAB) without GS, in Gunma university hospital. The RNA was subjected to RNA sequencing (RNA-seq) using NextSeq500 (Illumina) to evaluate the variations in gene expression between the AML groups with and without GS. The expression of integrin α7 (ITGA7) was evaluated using RT-qPCR and RNA extracted from the bone marrow mononuclear cells. Protein expression was confirmed using immunohistochemical staining of formalin fixed paraffin embedded (FFPE) specimens of the bone marrow clot. We also analyzed the clinical prognosis of these patients. As well, AML cell lines including PL21, THP1, and HL60 were evaluated for ITGA7 gene and protein expression using RT-PCR and flow cytometry, respectively. Cell proliferation assays were performed using the WST-8 cell proliferation cytotoxicity assay kit.
Results: Analysis of the RNA-seq results using Gene Set Enrichment showed that the genes expressed on the cell surface were significantly different in the group with GS (FDR q-value <0.005). We focused on ITGA7, which has a relatively higher expression in the GS group, because of its role in cell-to-cell interaction. RT-PCR analysis of the clinical specimens confirmed that the expression of ITGA7 was significantly higher in the GS group (p=0.00168) and AML M5 group (p=0.000108). Immunostaining of the FFPE specimen from the bone marrow also validated ITGA7 expression. Flow cytometry confirmed ITGA7 expression on PL21 and THP1 cell surfaces. ITGA7 has been shown to be expressed mainly in the muscle and is involved in cell proliferation, migration, and adhesion. Therefore, we examined PL21 and THP1 cells in a cell culture assay using a dish coated with the extracellular matrix laminin 211 isoform – a ligand of integrinα7β1. Proliferation of these cells was significantly enhanced in the presence of laminin 211 compared to that of laminin 411 or in an uncoated dish. However, ITGA 7 expression level was not associated with the prognosis of AML patients. No significant differences were seen in 64 patients, except for the rates of overall survival (p=0.918) and disease-free survival (p=0.398).
Summary/Conclusion: The proliferation of an AML cell line expressing ITGA7 was enhanced by laminin 211. However, the association of ITGA7 expression and the clinical prognosis was unclear. The formation of GS occurs more frequently in AML classified as M4 and M5 in the FAB classification than in other subtypes of AML. Clinically, these AML types are often accompanied by organ infiltration at diagnosis. Interaction of ITGA7 and laminin demonstrates the mechanism of infiltration and local growth of these AML subtypes.

PS946

THE ROLE OF STAT3B IN ACUTE MYELOID LEUKEMIA

P. Aigner[1,*], T. Mizutani[1], J. Horvath[1], H. Sill[2], K. Lind[2], V. Just[1], E. Casanova[1], D. Stoiber[1]

[1]Ludwig Boltzmann Institute for Cancer Research, Vienna, [2]Division of Hematology, Medical University of Graz, Graz, Austria

Background: Signal transducer and activator of transcription 3 (STAT3), a multifunctional transcriptional regulator, plays a central role in proliferation, differentiation and oncogenic transformation. It is expressed in two alternatively spliced isoforms, STAT3α and truncated STAT3β, which lacks the C-terminal transactivation domain. In addition to the C-terminal transactivation domain, which is crucial for the recruitment of nuclear cofactors, STAT3β is lacking the additional STAT3 phosphorylation site, serine 727, and instead carries a tail of seven unique amino acids. STAT3β recently gained attention as a powerful anti-tumorigenic molecule and protective prognostic marker. This has been shown in patients with esophageal squamous cell carcinoma, as well as in mouse models for breast, skin and colon cancer. Deregulated STAT3 signaling is frequently observed in leukemogenesis and generally associated with a poor clinical outcome in patients with acute myeloid leukemia (AML). Furthermore, it has been shown that the expression ratios of STAT3α and STAT3β change upon myeloid differentiation. However, the exact role of STAT3β in AML patients and during disease progression remains unknown.
Aims: The aim of our study is to gain a better understanding of STAT3β and its specific function in acute myeloid leukemia.
Methods: Hence, we analyzed more than 50 AML patient samples for *STAT3β/STAT3α* mRNA expression levels and its correlation with clinical prognosis and overall survival. Moreover, we generated an inducible *Stat3β* transgenic mouse model and crossed it with *Pten*-deficient mice, a previously described model for AML. In addition, we used fetal liver-derived stem cells from *Stat3β* transgenic mice, transduced with a retrovirus encoding for the MLL-AF9 translocation, in a transplant model for AML.
Results: We observed a correlation between clinical prognosis and *STAT3β/STAT3α* expression ratio. Specifically, we found elevated levels of *STAT3α* in all AML samples compared to healthy CD34-positive cells and only low levels of *STAT3β*. Similarly, we detected higher levels of *STAT3α* in patients with an adverse prognosis. A higher *STAT3β/STAT3α* ratio was further associated with a favorable clinical prognosis and a significantly increased overall survival.
In both *in vivo* models we could demonstrate that the expression of STAT3β impairs leukemogenesis and delays disease progression. In particular, we observed a significant decrease of leukemic cell infiltration into hematopoietic organs. The STAT3β-dependent delay in disease progression and leukemic infiltration resulted in a significant increase of overall survival. Additionally, *in vitro* colony formation assays with bone marrow cells (BMCs) derived from *Stat3β* transgenic mice, transformed with MLL-AF9, revealed lower numbers of colonies in *Stat3β* transgenic BMCs compared to the wild-type control.
Summary/Conclusion: In conclusion, our results indicate that STAT3β can serve as an anti-tumorigenic molecule in AML mouse models and the *STAT3β/STAT3α* ratio correlates with a more favorable outcome in AML patients. This study helps to unravel the role of STAT3-mediated signaling in the development of acute myeloid leukemia.

PS947

DISSECTING INTRA-TUMOR HETEROGENEITY IN ACUTE MYELOID LEUKEMIA THROUGH LEUKEMIA STEM CELL-ASSOCIATED MIRNA SIGNATURES AND SINGLE-CELL RNA TRANSCRIPTIONAL PROFILING

M. M. Naldini[1,*], F. Pavesi[2], A. Cammarata[3], M. Volpin[3], G. Casirati[2], G. Desantis[3], C. Caserta[1], A. Cosentino[3], D. Cittaro[4], E. Montini[3], F. Ciceri[2], B. Gentner[5]

[1]UNISR - SRTIGET, [2]Hematology and Bone Marrow Transplant Unit, San Raffaele Scientific Institute, [3]San Raffaele Telethon Institute for Gene Therapy, [4]Center for Translational Genomics and BioInformatics, San Raffaele Scientific Institute, [5]Hematology and Bone Marrow Transplant Unit, San Raffaele Telethon Institute for Gene Therapy, Milan, Italy

Background: Despite recent advances in risk-adapted treatment of AML, a large proportion of patients relapses. Recent findings suggest that relapse-promoting clones are already present at diagnosis and often show features of leukemic stem cells (LSC), which may persist through chemotherapy as minor subpopulations later leading to relapse. LSC-associated mRNA and miRNA signatures have shown promise in measuring LSC content in clinical samples.
Aims: To exploit miRNAs as new biomarkers of LSC and chemotherapy resistance in AML.
Methods: We have set up a droplet PCR assay for miRNA quantification in patient samples, as well as lentiviral reporter vectors to measure the activity of miR-126 (stem cell-associated miRNA), miR-21 and miR-22 (associated with chemotherapy resistance) in patient-derived xenografts (PDX) allowing prospective isolation of miRNA(high) AML subpopulations. Vector integration site (IS) analysis was used to identify single AML clones and track blast ontology in serial PDX transplantation. Single-cell RNA sequencing (scRNAseq) of AML blasts and residual HSPC at diagnosis was obtained by drop-seq technology.
Results: Based on previous data describing a panel of LSC-enriched and depleted miRNAs that correlate with clinical outcome, we verified the predictive power of the top 4 LSC miRNAs in an independent cohort of AML patients (n=55). Excluding CBF leukemia, higher miR-126 expression was associated with adverse ELN2017 risk. We investigated miR-126 and miR-21/22 activity in PDX by transducing patient blasts with miRNA reporters and transplanting them into NSG mice for prospective isolation of LSC enriched/miRNA(high) populations. miRNA(high) cells were enriched for CD34+ blasts (n=5 AML), and clonal tracking by vector IS analysis (n=1 disease) revealed higher sharing of miR-126(high) IS between primary and secondary PDX, further suggesting enrichment of leukemia-propagating cells within such population. We then sought to measure miR-126 expression in longitudinal patient samples at time-points putatively enriched for LSC, either early after chemotherapy (day 14) or at relapse. When sorting CD45dim blasts, we found increased levels of miR-126 with respect to the diagnosis samples. Furthermore, we sorted day 14 BM samples for residual AML based on leukemia-associated phenotype (LAIP+), and for non-leukemic HSPC (LAIP-CD34+CD90+) (n=5 pts). While we confirmed an increased LSC miRNA score in the LAIP+ blasts at day 14, we surprisingly found an even higher score within the HSPC compartment. We next performed scRNAseq on diagnostic blasts from 2 NPM1+AML patients: patient 1 achieved a long-lasting CR, while patient 2 had primary refractory disease. Since miRNAs cannot be evaluated with this technology, we calculated the extensively validated mRNA-based LSC17 score. Preliminary data show a higher LSC17 score in CD117+blasts from patient 2 than patient 1. Unexpectedly, the highest LSC17 score was detected within CD34+ cells from patient 1, which turned out NPM1(negative) and resulted in a multilineage graft when transplanted into NSG mice.
Summary/Conclusion: We show the feasibility of identifying LSC-enriched populations *in vivo* by LSC-associated microRNA reporters. We also highlight promiscuous expression of stem cell associated microRNA and mRNA signatures within LSC and residual healthy hematopoiesis in AML patients, hinting to shared transcriptional programs associated with stemness and underlining the need to separate blasts from regenerating HSPC for accurate interpretation of LSC signatures in post-treatment samples.

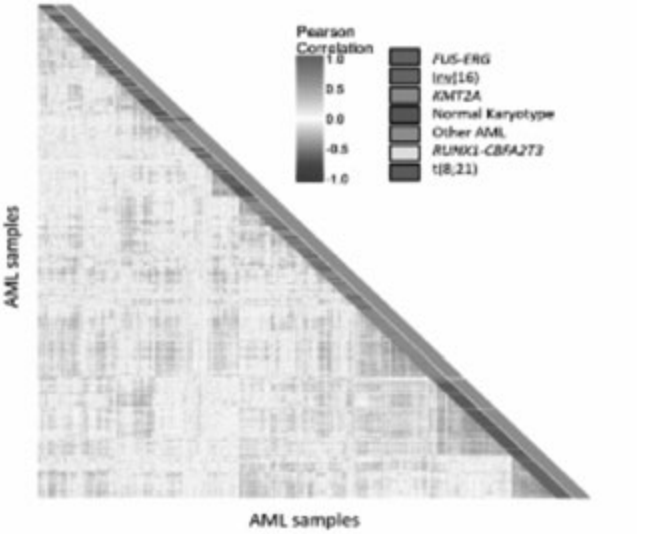
PS948

CLINICAL AND BIOLOGICAL CHARACTERISTICS OF TWO DISTINCT T(16;21) FUSIONS IN PEDIATRIC ACUTE MYELOID LEUKEMIA: A RETROSPECTIVE ANALYSIS BY THE I-BFM STUDY GROUP

S. Noort[1,*], M. Zimmermann[2], D. Reinhardt[3], W. Cuccuini[4], M. Pigazzi[5], J. Smith[6], R. E. Ries[6], T. A. Alonzo[7], B. Hirsch[7], D. Tomizawa[8], F. Locatelli[9], T. A. Gruber[10], S. Raimondi[11], E. Sonneveld[12], D.K. Cheuk[13], M. Dworzak[14], J. Stary[15], J. Abrahamson[16], N. Arad-Cohen[17], W. Balwierz[18], B. De Moerloose[19], H. Hasle[20], S. Meshinchi[6,21], M.M. van den Heuvel-Eibrink[22], C. M. Zwaan[1]

[1]Pediatric Oncology/Hematology, Erasmus MC/Sophia Childrens Hospital, Rotterdam, Netherlands, [2]Department of Pediatric Hematology/Oncology, Medical School Hannover, Hannover, [3]AML-BFM Study Group, Pediatric Hematology and Oncology, University Hospital Essen, Essen, Germany, [4]Department of Cytogenetics, Saint-Louis Hospital, Paris, France, [5]Women and Children's Health, Hematology-Oncology Laboratory, University of Padova, Padova, Italy, [6]Fred Hutchinson Cancer Research Center, Seattle, Washington, [7]Children's Oncology Group, Monrovia, California, United States, [8]Japan Children's Cancer Group, National Center for Child Health and Development, Tokyo, Japan, [9]Italian Association of Pediatric Hematology and Oncology, University of Pavia, Pavia, Italy, [10]Department of Oncology, [11]Department of Pathology, St Jude Children's Research Hospital, Memphis, Tennessee, United States, [12]Dutch Childhood Oncology Group, The Hague, Netherlands, [13]Department of Pediatrics and Adolescent Medicine, Queen Mary Hospital, University of Hong Kong, Hong Kong, Hong Kong, [14]Children's Cancer Research Institute and St. Anna Children's Hospital, Department of Pediatrics, Medical University of Vienna, Vienna, Austria, [15]Czech Pediatric Hematology/Oncology, University Hospital Motol and Charles University, Prague, Czech Republic, [16]Nordic Society for Pediatric Hematology and Oncology, Department of Pediatrics, Institution for Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, [17]Pediatric Hemato-Oncology Department, Ruth Rappaport Children's Hospital, Rambam Health Care Campus, Haifa, Israel, [18]Department of Pediatric Oncology and Hematology, Institute of Pediatrics, Jagiellonian University Medical College, Krakow, Poland, [19]Department of Pediatric Hematology-Oncology and Stem Cell Transplantation, Ghent University Hospital, Ghent, Belgium, [20]Pediatrics and Adolescent Medicine, Aarhus University Hospital, Aarhus, Denmark, [21]Department of Pediatrics, Seattle Children's Hospital, University of Washington, Seattle, Washington, United States, [22]Princess Maxima Center for Pediatric Oncology, Utrecht, Netherlands

Background: Despite the use of intensive chemotherapy and allogeneic stem cell transplantation (HSCT) in selected patients, children with acute myeloid leukemia (AML) have 5-year overall survival (OS) rates of about 65%. Apart from early clinical response, cytogenetic and molecular aberrations are the most reliable prognostic factors for survival. It is clinically important to identify prognostic subgroups to stratify patients in future trials. Studies on rare AML subtypes can be facilitated by international collaborations. Herein, we focus on AML with t(16;21) rearrangements, including 2 different translocations: t(16;21)(p11;q22) resulting in the *FUS–ERG* fusion, and t(16;21)(q24;q22) resulting in the *RUNX1–CBFA2T3* fusion.
Aims: To define clinical and biological characteristics of pediatric AML with t(16;21) rearrangements.
Methods: Clinical data from children with t(16;21) AML were collected from 14 study groups participating in the international Berlin–Frankfurt–Munster (I-BFM) network. Patients (age 0–18 years) diagnosed from January 1, 1995, to January 1, 2016, were included. The AML-BFM study group provided data for 1326 unselected patients diagnosed between 1997 and 2013 as a reference cohort. All karyotypes were centrally reviewed by 2 independent cytogeneticists according to the international system for human cytogenetic nomenclature 2005. For gene expression analysis, the Children's Oncology Group (COG) provided RNAseq data for 1035 patients in AAML1031.



Unsupervised hierarchical clustering of pediatric AML. Fractional counts were normalized to trimmed mean of m-values and counts per million mapped reads (CPM), log2 transformed and filtered for genes with at least 1 CPM in 5% of samples. The relative level of expression per gene in each sample was determined by mean centering the expression values, using the geometric mean. Pairwise sample correlations (Pearson's R) were used for hierarchical clustering.

Figure 1.

Results: Clinical data were collected from 54 patients with t(16;21): 32 with *FUS–ERG* and 22 with *RUNX1–CBFA2T3*. Patients with *FUS–ERG* had no predominant French–American–British (FAB) type, whereas

76.4% of those with *RUNX1–CBFA2T3* had a granulocytic FAB type (M1, M2) compared with 42.1% in the reference cohort (p=0.004). Event free survival, OS and cumulative incidence of relapse at 4 years were 51% [standard error (SE)=1%], 68% (SE=1%), and 32% (SE=1%), respectively, for the reference cohort. The corresponding numbers for *FUS-ERG* were 7% (SE=5%, p<0.0001), 21% (SE=8%, p<0.0001) and 74% (SE=8%, p<0.0001), and for *RUNX1–CBFA2T3* were 77% (SE=9%, p=0.06), 81% (SE=8%, p=0.34) and 0%, respectively. In multivariate analysis with age, white blood cell count at diagnosis, and cytogenetic risk group as co-variables and allogeneic HSCT as the time-dependent variable, presence of *FUS–ERG* and *RUNX1–CBFA2T3* were independent risk factors for EFS, with hazard ratios of 1.93 (p<0.0001) and 0.325 (p=0.025), respectively. Hierarchical clustering analysis of RNAseq data showed that *FUS–ERG* and *RUNX1–CBFA2T3* clustered separately. *RUNX1–CBFA2T3* clustered with t(8;21) cases (Figure 1). Comparison of gene expression of *FUS–ERG* and *RUNX1–CBFA2T3* with other AML cases revealed 1314 and 119 differentially expressed genes, respectively. Of differentially expressed genes of *RUNX1–CBFA2T3*, 63 overlapped with differentially expressed genes in t(8;21) AML.

Summary/Conclusion: We identified two clinically relevant, distinct subtypes of pediatric AML patients with different t(16;21) rearrangements: those with *FUS–ERG* had poor outcomes, and those with *RUNX1–CBFA2T3* had favorable outcomes. *RUNX1–CBFA2T3* should be considered a core binding factor (CBF) AML, as the fusion involves *RUNX1*. RUNX1 is a subunit of CBF, a transcription factor that is an essential regulator of normal hematopoiesis. Disruption of CBF subunits by cytogenetic rearrangements leads to CBF AML. Patients with *RUNX1–CBFA2T3* might benefit from inclusion in the standard-risk group, along with other CBF AMLs, whereas those with *FUS–ERG* should be included in the high-risk group.

PS949

NUP98-KDM5A FUSION GENE IS AN INDEPENDENT PREDICTOR OF POOR PROGNOSIS WITH A DISTINCT HOX GENE EXPRESSION PATTERN

S. Noort[1,*], T. A. Alonzo[2], J. Smith[3], R. E. Ries[4], R. B. Gerbing[2], E. Sonneveld[5], F. Locatelli[6], D. Reinhardt[7], A. Baruchel[8], J. Stary[9], M.M. van den Heuvel-Eibrink[10], C.M. Zwaan[1], S. Meshinchi[311]

[1]Pediatric Oncology/Hematology, Erasmus MC/Sophia Childrens Hospital, Rotterdam, Netherlands, [2]Children's Oncology Group, Monrovia, California, [3]Fred Hutchinson Cancer Research Center, Seattle, Washington, [4]Fred Hutchinson Cancer Research Center, Seattle, Washington, United States, [5]Dutch Childhood Oncology Group, The Hague, Netherlands, [6]Italian Association of Pediatric Hematology and Oncology, University of Pavia, Pavia, Italy, [7]AML-BFM Study Group, Pediatric Hematology and Oncology, Essen, Germany, [8]Pediatric Hematology-Immunology Department, University Hospital Robert Debré and Paris Diderot University, Paris, France, [9]Czech Pediatric Hematology/Oncology, University Hospital Motol and Charles University, Prague, Czech Republic, [10]Princess Maxima Center for Pediatric Oncology, Utrecht, Netherlands, [11]Department of Pediatrics, Seattle Children's Hospital, University of Washington, Seattle, Washington, United States

Background: *NUP98* rearrangements are recurrent genetic events in pediatric acute myeloid leukemia (AML). We identified *NPU98-NSD1* as a recurrent poor risk abnormality (Hollink *et al.*, Blood 2011). Another recurrent fusion is *NUP98-KDM5A*, which was found in pediatric acute megakaryocytic leukemia (AMKL) and associated with poor clinical outcome (de Rooij *et al.*, Nature Genetics 2017).

Aims: The aim of this study was to determine the incidence of *NUP98-KDM5A* in large pediatric AML cohort not restricted to AMKL, and to define the biological and clinical characteristics of this subgroup.

Methods: The study comprised a COG and a European cohort of patients, and included 975 patients from COG trials 03p1 and 0531 (Gamis *et al.*, Blood 2013) and 343 European pediatric AML patients, previously described by Balgobind *et al.* in Haematologica, 2011. *NUP98-KDM5A* rearrangements were detected either by paired-end RNA sequencing or RT-PCR. Patients with FAB M3 and Down AML were excluded from this study. The Cox proportional-hazard model was used for multivariate analysis, considering as co-variates age, white blood cell count, cytogenetic risk group, allogeneic hematopoietic stem cell transplant as time dependent variable and study group (COG vs European cohort). Gene expression analysis was performed using RNAseq data of patients enrolled in the AAML1031 trial.

Results: *NUP98-KDM5A* rearrangements were detected in 31 out of 1348 patients (2.3%). Three NUP98-KDM5A+ patients had to be excluded due to inconclusive analysis. There was no significant difference in prevalence between the COG and European cohorts. The median age of *NUP98-KDM5A* patients was significantly lower as compared to patients without this fusion gene (5.4 vs 9.4 yrs; p=0.017). *NUP98-KDM5A* occurred in all FAB types, as only 37% of the *NU98-KDM5A+* cases had an M7 FAB type. The 5-year EFS and OS of *NUP98-KDM5A+* patients was 28.6±18.9% and 33.0±20.2%. This was significantly lower as compared to the *NUP98-KDM5A-* patients with EFS of 48.1±2.8% (p=0.015) and OS of 63.9±2.8% (p=0.001). In multivariate analysis, *NUP98-KDM5A* was an independent risk factor for OS, but not for EFS, with hazard ratios of 1.89 (p=0.025) and 1.53 (p=0.122), respectively.

Gene expression analysis of RNAseq data of 1,035 patients, of which 16 *NUP98-KDM5A+* patients, revealed 2252 differentially expressed genes between *NUP98-KDM5A+* and *NUP98-KDM5A-* cases, of which 1542 upregulated genes. However, unsupervised hierarchical clustering did not cluster the majority of *NUP98-KDM5A+* cases together, but scattered them among samples with normal and other karyotypes. The top 3 most differentially expressed genes when comparing gene expression between NUP98-KDM5A+ and NUP98-KDM5A- cases were 3 *HOXB* genes, i.e. *HOXB5*, *HOXB6* and *HOXB9*. When we performed HOX-gene based clustering, several subgroups were revealed (Figure 1), separating the MLL-rearranged cases with a *HOXA* profile from the *NUP98-KDM5A* and *NUP98-NSD1* cases, characterized by *HOXA* and *B* expression.

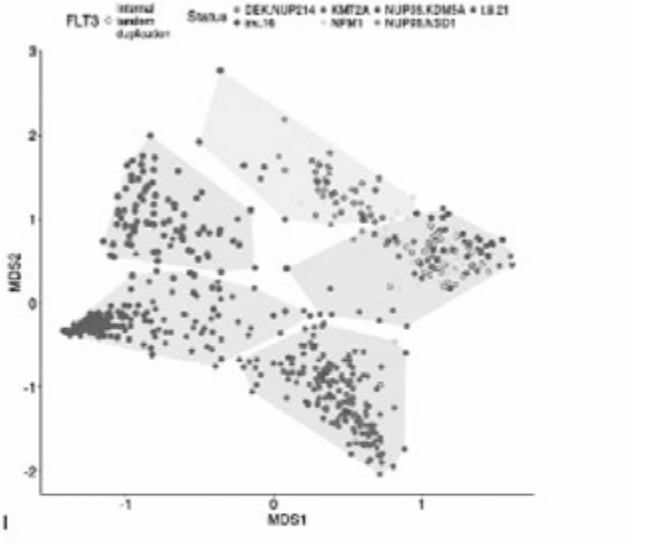


Figure 1: *HOXA and HOXB gene expression in pediatric AML. HOXA and HOXB gene expression was examined using principal coordinates analysis. Five distinct groups(clusters) were determined using k-means clustering and are depicted in convex hulls. Samples with very rare or unknown driving aberrations have been excluded for this analysis. The NUP98-KDM5A samples all cluster in the purple hull, together with NPM1 and DEK-NUP214, whereas most NUP98-NSD1 samples cluster separately.*

Figure 1.

Summary/Conclusion: This study shows that *NUP98-KDM5A* is an aberration that occurs in all FAB types and not exclusively in AMKL. *NUP98-KDM5A* is an independent risk factor for OS, but not for EFS, and identifies a relatively poor prognostic subgroup. Gene expression analysis revealed upregulation of *HOXB* genes, similar to results previously found in *NUP98-NSD1* rearranged AML (Hollink *et al.*, Blood 2011). However, *NUP98-NSD1* and *NUP98-KDM5A* positive patients do not cluster together in *HOX*-gene based clustering analysis.

PS950

TARGETED THERAPY FOR AML EXPRESSING SOMATIC OR GERMLINE MUTATION IN RUNX1

K. Bhalla[1,*], C. DiNardo[1], C. Mill[1], W. Fiskus[1]

[1]Leukemia, MD Anderson Cancer Center, Houston, United States

Background: RUNX1 is the DNA-binding subunit of the core binding factor complex and a transcription factor involved in normal and malignant hematopoiesis. Somatic mutations in RUNX1 commonly occur in secondary or *de novo* AML (~10%). Presence of mtRUNX1 confers relative therapy-resistance and poorer survival in patients with AML. Germ-line, intragenic mutations and deletions in RUNX1 cause the highly penetrant (~40%), autosomal dominant, Familial Platelet Disorder (FPD) that evolves into myeloid malignancy (FPD-MM), i.e., MDS or AML. Majority of mutant

(mt) RUNX1 are missense, large deletions or truncation-mutations, behaving mostly as loss of function mutations. RUNX1 transcription is controlled by a conserved +24-kb-enhancer within a super-enhancer (covering the intron 1 of RUNX1) that demonstrates high occupancy by the BET protein BRD4. Hi-C analysis showed high interaction scores between +24-kb-enhancer and P2 promoter of RUNX1.
Aims: Following knockdown of RUNX1 or BRD4, the effects on RUNX1 and its targets, as well as on survival of AML cells expressing mutant versus WT RUNX1 were determined.
Methods: Following knockdown of RUNX1 or BRD4, the effects on RUNX1 and its targets, as well as on survival of AML cells expressing mutant versus WT RUNX1 were determined.
Results: Our findings confirmed that, as compared to the luciferase-containing P2 promoter or the enhancer alone, the +24kb-enhancer-driven P2 promoter exhibited superior luciferase induction, representing increased transcriptional activity of RUNX1. We also determined that shRNA-mediated knockdown of RUNX1 depleted RUNX1 targets c-MYC and PU.1, as well as induced significantly more apoptosis of cultured cell line and patient-derived (PD) AML blast progenitor cells (BPCs) expressing mtRUNX1, as compared to the PD AML BPCs expressing wild-type (WT) RUNX1. Doxycycline-inducible, shRNA-mediated *in vivo* knockdown of RUNX1 in engrafted OCI-AML5 cells expressing mtRUNX1 markedly reduced AML burden and improved survival of the immune-depleted (NSG) mice. Knockdown of BRD4 (via shRNA) also attenuated expression of RUNX1 and its targets, as well as inhibited growth and induced apoptosis of OCI-AML5 cells. We also found that BETP-PROTACs (proteolysis targeted chimera) ARV-825 and ARV-771 (20 to 250 nM) caused degradation and depletion of BRD4, reduced BRD4 occupancy at the +24-kb-enhancer and P2 promoter of RUNX1, thereby repressing RUNX1 and its targets, and potently inducing apoptosis of AML BPCs expressing mtRUNX1. Treatment with ARV-771 (30 mg/kg SQ daily for 3 weeks also inhibited AML growth and improved survival of NSG mice engrafted with OCI-AML5 cells. CRISPR-Cas9 mediated editing of the +24-kb RUNX1 enhancer markedly depleted levels of RUNX1 in the surviving fraction of slow growing OCI-AML5 cells expressing mtRUNX1. By utilizing the RNA-seq mRNA signature from RUNX1-depleted AML cells we also queried for expression mimickers (EMs) through LINCS1000-CMap (Connectivity Mapping) analyses. This uncovered several novel agents as EMs, e.g., narciclasine, anisomycin, cinobufagin and fenbendazole. These EMs, at nanomolar levels, inhibited mRNA and protein expressions of RUNX1, c-Myc and PU.1 and induced more apoptosis of the AML cells expressing somatic or germ-line mtRUNX1, as compared to FPD hematopoietic progenitor cells (HPCs) that solely expressed mtRUNX1 or of CD34+ normal HPCs.
Summary/Conclusion: These findings highlight RUNX1-targeted novel agents that exert preferential lethal activity against AML BPCs expressing somatic or germline mtRUNX1.

PS951

DECIPHERING THE GENETIC LANDSCAPE OF ACUTE MYELOID LEUKEMIA WITH COMPLEX KARYOTYPE BY TRANSCRIPTOME ANALYSIS USING RNA SEQUENCING

F. G. Rücker[1,*], X. Gong[2], A. Dolnik[1], M. Hoyos[1], J. Biscocho[2], T.J. Blätte[1], J. Swoboda[1], A. S. Pollack[2], A. Turchiano[1], H. Döhner[1], K. Döhner[1], J.R. Pollack[2], L. Bullinger[1,3]

[1]Department of Internal Medicine III, University Hospital of Ulm, Ulm, Germany, [2]Department of Pathology, Stanford University, Stanford, United States, [3]Department of Hematology, Oncology and Tumorimmunology, Charité University Medicine, Berlin, Germany

Background: Acute myeloid leukemia with complex karyotype (CK-AML) represents approximately 15% of adult AML. CK-AML is the least understood cytogenetic subgroup at molecular level, except that about two-thirds of cases carry *TP53* alterations. In particular, as cytogenetic alterations are distinct among patients, it is unclear whether they are cause of leukemogenesis, or whether they merely reflect genomic instability.
Aims: We hypothesized that cytogenetic aberrations in CK-AML create gene fusions (GF) that, although not recurrent, nonetheless deregulate cancer genes contributing to leukemogenesis.
Methods: We performed Illumina paired-end (101bpx2) RNA-seq transcriptome analysis of 65 CK-AML to identify GF using multiple independent algorithms. GF were in part independently validated by array-based genomic profiling and/or long range PCR followed by long-read Oxford Nanopore sequencing technology.
Results: RNA-seq uncovered 55 high-evidence GF in 30 (46% of) CK-AML with up to four per case. Nearly all fusions were previously unreported in AML, except *RUNX1-MECOM*, *CBFA2T3-GLIS2*, *MN1-ETV6*, *and ETV6-MN1*. No GF were recurrent, though some genes recurred as 5' or 3' partners. About 35% were in-frame, encoding chimeric proteins. The remainder encoded either C-terminally truncated 5' fusion partners, or else N-terminally truncated (or rarely full-length) 3' fusion partners in instances where the 5' partner contributed only the 5'UTR. Many fusions were predicted to lead to overexpression or chimeric activation of known or putative novel cancer genes. Most frequently affected genes were *RUNX1* (n=5) leading to truncation in 4/5 fusions, *KMT2A*, and *MECOM* (n=3 each). Due to affected genes, fusions could be categorized into five functional clusters. Many fusions (n=17) contained at least one known AML gene (e.g. *RUNX1*, *MECOM*, *DEK*, *ETV6*, *KMT2A*) or oncogenes (e.g. *MYB*) and a novel fusion partner, clearly suggesting pathogenic relevance. Others were predicted to disrupt tumor suppressors (n=4; e.g. *TP53*, *NF1*). The majority of fusions comprised partners where neither was previously associated with AML, though many had plausible leukemic roles or targetable domains (n=34, e.g. cell surface receptors, kinases, enzymes). To elucidate the correlation between gene fusions (GF) and gene mutations in $TP53^{altered}$ CK-AML, we performed a targeted mutation calling for 36 known AML genes delineating only 1.1 mutations per $TP53^{altered}$ CK-AML (range: 0-6 mutations) and 2.6 mutations per $TP53^{unaltered}$ CK-AML (0-8 mutations) (p<.001), respectively. Compared to GF-/CK-AML, GF+/CK-AML exhibited significantly less gene mutations per case [1.4 (range: 0-4 mutations) vs 2.0 (0-8 mutations), p=.038] and was significantly correlated with chromothripsis (p=.001), a plausible mechanism underlying GF. GF seem not to be only of leukemogenic, but also prognostic relevance in $TP53^{altered}$ CK-AML since GF+ cases had significantly worse overall survival (p=.018).
Summary/Conclusion: Detailed molecular characterization of CK-AML revealed a high incidence of novel GF and demonstrated that $TP53^{altered}$ CK-AML is rather characterized by the acquisition of genomic aberrations leading to novel GF than the accumulation of gene mutations. While GF might play an important role in CK-AML pathogenesis, identifying GF could lead to more effective, personalized treatment strategies and provide valuable patient-specific biomarkers to track leukemic burden within the monitoring of disease remission and early prediction of relapse.

PS952

PHOSPHO-PROFILING LINKING AML-BIOLOGY AND RESPONSE PREDICTION IN PEDIATRIC MYELOID LEUKEMIA

M. Prchal-Murphy[1,*], A. Hoelbl-Kovacic[1], M. Dolezal[2], S. Fajmann[1], A. Schumich[3], M. Kauer[3], V. Sexl[1], M. Dworzak[3]

[1]Department for Biomedical Science, Institute of Pharmacology and Toxicology, VetMed university Vienna, [2]Department for Biomedical Science, Institute of Population Genetics, [3]St.Anna Children's Hospital and Children's Cancer Research Institute, Medical University of Vienna, Vienna, Austria

Background: Acute myeloid leukemia (AML) accounts for 15-20% of all pediatric leukemias. Treatment outcome has significantly been improved and reached 65-70% survival rate due to better risk classifications, advances in chemotherapy, stem cell transplantation and accessory treatment with signal interceptors. Nevertheless the high frequency of 25-30% in relapse is rooted in the genetic heterogeneity of the disease that brings a broad diversity in aberrant protein expression and activation. Those mainly pro-survival and proliferation-inducing factors get phosphorylated for their full activation. Proof of phosphorylation points at a disease-propagating role of a given factor and renders it a promising drug target. In adults, the inhibition of FLT-3 signaling by Midostaurin in combination with chemotherapy was recently shown to prolong event-free survival. In pediatric patients, a comparable breakthrough is still lacking. To identify new drug targets in pediatric AML, we analyzed a detailed picture of phospho-profiles in individual subtypes and cases of relapse.
Aims: We aimed at deriving a detailed picture of phospho-profiles in AML samples, at stratifying them according to individual subtypes of the disease and at identifying vulnerable nodes for future patient-tailored therapies.
Methods: We established a high-throughput FACS-based screening method and characterized phospho-profiles of cells derived from 116 pediatric AML patients. We compared basal and cytokine-induced phosphorylation patterns. Cytokine stimulation was included to evaluate influences provided by the *in vivo* milieu. Pathways that were identified to be deregulated were blocked with specific inhibitors *in vitro*. Their efficacy was determined by a FACS-based cytotoxicity assay. Furthermore we established a mouse xenograft model to amplify limited patient material and to validate the efficacy of inhibitors that were identified in the *in vitro* studies to block leukemic cell proliferation. In parallel, we correlated individual activation of proteins to the incidence of relapse.
Results: We demonstrate a novel high-throughput FACS-based screening method to determine AML sub-type specific alterations of signaling path-

ways. Independent from the AML subtype, the transcription factor STAT5 was prominently activated. Leukemic cell survival could be blocked by Midostaurin, a PKC - and Flt-3 inhibitor. Of note, we found that Midostaurin also blocks STAT5 activation. In line, high levels of pSTAT5 correlated with an increased cumulative incidence of relapse in pediatric patients.
Summary/Conclusion: The transcription factor STAT5 is over-activated in all AML subtypes tested. Activation of STAT5 negatively influences the incidence of relapse and can be blocked by Midostaurin. These data suggest that the applicability of Midostaurin extends to pediatric patients suffering from AML, irrespective of the AML subtype.

PS953

TOWARDS IMPROVING OF SURVIVAL IN AML BY REPURPOSING EXISTING DRUGS AS NOVEL SAMHD1 INHIBITORS

N. Tsesmetzis[1,*], S. Rudd[2], C. Paulin[2], K. Sanjiv[2], J. Kutzner[3], I.H. Myrberg[1], H. Axelsson[4], A. Rasti[2], R.M. Branca[5], E. Witta[2], J. Lehtiö[5], G. Rassidakis[6], U. Warpman-Berglund[2], K. Pokrovskaja Tamm[6], M. Heyman[1], S. Lehman[7,8], T. Lundbäck[4], T. Helleday[2], J.-I. Henter[1,9], T. Schaller[3], N. Herold[1,9]

[1]Childhood Cancer Research Unit, Department of Women's and Children's Health, [2]Science for Life Laboratory, Division of Translational Medicine and Chemical Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden, [3]Department of Infectious Diseases, Virology, University Hospital Heidelberg, Heidelberg, Germany, [4]Chemical Biology Consortium, [5]Clinical Proteomics Mass Spectrometry, Science for Life Laboratory, [6]Department of Oncology-Pathology, [7]Department of Medicine, Center of Hematology and Regenerative Medicine, Karolinska Institutet, Stockholm, [8]Department of Medical Sciences, Uppsala Universitet, Uppsala, [9]Paediatric Oncology, Theme of Children's and Women's Health, Karolinska University Hospital Solna, Stockholm, Sweden

Background: Despite highly toxic chemotherapy regimens, seven out of ten patients with acute myelogenous leukaemia (AML) still succumb to their disease. The antimetabolic nucleoside analogue cytarabine (ara-C) is, together with anthracyclines, the single most important drug in the treatment of AML, and its clinical efficacy directly correlates with AML blasts' ability to accumulate its active metabolite ara-CTP. We have previously shown that SAM and HD domain-containing protein 1 (SAMHD1) hydrolytically inactivates ara-CTP, conferring resistance to ara-C in primary AML blasts and animal models. More importantly, SAMHD1 expression is significantly associated with worse survival in paediatric and adult AML. Notably, SAMHD1 has no impact on the efficacy of induction therapy, but rather limits the outcome of high-dose ara-C consolidation courses. We verified these findings in a total of three adult and two paediatric patient cohorts (n=600).
Aims: Hence, combining ara-C with a SAMHD1 inhibitor promises to greatly improve outcome for children with AML during consolidation chemotherapy.
Methods: We therefore screened a large library of small molecules in a phenotypic screen to identify compounds that increase ara-C toxicity in SAMHD1-wildtype, but not SAMHD1-knockout cells. Activity was validated in AML mouse models. Using *in vitro* activity together with cellular thermal shift and cross-linking assays we furthermore addressed the mode of action of inhibition of SAMHD1 ara-CTPase activity (allosteric vs catalytic).
Results: Excitingly, we found two FDA- and EMA-approved substances for which patens have expired and for which extensive safety data exists in both adults and children. These drugs completely abolished SAMHD1-mediated ara-C resistance in primary AML samples and resensitised animal models of AML to ara-C.
Summary/Conclusion: We thus advocate clinical studies that prospectively test the ability of these novel SAMHD1-inhibitors to improve survival when combined with high-dose ara-C consolidation courses.

PS954

GENOMIC-DRIVEN AND FUNCTIONAL ALTERATIONS IN ENERGY, AMINO ACID AND LIPID METABOLISM IN ACUTE MYELOID LEUKEMIA BLASTS AND STEM PROGENITOR CELLS

G. Simonetti[1,*], C. Delpino[2], A. Padella[1], E. Fonzi[1], M. Pazzaglia[1], R. De Tommaso[1], M.C. Fontana[1], S. Bruno[1], C. Papayannidis[1], G. Marconi[1], A. Astolfi[3], E. Dan[1], B. Sinigaglia[1], M. Cavo[1], D. Remondini[4], G. Martinelli[5]

[1]Department of Experimental, Diagnostic and Specialty Medicine, "Seràgnoli" Institute of Hematology , University of Bologna, Bologna, Italy, [2]Departamento de Ingeniería Química, Universidad Nacional del Sur (UNS), Planta Piloto de Ingeniería Química – PLAPIQUI (UNS-CONICET), Bahía Blanca, Argentina, [3]Giorgio Prodi" Cancer Research Center, [4]Department of Physics and Astronomy, University of Bologna, Bologna, [5]Istituto Scientifico Romagnolo per lo Studio e Cura dei Tumori (IRST) IRCCS, Meldola, Italy

Background: Altered cellular metabolism participates to acute myeloid leukemia (AML) pathogenesis, predicts response to therapy and can be targeted. Mutated enzymes, oncogenes or tumor suppressors can drive metabolic dependency of patients' subgroups
Aims: To map potential connections between genetic lesions and metabolism in AML and identify metabolic alterations underlying druggable pathways.
Methods: Whole exome sequencing was performed on 78 AML. Variants were called by MuTect and Varscan2. Metabolites were quantified by mass spectrometry of bone marrow (BM) cells (35 CD34$^+$, 16 CD33$^+$ AML), CD34$^+$ cord blood and CD33$^+$ healthy blood cells (n=21, Metabolon) and compared by Welch's *t*-Test ($p<0.05$). Gene expression profiling was performed on 56 BM (AML and healthy donors, Affymetrix). Cell specific metabolic reconstructions derived with iMat and mCADRE algorithms were studied for data integration.
Results: Metabolism-related genes were mutated in 67% AML (47% of lesions rated as damaging). The most represented pathways were lipid (24% mutations) and nucleotide metabolism (11%), with mutations in the pyrimidine-related gene *AK9* and the pentose phosphate pathway (PPP) gene *H6PD*; bioenergetics pathway (9%), with the respiratory chain genes *NNT* and *ADCK3*; amino acid metabolism (8%), with the tryptophan-related gene *IDO2*; carbohydrate and glucose metabolism (6%), with altered *PKM* and *HK3*; response to stress (4%), with mutated *SOD2*. AML cells showed distinct metabolic profiles compared with healthy cells, while little differences were observed between AML subgroups. The top 30 ranking biochemicals suggested differences in lipid, nucleotide and amino acid metabolism between AML and controls, in line with genomic data. The bioenergetic pathway was also altered in CD34$^+$ AML, with reduced glycolytic intermediates (FDP, 3PG, PEP) and enzymes (GPI, PFKFB3). The Krebs cycle intermediates citrate, aconitate, succinate and malate were decreased in CD34+ AML. The PPP metabolite S7P and intermediates of purine/pyrimidine synthesis/salvage were increased, suggesting elevated demands in DNA/RNA synthesis and repair. Half AML cases displayed high 4-HNE-glutathione, that was undetectable in normal cells, indicating elevated detoxification of 4-HNE. 4-HNE-glutathione significantly correlated with GSH and GSSG levels. Moreover, CD34$^+$ and CD33$^+$ AML showed distinct glutamine/glutamate-dependence: increased NAAG and NAA in CD33$^+$ AML, along with GLS upregulation, and decreased NAAG and elevated glutamine in CD34$^+$ AML. To investigate the relations between genomic and metabolomic alterations, we sought to identify the most fitting metabolic model. Metabolites, reactions and genes annotated in BM, blood, CD14$^+$ cells and monocyte subsystems by mCADRE were largely overlapping and shared a core with an iMat hematological model. 69% of altered metabolites and 14% of differentially expressed genes were mapped in the RECON model: of them, 31% and 69% metabolites and 28% and 26% genes, on average, were present in mCADRE and iMAT models, respectively.
Summary/Conclusion: Mutations of metabolic genes are common in AML. Despite their heterogeneity, genomic-driven and functional metabolic alterations converge to common pathways. Dissecting the link between genomic and metabolic alterations, by simultaneous consideration of datasets for the construction of cell specific models may help define AML subtypes sensitive to antimetabolic therapies, as PPP inhibition and 4-HNE treatment.
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PS955

THE CALCIUM BINDING PROTEIN S100A8/S100A9 COMPLEX IS ASSOCIATED WITH DRUG RESISTANCE IN ACUTE MYELOID LEUKEMIA

M. Liu[1,*], R. Karjalainen[1], A. Kumar[1], A. Parsons[1], L. He[1], D. Malani[1], K. Porkka[2,3], C. A. Heckman[1]

[1]Institute for Molecular Medicine Finland (FIMM), [2]Hematology Research Unit Helsinki, University of Helsinki, [3]Department of Hematology, Helsinki University Hospital Comprehensive Cancer Center, Helsinki, Finland

Background: Despite the development of novel therapy options for acute myeloid leukemia (AML), the overall cure rate remains low with most patients succumbing to relapse or refractory disease. Better understanding of the molecular mechanisms responsible for drug resistance could lead to development of improved treatment strategies, as well as the means to monitor patients at risk for developing drug resistance. S100A8 and S100A9 are members of the S100 family of calcium-binding proteins. Expression of

S100A8 and *S100A9* have been studied in many different tumor types. In AML, expression of S100A8 is considered to be a poor prognostic indicator (Nicolas *et al.*, 2011). Although these proteins are known to have a role in myeloid cell development, little is known about their function in myeloid malignancies and treatment response.
Aims: In this study, we investigated the expression of *S100 genes* in RNA sequence data from AML patients with matching *ex vivo* drug sensitivity and resistance data. Integrating the datasets allowed us to identify associations between *S100A8* and *S100A9* and response to drugs currently being used or under development for the treatment of AML.
Methods: RNA-sequencing was used to determine *S100* gene expression in a cohort of AML samples (n = 112) and healthy individuals (n = 4). Sensitivity of MNCs from AML patient samples was assessed to 304 different small molecule inhibitors tested in 5 concentrations in a 10,000-fold concentration range by measuring cell viability after 72 h incubation using the CellTiter-Glo (CTG) assay. Free cytosolic calcium was measured using the calcium assay kit (BD Biosciences). Statistical dependence between gene expression and DSRT data was assessed by Pearson's correlation coefficient modelling.
Results: Among the S100 members, *S100A8* and *S100A9* were the most highly expressed genes in a cohort of AML samples (n = 112) compared with healthy individuals. Integrated analysis of *S100A8* and *S100A9* gene expression and *ex vivo* drug sensitivity data showed high and moderate correlation with the selective BCL2 inhibitor venetoclax and the FLT3 inhibitor quizartinib, respectively. Both drugs are in development for AML and have shown promising activity in clinical investigations. We further validated and confirmed the high expression of *S100A8* and *S100A9* by qRT-PCR in the venetoclax resistant samples. We also observed a similar association between *S100A8* and *S100A9* expression and venetoclax sensitivity in AML cell line models. Mechanistically, over-expression of *S100A8* and *S100A9* in venetoclax-resistant cell lines (OCI-AML3, SHI-1, SKM-1) was associated with failure to release free-cytosolic Ca^{2+} compared with venetoclax-sensitive cell lines (MOLM-13, Kasumi-1, ML-2). By binding free Ca^{2+}, the S100A8/S100A9 complex can inhibit mitochondrial membrane depololarization and the initiation of apoptosis, which may contribute to the development of venetoclax resistance in *S100A8* and *S100A9* high-expressing AML.
Summary/Conclusion: Our *ex vivo* and *in vitro* data show high correlation between the expression level of calcium binding proteins (S100A8/S100A9) with resistance to the BCL2 inhibitor venetoclax. This study suggests that S100A8/S100A9-mediated calcium pathway may play a role in the development of resistance to venetoclax in AML patients.
ML and RK equeal contribution.

PS956

Abstract withdrawn.

PS957

A HIGH RISK PEDIATRIC AML SUBTYPE PRESENTS A DIAGNOSTIC CHALLENGE-A REPORT FROM THE CHILDREN'S ONCOLOGY GROUP AAML0531 STUDY

S. Kahwash[1], L. Brodersen[2,*], T. Alonzo[3,4], R. Gerbing[3], A. Gamis[5], M. Loken[2], S. Meshinchi[3,6]

[1]Nationwide Children's Hospital, Columbus, [2]Hematologics, Inc, Seattle, [3]Children's Oncology Group, Monrovia , [4]University of Southern California, Los Angeles, [5]Children's Mercy Hospitals & Clinics, Kansas City, [6]Fred Hutchinson Cancer Research Center, Seattle , United States

Background: A new subtype of pediatric acute myeloid leukemia, RAM, identified by immunophenotype, has been previously reported from the Children's Oncology Group (COG) AAML0531 phase 3 study. Patients harboring RAM phenotype AML are predominantly under the age of 2, with an over representation of acute megakaryoblastic leukemia (AMKL), and a 3 year EFS of 16%. Patients harboring the chimeric fusion gene, CBFA2T3-GLIS2, share several of these features and both have recently been included as high-risk prognostic markers in the enhanced risk stratification strategy from COG.
Aims: To improve diagnostic testing algorithms among pediatric AML patients by properly identifying these rare yet high-risk patients by correlating RAM status, CBFA2T3-GLIS2 status, and morphologic features.
Methods: We correlated the RAM and CBFA2T3-GLIS2 status among 821 patients meeting eligibility requirements who participated in AAML0531. Although FAB classification was completed at diagnosis and centrally reviewed, the unique morphologic features of a patient with RAM phenotype overlapping with metastatic solid tumor malignancies were not yet described. Blast features include: 1) cohesive clumps showing nuclear molding, 2) bi- or multinucleation, 3) cytoplasmic blebs or detached cytoplasmic fragments, 4) lack of cytoplasmic granules and/or Auer rods, and 5) bone marrow architecture consisting of patchy islands of blasts with alternating areas of less affected hematopoietic bone marrow.
Blinded retrospective analysis assessing these morphologic features was completed for 72 patients that included those with RAM phenotype, positive CBFA2T3-GLIS2 status, randomly selected AMKL patients, and other randomly selected patients. Each of the morphologic features was scored with one point given for each of the above characteristics, with a point deducted if cytoplasmic granules or Auer rods were identified. Patients were stratified based on morphologic scores: ≥4 or, ≤3.
Results: Of the 821 patients, 26 patients (3.2%) harbored either RAM phenotype or CBFA2T3-GLIS2 fusion transcripts with 19/25 (61%) defined as RAM+ (no data for 1 patient), while 18/24 (75%) were CBFA2T3-GLIS2+ (no data for 2 patients). Data for both markers were available for 23 patients; where 11 patients were dual positive (48% RAM+/ CBFA2T3-GLIS2+), while 6 were RAM+/CBFA2T3-GLIS- (26%) and 6 were RAM-/CBFA2T3-GLIS2+ (26%).
Morphologic analysis revealed 20/72 patients had scores ≥4 while 53/72 patients were ≤3. 12/20 (60%) of patients with scores ≥4 harbored RAM phenotype, 8 of these were also CBFAT23-GLIS2+. No RAM-/CBFA2T3-GLIS2+ patients had scores ≥4. 5/20 patients with scores ≥4 had previously been classified as classic AMKL (and these were RAM-/CBFA2T3-GLIS2-). Morphologic identification of patients exhibiting RAM phenotype demonstrated a sensitivity of 80% [95% CI=51.9-95.7] and specificity of 86% [95% CI=74.2-93.7]. When morphologic identification of patients with RAM phenotype or CBFA2T3-GLIS2+ status is considered, the sensitivity decreased to 63% [95% CI=38.4-83.7], while the specificity remained at 86%.
Summary/Conclusion: Proper identification of these high-risk features is essential because of the poor prognosis of patients with RAM phenotype and/or the CBFA2T3-GLIS2 fusion transcript. Given the overlap of the morphologic features with metastatic solid tumors and other challenging acute leukemias, these data suggest that patients with these morphologic features should be screened for RAM phenotype and CBFA2T3-GLIS-2, particularly patients under the age of 5.

PS958

TARGETED COMBINATION THERAPY AGAINST ONCOGENIC PI3K/AKT/MTOR AND NFKB SIGNALLING PATHWAYS USING ACUTE MYELOID LEUKEMIA AS A CANCER MODEL

M. Estruch[1,*], K. Reckzeh[1], A. Søgaard Helbo[1], K. Theilgaard-Mönch[1]

[1]Finsen Laboratory, Biotech Research and Innovation Centre, Univesrity of Copenhagen, Copenahgen, Denmark

Background: Various cancer entities are addicted to aberrant activity of an "oncogenic" signalling pathway in order to maintain their malignant phenotype. As a consequence, cancer patients treated with therapeutics that effectively target such a pathway often demonstrate robust initial clinical responses. However, chronic treatment frequently gives way to relapse, due to various escape mechanisms including loss of negative feedback regulation promoting activation of a "bypass" signalling pathway, that render cancer cells independent of their primary oncogenic signalling pathway.
Aims: We aim to unravel the molecular mechanisms of a hitherto undescribed crosstalk of the PI3K/AKT/MTOR and NFKB signalling pathways and define how combinatorial as compared to individual targeting of these pathways will affect growth and survival of primary cancer cells, as a promising strategy to improve clinical responses to targeted therapies.
Methods: In order to identify small molecule inhibitors targeting PI3K/AKT/MTOR and NFKB signalling pathways, we use acute myeloid leukaemia (AML) as a cancer model and apply AML mouse models that accurately mimic the genetics of AML patients to conduct initial drug screening experiments that can be applied in preclinical trials using AML patient cells from our biobank in xenograft assays. Specifically, we are currently focussed on the inv(16) AML subtype showing poor (15-20%) survival rates. For this, our group has specifically implemented the inv(16)/KitD816Y mouse model mimicking the genetics of these AML patients.
Results: So far, we have identified PI3K inhibitors (BKM120 and CAL-101) that abrogate phosphorylation of PI3K substrates (Western Blot analysis) and inhibit growth and survival of clonogenic primary murine inv(16)/KitD816Y AML cells *in vitro*. Moreover, we have identified proteasome inhibitors (Bortezomib/Carfilzomib/Oprozomib) that abrogate NFKB translocation to the nucleus in primary murine inv(16) AML cells

(confocal microscopy) leading to inhibition of the constitutively active NFKB pathway as well as inhibition of survival and clonogenic growth of AML cells (CFC- assays). Significantly, co-inhibition of both PI3K/AKT/MTOR and NFKB signalling pathways demonstrated a strong synergistic inhibitory effect on growth and survival of clonogenic murine inv(16) AML cells *versus* normal clonogenic haematopoietic stem/progenitor cells (HSC/HPCs), suggesting potential crosstalk and the need of co-inhibition of oncogenic signalling pathways (*i.e.* PI3K/AKT/MTOR and NFKB) rather than inhibition of a single pathway to effectively kill AML cells. Based on these *promising results*, we are currently conducting *in vivo* studies in inv(16)/KitD816Y mouse model to assay the effects of the single versus the combinatorial treatment on decreasing AML cell progression and thereby promoting an increase on survival (Figure 1).

Summary/Conclusion: Significantly, the present results not only shed new light on how PI3K/AKT/MTOR and NFKB signalling pathways crosstalk in AML but also highlight the importance of identification and combinatorial therapeutic targeting of interconnected oncogenic signalling pathways in order to improve clinical outcome of patients suffering from AML as well as other cancer entities.

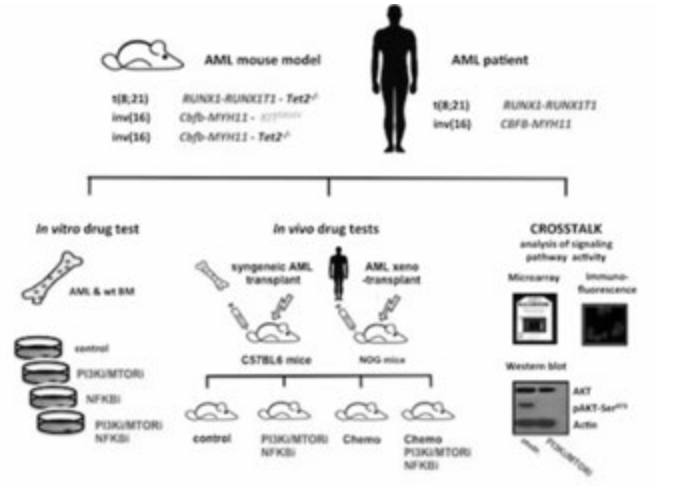

Figure 1.

PS959

IDENTIFICATION OF GENE COPY NUMBER ABERRATIONS AS PROGNOSTIC MARKERS OF SURVIVAL IN ACUTE MYELOID LEUKEMIA (AML) PATIENTS BY EXOME SEQUENCING OF LEUKEMIA DNA IN THE MAYO CLINIC AML EPIDEMIOLOGY COHORT

Y. Asmann[1,*], M. Heckman[1], N. Diehl[1], V. Sarangi[2], R. Ketterling[3], L. Sproat[4], L. Jiang[5], B. Eckloff[6], L. Finn[7], K. Robertson[8], J. Foran[9]

[1]Department of Health Sciences Research, Mayo Clinic, Jacksonville Florida, [2]Department of Health Sciences Research, [3]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester Minnesota, [4]Department of Oncology and Hematology, Mayo Clinic, Phoenix Arizona, [5]Department of Laboratory Medicine and Pathology, Mayo Clinic, Jacksonville Florida, [6]Medical Genome Facility, Mayo Clinic, Rochester Minnesota, [7]Oncology and Hematology, Ochsner Medical Center, New Orleans, LA, [8]Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester Minnesota, [9]Department of Oncology and Hematology, Cancer Center, Mayo Clinic, Jacksonville Florida, United States

Background: Chromosomal aberrations are the strongest genetic markers for AML prognosis. Studies have identified genomic abnormalities at whole chromosome, arm, and band levels that are associated with outcomes in AML patients. However little is known about the role of specific gene copy number (CN) aberrations.

Aims: We performed exome sequencing of AML DNA collected at the time of diagnosis from Mayo clinical epidemiology cohort and evaluated associations between gene level CN and survival after AML diagnosis.

Methods: The Mayo Clinic AML Epidemiology cohort comprises a highly annotated, unselected, and consecutive patients diagnosed and treated at Mayo Clinic Florida and Arizona. All patients received standard of care intensive remission induction chemotherapy with curative intent. We obtained AML DNA from remnant diagnostic cytogenetic pellets identified in 98 patients and performed exome sequencing . Approximately 100 million paired-end 100bp reads per sample were mapped to human reference genome build hg19 using BWA-MEM. The gene level copy numbers were called using patternCNV method. Separately for each gene, comparisons of survival after AML onset between CN neutral patients and patients with a gain, and also between normal patients and patients with a loss, were made using Cox proportional hazards regression models that were adjusted for chemotherapy regimen. Comparisons were made only if there were greater than five patients in both of the given comparison groups in order to avoid performing non-informative tests. In order to correct for the 38,138 total statistical tests that were performed, we utilized a false-discovery rate (FDR) correction (assuming a FDR of 5%), after which p-values < 7.90E-06 were considered as statistically significant. All statistical tests were two-sided.

Tables 1, 2 and Figure 1.

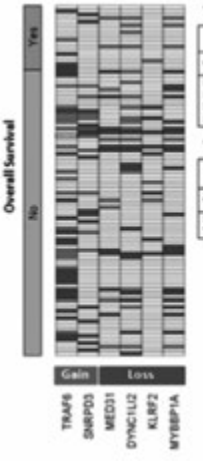

Table 1. Genes with Copy Number (CN) Loss as Prognostic Marker

Gene	Chr	Band	Patient Counts and CN Status: Loss	Neutral	Gain	Kaplan-Meier 12-month Survival: Loss	Neutral	HR (Loss vs. Neutral)	Adjusted p Values
DYNC1LI2	16	q22.1	12	70	18	8.0%	50.0%	8.40	2.410E-06
MYBBP1A	17	p13.2	20	73	5	5.0%	48.0%	4.25	1.310E-06
MED31	17	p13.1	10	80	8	0.0%	43.6%	5.73	8.330E-06
KLRF2	12	p13.31	8	87	3	0.0%	44.1%	6.68	8.670E-06

Table 2. Genes with Copy Number (CN) Gains as Prognostic Marker

Gene	Chr	Band	Patient Counts of CN Status: Loss	Neutral	Gain	Kaplan-Meier 12-month Survival: Neutral	Gain	HR (Gain vs. Neutral)	Adjusted p Values
SNRPD3	22	q11.23	14	74	10	45.9%	0.0%	6.86	7.030E-07
TRAF6	11	p12	24	56	18	50.9%	5.6%	4.96	1.680E-06

Figure 1. heat-map of the copy numbers from genes associated with 12-month survival. Rows are patients, and columns are genes. The red, white, and blue cells of the heat map indicate copy number gain, neutral, and loss, respectively. The copy number losses of 4 genes are associated with poor survival (the right 4 columns of the heat-map, and Table 1); and the copy number gains of 2 genes were associated with poor survival (the left 2 columns of the heat-map, and Table 2); and There were 18 patients (the top 18 rows of the heat-map) achieved and 80 patients (bottom 80 rows of the heat-map) failed 12-month survival.

Results: We found significant associations of CN losses of DYNC1LI2, MYBBP1A, MED31, and KLRF2 with poorer overall survival (OS) (Table 1). DYNC1LI2 is a microtubule-associated motor protein with unknown significance in AML. However the expression of DYNC1LI2 is correlated with BICD1, a potential biomarker for prognosis and therapy response in glioblastoma. MYBBP1A is a nucleolar transcriptional regulator involved in cell cycle control, and the dysregulation of MYBBP1A is functionally associated with RUNX1 in inducing leukemia. KLRF2 is reported to regulate MYC expression, and MED31 is a transcription cofactor with unknown function in AML. In addition, we observed significant associations of CN gains of SNRPD3 and TRAF6 with poorer OS after AML diagnosis (Table 2). Report showed that mice transplanted with TRAF6-expressing marrow progressed either to marrow failure or AML and acquired 5q-syndrome. This is intriguing since 5q deletion is a known marker for poor prognosis in AML. The role of SNRPD3 gain in AML is unclear but it is involved in local aggressiveness and metastatic behavior in soft tissue tumor (Figure 1).

Summary/Conclusion: Exome sequencing was for the first time successfully performed on archived diagnostic cytogenetic cell pellets, and we identified CN gain or loss of 6 genes as potential biomarkers located outside of the previous known large chromosomal aberration regions associated with survival after therapy for AML. Functional roles of these genes provided important insights into the mechanism of AML clinical outcome and will guide prospective studies to determine the mechanism AML prognosis and therapy response.

PS960

NEO2734, A NOVEL HIGHLY POTENT ORAL DUAL BET AND P300/CBP BROMODOMAIN INHIBITOR FOR TREATMENT OF HEMATOLGICAL MALIGNANCIES

B. Brown[1,*], C. Wahlestedt[2], D. Chiche[1], F. Giles[3]

[1]NEOMED Therapeutics 1 Inc., Montreal, Canada, [2]University of Miami, Miami, [3]Developmental Therapeutics Consortium, Chicago, United States

Background: The Bromodomain (BRD) and Extra-Terminal domain (BET) family of proteins are key regulators of epigenetic control. Modulation of epigenetic 'readers' such as BRDs is a recognized developmental therapeutics priority. Cyclic AMP response element binding protein (CREB)-binding protein (CBP) and E1A interacting protein of 300 kDa (EP300 or P300) are highly homologous BRD-containing transcriptional co-activators that regulate a number of important cellular events through their acetyltransferase activity. We have developed a series of molecules that exhibit dual inhibition of BET and CBP/P300 from which NEO2734 has emerged as lead clinical candidate.

Aims: To study NEO2734 a dual inhibitor of BET and CBP/P300 in AML and other hematolgical malignancies
Methods: NEO2734 was initially profiled against the BET inhibitor iBET762 in cellular assays and in multiple human cancer xenograft models including leukemia (MV-4-11).
Results: Table 1 shows NEO2734 *in vitro* antiproliferative activity against a variety of leukemia cell lines. NEO2734 has major activity at 10mg/kg (p.o.) in a leukemia xenograft model whereas iBET762 has minimal activity at 30mg/kg (p.o.) both dosed once daily for 18 days. For example, MV-4-11 xenograft mice were treated by oral gavage with NEO2734 (1 mg/kg, 3mg/kg and 10mg/kg) and the reference compound iBET762 (10 mg/kg and 30mg/kg) for 18 days. In this model, NEO2734 led to potent tumor regression in a dose dependent manner. Much weaker activity was observed for iBET-762 at either 10 or 30 mg/kg (Figure 1).

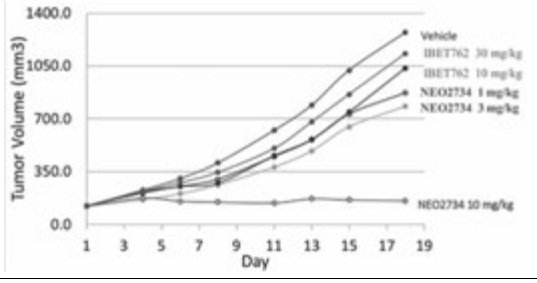

Figure 1.

Table 1.

Cell line	Tissue Type	IC50 (μM) (72h)		
		NEO2734	iBET762	Cisplatin
Molt-4	Blood	0.56	2.4	0.49
KG-1		0.11	0.58	1.9
THP-1		0.29	1	1.2
KHYG-1		0.18	1.1	0.46
Daudi		0.3	1.2	0.27
Raji		0.32	1.8	0.91
RL		0.13	0.84	2
U-937		0.28	1.1	1.2

Summary/Conclusion: NEO2734, an oral potent novel dual inhibitor of BET and CBP/P300, has pre-clinical activity in a spectrum of human hematoligical malignancies. Clinical studies are in preparation.

PS961

ACUTE MYELOID LEUKEMIA INDUCED TRANSFORMATION OF THE HUMAN BONE MARROW MICROENVIRONMENT PREDICTS CLINICAL OUTCOME

Y. Chen[1,2,*], Y. Zaun[1,2], L. Arnold[1,3], K. Weissenfels[1,2], K.W. Schmid[2,4], L. Klein-Hitpass[2,5], A. Squire[2,6], S. Bertram[2,4], U. Dührsen[1,2], M. Hanoun[1,2]

[1]Department of Hematology, University Hospital Essen, [2]University of Duisburg-Essen, [3]University Hospital Essen, Essen, Germany, [4]Institute of Pathology and Neuropathology, [5]Institute of Cell Biology (Cancer Research), [6]Institute for Experimental Immunology and Imaging and Imaging Center Essen (IMCES), University Hospital Essen, Essen, Germany

Background: Acute myeloid leukemia (AML) is an aggressive hematological malignancy which is characterized by a high relapse rate, suggesting that leukemic stem cells escape chemotherapeutic treatment. Recently, different murine models gave insight how leukemia infiltration alters the bone marrow microenvironment to reinforce disease. However, little is known about the bone marrow architecture in human. Further, if these alterations have an impact on clinical outcome in AML patients so far remained unsolved.
Aims: We aimed at dissecting the bone marrow architecture in human AML, characterize distinct niche constituents and uncover its clinical significance for the course of disease.
Methods: We combined immunohistochemical stainings with global gene expression analyses as well as protein analyses from human AML patients at first diagnosis and matching non-leukemic donors. To assess the bone marrow architecture, we automatically quantified CD271+ mesenchymal stem and progenitor cells (MSPCs) as well as reticular fibers on bone marrow sections. Bone marrow MSPCs were freshly isolated by fluorescence-activated cell sorting (FACS), herewith avoiding culture-induced artefacts and were used for microarray analyses (Clariom™ S Human Assay). The bone metabolism was evaluated by measuring serum osteocalcin levels. Final findings were correlated with clinical outcome.
Results: Here, we found that AML bone marrow infiltration induced a 2-fold increase in CD271+ MSPCs which correlated with loss of quiescence as indicated by gene expression analysis of freshly isolated bone marrow MSPCs. Increase in MSPCs in AML bone marrow significantly correlated with an increase in reticular fiber, an extracellular matrix protein produced by mesenchymal stroma cells (AML mean 4.50±2.28% vs. Control, 2.39±1.50%, p=0.0038), which negatively correlated with hemoglobin, thrombocytes and neutrophils. Alongside, MSPCs showed decreased expression of hematopoietic stem cell-regulating genes, particularly *CXCL12* and *KITLG*. Also the niche capacity of isolated bone marrow MSPCs from AML patients differed with respect to osteoblast differentiation as indicated by impaired expression of bone growth and mineralization related genes. To assess the bone metabolism in AML patients at first diagnosis we quantified serum osteocalcin levels. In fact, AML patients showed a significant reduced expression of osteocalcin indicating impaired bone turnover (AML mean 13.14±7.74ng/ml vs. Control 17.88±9.00ng/ml, p=0.0152). Ongoing *in vitro* studies demonstrated that AML cells specifically inhibit osteoblast mineralization. Strikingly, the level of osteocalcin expression proved to be of clinical significance. Patients with low osteocalcin level showed inferior median overall survival of 280 days, while the median overall survival of high osteocalcin level was not reached.
Summary/Conclusion: In summary, our data show how AML severely alters the human bone marrow architecture and the fate of MSPCs. These alterations proved to be of high clinical significance and allowed us to detect new prognostic markers. This increasing understanding of the human AML bone marrow niche might open the window for new niche-targeted therapies to eradicate leukemic stem cells and eventually decrease the high relapse rate in AML.

PS962

IMMUNOPHENOTYPIC PROFILING OF LEUKEMIC STEM CELLS TO TRACK FLT3-ITD POSITIVE, CHEMO-RESISTANT CLONES IN ACUTE MYELOID LEUKEMIA

T. Ottone[1,2,*], V. Alfonso[1], D. F. Angelini[2], G. Guerrera[2], L. Iaccarino[1], S. Lavorgna[1,2], M. Divona[1], M. Irno Consalvo[1], L. Cicconi[1], S. Travaglini[1], M. De Bardi[2], B. Neri[3], V. Martini[4], L. Fianchi[5], L. Battistini[2], A. Venditti[1], F. Buccisano[1], M. T. Voso[1], F. Lo-Coco[1,2]

[1]Department of Biomedicine and Prevention, University of Rome Tor Vergata, [2]Fondazione Santa Lucia, Laboratorio di Neuro-Oncoematologia, [3]Hematology Unit, Sant' Eugenio Hospital, Rome, [4]Hematology Unit, F. Spaziani Hospital, Frosinone, [5]Department of Hematology, Università Cattolica del Sacro Cuore, Roma, Italy

Background: Persistence of leukemia stem cells (LSCs) in AML patients achieving complete remission after chemotherapy is known to lead to disease recurrence and poor outcome. Therefore, the identification of LSCs driving resistance to therapy represents an important challenge. A widely accepted hypothesis is that LSCs reside within the CD34+/CD38- cell fraction and several groups have identified cell surface antigens preferentially expressed on LSCs. We recently demonstrated a strong correlation between the CD123/CD99/CD25+ population within CD34+ cells and the presence of the *FLT3*-ITD mutation, notoriously associated with higher risk of relapse in AML.
Aims: The aim of this study was to better characterize the LSCs of *FLT3*-ITD positive patients and to track, through flow-cytometry and semiquantitative-PCR, the expansion of mutated clones.
Methods: Bone marrow (BM) samples from 9 patients with *de novo* AML harbouring *FLT3*-ITD were analysed by 8-color flow cytometry. A sequential gating strategy was carried out to identify and purify the hematopoietic precursor fraction CD34/CD45/CD123+,CD38-, the LSCs enriched fraction CD34/CD123/CD99/CD25+, the CD34+ stem cell subset lacking CD123/CD99/CD25 expression, and T-lymphocytes. BM cells from UPN9 were sorted at diagnosis, at several follow-up time points during treatment and at the time of relapse. Cells were purified by a high-speed cell sorter. *FLT3*-ITD was monitoring was carried out through PCR followed by capillary electrophoresis.
Results: Enrichment of *FLT3*-ITD positive population, defined as a significantly higher *FLT3*-ITD mutation load, was observed within the CD34+ compartment of CD123/CD99/CD25+ cells, as compared to the MNC (p=0.001) and the lymphoid/myeloid precursors which showed a low or absent CD123/CD99/CD25 expression (p=0.004) (Figure 1A). In one patient with 2 different *FLT3*-ITD mutated clones at diagnosis (UPN1), we identified a LSC population defined by CD34/CD123/CD99/CD25+ with one ITD mutated clone in homozygous, likely originated by loss of heterozygosity (LOH) of the mutated allele (Figure 1B). Based on several studies indicating that LSCs are enriched in CD34+/CD38- cells, we investigated whether *FLT3*-ITD mutation was enriched in the LSCs CD34/CD123/CD99/

CD25+/CD38- compartment. As shown in Figure 1C, in UPN7 the CD34+/CD38- LSCs fraction, characterized by CD123/CD99/CD25 co-expression, represented the dominant *FLT3*-ITD mutated population in comparison with the CD34+/CD38+ counterpart. Finally, to trace the clonal evolution of LSCs carrying *FLT3*-ITD mutation, we sorted different BM-cell fractions at diagnosis and during follow-up from a patient (UPN9) who underwent disease relapse 8 months after the initial diagnosis. As shown in Figure 1D, a progressive increase of the *FLT3*-ITD allele burden was detected in the CD34/CD123/CD99/CD25+ subset as compared to the CD34+ cells lacking CD123/CD99/CD25 expression and the total MNC population. In this patient, a high *FLT3*-ITD mutant allele burden in CD34/CD123/CD99/CD25+ fraction was detected already at time of complete morphologic remission and 2 months before haematological relapse.
Summary/Conclusion: Our study shows that *FLT3*-ITD mutation represents a founding clone occurs at an early LSC level as defined by CD34/CD123/CD99/CD25+,CD38- immunophenotype. Prospective studies are needed to assess whether monitoring of this cell subset may allow early identification of patients at higher risk of relapse.

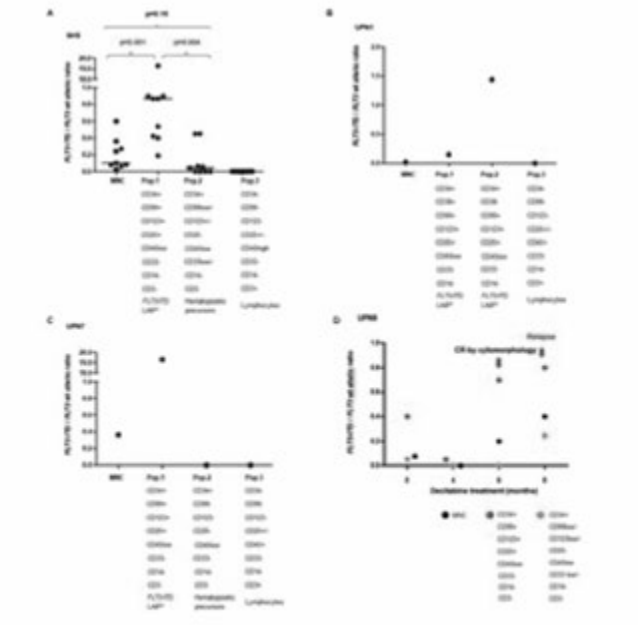

Figure 1 *FLT3*-ITD allele ratio analysed in several BM AML samples
(A) *FLT3*-ITD allelic ratio analysis in 9 AML patients collected at diagnosis and in particular in (B) UPN1, (C) UPN7 and (D) UPN9. The *FLT3-ITD* mutant allele burden, defined as the ratio of the area under the curve of mutant and wild type alleles, was performed both in total MNC and in several BM high-purified cells. The antibody combination used for cell sorting is indicated. *P* values were calculated by a paired Student's *t*-test.

Figure 1.

PS963

ASSESSMENT OF HIGH-RISK MOLECULAR MARKERS IN INTERMEDIATE RISK ACUTE MYELOID LEUKEMIA AS DEFINED BY 2010 ELN RISK CLASSIFICATION

E. Panina[1,*], F. G. Rücker[1], D. Anna[1], W. Daniela[1], T. Veronica[1], N. Jahn[1], S. Laura[1], M. Agrawal[1], T. J. Blätte[1], P. Peter[1], V. I. Gaidzik[1], J.K. Herzig[1], T. Kindler[2], J. Westermann[3], M. Lübbert[4], W. Schmiegel[5], S. Kremers[6], R. Greil[7], F. Thol[8], M. Heuser[8], A. Ganser[8], H. Döhner[1], L. Bullinger[3], K. Döhner[1]

[1]Internal Medicine III, University Hospital of Ulm, Ulm, [2]Third Department of Medicine, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, [3]Department of Hematology, Oncology and Tumor Immunology, Charité University Medicine, Campus Virchow-Klinikum, Berlin, [4]Department of Medicine, Division of Hematology and Oncology, University Hospital of Freiburg, Freiburg, [5]Department of Medicine, Haematology and Oncology, Ruhr-University of Bochum, Knappschaftskrankenhaus, Bochum, [6]Department of Hematology/Oncology, Caritas Krankenhaus Lebach, Lebach, Germany, [73]rd Medical Department, Paracelsus Medical University Salzburg, Salzburg Cancer Research Institute, Cancer Cluster Salzburg, Salzburg, Austria, [8]Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany

Background: In 2010 an international expert panel (European LeukemiaNet, ELN) published recommendations for diagnosis and management of acute myeloid leukemia (AML) including a risk stratification by genetics, subdividing AML in four risk groups. Emerging data on molecular markers in AML has led to an introduction of new stratification criteria by ELN in 2017 including the recommendation for screening of the high-risk (HR) molecular markers *ASXL1, RUNX1,* and *TP53* that have been shown to be associated with poor prognosis.
Aims: To assess the prevalence of the HR molecular markers *ASXL1, RUNX1,* and *TP53* in patients with newly diagnosed AML previously classified as intermediate-I and -II risk (inter-I/-II) based on the 2010 ELN criteria.
Methods: Using a next-generation targeted sequencing approach [HaloPlex HS (Agilent) on a Miseq (Illumina)], we performed a prospective analysis of all coding regions of *ASXL1*, *RUNX1*, and *TP53* in 200 newly diagnosed intermediate risk AML patients all enrolled in the AMLSG Biology and Outcome (BiO)-Project of the German-Austrian AML Study Group (AMLSG). Patient genetic features were as follows: Inter-I: normal karyotype n=124 (62%); inter-II: trisomy 8 (sole) n=12 (6%), nullisomy Y (sole) n=5 (2.5%), others n=59 (30%); *FLT3*-internal tandem duplication (*FLT3*-ITD^{+}) n=38 (19%), mutations in tyrosine kinase domain of *FLT3* (*FLT3*-TKD^{mut}) n=7 (3,5%), in *NPM1* n=32 (16%), in IDH1/2 19.5% (*IDH1*^{mut} *n=13*, *IDH2*^{mut} n=26); median age was 67 yrs (range: 21-89 yrs).
Results: Overall, 137 HR mutations in 98 patients (49%) were identified (one HR marker mutated, n=73; two HR markers mutated, n=25). Most frequently co-mutated partners were *ASXL1* and *RUNX1* accounting for 20/25 cases with two concurrent mutated genes, whereas co-mutations in *RUNX1/TP53* and *ASXL1/TP53* were seen in only 3/25 and 2/25 cases, respectively. Mutations in *ASXL1* occurred in 32% (64/200; inter-I: n=37, inter-II: n=27), followed by *RUNX1* in 22% (44/200; inter-I: n=23, inter-II: n=21), and *TP53* in 7.5% (15/200; inter-I: n=7, inter-II: n=8). Patients with mutation in one of the three HR markers exhibited lower WBC (median 5.92 vs 13.9, p=.031), lower hemoglobin value (median 8.6 vs 9.35 g/dl, p=.008), and lower LDH serum level (median 324 vs 510 U/l, p<.0001). Furthermore, HR markers correlated with male gender (66% HR+ vs 51% HR-, p=.044). HR marker mutations were inversely correlated with *FLT3*-ITD^{+} (4% vs 34%, p<.0001), *NPM1*^{mut} (1% vs 31%, p<.0001), and the genotype *NPM1*^{mut}/*FLT3*-ITD^{+} (1% vs 30%, p<.0001). Moreover, HR marker mutations were in trend inversely correlated with normal karyotype (60% vs 72%, p=.087). There were no correlations with regard to age, platelet count, *FLT3*-TKD, or *IDH1/2* mutation. Further analyses of cooperating mutations and survival data are currently ongoing.
Summary/Conclusion: In this prospective study, we show that 50% of AML patients classified by 2010 ELN criteria as intermediate risk AML harbor at least one HR marker. This high prevalence clearly demonstrates the clinical importance of HR marker screening by 2017 ELN criteria in this AML subgroup resulting in a shift of the prognostic stratification towards adverse risk. Screening for *ASXL1, RUNX1,* and *TP53* mutations in previously intermediate-risk patients with newly diagnosed AML allows the identification of those who potentially benefit from more intensive therapy or novel treatment approaches.

PS964

MOLECULAR PROLIFE BY NEXT GENERATION SEQUENCING OF ACUTE MYELOID LEUKEMIA WITH NORMAL KARYOTYPE: CLINICAL RESULTS FROM THE PROSPECTIVE TRIAL 02/06 OF THE NORTHERN ITALY LEUKEMIA GROUP (NILG)

O. Spinelli[1,*], S. Salmoiraghi[1], P. Zanghì[1], R. Cavagna[1], A. Michelato[1], K. Buklijas[1], L. Zannino[1], T. Intermesoli[1], F. Lussana[1], F. Delaini[1], E. Oldani[1], C. Caprioli[1], P. Stefanoni[1], G. Gianfaldoni[2], F. Marmont[3], D. Ferrero[3], E. Terruzzi[4], L. De Paoli[5], G. Rossi[6], E. Borlenghi[6], I. Cavattoni[7], M. Tajana[8], A. M. Scattolin[9], D. Mattei[10], P. Corradini[11], L. Campiotti[12], F. Ciceri[13], M. Bernardi[13], E. Todisco[14], A. Cortelezzi[15], S. Cortelazzo[7], E. Audisio[3], A. Bosi[2], B. Falini[16], C. Pavoni[1], R. Bassan[9], A. Rambaldi[1]

[1]Haematology and Bone Marrow Transplantation Unit, ASST PAPA GIOVANNI XXIII, Bergamo, [2]Azienda Ospedaliera Universitaria Careggi, Firenze, [3]A.O.U. Città della Salute e della Scienza, Torino, [4]Azienda Ospedaliera San Gerardo , Monza, [5]Azienda Ospedaliera ss. Antonio e Biagio e Cesare Arrigo, Alessandria, [6]ASST-Spedali Civili, Brescia, [7]Ospedale San Maurizio, Bolzano, [8]ASST Ospedale di Cremona, Cremona, [9]Ospedale dell'Angelo e SS. Giovanni e Paolo, Venezia Mestre, [10]Azienda Ospedaliera S.Croce e Carle, Cuneo, [11]Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, [12]University of Insubria, Varese, [13]IRCCS Ospedale San Raffaele, Milano, [14]IRCCS Istituto Clinico Humanitas, Rozzano (MI), [15]Fondazione IRCCS Ca' Granda Ospedale maggiore policlinico, Milano, [16]University of Perugia, Perugia, Italy

Background: Treatment strategies of Acute Myeloid Leukemia (AML) patients are based on clinical features as well as the molecular and cytoge-

netic profile of each patient. For patients with a normal karyotype (NK) the final risk classification is also defined by the identification of somatic mutations that have been recently described and impact on patient survival.
Aims: To describe the clinical impact of the gene mutation profile of NK AML patients treated within the prospective NILG trial 02/06 [ClinicalTrials.gov Identifier: NCT00495287].
Methods: The 02/06 trial enrolled a total of 572 patients, 250 of them having a NK AML. Molecular profiling was prospectively obtained with standard approach and subsequently with Next Generation Sequencing (NGS) on available material (207). Two commercial NGS kits were applied: Trusight Myeloid panel (Illumina) and Sophia Myeloid Solution (Sophia genetics) investigating 54 and 30 gene regions, respectively. Variants were identified with dedicated software and pathogenicity was verified in public repository.
Results: The median age of the 207 NK AML studied was 52 years (19-74) and 30% of them had >50.000 White Blood Cell (WBC) count. The 5-year overall survival (OS) and Disease Free Survival (DFS) were 52% and 51%, respectively. The most frequently mutated genes in this cohort were NPM1 (50%) followed by DNMT3A (38%), FLT3-ITD (26%), CEBPA (20%), (single allele mutation in 9% and double mutated in 11%), TET2 (16%), RUNX1 (15%), IDH2 (12%), FLT-TKD (11%), ASXL1, IDH1 (10%), PTPN11, SRSF2, MLL-PTD (9%), NRAS (8%), STAG2, BCOR (7%), GATA2, KRAS (6%), WT1 (5%), SF3B1, U2AF1 (4%), PHF6, RAD21 (3%), CBL, BCORL1, KDM6A, SMC3, CSF3R (2%), ETV6, EZH2, ZRSR2, SETBP1, CUX1, GATA1, SMC1A (1%), TP53, HRAS, JAK2, CDKN2A, FBXW7, GNAS, JAK3, KMT2A, MID88, NOTCH1 (<1%). We verified whether the incidence of co-occurring mutations was different among NPM1mutated (NPM1m) and NPM1 wild type (NPM1wt) groups of patients. DNMT3A, FLT3-ITD, FLT3-TKD, IDH1, PTPN11 and RAD21 mutations were more frequently detected in the NPM1m group ($p<0.05$) while double mutated CEBPA (CEBPAdm), RUNX1, IDH2, ASXL1, SRSF2, MLL-PTD, STAG2, BCOR, GATA2, WT1, SF3B1, U2AF1 mutations were mostly associated to the NPM1wt group. Univariate analysis showed that FLT3-ITD negatively affected the OS of both NPM1m and NPM1wt patients while DNMT3A, RUNX1, TET2, IDH1, NRAS, U2AF1, determined a worse OS in the NPM1wt group ($p<0.05$). In this latter, the presence of CEBPAdm was associated to a favorable clinical outcome while ASXL1 had an adverse effect, only in the absence of RUNX1 mutation. The 5-year OS of NPM1m group with or without FLT3-ITD were 38% and 72%, respectively ($p=0.0002$, Figure 1A), while the 5-year OS of NPM1wt group with or without any of the above described prognostically detrimental gene mutations were 26% and 72%, respectively ($p<.0001$, Figure 1B). The same results were obtained by multivariate analysis with the exception of IDH1 and NRAS that did not affect the outcome of NPM1wt patients. Interestingly, by univariate analysis allogeneic stem cell transplantation was associated to a better OS and DFS in both the NPM1m and NPM1wt cohort. By multivariate analysis, the beneficial effect of transplant was confirmed only in the NPM1m group.

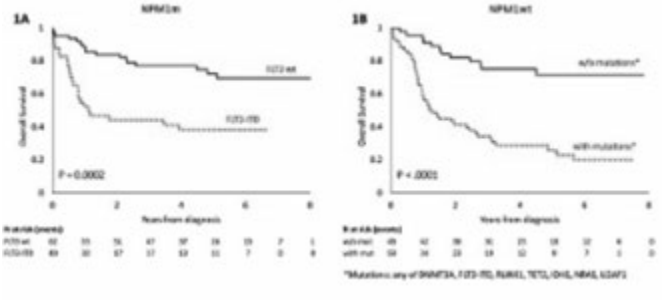

Figure 1.

Summary/Conclusion: Molecular profiling of NK AML is crucial for a precise definition of the relapse risk in AML patients, particularly those lacking the NPM1 mutation. High throughput NGS provides timely information on a growing set of genes and may improve treatment strategies.

PS965

SINGLE CELL SIGNALING PHARMACODYNAMICS AND CLONAL EVOLUTION IN A PHASE 1/2 CLINICAL TRIAL OF SELECTIVE AXL INHIBITOR BEMCENTINIB (BGB324) IN R/R ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROME

M. Hellesøy[1,*], S.-E. Gullaksen[2,3], R.B. Forthun[1], O.H.E. Fagerholt[2,3], B.S. Tislevoll[2,3], H. P. Brodal[1], R. Hovland[4], H. Reikvam[1], A.O. Kittang[1], J. Cortez[5], J. Skavland[2,3], J. Lorens[6], D. Micklem[6], M. Yule[6], S. Loges[7], B.T. Gjertsen[1,2,3]

[1]Department of Internal Medicine, Section of Hematology, Haukeland University Hospital, [2]Department of Clinical Science, [3]Centre for Cancer Biomarkers CCBIO, Department of Clinical Science, Faculty of Medicine and Dentistry, University of Bergen, [4]Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway, [5]Department of Leukemia, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, United States, [6]BerGenBio AS, Bergen, Norway, [7]Department of Oncology and Hematology with Sections BMT and Pneumology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Background: Overexpression and activation of Axl is found in many cancers, and is linked to increased proliferation, invasion and resistance to apoptosis. It is also a poor prognostic marker, linked to acquired resistance to chemotherapy and other anticancer therapies in many malignancies, including AML. Bemcentinib (BGB324) is a first-in-class orally available highly selective small molecule inhibitor of Axl, currently being investigated in a ph 2 clinical trial in patients with refractory/relapsed AML and MDS (BGBC003, NCT02488408). Here we report results from phospho-flow cytometry and mass cytometry analyses of PB and BM samples from the clinical trial.
Aims: We aimed to investigate the effect of bemcentinib on the Axl signaling pathway and on clonal evolution in treated patients.
Methods: The dose escalation part of the study evaluating bemcentinib monotherapy followed a standard 3+3 design. PB sampled at frequent intervals was analyzed by flow- and mass cytometry to investigate the effects of bemcentinib treatment on signaling proteins known to be downstream of Axl, such as pPLCγ, pAkt and pErk. Sequential BM samples were also analyzed by mass cytometry for extensive single cell immune profiling using a 35 marker panel. Clonal evolution was analyzed by TruSight myeloid panel (Illumina) sequencing of sequential BM samples.
Results: PhosphoFlow and CyTOF analyses of PB showed altered signaling in circulating leukemic blasts relative to pre-treatment in several proteins downstream of Axl, including pPLCγ1, pErk and pAkt. Changes in signaling relative to pre-treatment were seen within 4-24 hours of treatment start in all patients. The time point of signaling response correlated with drug exposure. Signaling responses were heterogeneous in the cohort examined. Two distinct patient groups were identified; one with high and one with low basal signal profiles in PB leukemic blasts pre-treatment. Patients with high basal signaling profiles tended to have reduced signaling with treatment, whereas patients with low basal profiles showed increased signaling with treatment. However, correlation to treatment response was not apparent. TruSight myeloid panel sequencing of sequential BM samples from 5 patients showed little or no clonal drift during treatment (median treatment time 5,5 mo, min 3 mo, max 27 mo), indicating that the clonal composition of the leukemic blast populations could be conserved during treatment. Deep single cell immune profiling of BM samples from 7 patients by mass cytometry revealed the presence of blast populations with composite immune phenotypes in all patients, with moderate drift during treatment. Blast immune phenotypic composition was more complex than what was reflected by the clonal composition. The mutational profiles will be validated via using exome sequencing.
Summary/Conclusion: Bemcentinib has unique pharmacodynamic properties, and signaling responses to exposure can be observed in peripheral blood leukemic blasts by phospho-flow and mass cytometry within hours of ingestion of the first treatment dose. Further studies may establish whether single cell signal profiling can discriminate responders from non-responders and provide information about dose-response. Bemcentinib appears to stabilize the clonal distribution in AML and MDS an effect that may be exploited in combination therapy with hypomethylating agents.

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FURTHER EVIDENCE FOR GPR56, BUT NOT CLL-1 (CLEC12A) AS A MARKER FOR LEUKEMIC STEM CELLS IN CD34-POSITIVE ACUTE MYELOID LEUKEMIA

S. Daga[1,2,*], A. Rosenberger[1,2], N. Krisper[1,2], B. Prietl[2], A. Reinisch[1], A. Zebisch[1], H. Sill[1], A. Wölfler[1,2]

[1]Division of Hematology, Medical University of Graz, [2]CBmed Center of Biomarker Research in Medicine, Graz, Austria

Background: Acute myeloid leukemia (AML) is characterized by a hierarchical cellular organization with a minor fraction of self-renewing leukemia stem cells (LSCs) at the apex of this hierarchy. The clinical importance of LSCs has been implied not only in leukemia initiation, but also in relapse

and drug resistance. Within CD34-positive leukemias, which comprise about three quarters of all AMLs, LSCs have been shown to predominantly reside in the $CD34^+38^-$ cell fraction. Several surface markers including GPR56 and CLL-1 have been reported to be up-regulated on $CD34^+38^-$ LSCs or to mark AML cells with high repopulating activity in immunocompromised mice. However, the immunophenotype of LSCs as well as their prognostic value still remain controversial.

Aims: In this study, we aimed for a detailed analysis of putative LSC markers using multi-color flow cytometry with a strictly standardized protocol and a focus on cellular compartments of AML specimens defined by their CD34/38 expression patterns. In addition, expression of distinct surface markers was correlated with expression of LSC-associated genes as well as with outcome of patients after intensive therapy.

Methods: Bone marrow or peripheral blood samples from 150 newly diagnosed AML patients were analyzed for 24 surface markers including several putative LSC markers using flow cytometry with a strictly standardized protocol including distinct backbone markers per tube allowing simultaneous expression analysis of all markers. Expression of 17 genes associated with leukemic stemness (LSC17 profile; Ng *et al.*, Nature 2016;540:433-437) was determined by qPCR after sorting CD34+38-AML cells according to their GPR56 and CLL-1 surface expression levels. Differences in fluorescence intensity as well as gene expression between specimens and/or sub-populations were calculated using the nonparametric Mann-Whitney U or Wilcoxon test. Overall survival (OS) was estimated using the Kaplan-Meier method.

Results: GPR56 and CLL-1 proved to be differentially expressed. While GPR56 exhibited an elevated expression in CD34-positive AML specimens (n=108), CLL-1 expression was higher in CD34-negative AMLs (n=42). Within CD34-positive AML samples, GPR56 levels were highest in the $CD34^+38^-$ LSC-containing subpopulation and lowest in the $CD34^-$ compartment ($p<0.001$). In contrast, CLL-1 was significantly up-regulated in the more mature $CD34^+38^+$ and $CD34^-$ leukemic cells as compared to the $CD34^+CD38^-$ compartment ($p<0.01$). All other markers tested were not differentially expressed among CD34/38 leukemic subpopulations. To determine whether GPR56 or CLL-1 marks cells with an LSC-associated gene expression signature, we sorted $CD34^+38^-$ leukemic cells according to their GPR56 as well as CLL-1 surface levels and determined the expression of genes included in the LSC17 panel. While nine out of 17 genes were significantly up-regulated in $GPR56^{hi}$ versus $GPR56^{lo}$ $CD34^+38^-$ leukemic cells, none of the genes was higher expressed in CLL-1^{hi} as compared to CLL-1^{lo} cells. Finally, in a cohort of 102 AML patients receiving intensive chemotherapy high GPR56 surface expression at diagnosis was associated with significantly lower OS ($p=0.0325$), while expression of CLL1 did not show prognostic significance.

Summary/Conclusion: Our data indicate that GPR56 may serve as a potential prognostic marker, but also as a promising marker for LSC activity in CD34-positive AML. In contrast, CLL-1 has limited potential as LSC marker in AML.

PS967

MOLECULAR ABNORMALITIES IN THERAPY-RELATED ACUTE MYELOID LEUKEMIAS

G. Balatzenko[1,*], B. Spassov[2], M. Jagurinoski[3], M. Stoychov[2], S. Ivanova[1], V. Madzharova[3], T. Dikov[3], Y. Petrov[4], M. Donchev[2], P. Ganeva[4], G. Arnaudov[4], K. Ignatova[2], M. Guenova[3]

[1]Laboratory of Cytogenetics and Molecular Biology, [2]Department of Clinical Hematology, [3]Laboratory of Hematopathology and Immunology, [4]Department of Hematopoietic Stem Cells Transplantation, National Specialized Hospital for Active Treatment of Hematological Diseases, Sofia, Bulgaria

Background: Therapy-related acute myeloid leukemias (t-AML) are one of the most serious long-term complications of anti-cancer treatment. These neoplasms are considered to be the direct consequence of genetic abnormalities induced by chemotherapy and/or radiotherapy. In contrast to *de novo* (dn) AML, t-AMLs are associated with resistance to standard chemotherapy and poorer overall prognosis. Despite that t-AMLs are a relatively frequent subtype of AML, accounting for approximately 10% of the cases, the molecular events, associated with the pathogenesis and unfavourable prognosis of t-AML are still poorly defined.

Aims: To analyze the spectrum of molecular abnormalities in adult patients (pts) with t-AML.

Methods: Over a 15 years period, 582 AML pts were tested for leukemia-associated abnormalities by RT-PCR. Among these, 52 (9.2%) pts (25 females; 27 males; at a median age of 60 years, range 22 - 83 years) had a history of antecedent chemotherapy/radiotherapy. The panel of routinely screened by RT-PCR molecular abnormalities in all pts included fusion transcripts: *BCR-ABL1, PML-RARA, RUNX1-RUNX1T1, CBFb-MYH11*; and *FLT3*-ITD. In addition, several other molecular abnormalities with a proven or suggestive role in malignant transformation were also tested in a significant proportion of pts, including: *KMT2A-MLLT3, KMT2A -MLLT4, DEK-NUP214, KMT2A*-PTD gene; aberrant overexpression of *Survivin, EVI1, BAALC, PRAME, MDR1* genes, and type A mutation of *NPM1* gene.

Results: In 15/52 (29%) pts with t-AML, recurrent fusion mRNAs were identified, as follows: 5 *RUNX1-RUNX1T1* (9.6%); 4 *CBFb-MYH11* (7.7%); 4 *PML-RARA* (7.7%); and 3 *KMT2A-MLLT3* (3.8%) . The incidence of the same abnormalities in dnAML was similar: 24/530 *RUNX1-RUNX1T1* (4.5%) ; 21/530 *CBFb-MYH11* (3.9%); 35/530 *PML-RARA* (6.6%); 4/235 *KMT2A-MLLT3* (1.7%). No significant differences were also found in regard to the incidence of *NPM1*-A mutation in t-AML and dnAML: 4/30 (13.3%) vs. 27/129 (20.9%). The frequency of *KMT2A*-PTD was only slightly higher in t-AML compared to dnAML: 4/27 (14.8%) vs. 10/228 (4.6%) [p=0.054]. In contrast, the incidence of *FLT3*-ITD was significantly lower in t-AML - 4/48 (8.3%) vs. in dnAML - 109/429 (25.4%) [p=0.038], while the frequency of *EVI1* gene overexpression was higher in t-AML compared to dnAML - 9/31 (29.0%) vs. 8/77 (10.4%) [p=0.037]. Results from an extended panel testing in 32 pts revealed 3 distinctive patterns of the molecular abnormalities: (i) 31.2% of pts were characterized by "favourable" fusion transcripts *PML-RARA*, *CBFb-MYH11*, or *RUNX1-RUNX1T1*, generally without any other molecular abnormalities, with the exception of *PRAME* gene overexpression in half of the cases; (ii) 25.0% of pts were characterized by the presence of unfavourable high *BAALC* and/or *EVI1* gene expression, generally in association with other unfavourable molecular abnormalities, such as *KMT2A*-PTD and *Survivin* gene overexpression; (iii) in the remaining 43.8% of pts no characteristic molecular profile was detected, with the exception of the elevated *Survivin* expression in about half of the cases (6/14). *MDR1* overexpression was found in 62.5% of pts, being the most common abnormality in t-AML. None of the pts was positive for *BCR-ABL1*, *KMT2A-MLLT4*, and *DEK-NUP214* mRNA.

Summary/Conclusion: t-AML pts are heterogeneous in regard to the spectrum of genetic abnormalities and in general, 3 groups of t-AMLs pts with a distinctive pattern of molecular abnormalities could be distinguished: with "favourable" fusion transcripts; with a high incidence of unfavourable *BAALC* and/or *EVI1* gene overexpression, and with no characteristic molecular profile.

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CD123 REDIRECTED NK92 CELLS FOR ACUTE MYELOID LEUKEMIA

L. Zhou[1,2,*], C. Zheng[2], X. Song[1]

[1]Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, United States, [2]Hematology, The First Affiliated Hospital of University of Science and Technology of China, Anhui provincial hospital, Hefei, China

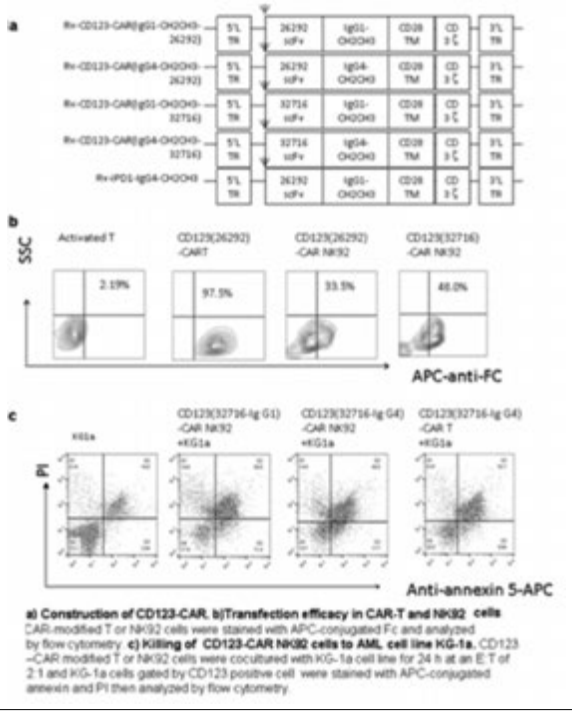
Background: Hematopoietic stem cell transplantation (HSCT) has been increasingly used as a curative treatment for acute myeloid leukemia (AML). However, relapse rates after HSCT in complete remission (CR) are reported between 30% and 70%. Currently, chimeric antigen receptor (CAR)-based T cells have been approved to able to treat ALL and lymphoma effectively in clinical studies. However, there are concerns that CAR-T therapy may induce severe cytokine release storm. By contrast, Natural killer (NK) cell-based cellular therapies have shown safety advantages in clinical studies, because of lack of in vivo clonal expansion and cytokine storm. Thus it would be desirable to develop CAR-NK cells targeting AML relapse. The human NK cell line, NK-92, that expresses a high affinity variant of the IgG Fc receptor (FcγrIII), has been demonstrated to kill leukemia primary blasts and leukemia cell lines *in vitro* and in clinical studies.hIgG1-CAR engineered NK92 cells, are likewise activated by FcγR binding resulting in cytokine secretion (cytokine storm) and lysis of monocytes and NK cells. To reduce the unwanted "off-target" effect, we designed a new CD123-CAR with human IgG4 CH2CH3 spacer domain without affecting CAR expressing and antigen binding.

Aims: To produce CD123-CAR-NK92 cells using NK92 cells engineered to express CAR for a specific AML cell-surface antigen CD123. Then to study the CD123-CAR-NK92 cell's specificity against CD123-positive AML cell lines *in vitro*.

Methods: We developed chimeric antigen receptors (CARs) containing a single polypeptide chain with an CD123-specific antibody-derived single chain fragment (scFv) and human IgG1 or IgG4 hinge-CH2CH3 spacer domain in the extracellular moiety for binding and combined CD28–CD3ζ signaling domain in the intracellular moiety for NK92 cells(hIgG1-CD123-CAR or hIgG4-CD123-CAR). We produced retrovial vector expressing hIgG1-CD123-CAR or hIgG4-CD123-CAR and retrovirus were transduced to NK92 cells. hIgG1-CD123-CAR or hIgG4-CD123-CAR engineered

NK92 cells were then co-cultured with CD123-positive AML cell lines including MOLM13, KG-1a. CD123-negative CML cell line K562 was used as negative control. The cytotoxicity of CD123-CAR NK92 was compared *in vitro* using flow cytometry.

Results: We successfully produced CD123-CAR-NK92 using NK92 cells engineered to express CAR for a specific AML cell-surface antigen CD123. We demonstrated that CD123-CAR-NK92 retained the specificity against CD123-positive AML cell lines MOLM13 and KG-1a *in vitro* (Figure 1).



a) Construction of CD123-CAR. b)Transfection efficacy in CAR-T and NK92 cells CAR-modified T or NK92 cells were stained with APC-conjugated Fc and analyzed by flow cytometry. **c) Killing of CD123-CAR NK92 cells to AML cell line KG-1a.** CD123 –CAR modified T or NK92 cells were cocultured with KG- 1a cell line for 24 h at an E:T of 2:1 and KG- 1a cells gated by CD123 positive cell were stained with APC-conjugated annexin and PI then analyzed by flow cytometry.

Figure 1.

Summary/Conclusion: Our results suggested that CD123-CAR-NK92 might be a valuable candidate to simultaneously prevent or treat relapse in AML HSCT recipients.

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THE DIFFERENTIAL EXPRESSION OF DNMT3A SPLICE VARIANTS AND THEIR PROGNOSTIC RELEVANCE IN ACUTE MYELOID LEUKEMIA

S. Beinicke[1,*], M. Jentzsch[1], J. Grimm[1], J. Häntschel[1], M. Bill[1], K. Goldmann[1], J. Schulz[1], M. Cross[1], G.-N. Franke[1], G. Behre[1], V. Vucinic[1], W. Pönisch[1], D. Niederwieser[1], S. Schwind[1]

[1]Hematology and Oncology, University Leipzig, Leipzig, Germany

Background: Acute myeloid leukemia (AML) is associated with aberrant DNA methylation patterns. DNA methyltransferase 3A (DNMT3A) catalyzes DNA methylation & is frequently mutated in AML. Five *DNMT3A* splice variants with the same catalytic domain & different N-terminus - important for DNA binding – are known. Different *DNMT3A* splice variants may lead to different DNA methylation patterns & influence the AML phenotype & outcome of AML patients (pts). Here we assessed the diagnostic expression of the two main *DNMT3A* splice variants *DNMT3A1* & *DNMT3A2* in AML pts.

Aims: To assess the *DNMT3A1* & *DNMT3A2* expression & analyze associations with clinical & molecular features & evaluate the prognostic relevance in AML pts.

Methods: We analyzed 146 pts who received allogeneic hematopoietic stem cell transplantation (HSCT; median age at HSCT 64 years [y]; range 32-76y) in complete remission (CR) or CR with incomplete peripheral recovery (CRi) after intensive chemotherapy. At diagnosis, cytogenetics & the mutation (mut) status of *CEBPA*, *DNMT3A*, *IDH1*, *IDH2*, *NPM1*, presence of *FLT3*-ITD & *EVI1* expression were assessed. At diagnosis, *DNMT3A1*, *DNMT3A2* & *ABL1* expressions were absolutely quantified in diagnostic bone marrow by digital droplet PCR (ddPCR). R's OptimalCutpoint package was used to determine optimal cut-offs according to *DNMT3A1*, *DNMT3A2* & *DNMT3A1*/*DNMT3A2* expression. Median follow up after HSCT for pts alive was 4.7y.

Results: Using ddPCR both main splice variants were detected in all pts, but *DNMT3A1* was higher expressed compared to *DNMT3A2* (p=0.02). No correlation of *DNMT3A1* & *DNMT3A2* expression was found (Pearson correlation 0.28). AML pts with higher expression of *DNMT3A1* or *DNMT3A2* associated with monosomal (*DNMT3A1* p=.02, *DNMT3A2* p=.002), complex (*DNMT3A1* p=.03, *DNMT3A2* p=.05) karyotypes & were less often *IDH2* mutated (*DNMT3A1* p=.07, *DNMT3A2* p=.002) at diagnosis. Pts with high *DNMT3A1* expression had by trend a higher platelet count (p=.07) & lower *EVI1* expression (p=.01). Pts with high *DNMT3A2* expression less often had a normal karyotype (p=.002) & more% bone marrow blasts (p=.02) &% blood blasts (p=.06) by trend & were less likely to be *NPM1* mutated (p=.001) at diagnosis. While pts with high *DNMT3A1* expression had a higher cumulative incidence of relapse (CIR, p=.05) & shorter overall survival (OS, p=.01), *DNMT3A2* expression did not associate with CIR (p=.36) or OS (p=.88). Combining the expression information for both splice variants pts with a high *DNMT3A1*/*DNMT3A2* expression ratio had lower% bone marrow blasts (p=.002) &% blood blasts (p=.007) at diagnosis. A high *DNMT3A1*/*DNMT3A2* expression ratio associated with higher CIR (p=.028, Figure 1A) & a trend for shorter OS (p=.055, Figure 1B). Using the Bayesian information criterion we found the model containing the *DNMT3A1*/*DNMT3A2* expression ratio to be the preferred model compared to a model with the *DNMT3A1* expression information. In multivariate analysis, a high *DNMT3A1*/*DNMT3A2* ratio was an independent factor for a higher CIR (Hazard ratio [HR] 2.09, Confidence Interval [CI] 1.0-4.25, p=.04).

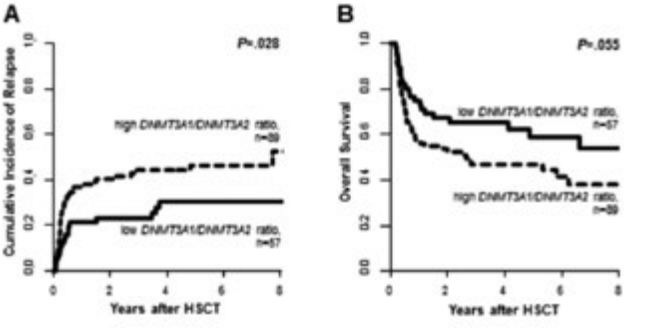

Figure 1.

Summary/Conclusion: In AML pts the two main *DNMT3A* splice variants are expressed at different levels. The differential expression of *DNMT3A* splice variants & their ratio may lead to aberrant DNA methylation patterns & specific AML phenotypes. Expression levels of *DNMT3A1* & *DNMT3A2* associate with distinct clinical & biological features. Especially a high *DNMT3A1*/*DNMT3A2* expression ratio was identified as a negative prognostic factor that independently associated with a higher CIR in AML pts.

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P53(D281G) MUTATION CAUSES CYTARABINE RESISTANCE VIA PROMOTING WARBURG EFFECT IN ACUTE MYELOID LEUKEMIA CELLS

K. M. Ng[1,*], C.-Y. Hu[1], H.-A. Hou[2], C.-Y. Chen[2], D.-L. Ou[3], H.-F. Tien[2], L.-I. Lin[1]

[1]Clinical Laboratory Sciences And Medical Biotechnology, National Taiwan University, [2]Internal Medicine, National Taiwan University Hospital, [3]Oncology, National Taiwan University, Taipei, Taiwan, Republic of China

Background: It is widely accepted that cancer cells tend to "ferment" glucose into lactate even in the presence of sufficient oxygen to support mitochondrial oxidative phosphorylation, so-called Warburg effect. However, the mechanistic links between p53 mutation-mediated Warburg effect and drug resistance in human acute myeloid leukemia (AML) remains to be established. We established a cytarabine-resistant human leukemic MV4-11 derivative (MV4-11-R) from parental MV4-11 (MV4-11-P) cells. The IC_{50} of cytarabine in resultant in MV4-11-R cells increases from 0.2 μM in MV4-11-P cells to 340.2 μM. Molecule alterations in several signaling pathways have been characterized, including p53. Exon sequencing and pyrosequencer analysis revealed that there were similar p53 R248W mutation burdens in MV4-11-P and -R cells; but D281G mutation increased from 1% in MV4-11-P cells to 41% in MV4-11-R cells.

Aims: To investigate the extent of D281G p53-associated metabolic alterations contribute to the cytarabine resistance in MV4-11-R cells.

Methods: The differential expression genes (DEGs) were examined using

RNA-Seq (Illumina NextSeq-500). Alterations of metabolites were profiled by using Liquid Chromatography-Time of Flight Mass Spectrometry (LC-TOF-MS) coupled with True ion pick (TIPick) algorithm in MV4-11-P and -R cells. Metascape resource and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analyses were performed to predict the biological functions of DEGs. Real-time quantitative PCR (RT-qPCR) were used to validate the RNA-seq results. To assess the effect of p53 D281G mutation on glycolysis and mitochondria respiration, the extracelluar acidification rate (ECAR) and oxygen consumption rate (OCR) were measured using Seahorse bio-analyzer.

Results: We identified a total of 869 DEGs between the MV4-11-P and -R cells. Among them, 210 DEGs were up-regulated, while 659 DEGs were down-regulated. Metascape enrichment analyses revealed the highest ranked pathway associated with down-regulated DEGs was p53-signaling pathway in MV4-11-R cells, in line with vanished wt p53 and decreased p21, a transcriptional target of wt p53. Moreover, KEGG mapper analyses showed that the highest ranked up-regulated DEGs are *ASNS, ASS1, CBS, GOT1, PSPH, PHGDH, UPB1, ALDOC, ARG2, SLC27A5, PTS, ME1, CYP27B1, PCK2, COQ3, and DGAT2*, relevant to the metabolic pathways. Metabolomic analyses revealed a total number of 56 increased and 25 decreased metabolites in MV4-11-R, compared to MV4-11-P, cells. Of them, three metabolites: malate (with 1.94x increase), glutamate (with 56410x increase) and serine (with 12083x increase) were the corresponding reaction product or intermediate of ME1, GOT1 and PSPH/PHGDH, respectively. Furthermore, MV4-11-R cells showed higher glucose uptake, GAPDH activity, lactate production and ATP content than MV4-11-P cells. Consistent with increased lactate production, ECAR analyses showed a significant increase in glycolysis, glycolytic capacity and glycolytic reserve in MV4-11-R cells. In addition, we showed increased basal mitochondrial and ATP-coupled respiration in MV4-11-R, compared to MV4-11-P, cells by OCR analyses. Lastly, decreased OCR/ECAR ratio indicated the relatively higher reliance on glycolysis in MV4-11-R cells.

Summary/Conclusion: In this study, transcriptomic and metabolomic profiles of cytarabine-sensitive and -resistant MV4-11 myeloid leukemia cells were analyzed. Our investigation highlights that the emerged p53 D281G mutation may contribute to the cytarabine-resistance of MV4-11-R cells via promoting Warburg effect.

PS971

SINGLE-CELL DNA ANALYSIS OF MUTATIONAL HETEROGENEITY IN ACUTE MYELOID LEUKEMIA TUMORS WITH HIGH-THROUGHPUT DROPLET MICROFLUIDICS

D. Eastburn[1,*], M. Pellegrino[1], A. Sciambi[1], S. Treusch[1], L. Xu[2], R. Durruthy-Durruthy[1], K. Gokhale[1], J. Jacob[1], T. Chen[1], W. Oldham[1], J. Matthews[3], H. Kantarjian[3], A. Futreal[3], K. Patel[3], K. Jones[1], J. Zehnder[2], K. Takahashi[3]

[1]Mission Bio, Inc., South San Francisco, [2]Stanford School of Medicine, Stanford, [3]UT MD Anderson Cancer Center, Houston, United States

Background: Current tumor sequencing paradigms are inadequate to fully characterize many instances of AML (acute myeloid leukemia). A major challenge has been the unambiguous identification of potentially rare and genetically heterogeneous neoplastic cell populations, with subclones capable of critically impacting tumor evolution and the acquisition of therapeutic resistance. Standard bulk population sequencing is often unable to identify rare alleles or definitively determine whether mutations co-occur within the same cell. Single-cell sequencing has the potential to address these key issues and transform our ability to accurately characterize clonal heterogeneity in AML

Aims: Our goal was to remove existing barriers to performing high-throughput single-cell DNA sequencing and make possible the routine characterization of genetic diversity within heterogeneous cancer cell populations. Insights provided by this type of analysis could ultimately lead to better patient stratification, disease monitoring and therapy selection.

Methods: We developed a novel multi-step microfluidic droplet workflow that enables efficient and massively-parallel single-cell PCR-based genomic barcoding for single-cell DNA sequencing applications. We demonstrate that the multi-step microfluidic approach is required for robust DNA amplification on thousands of individual cells per run with high coverage uniformity and low allelic dropout of targeted genomic loci. To apply our single-cell sequencing technology to human tumor samples, we developed a targeted panel to partially sequence 26 genes frequently mutated in acute myeloid leukemia (AML) including *TP53, DNMT3A, FLT3, NPM1, NRAS, IDH1* and *IDH2*.

Results: Using this panel, we were able to sensitively identify SNV and indel-defined clones within AML samples and assess their distribution at the time of diagnosis, remission and relapse. In total, more than 35,000 cells were successfully genotyped for variant alleles likely to play a role in the progression of the disease. Significant clonal remodeling with both expansion and contraction of clones was seen when comparing the diagnosis and relapse samples in some patients. Additionally, our approach was able to detect rare clones during complete remission (CR) and identified clonal populations that were missed with bulk sequencing inference. We also used single cell SNVs to monitor host and donor cell populations during bone marrow transplantation (BMT), which allowed us to accurately evaluate engraftment and disease relapse.

Summary/Conclusion: Collectively, our single-cell data indicates that clonal populations inferred from VAFs obtained from bulk sequencing data may not fully resolve the heterogeneity within tumors; moreover, the single-cell nature of our approach enabled the sensitive and unambiguous identification of multiple co-occurring mutations within subclones that is not possible with bulk measurements. Our results also show a greater degree of heterogeneity in AML tumor samples than is commonly appreciated with traditional sequencing paradigms and demonstrate the value of single-cell analysis for AML. Additionally, our single-cell method can be applied to build custom panels of interest and to solid tumor specimens.

PS972

CANDIDATE FDA-APPROVED DRUGS THAT MAY MIMIC HOXA CLUSTER DELETION IN MLL-AF9 LEUKEMIA

L. Kettyle[1,*], C.-É. Lebert-Ghali[2], J. Bijl[3], D. Simpson[4], K. Mills[1], G. Sauvageau[5], A. Thompson[16]

[1]CCRCB, Queen's University Belfast, Belfast, United Kingdom, [2]Division of Hematology, Maisonneuve-Rosemont Hospital Research Center, Montreal, QC, Canada, [3]Division of Hematology, Maisonneuve-Rosemont Hospital, Montreal, Canada, [4]Centre for Experimental Medicine, Queen's University Belfast, Belfast, United Kingdom, [5]Institute for Research in Immunology and Cancer, University of Montreal, Montreal, Canada, [6]Cancer and Stem Cells, University of Nottingham, Nottingham, United Kingdom

Background: Gene rearrangements involving *MLL*, termed *MLLr*, are among the most potent oncogenic drivers of leukemia. *HOX* genes are well established as downstream targets of MLL and their high expression is a hallmark of high-risk, refractory AML.. Whilst several studies indicate a need for *HOXA* expression in the establishment of MLLr leukemia, their absolute requirement for disease progression and maintenance is less clear. Currently, there are no specific therapies approved for MLLr-AML and identifying target and pathway dependencies and candidate drugs is critical to improving clinical outcome for these patients. The HOXA-axis is a potential target in MLLr-AML if candidate drugs that mimic HOXA repression/deletion can be identified.

Aims: 1. Determine the criticality of *HoxA* to disease maintenance in established leukemia. 2. Identify FDA-approved drugs with the potential to mimic *HoxA* deletion for repurposing in AML

Methods: Transgenic mice: *Hoxaflox/WT* mice were backcrossed to generate *Hoxaflox/flox* mice. These were subsequently crossed into the MxCre background to generate compound *MxCre$^{+/}$ Hoxaflox/flox* mice.

Generation of Leukemia: Haematopoietic stem and progenitor cells enriched from bone marrow were transduced with MLL-AF9 virus by spinoculation. Colonies were grown and serially re-plated in Methocult M3434 prior to being transplanted intravenously into sub-lethally irradiated recipient mice.

Deletion of the Hoxa cluster and analysis: Deletion of the *Hoxa* cluster was achieved *in vitro* by IFNα, *ex vivo* by direct exposure to *Cre*-recombinase and *in vivo* by IP injection of PolyI:C. Treated leukemic cells were subsequently analyzed for cell/colony growth and gene expression by RNAseq. The *Hoxa-del* signature was further analyzed by Ingenuity® Pathway Analysis (IPA) and connectivity mapping software to identify key processes and FDA-approved drugs within the LINCS database.

Results: Aggresssive MLL-AF9 models were generated and homozygous deletion of the *HoxA* cluster was not tolerated by the leukemia cells. A *Hoxa-del* signature including Cxcl12, Tgfb2, Notch4 and Mmp9 was obtained. Analysis of the *Hoxa-del* signature identified cell cycle, polo-like kinase activity, DNA damage regulation, and aryl hydrocarbon receptor signaling as associated processes and canonical pathways. Further bioinformatics analysis using connectivity mapping identified candidate FDA-approved drugs, including Albendazole and Homoharringtonine for potential repurposing in *HOXA* expressing leukemias.

Summary/Conclusion: *Hoxa* deletion, within a MLL-AF9 leukemia background, resulted in reduced proliferation, colony formation and repopulating ability in transplanted mice. Surviving leukemic cells retained at least one copy of the *Hoxa* cluster, indicating dependency, in contrast to the normal hematopoiesis setting in which biallelic deletion of the *Hoxa* locus is

tolerated [Lebert-Ghali *et al.* Blood 2016; 127(1):87-90]. Comparative RNA-seq analysis identified a unique *Hoxa-del* signature, further bioinformatics analysis of which identified a number of FDA-approved drugs for potential repurposing in MLL-AF9 leukaemias.
In conclusion our findings demonstrate, for the first time, dependency on the *Hoxa* locus for the maintenance of MLL-AF9 leukemias and in the absence of direct inhibitors identifies candidate FDA-approved drugs for further pre-clinical validation and redeployment to this highly refractory disease.

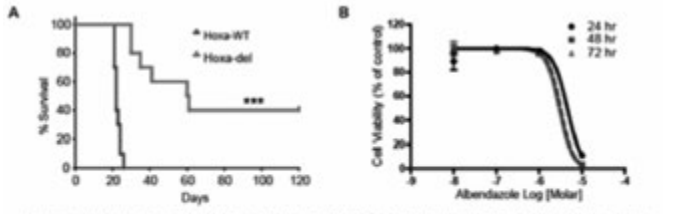


Figure. A Kaplan meier plot demonstrating that deletion of the Hoxa cluster in MLL-AF9 leukemia results in significantly improved survival compared to wild type in transplantation models (A). A dose response curve for the connectivity mapped candidate FDA-approved drug Albendazole shows anti-leukemia properties in MLL-AF9 primary cell lines in the uM range (B).

Figure 1.

PS973

IMPACT OF NPM1 MRD STATUS AFTER TWO CYCLES OF INTENSIVE CHEMOTHERAPY TO INFORM TYPE OF POST-REMISSION THERAPY: A STUDY OF THE AML STUDY GROUP (AMLSG)

S. Kapp-Schwoerer[1,*], A. Corbacioglu[1], D. Weber[1], V.I. Gaidzik[1], F.G. Rücker[1], P. Paschka[1], J. Krönke[1], M.-V. Teleanu[1], G. Göhring[2], B. Schlegelberger[2], W. Fiedler[3], G. Wulf[4], H. R. Salih[5], M. Lübbert[6], T. Kindler[7], K.S. Götze[8], L. Bullinger[9], K. Mayer[10], D. Wolf[10], T. Schroeder[11], H. Salwender[12], E. Koller[13], M. Bentz[14], M. Ringhoffer[14], H.A. Horst[15], J. Krauter[16], R.F. Schlenk[1,17], F. Thol[18], M. Heuser[18], A. Ganser[18], H. Döhner[1], K. Döhner[1]

[1]Department of Internal Medicine III, University Hospital of Ulm, Ulm, [2]Institute of Cell and Molecular Pathology, Hannover Medical School, Hannover, [3]Hubertus Wald University Cancer Center, University Medical Centre Hamburg-Eppendorf, Hamburg, [4]Department of Hematology and Oncology, Georg-August-University Göttingen, Göttingen, [5]Department of Internal Medicine II, University Hospital of Tübingen, Tübingen, [6]Department of Hematology, Oncology and Stem Cell Transplantation, University of Freiburg Medical Center, Freiburg, [7]Department of Internal Medicine II, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, [8]III. Department of Medicine, Hematology and Medical Oncology, Technical University of Munich, Munich, [9]Department of Hematology, Oncology and Tumor Immunology, Campus Virchow Klinikum, Charité University Hospital, Berlin, [10]Department of Hematology, Oncology, University Hospital Bonn, Bonn, [11]Department of Hematology, Oncology, and Clinical Immunology, Heinrich-Heine-University Duesseldorf, Duesseldorf, [12]Department of Hematology, Oncology, Asklepios Hospital Altona, Hamburg, Germany, [13]Department of Internal Medicine III, Hanuschkrankenhaus, Vienna, Austria, [14]Department of Internal Medicine III, Municipal Hospital of Karlsruhe, Karlsruhe, [15]Department of Internal Medicine II, University Hospital Schleswig Holstein, Campus Kiel, Kiel, [16]Department of Internal Medicine III, Municipal Hospital of Braunschweig, Braunschweig, [17]Nationales Centrum für Tumorerkrankungen (NCT) Heidelberg, Heidelberg, [18]Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany

Background: *Nucleophosmin1* mutated (*NPM1*^{mut}) based minimal residual disease (MRD) monitoring allows the early identification of patients (pts) with acute myeloid leukemia (AML) at high risk of relapse. However, its predictive value, in particular with regard to the type of post-remission therapy [high-dose cytarabine, (HIDAC) versus (vs) allogeneic stem cell transplantation, (alloSCT)] has not been investigated yet.
Aims: To exploratory evaluate the predictive value of the *NPM1*^{mut} MRD status after two cycles of treatment with respect to the type of post-remission therapy (HIDAC vs alloSCT) in a large cohort of younger AML pts [18 to 60 years (yrs)] harboring *NPM1*^{mut}. Indication for alloSCT was performed according to institutional algorithms.
Methods: In total, 611 *NPM1*^{mut} pts were enrolled in one of four AMLSG treatment trials. Treatment comprised 1 to 2 cycles of induction therapy followed by HIDAC (n=363, 59%), autologous (n=19, 3%) or alloSCT (n=162, 27%). Overall, 3527 bone marrow (BM) and 2812 peripheral blood (PB) samples were analyzed at diagnosis, during treatment and follow-up (FU). *NPM1*^{mut} transcript levels (ratio of *NPM1*^{mut}/*ABL1* transcripts×10^4) were determined by RQ-PCR with a sensitivity of 10^{-5} to 10^{-6}; the median FU for all pts was 3.2 yrs.
Results: We first compared the outcome of pts (n=63) who achieved RQ-PCR-negativity (RQ-PCR^{neg}) in the BM after two cycles of intensive chemotherapy with respect to the type of post-remission therapy (HIDAC vs alloSCT). Here, 4-yr overall survival (OS) was significantly superior in pts treated with HIDAC (100% vs 46%; p<.00001) compared to pts who received alloSCT; median survival was not reached for pts after HIDAC and was 3.06 yrs in pts after alloSCT. When stratifying pts according to the 2017 European LeukemiaNet (ELN) risk stratification with regard to *FLT3*-ITD mutation (wildtype / allelic ratio < 0.5 vs allelic ratio ≥ 0.5) this survival benefit was sustained. In addition, there was no significant difference in the cumulative incidence of relapse (CIR) at 4 yrs between HIDAC and alloSCT (p=.18). The predictive value of the MRD status was also observed when only PB samples (129 pts) were analyzed. However, the results were slightly different when looking at pts being RQ-PCR-positive (RQ-PCR^{pos}) in the BM after two treatment cycles (n=332). Here we still found a significant difference in OS in favor of the HIDAC group (68% vs 54% at 4 yrs, p=.0001) but when stratifying according to the *FLT3*-ITD status this difference only remained significant in pts with wildtype / allelic ratio < 0.5 while OS in pts with an allelic ratio ≥ 0.5 was equivalent for the two groups (HIDAC 53% vs alloSCT 49% at 4 yrs; p=.89). This was mainly due to a higher 4-yr CIR rate in RQ-PCR^{pos} pts which was 47% in HIDAC pts vs 22% in the alloSCT group (p=.007). In pts with *FLT3*-ITD wildtype / allelic ratio < 0.5 CIR at 4 yrs was 46% for HIDAC pts vs 21% for the alloSCT group (p=.03); alloSCT treatment was also associated with a significantly lower CIR in pts with an allelic ratio ≥ 0.5 (alloSCT 26% vs HIDAC 50%; p=.04). Again, the predictive value of the MRD status could be confirmed when PB (164 pts) samples were analyzed.
Summary/Conclusion: In this retrospective exploratory analysis of *NPM1*^{mut} AML pts, outcome of pts who became MRD negative or remained positive but had a 2017 ELN favorable risk profile was superior after HIDAC consolidation versus alloSCT. Our data provide strong support for the importance of *NPM1*^{mut} MRD monitoring after two cycles of intensive chemotherapy to inform type of post-remission therapy.

PS974

MUTATIONAL LANDSCAPE OF RELAPSED ACUTE PROMYELOCYTIC LEUKEMIA

L. Iaccarino[1,*], T. Ottone[1], V. Alfonso[1], L. Cicconi[1], M. Divona[1], S. Lavorgna[1], A. Ferrantini[1], C. Baer[2], M. Usai[3], F. Forghieri[4], M.T. Voso[1], T. Haferlach[2], F. Lo Coco[1]

[1]Biomedicine and Prevention, University of Tor Vergata, Rome, Italy, [2]Munich Leukemia Laboratory, Munich, Germany, [3]Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, [4]Department of Surgical and Medical Sciences, University of Modena and Reggio Emilia, Modena, Italy

Background: Acute promyelocytic leukemia (APL) is characterized by the unique PML/RARA oncoprotein, whose role in disease pathogenesis and diagnostics is well established. Additional genetic abnormalities have also been detected in APL both at diagnosis and at time of relapse; however, their biologic and clinical significance remains unclear. Recent reports, including one from our group, suggested that mutations in *PML* and *RARA* genes play an important role in development of resistance to arsenic trioxide (ATO) and/or all-*trans* retinoic acid (ATRA).
Aims: We hypothesize that deciphering the molecular mechanisms involved in APL relapse and resistance may be important for the early identification of patients in need of salvage pre-emptive therapy.
Methods: An NGS-based approach was used to analyze a total of 30 APL patients who underwent disease relapse after treatment (15 following ATRA-ATO, and 15 after ATRA-chemotherapy). The NGS assay included both a 31-target gene panel and a customized assay to specifically identify mutations in *PML* and *RARA*. Twenty one patients in continuous complete remission (CCR) at a median follow-up of 42 months (range 12-85), were analysed as controls.
Results: A high prevalence of mutations in *PML* and *RARA* genes was found in relapse samples (8/30, 27%). Comparing the initial mutation burden of patients who relapsed with those who remained in CCR, we found that the number of concomitant mutations *per* patient was significantly higher in the former group (median n=2 per patient) compared to controls (median n=1 per patient, p=0.02). Moreover, APL patients with multiple relapses after ATRA-ATO showed a significantly higher number of mutations (median n=2 per patient, p=0.04), and an accumulation of mutations during disease progression. In particular, we detected in relapsing patients alterations associated with clonal hematopoiesis such as *ASXL1, DNMT3A, JAK2, SRSF2, TET2*

and mutations in *TP53* (Figure 1A). *PML* and *RARA* mutations were mutually exclusive with *FLT3*-mutations. The identified mutational patterns suggested different models of disease progression. In 8 patients, relapse apparently derived from a mutated subclone present at diagnosis. In particular, we observed the co-occurrence of at least one driver mutation in addition to *PML/RARA,* which was detected both at disease onset and relapse (Figure 1B). In contrast, in 3 patients, relapse probably emerged from *PML*-mutated ATO-resistant subclones likely arising under selective pressure of ATO. During clonal selection, these subclones may acquire mutations in genes, such as *ASXL1, DNMT3A, JAK2, SRSF2, TET2, TP53, WT1*, which confer advantages in self-renewal and proliferation (Figure 1C).

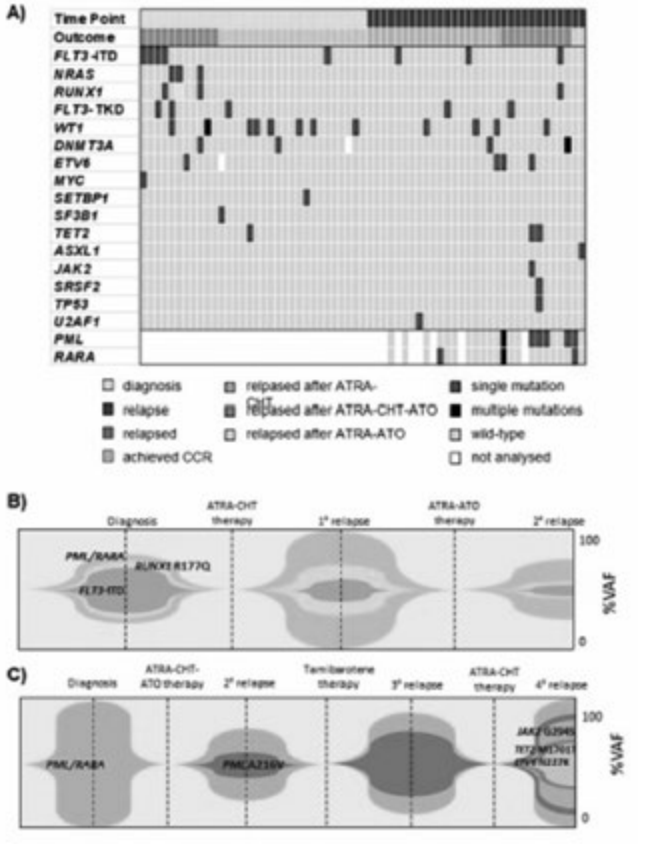


Figure 1.

Summary/Conclusion: The two proposed mechanisms of disease relapse could explain why *PML* and *RARA* mutations in our series were mutually exclusive with driver mutations, such as *FLT3*-ITD and TKD. Deep molecular sequencing at the time of relapse in APL may help to identify patients with a high mutational burden, and/or with PML-mutated subclones resistant to ATO. This may help informing treatment decisions, including the choice anti-CD33 antibodies or allogeneic stem cell transplantation for cases with unfavorable genetic profile.

PS975

THE ROLE OF THE TRANSCRIPTION FACTOR GFI1 IN LEUKEMIA GENOME STABILITY AND EVOLUTION

D. Frank[1,2,*], T. Liehr[3], U. Pascheberg[4], U. Dührsen[1], C. Khandanpour[1,2]

[1]Department of Hematology, University Hospital Essen, Westdeutsches Tumorzentrum, Essen, [2]Medical Clinic A, University Hospital Muenster, Muenster, [3]Institute of Human Genetics, Jena, [4]MVZ Dr. Eberhard & Partner, Dortmund, Germany

Background: Genetic and cytogenetic aberrations are one of the key players in the development of leukemia. Our team is interested to find new pathways with participation of GFI1 which regulate the genetic stability of leukemia. Growth Factor Independence 1 (GFI1) is a transcriptional repressor which regulates the proliferation and differentiation of hematopoietic stem cells (HSCs). We have previously shown that less expression levels of Gfi1 promotes the development of *myelodysplastic syndrome* (MDS) and acute myeloid leukemia (AML) in patients as well as in murine models. Low expression of GFI1 leads to failure to repress efficiently genome wide GFI1 target genes; among them a number of AML related oncogenes. Additional, a single nucleotide polymorphism variant of GFI1, named GFI1-36N (whereby the Serine on position 36 is exchange with an Asparagine) also contributes to the development of MDS and AML by failing to induce certain genome wide epigenetic changes. AML patients which feature a low expression level of GFI1 or presence of GFI1-36N have a poor prognosis. A low expression level and presence of GFI1-36N can be detected in around 30% of all AML patients: Our group reanalyzed the available genomic data of MDS and AML patients with low expression of GFI1 or expression of GFI1-36N. We found that this patient cohort showed significantly more frequent mutation and cytogenetic aberrations (around 50% had a complex karyotype, meaning more than 3 chromosomes were altered) than MDS/AML patients with increased "wild type" level of GFI1-36S. This indicates that low expression level of GFI1 or presence of GFI1-36N are associated with genomic instability.
Aims: Our aim was to determien which role GFI1might play in the regulation of genome stability.
Methods: Using different methods such as comet assay, yH2Ax staining, gene expression analysis and murine models of human AML we examined the role of GFI1 in DNA repair and stability.
Results: . To investigate the DNA repair and DNA damage after irradiation (X-rays) we using different methods such as comet assay and γH2AX assay. We could show that reduced level of GFI1 and presence of GFI1-36N impedes the DNA repair and increase the DNA damage after irradiation. On molecular level, low expression of GFI1 or presence of GFI1-36N is associated with a reduction of the homologous and non-homologous DNA repair capacity. Moreover we found that GFI1 regulates activation of different DNA repair pathways by inducing demethylation and therefore activate PRMT1 or MGMT. On a functional level, we serially transplanted murine AML cells expressing either hGFI1-36N or low level of hGFI1-36S or normal level of hGFI1-36S. After four rounds the leukemic cells with low level of GFI1-36S or presence of GFI1-36N showed a significantly higher number of cytogenetic aberrations and mutation load than the leukemic GFI1-36S control cells. In addition we treated GFI1-36N expressing, low GFI1-36S expressing and GFI1-36S leukemic cells with Temozolomide a drug which affects the MGMT pathway and is used in glioblastoma. We could see that the leukemic cells with low level of GFI1-36S and presence of GFI1-36N are susceptible to low doses of Temozolomide compared to the leukemic GFI1-36S control cells.
Summary/Conclusion: In summary, low expression level of GFI1 as well as expression of GFI1-36N leads to a clonal genetic evolution of about 30% of all human AML cases by impeding DNA-repair and GFI1 could be a new target in leukemia therapy.

PS976

VEGFC ANTIBODY TREATMENT: A NEW DIFFERENTIATION THERAPY FOR AML

K. R. Kampen[1,2,*], F. J. Scherpen[2], H. Mahmud[2], A. Ter Elst[2], A.B. Mulder[3], V. Guryev[4], H. J. Verhagen[5], K. De Keersmaecker[1], L. Smit[5], S.M. Kornblau[6], E. S. de Bont[2]

[1]Laboratory for Disease Mechanisms in Cancer, Oncology, KU leuven, Leuven, Belgium, [2]Pediatric Oncology, [3]Department of Laboratory Medicine, [4]European Research Institute for the Biology of Ageing, University Medical Center Groningen, Groningen, [5]Department of Hematology, VU University Medical Center, Cancer Center Amsterdam, Amsterdam, Netherlands, [6]Department of Leukemia , M.D. Anderson Cancer, Houston, Texas, United States

Background: High VEGFC levels were identified as an independent prognostic factor in AML and associated with decreased complete remission rates and a reduced survival. Exogenous VEGFC can protect AML cells from chemotherapy induced apoptosis. We previously showed that endogenous VEGFC expression is associated with decreased drug responsiveness in childhood AML.
Aims: We hypothesized that VEGFC is an important autocrine growth factor involved in CD34+ AML blast maintenance.
Methods: Reverse Phase Protein Array analysis. Immunoblot analysis. Genetic shRNA interference. Quantitative RT-PCR. NSG systemic AML PDX xenografts. Immunohistochemistry. Flow cytometry.
Results: The VEGFC/KDR axis was shown to be selectively expressed by AML blasts. The CD34+ and CD34- cell populations within primary AML patient samples expressed significantly higher levels of VEGFC as compared to NBM controls and associated with elevated KDR membrane protein expression levels, while FLT-4 membrane protein expression was absent. These findings, challenged us to explorer VEGFC targeting effects on AML cell functions by using monoclonal antibody treatment (30 µg/mL). VEGFC targeting antibody treatment reduced the expansion potential of primary CD34+ AML

blasts by 25-50% reduction in short-term and long-term colony forming cell assays and especially in clonal limiting dilution assays. Interestingly, VEGFC antibody treated induced a significant 3-fold enahnced differentiation along the myelocytic lineage. Differentiation marker analysis confirmed that increasing percentages of cells stained positive for CD38, CD11b and CD14 or CD15, while CD34 percentages were decreased. In addition, VEGFC antibody treated cultures presented increased percentages of apoptotic cells. FLT3-ITD fragment analysis showed identical ratios of heterozygous FLT3-ITD mutant cells in control and VEGFC antibody treated cultures, supporting that anti-VEGFC treatment affects the leukemic clone. Proteomics analysis revealed that VEGFC antibody treatment reduced proliferative signals via inhibiting the phosphorylation of Erk and STAT5. The VEGFC antibody treatment induced differentiation phenotype was shown to be controlled by FOXO3A suppression, as FOXO3A overexpression could repress the VEGFC antibody treatment induced differentiation. *In vivo* targeting of VEGFC in an EVI1 ASXL1 AML PDX model is characterized by reduced circulating white blood cell counts, decreased splenic AML blast infiltration, and induced myelomonocytic differentiation in the bone marrow (Figure 1).

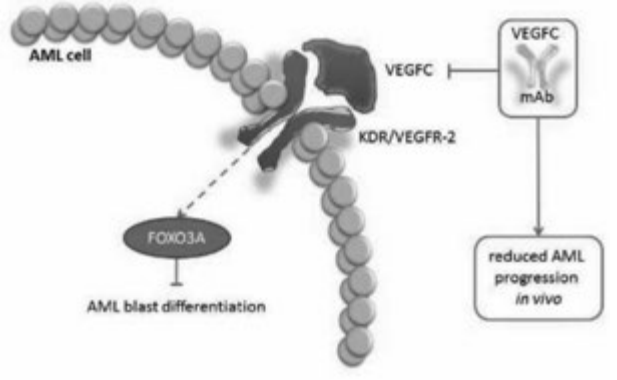


Figure 1.

Summary/Conclusion: This study shows that VEGFC antibody therapy suppresses the AML progression *in vivo* via enforced myelocytic differentiation.

Acute myeloid leukemia – Clinical

PS977

EVALUATION OF THE DRUG INTERACTION POTENTIAL OF PRACINOSTAT, A NOVEL HISTONE DEACETYLASE (HDAC) INHIBITOR, WITH CYP1A2 INDUCER CIGARETTE SMOKING AND CIPROFLOXACIN, A CYP1A2 INHIBITOR

A. Bernareggi[1,*], O. Moreno[2]

[1]R&D Innovation and Strategy, Helsinn Healthcare, Lugano , Switzerland, [2]Research & Development, MEI Pharma, San Diego, United States

Background: Pracinostat (PRAN) is a novel oral HDAC inhibitor in Phase 3 clinical development for patients with acute myeloid leukemia (AML). *in vitro* metabolism studies indicate that PRAN is metabolized mainly by CYP1A2 and CYP3A4, making it susceptible to drug-drug interactions (DDIs) when co-administered with relevant inhibitors or inducers.
Aims: Two studies were conducted to determine the potential risk for DDIs when PRAN was co-administered with a representative CYP1A2 inducer and inhibitor. As tobacco smoke contains substances, such as polycyclic aromatic hydrocarbons which are potent inducers of CYP1A2, Study 1 evaluated the effect of cigarette smoking on the pharmacokinetics (PK) of PRAN. Study 2 evaluated the effect of ciprofloxacin, a strong CYP1A2 inhibitor, on the PK of PRAN.
Methods: Study 1 was an open-label, single-dose, parallel study in 28 adult healthy males/females (14 non-smokers in Cohort 1 and 14 moderate-to-heavy smokers in Cohort 2). A single 60 mg oral dose of PRAN was administered, with blood samples collected for PK evaluation at specified times through 48 hours post-dose. Study 2 was an open-label, 2-period crossover study in 16 nonsmoking adult healthy males/females, with a 10-day washout period between periods. In Period 1 subjects received a single 60 mg dose of PRAN with PK blood sampling for 48 hours post-dose; in Period 2, subjects received 500 mg ciprofloxacin BID for 7 days with a single dose of PRAN on the morning of Day 4 with PK sampling for 96 hours after the PRAN dose.
Results: In Study 1, peak concentration (C_{max}), and exposure to last measurable concentration (AUC_{0-t}) and to infinity ($AUC_{0-\infty}$) of PRAN were 52%, 57% and 55% lower, respectively, in smokers (Table). In Study 2, PRAN C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and $t_{1/2}$ were comparable following administration of PRAN alone or in combination with ciprofloxacin (Table 1).
A single dose of PRAN, when administered alone in both studies or in combination with ciprofloxacin in Study 2 was well-tolerated.

Table 1.

PRAN PK Parameters	Study 1, Means (SD)		Study 2, Means (SD)	
	Non-Smokers	Smokers	PRAN	PRAN + Ciprofloxacin
C_{max} (ng/mL)	108.2 (32.2)	51.6 (18.1)	97.3 (24.8)	97.5 (35.0)
t_{max} (hr) median (min, max)	1.3 (0.99, 1.5)	1.3 (1.0, 2.0)	1.50 (0.99, 2.0)	2.0 (1.5, 3.0)
AUC_{0-t} (ng*hr/mL)	599.5 (198.0)	257.6 (92.5)	623.6 (130.1)	690.1 (148.1)
$AUC_{0-\infty}$ (ng*hr/mL)	609.2 (198.7)	271.5 (87.7)	637.3 (131.5)	702.2 (14.2)
$t_{1/2}$ (hr)	7.6 (1.5)	6.4 (2.0)	9.6 (1.6)	10.5 (2.9)

Summary/Conclusion: The results of Study 1 suggest that exposure to PRAN is reduced in smokers, which may result in reduced benefit to patients who choose to smoke while receiving treatment. In the ongoing Phase 3 AML trial, smokers are excluded. There was no indication of DDI when PRAN and ciprofloxacin, a strong CYP1A2 inhibitor, were co-administered.

PS978

SINGLE CENTRE REAL-LIFE EXPERIENCE OF HYPOMETHYLATING AGENTS WITH OR WITHOUT DLI AS SALVAGE THERAPY FOR ACUTE MYELOID LEUKEMIA RELAPSING AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

D. Lazzarotto[1,*], A. Candoni[1], C. Filì[1], M.E. Zannier[1], M.V. Dubbini[1], N. Rabassi[1], A. Sperotto[1], M. Cerno[1], A. Geromin[1], M. Battista[1], F. Patriarca[1], R. Fanin[1]

[1]Division of Hematology and Stem Cell Transplantation, Azienda Sanitaria Universitaria Integrata di Udine, Udine, Italy

Background: Acute Myeloid Leukemia (AML) relapsing after allogeneic stem cell transplantation (Allo-SCT) carries a very poor prognosis and there are very few therapeutic options outside clinical trials, even nowadays.
Aims: We wanted to analyze the outcome of patients with AML relapsing after Allo-SCT and treated with hypomethylating agents (HMA) with or without Donor Lymphocytes Infusions (DLI).
Methods: We report the outcome of 19 patients (pts) with AML relapsed after Allo-SCT (89% with cytologic relapse) and treated with HMA, either Decitabine or Azacytidine, with or without DLI.
Results: Eleven cases (58%) had an unfavorable karyotype at diagnosis and 2 (11%) cases were FLT3-ITD positive. Median age was 51 years (range 24–66), 12 were male and 7 were female; 4/19 pts underwent Allo-SCT from an HLA-identical sibling donor, 13 from a Matched Unrelated donor (MUD) and 2 from an Haploidentical donor. The median time from SCT to relapse was 6,9 months (range 2,2–72,5), 17/19 pts had a cytologic relapse (with a bone marrow average blast count of 40%), while 2/19 had a molecular relapse. Twelve pts (63%) received salvage therapy with Azacytidine while 7 (37%) received Decitabine. Nine cases (47%) received concomitant HMA and Donor Lymphocytes Infusions (DLI) according to DLI availability. Pts received a median of 2 cycles of HMA therapy (range 1-9) and a median of 3 DLI doses (range 1-9) with a median dosage of $3x10^6$/kg (range 0,05 $x10^6$/kg – 35 $x10^6$/kg). After a median follow-up of 3,1 months (range 1-38) from the beginning of the HMA therapy, 14 (74%) pts died, of these 10 (71%) due to AML and 4 (29%) due to TRM. Five pts (26%) are alive, of these 3 (60%) in complete remission (CR) and 2 with active, but stable, disease. One pts received a second Allo-SCT procedure and maintains a CR status. No pt experienced a GvHD > grade I after DLI. The median OS of this population was 6,7 months with a 1-year OS probability of only 18%. We observed a significant survival improvement in pts that received concomitant DLI (median OS 9,3 months vs 2,2 months, p=0,05) and in pts that achieved a CR with HMA (median OS 12,3 months vs 2,9 months, p=0,01). The concomitant use of DLI was the only variable to retain statistical significance in multivariable analysis (p=0,03). Notably, the type of HMA and the timing of relapse from the previous Allo-SCT did not influence OS.
Summary/Conclusion: Our real-life experience suggests that salvage therapy with HMA is feasible and safe. We observe a clear OS benefit in pts who received a combination of HMA and DLI suggesting a synergistic effect. Prospective studies are needed to explore the benefit of HMA combinations with DLI and/or new drugs for AML relapsing after Allo-SCT. Given the unfavorable probability of OS at 1-year of this population (18%) we strongly suggest using this therapeutic approach early after Allo-SCT as a preemptive strategy to prevent cytologic relapse.

PS979

CONSOLIDATION CHEMOTHERAPY PREVENTS RELAPSE BY INDIRECTLY REGULATING BONE MARROW ADIPOGENESIS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

H. Liu[1,*], Y. Zhai[2], W. Zhao[3], J. Shi[4], Z. Li[5]

[1]Hematology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, [2]Hematology , Shanghai Jiao Tong University School of Medicine Affiliated Tongren Hospital, [3]Hematology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital South Campus, [4]Hematology, [5]Blood Transfusion, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China

Background: Consolidation chemotherapy is still the mandatory strategy to prevent relapse of acute myeloid leukaemia (AML) during complete remission (CR), including those patients acquiring molecular level elimination of leukemia cells. This implicates that an working mechanism may exist in addition to killing residual leukemia cells using chemotherapy.
Aims: As the most abundant stromal component in bone marrow (BM), marrow adipocytes have been previously identified to be the promoter in leukemogenesis. We hypothesized that consolidation chemotherapy may regulate adipogenesis to maintain remission.
Methods: A retrospective study of BM biopsies from 80 patients with AML in remission and 71 control subjects was applied to quantitatively analyse marrow adipocyte volume. Toxicity tests were used to assess the effect of chemotherapy drugs on BM cells. The possible mechanisms by which chemotherapy regulated the reduced marrow adipocyte content were investigated using antibody neutralization experiments, with an emphasis on growth differentiation factor 15 (GDF15), and a correlation analysis was performed to assess the relationship between the GDF15 level and the adipocyte volume in AML-CR patients.
Results: Our retrospective analysis indicated the marrow adipocyte volume in AML patients after consolidation chematherapy was significantly lower than that in the controls ($p<0.001$). Moreover, the patients with an adipocyte content lower than 21.5% exhibited a longer relapse-free survival ($p<0.001$). However, the chemotherapeutic drug cytarabine was neither sufficient to kill the mescenchymal stem cells (MSCs) or MSCs-derived adipocytes, nor was it directly inhibit the adipogenic capability of MSCs *in vitro*. Instead, the condition medium from cytarabine -treated hematopoietic cells (HCs), which was the main ingredient in BM during CR period, partially blocked the adipogenic differentiation of MSC. RNAseq analysis showed that transcription of GDF15 was higher in treated HCs than the controls. The antibody against GDF15 neutralized the effects of cytarabine-treated HCs, whereas rhGDF15 treatment potentiated the effects. Finally, a higher GDF15 level in bone marrow (BM) aspirates was associated with the reduced marrow adipocyte content in AML patients after consolidation chemotherapy, suggesting that GDF15 secreted by chemotherapy-induced HCs plays an important role in adipogenesis.
Summary/Conclusion: chemotherapy indirectly inhibits adipogenesis by promoting secretion of GDF15 from HCs in BM , which strengthens consolidation chemotherapeutic efficacy in AML patients during CR.

PS980

CONTINUING ENASIDENIB TREATMENT FOR PATIENTS WITH MUTANT-IDH2 RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA (R/R AML) WITH STABLE DISEASE MAY RESULT IN IMPROVED RESPONSES AND SURVIVAL OVER TIME

E.M. Stein[1,2,*], R.M. Stone[3,4], D.A. Pollyea[5,6], G.J. Roboz[2,7], J.K. Altman[8], C. D. DiNardo[9], S. de Botton[10,11], A. Tosolini[12], N. Tu[12], A.S. Swern[12], J. VanOostendorp[12], S.V. Agresta[13], A.T. Fathi[3,14]

[1]Memorial Sloan Kettering Cancer Center, [2]Weill Cornell Medical College, New York, [3]Harvard Medical School, [4]Dana-Farber Cancer Institute, Boston, [5]University of Colorado School of Medicine, [6]University of Colorado Denver, Aurora, [7]New York Presbyterian Hospital, New York, [8]Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, [9]The University of Texas MD Anderson Cancer Center, Houston, United States, [10]Gustave Roussy, Villejuif, France, [11]Univ Paris Sud, Université Paris-Saclay, Le Kremlin-Bicêtre, [12]Celgene Corporation, Summit, [13]Agios Pharmaceuticals, Inc., Cambridge, [14]Massachusetts General Hospital Cancer Center, Boston, United States

Background: Enasidenib (AG-221) is a small-molecule, oral inhibitor of mutant IDH2 (mIDH2) proteins. The clinical efficacy of enasidenib is derived in part by differentiation of immature leukemic cells. Unlike cytotoxic therapies, lower-intensity therapies, including differentiating agents such as enasidenib, can induce first responses months after treatment (Tx) initiation.
Aims: Investigate efficacy outcomes for patients (pts) with R/R AML in the phase 1/2 AG221-C-001 study (NCT01915498) who maintained stable disease (SD) during early Tx with enasidenib.
Methods: Response and overall survival (OS) were assessed for pts with m*IDH2* R/R AML who received enasidenib 100 mg/day and maintained SD, defined by the European LeukemiaNet (Döhner, *Blood*, 2017) as no formal IWG response and no evidence of progressive disease (PD) during the first 90 days on-study.
Results: Of 214 pts with R/R AML who received enasidenib 100 mg/day, 82 pts (38%) maintained SD during the first 90 days of Tx. As of data cutoff (1-Sep-2017), 25 pts (30%) achieved an IWG-defined response after day 90 ("Late Responders"), including 16 pts who attained complete remission. Twenty-nine pts (35%) retained SD at all subsequent response assessments ("SD Only"), and 28 pts (34%) developed PD after day 90 (Table 1). Median time to response for Late Responders was 121 days (range 91-286). Median Tx duration for Late Responders was 259 days, for SD Only pts was 164 days, and for pts who developed PD after day 90 was 107 days. At baseline, Late Responders were less likely than pts who later developed PD to have received >2 prior anti-cancer Tx (8% *vs* 32%, respectively) or to have poor-risk cytogenetics (8% *vs* 43%). Seven Late Responders (28%) went to transplant. Median OS for Late Responders was 15.1 months (95%CI 10.7, not reached [NR]), for SD Only pts was 9.0 months (7.7, 11.4), and for pts who developed PD after day 90 was 5.8 months (5.4, 8.3); estimated 1-year survival rates were 57.7%, 23.9%, and 8.2%, respectively. Risk of death was significantly reduced in Late Responders by 59% compared with SD Only pts (hazard ratio [HR] 0.41; 95%CI 0.21, 0.82), and by 79% compared with pts who later developed PD (HR 0.21; 0.10, 0.42). Similarly, maintaining SD after day 90 was associated with a significant 51% reduction in risk of death vs early SD followed by PD (HR 0.49; 0.28, 0.87).

Table 1.

Outcomes after day 90 for patients with m*IDH2*-R/R AML receiving enasidenib 100 mg/day with sustained stable disease during the first 90 days of Tx (N=82)	
	n (%)
CR + CRp	20 (24.4)
CR	16 (19.5)
CRp	4 (4.9)
Partial remission	3 (3.7)
Morphologic leukemia-free state	2 (2.4)
Stable disease	29 (35.4)
Progressive disease / not evaluable / unknown	28 (34.1)
CR, Complete remission; CRp, CR with incomplete platelet recovery	

Summary/Conclusion: SD may represent sustained but controlled proliferation of leukemic cells that, in some cases, later differentiate and lead to clinical responses. In the first 90 days of enasidenib Tx, 38% of pts with m*IDH2* R/R AML maintained SD. Of them, almost one-third responded after day 90 during continued Tx. Late Responders had a significant OS benefit compared with pts with no later response. However, pts who maintained SD at all response evaluations received a median of ~5.5 months of enasidenib Tx and had a median OS of 9 months. These data suggest pts who sustain SD during early enasidenib Tx should continue Tx for at least 6 cycles or until PD. SD during early enasidenib therapy does not predict Tx failure, and pts who maintain SD may benefit from continuing enasidenib Tx.

PS981

PH II TRIAL WITH SELECTIVE ORAL AXL INHIBITOR BEMCENTINIB (BGB324) IN RELAPSED/REFRACTORY AML AND MDS: IDENTIFICATION OF PREDICTIVE AND PHARMACODYNAMIC BIOMARKER CANDIDATES ASSOCIATED WITH PT BENEFIT

S. Loges[1,*], B. T. Gjertsen[2], M. Heuser[3], J. Chromik[4], C. E. Vigil[5], P. Paschka[6], I. Ben-Batalla[1], N. Akyüz[1], D. Micklem[7], R. Holt[7], A. Brown[7], M. Yule[7], J. Lorens[7], M. Kebenko[1], M. Janning[1], M. Binder[1], W.M. Friedler[1], J. Cortes[8]

[1]University Medical Center Hamburg- Eppendorf, Hamburg, Germany, [2]Department of Clinical Science, Translational Hemato-Oncology group, University of Bergen, Bergen, Norway, [3]Hamatologie, Hamostaseologie, Onkologie und, Stammzelltransplantation, Medizinische Hochschule Hannover, Hannover, [4]Frankfurt Medizinische Klinik II, Hamatologie/Onkologie, Johann-Wolfgang-Goethe Universität , Frankfurt/Main, Germany, [5]University of Iowa Hospitals and Clinics, Iowa City, United States, [6]Universitätsklinikum Ulm, Ulm, Germany, [7]BerGenBio ASA, Bergen, Norway, [8]The University of Texas M.D. Anderson Cancer Center, Houston, United States

Background: Bemcentinib is a first-in-class, selective, orally bioavailable, Axl kinase inhibitor which is being evaluated as a therapy for solid tumours and myeloid malignancies in multiple phase II clinical trials. Bemcentinib has shown anti-leukaemic activity and immune activation in pre-clinical models of AML and other cancers and selective blockade of Axl via bemcentinib in patients with relapsed / refractory AML and int-2 and high-risk MDS is currently being evaluated in the Phase II trial BGBC003 (NCT02488408).
Aims: To identify the safety and tolerability of BGB324 both as a single agent and in combination with cytarabine. To identify biomarkers for monitoring target inhibition, to collect evidence of immune activation and to discover and validate predictive biomarkers which may be used as companion diagnostics
Methods: The dose escalation part of the study evaluating bemcentinib monotherapy followed a standard 3+3 design, cohort expansion was carried out the recommended phase 2 dose. Plasma protein biomarker levels were measured using the DiscoveryMap v3.3 panel (Myriad RBM) at pre-dose and after one cycle of treatment. Gene expression analysis was carried out on RNA extracted from BM-MNCs by qPCR using TaqMan. Levels of sAxl were measured in patient peripheral blood plasma as well as bone marrow plasma using a custom ELISA assay. The presence of phosphorylated Axl was measured using Western blotting and a custom Luminex assay. The TCRß repertoire was quantified by NGS of DNA isolated from PBMNCs using an Illumina MiSeq sequencer. TCRß genes and the IGH repertoire were analysed with BIOMED2-TCRß-A and –B and BIOMED2-FR1/-FR3 primer pools, respectively. Using genomic DNA as template, the amplicons were tagged with Illumina adapters and indices in two consecutive PCR reactions. Demultiplexing and FastQ formated data output was generated by the MiSeq reporter. Analysis of TCRß and IGH data was performed on a Microsoft Cloud using our in-house analysis pipeline Pippa.
Results: A loading dose of 400 mg on days one to three followed by 200 mg daily thereafter has been established as safe and recommended phase 2 dose. Most adverse events were mild or moderate in severity congruent with bemcentinib monotherapy being well-tolerated in this patient population. 2 pts (6%) achieved complete responses with incomplete recovery of peripheral counts (CRi) and 5 (14%) achieved partial responses (PR). 8 pts (23%) reported disease stabilisation for more than 4 months. Immune activation was observed by T- and B-cell receptor diversification. PhosphoFlow analysis of AXL and downstream signaling intermediates demonstrated target pathway inhibition. Levels of plasma soluble AXL (sAxl) and angiogenin as well as bone marrow SLFN-11 expression correlated with pt benefit. sAxl levels were strongly correlated in bone marrow plasma and blood plasma.
Summary/Conclusion: Bemcentinib is well tolerated in relapsed / refractory MDS and AML pts and exhibits anti-leukemic activity through multiple mechanisms including immune modulation. Pt benefit (CRi/PR/SD > 4 mths) is predictable by measurement of plasma markers soluble AXL and angiogenin. These predictive biomarker candidates may be developed as companion diagnostics to select patient populations that are more likely to respond to bemcentinib treatment. In addition, the identification of Axl and Axl-mediated pathways as predictive biomarkers, demonstrates the 'on-target' activity of bemcentinib.

PS982

CYTOGENETIC AND MOLECULAR DRIVERS OF OUTCOME WITH VENETOCLAX-BASED COMBINATION THERAPIES IN TREATMENT-NAÏVE ELDERLY PATIENTS WITH AML

S. Strickland[1,*], B. Chyla[2], R. Popovic[2], A. Bhathena[2], M. Dail[3], Y. Sun[2], A. Wei[4], W. Fiedler[5], K. Pratz[6], J. Hayslip[2], J. Potluri[2], C. DiNardo[7], D. Pollyea[8]

[1]Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN, [2]AbbVie, Inc, North Chicago, IL, [3]Genentech, Inc, South San Francisco, CA, United States, [4]The Alfred Hospital and Monash University, Melbourne, Australia, [5]Hubertus Wald University Cancer Center, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [6]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD, [7]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX, [8]University of Colorado School of Medicine, Aurora, CO, United States

Background: Acute myeloid leukemia (AML) is highly heterogeneous and tumor-related genetic abnormalities have been shown to be key prognostic factors affecting outcomes to standard treatments. Although AML treated with low-intensity therapies has a poor prognosis, recent studies suggest higher response rates may be achievable in combination with venetoclax (Ven)—an oral BCL-2 inhibitor. Therefore, identifying cytogenetic and molecular factors that influence outcome to these novel combinations is of high importance.
Aims: To evaluate the impact of molecular profiles in elderly, untreated AML patients in response to front-line Ven combination therapies.
Methods: This analysis includes data from two open-label multicenter trials assessing the safety and efficacy of Ven in combination with azacitidine or decitabine (NCT02203773; phase 1b; data cutoff July 7, 2017), or low-dose cytarabine (NCT02287233; phase 1/2; data cutoff August 15, 2017) in treatment-naïve patients with AML. Patients (≥65 years) were classified into ten molecular subgroups based on cytogenetic or molecular mutations identified using next-generation sequencing in baseline bone marrow or blood samples (Table 1). Response to Ven combination therapies was evaluated in patients with intermediate and poor cytogenetic risk, as well as in patients within the three most common molecular subgroups: those with mutated chromatin/RNA-splicing genes, those with mutated *TP53*/aneuploidy, and those with *NPM1* mutation. Determination of minimal residual disease (MRD) used standardized multicolor flow cytometry, centrally analyzed.
Results: Patients with intermediate cytogenetic risk had higher rates of CR/CRi than those with poor risk, regardless of Ven therapy combination (Table 2). There was no significant difference in rates of CR/CRi across Ven combinations among subgroups with concordant cytogenetic risk. Rates of CR/CRi were high (range: 68–100%) for patients with chromatin spliceosome, or *NPM1* mutations across all Ven doses and combination backbones. In comparison, those with chromosomal aneuploidy and/or *TP53* mutations had lower rates of CR/CRi (50–55%). Of patients with CR/CRi and available MRD data, 43% (34/80) and 32% (12/38) achieved MRD below 10^{-3} threshold when treated with Ven + Aza/Dec and Ven + LDAC, respectively. Additional univariate and multivariate analyses are ongoing to determine significant drivers of Ven therapeutic response in elderly AML patients. OS will be presented at the meeting.

Table 1.

Genomic subgroup*	Patients, n (%)	Class-defining mutations
Mutated chromatin, RNA splicing genes, or both	71 (37)	*RUNX1, ASXL1, BCOR, STAG2, EZH2, SRSF2, SF3B1, U2AF1, ZRSR2, or MLL*[PTD]
TP53 mutations, chromosomal aneuploidy, or both	65 (34)	TP53, Complex karyotype, -4/4q, -5q, -5/5q, -7/7q, -17/17p, -12/12p, -18/18q, -20/20q, +11/11q, +13, +21, or +22
NPM1 mutation	27 (14)	*NPM1*
MLL fusion genes; t(x;11)(x;q23)	6 (3)	t(x;11q23)
Biallelic *CEBPA* mutations	5 (3)	*CEBPA*[biallelic]
Inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *GATA2, MECOM (EVI1)*	4 (2)	inv(3)
IDH2[R172] mutation and no other class-defining lesions	1 (1)	*IDH2*[R172]
t(6;9)(p23;q34); *DEK-NUP214*	1 (1)	t(6;9)
Inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*	0	inv(16)
Driver mutations but no detected class defining lesions	8 (4)	–

Total N = 191; 3 patients had no detected driver mutations

* Subgroups based on Papaemmanuil *et al*. N Engl J Med. 2016 Jun 9;374(23):2209-2221

Table 2.

Outcomes after day 90 for patients with m*IDH2*-R/R AML receiving enasidenib 100 mg/day with sustained stable disease during the first 90 days of Tx (N=82)	
	n (%)
CR + CRp	20 (24.4)
CR	16 (19.5)
CRp	4 (4.9)
Partial remission	3 (3.7)
Morphologic leukemia-free state	2 (2.4)
Stable disease	29 (35.4)
Progressive disease / not evaluable / unknown	28 (34.1)
CR, Complete remission; CRp, CR with incomplete platelet recovery	

Summary/Conclusion: Mutated chromatin and RNA splicing genes, chromosomal aneuploidy or *TP53* mutations, and *NMP1* mutations were identified as the most common molecular drivers in treatment-naïve elderly AML patients. Preliminary data suggest that venetoclax combination therapies induce high rates of response compared to historical rates of response to standard chemotherapy; this includes all tested cytogenetic risk and molecular subgroups, including those with *TP53* mutations.

PS983

RANDOMIZED OPEN-LABEL, PHASE III MULTICENTER TRIAL TO EVALUATE AZACITIDINE POST-REMISSION THERAPY IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA

E. N. Oliva[1,*], B. Martino[1], P. Salutari[2], C. Alati[1], E. Mauro[3], A. Cortelezzi[4], D. Capelli[5], P. Niscola[6], C. Selleri[7], A. Volpe[8], N. Cascavilla[9], P. Musto[10], E. Vigna[11], D. Mannina[12], G. Specchia[13], M. V. Dubbini[14], M. E. Zannier[14], P. Di Bartolomeo[2], F. Di Raimondo[3], A. Freyrie[4], P. Leoni[5], A. Candoni[14]

[1]Hematology Division, Grande Ospedale Metropolitano Bianchi Melacrino Morelli, Reggio Calabria, [2]Department of Hematology, Presidio Ospedaliero Spirito Santo, Pescara, [3]Division of Hematology, Policlinico- OVE, University of Catania, Catania, [4]Department of Hematology, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico and University of Milan, Milan, [5]Clinica di Ematologia, Ospedali Riuniti, Ancona, [6]Hematology Unit, S.Eugenio Hospital, Roma, [7]UOC di Ematologia e Trapianto di cellule staminali emopoietiche, University Hospital, Salerno, Salerno, [8]Hematology Unit, A.O. San Giovanni Moscati, Avellino, [9]Hematology Unit, Casa Sollievo della Sofferenza IRCCS, San Giovanni Rotondo, [10]Scientific Direction, IRCCS-CROB, Rionero in Vulture (Pz), [11]Hematology Division, Ospedale L'Annunziata, Cosenza, [12]Division of Hematology, Azienda Ospedaliera Papardo, Messina, [13]U.O. Ematologia con Trapianto, Università degli Studi Di Bari, Bari, [14]Division of Hematology and SCT, ASUIUD, University of Udine, Udine, Italy

Background: In elderly patients with acute myeloid leukemia (AML), complete remission (CR) rate following intensive chemotherapy is approximately 45%, considerably lower than in younger patients, with a shorter duration of remission and high treatment-related mortality. Median survival is 7 to 12 months. Several studies have suggested that maintenance therapy may improve survival.

Aims: This phase III, prospective, randomized, multicenter trial assesses the efficacy of post-remission treatment with Azacitidine (Aza) versus best supportive care (BSC) in 54 AML patients >60 years of age in CR after induction and consolidation chemotherapy. Primary endpoint is the difference in disease free survival (DFS) at 2 and 5 years between arms; secondary endpoints are the difference in overall survival (OS) at 2 and 5 years, the number and length of hospitalizations and quality of life in the 2 arms in the 2-year post-remission period.

Methods: Newly diagnosed AML patients with >30% blasts, "*de novo*" or evolving from myelodysplastic syndrome and fit for intensive chemotherapy have been included. Induction consisted of two courses of "3+7" (Daunorubicin 40 mg/m^2 daily days 1-3 and cytarabine 100 mg/m^2 daily continuous IV infusion days 1-7). Patients in CR received cytarabine 800 mg/m^2 3 hour infusion bid days 1-3 and were randomized 1:1 to recieve BSC or Aza according to the following schema: 50 mg/m^2 s.c. or i.v. for 7 days every 28 days and dose increase after 1st cycle to 75 mg/ m^2 for further 5 cycles, followed by cycles every 56 days for 4.5 years.

Results: Enrollment has been completed with 149 patients of median age 69 years, interquartile range (IQR) 65-74 years, male/female 78/71. During chemotherapy, 59 patients were relapsed/refractory, 22 died, 10 refused to continue, 3 were excluded for protocol violation, and 1 was lost to follow-up. Fifty-four randomized patients (27 Aza, 27 BSC) are included in this interim analysis. At 2-years, the median period of observation was 15 months (IQR: 6-24 months) with 18 patients in CR. Nineteen BSC patients (median DFS 5 months) have relapsed versus 17 Aza patients (median DFS 11 months) (p=0.23, see Figure 1a). Twenty patients have died, all after relapse: 10 in BSC arm (median OS not reached) versus 10 in AZA arm (median OS not reached) (p=0.40, see Figure 1b). Age modified the effect of Aza on both relapse (P for effect modification=0.001) and mortality (P for effect modification=0.01), its effect versus BSC being not significant in patients in the 1st (<65 years) and 2nd (65-73 years) age tertiles (relapse, p=0.38 and p=0.47, respectively; mortality, p=0.23 and p=0.81, respectively) but highly significant in patients in the highest age tertile (>73 years) (relapse, p<0.001, Figure 1c; mortality, p=0.015, Figure 1d). Cytogenetics and minimal residual disease did not modify the effect of AZA on survival. At 4.5 years from randomization (median 15 months, IQR: 6-32 months), 15 patients are in CR and the effect modification by age on the effect of AZA *versus* BSC is confirmed with AZA showing significant efficacy only in patients >73 years of age (relapse, p<0.001; mortality, p=0.005). Grade 3-4 adverse events (mainly neutropenia) were more frequent in the AZA (18%) than in the BSC arm (0%)(p=0.051).

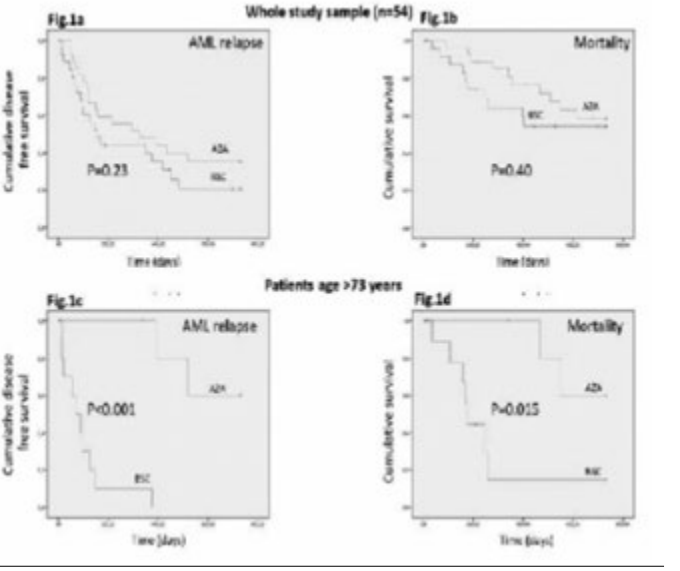


Figure 1.

Summary/Conclusion: Preliminary results indicate that in elderly AML patients receiving standard induction-consolidation chemotherapy, 5-Aza post-CR is well-tolerated and significantly prolongs DFS and OS. The trial is ongoing and may provide further insight on the impact of post-remission 5-Aza treatment on OS in this elderly population.

PS984

A PHASE 2B OF ERYASPASE IN COMBINATION WITH LOW-DOSE CYTARABINE AS FIRST-LINE THERAPY IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA (ENFORCE - NCT01810705)

X. Thomas[1,*], F. Orsini-Piocelle[2], P. Chevallier[3], E. Tavernier-Tardy[4], J. Marolleau[5], M. Hunault[6], B. de Renzis[7], C. Bonmati[8], M. Ledoux[9], F. Larosa[10], G. Guillerm[11], S. Chantepie[12], M. Sanz-Alonso[13], K. Porkka[14], F. Pane[15], B. Gjertsen[16], B. Quesnel[17], Y. Hichri[18], E. Jourdan[19], S. Lepre-

tre[20], C. Recher[21], T. Leguay[22], T. Cluzeau[23], B. Steffen[24], J. Bertrand[25], A. Hamm[26], R. Kay[27], I. El-Hariry[25]

[1]Centre hospitalier Lyon Sud, Pierre-Bénite, [2]Centre Hospitalier de la Région d'Annecy, Pringy, [3]Hôtel Dieu, Nantes, [4]Institut de Cancérologie de la Loire, Saint-Priest-en-Jarez, [5]Groupe Hospitalier Sud, Amiens, [6]Centre Hospitalier Universitaire d'Angers, Angers, [7]Centre Hospitalier Universtaire Estaing, Clermont-Ferrand, [8]Hôpital de Brabois, Vandoeuvre-lès-Nancy, [9]Hopitaux Universitaires de Strasbourg, Strasbourg, [10]Hôpital Jean Minjoz, Besançon, [11]Centre Hospitalier Universitaire Morvan, Brest, [12]Centre Hospitalier Côte de Nacre, Caen, France, [13]Hospital Universitario y Politécnico la Fe, Valencia, Spain, [14]Helsinki University Hospital, Helsinki, Finland, [15] Universita' di Napoli Federico II, Napoli, Italy, [16]University of Bergen & Haukeland University Hospital, Bergen, Norway, [17]Hôpital Claude Huriez, Lille, [18]Hôpital Saint Eloi, Montpellier, [19]Groupe Hospitalo-Universitaire Carémeau, Nimes, [20]Centre Henri Becquerel, Rouen, [21]Institut Universitaire du Cancer de Toulouse, Toulouse, [22]Hôpital Haut-Lévèque, Pessac, [23]Hôpital L'archet [1], Nice, France, [24]University Hospital Frankfurt, Frankfurt, Germany, [25]Erytech Pharma, Lyon, France, [26]Cytel, Inc, Boston, United States, [27]Cardiff University, Cardiff, United Kingdom

Background: L-asparaginase (ASNase) is a key drug in the treatment of ALL. ASNase therapy aims to lower serum asparagine (ASN) levels, which in turn inhibits the protein synthesis, resulting in cell cycle arrest and apoptosis.

A synergistic effect of ASNase has been demonstrated in both children and adult patients with acute myeloblastic leukemia (AML) when given in combination with chemotherapy and especially cytarabine. However, L-asparaginase is poorly tolerated in elderly patients due to severe toxicities.

Eryaspase, ASNase encapsulated in red blood cells (RBCs) is an investigational product under development. Following infusion, ASN is actively transported into RBCs, where it is hydrolyzed by the encapsulated ASNase.

Aims: The primary endpoint was overall survival (OS). The key secondary endpoints included progression free survival (PFS), objective response rate (ORR), toxicity as well as pharmacokinetic and pharmacodynamic profile of eryaspase.

Methods: This open-label, multicenter phase 2 study enrolled 123 patients (65 to 85 years-old) with newly diagnosed AML and unfit for intensive chemotherapy, randomized 2:1 to receive subcutaneous low-dose cytarabine – LDAC (D1 to D10 of 4-wk regimen) in combination with eryaspase (100 U/Kg Day11) versus LDAC alone.

Results: The median age of the patients enrolled in this study was 78 years. Baseline characteristics were well balanced between the two treatment arms. The adjusted OS Hazard Ratio (HR) was 0.98 (95% CI; 0.70, 1.61). The adjusted PFS HR was 1.15 (95% CI; 0.75, 1.76) and the ORR was 25.3% and 22.5% in the eryaspase and control arms, respectively (p=0.74).

Forest plots for OS and for PFS showed relatively consistent results across the population as a whole.

The incidence of adverse events (AE) was similar between both groups except for hypersensitivity reactions, coagulopathic, pancreatic and hepatic events that occurred at a higher frequency during in the eryaspase arm. These events are generally considered to be class-effects relevant to asparaginase and were consistent with previously reported AEs for eryaspase.

Summary/Conclusion: This is the largest prospective study to date to evaluate the efficacy of ASNase in elderly AML patients. The addition of eryaspase did not improve survival in this difficult-to-treat patient population. Patient selection is likely the most important factor. The study enrolled unfit patients, who remained briefly on treatment to achieve a potential drug effect. Individualized risk stratification based on multiparameter assessment tools and comorbidity burden, should be considered for older adults with AML participating in clinical trials.

PS985

AN EVALUATION OF CONTROL REGIMENS IN A GROUP OF HIGH RISK OLDER PATIENTS WITH ACUTE MYELOID LEUKAEMIA FROM TWO COOPERATIVE GROUPS: DATA FROM THE UK NCRI AND DUTCH HOVON STUDIES

R. Hills[1,*], G. Ossenkoppele[2], A. Burnett[3], B. Lowenberg[4], G. Stussi[5], D. Breems[6], P. Gradowska[4], N. Russell[7]

[1]Centre for Trials Research, Cardiff University, Cardiff, United Kingdom, [2]Department of Haematology, VU University Medical Center, Amsterdam, Netherlands, [3]Blackwaterfoot, Isle of Arran, United Kingdom, [4]Department of Haematology, Erasmus Medical Center, Rotterdam, Netherlands, [5]Department of Haematology, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland, [6]Department of Haematology, Hospital Network Antwerp, Antwerp, Belgium, [7]Department of Haematology, Nottingham University Hospitals, Nottingham, United Kingdom

Background: Outcomes for older patients with AML with high risk features are poor, even with intensive chemotherapy (typically ara-C plus an anthracycline). The multifactorial Wheatley index found 1-year survival with standard chemotherapy of only 30% in high risk patients. There is a need for better treatments in this population. A recent phase 3 trial of CPX-351, a liposomal daunorubicin/ara-C combination, found significant benefit in a high-risk population with prior MDS or CMML, therapy related AML, or with an MDS-related karyotype (median survival 9.6 vs 6.0 months p=0.005). Enrolment was at US and Canadian sites: we therefore examined the European experience to contextualise the results when placed against European standard of care.

Aims: To evaluate the outcomes of high-risk patients aged 60-75 enrolled in successive European AML trials from the UK NCRI and Dutch HOVON Group.

Methods: Patients treated with standard anthracycline/ara-C chemotherapy recruited to collaborative group trials since 2002 were included if they satisfied the eligibility criteria corresponding to those for the Phase III CPX-351 trial: age 60-75, WHO/ECOG performance status 0-2, and exhibiting one of the adverse risk features above. NCRI patients were treated with daunorubicin/ara-C for two courses in a 3+10/3+8 schedule; HOVON patients received daunorubicin/ara-C or idarubicin/ara-C in a 3+7 schedule for course 1 and regimens based upon ara-C 1g/m^2 for course 2. Patients were hierarchically grouped by type of AML: therapy-related, prior MDS, prior CMML, and MDS-like karyotype. Survival was evaluated using the Kaplan-Meier method and compared using logrank tests and Cox regression for multivariate analyses. Median follow-up was 61.3 months for NCRI and 107.5 months for HOVON.

Results: A total of 669 patients (NCRI 465 patients in 2 trials, HOVON 204 patients in 4 trials) were identified. Median age was 65 years (NCRI) vs 66 years (HOVON, p=0.5); 61% were male (63% MRC vs 56% HOVON, p=0.13); and median WBC was 5.0x10^9/L (5.1 vs 4.4 p=0.2). Distribution of disease type was similar between groups (p=0.9); overall 18% had t-AML; 23% prior MDS; 4% prior CMML; and 55% an MDS-like karyotype. Remission rates CR/CRi were 59% for NCRI and 47% for HOVON patients (OR 0.61 (0.44-0.85) p=0.004). However, overall survival was similar between trial groups (median OS 8.9 vs 8.3 m; 1-year OS 40% vs 38%; 3 year OS 13% vs 13%, HR 0.96 (0.81-1.14) p=0.6). In the entire cohort, there was no significant difference in survival by type of AML (p=0.14). In a multivariable analysis with age, sex, white blood cell count, type of AML and study group as candidate variables, only white cell count (HR 1.20 (1.05-1.37) per 10-fold increase, p=0.03) and type of AML (p=0.05) were significantly prognostic with patients with MDS-like karyotype performing worst. Transplant rates were similar between trial groups with 15% of NCRI and 17% of HOVON patients receiving stem cell transplantation. In a landmark analysis at 120 days, SCT gave better survival (34% vs 15% at 3 yrs, p<.0001) (Figure 1).

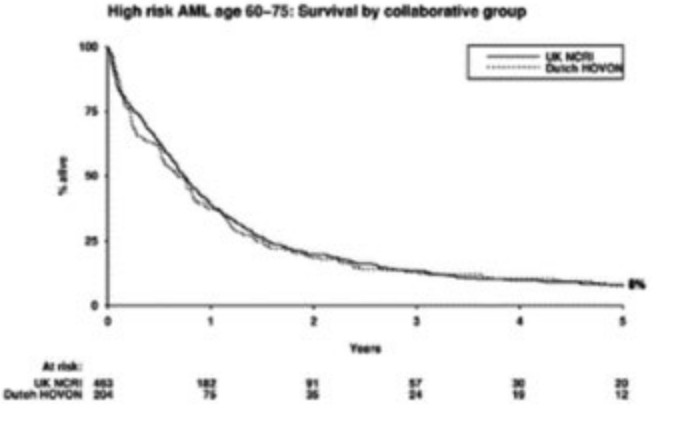


Figure 1.

Summary/Conclusion: Despite the different chemotherapy regimens used by two large European groups, survival in this group of patients is equally poor. In particular, a 10+3 regimen, produces more remissions, does not show better survival. Median survival is approximately 9 months, and continues to decrease with only 13% alive at 3 years, about half that in the pivotal CPX-351 trial. Although the median survival is similar to the pivotal trial, we do not see evidence of a plateau. Stem cell transplant is associated with prolonged survival.

PS986

TP53 ALTERATIONS AND MONOSOMAL KARYOTYPE IN OLDER UNFIT PATIENTS WITH ACUTE MYELOID LEUKEMIA TREATED WITH DECITABINE

H. Becker[1,2,*], D. Pfeifer[1,2], G. Ihorst[2,3], B. Rüter[1], L. Bullinger[4,5], B. Hackanson[1,2,6], U. Germing[7], A. Kündgen[7], U. Platzbecker[8], K. Döhner[4], A. Ganser[9], P. Wijermans[10], H. Döhner[11], J. Duyster[1,2], M. Lübbert[1,2]

[1]Department of Medicine I, Medical Center - University of Freiburg, [2]Faculty of Medicine, University of Freiburg, [3]Clinical Trials Unit, Medical Center - University of Freiburg, Freiburg, [4]University of Ulm, Ulm, [5]Charite University Hospital, Berlin, [6]Interdisciplinary Cancer Center Augsburg, Augsburg, [7]Heinrich-Heine-University, Düsseldorf, [8]University Hospital Dresden, Dresden, [9]Hannover Medical School, Hannover, Germany, [10]Haga Ziekenhuis, The Hague, Netherlands, [11]University of Ulm, Freiburg, Germany

Background: Monosomal karyotype (MK+), particularly in case of multiple monosomies (MK2+), and *TP53* mutations have been described to be predictive for a favorable response to decitabine (DAC) in acute myeloid leukemia (AML) (Lübbert *et al.* Haematologica 2012;97:393; Welch *et al.* NEJM 2016;375:2023).
Aims: To assess clinical features and outcome associated with gene and chromosome alterations of *TP53* in AML patients treated with DAC within a phase II trial.
Methods: Briefly, 227 AML patients >60 years ineligible for intensive therapy were enrolled (Lübbert *et al.* Haematologica 2012;97:393). Patients received DAC 3x15 mg/m^2/day on 3 days, every 6 weeks. Patients with antileukemic effect (ALE) or stable disease after course 1 additionally received all-trans retinoic acid (ATRA, 45 mg/m^2/day) for 28 days in course 2; those with complete remission (CR), partial remission (PR) or ALE after course 4 qualified for maintenance with DAC 20 mg/m^2/day on 3 days, every 6-8 weeks. Karyotypes were centrally reviewed (A. Hagemeijer, University Leuven). Sequencing was performed using Illumina TruSight panels. Subclones were defined as cell fractions >20% smaller than the major clone.
Results: Gene mutations were assessed in 45 patients with material available. Median patient age was 75 years, 27 had AML secondary to MDS. Patients harbored a median of 3 mutations (range, 0-9); 9 patients had >1 mutation in a given gene. Most frequently mutated were: *ASXL1* (29%), *RUNX1* (24%), *SRSF2* (24%), *IDH1/2* (22%), *TET2* (18%), *TP53* (18%). Of the 8 *TP53* mutated patients 4 were MK+, two of whom had a -17. *TP53* mutated patients had received a median of 1 DAC course (range 1-6), *TP53* wild-type (wt) patients 2 (range 1-11) (p=.22); 24 patients had additionally received ATRA. Patients with *TP53* mutations had similar CR/PR/ALE rates (38% vs 50%) but shorter OS than those with *TP53* wt (p=.036; median 1.8 vs 4.4 months). Moreover, patients with a subclone (n=9) had shorter OS than those without (n=24; p=.05), while a subclone was present in only one *TP53* mutated patient; *TP53* mutations were all present in the major clone. Next, we sought to evaluate the impact of chromosomal loss of *TP53* (ie, -17/del(17p)) in the context of MK+. Of 178 patients with cytogenetics available, 38 were MK+. Of these, 22 were MK2+, while 16 had one monosomy and ≥1 structural aberration (MK1). Overall, 17 patients had a -17, all of whom were MK+ and had a complex karyotype (CK+). Among the MK+ patients, those with -17 differed from those without -17 by higher platelet counts (p=.01), by trends for lower blood blasts but higher marrow blasts and by more often being MK2+ (p=.05). We evaluated the outcome according to -17 in all patients with cytogenetic data and restricted to CK+ and MK+ patients. Patients with -17 had higher rates of CR/PR/ALE than those without in all subsets (82% vs 41-53%). However, the favorable responses did not translate into prolonged OS. Considering only patients who received ATRA in addition to DAC, there were also no OS differences according to -17. We previously observed that MK2+ patients treated with DAC had better outcome than MK1 patients. Thus, to further dissect the impact of -17, we compared the outcomes of MK2+ patients with -17 (n=13) and those without (n=9) but observed no differences in response rates or OS.
Summary/Conclusion: *TP53* mutations did not impact response, but were associated with shorter OS. In contrast, -17 was associated with favorable responses, even among MK+ patients, but not among MK2+ patients. Our data suggest that monosomies rather than *TP53* alterations may sensitize for DAC treatment.

PS987

IDENTIFYING A GENETICALLY DEFINED RISK GROUP OF PEDIATRIC AML PATIENTS THAT CANNOT BE RESCUED AT ALL AFTER THE FAILURE OF THE FIRST LINE TREATMENT

N. Niktoreh[1,*], C. Walter[1], M. Zimmermann[2], N. von Neuhoff[1], M. Rasche[1], U. Creutzig[2], H. Hanenberg[1], D. Reinhardt[1]

[1]Department of Pediatrics III, Pediatric Hematology and Oncology, University Children's Hospital Essen, University of Duisburg-Essen, Essen, [2]Department of Pediatric Hematology and Oncology, Hannover Medical School, Hannover, Germany

Background: Pediatric acute myeloid leukemia (AML), a rare and heterogeneous disorder, is currently treated with intensive risk-adaptive chemotherapy regimens combined with myeloablative therapies. These treatments are assigned based on various clinical and cytogenetic characteristics of patients at the time of diagnosis which define risk of the disease. Genetic alterations of the leukemic blasts at initial diagnosis are one of the most important indicators currently used for the risk group stratification in pediatric AML. Mutations in the *WILMS TUMOR 1 (WT1)* gene and the internal tandem duplication of the juxta-membrane domain of *FLT3* gene (*FLT3-ITD*) frequently co-occur in myeloid blasts of children at diagnosis.
Aims: Two pediatric treatment optimization studies demonstrated before that this genetically defined subgroup of patients had poor survival when treated on protocols before 2005. To reevaluate this finding in newer protocols, 346 patients (<18 years) treated in Germany since 2005 in the BFM-AML 2004 trial (ClinicalTrials.gov Identifier: NCT00111345) or AML-BFM Registry 2012 (EudraCT number: 2013-000018-39) were included in this report.
Methods: We retrospectively reviewed medical records of patients with 0–18 years of age with *de novo* AML (excluding FAB M3 and Down Syndrome) and identified patients with *WT1* mutations and *FLT3-ITD* at the time of diagnosis. As confirmation, material from patients with *WT1* and *FLT3-ITD* was re-analyzed by next-generation sequencing using the TruSight Myeloid Panel (Illumina).
Results: We identified 45 (13%) patients with 60 *WT1* mutations and 52 (15%) with *FLT3-ITD.* Other mutations in *NPM1*, *NRAS* and *c-KIT* were present in 9, 18 and 10% of patients' blasts, respectively; however, *WT1* and *FLT3-ITD* frequently co-occurred with each other and predominantly were detected in older children with blasts belonging to the FAB M1/M2 subtype and having normal cytogenetics. Analysis of the patient outcomes showed that *WT1* and *FLT3-ITD*, but not *NRAS*, *NPM1* or *c-KIT* mutations significantly reduced the probability of 3-year overall survival (OS) as single factors. However, presence of both mutated *WT1* and *FLT3-ITD* resulted in a 3-year event-free survival of 34±12% compared to 67±3% for patients with none of these mutations (p=0.0009) and 56±9% or 70±10% for patients with only *FLT3-ITD* (p=0.08) or *WT1* mutation (p=0.013), respectively. Importantly, the probability of 3-year OS in patients with co-occurrence of *WT1* and *FLT3-ITD* mutations was only 35±12% compared to patients without these mutations (84±2%, p<0.0001), patients with only mutated *WT1* (91±6%, p=0.0004) or only *FLT3-ITD* (77±8%, p=0.004). This poor survival outcome demonstrated that also our current second line treatment completely failed to rescue at least some of these patients and our multivariate analysis identified the effect of these two factors to be dependent on each other (Figure 1).

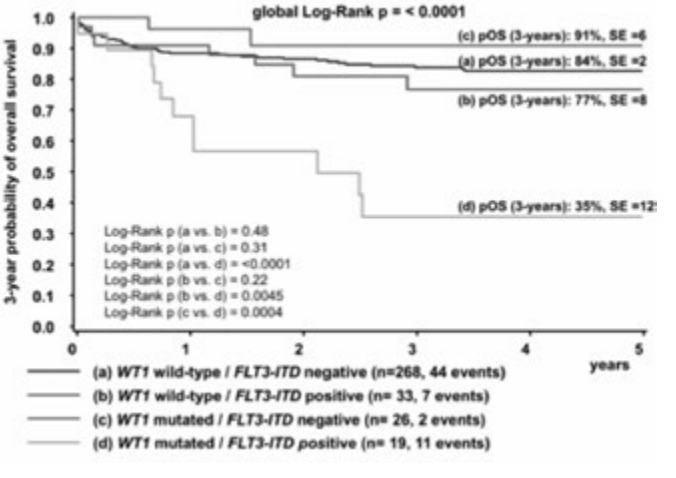


Figure 1.

Summary/Conclusion: We conclude that co-occurrence of *WT1* mutation and *FLT3-ITD* is still predictor of poor response in contemporary first and especially second line treatment for pediatric AML.

PS988

A PHASE II STUDY OF THE AURORA A KINASE INHIBITOR ALISERTIB COMBINED WITH 7+3 INDUCTION CHEMOTHERAPY IN PATIENTS WITH HIGH-RISK ACUTE MYELOID LEUKEMIA

A. Brunner[1], T. Blonquist[2], D. DeAngelo[2], M. McMasters[3], E. Winer[2], G. Hobbs[1], P. Amrein[1], H. Hock[1], D. Steensma[2], J. Garcia[2], M. Luskin[2], R. Stone[2], K. Ballen[4], J. Rosenblatt[3], D. Avigan[3], S. McAfee[1], J. Moran[1], J. Foster[1], C. Bertoli[1], M. Bergeron[1], K. McGregor[1], K. Fishman[1], M. MacRae[1], M. Burke[1], T. Behnan[1], T. Som[1], A. Ramos[1], J. Lombardi Story[1], C. Connolly[1], D. Neuberg[2], Y.-B. Chen[1], T. Graubert[1], A. Fathi[1,*]

[1]Massachusetts General Hospital, [2]Dana-Farber Cancer Institute, [3]Beth Israel Deaconess Medical Center, Boston, [4]University of Virginia, Charlottesville, United States

Background: Increased expression of aurora A kinase occurs in hematologic malignancies, including myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). We previously conducted a phase I study of alisertib combined with "7+3" induction chemotherapy as upfront treatment of AML; this combination appeared to be particularly promising for the patient cohort with high-risk disease, who typically have poor induction outcomes and remission rates of 45% or less. We therefore conducted a phase II study of alisertib and induction chemotherapy in patients with high-risk AML.
Aims: To evaluate the efficacy of alisertib combined with conventional induction in high-risk AML.
Methods: We enrolled patients with high-risk AML, defined as those with adverse-risk karyotype (as per European Leukemia Net Guidelines), secondary AML, or aged ≥ 65 years, in a phase II study. We used a Simon two-stage design, assuming a null composite remission rate (complete remission [CR] and complete remission with incomplete count recovery [CRi]) rate of 45% with induction chemotherapy alone. Patients with favorable cytogenetics were removed prior to day 8 and replaced. All patients received 7+3 induction (continuous infusion cytarabine 100mg/m^2 on days 1-7 [D1-7] and idarubicin 12mg/m^2 [or daunorubicin 60mg/m^2] D1-3). On D8-15, alisertib 30mg BID was administered. All underwent a mid-induction bone marrow biopsy to assess for residual disease, which, if present, was treated with 5+2 re-induction without alisertib. Following remission, patients could receive up to 4 consolidation cycles with cytarabine (3g/m^2 BID D1,3,5 for age <60 and 2g/m^2 daily D1-5 for age ≥60) followed by alisertib on D6-12. Patients could come off study for stem cell transplant (SCT).
Results: 39 eligible patients were treated on study. The median age was 68 (range 33-83); 25 (64%) were male, and 33 (85%) were Caucasian. Twenty one patients (51%) had secondary AML (18 with antecedent MDS, 2 with antecedent CMML, and 1 with antecedent MPN). Two (5%) had therapy related AML. Thirteen (33%) exhibited adverse risk karyotype. *FLT3* mutations were seen in 7 (18%), *NPM1* in 7 (18%), *IDH1* in 5 (13%), *IDH2* in 5 (13%), *CEBPA* in 3 (8%), and *TP53* mutations in 4 (10%) patients. 33 patients (84%) demonstrated an ablated marrow at mid-treatment, and six (15%) received re-induction at mid-treatment. 8 patients (21%) were refractory to induction, and five (13%) died prior to response assessment due to infection or bleeding. The 30-day and 60-day mortality were 8% and 13%, respectively. Patients experienced expected grade 4 toxicities of leukopenia, anemia, thrombocytopenia, and febrile neutropenia; no new safety signals were detected. The CR+CRi rate was 64% (2-stage 95% CI 47.45-79.44%) with 20 patients (51%) achieving CR and 5 (13%) achieving CRi. One (3%) patient achieved a partial remission. Based on this composite remission rate, the combination was deemed effective per study design. The CR+CRi rate for P53 mutated patients was 75% (3 of 4). Survival data is maturing and is expected to be available at time of presentation. Four patients have relapsed and 17 patients have died to date. 16 patients (41%) have gone on to SCT. Ten have received at least 1 cycle of consolidation.
Summary/Conclusion: Alisertib, a novel aurora A kinase inhibitor, combined with conventional induction, is efficacious and demonstrates a promising rate of remission, particularly among patients with high-risk AML. Larger randomized studies are under consideration to better assess the promise of this novel combination.

PS989

ADMINISTRATION OF BPX-501 CELLS FOLLOWING ALPHA/BETA T-CELL AND B-CELL-DEPLETED HLA HAPLOIDENTICAL HSCT (HAPLO-HSCT) IN CHILDREN WITH ACUTE MYELOGENOUS LEUKEMIA

N. Kapoor[1,*], P. Merli[2], M. Algeri[3], V. Aquino[4], D. Jacobsohn[5], S. Naik[6], E. Nemecek[7], P. Woodard[8], F. Locatelli[3]

[1]Hematology, Oncology, Blood and Marrow Transplantation, Children's Hospital Los Angeles, Los Angeles, United States, [2]Department of Onco-Hematolgy and Cell and Gene Therapy, [3]Department of Onco-Hematology and Cell and Gene Therapy, Ospedale Pediatrico Bambino Gesu, Rome, Italy, [4]Pediatric Hematology-Oncology, University of Texas-Southwestern, Dallas, [5]Blood and Marrow Transplantation, Children's National Medical Center, Washington, DC, [6]Bone Marrow Transplant/Stem Cell Transplant Program, Texas Children's Hospital, Houston, TX, [7]Pediatric Bone Marrow Transplantation Program, Oregon Health Sciences University, Portland, OR, [8]Clinical Development, Bellicum Pharmaceuticals, Inc., Houston, TX, United States

Background: Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) is an established treatment for children with either high-risk or relapsed Acute Myelogenous Leukemia (AML). HSCT from a partially-HLA matched (haplo) relative is a suitable option for children who lack an HLA-matched donor.
Aims: To evaluate the safety and efficacy of BPX-501 T cells administered after ab T-cell and B-cell depleted haplo HSCT in pediatric patients with AML. In particular, we investigated whether BPX-501 cells administered post-haplo HSCT can enhance immune reconstitution, reduce the risk of infections and of relapse in children with AML.
Methods: Thirty-eight (38) patients (EU:25, US:13; 21 males, 17 females; median age 7.9 years) with AML were enrolled in a multicenter, prospective trial utilizing ab-T-cell and B-cell-depleted haplo-HSCT, followed by infusion of donor lymphocytes genetically modified to include the inducible caspase 9 (iCasp9) suicide gene (BPX-501 T cells). Conditioning regimens included, TBI (n=19; 50%), busulfan (n=15; 39.5%) and other regimens (n=4; 10.5%). Patients received rabbit anti-thymocyte globulin either as Grafalon/Neovii® or Thymoglobulin/Sanofi® in the EU and US, respectively. The median HSCT CD34+ and ab TCR+ cell doses were 12×10^6 /kg and 0.4×10^5 /kg, respectively. The donor was a parent in 31 children (81.6%), a sibling in 5 (13.2%), and a half-sibling in the remaining 2 (5.3%). The median time to BPX-501 infusion was 18.5 days (range 12-147).
Results: The median time for neutrophil and platelet engraftment was 13 and 11 days, respectively. The median time to discharge was 23.5 days, with 61% (23/38) of patients not requiring re-hospitalization. The median follow-up period was 374 days (range, 26-1024). Eleven patients developed Grade I-IV aGVHD (cumulative incidence [CI] 31.9% [95% confidence interval (CoI): 16.3-47.5]). Six patients developed Grade II-IV aGVHD (CI 17.7% [95% CoI: 4.8-30.6]). Two patients developed Grade III-IV aGVHD (CI 6.7% [95% CoI: 0-15.7]). There were no cases of cGVHD. Four patients received rimiducid for aGVHD not responsive to standard treatment and 3 had complete resolution 4-31 days after rimiducid. One patient died after HSCT, translating into a TRM CI of 2.8% [95% CoI: 0.0-8.2]. Two patients relapsed (CI 6.1% [95% CoI: 0-14.2]) post-HSCT. In patients that received a chemo-based (n=19) or a TBI-based conditioning regimen (n=19), the relapse-free survival (RFS) was 100% and 84.2%, respectively . CR1 (n=13) and CR2 (n=25) AML patients exhibited a RFS of 91.7% [95% CI: 76.1-100.0] and 91.3% [95% CI: 79.8-100.0], respectively. One patient expired, leading to a 1-year overall survival probability of 97.2%. BPX-501 cells expand after infusion and persist through the duration of follow-up.
Summary/Conclusion: Although longer follow-up is needed to validate these data, current results suggest that ab T-cell and B-cell depleted haplo-HSCT followed by BPX-501 represents and effective therapeutic strategy to achieve high curability rates in childhood AML.

PS990

NATIONAL EARLY WARNING SCORE (NEWS), QUICK SEQUENTIAL (SEPSIS-RELATED) ORGAN FAILURE ASSESSMENT (QSOFA) AND MICROBIOLOGICAL EVALUATION IN FEBRILE NEUTROPENIA OF ACUTE MYELOID LEUKEMIA

B. Nicolino[1,*], E. Audisio[1], S. Aydin[1], A. Busca[2], S. D'Ardia[1], C. Dellacasa[2], C. Frairia[1], G. Iovino[2], U. Vitolo[1]

[1]Hematology, [2]Bone Marrow Transplant Unit, AOU Città della Salute e della Scienza di Torino, Turin, Italy

Background: Chemotherapy induced neutropenia in acute myeloid leukemia (AML) patients is at high risk for life threatening infections. Early diagnosis and prompt interventions are associated with better outcomes that, however, is hampered by a lack of diagnostic tools. Recently, the quick Sequential (Sepsis-related) Organ Failure Assessment (qSOFA) as well as the National Early Warning Score (NEWS) were proposed predicting in-hospital mortality.
Aims: The aim of the present study was to analyze the predictive validity of the NEWS and the qSOFA in a large cohort of homogeneous AML patients during neutropenia.
Methods: The NEWS is a warning clinical score that evaluates seven clinical parameters. The qSOFA score consists of three parameters, each allocated with one point: respiration rate of ≥22/min, altered mental status and sys-

tolic blood pressure of ≤ 100 mmHg. The ability of the NEWS and the qSOFA score on prediction of early mortality was analyzed by calculating the area under the curve using a logistic regression model (AUROC). Further, we assess the incidence and microbiological spectrum of febrile neutropenia.

Results: A total of 1048 neutropenic episodes in 334 consecutive adult patients were analyzed. After induction chemotherapy, a complete remission was achieved in 238 patients (71%); 74 were resistant (22%) and 22 died (7%). In 418 cycles no fever was observed; fever of unknown origin (FUO) occurred during 230 neutropenia periods; clinically documented infection was noted in 106 aplasia courses, whereas a microbiologically documented infection was evident in 272 cases and an invasive fungal infection (IFI) in 22. We analized the risk for life threatening infections using NEWS and qSOFA. Both scores were evaluated during a total of 2792 days of neutropenic fever. In addition, clinical conditions such as septic shock, amine necessity, ventilation support, intensive care unit (ICU) admission and death were analyzed. In our study, the determination of the NEWS and qSOFA significantly predicted ICU admission on the same day (NEWS AUROC 0.917, qSOFA AUROC 0.916) as well as death on the day of score determination (NEWS AUROC 0.984, qSOFA AUROC 0.969). Further, both scores were able to significantly predict ICU admission after 24 hours ((leg-1 analysis) NEWS AUROC 0.929, qSOFA AUROC 0.913) and as well as death after 24 hours (NEWS AUROC 0.928, qSOFA AUROC 0.887). Further, analyzing the microbiological spectrum of the infections, a significant difference was documented. Incidence of Gram positive bacteria were significantly more evident during the first induction cycle compared to Gram negative bacteria, that were significantly increased after consolidation treatments (66/119 versus 67/93), p=0.001.

Summary/Conclusion: To the best of our knowledge, the present analysis is the first validation of the NEWS and the qSOFA during neutropenia in a large homogeneous AML patient cohort. The NEWS and qSOFA may be valid tools to evaluate critical patients in intensive chemotherapy induced aplasia. Furthermore, the pattern of infections changed during the ongoing chemotherapeutic treatment. This may be attributed to the prophylactic and therapeutic use of antibiotics that could select the microflora.

PS991

COMPARISON BETWEEN MULTIPARAMETRIC FLOW CYTOMETRY AND RT-QPCR ASSAY IN EVALUATING MINIMAL RESIDUAL DISEASE BEFORE ALLOGENEIC STEM CELL TRANSPLANTATION IN NPM1 ACUTE MYELOID LEUKEMIA

C. Caprioli[1,*], F. Lussana[1], P. Stefanoni[1], C. Pavoni[1], O. Spinelli[1], K. Buklijas[1], A. Michelato[1], P. Zanghì[1], G. Borleri[1], A. Algarotti[1], C. Micò[1], A. Grassi[1], T. Intermesoli[1], A. Rambaldi[1,2]

[1]Hematology and Bone Marrow Transplant Unit, Ospedale Papa Giovanni XXIII, Bergamo, [2]Department of Oncology and Hematology, Università degli Studi di Milano, Milan, Italy

Background: Nucleophosmin (NPM1) is the most commonly mutated gene in acute myeloid leukemia (AML), occurring in 30% of cases. NPM1 transcript is also a stable marker when used to track minimal residual disease (MRD) by molecular analysis. Recent studies found an association between persistence/reoccurrence of NPM1 transcript and risk of relapse along with lower survival. Moreover, NPM1 persistence before allogeneic stem cell transplantation (alloHSCT) has been associated with a higher risk of relapse. However, other studies showed that similar conclusions could be drawn when pre-alloHSCT MRD is determined by multiparametric flow cytometry (MFC) regardless of molecular classification. To date there is no direct comparison that tested efficacy between pre-alloHSCT MRD by MFC and molecular analysis in predicting relapse risk in patients with NPM1 mutated AML.

Aims: To compare sensitivity and concordance of pre-alloHSCT MRD by MFC and molecular analysis and to evaluate the efficacy of combining the two methods in predicting leukemia free survival (LFS) and overall survival (OS).

Methods: Twenty-seven consecutive NPM1 AML patients who received alloHSCT in first or second complete hematologic remission between 2011 and 2017 were retrospectively analyzed. Pre-alloHSCT MRD determination by both MFC and molecular analysis on bone marrow aspirate was available for the whole cohort. Multiparametric flow cytometry MRD was performed using a leukemia-associated immunophenotype approach (8-color flow cytometry with a 8-antibody panel); the sensitivity level was 0.1% and MRD positivity was defined as any level of residual disease. Molecular analysis of MRD was performed using a mutation-specific RT-qPCR assay, with MRD levels expressed as percentage (ratio of the NPM1 copies to housekeeping gene ABL copies×100); the sensitivity level was 0.01% and MRD positivity was defined as any level above 0.01%. Combining the two methods, MRD was empirically divided into three levels: MRD^{high} (MRD detected by MFC or NPM1 ≥0.1%); MRD^{low} (MFC negative and NPM1 <0.1% but >0.01%); and $MRD^{negative}$ (MFC negative and NPM1 ≤0.01%). LFS and OS were estimated using the Kaplan-Meier method.

Results: At the time of alloHSCT, 23 patients were in first complete remission and 4 in second complete remission. The median age was 57 years (range 23-65). The proportion of patients achieving MRD negativity by molecular analysis was significantly lower than that of patients with MRD negativity by MFC (30% versus 74%, p<0.001). Sensitivity of molecular analysis was superior in comparison with MFC (90% versus 60%, p=0.0018). Concordance between the two methods was low (Cohen's kappa coefficient=0.13; p=0.15). No difference was observed in 3-year LFS and OS according to MRD negativity determined by molecular analysis and MFC, respectively. By combining the two methods, 15 patients had MRD^{high} (7 detected by MFC), 5 had MRD^{low}, and 7 had $MRD^{negative}$. Patients with MRD^{high} had a poor 3-year LFS as compared to patients with MRD^{low} and $MRD^{negative}$ (p=0.0078), while no significant difference was reported comparing MRD^{low} and $MRD^{negative}$ (p=0.3173) (Figure 1).

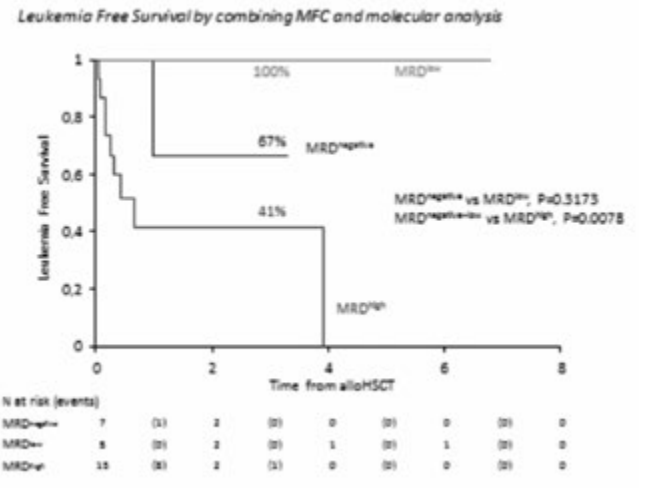


Figure 1.

Summary/Conclusion: Although molecular analysis resulted in a better sensitivity than MFC, pre-alloHSCT MRD negativity determined by molecular analysis and MFC showed similar 3-year LFS and OS. Achieving low-level or negative pre-alloHSCT MRD by combining MFC and molecular analysis is associated with an improved LFS compared to persistence of high levels.

PS992

MRD STATUS AT TRANSPLANTATION IN SECOND OR THIRD CR IS THE STRONGEST FACTOR AFFECTING THE PROBABILITY OF RELAPSE IN AML PATIENTS

F. Guolo[1,*], P. Minetto[1], M. Clavio[1], N. Colombo[1], A. Kunkl[2], S. Matarese[1], E. Carminati[1], A. Todiere[1], D. Guardo[1], C. Di Grazia[3], A. M. Raiola[3], F. Ballerini[1], M. Miglino[1], E. Angelucci[3], R. M. Lemoli[1], M. Gobbi[1]

[1]Clinic of Hematology, Department of Internal Medicine (DiMI), University of Genoa, Policlinico San Martino, IRCCS per l'Oncologia, [2]Unit of Flow Cytometry, Department of Pathology, [3]Hematology Unit and Bone Marrow Transplantation, Policlinico San Martino, IRCCS per l'Oncologia, Genoa, Italy

Background: Allogeneic stem cell transplantation (HSCT) offers the greatest chance of cure for patients with acute myeloid leukemia (AML). Minimal residual disease status (MRD) before HSCT has been reported to predict relapse risk in patient transplanted in 1st complete remission (CR1). However, the role of pre-transplant MRD in AML patients undergoing HSCT beyond first CR has not been clearly established.

Aims: To analyze the role of pre-HSCT MRD in predicting post-transplant relapse risk in AML patients transplanted beyond 1st CR; to analyze whether MRD status at 1st CR influences the probability of achieving a subsequent MRD negative CR.

Methods: We retrospectively analyzed the outcome of 92 consecutive AML patients receiving allo-ASCT in 2nd or 3rd CR. MRD was evaluated on pre-HSCT marrow samples by multicolor flow cytometry (MFC) and WT1 expression. Pre-HSCT marrow samples were analyzed for MFC and WT1 expression for MRD evaluation. Median age at transplant was 45 years

(range 18-62). Disease status was CR2 in 63 (68%) and CR3 in 29 patients (32%). ELN risk at diagnosis was low in 28 (30%), intermediate (INT) in 44 (48%) and high in 20 (22%) patients. Sixty-six (71%) received myeloablative conditioning, whereas 26 patients (29%) received reduced intensity conditioning. Stem cell source was HLA-identical sibling in 18 (20%), haploidentical (HAPLO) in 24 (26%) and alternative donor in 50 (54%). Median follow-up was 64 months (95% CI 39.8 – 88.2 months). A positive MFC MRD was defined by the presence of at least $1x10^{-3}$ residual leukemic cells by four or eight (since 2011) color flow-cytometry. WT1 copy number/Abl copy number $250x10^4$ was used as cut-off value.

Results: Relapse occurred in 30 patients (33%). Three-year overall survival (OS) was 47.9% (median 19 months). The survival probability was significantly affected by donor source (better for HAPLO, p<0.05), ELN risk at diagnosis (better for ELN low risk, p<0.01), MRD status before HSCT measured with any method (p <0.01 for WT1-based MRD, p<0.03 for MFC based MRD) and was better for CR2 compared to CR3 patients (p<0.05). Pre-HSCT MRD negative patients showed a lower rate of relapse regardless of risk at diagnosis (2-years OS of 62.2% and 52.7% among MFC MRD negative patient with ELN risk low vs INT/high, Figure 1). In competitive risk analysis MRD evaluation with any method was a strong predictor of cumulative incidence (CI) of relapse (p<0.01 and p<0.03, respectively, for WT1 and MFC MRD). Multivariate analysis showed that donor source and MRD evaluation both significantly influenced CI of relapse (p <0.05 and <0.01, respectively). Interestingly, among 30 INT patients transplanted in 2nd or further CR, all 13 patients who were MRD negative after 1st induction, were able to obtain a 2nd MRD negative CR before HSCT. In this cohort, MRD negativity after induction was the strongest factor related to the probability of achieving a 2nd MRD negative CR.

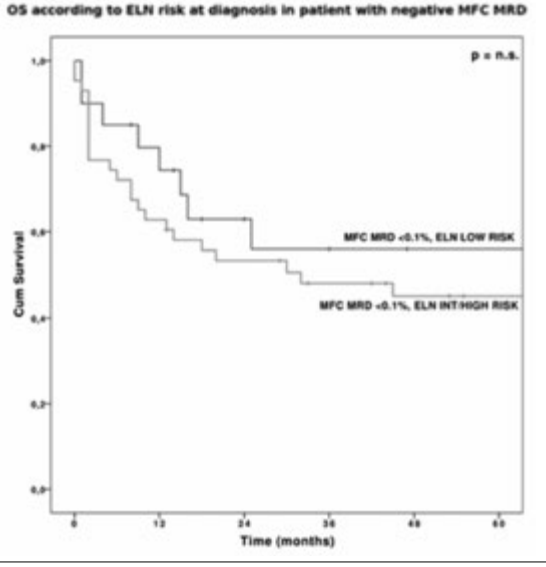


Figure 1.

Summary/Conclusion: Pre transplant MRD evaluation by both MFC and WT1 is a reliable predictor of relapse risk overcoming ELN risk assessment at diagnosis. Pre-HSCT MRD negative patients have a significantly lower risk of relapse. Furthermore, our result suggests that HAPLO donor source has a positive impact in reducing relapse rate in advanced AML patients. Our data show that all non-transplanted INT patients relapsing after the achievement of a 1st MRD negative were able to obtain a 2nd MRD negative CR. Postponing HSCT in INT patients with optimal response to frontline induction may be therefore a safe option.

PS993

THE CXCR4 INHIBITOR BL-8040 IN COMBINATION WITH CYTARABINE RESULTS IN A SIGNIFICANTLY EXTENDED OVERALL SURVIVAL OF RELAPSED/REFRACTORY AML PATIENTS

G. Borthakur[1,*], M. S. Tallman[2], Y. Ofran[3], J. Foran[4], G. Uy[5], J. Dipersio[5], A. Nagler[6], J. Rowe[7], M. M. Showel[8], J. K. Altman[9], A. Peled[10], M. Abraham[10], Y. Pereg[11], A. Vainstein[11], G. Oberkovitz[11], T.M. Lustig[11], S. Brawer[11], O. Bohana-Kashtan[11], J. E. Cortes[1], M. Andreef[1]

[1]MD Anderson Cancer Center, Houston, [2]Memorial Sloan Kettering Cancer Center, New York, United States, [3]Rambam Medical Center, Haifa, Israel, [4]Mayo Clinic, Jacksonville, [5]Washington University of St Louis, St Louis, United States, [6]Sheba Medical Center, Ramat Gan, [7]Shaarei Tzedek Medical Center, Jerusalem, Israel, [8]Johns Hopkins University, Baltimore, [9]Northwestern University, Chicago, United States, [10]The Hebrew University of Jerusalem, Jerusalem, [11]BioLineRx, Modiin, Israel

Background: CXCR4 plays a key role in the retention and survival of human acute myeloid leukemia (AML) blasts in the bone marrow (BM) microenvironment. Interaction of AML blasts with the leukemic microenvironment is considered an important mediator of resistance to chemotherapy, disease relapse and poor survival. Blockade of the CXCR4/CXCL12 pathway using the high-affinity CXCR4 antagonist BL-8040 interrupts this interaction, thereby mobilizing and increasing the sensitivity of leukemic blasts to chemotherapy as well as inducing a direct anti-leukemic effect.

Aims: To assess the safety and efficacy of BL-8040 combined with high-dose Cytarabine (HiDAC) in adult patients with relapsed/refractory (r/r) AML.

Methods: A Phase IIa study (NCT02073019) consisting of two cohorts: (i) dose-escalation (3+3 design, 6 escalating doses, range 0.5-2.0 mg/kg) and (ii) expansion at the selected dose of 1.5 mg/kg. Patients with r/r AML (salvage 1/2) were treated daily with BL-8040 monotherapy (SC) for two days followed by combined administration of BL-8040 and HiDAC (IV; 1.5g or 3.0 g/m^2/d,
based on age) for 5 days for 1-2 cycles. Efficacy endpoints included response rate (CR/CRi), overall survival (OS), duration of response (DOR) and event free survival (EFS).

Results: 42 patients, median age 61 (33-75) years were enrolled; 19 patients (45%) were refractory and 23 (55%) were relapsed. Overall, 26% (11/42) had secondary AML (from prior MDS), and 17% (7/42) were patients that relapsed after stem-cell transplantation. 81% (34/42) of patients had high-risk features that included primary refractory to one or two inductions or previous CR duration of less than 12 months. Twelve subjects (28.6%) were refractory to a single induction and seven were refractory to 2 or more inductions. Eight subjects relapsed after a complete response that lasted less than a year after diagnosis and primary induction and seven relapsed after a complete response for less than a year after salvage chemotherapy following first relapse. Median follow-up time was 213 days (1-958). BL-8040 in combination with HiDAC was safe and well tolerated. The CR/CRi rate for all dosing levels was 29% (12/42) and median OS (Kaplan-Meier estimate) was 9.1 months with 1-year and 2-year survival rates of 32.4% and 18.9%, respectively. In patients receiving the dose selected for expansion, 1.5 mg/kg (23 patients, 54.8%), the response rate (CR/CRi) was 39% (9/23) and median OS was 9.2 months with 1-year and 2-year survival rates of 31.6% and 21.1%, respectively. Median OS for responding (9/23 with CR/CRi) patients at the 1.5 mg/kg dose was 16.7 months, with 1- and 2-year survival rates of 50% and 37.5%, respectively. Median DOR for these patients was 10.1 months and EFS at 1 year and 2 years was 37.5% and 12.5%, respectively.

Summary/Conclusion: This study demonstrates that BL-8040 administered at a dose of 1.5 mg/kg in combination with HiDAC significantly improved overall survival of r/r AML patients compared with historical data[1] (overall survival of 6.1 months) for HiDAC alone. This supports continued clinical development of BL-8040 in frontline and early salvage AML patients.

Reference

1. Ravandi et. al., Vosaroxin plus cytarabine versus placebo plus cytarabine in patients with first relapsed or refractory acute myeloid leukaemia (VALOR): a randomised, controlled, double-blind, multinational, phase 3 study. Lancet Oncol. 16: 1025 (2015).

PS994

CELL-FREE DNA MONITORING OF MINIMAL RESIDUAL DISEASE IN AML USING A TARGETED NGS GENE PANEL

Z. Xie[1,*], L. Chamberlain[1], A. Carson[1], V. McClain[1], O. Kiya[1], B. Patay[1], M. Blankfard[1], T. Stenzel[1], J. Miller[1,2]

[1]Invivoscribe Inc., [2]LabPMM LLC, San Diego, United States

Background: We have developed a sensitive NGS gene panel (MyMRD™), which identifies pathogenic variants in acute myeloid leukemia (AML) establishing remission status. This panel targets mutation hotspots in 23 genes associated with AML. It identifies driver mutations that cause relapse in >90% of all AML patients, as well as common drivers in other myeloid neoplasms (MPN) and myelodysplasic syndromes (MDS). We have leveraged data from literature that suggest cell-free DNA (cfDNA) isolated from blood plasma of cancer patients contains tumor-derived DNA fragments with a molecular profile similar to that of bone-marrow tumor cells, and that DNA obtained from plasma provided a more accurate assessment of disease burden than testing circulating leukocytes, and results correlated

with disease burden. Therefore we set out to investigate whether the MyMRD assay, originally developed for genomic DNA analysis, could be applied to cfDNA to assess mutations at a level comparable to testing of genomic DNA and developed the MyMRD cfDNA assay for characterization and residue disease monitoring of targeted variants.
Aims: To establish a sensitive, reliable, targeted NGS gene panel assay for cell-free DNA to detect and monitor at least one driver mutation in >90% of acute myeloid leukemias.
Methods: Cell-free DNA was extracted from fresh, frozen, and synthetic plasma using commercially available kits. To overcome the limitation of testing cfDNA, DNA fragments with size similar to cfDNA were generated for initial feasibility studies during assay development. Genomic DNA was fragmented by sonication and the DNA fragments were selected for size in the range of 50- 400bp (mean size ~160bp). Whole genome libraries, generated from cfDNA and DNA fragments, were hybridized with MyMRD probes. Enriched libraries were sequenced with the MiSeq®. Sequencing data was analyzed using proprietary Invivoscribe MyInformatics™ software.
Results: The linearity and limit of detection (LOD) of the MyMRD cfDNA assay were assessed using data obtained from DNA fragments generated from contrived cell lines with a range of variant allele frequencies (VAFs). The assay shows strong linearity (R^2=0.975 – 0.998) in the entire range of VAFs (0.1– 20%) tested. With DNA input of 25ng, the LOD was established to be 0.5% for targeted SNV sites, and 1% for targeted indel variant sites. When the assay was applied to plasma reference samples, mutations were successfully identified with expected VAFs. The cfDNA extracted from clinical samples, fresh or frozen plasmas, were also tested with the MyMRD cfDNA assay and quality data were generated.
Summary/Conclusion: This MyMRD gene panel is a sensitive, reliable assay that provides monitoring of residual disease using cfDNA. The assay is shown to detect clinically important driver variants and has excellent linearity and LOD for targeted variant sites. This assay can potentially replace invasive BM sampling and provide an alternative test for longitudinal genetic monitoring of patients receiving targeted therapy.

PS995

INTRA-PATIENT DOSE-ESCALATION STUDY OF CRENOLANIB MAINTENANCE THERAPY IN PATIENTS WITH FLT3 MUTANT AML WHO HAD UNDERGONE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT (ALLO-HSCT)

B. Oran[1,*], S. Ciurea[1], D. Marin[1], J. McCarty[1], Q. Bashir[1], S. Ahmed[1], A. Olson[1], U. Popat[1], Y. Nieto[1], P. Kebriaei[1], E. Shpall[1], T. Agrawal[2], R. Champlin[1]

[1]Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, [2]AROG Pharmaceuticals, Inc., Dallas, United States

Background: FLT3 inhibitors (like sorafenib and midostaurin) have been administered as maintenance therapy post allo-HSCT to reduce persistent relapse risks in FLT3-mutant AML patients. Recent studies suggest that inhibition of FLT3 can downregulate dendritic cell proliferation and increase cytotoxic $CD8^+$ T-cell function in allo-HSCT recipients thus increasing graft-versus-leukemia (GVL) effect (Lau *et al. J. Exp. Med.*, 2016). Some FLT3 inhibitors including crenolanib have shown increased IL-15 production resulting in elevated GVL activity, thus reducing the risk of post-transplant relapse (Mathew *et al. Nat. Med.*, 2018).
Only reduced doses of both sorafenib and midostaurin have been found to be tolerable in the post-HSCT setting. Crenolanib is a highly potent and selective FLT3-targeted TKI that has activity as a single-agent and combined with chemotherapy in patients with FLT3-ITD and/or FLT3-TKD mutations. Given the favorable safety profile of crenolanib at 100 mg TID (300 mg daily) and promising clinical benefits in AML patients, crenolanib has been used as maintenance therapy in the post-transplant setting. We here report the outcomes of safety and tolerability of crenolanib maintenance in FLT3 mutant AML patients with prior allo-HSCT (NCT02400255).
Aims: To assess the tolerability of crenolanib maintenance in the post allo-HSCT AML patients and evaluate the appropriate dose for such patient population.
Methods: A study of crenolanib maintenance therapy was performed in patients (age ≥18) with FLT3 mutant AML who had undergone HSCT. Initially, patients were treated with 80 mg TID (240 mg daily) which was subsequently changed to an intra-patient dose-escalation, in which patients received crenolanib starting at a dose of 60 mg BID for a month and then escalated to 80 mg BID and finally 80 mg TID as tolerated. Regular assessment of chemistry and hematologic laboratory values were performed to ensure safety of crenolanib in this patient population.
Results: 21 patients, who received a variety of prior treatments and graft sources, have been enrolled to date. 4 patients received an initial dose of 80 mg TID, but due to early tolerability issues dosing was changed to an intra-patient escalation, after which crenolanib was well tolerated at the initial dose of 60 mg BID as well as the next dose level of 80 mg BID. 16 patients have discontinued treatment: 7 due to patient choice, 5 due to disease progression and 4 due to investigator decision. Of 5 progressions, 4 were MRD positive prior to HSCT and 2 had received haplo transplant, with a median time to progression of 17 days (range: 7-29 days). Side effects were predominantly grade 1 and 2 with the most common (regardless of attribution) being nausea (62%), vomiting (38%), and diarrhea (33%). Of the 6 patients escalated to 80 mg TID, 4 tolerated multiple cycles. Currently, five patients remain on treatment and the median duration of survival follow-up is 433 days (Table 1).

Table 1.

Patient Characteristics	n = 21
Age, median (range)	54 (31-74)
FLT3 mutational status	
FLT3-ITD	16
FLT3-ITD+/NPM1+	8
FLT3-ITD+/NPM1-	5
FLT3-TKD	5
Disease status prior to HSCT	
CR	7
CRi	11
Residual disease	2
Unknown	1
Conditioning regimen	
Myeloablative	15
Reduced intensity	5
Unknown	1
Donor type	
Match-related donor	9
Match-unrelated donor	10
Haploidentical	2
Crenolanib start date post-HSCT, days, median (range)	80 (50-98)
Days on maintenance, median (range)	208 (4-699)

Summary/Conclusion: These interim results suggest that crenolanib can be safely given at a dose of 160 mg to 240 mg total daily in the post-HSCT setting. Two randomized phase III trials have been initiated to investigate the efficacy of crenolanib with chemotherapy vs chemotherapy alone in R/R FLT3 mutated AML as well as crenolanib vs midostaurin following chemotherapy in newly diagnosed FLT3 mutated AML (NCT03250338, EudraCT 2017-001600-29; NCT03258931). Based on these results, post HSCT crenolanib maintenance will be offered at 100 mg BID (200 mg daily) in both trials.

PS996

CONSOLIDATION THERAPY WITH PONATINIB IN COMBINATION WITH CYTARABINE IN PATIENTS FOR FLT3-ITD ACUTE MYELOID LEUKEMIA IN FIRST COMPLETE REMISSION

P. Rousselot[1,2,3,*], T. Leguay[3,4], A. Vekhoff[3,5], T. Cluzeau[3,6], E. Lengline[3,7], H. Farhat[1], D. Caillot[3,8], M. Hunault[3,9], J. V. Malfuson[3,10], S. Chantepie[3,11], E. Tarvernier[3,12], B. Gruson[3,13], F. José[14], P. Peterlin[15], C. Preudhomme[3,16], H. Dombret[3,7], A. Pigneux[3,17]

[1]Centre Hospitalier de Versailles, Le Chesnay, [2]INSERM U[1173]Université Versailles Saint-Quentin-en-Yvelines, Université Paris-Saclay, Paris Saclay, [3]French AML group, Paris, [4]Centre Hospitalier Haut-Lévêque, Pessac, [5]Hôpital Saint-Antoine, Paris, [6]Hôpital Larchet, Nice, [7]Hôpital Saint-Louis, Paris, [8]CHU de Dijon, Dijon, [9]CHU d'Angers, Angers, [10]Hôpital d'Instruction des Armées, Percy, [11]Centre Hospitalier Côte-de-Nacre, Caen, [12]CHU de Saint-Etienne, Saint-Etienne, [13]CHU d'Amiens, Amiens, [14]CH de Valenciennes, Valenciennes, [15]CHU de Nantes, Nantes, [16]Hôpital Huriez, Lille, [17]Hôpital Haut-Lévêque, Pessac, France

Background: Ponatinib is a third-generation tyrosine kinase inhibitor targeting the BCR-ABL tyrosine kinase domain. Despite low activity against FLT3 based on the IC50 value (FLT3 IC50: 12.6 nM), ponatinb has been reported to have significant activity against the MV4-11 cell line which harbors an FLT3-ITD activating mutation. Primary blasts from acute myeloid leukemia (AML) patients were also tested and ponatinib reduced their viability whereas no activity was shown on FLT3-ITD-negative blast cells.
Aims: We aim to combine ponatinib with cytarabine consolidations in FLT3-

ITD AML patients in first complete remission.
Methods: AML patients in first CR were eligible after signed informed consent if they had intermediate risk caryotype with FLT3-ITD allelic ratio >10%, normal pancreatic and hepatic functions, low cardiovascular risk, QTc > 470 ms and no CNS involvement. Patients aged 18-60y received high-dose ARA-C (HDAC, 3 g/m²/12h at D1, D3 and D5, 2 courses, cohort A) and patients aged over 60y received intermediate-dose ARA-C (IDAC, 1.5 g/m²/12h D1, D3 and D5, 3 courses, cohort B). Ponatinib was given continuously during the consolidation phases. Three dose levels of ponatinib were tested following a classical phase I – II dose escalation schedule (3-3-3) in each age group: 15 mg/d, 30 mg/d and 45 mg/d. Allogenic hematopoietic stem cell transplant was allowed for eligible patients. Primary end-point was dose limiting toxicity, secondary end-points were OS, RFS.
Results: Forty patients were included from April 2014 to October 2017, 24 in cohort A (median age 51.5y) and 16 in cohort B (median age 64y). Dose escalation of ponatinib in cohort A resulted in 1 dose limiting toxicity (DLT) (cardiac failure) at 45 mg/d. Thus, the recommended ponatinib daily dose was 30 mg/d for the remaining 13 patients and no further DLT was recorded. Nineteen patients received ponatinib during the second consolidation and 8 patients were allografted. Eight patients relapsed all during the first 15 months post inclusion. Kaplan-Meier (KM) estimate for OS at 12 and 24 months were 71.3% (95% CI: 44.1-86.9) and 59.4% (95% CI: 33.1-78.3) respectively. Median EFS was 23.8 months. KM estimate for EFS at 12 and 24 months were 51.7% (95% CI: 27-71.6) and 43% (95% CI: 18.8-65.3) respectively. Dose escalation of ponatinib in cohort B resulted in 2 DLTs at 30 mg/d (atrial fibrillation and pulmonary embolism). Thus, the recommended ponatinib daily dose was 15 mg/d for the remaining patients and only 1 delayed hematopoietic recovery was further observed. Twelve patients received ponatinib during the second consolidation and 2 patients were allografted. Five patients relapsed all during the first 9 months post inclusion. Median OS was 14.2 months. Kaplan-Meier (KM) estimate for OS at 12 and 24 months were 63% (95% CI: 32.3-82.8) and 36% (95% CI: 11.6-61.6) respectively. Median EFS was 14.2 months. KM estimate for EFS at 12 and 24 months were 52.5% (95% CI: 25.2-73.8) and 42% (95% CI: 17.4-68.3) respectively.
Summary/Conclusion: The combination of ponatinib and high or intermediate dose cytarabine is feasible and safe in patient with FLT3-ITD AML in first CR. The determined ponatinib daily dose is 30 mg/d in patients aged 18-60y and 15 mg/d for patients aged over 60y. Median overall survival was 21.7 months for all patients included in the study.

PS997

PHASE 1/1B STUDY OF PEVONEDISTAT (PEV) AS A SINGLE AGENT OR COMBINED WITH AZACITIDINE (AZA) IN EAST ASIAN PATIENTS (PTS) WITH ACUTE MYELOID LEUKEMIA (AML) OR MYELODYSPLASTIC SYNDROME (MDS)

S.-J. Wu[1,*], J.-W. Cheong[2], Y. Onishi[3], H. Iida[4], Y. Kobayashi[5], H.-J. Kim[6], T.-J. Chiou[7], X. Zhou[8], H. Faessel[8], Y. Yuan[8], F. Sedarati[8], D. V. Faller[8], A. Kimura[9], H. Handa[10]

[1]National Taiwan University Hospital, Taipei, Taiwan, Republic of China, [2]Severence Hospital, Yonsei University Health System, Seoul, Korea, Republic Of, [3]National University, Corporation Tohoku University, Tohoku, [4]National Hospital Organization, Nagoya Medical Center, Nagoya, [5]International University of Health and Welfare, Mita Hospital, Tokyo, Japan, [6]Chonnam National University, Hwasun Hospital, Hwasun, Korea, Republic Of, [7]Taipei Veterans General Hospital, Taipei, Taiwan, Republic of China, [8]Millennium Pharmaceuticals Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, MA, United States, [9]Takeda Pharmaceutical Company Limited, Osaka, [10]Gunma University Hospital, Maebashi, Japan

Background: PEV, a first-in-class NEDD8-activating enzyme inhibitor, has demonstrated synergistic activity with AZA in preclinical AML models. The clinical safety and tolerability profile of PEV administered alone or in combination with AZA was shown in phase 1/1b studies of Western pts with AML/MDS.
Aims: The primary objectives of this phase 1/1b trial (NCT02782468) were to assess safety/tolerability and pharmacokinetics (PK) of PEV as a single agent (PEV arm) or combined with AZA (PEV+AZA arm) and to determine the recommended phase 2/3 dose (RP2/3D) of PEV in the PEV+AZA arm in East Asian pts with AML/MDS. Response evaluation was a secondary objective.
Methods: This 3+3 dose escalation and expansion study enrolled adult East Asian pts with WHO-defined AML or higher-risk MDS (IPSS-R very high/high/intermediate risk). Pts receiving PEV had relapsed/refractory (R/R) AML/MDS and pts receiving PEV+AZA had R/R or untreated disease and were unfit for intensive chemotherapy. In the PEV arm, pts received PEV 25 or 44 mg/m² IV on days 1, 3 and 5 (21 day cycle). Pts in the PEV+AZA arm received PEV 10 or 20 mg/m² IV on days 1, 3 and 5 + AZA 75 mg/m² IV or SC on days 1–5 and 8–9 (28 day cycle). Treatment was given until progressive disease or discontinuation for any reason. Informed consent was provided. PK samples were collected pre- and post-dose at various timepoints on days 1 and 5, and post-dose on days 2, 3, 6 and 7 of cycle 1. Best overall response was assessed by investigators according to International Working Group (IWG) criteria for AML or modified IWG criteria for MDS.
Results: As of 1-Nov-2017, 20 pts were enrolled in East Asian countries (safety population): 10 received single-agent PEV (25 mg/m², n=3; 44 mg/m², n=7) and 10 PEV+AZA (PEV 10 mg/m², n=3; PEV 20 mg/m², n=7); overall median treatment exposure was 3 (range: 1–19) cycles. Median age was 75 (range: 47–84) years in the PEV arm and 67 (range: 59–76) years in the PEV+AZA arm. In each arm, 9 pts (90%) were male, 4 (40%) had *de novo* AML, 4 (40%) had secondary AML (including 3 pts in the PEV arm and 2 pts in the PEV+AZA arm who had AML secondary to prior MDS) and 2 (20%) had *de novo* MDS. Most pts had received prior chemotherapy (13/16 AML and 3/4 MDS pts). There was 1 pt with dose-limiting toxicities in cycle 1 of grade 3 atrial fibrillation and grade 3 tumor lysis syndrome in the PEV 20 mg/m² +AZA arm. PEV RP2/3D in the PEV+AZA arm was 20 mg/m², identical to the RP2/3D determined for Western pts. All pts experienced grade ≥3 adverse events (AEs) (drug related in 4 PEV and 8 PEV+AZA pts). Nine (90%) PEV pts and 5 (50%) PEV+AZA pts had serious AEs, and 3 (30%) pts in each arm discontinued study due to AEs. Common any-grade AEs are shown in the Table 1. Three on-study deaths were reported (not treatment related): 1 PEV pt (AML) and 2 PEV+AZA pts (pneumonia; acute kidney injury). PEV PK were consistent with those observed in Western pts. In the PEV+AZA arm, all 5 evaluable AML pts (1 *de novo*, 1 relapsed/refractory, and 3 secondary AML) responded to treatment and 1 MDS pt had marrow CR. Responses were not observed in the PEV arm.

Table 1.

	PEV arm			PEV+AZA arm			All pts
	25 mg/m²	44 mg/m²	Total	10 mg/m²	20 mg/m²	Total	
Any-grade AEs (incidence ≥25%), n (%)							
Evaluable pts	*n=3*	*n=7*	*n=10*	*n=3*	*n=7*	*n=10*	*N=20*
Constipation	1 (33)	3 (43)	4 (40)	2 (67)	4 (57)	6 (60)	10 (50)
Pneumonia	1 (33)	6 (86)	7 (70)	1 (33)	0	1 (10)	8 (40)
Febrile neutropenia	2 (67)	4 (57)	6 (60)	0	1 (14)	1 (10)	7 (35)
Nausea	1 (33)	3 (43)	4 (40)	1 (33)	2 (29)	3 (30)	7 (35)
Vomiting	1 (33)	1 (14)	2 (20)	1 (33)	4 (57)	5 (50)	7 (35)
Diarrhea	1 (33)	2 (29)	3 (30)	0	3 (43)	3 (30)	6 (30)
Fatigue	1 (33)	2 (29)	3 (30)	2 (67)	1 (14)	3 (30)	6 (30)
Stomatitis	0	4 (57)	4 (40)	1 (33)	1 (14)	2 (20)	6 (30)
Increased AST	1 (33)	1 (14)	2 (20)	1 (33)	2 (29)	3 (30)	5 (25)
Dyspnea	0	2 (29)	2 (20)	1 (33)	2 (29)	3 (30)	5 (25)
Insomnia	1 (33)	3 (43)	4 (40)	0	1 (14)	1 (10)	5 (25)
Platelet count decreased	0	1 (14)	1 (10)	1 (33)	3 (43)	4 (40)	5 (25)

AE, adverse events; AST, aspartate aminotransferase; AZA, azacytidine; PEV, pevonedistat; pts, patients.

Summary/Conclusion: Single-agent PEV treatment in East Asian pts with AML/MDS appeared to be tolerable, with similar safety and PK profiles to those of Western pts. Addition of PEV to AZA did not result in additional toxicity and PEV RP2/3D was identical to that of Western pts. Substantial objective clinical responses were seen in East Asian pts with AML treated with PEV+AZA and were also consistent with those of Western pts with AML.

PS998

ANALYSIS OF PHASE I AND PILOT PHASE II DATA REVEAL 2,000 MG/M2 AS THE OPTIMAL DOSE OF CPI-613 IN COMBINATION WITH CYTARABINE AND MITOXANTRONE FOR ELDERLY PATIENTS WITH RELAPSED OR REFRACTORY AML

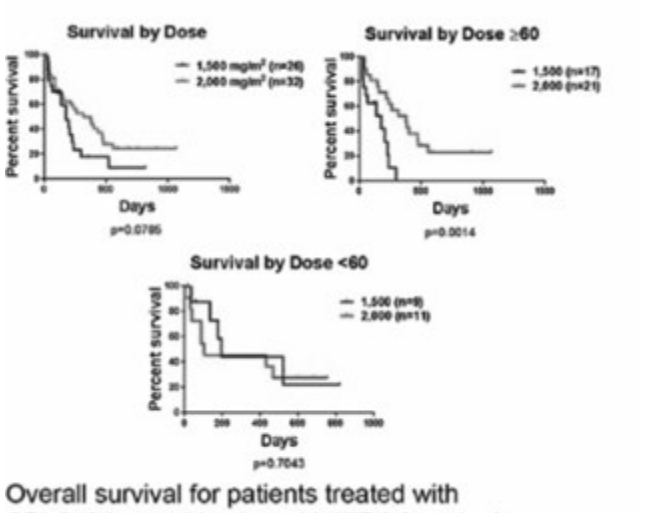
T. Pardee[1,2,*], S. Isom[3], K. Hu[2], L.W. Boteju[2], A.R. Boteju[2], R. Shorr[2], S. Luther[2], L. R. Ellis[1], D.S. Howard[1], R. Bhave[1], M. Manuel[1], S. Dralle[1], S. Lyerly[1], B.L. Powell[1]

[1]Internal Medicine, Section on Hematology and Oncology, Wake Forest Baptist Health, Winston-Salem, [2]Rafael Pharmaceuticals, Cranbury, [3]Department of Biostatistical Sciences, Wake Forest Baptist Health, Winston-Salem, United States

Background: CPI-613 is a first in class agent that inhibits pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. In a phase I trial for relapsed AML patients CPI-613 combined with high dose cytarabine (HiDAC) and mitoxantrone was particularly effective in elderly patients.
Aims: To determine the optimal dose of CPI-613, demographics, response, survival and PK data for various dosing cohorts from the pooled dataset of phase I and II studies were analyzed.
Methods: All patients included in the analysis were treated with identical doses and schedules of HiDAC and Mitoxantrone as published. Only the doses of CPI-613 varied.
Results: The two largest cohorts of CPI-613 were 1,500 (n=26) and 2,000 mg/m^2 (n=32). Demographics for each group are shown. Response rates (CR+CRi) were similar in patients treated with 2,000 or 1,500 mg/m^2 at 47% vs 50% (Table 1). However, the median overall survival for patients treated with 2,000 was 11.4 vs 5.9 months with a p value of 0.0785 (Figure 1). To determine which age group contributed to this difference, data from patients ≥60 years of age was analyzed independently. In this group the response rate for patients treated with 2,000 was 57% vs 35% (Table 1). Survival was significantly increased for patients treated with 2,000 at 12.4 vs 5.7 months (Figure 1). In contrast, there was no significant difference in younger patients (<60 years, Figure 1). Additionally, 24% of older patients treated with 2,000 went on to receive an allogeneic stem cell transplant compared to none treated with 1,500. PK data showed the Cmax values on day 5 of dosing 1,500 and 2,000 mg/m^2 were approximately dose-proportional at 30.4 (SD±13.8) and 38.0 (SD± 26.1) mM, respectively.

Table 1. Demographics and Response.

Age	All	All	≥60 y/o	≥60 y/o
Dose of CPI-613 (mg/m^2)	2,000 (n=32)	1,500 (n=26)	2,000 (n=21)	1,500 (n=17)
Demographics				
Male	56% (18/32)	58% (15/26)	66% (14/21)	71% (12/17)
Median Age (range)	62.5 (21-76)	64.5 (28-79)	68 (60-75)	68 (60-79)
Line of Salvage (range)	1 (1-4)	1 (1-3)	1 (1-2)	1 (1-3)
Refractory to Initial Therapy	25% (8/32)	31% (8/26)	29% (6/21)	18% (3/17)
Duration of CR1 (months)	9	8	10	7.5
Cytogenetic Risk				
Poor	44% (14/32)	46% (12/26)	43% (9/21)	41% (7/17)
Intermediate	47% (15/32)	50% (13/26)	48% (10/21)	49% (10/17)
Good	9% (3/32)	0% (0/26)	9% (2/21)	0% (0/17)
Unknown	0% (0/32)	4% (1/26)	0% (0/21)	0% (0/17)
Outcomes:				
Median Survival (months)	11.4	5.9	12.4	5.7
Response (CR+CRi)	47% (15/32)	50% (13/26)	57% (12/21)	35% (6/17)
Went on to Transplant	25% (8/32)	12% (3/26)	24% (5/21)	0% (0/17)



Overall survival for patients treated with CPI-613 in combination with HiDAC and mitoxantrone.

Figure 1.

Summary/Conclusion: These data suggest that there was a dose response relationship for CPI-613 when given in combination with HiDAC and mitoxantrone for older patients with relapsed or refractory AML. The results obtained using a dose of 2,000 mg/m^2 are encouraging in this poor prognostic group.

PS999

CLADRIBINE ADDED TO STANDARD INDUCTION CHEMOTHERAPY FOR ACUTE MYELOID LEUKAEMIA, 4-YEAR EXPERIENCES FROM SLOVENIA

S. Anžej Doma[1,*], M. Škerget[1], S. Zver[1]

[1]*Hematology, University Medical Centre Ljubljana, Ljubljana Slovenia*

Background: Polish Adult Leukemia group demonstrated that addition of cladribine to standard daunorubicin + cytarabine induction chemotherapy for acute myeloid leukaemia (AML) increases remission rate and prolongs overall survival. In Slovenia we have been using this induction protocol since 2014 for all patients eligible for intensive chemotherapy aged 65 or less.
Aims: The study was performed to assess the efficacy and possible drawbacks of the daunorubicin+cytarabine+cladribine induction protocol; data on our patients' characteristics, remission induction rate, relapse rate and overall survival are presented.
Methods: We included all consecutive patients diagnosed with AML between April 2014 und February 2018, aged 65 or less, who were eligible for intensive chemotherapy according to ECOG performance status, echocardiography and spirometry. Exclusion criteria were severe organ impairment and acute promyelocytic leukaemia. The induction regimen consisted of daunorubicin 60 mg/m^2 on days 1,3 and 5, continuous infusion of cytarabine 200 mg/m^2 from day 1 to 7 and cladribine 5 mg/m2 in a 3-hour infusion on days 1 through 5. Consolidation treatment consisted of 2 or 3 courses of cytarabine and eligible patients with unfavorable characteristics (monosomal/complex kariotype, FLT3 ITD or KMT2A rearrangements, therapy-related AML) with a sibling or matched unrelated donor proceeded with alogenic bone marrow transplantation. The efficacy of induction treatment was assessed by bone marrow aspiration in the aplastic phase, preferably between days 7 and 10 after the last day of induction chemotherapy, and before the first consolidation therapy.
Results: 69 patients with AML (35 women, 34 men) were treated with daunorubicin+cytarabine+cladribine induction chemotherapy at our hematology department between April 2014 and February 2018. Median age was 55 years (range 20 to 65 years). 38/69 (55%) patients had unfavorable cytogenetics or therapy-related AML. 53/69 (77%) patients achieved remission after induction chemotherapy. With a median follow-up of 11 months (1-44 months), the probability of 1-year OS was 71%, and the median survival was not reached. 11 patients relapsed. There were no significant differences between patients with unfavorable characteristics versus the others. The most serious side effect, attributed to cladribine, was noncardiogenic pulmonary oedema, observed in 5/69 (7%) patients during the induction chemotherapy (Figure 1).

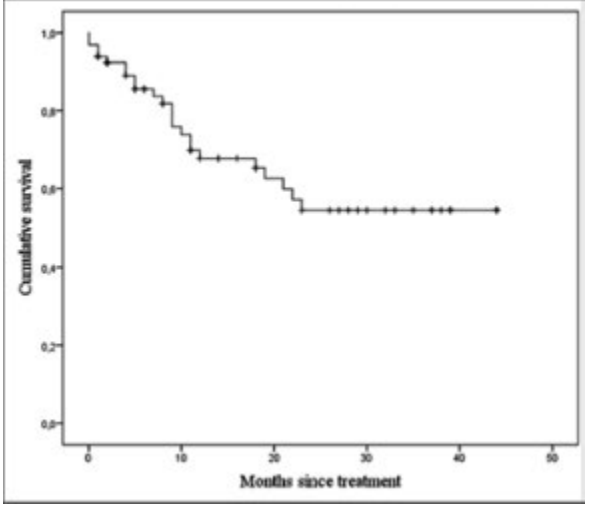


Figure 1.

Summary/Conclusion: Induction chemotherapy for AML, consisting of daunorubicin, cytarabine and cladribine results in a high remission rate (77%) and probability of 1- year OS of 71%. For predicting a long-term overall survival, further follow up is needed.

PS1000

DECITABINE AS SINGLE AGENT FOR TREATMENT OF NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA (AML) IN ELDERLY PATIENTS: A RETROSPECTIVE, MULTICENTER REAL LIFE STUDY OF THE "RETE EMATOLOGICA PUGLIESE" (REP)

M. P. Loglisci[1,*], C. Buquicchio[2], P. Carluccio[3], G. Guaragna[4], G. Rossi[5], G. Greco[6], L. Aprile [7], G. Tarantini[2], G. Specchia[3], D. Pastore[4], N. Cascavilla[5], V. Pavone [6], P. Mazza[7], N. Di Renzo[1]

[1]Hematology, PO "V.Fazzi" , Lecce, [2]Hematology, PO "Mons. Dimiccoli", Barletta, [3]Hematology, AO Policlinico, Bari, [4]Hematology, PO "Di Summa", Brindisi, [5]Hematology, IRCCS "Casa Sollievo della Sofferenza", S.Giovanni Rotondo -FG, [6]Hematology, Ospedale Cardinale Panico, Tricase - Lecce, [7]Hematology, PO "G. Moscati", Taranto, Italy

Background: Standards of care for elderly AML patients unfit for intensive chemotherapy remain still to be defined. Decitabine, a DNA hypomethylating agent, has been recently approved for use in older adults with AML not eligible for standard chemotherapy and is now widely accented as standard treatment. However, its efficacy has not yet been assessed in a real life setting.
Aims: The aim of present study was to evaluate efficacy and toxicity of decitabine given as single agent in unselected previously untreated elderly patients with AML *de novo* or secondary/therapy-related to MDS.
Methods: One hundred and thirty-five patients with *de novo* (n=62) or secondary/therapy-related (n=73) AML were diagnosed between Sep 2013 and Jan 2018 in seven hematological departments of the "Rete Ematologica Pugliese". Patient was included in this study if 60 years or older, diagnosis of *de novo* or secondary/therapy-related AML and if treated with decitabine in a schedule of 20 mg/m^2 for five days every 4 weeks. Patients previously treated with HMA were not included. The median age was 76 years (range, 63 to 89 years) and 84 (62%) male. The median baseline BMblast percentage was 52% (range,10-90), median hemoglobin level 7.4 g/dL (range, 5.3-12.0), WBC count 12.8 mL (range, 0.8-248) and platelet count 47.000 mL (range, 2.000-380.000). Baseline karyotype analysis was performed in 90 (66%) of cases and based on cytogenetic risk group 12 cases (13%) were classified as favorable, 50 (55%) as intermediate and 28 (31%) as poor-risk. Molecular genetic profile was performed at diagnosis in 83 (61%) of cases and mutation of FLT3 or NPM1 was found in 12 (14%) and 10 (12%) of patients respectively. One-hundred and twenty patients (88%) presented comorbidity including previous myocardial infarction (6%), COPD (8%), atrial fibrillation (6%), hypertension with or without cardiomyopathy (25%) and positive serology for B or C viruses (24%). The median number of cycles delivered was 4 (range, 1-23).
Results: Seventy-five (55%) patients received a minimum of four cycles and were evaluated for response. According to AML response criteria the complete response rate was 20% (n=15) and partial response 38% (n=29) with an overall response (CR+PR) rate of 58%; in addition an hematologic improvements and stable disease was recorded in 6 (8%) and 2 (3%) of patients respectively. One hundred and two (75%) patients were admitted to the hospital to start treatment or for treatment related toxicity. The median duration of hospitalization was 15 days (range, 5-92 days). All patients experienced myelosuppression; the most commonly reported serious adverse events were febrile neutropenia (57%), grade 3-4 documented infection (9%), major bleeding (10%), atrial fibrillation (5%) and pleural effusion (2%). After a median follow-up of 10 months (95% CI, 6-14 months) the median overall survival in the intent to treat population was 7.0 months (95% CI, 6-8 months) from the start of decitabine treatment (Figure 1). At 12-months 31% (95% CI, 21-41%) of patients included in the analysis are alive.

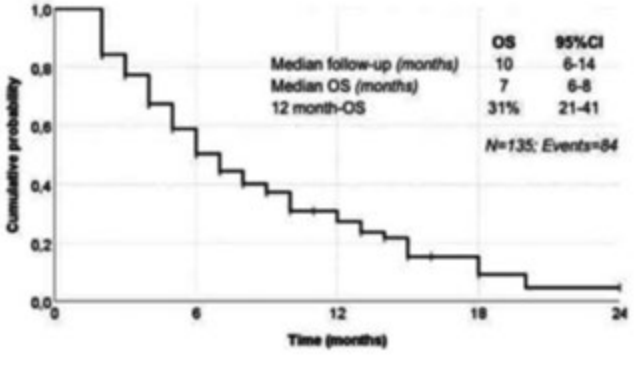


Figure 1.

Summary/Conclusion: The preliminary results of this retrospective, multicenter study of REP confirm, even in the real life setting, the reported ORR and OS rate of decitabine given as single agent for the treatment of elderly patients with AML ineligible for conventional chemotherapy.

PS1001

FLUDARABINE, HIGH DOSE CYTARABINE AND IDARUBICIN-BASED INDUCTION (FLAI) IS HIGHLY EFFECTIVE IN AML WITH MUTATED NPM1 WITH CONCOMITANT FLT3-ITD MUTATION IRRESPECTIVELY OF FLT3-ITD ALLELIC BURDEN

P. Minetto[1,*], A. Candoni[2], F. Guolo[1], M. Clavio[1], M. Miglino[1], N. Colombo[1], A. Kunkl[3], M. E. Zannier[2], M. V. Dubbini[2], D. Guardo[1], F. Ballerini[1], R. Fanin[2], R.M. Lemoli[1], M. Gobbi[1]

[1]Clinic of Hematology, Department of Internal Medicine (DiMI), University of Genoa, Policlinico San Martino, IRCCS per l'Oncologia, Genoa, [2]Division of Hematology and SCT, University of Udine, Udine, [3]Unit of Flow Cytometry, Department of Pathology, , Policlinico San Martino, IRCCS per l'Oncologia, Genoa, Italy

Background: The presence of FLT3 "internal tandem duplication" (FLT3-ITD) mutation is associated with poor prognosis in acute myeloid leukemia (AML). However, the concomitant presence of NPM1 mutation (NPM1-mut) mitigates the negative prognostic impact given by FLT3-ITD, which is also modulated by FLT3-ITD/wild type allelic ratio. NPM1 and FLT3 mutational status assessment is strongly recommended for risk stratification at diagnosis by the last ELN 2017 guidelines.
Aims: To investigate the efficacy of an intensive fludarabine, high dose cytarabine and Idarubicin based induction regimen (FLAI) as frontline treatment for fit, *de novo* AML patients according to NPM1 and FLT3-ITD mutational status.
Methods: One-hundred and one consecutive AML patients treated in two Hematology centers of Northern Italy were retrospectively included in this study: 21 carrying isolated FLT3-ITD, 22 concomitant FLT3-ITD and NPM1-mut and 58 isolated NPM-1 mut. Median age was 52 yrs (range: 17-68 years). All patients received FLAI induction (fludarabine 30 mg/sqm and ARA-C 2g/sqm on days 1 to 5 plus idarubicin 10 mg/sqm on days 1-3-5). Before 2017 patients with isolated FLT3-ITD mutation were scheduled to receive allogeneic bone marrow transplantation (BMT) in first complete remission. In most cases minimal residual disease has been evaluated on marrow samples using multicolor flow-cytometry (MFC) and NPM1 expression levels. Negative MFC-MRD was defined by the presence of less than 25 clustered leukemic cells/105 total events (threshold of 0.025% residual leukemic cells). NPM1 mutation (NPM1-A, B and D) was measured using Muta Quant Kit Ipsogen from Qiagen. FLT3-ITD allelic burden was available in 27/43 of FLT3-ITD patients.
Results: Two patients (2%) died of infections and two during the second induction course. Overall 60-days mortality was 4%. After two induction cycles, 87 patients achieved CR (87%). Thirty-three/101 (33%) CR patients underwent BMT in first CR. After a median follow up of 71 months, 3-year overall survival (OS) was 52.3% (median 65 months). OS duration was significantly longer in NPM1 mutated patients ($p < 0.05$) and in in patients achieving MRD-negative status after induction (both MFC and NPM1 based MRD) after induction ($p < 0.001$). Overall survival (OS) was comparable in patients with isolated NPM1 mutation and in those with concomitant NPM1/FLT3-ITD mutation (3-year OS 57.6% and 51.7%, respectively, p= n.s), regardless of FLT3-ITD allelic burden. Patients with isolated FLT3-ITD mutation had a significantly worse prognosis (3-year OS 38.7%, $p < 0.05$). Multivariate analysis showed that persistence of MRD (by any method, $p < 0.05$) was the strongest predictor of outcome. Interestingly, performing BMT in first CR did not impact OS both in univariate and multivariate analysis (Figure 1).

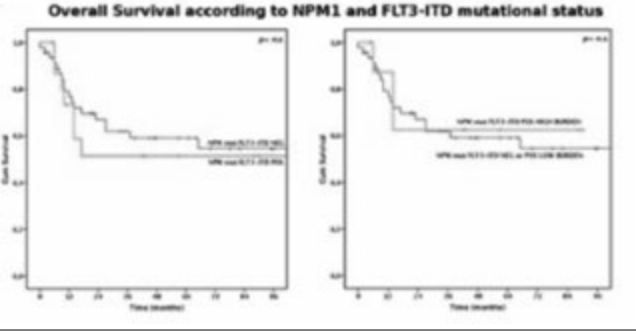


Figure 1.

Summary/Conclusion: Despite the potential bias due to the retrospective nature of the analysis, our data seem to indicate that FLAI displays a strong anti-leukemic efficacy in patients carrying NPM1 mutation irrespectively of FLT3-ITD mutational status. The synergism of fludarabine and cytarabine might increase the chemosensitivity of NPM1 mut leukemic cells, thus overcoming the negative impact of FLT3-ITD.

PS1002

CLINICAL CHARACTERISTICS AND PROGNOSIS OF 34 CASES OF ACUTE MYELOID LEUKEMIA WITH FLT3 INTERNAL TANDEM DUPLICATION AND MLL GENE REARRANGEMENT

J. Zhou[1], X. Zhang[1], Y. Zhao[1], J. Yang[1], J. Zhang[1], X. Cao[1], Y. Lu[1], D. Liu[1], F. Lv[1], J. Ouyang[1,*], P. Lu[1], D. Lu[1]

[1]Hematology, Hebei Yanda Ludaopei Hospital, Langfang Shanghe city Hebei Province, China

Background: The internal tandem duplication (ITD) of the juxtamembrane region of the FLT3 receptor tyrosine kinase gene and MLL gene rearrangement have been described in acute myeloid leukemia (AML) patients. These alterations have been associated with a poor prognosis. The data about the AML patients with both FLT3-ITD and MLL gene abnormalities is scarce. In our study, we retrospectively analysis of clinical features and prognosis in 34 AML patients with both FLT3-ITD and MLL gene abnormalities.
Aims: To analyze the clinical characteristics and prognosis of 34 cases of AML with FLT3 internal tandem duplication(FLT3-ITD) and MLL gene rearrangement.
Methods: The clinical data of 34 AML patients with FLT3-ITD and MLL gene rearrangement was compared and analyzed for the therapeutic efficacy, influencing factors when treated with chemotherapy, chemotherapy combined with targeted therapy or allogenic hematopoietic stem cell transplantation (allo-HSCT).
Results: Of the thirty-four cases with median age 41 (4-71), 63.6% presented with white blood cells (WBC) greater than 30×10^9/L , 39.4% greater than 50×10^9/L respectively on admission. M5 (35.3%) made up the highest proportion. The cytogenetic abnormality reached 63.64%, of which the complex cytogenetic abnormality accounted for 12.12%. Eleven patients (32.35%) had both FLT3-ITD and MLL gene abnormalities. In addition to FLT3 and MLL abnormalities, 23 patients (67.65%) had one or more other gene abnormalities (multiple gene abnormalities). Of the 34 cases, 29.4% patients went into complete remission (CR) after two courses of chemotherapy. 20.6% (7 patients) went into CR after 3 or more courses of chemotherapy. The rate of early recurrence in the CR of this group of patients was 52.9%. Patients with WBC> 50×10^9/L, cytogenetic abnormalities and multiple gene abnormalities had a lower remission rate (5.43% -19.04%) after two courses of chemotherapy. The CR rate for the patients with more than three gene abnormalities was 0.00%. The total 2-year overall survival (OS) in the 34 patients was 28.8% (95% CI 13.5% -46%) and the disease-free survival (DFS) was 27.1% (95% CI 12.5% -44%). Of the 18 patients treated with chemotherapy alone or chemotherapy combined with targeted therapy, 17 cases died within 2 years and 1 lost follow-up after giving up treatment. For the 16 patients received allo-HSCT, the three-year OS was 43.4% (95% CI 13.7% to 70.4%) and DFS 42.7% (95% CI 13.4% to 69.7%).
Summary/Conclusion: AML patients with FLT3-ITD and MLL gene rearrangement often presented with M5, accompanied by hyperleukocytosis, cytogenetic or multiple gene abnormalities. Those patients were observed to have low response rate and high early recurrence when treated with chemotherapy without allo-HSCT. Patients had multiple gene abnormalities may be an important poor prognostic factor. Allo-HSCT is an effective treatment which could significantly improve the prognosis and survival of AML patients with FLT3-ITD and MLL gene abnormalities.

PS1003

PROGNOSTIC RELEVANCE OF FLT3, NPM1, DNMT3A, IDH1/2 GENE MUTATIONS IN ACUTE MYELOID LEUKEMIA PATIENTS

E. Motyko[1,*], I. Martynkevich[1], O. Blau[2], L. Polushkina[1], L. Martynenko[1], M. Bakai[1], N. Cybakova[1], I. Ruzhenkova[1], E. Kleina[1], N. Pavlenko[1], A. Radzhabova[1], C. Baldus[2], I. Blau[2], S. Voloshin[1], A. Chechetkin[1]

[1]Russian Research Institute of Hematology and Transfusiology, Saint-Petersburg, Saint-Petersburg, Russian Federation, [2]Charité University School of Medicine, Berlin, Germany

Background: Acute myeloid leukemia (AML) is a heterogeneous disease characterized by differentiation arrest and uncontrolled clonal proliferation of immature hematopoietic precursors. The prognosis of AML is variable, partly based on clinical features (patient age, medical comorbidities, performance status) and also on underlying genetic characteristics (cytogenetic and molecular aberrations). The development of new classification strategy is an important step in understanding the molecular complexity of AML and also leads to new targeted therapy.
Aims: To analyze the prognostic effect of typical mutations in AML patients.
Methods: The study included 620 AML patients, treated in Saint-Petersburg and Berlin. Cytogenetic analysis was performed using standard GTG-method. Screening of mutations in genes *FLT3, NPM1, DNMT3A, IDH1/2* was realized by PCR, DNA fragment analysis and sequencing.
Results: Mutations were found in 343/620 (55.3%) AML patients and were more often detected in patients with normal karyotype (NK) (220/328 (67.1%)) (p=0.001). Single gene mutations were revealed in 199/343 (58.0%) patients. The presence of *FLT3*-ITD mutation was associated with adverse overall survival (OS) and relapse-free survival (RFS): 11.3 and 15.8 months (*FLT3*-ITD-), p=0.005; 10.0 and 13.3 months (*FLT3*-ITD +), p=0.009, respectively. Increased *FLT3*-ITD allelic ratio (≥0.5) correlated with low OS (p=0.028). Patients with single *NPM1* mutation reached significantly better OS and RFS compared to other patients (27.4 and 13.9 months, p=0.040, 19.3 and 12.0 months, p=0.049, respectively). The negative influence of *DNMT3A* gene mutations on OS was not significant: 12.0 (*DNMT3A*+) and 15.0 (*DNMT3A*-) months (p=0.112). The presence of *IDH1*+ mutation (R132) correlated with better OS (p=0.092), whereas polymorphism rs11554137 in *IDH1* gene - with worsening of outcome in the group of patients with NK (p=0.186). In 144 patients various combinations of 2 to 5 mutations were detected. It was found that mutations in *FLT3* (*FLT3*-ITD), *NPM1, DNMT3A* and *IDH2* genes were significantly more often detected in combination with other mutations, than alone (p=0.001). The most frequent combinations were: *NPM1+/FLT3*-ITD+ (20.8%), *NPM1+/FLT3*-ITD+/*DNMT3A*+ (8.3%) and *FLT3*-ITD+/*DNMT3A*+ (8.3%). Patients with single mutation had significantly longer OS compared with patients with 2 mutations (18.1 and 12.2 months, p=0.003). For patients with *NPM1*+ the most unfavorable OS was found in the presence of *FLT3*-ITD (27.4 and 9.2 months, p=0.019), and a combination of *FLT3*-ITD+/*DNMT3A*+ (27.4 and 14.6 months, p=0.141). Patients with *DNMT3A*+ tended to have lower OS in the presence of *FLT3*-ITD mutation (17.3 and 7.1 months, p=0.074).
Summary/Conclusion: Mutations in analyzed genes are frequent in intermediate risk group of AML patients. They significantly affect the prognosis. Our data support the importance of the type of mutation, its allele ratio and the presence of additional mutations. The presence of 2 mutations significantly reduces OS compared to patients with one mutation. The worst prognosis was found in patients with following mutations combinations: *NPM1+/FLT3-ITD+, NPM1+/FLT3*-ITD*+/DNMT3A+, DNMT3A+/FLT3-ITD+*. Complex analysis of genetic aberrations in AML patients provides the most accurate prognosis prediction and planning of targeted therapy.

PS1004

IMPACT OF BCL2 AND ABCG2 OVEREXPRESSION IN ACUTE MYELOID LEUKEMIA

M. Tiribelli[1,*], M. Cavallin[1], S. Di Giusto[1], E. Toffoletti[1], D. Lazzarotto[1], R. Fanin[1], D. Damiani[1]

[1]Division of Hematology and BMT, Azienda Sanitaria Universitaria Integrata di Udine, Udine, Italy

Background: ABCG2, the last discovered member of ABC transporter family, is frequently overexpressed in acute myeloid leukemia (AML). A high expression at diagnosis has been associated with increased AML relapse after conventional chemotherapy and after stem cell transplantation. In the last years the ABCG2 substrate spectrum has greatly increased, including so called "target drugs" directed against molecular markers of hematologic malignancies but often with non-univocal effects. Among these drugs interacting with ABCG2 there are also Bcl2 inhibitors, recently approved for lymphoproliferative disorders but under active investigation also in AML, where their use appears rationale due to the negative role of Bcl2 overexpression. Data on cell lines suggest that at least venetoclax could be an ABCG2 inhibitor, so potentially sensitizing cell to chemotherapeutics.
Aims: To evaluate the incidence of concomitant ABCG2 and Bcl2 overexpression in AML, to assess their impact on response to therapy and to possibly identify subsets of patients with the highest drug sensitivity.
Methods: ABCG2 and Bcl2 expression was evaluated by flow cytometry in 290 adult patients with non-promyelocytic AML. Logistic regression was employed to assess their association with other clinical and biological features and with response rate. Leukemia free survival (LFS) and overall survival (OS) in the different subgroups were compared by log-rank test.

Results: ABCG2 overexpression was found in 116/290 patient (40%) and Bcl2 was overexpressed in 190/290 (65%) patients. Eighty-one patients (28%) were double positive (DP), 65 (22%) were double negative (DN) and 144/290 (50%) expressed either ABCG2 or Bcl2. WBC count ad diagnosis was higher in DP patients (mean $52\pm78\times10^9$/L vs $30\pm35\times10^9$/L in other groups; p=0.04) and DN patients were younger (mean age 52±11 years vs 62±14 years in other groups; p=0.02). No patient with favorable karyotype was observed among DP cases compared to 16 patients in other groups (0% vs 18%; p=0.003). CD34-negativity was significantly more common in DN patients (32/56, 57% vs 92/224, 41%; p=0.03). No difference was observed in CD56 expression, frequency of Flt3-ITD mutation and NPM1 mutation. No difference in OS was observed according to Bcl2 and/or ABCG2 expression. Considering disease relapse, Bcl2 expression "per se" did not affect LFS in ABCG2 negative cases but have a negative impact in ABCG2 positive patients. So, we can identify 3 groups with significantly different LFS: 3-years LFS was 25% (95%CI: 9-40) in ABCG2+/Bcl2+ patients, compared to 46% (95%CI: 24-68) in ABCG2+/Bcl2- patients and 61% (95%CI: 48-74) in ABCG2- patients, irrespectively of Bcl2 (p=0.008) (Figure 1).

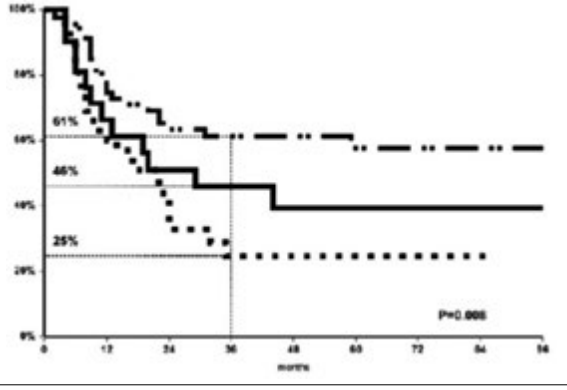


Figure 1. Leukemia free survival.

Summary/Conclusion: The effect of ABCG2 and Bcl2 concomitant overexpression in AML has never been described. Our data suggest an additive negative impact of Bcl2 overexpression in ABCG2 patients. Further *in vitro* data are needed to investigate the effect of Bcl2 inhibitors on ABCG2 efflux activity, to prove the hypothesized sensitizing action of Bcl2 inhibitors and to identify patients who may maximally benefit from combination therapy to reduce the risk of AML recurrence.

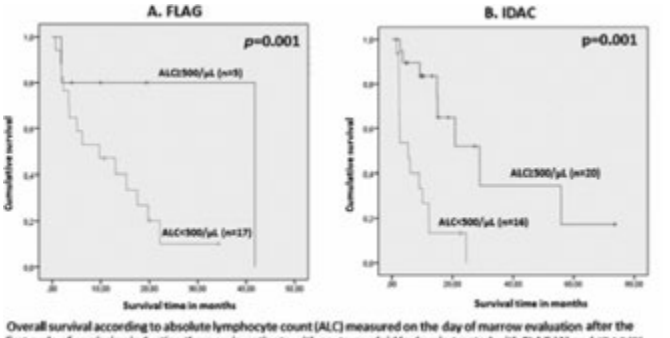
PS1005

ABSOLUTE LYMPHOCYTE COUNT RECOVERY AFTER INDUCTION REMISSION THERAPY IS A STRONG PREDICTOR OF OVERALL SURVIVAL IN ADULT ACUTE MYELOID LEUKAEMIA

J. Infante[1,*], H. Martins[1], S. Valle[1], G. Esteves[1], J. Raposo[1]

[1]Serviço de Hematologia e Transplantação de Medula Óssea, Centro Hospitalar Lisboa Norte - Hospital de Santa Maria, Lisboa, Portugal

Background: Acute Myeloid Leukaemia (AML) carries a dismal prognosis, with an estimated 5-year overall survival (OS) of 30%. Achievement of a complete response (CR) after remission induction therapy (IT) is an independent prognostic marker for OS in AML. Response criteria include evaluation of the peripheral blood for neutrophil and platelet recovery only, with no defined significance for lymphocyte recovery.
It has been proposed that an adequate absolute lymphocyte count (ALC) after IT may reflect a deeper marrow leukaemia cell burden reduction with restoration of hematopoietic function, and that these lymphocytes may exert an antileukemic effect and prevent serious infections. Whether lymphocyte recovery after IT has an impact on prognosis is unclear, and few studies have focused on the subject.
Aims: To determine the impact of ALC recovery after a first cycle of IT on OS in adults with newly diagnosed AML.
Methods: Between 2012 and 2017, 64 patients with previously untreated acute myeloid (non-promyelocytic) leukaemia received IT at our center – 36 received IDAC (idarubicin 12 mg/m^2×3 days plus cytarabine 100 mg/m^2×7 days) and 18 received FLAG (fludarabine 30 mg/m^2×5 days plus cytarabine 2 g/m^2×5 days). Patients who died during aplasia were excluded (n=10). Baseline patient characteristics and performance status, cytogenetic risk, type of treatment, response to treatment and the ALC on the day of morphologic marrow evaluation (between days 21-30 after first cycle of IT) were collected. All patients were followed until death or until the end of the observation period on February 22nd 2018.
In each treatment group, patients were separated according to the ALC: less than 500/µL (n=14 with FLAG, n=16 with IDAC) and 500/µL or more (n=4 with FLAG, n=20 with IDAC). Survival analysis with Kaplan-Meier curves was performed for each group and multivariate survival analysis using Cox's regression model was performed.
Results: Median age of this population was 62 years (range, 17-79). CR and CR with incomplete hematologic recovery rates were 36% and 19% in the IDAC group, and 28% and 17% in the FLAG group, respectively.
On univariate analysis, an ALC<500 cells/µL was significantly associated only with an ECOG performance status of 1 (p=0.01) versus 0 in the IDAC-treated group; there was no association with response to induction nor cytogenetic risk groups. Median follow-up time was 22.55 months. Overall, median survival was 15.32 months for FLAG-treated patients and 12.20 months for IDAC-treated patients (p=0.839). IDAC-treated patients had a median survival of 28.99 months if N>500/µL and 5.33 months if <500/µL. FLAG-treated patients had a median survival of 41.82 months if N>500/µL and 12.99 months if <500/µL (Figure 1). Difference between survival curves was statistically significant (log-rank test p<0.001), before and after adjusting for treatment protocol. After adjusting for high-risk cytogenetics, treatment protocol and achievement of a CR/CRi using a Cox Regression model, the only independent predictor of death was an ALC<500/µL after IT (HR 7.45, 95% CI 2.00-27.83).



Overall survival according to absolute lymphocyte count (ALC) measured on the day of marrow evaluation after the first cycle of remission induction therapy, in patients with acute myeloid leukemia treated with FLAG (A) and IDAC (B).

Figure 1.

Summary/Conclusion: An ALC<500/µL after the first cycle of IT independently predicts an inferior OS, independently of treatment with IDAC or FLAG. Adjusting for validated predictors of reduced survival in the literature, the ALC was still the only parameter to predict OS. These results suggest that the ALC after IT, a widely available and inexpensive parameter, could be useful in establishing prognosis early in the course of treatment of AML.

PS1006

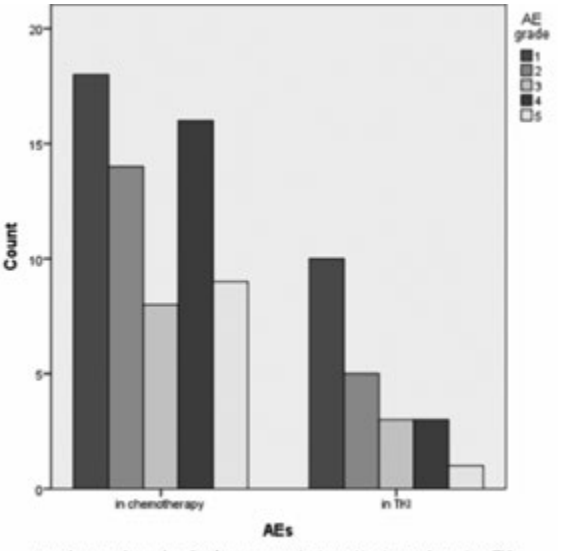
TYROSINE KINASE INHIBITORS (TKI) IN RELAPSED/REFRACTORY (RR) PATIENTS WITH FLT3-ITD POSITIVE ACUTE MYELOID LEUKEMIA (AML) CONFER BETTER SURVIVAL THAN CHEMOTHERAPY, DUE TO A BETTER SAFETY PROFILE

G. Marconi[1,*], S. De Polo[1], E. Ottaviani[1], S. Paolini[1], M.T. Bochicchio[1], J. Nanni[1], L. Bertamini[1], M. C. Abbenante[1], C. Sartor[1], S. Parisi[1], A. Talami[1], M. Olivi[1], S. Ragaini[1], M. Raffini[1], C. Baldazzi[1], N. Testoni[1], M. Cavo[1], A. Curti[1], G. Martinelli[1,2], C. Papayannidis[1]

[1]Istituto di Ematologia e Oncologia Medica "Lorenzo e Ariosto Seràgnoli", bologna, [2]Istituto Scientifico Romagnolo Per Lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy

Background: Approximately 20-30% of AML patients harbor internal tandem duplication (ITD) of FLT3 gene. FLT-ITD mutations are associated with a poor prognosis, due to a high relapse rate. Several drugs have been developed to inhibit FLT3. However, R/R FLT3-ITD AML patients still represent an unmet clinical need.
Aims: Since no prospective randomized studies comparing the role of chemotherapy and TKIs in R/R FLT3 ITD AML patients have been conducted, our aim is to assess outcome, safety and duration of hospitalizations in two retrospective groups of patients, referred to or diagnosed at our Institution, and treated with TKIs or chemotherapy, respectively.
Methods: We retrospectively collected and analyzed clinical and biological data of 58 consecutive FLT3-ITD AML patients, treated at our Institution

from 2004 to 2017 with chemotherapy (3+7 like regimens; 3+7 like regimens with the addition of a third agent; fludarabine based regimens) and/or single agent TKIs (Sorafenib, Ponatinib, Quizartinib, Gilteritinib, Midostaurin). All the patients underwent any kind of therapy after informed consent was signed.



number and grade of adverse events in patients undergoing TKI compared with patients treated with chemotherapy (p=.049).

Figure 1.

Results: We compared patients who received at least once in their life, as salvage treatment, a TKI inhibitor ("TKI" group; N=36) with patients that were treated exclusively with conventional cytotoxic ("conventional group"; N=22). The median age of the entire population was 59 years (range 17-74); there were no significant differences in patient age, white blood cells count, platelet count and ELN risk at diagnosis between the two groups. Fifty-one out of 58 patients (86%) relapsed after (N=22; 36%) or were refractory (N=29; 50%) to the first course of induction chemotherapy. Second-line therapy included salvage chemotherapy (N=32/51, 63%), a TKI as single agent (N=12/51, 23%) or best supportive therapy (N=14%, 7/51). Forty-one patients experienced a 2nd relapse, or were persistently refractory, and of these 18 received a single agent TKI as salvage treatment. Six patients received a TKI in 3rd or further relapse.Standard chemotherapy compared with TKIs did not show an increased efficacy in terms of CR (25% vs 16.7%), and it was not a better bridge-to-transplant option. However, among R/R patients, we observed an advantage in terms of OS for patients of the "TKI" group compared with "conventional" group (median OS from R/R of 10 months [95% CI, 5.89-14.12] and 4 months [95% CI, 3.12- 4.90], respectively; p= .017). Finally, as far as toxicity is concerned, patients in "TKI" group experienced a lower number of AEs during treatment with TKIs (1.63 mean AEs in each TKI line vs 3.03 mean AEs in each chemotherapy line, excluding stem cell transplant; p< .001; grade III-IV 6/23 and grade V 2/23 with TKI; grade III-IV 24/66 and grade V 12/66 with chemotherapy). Furthermore, AEs during TKI therapy were less severe if compared with AEs during chemotherapy (Figure 1, p= .049). We also noted a trend toward less day spent in hospital per month by patients during TKI treatment, compared to patients treated with standard chemotherapy (including post chemotherapy remission period): 10.5 days and 16.7 days in the two groups, respectively.

Summary/Conclusion: Our study, even if in a retrospective set, reports a survival advantage of TKI in R/R FLT3 ITD AML patients, compared with conventional approaches. Such an advantage is due to the lower number and grade of AEs of "TKI" group. For their safety profile, TKIs are probably a better option to bridge patients to transplant, thanks to a lower risk of toxicity. *Supported by: ELN, AIL, AIRC, FP7 NGS-PTL project; GM and SDP equally contributed*

PS1007

ADDITION OF BORTEZOMIB TO AML-LIKE TREATMENT FOLLOWED BY NMA ALLO-SCT IN PATIENTS WITH BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM

E. Reuben Tolley[1,*], E. Clasen-Linde[2], H. Sengeløv[1], L. Smidstrup Friis[1], I. M. Schjødt[1], P. Kampmann[1]

[1]Dept. of Hematology, [2]Dept. of Pathology, Rigshospitalet, Copenhagen O, Denmark

Background: Blastic plasmacytoid dndritic cell neoplasm (BPDCN) is an aggressive, rare hematopoietic malignancy derived from CD4+ CD56+ precursors of plasmacytoid dendritic cells. The skin is the most common site (64-100%) but involvement of the bone marrow and peripheral blood (60-90%) and lymph nodes (40-60%) is frequent. The BPDCN immunohistophenotype is characterized by the co-expression of CD4, (CD43, CD45RA,) and CD56 and associated antigens CD123, CD303 (BDCA2), TCLA1, CD2AP, SPIB, MT1 as well as negativity in regard to specific myeloid, T- and B-cell markers. Genetic profiling finds complex karyotypes with 2/3 of the patients but no specific chromosomal aberrations. Gene expression however shows noticeable alterations including aberrant activation of the NF-kappaB pathway. The prognosis is poor with a median survival of 10.0-19.8 months. Transplant-eligible patients are treated with typically standardized ALL or AML-regimes followed by allogenic stem cell transplantation (allo-SCT) in first CR. The majority of patients show a clinical response to treatment, but approximately 1/3 of the patients relapse despite allo-SCT. There is no consensus regarding standard treatment. Novel treatment options have suggested targeting of the nuclear factor-kappa B pathway and recent murine studies have shown that treatment with Bortezomib significantly increased the animals' survival.
Aims: In this article we present three cases of BPDCN with different clinical presentations. In all cases treatment with standardized AML-regimes was combined with off-label use of Bortezomib + Dexamethasone in an adapted Multiple Myeloma dosing schedule before undergoing non-myeloablative allogeneic hematopoietic stem cell transplantations (NMA allo-SCT).
Methods: Patient 1 had primarily received standard AML-regime treatment, but relapsed in CNS. The relapse brought in remission with triple-IT and Bortezomib before undergoing NMA allo-SCT. Patient 2 was given AML induction therapy combined with Bortezomib + Dexamethasone and prophylactic Triple IT treatment. This was repeated after reinduction and consolidation with Cytosar before undergoing NMA allo-SCT. Patient 3 received AML induction combined with Bortezomib + Dexamethasone and Triple It. Patient 3 relapsed and was brought into remission with one serie FLAG-Mitox followed by one serie NOPHO high risk Block A regime before NMA allo-SCT.
Results: All three patients are in CR 3, 2,5 and 2 years after NMA allo-SCT respectively and thus still alive past the time of median survival for BPDCN reported in literature. (Table 1).

Table 1.

Patient	Age	Sex	Location	Immunophenotype	Treatment	Relapse	Complications
Pt 1	60	M	Lymph nodes/ Bone marrow	Pos: CD 4+, CD 56+ Neg: myeloid, B and T-cell markers Not tested: CD123, CD303 (BDCA2), TCLA1, CD2AP, SPIB, MT1	1. DA 3+10 2. DA 3+8 3. Consolidation, multiple triple IT, Bortezomib+ Dexamethasone 4. NMA Allo-SCT	Yes, before Allo-SCT. CNS involvement. Remission on Triple IT and Bortezomib	Pre transplant: Parasthesis bilat in lower extremities Post transplant: Skin: GvH stadium II, complete remission
Pt 2	64	F	Skin	Pos: CD 4+, CD 56+, CD 123+ Neg: Myeloid, B- and T-cell markers Not tested: CD303 (BDCA2), TCLA1, CD2AP, SPIB, MT1	1. DA 3+10, Prophylactic Triple IT, Bortezomib + Dexamethasone 2. DA3+8 Prophylactic Triple IT, Bortezomib+ Dexamethasone 3. Consolidation, Prophylactic Triple IT, Bortezomib + Dexamethasone 4. Consolidation, Prophylactic Triple IT 5. NMA Allo-SCT	No	Pre transplant: Parasthesis of lower extremities Post transplant: Cronic oral GvH and skin GvH with sclerodermia
Pt 3	64	F	Bone marrow	Pos: CD 4+, CD 56+, CD 123+ Neg: Myeloid, B- and T-cell markers Not tested: CD303 (BDCA2), TCLA1, CD2AP, SPIB, MT1	1. DA 3+10, Prophylactic Triple IT, Bortezomib + Dexamethasone 2. DA3+8 Prophylactic Triple IT, Bortezomib+ Dexamethasone 3. FLAG-Mitox 4. NOPHO block A 5. NMA Allo-SCT	Yes, before Allo-SCT. BM. 1 curis of FLAG-Mitox and 1 curis of NOPHO block A CR before ITC	Post transplant: Skin: Chronic GvH

Summary/Conclusion: We conclude that addition of Bortezomib + Dexamethasone to AML-Like induction and consolidating regimens followed by Non-Myeloablative Allogeneic Hematopoietic Stem Cell Transplant is feasible with acceptable peripheral neuropathic toxicity.

PS1008

BLAST TRANSFORMATION IN MYELODYSPLASTIC AND MYELOPROLIFERATIVE NEOPLASMS - SIMILARITIES AND DIFFERENCES - A REVIEW OF 100 CASES

M. Jagurinoski[1,*], M. Stoychov[1], Y. Petrov[1], V. Madzarova[1], S. Angelova[1], G. Balatzenko[1], B. Spassov[1], M. Guenova[1]

[1]Specialized Hospital for Active Therapy of Hematological Diseases, Sofia, Bulgaria

Background: Myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN), i.e. chronic myeloid leukemia (CML) and Ph(-)MPNs, are clonal disorders of hematopoiesis characterized by high rates of genetic alterations and an increasing predisposition to transform to acute leukemia (AL).
Aims: To compare the main features of pts with blast transformation (BT) of MDS or MPN with *de novo* acute myeloid leukemia with myelodysplasia-related changes (AML-MDS), and to assess survival after BT.
Methods: Our study includes 100 pts, 60 men/40 women: BT of Ph(-)MPNs (n=20), CML (n=18),MDS (n=30), AML-MDS (n=32). Demographic, morphological, phenotypic, cytogenetic and survival data were evaluated.
Results: The comparative analysis did not show significant differences in gender and age within disease entities, except for the younger age in CML (p=0.005). There was no difference in Hb levels (mean 82.9±18.8 g/l) and blast% (mean45.3±20.6), while BT in Ph(-)MPNs and CML was characterized by signficantly higher mean PLT counts and the mean WBC count was higher in CML. Morphologically, as opposed to AML-MDS (96.8%) and MDS (96.4%), dysplasia in ≥ 2 lineages was found in only 33.3% and 44.4% of CML and MPN, respectively (p=0.000). The blast cell phenotype was generally myeloid, except 4 CML lymphoid blast crises(BC). On average, 67.4% of all groups (incl. all myeloid CML BC) had aberrant co-expression of 1-4 lymphoid-associated markers, most commonly CD7, CD19, CD56, CD22. The genetic profile demonstrated significant differences as well. Higher incidence of abnormal karyotypes was found in AML-MDS compared to BT of MDS and CML aside of Ph' chromosome (p=0.042). Chromosomal aberrations were found in 80% with Ph(-)MPNs, however the number of successful karyotypes was low. The molecular pattern also differed within the groups. The incidence of *FLT3-ITD* was higher in AL after MDS compared to AML-MDS and MPN, while *EVI1* overexpression was found at the time of BT in a high proportion of CML and Ph(-)MPN. No significant difference was observed in regard to *NPM1* mutations. The mean time to BT was signficantly shorter in MDS (12.5±23.0 mo) compared to MPN (32.4±34.5 mo) and CML (34.1±25.2 mo) (p=0.033) and the mean overall survival after the BT was poorer in MDS and MPN compared to CML BC and *de novo* AML-MDS (Table 1).
Summary/Conclusion: The main clinical and laboratory characteristics of AL occurring *de novo* or based on a BT of a previous MDS or MPN highlights both similarities and certain differences in the biology and clinical course and may require a different therapeutic approach in future. However, the outcome of pts with BT after MDS or MPN is poor and attempts should concentrate on early identification of pts at risk for disease progression.

Table 1.

Variables	Ph(-) MPN	CML	MDS	De novo AML-MDS	P
Age years (mean, SD)	64.8±10.5	49.2±14.1	61.2±16.9	62.8±13.9	0.005
WBC x10⁹/L (mean, SD)	63.9±61.9	227.3±426.3	25.8±42.7	16.1±33.3	0.002
PLT x10⁹/L (mean, SD)	149.1±191.9	238.9±221.3	76.2±100.6	93.5±104.4	0.005
Abnormal karyotype (% pts)	80%	50%	40%	70%	0.042
FLT3-ITD(+) (% pts)	8.3%	NA	24.1%	3.2%	0.056
EVI1 (+) (% pts)	42.6%	80%	10.3%	6.4%	0.023
NPM1 mut(+) (% pts)	14.3%	NA	16.7%	28.6%	NS
OS mo (mean, SD)	13.1±4.2	52.7±24.0	13.5±3.4	20.9±4.9	NS

PS1009

QUALITY OF LIFE IN ACUTE LEUKEMIA PATIENTS WITH COMORBID ISCHEMIC HEART DISEASE

T. Lymanets[1,*], I. Skrypnyk[1], G. Maslova[1], I. Gusachenko[2]

[1]Chair of Internal Medicine #1, Ukrainian Medical Stomatological Academy, [2]Hematology Department, Poltava Regional Clinical Hospital n.a.M.V. Sklifosovsky, Poltava, Ukraine

Background: Due to significant advances in the treatment of oncohematological patients their survival rates, life expectancy and progression-free period have been greatly improved. The study of quality of life (QoL) parameters in patients with acute leukemia during the induction chemotherapy (ICT) has aquired special interest. Patients with comorbid ischemic heart disease (IHD) are at extremely high risk of anthracycline cardiotoxicity development that may significantly reduce their QoL.
Aims: To assess quality of life before and after induction chemotherapy in acute leukemia patients with comorbid ischemic heart disease depending on prescribed cardioprotective therapy.
Methods: The study involved 66 patients with newly diagnosed acute leukemia (acute lymphoblastic leukemia - 7, acute myeloblastic leukemia - 59 patients) and comorbid IHD, treated with ICT at Hematology Department, Poltava Regional Clinical Hospital n.a.M.V. Sklifosovsky, Ukraine. The cohort consisted of 34 (51.5%) males and 32 (48.5%) females, age of 54-72 years, ECOG I-II. The duration of IHD ranged from 3 to 15 years. QoL of patients was assessed using a questionnaire SF-36 V2 counting the physical and mental components of health before treatment and after ICT before remission consolidation. The study was approved by the local ethical committee and all patients gave a written consent before they were included in the study. Patients were divided into two groups: I (n=36)-patients treated with ICT; II (n=30)-patients, whom during the ICT L-arginine was prescribed.
Results: At baseline, all patients had significantly lower QoL, both physical and mental components of health, compared with healthy respondents (Table 1). The newly diagnosed acute leukemia in combination with currently existing IHD had a great influence on different aspects of subjective well-being. At follow-up, we noticed a significant improvement of physical functioning, vitality, general and mental health in patients of group I; and role physical, vitality, bodily pain and role emotional QoL parameters' improvement in patients of group II. The average physical status indicators in patients of groups I and II did not significantly change. At the same time, the mental status of patients improved: in group I in 1.3 times (37.3±2.82 vs 28.3±2.37 before ICT, p<0.05), in group II – in 1.4 times (37.6±3.46 vs 27.5±3.04 before ICT; p<0.05). Differences between groups were not statistically significant on all scales. However, in comparison with the data of practically healthy individuals, QoL of patients with acute leukemia after ICT remained significantly lower.

Table 1.

Quality of life in patients with acute leukemia and comorbid ischemic heart disease

	practically healthy (n=18)	I (n=36)		II (n=30)	
		before ICT	after ICT	before ICT	after ICT
SF-36 V2 Norm-Based Scales					
PF (physical functioning)	55.3±2.81	34.5±2.74*	41.7±1.36*√	36.4±2.01*	41.9±2.79*
RP (role physical)	54.3±1.25	32.0±2.18*	37.2±2.15*	34.2±1.26*	41.7±2.84*√
BP (bodily pain)	57.6±3.71	39.4±1.85*	44.2±3.72*	38.6±1.45*	44.7±1.04*√
GH (general health)	57.9±3.54	26.8±2.25*	32.3±1.06*√	29.3±2.13*	33.1±2.29*
VT (vitality)	60.4±2.41	33.8±1.31*	43.3±2.96*√	30.6±2.55*	42.1±2.25*√
SF (social functioning)	51.1±2.44	29.5±2.39*	35.2±2.71*	29.8±2.70*	37.1±2.95*
RE (role emotional)	49.9±2.38	28.4±2.56*	36.2±3.80*	31.5±2.99*	40.9±3.07*√
MH (mental health)	53.8±3.45	28.9±3.26*	38.7±2.06*√	27.5±3.62*	35.9±3.32*
SF-36 V2 summary scores					
PCS (physical component summary)	57.5±1.26	36.4±2.34*	40.8±2.60*	37.9±2.45*	42.5±2.65*
MCS (mental component summary)	51.6±3.29	28.3±2.37*	37.3±2.82*√	27.5±3.04*	37.6±3.46*√

Note: significant differences, p<0.05: *– between indicators of healthy persons and in the group; √ – between indicators before and after induction chemotherapy.

Summary/Conclusion: The QoL assessment in patients with acute leukemia and ischemic heart disease during induction chemotherapy is an important component of oncological patient's management, which allows to individualize the approach to each patient in the presence of this type of comorbidity. Psychological and supportive therapy during induction chemotherapy could improve QoL of these patients.

Aggressive Non-Hodgkin lymphoma – Clinical

PS1010

BIOMARKER ANALYSIS OF TISAGENLECLEUCEL PRE-INFUSION BIOPSIES OF ADULT PATIENTS WITH RELAPSED OR REFRACTORY (R/R) DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

S. Agoulnik[1], U. Jäger[2,*], C. S. Tam[3], J.P. McGuirk[4], S.R. Foley[5], S.M. Jaglowski[6], C. Andreadis[7], P. J. Ho[8], P. Borchmann[9], E. K. Waller[10], S. Mielke[11], S.J. Schuster[12], R.T. Maziarz[13], V. Bachanova[14], K. van Besien[15], D. Zheng[16], F. Tai[17], L.B. Pacaud[17], R. Awasthi[18], T. Tran[19], N. Dakappagari[19], Ö. Anak[20], S. Pantano[20], M.R. Bishop[21]

[1]Novartis Precision Medicine, Cambridge, United States, [2]Dept. of Medicine I, Div. of Hematology and Hemostaseology, Medical University of Vienna, Vienna, Austria, [3]Peter MacCallum Cancer Centre, St Vincent's Hospital and University of Melbourne, Melbourne, Australia, [4]Department of Blood and Bone Marrow Transplant, The University of Kansas Medical Center, Kansas City, United States, [5]Juravinski Hospital and Cancer Centre, McMaster University, Hamilton, Canada, [6]The James Cancer Hospital and Solove Research Institute, The Ohio State University Comprehensive Cancer Center, Columbus, [7]Department of Hematology and Blood and Marrow Transplant, University of California San Francisco Helen Diller Family Comprehensive Cancer Center, San Francisco, United States, [8]Royal Prince Alfred Hospital and Department of Medicine, The University of Sydney, Sydney, Australia, [9]Department of Haematology and Oncology, University Hospital of Cologne, Cologne, Germany, [10]Winship Cancer Institute of Emory University, Bone Marrow and Stem Cell Transplant Center, Atlanta, United States, [11]Würzburg University Medical Center, Center for Allogeneic Stem Cell Transplantation, Würzburg, Germany, [12]Lymphoma Program, Abramson Cancer Center University of Pennsylvania, Philadelphia, [13]Oregon Health & Science Knight Cancer Institute, Center for Hematologic Malignancies, Portland, [14]Department of Medicine, Division of Hematology, Oncology and Transplantation, University of Minnesota, Minneapolis, [15]Weill Cornell Medicine/New York-Presbyterian Hospital, New York, [16]Novartis Institutes for BioMedical Research, Cambridge, [17]Novartis Pharmaceuticals Corporation, [18]Novartis Institutes for BioMedical Research, East Hanover, [19]Navigate BioPharma Services, Inc, Carlsbad, United States, [20]Novartis Pharma AG, Basel, Switzerland, [21]Hematopoietic Cellular Therapy Program, The University of Chicago Medicine, Chicago, United States

Background: The global trial JULIET (NCT02445248) is evaluating the efficacy and safety of tisagenlecleucel in adult patients (pts) with R/R DLBCL. Tisagenlecleucel is an autologous chimeric antigen receptor (CAR)–expressing T-cell therapy targeting CD19+ cells.
Aims: To explore the potential correlation of tisagenlecleucel efficacy with CD19 and immune checkpoint protein expression in pre-infusion biopsies of patients with DLBCL.
Methods: Exploratory analyses were conducted in archival formalin-fixed, paraffin-embedded tumor tissue samples collected months prior to infusion. 92 patients were evaluated for efficacy as of September 6, 2017; 81 had baseline biopsy results included in this analysis. Of these samples, the majority were collected within 1-year pre-infusion (8 from 1-3 months, 26 from 3-6 months, and 25 from 6 months to 1 year). Quantitative immunofluorescence and image analyses by AQUA Technology were used to evaluate relative levels of CD19, presence of total PD-L1+ cells, and frequency of T cells and non–T cells positive for immune checkpoint molecules (PD-1, LAG3, TIM3). PD-1/PD-L1 interaction scores, defined as the proportion of PD-1–positive cells colocalized with PD-L1–positive cells, were also derived. Results were assessed in the context of response to tisagenlecleucel to investigate a potential relationship between tissue biomarkers and efficacy.
Results: Response to tisagenlecleucel was observed in pts whose tumor samples showed unequivocal CD19-positive expression (best overall response rate [ORR], 49% [95% CI, 34%-64%]) and pts with low/negative CD19 expression (best ORR, 52% [95% CI, 31%-73%]). No apparent differences were observed among the best overall response (BOR) groups (CR, PR, SD, PD, unknown) in median or mean levels of the percentages of PD-L1+, PD-1+, LAG3+, or TIM3+ cells. Similarly, no apparent differences were observed between BOR groups in median and mean levels of: proportions of PD-1+, LAG3+, and TIM3+ T cells and PD-1/PD-L1 interaction scores.
Despite no apparent differences, some markers showed higher expression in a small subset of patients in whom tisagenlecleucel had reduced efficacy. The 5 patients with the highest PD-1/PD-L1 interaction score did not respond to tisagenlecleucel or relapsed by month 3. Likewise, the 10 patients with the highest proportion of LAG3+ T cells did not respond to tisagenlecleucel or relapsed by months 3-6 (by objective response criteria or according to clinical criteria). Furthermore, of 8 pts with the highest percentage (> 30%) of PD-1+ cells (in areas selected based on high PD-L1 expression), only 2 responded to tisagenlecleucel, and 1 progressed at month 3. Interestingly, the majority of PD-1+ cells in these 8 patients were not T cells, as the percentage of T cells was much lower than the percentage of PD-1+ cells. Additional analysis correlating tissue biomarkers with efficacy will be presented.
Summary/Conclusion: These preliminary exploratory biomarker data indicate similar response rates across all CD19 expression levels. Furthermore, a small subset of pts with the highest levels of PD-1/PD-L1 interaction score, PD-1+ cells, and high proportion of LAG3+ T cells (among T cells present) seem to not respond to tisagenlecleucel or have early relapse. These preliminary observations require further investigation but nonetheless raise the possibility that patients with a high PD-1/PD-L1 interaction score, a high proportion of PD-1+ cells, and/or a high proportion of LAG3+ cells present among T cells, may experience reduced tisagenlecleucel efficacy.

PS1011

ACALABRUTINIB IN COMBINATION WITH THE PI3Kδ INHIBITOR ACP-319 IN PATIENTS WITH RELAPSED/REFRACTORY B-CELL MALIGNANCIES

P. M. Barr[1,*], S.D. Smith[2], M. Roschewski[3], S.M. O'Brien[4], J. Sharman[5], J.M. Melear[6], A. Hamdy[7], R. Izumi[7], J.G. Slatter[7], N. Chernyukhin[7], E. Bibikova[7], M. Yin[7], T. Chen[7], S. E. Spurgeon[8]

[1]Wilmont Cancer Institute, University of Rochester Medical Center, Rochester, [2]University of Washington/Fred Hutchinson Cancer Research Center, Seattle, [3]National Cancer Institute, Bethesda, [4]Chao Family Comprehensive Cancer Center, University of California-Irvine, Irvine, [5]Willamette Valley Cancer Institute and Research Center, US Oncology Research, Springfield, [6]Texas Oncology, Austin, [7]Acerta Pharma, Redwood City, [8]Center for Hematologic Malignancies/Knight Cancer Institute, Oregon Health and Sciences University, Portland, United States

Background: Bruton tyrosine kinase (BTK) is a clinically validated target in B-cell malignancies. Acalabrutinib is a selective, covalent inhibitor of BTK. Preclinical studies show synergy with combined BTK and PI3K inhibition in B-cell lymphoma (Griner *PNAS* 2014).
Aims: Acalabrutinib was evaluated in combination with the PI3Kδ inhibitor ACP-319 in patients with relapsed/refractory B-cell malignancies.
Methods: Patients with relapsed/refractory B-cell malignancies, ≥1 prior therapy and ECOG PS ≤2 received oral acalabrutinib 100 mg bid and ACP-319 in escalating doses (25/50/100 mg bid [n=6 per dose]; Part 1) or 50 mg bid (expansion cohort; Part 2) until progressive disease or unacceptable toxicity. The primary endpoint was safety (including dose-limiting toxicity [DLT] in Part 1). Secondary endpoints were pharmacokinetics/pharmacodynamics, overall response rate (ORR), duration of response (DOR) and progression-free survival (PFS).
Results: Eighteen patients were enrolled in Part 1: 8 had chronic lymphocytic leukemia/small lymphocytic leukemia (CLL/SLL), 3 had follicular lymphoma, 3 had mantle cell lymphoma, 3 had diffuse large B-cell lymphoma (DLBCL) and 1 had Waldenstrom macroglobulinemia. Part 1 patients had a median age of 65 y (range 48-77); all had ECOG PS ≤1. One patient had a DLT in the ACP-319 50-mg group (maculopapular rash) and 2 had DLTs in the 100-mg group (maculopapular rash; febrile neutropenia, diarrhea and pneumonitis). The maximum tolerated dose was defined as ACP-319 50 mg bid with acalabrutinib 100 mg bid. Patients with CLL/SLL were switched to acalabrutinib monotherapy in 2016 due to reduced benefit:risk ratio with added ACP-319. Herein, 25 patients with DLBCL (Part 1, n=3; Part 2, n=22) were analyzed; by immunohistochemistry (Hans algorithm), 9 had germinal center B-cell (GCB) and 16 had non-GCB DLBCL. Median age was 70 y (range 55-90) and the median number of prior therapies was 2 (range 1-5). The most common adverse events (AEs; ≥40% of patients) were increased AST/ALT (48%/52% [Grade ≥3, 28%/20%]), diarrhea (52% [12%]), fatigue (40% [0%]) and rash (40% [12%]). Twenty-two patients discontinued (5 discontinued acalabrutinib and 7 discontinued ACP-319 due to AEs); no deaths were due to AEs. ORR is shown in the Table 1. ACP-319 exposure was dose proportional with higher/variable exposure to an inactive metabolite. Acalabrutinib exposure was slightly higher at ACP-319 100 (vs 25/50) mg bid. Median BTK occupancy at trough was 95%; p-AKT inhibition was ACP-319 dose dependent.

Table 1.

	DLBCL	
	GCB n=9	Non-GCB n=16
ORR (≥ PR), n (%)	0	10 (63)
95% CI	–	35, 85
Complete response	0	4 (25)
Partial response (PR)	0	6 (38)
Median, months		
Time on study (range)	2.4 (0.8, 10.4)	4.0 (0.8, 29.7)
DOR (95% CI)	–	NR (1.8, NR) range, 0.03* – 18.4*
PFS (95% CI)	1.8 (1.7, 10.4)	5.5(1.6, NR)

*Censored. NR, not reached.

Summary/Conclusion: The combination of acalabrutinib + ACP-319 was tolerable with manageable AEs; response rate was high in patients with non-GCB DLBCL.

PS1012

TIME TO RELAPSE OF MANTLE CELL LYMPHOMA AFTER INTENSIVE HIGH DOSE CYTARABINE CONTAINING REGIMENS DEFINES PATIENTS RISK FOR DEATH: AN ANALYSIS FROM CENTERS OF THE FONDAZIONE ITALIANA LINFOMI

C. Visco[1,*], M. C. Tisi[1], A. Evangelista[2], A. Di Rocco[3], V.R. Zilioli[4], S. Hohaus[5], R. Sciarra[6], A. Re[7], C. Tecchio[8], A. Chiappella[9], L. Morello[10], G. Gini[11], L. Nassi[12], T. Perrone[13], A.L. Molinari[14], A. Fabbri[15], M.C. Cox[16], E. Finolezzi[17], S. Ferrero[18], B. Puccini[19], I. Alvarez De Celis[20], A. Arcari[21], D. Marino[22], M. Merli[23], F. Piazza[24], M. Gentile[25], M. Pelosini[26], G. Loseto[27], M. Ruggeri[1], M. Martelli[3], U. Vitolo[9]

[1]Cell Therapy and Hematology, San Bortolo Hospital, Vicenza, [2]Clinical Epidemiology, Città della Salute e della Scienza and CPO Piemonte, Torino, [3]Cellular Biotechnologies and Hematology, 'Sapienza University', Rome, [4]Hematology, ASST Grande Ospedale Metropolitano Niguarda, Milan, [5]Policlinico Gemelli Foundation, Institute of Hematology, Catholic University of the Sacred Heart, Rome, [6]Haematology Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia, [7]Hematology, Spedali Civili, Brescia, [8]Medicine, Section of Hematology and Bone Marrow Transplant Unit, University of Verona, Verona, [9]Hematology, Citta' della salute e della scienza university hospital, Torino, [10]Hematology, Humanitas Clinical and Research Center, Rozzano, [11]Haematology Clinic, Department of Clinical and Molecular Sciences, Marche Polytechnic University, Ospedali Riuniti di Ancona, Ancona, [12]Hematology Department, Azienda Ospedaliero-Universitaria Maggiore della Carità, Novara, [13]Department of Emergency and Organ Transplantation, Hematology Section, University of Bari, Bari, [14]Hematology, Ospedale degli Infermi, Rimini, [15]Unit of Hematology, Azienda Ospedaliera Universitaria Senese & University of Siena, Siena, [16]Hematology unit, AOU Sant'Andrea, Rome, [17]Hematology, Transfusional Medicine and Biotechnologies, UOSD "Centro Diagnosi e Terapia dei Linfomi", PO Santo Spirito, Pescara, [18]Molecular Biotechnologies and Health Sciences, University of Torino/AOU "Città della Salute e della Scienza di Torino", Torino, [19]Hematology, University of Firenze, Firenze, [20]Hematology Unit, AUSLL/IRCCS Santa Maria Nuova Hospital, Reggio Emilia, [21]Hematology and Bone Marrow Transplant Unit , "Guglielmo da Saliceto" Hospital, Piacenza, [22]Medical Oncology [1], Veneto Institute of Oncology IOV IRCCS, Padova, [23]Hematology, Ospedale di Circolo e Fondazione Macchi, University of Insubria, Varese, [24]Hematology, Department of Medicine, University of Padova, Padova, [25]Onco-hematology, Hematology Unit, AO of Cosenza, Cosenza, [26]Clinical and Experimental Medicine, Section of Hematology, University of Pisa, Pisa, [27]Hematology and Cell Therapy Unit, IRCCS Istituto Tumori "Giovanni Paolo II", Bari, Italy

Background: Most patients with mantle cell lymphoma (MCL) respond to initial therapy, but inevitably experience relapse or progression of disease (POD) despite the use of very effective initial chemoimmunotherapy including high-dose cytarabine (HDAraC).
Aims: The goal of this analysis was to characterize the outcomes in patients with MCL following POD and to identify if time to progression may define a subgrouop of patients at high risk of death.
Methods: We retrospectively analyzed data from 188 patients with MCL that were refractory or had first relapse after induction regimens including rituximab and HDAraC followed by high-dose chemotherapy and autologous stem cell transplant, if appropriate. These 188 patients were retrieved from 418 patients with MCL treated upfront with intensive regimens that were consecutively recruited from 26 centers of the Fondazione Italiana Linfomi between 2007 and 2016. Primary outcome was the overall survival from time of POD (OS-2).
Results: Based on a linear regression model showing the relationship between risk of death (Log relative hazard) and time to first relapse or progression in 188 patients with MCL (Figure 1A), two groups were defined: patients with early POD 2 years or less after diagnosis (n=90, 48%) and those with late POD (n=98, 52%). Overall, OS-2 was 34 months (range 25-42), with early POD being the most powerful adverse factor (12 months versus not reached in the later POD group, p<0.0001, hazard ratio, 3.90; 95% CI, 2.16 to 7.06; Figure 1B). This significant impact on survival was independent of other known prognostic variable, and maintained after adjusting for MCL International Prognostic Index, tumor kinetics, morphological variant, presence of B-symptoms, different induction regimens, or allogeneic stem cell transplantation. Median overall survival from time of initial diagnosis (OS-1) was 76 months (range 64-88), 27 months in the early POD group and 127 months in remaining patients. Forty-one patients (22%) underwent allogeneic stem cell transplant (AlloSCT) during study follow-up. A significantly effect modification of AlloSCT according to POD time was detected (interaction test P .012), with early POD patients that benefited most of the procedure.

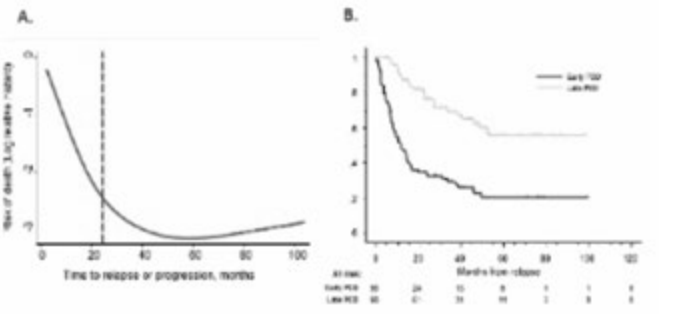


Figure 1.

Summary/Conclusion: In patients with MCL who received upfront HDAraC containing regimens, POD within 2 years after diagnosis was associated with poor survival and should be validated as a standard endpoint of chemoimmunotherapy trials of untreated MCL. Contrarily, late POD patients had an unexpectedly long life expectancy in the modern treatment era.

PS1013

CC-122-DLBCL-001: PHASE IB STUDY OF CC-122 PLUS RITUXIMAB IN PATIENTS WITH CHEMO-REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA

V. Ribrag[1,*], J. Kuruvilla[2], U. Vitolo[3], J.B. Kaplan[4], J.C. Chandler[5], A. Santoro[6], P. Corradini[7], I.W. Flinn[8], L. Nastoupil[9], P.A. Cassier[10], R. Sangha[11], V.P. Kenkre[12], R. Advani[13], I. Isufi[14], T. Witzig[15], S. Uttamsingh[16], M. Petrarca[16], P.R. Hagner[17], A.K. Gandhi[17], X. Wu[18], A. Risueño[19], K. Hege[16], M. Pourdehnad[16], J.C. Chavez[20]

[1]Institut Gustave Roussy, Villejuif, France, [2]Division of Medical Oncology and Hematology, University of Toronto, Princess Margaret Cancer Centre, Toronto, Canada, [3]Azienda Ospedaliera Universitaria Città della Salute e della Scienza di Torino, Torino, Italy, [4]Northwestern University, Feinberg School of Medicine, Chicago, [5]West Cancer Center, Memphis, United States, [6]Instituto Clinico Humanitas, Rozzano, [7]IRCCS Istituto Nazionale dei Tumori, Milano, Italy, [8]Sarah Cannon Research Institute, Nashville, [9]Department of Lymphoma and Myeloma, UT MD Anderson Cancer Center, Houston, United States, [10]Centre Leon Berard, Lyon, France, [11]Cross Cancer Institute, Edmonton, Canada, [12]Division of Hematology/Oncology, University of Wisconsin, Madison, [13]Stanford Cancer Institute, Stanford, [14]Yale Cancer Center, New Haven, [15]Mayo Clinic, Rochester, [16]Celgene Corporation, San Francisco, [17]Celgene Corporation, Summit, [18]Celgene Corporation, Berkeley Heights, United States, [19]Celgene Institute for Translational Research Europe, Seville, Spain, [20]H. Lee Moffitt Cancer Center & Research Institute, Tampa, United States

Background: Patients (pts) with diffuse large B-cell lymphoma (DLBCL) unresponsive to standard therapies have limited curative options (Crump *et al.*, *Blood* 2017). Novel therapeutics are being developed based on the improved understanding of the biology of DLBCL. CC-122 is a cereblon-modulating agent that promotes degradation of the hematopoietic transcription factors Aiolos and Ikaros (Hagner *et al.*, *Blood* 2015). CC-122 has shown promising activity in combination with obinutuzumab or rituximab in relapsed or refractory DLBCL and follicular lymphoma (FL) (Michot *et al.*, ASH 2017; Ribrag *et al.*, ASH 2017).
Aims: To report updated results of the safety and efficacy of CC-122 in combination with rituximab in chemo-refractory DLBCL pts from Arm D (expansion phase) of the CC-122-DLBCL-001 study.
Methods: CC-122-DLBCL-001 is a phase Ib dose escalation/expansion study of CC-122, CC-223, and CC-292 given orally as doublets and triplets in combination with rituximab in chemo-refractory pts (NCT02031419; Ribrag *et al.*, ASH 2016). Chemo-refractory DLBCL is defined as: duration of stable disease ≤ 12 months, progressive disease as the best response to the last chemotherapy containing regimen, or disease progression or recurrence ≤ 12 months of prior autologous SCT. In the dose-escalation phase (3+3), pts received CC-122 at 1, 2, 3, or 4 mg orally daily (QD) or 5 days per week (5/7d), with a fixed dose of intravenous rituximab 375 mg/m^2 once every 28 days. The dose expansion cohort received 3 mg CC-122 QD 5/7 d as a formulated capsule plus rituximab. The primary endpoints were to determine safety, NTD, MTD, and RP2D. Secondary endpoints included PK, PD, and preliminary efficacy assessments. Gene expression profiling identified the cell-of-origin subset of DLBCL. A novel gene expression classifier was developed from a public DLBCL dataset and applied using NanoString profiling on tumor samples to assess enrichment for CC-122 respon-

ders (Risueño *et al.*, ASH 2017).
Results: As of January 10, 2018, a total of 27 DLBCL pts were enrolled in Arm D expansion. The median age was 63 years (range, 33-84), 20 (74%) were male, 22 (81%) had stage III/IV disease. The median number of prior systemic anticancer therapies was 3 (range, 2-6). One pt had a DLT due to grade 4 neutropenia during cycle 1. Two pts (7%) had at least one dose reduction (4% due to AEs) and 14 pts (52%) had at least one dose interruption (44% due to AEs). The most common AEs (all grades) were neutropenia (48%), fatigue, and peripheral edema (30% each). Grade 3/4 AEs were neutropenia (44%), fatigue and decreased neutrophil count (11% each), and anemia, abdominal pain, and pain in extremity (7% each). Nine pts (33%) had SAEs, including infections and vascular disorders (7% each). The objective response rate (ORR) was 41% with a complete response (CR) rate of 22%. The median duration of response (mDOR) was not yet reached. Median treatment duration was 2.0 months (range, 0-15, Q1 1.5, Q3 8.9). Subgroup efficacy analyses are shown in Table 1. Gene expression classifier positive pts had a CR rate of 50% and 6-mos PFS rate of 50% compared with 13% CR rate and 16% 6-mos PFS rate in gene expression classifier negative pts.

Table 1.

Subgroup Efficacy Analyses in Arm D (CC-122 + R) (n = 27)

DLBCL Type	n	ORR n (%) (95% CI)	CR n (%) (95% CI)	6-mo PFS rate % (95% CI)
All	27	11 (41) (22-61)	6 (22) (9-42)	32 (15-50)
DLBCL NOS	19	9 (47) (24-71)	5 (26) (9-51)	35 (15-56)
DLBCL NOS COO				
ABC	2	1 (50) (1-99)	1 (50) (1-99)	50 (1-91)
GCB	9	5 (56) (21-86)	3 (33) (8-70)	33 (8-62)
Unclassified	3	1 (33) (1-91)	0 (0) (0-71)	NR (NR-NR)
DLBCL NOS Gene Classifier				
Gene classifier +	6	3 (50) (12-88)	3 (50) (12-88)	50 (11-80)
Gene classifier -	8	4 (50) (16-84)	1 (13) (0-53)	16 (1-49)
Gene expression unknown*	5	2 (40) (5-85)	1 (20) (1-72)	40 (5-75)

Data cutoff was January 10, 2018.
ABC, activated B-cell; CI, confidence interval; COO, cell-of-origin; CR, complete response; GCB, germinal center B-cell; PFS, progression-free survival; NOS, not otherwise specified; NR, not reached; ORR, overall response rate
*Patients defined as classifier unknown were due to failure to meet pathology requirements for gene classifier evaluation.

Summary/Conclusion: In pts with chemo-refractory DLBCL, CC-122 combined with rituximab demonstrated manageable toxicities, with a low incidence of severe AEs. This combination showed favorable clinical activity and may represent an important treatment option in a difficult to treat pt population. Notably, gene expression classifier positive pts had encouraging clinical activity.

PS1014

EXTRACELLULAR CIRCULATING DNA IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS: BIOLOGICAL CORRELATES AND PROGNOSTIC IMPACT

D. Dujmovic[1,*], S. Bašić Kinda[1], P. Rončević[1], A. Samardžić[1], I. Radman[1], V. Škaro[2], G. Lauc[2], K. Dubravčić[3], I. Aurer[1]

[1]Hematology, UHC Zagreb, [2]Molecular biology, PMF Zagreb, [3]Molecular biology, UHC Zagreb, Zagreb, Croatia

Background: Extracellular circulating DNA (cfDNA) can be found in small quantities in plasma of healthy persons, but higher concentrations are found in various disorders including malignant and autoimmune diseases, myocardial infarction, trauma, inflammation and complications of pregnancy. In patients with tumors, cfDNA is of tumor origin. Although it has been studied extensively in solid cancers, there is a dearth of information on cfDNA in hematologic neoplasia.
Aims: The goal of this study was to determine the concentration of cfDNA in patients with lymphoma, its relation to demographic, biologic and clinical patient and tumor characteristics and to investigate its potential role as a marker of disease activity, prognosis or response to treatment.
Methods: The study was performed in 47 patients with diffuse B-large cell lymphoma from 2010-2013 lymphoma treated according to standard guidelines (Figure 1). cfDNA concentration was determined using quantitative real-time PCR before and at the end of treatment.
Results: In DLBCL patients cfDNA concentration is somewhat higher than in healthy persons (median 24.6 ng/ml vs. 12.1 ng/ml), range from 0.45 ng/ml – 1675 ng/ml. In all patients cfDNA concentration correlated with unfavorable prognostic characteristics: age, LDH, beta-2 microglobulin, disease stage and IPI but not with Ki-67, gender or presence of extranodal disease. Correlation with beta-2 microglobulin was strongest. The conctration of cfDNA had no impact on survival(both OS and PFS). (Figure 1). In multivariate analysis, cfDNA concentration was not an independent prognostic factor. In the vast majority of patients cfDNA concentrations at the end of treatment were lower than at the beginning but neither the absolute nor relative decline correlated with treatment outcomes.

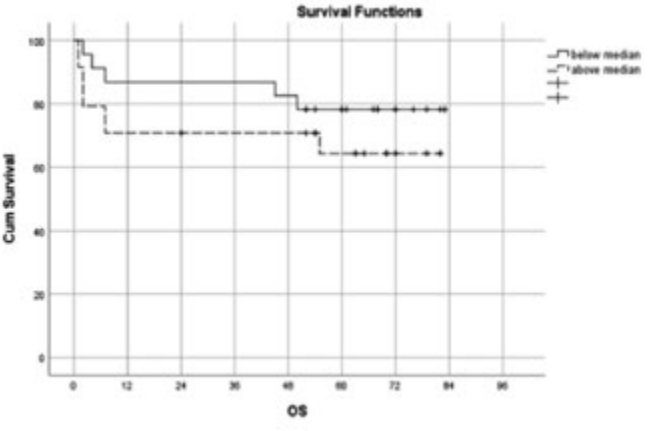


Figure 1.

Summary/Conclusion: In patients with diffuse large cell lymphoma, cfDNA concentration prior to treatment start correlates with negative prognostic factors, dominantly with those related to the total tumor mass. However, measuring cfDNA does not add independent clinically meaningful information and seems to be neither of diagnostic nor of prognostic value.

PS1015

ACALABRUTINIB MONOTHERAPY IN PATIENTS WITH RELAPSED/REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) IN THE PHASE 1B ACE-LY-002 STUDY

M.J.S. Dyer[1,*], S. de Vos[2], J. Ruan[3], C. Flowers[4], K. Maddocks[5], S. Rule[6], A. Hamdy[7], R. Izumi[7], J.G. Slatter[7], J. Cheung[7], M.M. Frigault[7], H. Wei[7], S. Mourya[7], N. Hunder[7], N.H. Fowler[8]

[1]The Ernest and Helen Scott Haematological Research Institute, University Hospitals of Leicester NHS Trust, Leicester, United Kingdom, [2]UCLA, Division of Hematology/Oncology, David Geffen School of Medicine, Los Angeles, [3]Joan and Sanford I. Weill Medical College of Cornell University, New York, [4]Emory University School of Medicine, Atlanta, [5]The Ohio State University Comprehensive Cancer Center, Columbus, United States, [6]Derriford Hospital, Plymouth, United Kingdom, [7]Acerta Pharma, Redwood City, [8]The University of Texas MD Anderson Cancer Center, Houston, United States

Background: DLBCL is an aggressive non-Hodgkin lymphoma, with poor prognosis in relapsed/refractory patients. Current therapy for relapsed/refractory DLBCL typically yields low overall response rates (ORRs) and short remissions. Acalabrutinib is a highly selective, potent, covalent Bruton tyrosine kinase inhibitor with minimal off-target activity.
Aims: To evaluate acalabrutinib as monotherapy in patients with relapsed/refractory *de novo* DLBCL in the Phase 1b ACE-LY-002 study.
Methods: Eligible patients aged ≥18 y, with Eastern Cooperative Oncology Group performance status (ECOG PS) ≤2 and confirmed relapsed/refractory non-germinal center (GCB) type DLBCL (assessed by local immunohistochemistry) were enrolled. Patients received oral acalabrutinib 100 mg bid until progressive disease (PD) or unacceptable toxicity. The primary endpoint

was safety; secondary endpoints included pharmacokinetics and pharmacodynamics as well as investigator-assessed ORR (according to Lugano criteria), duration of response (DOR) and progression-free survival (PFS).
Results: In total, 21 patients were enrolled. To their most recent therapy, 11 patients were relapsed (partial response or better [≥PR] followed by PD) and 10 were refractory (no response or stable disease). Median age was 64 y (range 32-84); 86% had ECOG PS ≤1; 57% had extranodal disease; 81% had Ann Arbor stage III/IV. Median number of prior therapies was 3 (range 1-5). Median time on study was 3.9 months (range 0.8-22.5), with 1 patient continuing therapy. PD (81%) was the most common reason for treatment discontinuation. Two patients discontinued treatment due to adverse events (AEs) that were considered unrelated to treatment. Pharmacokinetic parameters were similar to previous studies, with rapid absorption and clearance; median steady-state BTK target occupancy was 97% - 99% (n=5). ORR (≥PR) for all patients was 24% (5/21; 19% complete response [CR]). NanoString subtyping conducted on 15 patients revealed 5 GCB, 9 activated B-cell (ABC), and 1 unclassified DLBCL. Characteristics of the 5 responders are listed in the Table 1. Common AEs (any grade) were diarrhea (43%), fatigue (43%), anemia (29%), cough (29%) and dizziness (29%); common Grade 3/4 AEs were anemia (24%), fatigue (10%) and abdominal pain (10%). Three patients had Grade 5 AEs (respiratory failure, meningeal progression and sepsis); none were drug related. No atrial fibrillation, hypertension, tumor lysis syndrome or Grade ≥3 bleeding AEs occurred.

Table 1.

Patient	Subtype by Nanostring	Relapsed/ refractory	Prior therapies, #	Best response	DOR, mo	PFS, mo
1	ABC	Refractory	3	CR	0.7*	2.6*
2	ABC	Relapsed	2	CR	13.7*	15.5*
3	ABC	Relapsed	2	PR	1.8	3.7
4 (ongoing)	GCB	Refractory	3	CR	15.9*	20.3*
5	Missing	Relapsed	4	CR	1.9	3.8

*Censored.

Summary/Conclusion: Acalabrutinib monotherapy was tolerable and had activity with durable remission in difficult-to-treat DLBCL patients including refractory patients.

PS1016

LENALIDOMIDE PLUS BENDAMUSTINE-RITUXIMAB DOES NOT OVERCOME THE ADVERSE IMPACT OF TP53 MUTATIONS IN MANTLE CELL LYMPHOMA

C.W. Eskelund[1,2,*], A. Albertsson-Lindblad[3], A. Kolstad[4], A. Laurell[5], R. Räty[6], L.B. Pedersen[2], C. Geisler[2], M. Jerkeman[3], K. Grønbæk[1,2]

[1]Biotech Research and Innovation Centre, [2]Rigshospitalet, Copenhagen, Denmark, [3]Skåne Universtity Hospital, Lund, Sweden, [4]Oslo University Hospital, Oslo, Norway, [5]Uppsala University Hospital, Uppsala, Sweden, [6]Helsinki Universty Hospital, Helsinki, Finland

Background: Despite the general improvement in the outcomes for patients with mantle cell lymphoma (MCL), recent studies have demonstrated that *TP53* mutations still designate approximately 10% of patients to a very high-risk sub-group in need of novel treatment strategies. Lenalidomide has shown high response rates in MCL in combination with rituximab, and preliminary experiences from chronic lymphocytic leukemia show promising effects in high-risk patients, including cases with *TP53* aberrations.
Aims: Thus, in this study we aimed at exploring the additive effect of lenalidomide to chemo-immunotherapy in *TP53* mutated patients.
Methods: The study cohort consisted of 50 elderly (or younger and frail) patients from the previously published frontline trial, MCL4 "Lena-Berit" (Albertsson-Lindblad, Blood, 2016), and of these pre-treatment DNA was available for 46 patients. The regimen consisted of an induction phase of bendamustin, rituximab and lenalidomide for 24 weeks, followed by lenalidomide maintenance for another 32 weeks. Pre-treatment bone marrow (BM, n=39) or peripheral blood (PB, n=7) DNA samples were analysed for the common deletions (chr17p13 (*TP53*) and chr9p21 (*CDKN2A*)) by droplet digital PCR (ddPCR) and point mutations and smaller deletions/insertions (indels) by targeted DNA sequencing on the Ion Torrent platform as previously described (Eskelund, Blood, 2017).
Results: After a median FU of 45 months, median overall (OS) and progression-free (PFS) survival were 69 months and 42 months, respectively. A drawback to the regimen was the large number of adverse events, especially infections and secondary malignancies. To date, 9 secondary malignancies have been reported (not including non-invasive skin cancers).
Most common mutations were *ATM* (34%), *KMT2D* (18%) and *TP53* (14%), and deletions of *TP53* and *CDKN2A* were detected in 20% and 22%, respectively. *TP53* (n=6) mutations were associated with significantly shorter median OS (25 months (95% CI: 6.6-43.4) versus 69 months (95% CI: 67.0-70.7), p<0.0001), PFS (10 months (95% CI: 0-22.9) versus 42 months (95% CI: 21.8-62.2), p=0.001) and time to relapse/progression (10 months (95% CI: 0-22.9) versus 58 months (95% CI: 35.7-80.3), p<0.0001) (Figure 1), and none of *TP53*-mutated patients achieved MRD negativity in both BM and PB at any time during treatment. Cases with deletions of both *TP53* and *CDKN2A* showed a trend towards inferior outcomes; however this was not significant.

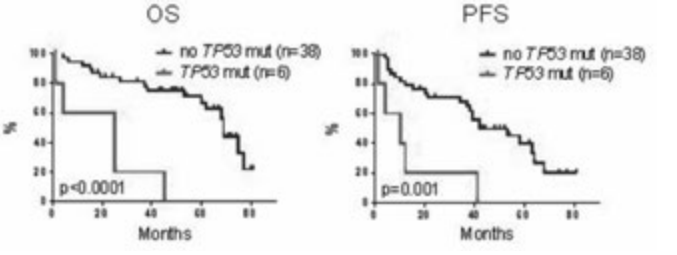


Figure 1.

Summary/Conclusion: Our results confirm the poor prognostic impact of *TP53* mutations in MCL, despite the addition of lenalidomide to rituximab-bendamustine. Trials evaluating novel agents in relation to *TP53* status are highly warranted to improve outcome in these MCL patients.

PS1017

OUTCOME OF PATIENTS WITH RELAPSED/REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) NOT ELIGIBLE FOR AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT)

R. Pytlik[1,2,*], K. Polgárová[1], A. Janíková[3], D. Belada[4], V. Procházka[5], H. Mociková[6], D. Šálek[3], M. Šimkovič[7], T. Papajík[5], P. Otáhal[18], P. Blahovcová[1], K. Benešová[1], M. Trněný[1]

[11]st Department of Medicine, [1]st Medical Faculty, Charles University, [2]Department of Cellular Therapy, Institute of Hematology and Blood Transfusion, Prague, Praha [2], [3]Department of Medicine, Hematology and Oncology, Medical Faculty, Masaryk´s University Brno, Brno, [4]IV. Department of Medicine - Hematology, Medical Faculty Hradec Králové, Charles University, Hradec Králové, [5]Department of Hemato-oncology, Medical Faculty, Palackého University, Olomouc, Olomouc, [6]Department of Medicine - Hematology, [3]rd Medical Faculty, Charles University, Prague, Praha [10], [7]IV. Department of Medicine - Hematology, Medical Faculty Hradec Králové, Charles University, Hradec Králové, [8]Department of Immunohematology, Institute of Hematology and Blood Transfusion, Prague, Praha [2], Czech Republic

Background: Relapsed/refractory DLBCL has poor prognosis, especially when patients cannot be salvaged by high dose therapy with SCT (age, comorbidities). There is no standard of treatment for these patients, therefore, the optimal combination of drugs, as well as intensity of next-line therapy have to be solved.
Aims: To describe the outcome of patients in Czech Lymphoma Study Group (CLSG) database who were refractory to or relapsed after R-CHOP treatment and found ineligible for SCT.
Methods: This analysis was a part of the NiHiL project (GovTrial No: NCT03199066). Patients with DLBCL progressing on or after treatment with R-CHOP (including reduced intensity R-CHOP or R-miniCHOP) between 2006 and 2016 who did not receive transplantation were identified. Primary progression was defined as progression ≤ 3 months, early relapse as progression 3-12 months, and late relapse > 12 months after completion of R-CHOP. Intensive second-line therapy was defined as a salvage regimen with ≥3 cytotoxic drugs, including high-dose dexamethasone. Palliative second-line treatment was defined as a regimen with ≤ 2 cytotoxic drugs. Categorical data were compared by chi-square tests, numerical data with Mann-Whitney U tests, survival curves were constructed according to Kaplan-Meier method and compared by log-rank test.
Results: Of 355 R/R DLBCL patients, 55 (15%) received autologous SCT and 1 (0.3%) allogeneic SCT. Median age of 299 patients, who were not transplanted, was 71 years (32-91). 48% of them were males, 64% had clinical stage III-IV, 65% had elevated LDH, 41% had ECOG performance status ≥ 2 and 62% had secondary IPI 3-5. There were 37% primary progressions, 30% early and 33% late relapses, with no significant differences in characteristics among these three subgroups. 41% of patients received

intensive salvage treatment at relapse, 130 patients (44%) palliative treatment and 47 patients (16%) best supportive care only. 52% of patients received rituximab (Rx). Median PFS for the whole group was 4.7 and median OS 8 months, with significant survival differences among primary progressions, early and late relapses (OS 4.3 v. 8.3 v. 14.5 months, p=0.0007, PFS 3.2 v. 5.0 v. 9.2 months, p=0.0001). Survival differences were not statistically significant among age subgroups ≤60, 61-70 and >70 years (OS 14.1 v. 6.7 v. 8.6 months, p=0.13, PFS 4.1 v. 4.1 v. 6.2 months, p=0.50). When patients receiving best supportive care only were excluded, there were no significant differences between intensive and palliative second-line treatment subgroups (OS 9 v. 8.4 months, p=0.7927, PFS 5 v. 5 months, p=0.6245). When patients receiving only best supportive care were excluded, Rx improved only PFS (5.5 v. 3.2 months, p=0.04) but not OS (10.9 v. 4.9 months, p=0.07) compared to treatment without Rx.

Summary/Conclusion: In our registry-based population of R/R DLBCL patients, 85% did not receive SCT, and their prognosis was dismal. Intensive second-line treatment did not bring survival benefit in transplant ineligible patients. Addition of rituximab to second-line therapy significantly improved their progression-free but not overall survival.

This study was supported by grants AZV R 16-31092A and AZV R 15-34498A

PS1018

WOMEN WITH DIFFUSE LARGE B CELL LYMPHOMA BENEFIT MORE FROM RITUXIMAB-CONTAINING CHEMOTHERAPY

H.-H. Huang[1,*], F.-Y. Hsiao[2,3,4], L.-J. Chen[1], H.-M. Chen[5], B.-S. Ko[1]

[1]Division of Hematology, Department of Internal Medicine, National Taiwan University Hospital, [2]Graduate Institute of Clinical Pharmacy, [3]School of Pharmacy, [4]Department of Pharmacy, [5]Health Data Research Center, National Taiwan University, Taipei City, Taiwan, Republic of China

Background: Diffuse large B cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma. The treatment response and overall survival improved after incorporating rituximab with chemotherapies. Yet, available evidence as to whether women and men may benefit similarly from rituximab have not been adequately addressed, particularly in the real-world setting.

Aims: To examine gender differences in the clinical outcomes of rituximab in DLBCL patients using the Taiwan Cancer Registry Database and National Health Insurance Research Database.

Methods: All DLBCL patients aged 20 years and older during 2009 to 2013 were identified (n=4490; women=2048). Cox proportional hazard models were used to compare the results.

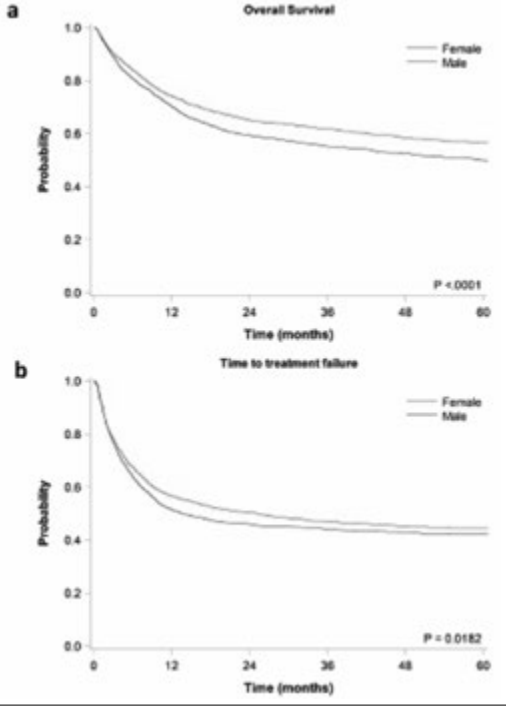


Figure 1.

Results: The baseline characteristics were similar between women and men with DLBCL, except that women had lower Charlson Comorbidity scores (CCS), and that fewer women underwent R-CHOP. In the survival analysis, women had better overall survival (OS) and longer time to treatment failure (TTF). The multivariate analysis of OS showed that the female gender remained to be an independent favorable prognostic factor regardless of Ann Arbor stages, age, treatments, CCS, and practice settings. In the subgroup analysis, the female advantage was only significant in the patients receiving rituximab-CHOP chemotherapy instead of in those receiving other rituximab-containing or non-rituximab therapies. This advantage diminished when rituximab dose was higher. (Figure 1).

Summary/Conclusion: From our population-based study, women demonstrated more survival benefits from the use of rituximab-containing induction chemotherapies for DLBCL.

PS1019

HIGH RISK AGGRESSIVE B-CELL LYMPHOMAS IDENTIFIED BY FISH: A MULTICENTRIC RETROSPECTIVE STUDY FROM CENTERS OF THE FONDAZIONE ITALIANA LINFOMI

M. C. Tisi[1,2,*], S. Ferrero[3], I. Dogliotti[3], S. Rattotti[4], M. Novo[5], C. Tecchio[6], F. Gherlinzoni[7], G. Carli[1], M. Balzarotti[8], D. Marino[9], A. Romano[10], L. Flenghi[11], M. Pelosini[12,13], A. Di Napoli[14], M. Zanni[15], E. Finolezzi[16], T. Calimeri[17], F. Mosna[7,18], A. Di Rocco[19], G. Tomei[20], N. Sgherza[21], J. Olivieri[22,23], A. Fama[24], M. Giannoccaro[25], A. M. Mazzone[26], C. Visco[1], G. Loseto[27], F. Zaja[23]

[1]Cell Therapy and Hematology, San Bortolo Hospital, Vicenza, [2]Institute of Hematology, Catholic University of the Sacred Heart, Rome, [3]Division of Hematology [1], Department of Molecular Biotechnologies and Health Sciences, University of Torino/AOU "Città della Salute e della Scienza di Torino", Torino, [4]Department of Hematology-Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia, [5]Division of Hematology [2], AOU "Città della Salute e della Scienza di Torino", Torino, [6]Medicine, Section of Hematology and Bone Marrow Transplant Unit, University of Verona, Verona, [7]Hematology, Dept. of Specialty Medicine, Ospedale Ca Foncello, ALSS[2], Treviso, [8]Hematology Unit, Humanitas Cancer Center, IRCCS Rozzano, Milano, [9]Medical Oncology [1], Veneto Institute of Oncology IOV IRCCS, Padova, [10]Hematology, AOU Policlinico Vittorio Emanuele, Catania, [11]Hematology, O. Santa Maria della Misericordia, Perugia, [12]Clinical and Experimental Medicine, Section of Hematology, University of Pisa, Pisa, [13]Hematology, Ospedale di Livorno-USL Nord-Ovest Toscana, Livorno, [14]Department of Clinical and Molecular Medicine, Roma Sapienza University, Sant'Andrea Hospital, Rome, [15]Hematology, AO SS Antonio e Biagio, Alessandria, [16]Hematology, Transfusional Medicine and Biotechnologies, UOSD "Centro Diagnosi e Terapia dei Linfomi", PO Santo Spirito, Pescara, [17]Unit of Lymphoid Malignancies, Department of Onco-Haematology, IRCCS San Raffaele Scientific Institute, Milano, [18]Hematology and BMT Unit, Ospedale Centrale San Maurizio, ASDAA, Bolzano, [19]Cellular Biotechnologies and Hematology, 'Sapienza University', Rome, [20]Hematology, Ospedale di Ivrea-ASL TO[4], Ivrea, [21]Hematology, IRCCS Ospedale Casa Sollievo Sofferenza , S. G. Rotondo, [22]Hematology, Ospedale di Civitanova Marche, Civitanova Marche, [23]Clinica Ematologica, Università degli Studi di Udine, Udine, [24]Ematologia, Azienda Unità Sanitaria Locale di Reggio Emilia – IRCCS, Reggio Emilia, [25]Hematology, ASL Le/[1] P.O. Vito Fazzi, Lecce, [26]Hematology, ASL Taranto, Taranto, [27]Hematology and Cell Therapy Unit, IRCCS Istituto Tumori "Giovanni Paolo II", Bari, Italy

Background: Double-hit lymphomas (DHL) are high-risk, non-Hodgkin B-cell lymphomas with dismal prognosis. The definition of DHL based on MYC, BCL2 and BCL6 abnormalities by FISH analysis is debated, as well as the optimal treatment strategy.

Aims: This multicentric, retrospective study aimed to characterize the clinical landscape of DHL in Italy, describing the current diagnostic paradigms and the preferred therapeutic choices.

Methods: Patients (pts) with ewly diagnosed diffuse large B-cell lymphoma (DLBCL) or "unclassifiable" aggressive B-cell lymphoma (BCLU) with at least one FISH alteration on either MYC, BCL2 or BCL6 loci were included. The main endpoints were clinical response rate, progression-free survival (PFS) and overall survival (OS).

Results: A total of 124 MYC aberrant pts (2010-2016) with DLBCL [42 BCLU and 82 DLBCL NOS] were screened by FISH among 30 Institutions of the Fondazione Italiana Linfomi. 109 pts resulted MYC translocated by FISH analysis. Of these pts, 83/109 (76%) were also studied for BCL2 and 58/109 (53%) for BCL6. Thus, out of the 109 MYC translocated pts, 60/109 (55%) were classified as DHL [41/60 (68%) MYC/BCL2, 19/60 (32%) MYC/BCL6] and 5/109 (4%) as "triple hit" lymphoma [(THL) - MYC/BCL2/BCL6]. 18/109 (17%) pts showed only a single translocation on MYC (S-MYC). The other 26/109 (24%) resulted not clearly classifiable (studied by FISH for MYC only). Thus, further analysis was focused on the series of 83 S-MYC/DHL/THL pts. Median age was 62 years, (19-84), 50 males. Thirty-one (37%) showed ECOG-PS≥2, 64 (77%) stage >2, 44 (53%)

>1 extranodal site, 59 (71%) elevated LDH and 48 (58%) bulky disease (> 6 cm). Therefore, 30 pts (36%) had intermediate and 53 (64%) had high IPI risk group. Fifty pts (69%) were defined as GCB according to the Hans algorithm. The most common upfront treatments were intensive regimens (R-hyperCVAD/MA, BFM, CODOX-M/IVAC or front line HSCT, n=37), median age 62, followed by R-CHOP (n=31), median age 61, and DA-EPOCH-R (n=11), median age 64. Four patients received palliative regimens. The median follow up of survivors was 27.7 months. The CR rate was 57% in the overall series and 42% in DHL/THL group. The 2 years PFS and OS of the overall series were 55% (95% CI 43-65) and 59% (95% CI 47-69), respectively; in the DHL/THL group the 2 years PFS and OS were 50% (95% CI 36-61) and 56% (96% CI 42-67), respectively. According to received treatment, the 2 years PFS of the overall series was 65% (95% CI 47-78) in pts who received intensive treatments, 48% (95% CI 29-65) in pts who received R-CHOP, and 40% (95% CI 11-68) in pts who received DA-EPOCH-R (p=0.05). This trend was confirmed in the DHL/THL group, with a 2 years PFS of 66% (95% CI 45-80) vs 42% (95% CI 22-61), vs 28% (95% CI 4-59), respectively (p=0.02), (Figure 1). B-symptoms (p=0.04), ECOG PS≥2 (p=0.005), bone marrow invasion (p=0.02), elevated LDH (p=.0.03) and high risk IPI (p=0.03) were associated to shorter PFS in univariate analysis, while only ECOG PS≥2 was independent predictor of PFS in multivariate analysis (p=0.01).

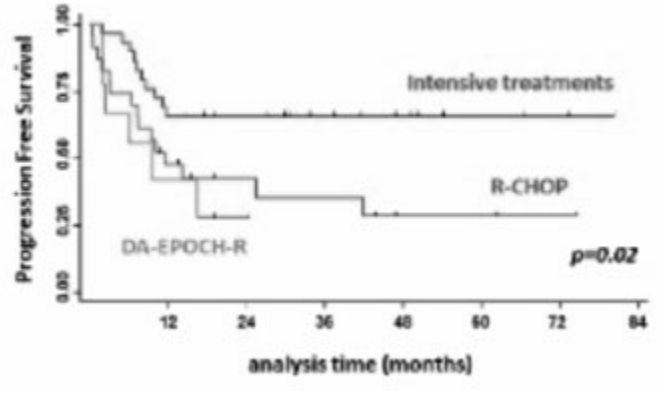


Figure 1. PFS in 65 DHL/THL pts according to received treatment.

Summary/Conclusion: This is the first, multicenter, retrospective study collecting DHL cases in Italy. Despite a fully annotated FISH profile is not always available in all centers, our preliminary data confirms that R-CHOP is no more the standard for these high-risk pts and suggest that intensified treatments might induce a PFS advantage. An extension of the series is needed to strengthen the data.

On behalf of the Fondazione Italiana Linfomi Postgraduate Master course.

PS1020

RITUXIMAB, BENDAMUSTINE AND CYTARABINE (R-BAC) IN PATIENTS WITH RELAPSED-REFRACTORY AGGRESSIVE B- CELL LYMPHOMA

M. C. Tisi[1,*], R. Paolini[2], F. Piazza[3], C. Tecchio[4], E. Ravelli[5], B. Famengo[6], E. S. D'Amore[6], G. Carli[1], O. Perbellini[1], M. Ruggeri[1], C. Visco[1]

[1]Cell Therapy and Hematology, San Bortolo Hospital, Vicenza, [2]Oncohematology, Santa Maria della Misericordia Hospital, Rovigo, [3]Hematology and Clinical Immunology Unit, Department of Medicine, University of Padua, Padova, [4]Medicine, Section of Hematology and Bone Marrow Transplant Unit, University of Verona, Verona, [5]Hematology, Valduce Hospital, Como, [6]Department of Pathology, San Bortolo Hospital, Vicenza, Italy

Background: Relapsed or refractory (R/R) aggressive lymphomas (i.e. diffuse large B-cell lymphoma -DLBCL-) have poor outcome, especially if not candidate to autologous stem cell transplant (ASCT). In this setting no standard therapy exists. Among salvage regimens that may be used, the combination of rituximab and bendamustine was associated to promising overall response rates (OR, 32-63%), but progression-free survival (PFS) was in the range of 3 to 8 months.

Aims: In this multicenter pilot study we evaluated the safety and efficacy of rituximab, bendamustine and cytarabine (R–BAC), as salvage treatment in R/R aggressive B-cell lymphomas not eligible to ASCT.

Methods: The study enrolled previously treated, histologically confirmed DLBCL, either de-novo or transformed from low grade B-cell lymphoma (t-DLBCL), and primary mediastinal B-cell lymphoma (PMBL), treated with the R-BAC regimen between November 2012 and September 2017 in four Centers. The R-BAC consisted of rituximab (R, 375 mg/m^2 intravenously [IV], day1), bendamustine (B, 70 mg/m^2 IV, days 2 and 3), and cytarabine (500 mg/m^2, IV on days 2 to 4) every 21/28 days, up to 6 cycles. The primary end-point was the complete remission rate (CR). Secondary end-points included OR, PFS, overall survival (OS), duration of response (DOR), and safety. Response was assessed according to IWG 2007 criteria.

Results: Thirty two patients (20 DLBCL, 11 t-DLBCL, 1 PMBL), aged 21-84 years (median 68), were enrolled and treated. All patients had received anthracycline containing induction therapy (R-CHOP or R-CHOP-like), six (19%) had previous ASCT, and median number of previous treatment was 2 (range 1-4). Median time from initial lymphoma diagnosis to enrollment was 22 months (4-120). Overall, 47% had relapsed disease, and 53% had refractory disease, with 35% of patients being refractory both to R-CHOP and R-DHAP (double refractory).

Patients received a median of 4 cycles of R-BAC (2-6). Overall, OR was 66%, and CR was 38%. Tumor shrinkage was observed in 78% of patients (25/32), as shown in the Waterfall plot in Figure 1A. Among different histologies, OR was 75% in DLBCL (CR 45%), and 55% in t-DLBCL (CR 27%). The only patient with PMBC had a transient promising response that was then classified as stable disease. The median PFS and OS were 13.9 months, and 17.3 months, respectively (Figure 1B). Median duration of response was 16 months. Treatment was well tolerated, with main toxicity being hematological, as expected. Treatment was discontinued before cycle 4 in 15 patients (47%), due to toxicity/adverse events in two patients, progressive disease in nine patients, and other reasons in four patients. Twenty patients (63%) had to delay or reduce treatment dose at least once along cycles, however, delays lasted < 14 days. Refractory disease (p=0.03), shorter time since prior anti-lymphoma treatment (p=0.007) and non-GCB cell-of-origin (p=0.01) were the only variables associated to a significantly inferior PFS in univariate analysis.

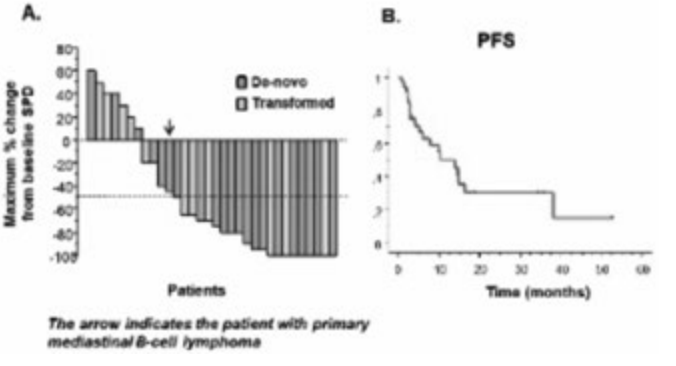


Figure 1.

Summary/Conclusion: R-BAC had promising activity with an acceptable toxicity profile in this small pilot trial on heavily pretreated R/R aggressive lymphomas. Our results suggest that R-BAC should be further investigated in this setting.

PS1021

A PROPENSITY SCORE ANALYSIS SHOWS THAT RITUXIMAB DOSE-ADJUSTED EPOCH IMPROVES PROGRESSION-FREE SURVIVAL OF YOUNG PATIENTS AFFECTED BY DOUBLE EXPRESSOR DIFFUSE LARGE B -CELL LYMPHOMAS

A. Guidetti[1,*], A. Dodero[1], A. Tucci[2], M. Novo[3], L. Devizzi[1], L. Farina[1], G. Pruneri[4], F. Barretta[5], R. Miceli[5], V. Monti[4], G. Dagrada[4], M. Pennisi[1], M. Dichio[1], A. Mussetti[1], C. Carniti[1], G. Rossi[2], P. Corradini[1]

[1]Hematology , Fondazione IRCCS Istituto Nazionale dei Tumori Milano , Milano, [2]Hematology , Azienda Ospedaliera Spedali Civili Brescia, Brescia, [3]Hematology, Azienda Ospedaliero Universitaria Citta' della Salute e della Scienza di Torino, Torino, [4]Pathology, [5]Department of Clinical Epidemiology and Trial Organization, Fondazione IRCCS Istituto Nazionale dei Tumori Milano , Milano, Italy

Background: A large portion of diffuse large B-cell lymphomas (DLBCL), ranging from 20% to 40%, has the double expression of MYC and BCL2 proteins (DEL). A subset of these patients have also Double Hit or Triple Lymphomas (DHL/THL) with concurrent chromosomal rearrangements involving the MYC and BCL2 and/or BCL6 genes. These lymphomas have poor outcome following standard R-CHOP chemotherapy. The optimal

induction regimen for DEL and DHL/THL has not been established yet. Preliminary data suggested a high complete response rate following Dose Adjusted EPOCH plus Rituximab (R-DAEPOCH).
Aims: The purpose of this study was to retrospectively compare survival outcome of DEL and DH/TH DEL pts treated with RDAEPOCH or RCHOP. A propensity score analysis (PS) was used to identify a control RCHOP group matched 1:1 by age, stage and IPI.
Methods: Eligible patients (n=121) had biopsy proven diagnosis of *de novo* DEL and were treated between January 2010 and December 2017. DEL were defined by immunohistochemical analysis with MYC expression ≥40% and BCL2 ≥ 50% of tumor cells. Fluorescence in situ hybridization (FISH) analysis for MYC, BCL2 and BCL6 was performed in all cases. Assignement of tumors to germinal center B-cell like (GCB) DLBCL or non GCB was done according to Hans algorithm. Fifty-eight pts were consecutively treated with R-DAEPOCH (RDA cohort) and their outcome was compared with a cohort of 63 patients treated with R-CHOP. Assignement to different treatment was based on istitutional practice at three Italian Hematological Divisions.
Results: After a median follow-up of survivors of 20 months (range, 6-100), estimated 3 years PFS and OS was 66% (95%CI:48%>79%) and 69% (95%CI: 35%>87%) in RDA cohort and 49% (95%CI: 36%>61%) and 62%(95%CI: 48%>73%) in R-CHOP cohort, respectively. Toxic deaths were observed in both groups (n=1 RDA, n=2 R-CHOP). Propensity score matching identified 45 pts pairs: median age was 56 (range, 28-78) and 63 (range, 33-84), stage III/IV were 37 (82%) and 39 (87%) and IPI 3-5 were 25 (56%) and 21 (47%), non-GCB pts were and 22 (49%) and 27 (60%), DH/TH pts were 9 (20%) and 1 (2%), in R-DA and R-CHOP group respectively. Considering the 90 pts (45 pairs), no statistically significant difference in 3-years PFS (66% versus 44%, p=0.102) and OS (65% versus 60%, p=0.149) were found in pts treated with RDA and R-CHOP, respectively. Younger patients (≤65 years) (n=59) had a significant advantage from the treatment with RDA in terms of PFS (p= 0.00873) and a trend for OS (88% versus 56%, p=0.05). Of note, the estimated 3-years OS was 73% and 58% in the pts who receive RDAEPOCH with DEL or DEL and DHL/THL, respectively.
Summary/Conclusion: RDAEPOCH is an active and safe regimen for DEL. In comparison to R-CHOP, we reported a significative advantage in term of PFS in patients aged below 65 yrs. This result is probably related to the better dose escalation achieved in the young population. Randomized trials are needed to confirm these results.

PS1022

GENETIC EVIDENCE IMPLYING THE COMMON PRECURSOR CELLS FOR PRIMARY AND EXTRA-CENTRAL NERVOUS SYSTEM RELAPSED TUMORS IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

K. Hattori[1,*], M. Sakata-Yanagimoto[1], T. Nanmoku[2], Y. Suehara[2], R. Matsuoka[2], M. Noguchi[2], Y. Yokoyama[2], T. Kato[2], N. Kurita[2], H. Nishikii[2], N. Obara[2], S. Takano[2], E. Ishikawa[2], A. Matsumura[2], M. Muratani[2], Y. Hasegawa[2], S. Chiba[2]

[1]Hematology, [2]University of Tsukuba, Tsukuba, Japan

Background: Primary central nervous system lymphoma (PCNSL) is a rare form of diffuse large B cell lymphoma that arises within the brain or the eyes. Recent exome sequencing studies have revealed that the L265P *MYD88* mutation is highly prevalent in patients with PCNSL (38-85.4%). This mutation, but not other mutations found in PCNSL tumors, was previously reported to be detected in peripheral blood mononuclear cells (PBMNCs). This finding may imply the presence of clonal expansion of B cells harboring the L265P *MYD88* mutation at outside the central nervous system (CNS) compartment.
Aims: We explored this possibility by examining paired primary PCNSL and extra-CNS relapse tumors, and bone marrow mononuclear cells (BMMNCs).
Methods: Targeted sequencing was performed for 39 genes in 5 pairs of primary intra-CNS and relapsed extra-CNS tumors using the Lymphopanel (Dubois, Clin Cancer Res 2016) and our in-house panel (Hattori, Br J Haematol 2017).Whole exome sequencing (WES) was also performed on 1 pair of primary and relapsed tumors and BMMNCs.
Results: The mean coverages using these panels were 1310×(range, 379 to 3099) and 1563×(range, 1084 to 1990), respectively. Targeted sequencing in 5 primary intra-CNS tumors and relapsed extra-CNS tumors identified 60 and 68 gene mutations, respectively. The mean coverage in WES was 81.1×(range, 64.9 to 99.1). WES in primary and relapsed tumors identified 45 and 10 mutations, respectively. By combining the results from targeted sequencing and WES, recurrent mutations were found in *MYD88* (4/5, 80%), *CD79B* (4/5, 80%), *PIM1* (3/5, 60%), *KMT2D* (3/5, 60%), *GNA13* (2/5, 40%), *CARD11* (2/5, 40%), and *PRDM1* (2/5, 20%) in either or both of the primary intra-CNS and relapsed extra-CNS tumors. The shared genetic alterations was present in all the 5 pairs. Especially, the *MYD88* mutations, L265P in 3, and P258L in 1, were shared by the 4 paired tumors. On the other hand, somatic mutations in other genes, such as *GNA13*, *CREBBP*, *BTG2*, and *TNFAIP3* were specific to either primary or relapsed tumors.IgH rearrangement status of paired tumors was examined to determine the clonal evolution from common precursor cells (CPCs). Because of the limitation of the quality of DNAs, the procedure was successful only in one paired tumors. The intra- and the extra-CNS tumors of this patient showed the monoclonal bands. Direct sequencing of the clonal bands confirmed that both tumors shared the same VDJ sequences. These data suggest that intra-CNS and extra-CNS tumors may originate from CPCs having the identical VDJ usage.In order to confirm the presence of pre-lymphoma cells with the L265P *MYD88* mutations, tumor-free BMMNCs or PBMNCs from patients whose tumors had this mutation were also studied. This mutation was detected with droplet digital PCR assay in 10 out of 24 tumor-free BMMNCs or PBMNCs, most of which were stemming from patients without extra-CNS relapse.
Summary/Conclusion: In this study, the analysis of 5 PCNSL patients who relapsed at extra-CNS sites suggest a branched pattern evolution from the *MYD88*-mutated CPCs to either primary PCNSL or relapsed extra-CNS tumors, supporting the concept of CPCs. Additionally, from the result of the analysis for the BMMNCs of CNS lesion-only patients, the possibility of the presence of pre-lymphoma cells in the extra-CNS compartment was also raised. Based on the result of these combinational analyses, it should be reasonable to consider that the pre-lymphoma cells outside of CNS can develop into primary intra-CNS tumors and occasionally into relapsed extra-CNS tumors.

PS1023

REAL-TIME CELL-OF-ORIGIN SUBTYPE IDENTIFICATION BY GENE EXPRESSION PROFILING IN PATIENTS WITH ABC-TYPE DLBCL IN THE PHASE III TRIAL OF LENALIDOMIDE + R-CHOP (R2-CHOP) VS PLACEBO + R-CHOP (ROBUST)

A. Chiappella[1,*], U. Vitolo[1], T. E. Witzig[2], D. W. Scott[3], M. Spina[4], R.D. Gascoyne[3], L. Zhang[5], J. Russo[5], J. Kang[5], J. Zhang[5], Y. Xu[5], G.S. Nowakowski[2]

[1]Division of Hematology, Città della Salute e della Scienza Hospital and University, Torino, Italy, [2]Division of Hematology, Mayo Clinic, Rochester, United States, [3]Centre for Lymphoid Cancer, British Columbia Cancer, Vancouver, Canada, [4]Division of Medical Oncology A, National Cancer Institute, Aviano, Italy, [5]Celgene Corporation, Summit, United States

Background: Activated B-cell-like (ABC) diffuse large B-cell lymphoma (DLBCL) is a subtype associated with inferior outcomes. Gene expression profiling (GEP) is the gold standard in identification of ABC-type DLBCL. In previously reported phase II studies, the combination of lenalidomide + R-CHOP (R^2-CHOP) provided efficacy based on cell-of-origin (COO) in patients with DLBCL (Vitolo, *Lancet Oncol* 2014; Nowakowski, *J Clin Oncol* 2015).
Aims: The goal of this analysis was to investigate the feasibility of performing real-time COO assessments using GEP in a large, global, randomized study.
Methods: ROBUST is a global, multicenter, randomized, double-blind, phase III study comparing R^2-CHOP vs placebo + R-CHOP in patients with previously untreated ABC-type CD20+ DLBCL (NCT02285062). ROBUST methods were previously described, and patients provided informed consent (Nowakowski, *Fut Oncol* 2016). Formalin-fixed paraffin-embedded excisional/surgical or core needle biopsy samples (Storhoff, *Blood* 2015) were analyzed by central pathology using the NanoString Lymphoma Subtyping Test (LST; Wallden, *JCO* 2015), based on the Lymph2Cx GEP assay (Scott, *Blood* 2014). Turnaround time was defined as the number of days between receipt of the central pathology sample and results being provided to the study site.
Results: 2093 patients were screened from January 21, 2015 to August 3, 2017; 570 patients were enrolled in ROBUST. Samples were analyzed in three central pathology labs in China, USA and the UK. Of 2110 submitted samples (including 1 or more sample per patient), 1798 were successfully tested; 312 (15%) samples could not be processed for technical reasons (incorrect/insufficient slides or blocks, or low tissue RNA concentration and/or purity). Mean turnaround time was 2.4 days. Among successfully tested samples, COO was 788 (44%) ABC and 1010 (56%) non-ABC. The ABC-type DLBCL rate according to geographic region of origin was 60% (241/404) from China/Japan/South Korea/Taiwan; 40% (441/1105) from

Russia/Europe/Middle East; and 37% (106/289) from North America/Australia/New Zealand.
Summary/Conclusion: In the phase III ROBUST study, real-time COO assessment from multiple global regions was feasible with a short turnaround time, which minimizes the delay in patients receiving treatment. The proportion of patients with ABC-type DLBCL was similar to previous reports in the literature. Our findings impact the size estimation and design of future studies utilizing COO as a biomarker in newly diagnosed DLBCL, which provides a significant advance in precision medicine for patients with DLBCL.

PS1024

EXCELLENT OUTCOMES OF OLDER PATIENTS WITH PCNSL USING R-MPV/ARA-C IMMUNOCHEMOTHERAPY WITHOUT WHOLE BRAIN RADIOTHERAPY (WBRT)

M. Tatarczuch[1,2,*], E. Paul[34], M. Gilbertson[2], G. Gregory[25], A. Bazargan[6], H. Quach[4], R. Filshie[4], M. Ku[4], C. Tam[4], S. Opat[27]

[1]Medicine, Monash University, [2]Haematology, Monash Health, [3]Medicine, University of Melbourne, [4]Haematology, St. Vincent's Hospital, [5]School of Clinical Sciences at Monash Health, Monash University, Melbourne, [6]Haematology, St. Vincent's Haematology, Melbourn, [7]School of Clinical Sciences at Monash Health, Monash University, Monash, Australia

Background: Primary CNS lymphoma (PCNSL) is a rare and poor-prognostic disease. While randomised studies including IELSG32 have confirmed chemoimmunotherapy regimens containing methotrexate, cytarabine and rituximab as standard of care, the role of ASCT or WBRT consolidative strategies are associated with significant toxicities and questionable benefit. Furthermore, many PCNSL patients are precluded from ASCT or WBRT due to age and co-morbidities.
Aims: This study seeks to assess outcomes of R-MPV/Ara-C chemotherapy in older (≥60yo) PCNSL patients.
Methods: Patients presenting with PCNSL (WHO Criteria: 2008) and treated with curative intent using R-MPV/Ara-C (Morris PG *et al.* J Clin Oncol. 2013;31(31):3971-9) were identified at 2 university hospitals: Monash Health (November 2009-December 2016) and St. Vincent's Hospital (June 2003-October 2012). Patients were separated according to age (i.e >60 vs ≤60yo). Risk-stratification was as per IELSG criteria. In the >60yo cohort, consolidative WBRT was omitted for patients in complete remission (CR) as per institutional practices. Only cases with biopsy-proven PCNSL and adequate information (i.e. baseline characteristics, treatment and outcome) were included. Outcome analysis was restricted to the rituximab cohort. Overall survival (OS) and progression free survival (PFS) were modelled by Cox regression. The Mann-Whitney and Fischer's exact test were used to calculate p-values for continuous and discrete variables, respectively.
Results: 44 patients were identified, 38 received R-MPV/ARA-C as per protocol (excluded: received other chemotherapy regimens: 6). Of these, 27 (71%) were >60yo (median: 67.5, range 38-86, p<0.0001) and 74% were intermediate-risk as per IELSG criteria (p>0.999). Therapy was well tolerated; 78% of older patients completed MTX therapy (vs 100%, p= 0.154), but with more frequent dose-attenuation (48% vs 9%, p: 0.003). WBRT was rarely used in the older cohort (11% vs 91%, p <0.0001). Despite this, older patients had excellent response rates (OR 89% vs 100%, p:0.54), 5-year PFS (65% vs 81%, p=0.54], and OS (65% vs 81%, p=0.56) [Figure 1]. There were 8 deaths, 6 of which were >60yo (median age: 70yo [57-83]). Neurotoxicity was reported in 5 (19%) patients, 3 of whom received WBRT, and contributed to the death of 1 patient.

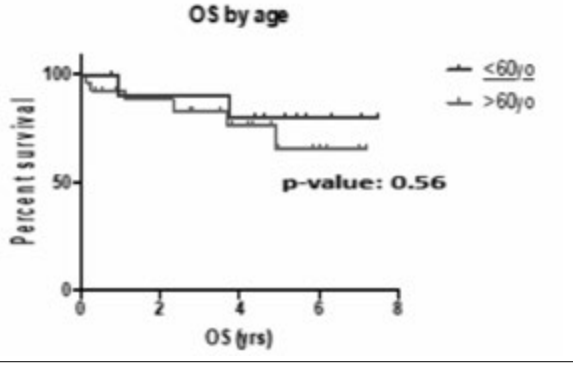


Figure 1.

Summary/Conclusion: Older patients treated with R-MPV/Ara-C without WBRT have excellent outcomes. Age and comorbidity should not exclude patients from receiving potentially curative immunochemotherapy.

PS1025

FIRST REPORT ON A SUCCESSFUL SCREENING PROGRAM FOR MYC REARRANGEMENTS AND A PROSPECTIVE CLINICAL TRIAL BASED ON MYC REARRANGEMENT IN NEWLY DIAGNOSED DLBCL PATIENTS IN THE NETHERLANDS

M. Chamuleau[1,*], M. Nijland[2], N. Lamers[3], N. Hijmering[4], K. Bakunina[3], J. Zijlstra[1], O. Visser[5], J. Vos[6], H. Berenschot[7], R. Boersma[8], M.J. Kersten[9], D. de Jong[4]

[1]Department of Hematology, VU University Medical Center, Amsterdam, [2]University Medical Center Groningen, Groningen, [3]HOVON Data Center, Rotterdam, [4]Department of Pathology, VU University Medical Center, Amsterdam, [5]Isala Clinics, Zwolle, [6]St Antonius Hospital, Nieuwegein, [7]Albert Schweitzer Hospital, Dordrecht, [8]Amphia Hospital, Breda, [9]Department of Hematology, Academic Medical Center, Amsterdam, Netherlands

Background: Patients with B-cell lymphoma that harbor a *MYC* rearrangement (Diffuse Large B-cell Lymphoma (DLBCL) or B-cell lymphoma with features intermediate between DLBCL and Burkitt lymphoma (BCL-U)) have a dismal prognosis following standard first-line treatment. Intensifying chemotherapy prolongs disease free survival when compared to R-CHOP. However, relapses often occur and overall survival has not been improved, hence justifying trials with MYC targeting drugs. Lenalidomide has demonstrated to down-regulate MYC and its target genes in *MYC* rearranged B-cells. Although lenalidomide seems mainly effective in ABC subtype DLBCL, the ability of MYC downregulation provides a rationale for lenalidomide administration in *MYC* rearranged lymphoma, that are usually of the GCB subtype.
Aims: To set up a *MYC* FISH screening program for all newly diagnosed DLBCL patients which allows patients with a *MYC* rearrangement to be included in a prospective interventional clinical trial to improve their prognosis.

Table 1. Clinical characteristcs of the total 32 eligible patients in the HOVON 130 study.

	N (%)
Total	82 (100)
Age	
median (range) in years	63 (29-82)
Sexe	
male	56 (68)
female	26 (32)
WHO	
0 or 1	75 (92)
2	5 (6)
3	2 (2)
Pathology diagnosis	
DLBCL	76 (93)
BCL-U	2 (2)
yet unknown	4 (5)
Ann Arbor stage	
I-II	13 (16)
III	12 (15)
IV	57 (69)

Methods: A screening program for all newly diagnosed DLBCL patients in which performing *MYC* FISH is advocated (HOVON 900) has been set

up. Patients with a proven *MYC* rearrangement were subsequently included in a clinical trial *(HOVON 130: A phase II non randomized study evaluating the effect of the addition of lenalidomide to R-CHOP for patients with newly diagnosed MYC positive DLBCL and BCL-U)*. During the screening period administration of one cycle of R-CHOP21 is allowed. After being diagnosed with a *MYC* rearrangement, lenalidomide 15 mg on day 1-14 of R-CHOP21 is added. Patients receive 6 cycles of R-CHOP21 plus lenalidomide plus an additional 2 cycles of rituximab monotherapy. Intrathecal prophylaxis is given to all patients. Primary endpoint of the study is centrally reviewed end-of-treatment complete metabolic remission rate on ^{18}F-FDG PET-CT scan.

Results: Screening database (HOVON 900): Between Aug 2015 and Feb 2018, 816 newly diagnosed DLBCL patients have been registered. Median age at diagnosis was 67 years (18 to 88 years). *MYC* FISH analysis was successful in 73% of patients. Logistics showed that within 2-3 weeks *MYC* FISH results were available. Clinical trial (HOVON 130): 85 patients with a *MYC* rearrangement were included. After central pathology review, 3 patients were declared ineligible. Additional FISH analysis on *BCL2* and *BCL6* rearrangements revealed that 33% were *MYC* single hit, 55% were double hit (*MYC* and *BCL2* or *BCL6*) and 12% were triple hit (*MYC* and *BCL2* and *BCL6*) (analysis of 69 patients, to be completed). Clinical baseline characteristics of the 82 eligible patients are shown in Table 1. A planned interim analysis (after 27 patients finished treatment) showed grade 2 toxicity in 41%, grade 3 in 33% and grade 4 in 15% of patients. Most common toxicities were polyneuropathy (44%), infections (33%) and gastrointestinal disorders (30%). 16 severe adverse events were reported (all hospitalizations due to infections). The DSMB concluded that no safety issues prohibit continuation of the trial. Efficacy results are blinded until the last patient finished treatment (expected Q3 2018). Updated demographic results will be available in June 2018.

Summary/Conclusion: A successful screening program for *MYC* rearrangements in the Netherlands enables newly diagnosed DLBCL patients with a proven *MYC* rearrangement to switch to a prospective biomarker-based clinical trial that evaluates the addition of lenalidomide to standard R-CHOP, demonstrating feasibility of this program.

PS1026

A NOVEL IMMUNOHISTOCHEMICAL PROGNOSTIC SCORE BASED ON MYC, BCL2 AND BCL6 EXPRESSION IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL): RETROSPECTIVE ANALYSIS OF *DE NOVO* DLBCL TREATED WITH RITUXIMAB-CHOP

M. Novo[1,*], A. Castellino[1], C. Ciochetto[1], F. vittone[2], B. Botto[1], F. Cavallo[3], A. Chiappella[1], A. Evangelista[4], P. Ghione[3], M. Nicolosi[1], E. Santambrogio[1], M. S. Scalzo[2], G. Ciccone[4], R. Chiarle[5], D. Novero[2], A. Zamò[2], U. Vitolo[1]

[1]S.C. Hematology, [2]Patology Unit, AOU Città della Salute e della Scienza, Torino, Italy, [3]S.C. Hematology, Università degli Studi di Torino, Torino, Italy, [4]Unit of Clinical Epidemiology, AOU Città della Salute e della Scienza, Torino, Italy, Torino, Italy, [5]Patology Unit, Boston Children's Hospital and Harvard Medical School, Boston, United States

Background: The dismal prognosis of Double-Hit Lymphoma (DHL) is well known, whereas the prognostic impact of *MYC*, *BCL2* and *BCL6* expression is still contradictory.

Aims: The aim of the study was to assess the prognostic role of *MYC*, *BCL2*, and *BCL6* expression in a retrospective cohort of de-novo DLBCL, treated with R-CHOP between January 2003 and December 2013.

Methods: *BCL2*, *BCL6* and *MYC* expression were evaluated by Immunohistochemistry (IHC) using Tissue Micro Arrays (TMA) technique; cases were deemed positive for *MYC*, *BCL2* or *BCL6* if >40%, >40% or >25% of cells stained positive, respectively. Fluorescent in situ hybridization (FISH) analysis for *MYC* and *BCL2* were analyzed with dual color break apart probes on TMA samples. Survival analysis of Progression Free Survival (PFS) and Overall Survival (OS) were performed using Kaplan-Meier method and compared amongst groups with log-rank and Cox model. In a pilot series on 56 patients, *MYC* and *BCL2* overexpression and *BCL6* lack of expression showed prognostic relevance in a Cox multivariate regression model adjusted for IPI and age with PFS as endpoint; established that these 3 variables contributed with different risk (Hazard Ratio 2.23 for *MYC*+, 2.29 for *BCL2*+ and 1.67 for *BCL6*-), an IHC sum additive score of 0-5 was calculated assigning an individual risk of 2 points for *MYC* or *BCL2* positivity and 1 point for *BCL6* negativity stratifying patients in 3 risk groups; Low-[0-1 point] (no *BCL2* or *MYC* overexpression, with or without *BCL6* lack expression), Intermediate-[2 points] (*MYC* or *BCL2* single overexpression) and High-risk [≥3 points] (*MYC*+/*BCL6*-, *BCL2*+/*BCL6*-, *MYC*+/*BCL2*+, *MYC*+/*BCL2*+/*BCL6*-).

Results: Of 297 DLBCL included into the study, 265 were evaluable for survival analysis; median age was 65 years (range 20-90); 152 were evaluable by IHC and 100 by both IHC and FISH. No bias selections were observed within the two groups. Among 100 patients investigated by FISH, 8 DHL cases were recorded (8%); with a median follow up of 60 months, 5-year PFS was 66% in DLBCL and 25% in DHL [HR 3.05 (95% CI: 1.26-7.36, p 0.013)] and 5-year OS was 80% and 25% respectively [HR 3.63 (95% CI:1.48- 8.91 p 0.005)]. Excluding the 8 DHL patients, the remaining 144 patients with complete IHC data were distributed as follows based on IHC score: low risk 12 (8%), intermediate risk 59 (41%) and high risk 73 (51%). 5-year PFS rates were significantly different across the three groups: 91% vs 68% vs 48% (p=0.014) respectively (HR per unit increase 1.63 (95% CI: 1.14 -2.55) p 0.032), (Figure 1).

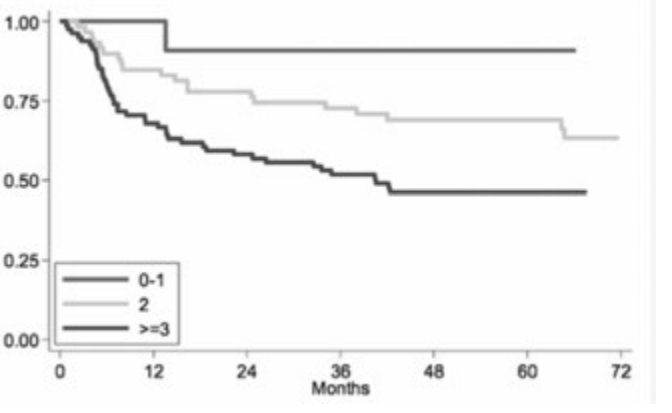


Figure 1. IHC score 5y-PFS on 144 patients (DHL excluded).

Summary/Conclusion: Our data showed that in a large cohort of DLBCL homogeneously treated with R-CHOP, excluding DHL cases, our simple and reproducible score based on *MYC*, *BCL2* and *BCL6* IHC expression had a prognostic value and was able to identify three groups at different outcome. This score is useful to select patients at poorer prognosis even if not DHL, that may take advantage of a more intensive chemotherapy regimen or combination of novel drugs.

PS1027

ASSESSMENT OF BONE MARROW INFILTRATION AND MINIMAL RESIDUAL DISEASE BY MULTIDIMENSIONAL FLOW CYTOMETRY IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

M. Baile[1,*], S. Barrena[2], J. Sancho[3], C. Grande[4], R. Fernández[5], A. Batlle[6], M. Peñarrubia[7], F. Peñalver[8], J. Hernández-Rivas[9], J. Guinea[10], J. Pérez[1], M. Vidriales[1], A. Martín[1]

[1]Hematology, Hospital Universitario de Salamanca/IBSAL, [2]Citometry, Cancer Research Center and University of Salamanca (IBMCC-USAL-CISC, SALAMANCA, [3]Hematology, Hospital Germans Trias i Pujol / ICO-IJC, Barcelona, [4]Hospital Universitario [12] de Octubre, Madrid, [5]Hematology, Hospital de Cabueñas, Gijón, [6]Hematology, Hospital Universitario Marqués de Valdecilla, Santander, [7]Hematology, Hospital Clínico de Valladolid, Valladolid, [8]Hematology, Hospital Universitario Fundanción de Alcorcón, [9]Hematology, Hospital Infanta Leonor, Madrid, [10]Hematology, Hospital Universitario de Araba, Vitoria, Spain

Background: Multidimensional flow cytometry (MFC) has not yet shown a high clinical value in patients with diffuse large B-cell lymphoma (DLBCL), probably due to the phenotypic heterogeneity of these lymphomas. In the present study, we have evaluated bone marrow (BM) infiltration of in patients with DLBCL using MFC.

Aims: 1) evaluating its prognostic impact compared to other techniques such as histology or PET; 2) determine the specific phenotypic pattern of the tumor cell of each patient for subsequent follow-up of minimal residual disease (MRD) after treatment.

Methods: We centrally evaluated BM samples from patients included in the multicenter randomized phase 2 clinical trial GEL-R-COMP-2013 (EudraCT: 2013-001065-17), that compares R-CHOP versus R-COMP in patients with newly diagnosed DLBCL or follicular lymphoma (FL) grade 3b. High resolution (8 colors) and high sensitivity (more than 1 million cells analyzed) direct immunofluorescence techniques were used, following the protocols defined by EuroFlow (Leukemia 2012, 26: 1908-1975). In order to further characterize the tumor population, an automatic analysis was

carried out in addition to the manual analysis, using the Compass III tool, available in the Infinicyt analysis software (Cytognos, S.L.).
Results: From a total of 91 patients included in the clinical trial, 56 participated in the present study (50 DLBCL and 6 FL grade 3b). The median age was 75.5 years (61-86) and the R-IPI was: 0 in 11% of patients, 1-2 in 32% and 3-5 in 57%. BM infiltration was detected by MFC in 20 out of 56 (36%) patients at diagnosis and 0 out of 13 at post-treatment evaluation. In 10 patients, infiltration was <1% of total BM cellularity. Regarding the phenotypic characterization, concordant BM infiltration by DLBCL was detected in only 5 cases. In contrast, discordant infiltration was detected in 15 (27%) patients, with a very heterogeneous phenotype: 4 low grade FL, 2 chronic lymphocytic leukemia, 5 marginal zone lymphoma and 4 small cell lymphoma with non-specific phenotype. Preliminary analysis of the prognostic impact of BM infiltration by MFC is shown in Table 1. The presence of concordant BM infiltration was associated with lower response rates and worse event-free survival, although the differences were not statistically significant, possibly due to the low number of cases. On the other hand, discordant BM infiltration had no significant prognostic influence.

Table 1.

Prognostic factor	n	CR (%)	p	OR (%)	p	EFS (%)	p	OS (%)	p
Bone Marrow infiltration by MFC	5	20		60		20		70	
- Concordant	15	67		100		50		64	
- Discordant	36	67	0,1	83	0,8	52	0,1	72	0,6
- Not infiltrated									
R-IPI									
- 0	6	**83**		**100**		67		**83**	
- 1-2	17	**88**		**100**		60		**100**	
- 3-5	30	**47**	**0,008**	**73**	**0,0016**	41	0,06	**50**	**0,03**

CR: Complete Response; OR: Overall Response; EFS: Event Free Survival; OS: Overall Survival.

Summary/Conclusion: High resolution MFC allowed the detection of small clonal populations in BM in a very high proportion of patients with newly diagnosed DLBCL. In most cases, infiltration was discordant with tumor histology and, apparently, had no prognostic relevance. The treatment administered was very effective in eradicating these populations, since MRD was not detected in any of the cases. We plan to correlate MFC findings with those of histology and PET, also centrally reviewed, as well as to perform molecular studies on tumor and BM samples to assess their clonal relationship.

PS1028

ON THE APPLICABILITY OF RHOA GLY17VAL POINT MUTATION TESTING FOR A DIFFERENTIAL DIAGNOSIS OF ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA

Y. Sidorova[1,*], N. Chernova [1], M. Sinitsyna[1], T. Moiseeva[1], L. Al-Radi[1], M. Aleksenko[1], O. Glinshchikova[1], S. Smirnova[1], N. Ryzhikova[1], E. Nikulina[1], E. Zakharko[1], E. Rybkina[1], V. Dvirnyk[1], A. Kovrigina[1], E. Zvonkov[1], A. Sudarikov[1]

[1]National Research Center for Hematology, Moscow, Russia, Moscow, Russian Federation

Background: Angioimmunoblastic T-cell lymphoma (AITL) is a T-cell lymphoma, characterized by abundant polymorphocellular infiltrate of lymph nodes with the small number of tumor CD4+ T-cells. Despite the use of various diagnostic methods, including immunohistochemistry and PCR-based clonality testing, AITL could often be misdiagnosed as reactive processes and other lymphomas, including Hodgkin's lymphoma and diffuse large B-cell lymphoma (DLBCL). Recently discovered point somatic RHOA Gly17Val mutation is present in 53-71% of angioimmunoblastic T-cell lymphomas, still it is not completely clear if this marker specific for AITL exclusively. Data on the presence of this point mutation in Hodgkin's lymphoma or DLBCL are limited.
Aims: To define if RHOA Gly17Val is a specific marker for angioimmunoblastic T-cell lymphoma.
Methods: The study included 62 patients diagnosed with AITL, established on the basis of WHO criteria. The ratio m/f - 37/25, the median age was 62 years (29-87). The control group included patients with Hodgkin's lymphoma <35 years old (n 35), ≥35 years old (n 25), DLBCL (n 15), reactive lymphadenitis (n 14), and other indolent B-cell lymphomas (n 20). Gly17Val mutation was analyzed in lymph nodes by quantitative allele-specific (qAS) TaqMan Real-Time PCR assay.
Results: RHOA Gly17Val mutation was detected in 53% of patients with AITL (32 of 62). Study of this mutation in the control group of patients with Hodgkin's lymphoma, DLBCL, reactive lymphadenitis, indolent B-cell lymphomas (n 109) showed a negative result, except for 5 patients (Figure 1). In two patients, males, 38 and 65 yo, with Hodgkin's lymphoma cell selection approach have shown that the CD4+ peripheral blood population contains cells with RHOA Gly17Val mutation. Flow cytometry of blood revealed a population of $CD3_{dim}$ CD5+ CD4+ CD10+/- CD279+ (2.251% of lymphocytes and 0.295% of all events) in one patient and CD3+ CD4+ CD10+ CD279- (0.2% of lymphocytes and 0.04% of all events) in another indicating AITL to exist as second tumor in these cases. In addition, clonal rearrangements of TCRG and TCRB in the lymph nodes were detected in these two patients. In the third case diagnosed as DLBCL (female, 63 yo) we revealed a small number of cells (0.24%) with an RHOA mutation in the lymph node sample, with no clonal rearrangements of TCRG, TCRB. Now the patient is in remission and under observation. In the fourth patient (female, 50 yo), DLBCL developed 7 months after the diagnosis of AITL. In the first and the second biopsies of lymph nodes, there is practically the same number of cells with a RHOA mutation - 1.5% and 2%, as well as the same clone of cells with clonal rearrangement of IgH, which indicates the initial presence of composite lymphoma in this patient. In patient # 5 diagnosed as reactive lymphadenitis in 2008 (female, 58 y.o.) cells with the RHOA Gly17Val mutation were detected in the lymph node at the amount of 1.5%. In the biopsy of the lymph node in 2017, 29% of the cells with the RHOA Gly17Val mutation were detected. The T-cell receptor clonal rearrangements coincided in both lymph nodes, so it can be an argument that this case of reactive lymphadenitis was the onset of AITL. Thus, in 4 out of 5 cases positive for RHOA Gly17Val point mutation, the presence of AITL as the primary or second tumor was demonstrated.

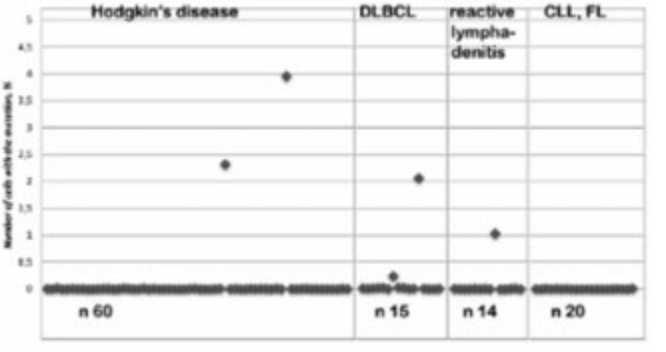


Figure 1.

Summary/Conclusion: Somatic RHOA Gly17Val point mutation is a specific marker of AITL in differential diagnosis with Hodgkin's lymphoma, reactive lymphadenitis and DLBCL and should be used as a screening method in a group of patients older than 35 years.

PS1029

THE ADDITION OF ROMIDEPSIN TO CHOEP FOLLOWED BY HIGH-DOSE CHEMOTHERAPY AND TRANSPLANTATION IS FEASIBLE IN UNTREATED PERIPHERAL T-CELL LYMPHOMAS: RESULTS OF PHASE IB FIL-PTCL13

A. Chiappella[1,*], C. Carniti[2], A. Evangelista[3], M.G. Cabras[4], A. Re[5], F. Salvi[6], V. Stefoni[7], A. Santoro[8], A. G. Congiu[9], A. Dodero[2], D. Gioia[10], S. A. Pileri[11], G. Ciccone[3], P. Corradini[2]

[1]On behalf of Fondazione Italiana Linfomi, Hematology, Città della Salute e della Scienza di Torino Hospital and University, Torino, [2]On behalf of Fondazione Italiana Linfomi, Division of Hematology and Bone Marrow Transplant, Fondazione IRCCS Istituto Nazionale dei Tumori; Department of Oncology and Hemato-oncology, University of Milan, Milano, [3]On behalf of Fondazione Italiana Linfomi, Unit of Clinical Epidemiology and CPO, Città della Salute Hospital and University, Torino, [4]On behalf of Fondazione Italiana Linfomi, Hematology, Ospedale A. Businco Cagliari, Haematology & Transplant Centre Wilma Deplano, Cagliari, [5]On behalf of Fondazione Italiana Linfomi, Hematology, ASST Spedali Civili di Brescia, Brescia, [6]On behalf of Fondazione Italiana Linfomi, Hematology, Azienda Ospedaliera SS Antonio e Biagio e Cesare Arrigo, Alessandria, [7]On behalf of Fondazione Italiana Linfomi, Hematology, Istituto L e A Seragnoli, Policlinico S. Orsola-Malpighi, Bologna, [8]On behalf of Fon-

dazione Italiana Linfomi, Hematology, Istituto Clinico Humanitas and IRCCS, Rozzano, [9]On behalf of Fondazione Italiana Linfomi, Division of Hematology and Bone Marrow Transplantation, Ematologia Ospedale Policlinico San Martino, Genova, [10]On behalf of Fondazione Italiana Linfomi, Pharmacovigilance, Secretary Fondazione Italiana Linfomi, Alessandria, [11]On behalf of Fondazione Italiana Linfomi, Haematopathology Unit, Istituto Europeo di Oncologia, Milano, Italy

Background: Peripheral T-cell lymphoma (PTCL) is a heterogeneous disease at dismal prognosis. The addition of etoposide to cyclophosphamide-doxorubicin-vincristine regimen (CHOEP) followed by a consolidation with autologous stem cell transplantation (autoSCT) can be considered a standard strategy in young patients with chemo-sensitive disease. However, 25-30% of patients are not eligible to transplant due to primary refractoriness or early progression. Romidepsin is a histone deacetylase inhibitor, a non-cross resistant agent that showed antitumor activity in PTCL and a manageable toxicity profile in combination with chemotherapy. On these bases, we designed the FIL-PTCL13 phase Ib/II study (NCT02223208). Herein, we report the phase I part of the study.
Aims: Aims of FIL-PTCL13 were to define the maximum tolerated dose (MTD) of romidepsin (Ro) when administered in combination with CHOEP in the treatment of PTCL patients' candidate to stem cell transplant, and to evaluate safety and efficacy of this combination.
Methods: Inclusion criteria were: stage II-IV patients aged 18-65, with newly diagnosis of PTCL not otherwise specified, angioimmunoblastic, ALK negative. Treatment scheme was: an induction with 6 courses of CHOEP every 21 days combined with Ro at the allocated dose, at day 1 and 8 of each cycle (Ro-CHOEP); responsive patients continued the program with one course of cisplatin, citarabine, desamethasone (DHAP) followed by stem cell harvest. Patients in complete remission after Ro-CHOEP proceeded to autoSCT; patients in partial remission and with an available donor, were sent to upfront alloSCT. Romidepsin dose allocation for sequential cohorts of 3 patients at each dose was defined according to the Continual Reassessment Method (O'Quigley and Zohar, 2006). The MTD of Ro was defined as the dose that achieves a dose-limiting toxicity (DLT) in 33% of patients reported during the first 2 courses of Ro-CHOEP. Four dose levels were tested, namely 8, 10, 12 and 14 mg/ms. According to expert opinions, the first administered dose was 12 mg/ms. Toxicities were classified according to the NCI Common Terminology Criteria for Adverse Events, version 4.0.
Results: From September 2014 to July 2017, 21 patients were enrolled into the phase I. Clinical characteristics were: median age 57 years (IQR 53;61); IPI risk ≥ 3 8 (38%); bone marrow involvement 6 (29%); stage III-IV 18 (86%). As for the protocol, the first 3 patients were treated with Ro at 12 mg/ms, and no DLTs were observed; the subsequent 6 cohorts were treated with Ro at 14 mg/ms. Nine DLTs were reported in 7 patients: 3 events of grade (g)3 mucositis and one event of g3 maculopapular rash, g3 fatigue, g3 fever, g3 respiratory failure, g3 typhlitis and g4 neutropenic fever, respectively. The observed toxicity was 35.2% (95% Credibility Interval: 17.1%>56.5%) and prompted to define 14 mg/ms the recommended dose of Ro in addition to CHOEP. No unexpected toxicities and no toxic deaths were reported into the phase I part of the trial. The addition of Ro during induction did not impact the harvest, with a median of 4.3×10 (IQR 3.4-5.71) peripheral blood CD34-positive cells/Kg collected. On September 2017, the phase II of the trial was opened. All enrolled patients included into phase I part completed the study and preliminary efficacy analyses are ongoing.
Summary/Conclusion: The phase Ib FIL-PTCL13 part of the study defined Romidepsin 14 mg/ms on day 1 and day 8 as the MTD, when administered in combination to CHOEP as induction in untreated T-cell lymphoma patients candidate to stem cell transplant.

PS1030

A PHASE I, MULTICENTER, OPEN-LABEL DOSE-ESCALATION STUDY OF CC-122, A NOVEL CEREBLON-MODULATING AGENT, IN ADULT JAPANESE PATIENTS WITH ADVANCED NON-HODGKIN LYMPHOMA OR SOLID TUMOR

K. Yamamoto[1,*], T. Chou[2], T. Doi[3], Y. Terui[4], H. Kato[1], T. Hirose[2], S. Seo[5], Y. Ogaki[6], H. Fujimoto[6], T. Ohtsu[6], K. Hatake[7]

[1]Department of Hematology and Cell Therapy, Aichi Cancer Center Hospital, Nagoya, [2]Department of Hematology Oncology, Niigata Cancer Center Hospital, Niigata, [3]Department of Experimental Therapeutics, National Cancer Center Hospital East, Chiba, [4]Department of Hematology Oncology, The Cancer Institute Hospital of Japanese Foundation for Cancer Research, Tokyo, [5]Department of Hematology and Oncology, National Cancer Center Hospital East, Chiba, [6]Celgene K. K., Tokyo, [7]Department of Hematology, International University of Health and Welfare, Mita, Tokyo, Japan

Background: Treatment for relapsed/refractory non-Hodgkin lymphoma (R/R NHL) continues to be a challenge, with many patients (pts) relapsing despite the availability of new treatment options. CC-122 is a novel cereblon-modulating agent that degrades the hematopoietic transcription factors Ikaros and Aiolos. CC-122 exerts antiproliferative, antiangiogenic, and immunomodulatory effects in solid and hematologic malignancies (Hagner *Blood* 2015; Gandhi *Clin Lymphoma, Myeloma & Leukemia* 2013; Carpio ASH 2017).
Aims: The current phase I CC-122-ST-002 study evaluates the safety and efficacy of CC-122 in Japanese pts with advanced NHL or solid tumors (NCT02509039).
Methods: Pts age ≥ 20 years, with ECOG PS 0-2, and documented diagnosis of advanced solid tumors (cohort 1 only) or NHL (all cohorts) were eligible for the study, including those who had progressed on or were ineligible for standard anticancer therapy. Pts received oral CC-122 active ingredient in capsule (AIC) 2.0 mg/day (cohort 1, n=3), 3.0 mg/day (cohort 2, n=3), 4.0 mg/day (cohort 3, n=3), or 3 mg/day of formulated capsules (FC) (cohort 4, n=6). All cohorts received CC-122 on 5 consecutive days of 7 days per week in a 3+3 design until progression, unacceptable toxicity, or pt/physician withdrawal. The primary endpoints were to assess safety (per NCI CTCAE v4.03) and to determine dose-limiting toxicity (DLT), maximum tolerated dose (MTD) or recommended phase II dose (RP2D), and pharmacokinetics (PK) of CC-122.
Results: As of data cut-off of 30 Mar 2017, 15 pts, 14 with NHL (5 DLBCL, 5 FL, 4 peripheral T-cell lymphoma) and 1 with a solid tumor (esophageal carcinoma) participated in the study. The median age was 64 years (range, 48-78), and 9 (60%) were male. The median number of prior systemic anticancer therapies was 3 (range, 1-10). Pts received CC-122 for a median of 29 weeks (range, 4-82). DLTs were reported in 1 pt (cohort 4) and included grade 1 facial edema, grade 1 pharyngeal edema, and grade 1 tumor flare reaction. Grade ≥ 3 adverse events (AEs) occurred in 11/15 pts (73%). The most frequently reported grade ≥ 3 AEs were neutrophil count decreased (5/15, [33%]), and lymphocyte count decreased (3/15, [20%]). Three pts experienced serious AEs (SAE); 1 SAE of pulmonary eosinophilia was suspected to be related to CC-122. The most common AEs (≥ 30%) were maculo-papular rash (53%), lymphocyte count decreased (47%), constipation (40%), neutrophil count decreased (40%), upper respiratory tract infection (40%), platelet count decreased (33%), and white blood cell count decreased (33%). No AEs led to discontinuation of CC-122. Seven pts discontinued treatment due to progressive disease. Two pts died within 30 days of the last dose of CC-122, both due to progressive disease. ORR for efficacy-evaluable NHL pts (n=13) was 54%, with 31% complete response. The best response for the single solid tumor pt in the study was progressive disease. Median time to response was 6.3 weeks (range, 5.9-22.0). Median DOR was not reached. Median progression-free survival was 46.1 weeks (95% CI, 13.9-NR). At steady state, PK showed that the 4 mg AIC cohort had the highest exposure followed by the 3 mg FC, 2 mg AIC and 3 mg AIC cohorts (Figure 1).

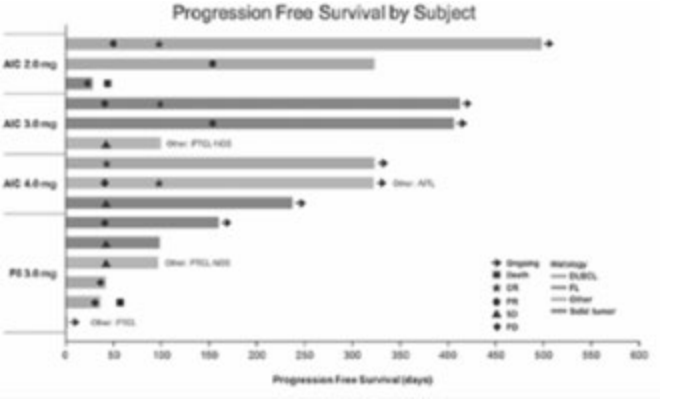


Figure 1.

Summary/Conclusion: The safety and PK results observed in this study are consistent with the known profile of CC-122 and the 3.0-mg dose using FC was considered safe and tolerable. Overall, the CC-122-ST-002 study showed preliminary efficacy with CC-122 in Japanese patients with previously treated NHL.

PS1031

GLOBAL LONGITUDINAL STRAIN (2D-GLS) IN LYMPHOMA PATIENTS TREATED WITH CHEMOTHERAPY +/- MEDIASTINAL RADIOTHERAPY: EARLY SUBCLINICAL CARDIOTOXICITY IN THE CARDIOCARE PROSPECTIVE OBSERVATIONAL STUDY

B. Botto[1,*], M. Levis[2], L. Orsucci[1], V. De Luca[3], S. Bartoncini[2], M. Giorgi[4], A. Chiappella[1], S. Ferrero[5], A. Fava[4], F. Cavallo[5], D. Caracciolo[5], S. Vicentini[4], A. Gastino[3], G. Furfaro[2], M. Nicolosi[1], P. Pregno[1], G. Priolo[1], A.R. Filippi[3], U. Ricardi[3], U. Vitolo[1]

[1]Department of Hematology, [2]Department of Radiation Oncology, A.O.U. Città della Salute e della Scienza, [3]Department of Oncology, Radiation Oncology, University of Torino, [4]Department of Cardiology, A.O.U. Città della Salute e della Scienza, [5]Department of Hematology, University of Torino, Torino, Italy

Background: Treatments-related cardiotoxicity is a critical issue in long term lymphoma survivors, particularly at young age, and its early identification is important to prevent clinically relevant cardiac events. Complete echocardiographic assessment including 2-dimension global longitudinal strain (2D-GLS), seems to be an effective tools in detecting preclinical systolic changes to the cardiac funtion even when the ejection fraction is preserved.
Aims: The aim of CARDIOCARE study is to investigate early detection of subclinical chemo and radiation-induced changes in left ventricular function using 2D-GLS
Methods: CARDIOCARE is an ongoing monocentric prospective observational study, approved by the ethic committee of our hospital (approval number: CS/370); the planned accrual will finally include 100 patients, of which 50 treated with chemotherapy alone (CT-alone) and 50 treated with combined modality treatment (CMT) including chemotherapy + mediastinal radiotherapy. Patients aged 18-70, affected with either Hodgkin or diffuse large B-cell (DLBCL) or primary mediastinal lymphomas (PML) were eligible. Patients received a complete echocardiographic assessment including 2D-GLS at baseline, after chemotherapy, after radiotherapy (if planned), and 3 months after end of treatment. Paired samples T test correlations were applied to evaluate GLS changes at each time-point. The cumulative dose of anthracycline and the adsorbed radiation dose of whole heart and cardiac substructures (coronaries, chambers and valves) were assessed for each patient. All patients signed an informed consent before the enrollment.
Results: Fifty-two patients (24 in CT-alone group and 28 in CMT group), out of 65 enrolled to date, completed the observational program and were included into this analysis. No patients experienced a significant reduction of the left ventricular (LV) ejection fraction during the entire treatment period. Median dose of anthracycline was 500 mg in CT arm vs 400 mg in CMT arm. A marginal reduction of 2D-GLS was seen after chemotherapy for patients in CT-alone arm ($GLS_{baseline}$: -19.24 vs $GLS_{post\text{-}CT}$: -18.42, p =0.06), but not for patients in CMT arm ($GLS_{baseline}$: -20.08 vs $GLS_{post\text{-}RT}$: -20.05, p=0.94). In the whole series a marginal reduction of GLS after CT was observed also for patients with Age >40 ($GLS_{baseline}$: -19.34 vs $GLS_{post\text{-}CT}$ -17.93 p =0.056) and receiving >4 cycles ($GLS_{baseline}$: -19.55 vs $GLS_{post\text{-}CT}$: -18.68 p =0.055). After mediastinal RT, a significant reduction of 2D-GLS was found in patients receiving: *A) maximum dose* to interventricular septum >10 Gy ($GLS_{baseline}$: -20.10 vs $GLS_{post\text{-}RT}$: -18.67 p =0.006), to lateral wall of the LV >8 Gy ($GLS_{baseline}$: -20.31vs $GLS_{post\text{-}RT}$: -18.53 p=0.002) and to whole LV >11 Gy ($GLS_{baseline}$: -20.37 vs $GLS_{post\text{-}RT}$: -19.1, p=0.002); *B) mean dose* to whole heart >4.5 Gy ($GLS_{baseline}$: -20.9 vs $GLS_{post\text{-}RT}$: -18.39, p=0.007) and mean dose ≥2 to interventricular septum ($GLS_{baseline}$: -20.21 vs $GLS_{post\text{-}RT}$: -18.45, p=0.01), to lateral wall of the LV ($GLS_{baseline}$: -18.84 vs $GLS_{post\text{-}RT}$: -18.84,p= 0.02) and to whole LV ($GLS_{baseline}$: - 20.17vs $GLS_{post\text{-}RT}$: - 18.58,p=0.006).
Summary/Conclusion: 2D-GLS seems a promising tool to detect early cardiotoxicity in lymphoma patients. Preliminary results suggest a correlation of both anthracyclines and radiation dose with preclinical heart damage. The completion of CARDIOCARE study, and a future correlation with clinical events are needed to support and strengthen these preliminary assumptions.

PS1032

CLINICAL FEATURES, TREATMENT AND PROGNOSTIC FACTORS FOR EXTRANODAL NATURAL KILLER/T-CELL LYMPHOMA, NASAL TYPE, IN THE SPANISH POPULATION. A STUDY FROM THE SPANISH GROUP GELTAMO

E. Gonzalez Barca[1,*], J.L. Bello[2], C. Panizo[3], A. Monter[4], R. Cordoba[5], P. Gomez[6], M. Bastos[7], S. Gonzalez de Villambrosia[8], M.-J. Sayas[9], H.D. Luzardo[10], M. C. Viguria[11], J. Perez de Oteyza[12], M. Roig[13], E. Cabezudo[14], R. Oña[15], J. A. Queizan[16], M. Encuentra[17], A. Lopez[18]

[1]Hematology, Institut Català d'Oncologia Hospitalet, IDIBELL, Universidad de Barcelona, Barcelona, [2]Hematology, Hospital Clínico Universitario , Santiago de Compostela, [3]Hematology, Clinica Universidad de Navarra, IdiSNA, Pamplona, [4]Hematology, Hospital de Sant Pau, Barcelona, [5]Hematology, Fundación Jimenez Díaz, [6]Hematology, Hospital La Paz, [7]Hematology, Hospital Gregorio Marañon, Madrid, [8]Hematology, Hospital Marques de Valdecilla, Santander, [9]Hematology, Hospital Dr Peset, Valencia, [10]Hematology, Hosptial Dr Negrín Las Palmas, Gran Canaria, [11]Hematology, Complejo Hospitalario de Navarra, Pamplona, [12]Hematology, Hospital Universitario Sanchinarro, Madrid, [13]Hematology, Consorcio Hospital General Universitario de Valencia, Valencia, [14]Hematology, Hosptial Sant Joan de Deu de Manresa, Barcelona, [15]Hematology, Hospital MD Anderson, Madrid, [16]Hematology, Hospital General de Segovia, Segovia, [17]Clinical Trial Unit, Institut Català d'Oncologia Hospitalet, IDIBELL, [18]Hematology, Hospital Valle de Hebron, Barcelona, Spain

Background: Extranodal natural killer (NK)/T-cell lymphoma, nasal type (ENKTL-NT) is a rare disease associated with Epstein-Barr virus (EBV) and is much more common in Asia and Latin America than in western countries. Data on disease presentation and outcome from European cases are very limited.
Aims: To analyze the clinical characteristics at diagnosis, treatment and outcome of a large retrospective series of Spanish patients diagnosed with ENKTL-NT.
Methods: Fifty-six patients with ENKTL-NT diagnosed from 2000 to 2016 were identified in 16 academic centers in Spain. Clinical data were collected retrospectively. Survival curves were estimated by the Kaplan-Meier method and compared using the Log-Rank test. Variables included in the univariate analysis were: race, gender, age, previous sinusitis, nasal localization, Ann Arbor stage, ≥2 extranodal sites, ECOG, B-symptoms, LDH, beta2-microglobulin, albumin and C-reactive protein. Multivariate analyses were performed by Cox proportional hazard regression model.
Results: Clinical characteristics at presentation: median age 51 yr (limits 23-89), Caucasian 42, previous history of chronic sinusitis 13 (23%), localized nasal involvement 27 (49%), Ann Arbor I/II 31/53 (57%), B symptoms 16/55 (29%), ≥2 extranodal sites 20/53 (38%), ECOG 0-1 36/50 (72%), elevated LDH 21/49 (43%), elevated beta2-microglobuline 13/31 (42%), elevated C-reactive protein 14/24 (58%), low albumin 17/30 (57%). Fifty-two patients received active treatment, 24 (46%) with chemotherapy (CT), 25 (48%) with CT+radiotherapy (RT), 3 with RT. Median RT dose was 50 Gy. CT with high doses of L-asparaginase (SMILE / Sandwich) was used in 18 (36%), CHOP/CHOP-like in 25 (50%) and intensive regimens ICE/PROMACE in 5 (10%). Nine patients proceed to a stem-cell transplant after CT in first line (8 auto/1 allo). Response rate was evaluable in 48 patients [by PET/TC in 21 (44%)]: CR 25 (52%), PR 6 (13%), progression 17 (35%). With a median follow-up of 37 months for alive patients, median OS and PFS were 14.3 months (95% CI 8.9-19.6). Causes of death: progression 25 (71%), toxicity 7 (20%), secondary neoplasms 3 (9%). In the univariate analysis, the following variables grant worse prognosis: age≥60 yrs, extra-nasal localization, Ann Arbor III-IV, B symptoms, ECOG 2-4, elevated LDH, elevated beta2-mycroglobulin, low albumin level. In the multivariate analysis (including all the previous variables except albumin and beta2-microglobulin due to the high number of missing data), B symptoms (OR 2.9, 95% CI 1.3-6.6, p=0.009) and Ann Arbor III-IV (OR 2.4 95% CI 1.1-5.3, p=0.03) were significantly related with worse OS. There was a trend to a better outcome in patients treated with high dose L-asparaginase (p=0.09) (Figure 1).

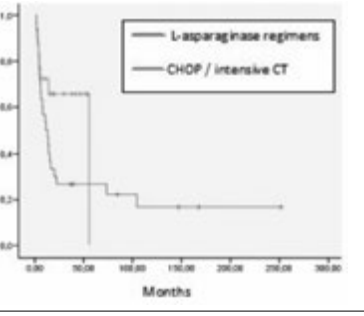


Figure 1. Overall Survival of ENKTL-NT patients according to initial theraphy.

Summary/Conclusion: In this European population mostly Caucasian, ENKTL-NT is diagnosed with a median age of around 50 yrs, and one fourth of the patients had a previous history of sinusitis. These patients have a very poor survival, being the main cause of death lymphoma progression. B symptoms and advanced stage disease at diagnosis are independent factors associated to worse outcome. The use of high dose of L-asparaginase seems to improve the outcome of these patients, although longer follow-up is needed.

PS1033

ARE OUTCOMES IMPROVING IN POST TRANSPLANT LYMPHOPROLIFERATIVE DISORDER? A RETROSPECTIVE ANALYSIS OF THE LAST 10 YEARS

N. Denny[1,*], J. Norman[1], M. Morton[2], S. Burns[1]

[1]Department of Haematology, [2]Department of Renal Medicine, Manchester University Foundation Trust, Manchester, United Kingdom

Background: Post transplant lymphoproliferative disorder (PTLD) is a spectrum of lymphoid and plasmacytic proliferations diagnosed in the context of previous solid organ (SOT) or haemopoietic stem cell transplantation (HSCT). The incidence varies between 1-20% depending on the transplanted organ. Many are driven by EBV infection. Understanding of PTLD is limited due to its heterogeneity and rarity. Our centre performs over 200 kidney and simultaneous kidney pancreas (SPK) transplants per year.
Aims: To review the local management of PTLD over the previous 10 years and to contribute to the understanding of this disorder.
Methods: Data was collected for all new PTLD patients diagnosed between 01/01/2007 and 31/12/2017 at Manchester Royal Infirmary. Patient notes were reviewed and the following details were collected; patient demographics, transplant details, type of PTLD (WHO 2016 classification), staging including organs involved, PS, EBV status, management and outcome. Statistics were performed using XLSTAT.
Results: 59 patients were diagnosed with a PTLD and 8 were excluded as the diagnosis was incorrect or they were treated at an external hospital. 51 patients were included in the final analysis with a mean age 47 years and 72.5% were male. 49 received a renal transplant and 2 SPK. 41 patients had 1 transplant, 9 had 2 transplants and 1 had 3 transplants. Mean age at time of transplant was 38 years old. Median time to PTLD diagnosis was 86 months. 43% of patients presented with GI symptoms, 27% with ENT symptoms and 10% with constitutional symptoms alone. Histology and site of disease are shown in Table 1; 66% of cases were extranodal and 61% were monomorphic. 70% of patients were EBV PCR positive in peripheral blood at diagnosis and a similar proportion of tissue specimens were EBV positive. Polymorphic PTLD occurred significantly earlier post transplant than monomorphic PTLD (p=0.04) and was strongly associated with positive EBV PCR. 37 patients had a R-IPI score 2 or less, 11 a R-IPI score of 3 or more and 3 primary CNS disease. Logistic regression analysis found a high R-IPI score/primary CNS disease to be significantly associated with death (p=0.004). Median time from diagnosis to starting treatment was 30 days and median follow up was 32 months. All patients had reduction in immunosuppression; in 10 this was the sole treatment, 26 received chemotherapy (81% RCHOP) and 7 received Rituximab. 2 out of 3 patients with CNS PTLD underwent surgical resection and WBRT as their primary treatment. There were 15 deaths during the 10 year period; 6 patients received no treatment/failed to complete treatment, 3 had primary CNS lymphoma and 6 completed treatment. Kaplan Meier analysis showed a 2 year OS of 75%. 44 patients completed treatment; 39 achieved CR, 2 had refractory disease and 3 had unknown response. Of the 39 patients achieving CR; 10 patients relapsed and 1 was lost to follow up. Kaplan Meier analysis showed a 2 year PFS of 80%.

Table 1.

Histology of PTLD	Number of patients (n=51)
Early IM like	2
Early plasmacytic	2
Polymorphic	13
Monomorphic	31
Hodgkin's	2
T-cell anaplastic	1

Site of disease	Number of patients (n=51)
Nodes	17
Extranodal	34
GI	13
Multiple	10
Liver	3
Oral	3
CNS	3
Adrenal	1
BM	1

Summary/Conclusion: In conclusion, our study shows that the majority of patients with PTLD present with extranodal disease and a significant proportion have a moderate to high R-IPI score. Nevertheless, with collaborative haematology/renal approach, controlled reduction in immunosuppression and Rituximab based chemotherapy, a 2 year OS of 75% can be achieved. Unfortunately, a significant minority of patients succumb at presentation or early in their treatment course highlighting the need to develop robust tools for monitoring SOT patients and early diagnosis of PTLD patients.

PS1034

PHASE II STUDY WITH OBINUTUZUMAB-DHAP IN RELAPSED/REFRACTORY DLBCL PATIENTS BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION: A STUDY FROM THE FONDAZIONE ITALIANA LINFOMI (GIOTTO STUDY)

L. Rigacci[1,*], B. Puccini[1], C. Visco[2], R. Battistini[3], L. Arcaini[4], M. Gotti[5], M. C. Tisi[2], L. Mannelli[1], M. Mian[6], M. Martelli[7], L. Flenghi[8], S. Pozzi[9], F. Palombi[10], A. Pascarella[11], A. Evangelista[12], C. Peracchio[13]

[1]Haematology, AOU Careggi, Firenze, [2]Cell Therapy and Hematology, San Bortolo Hospital, Vicenza, [3]Hematology, AO San Camillo Forlanini, Roma, [4]Molecular Medicine and Hematology Division , University of Pavia and IRCCS Policlinico S Matteo, [5] Hematology Division, IRCCS Policlinico S Matteo, Pavia, [6]Hematology-CBMT and Internal Medicine V, Hematology and Oncology, Hospital of Bolzano and Medical University of Innsbruck, Bolzano, [7]Hematology, University La Sapienza, Roma, [8]Hematology, Azienda Ospedaliera, Perugia, [9]Hematology, Medicina Diagnostica Clinica, Modena, [10]Hematology, IFO Regina Elena, Roma, [11]Hematology, Ospedale dell'Angelo, Venezia-Mestre, [12]Epidemiology , Unit of Clinical Epidemiology and CPO Piemonte, Torino, [13]Clinical Trial, Fondazione Italiana Linfomi, Alessandria, Italy

Background: Salvage immune-chemotherapy followed by autologous stem cell transplantation (ASCT) is the standard second line treatment for relapsed and refractory Diffuse Large B Cell Lymphoma (DLBCL). The initial exposure to Rituximab may increase the difficulty of salvaging patients who fail first line therapy, particularly refractory patients had poor clinical outcomes.
Aims: The aim of this phase II study (GIOTTO study, Eudract: 2013-004014-17) was to evaluate the efficacy and safety of the new anti-CD20 antibody (Obinutuzumab) in association with DHAP as induction therapy followed by high dose chemotherapy BEAM or FEAM with ASCT in patients with relapsed/refractory DLBCL.
Methods: The primary objective was to assess whether the treatment achieves an absolute increase of the complete remission (CR) proportion of at least 20% (hypothesizing from 30% to 50%) with respect to the standard treatment with an acceptable extra-hematological toxicity grade 3-4 of 0.25 (unacceptable extra-hematological toxicity=0.40). The CR rate was evaluated by PET scan after four cycles of GA101-DHAP before ASCT according to Cheson criteria from 2007. According to a Briant & Day design an interim analysis was necessary after the enrollment of 29 patients. To continue the study, at interim analysis at least 10 patients were required to obtain a CR while non-haematological toxicities of grade>=3 did not have to occur in more than 19 patients.
Results: From June 2014 to June 2017, 29 patients were enrolled; according to clinical characteristics 17 patients were refractory to first line therapy and 12 were relapsed. Five patients progressed or stopped due to an adverse event after the first GA101-DHAP cycle, 24 patients reached the intermediate evaluation and 9 were withdrawn from the study for the following reasons: 7 for progressive disease, 1 for physician decision and 1 for other reason. Fifthteen patients performed pre-ASCT evaluation, and 7 of them did not perform ASCT: 6 for progressive disease and 1 for adverse event. Finally eight patients underwent ASCT of whom 7 patients were in complete remission and 1 had stable disease. Overall, before ASCT, 6 patients obtained a CR (6/29=21%), 4 a partial remission, 1 stable disease and 13 progressive disease. Of note, 4 out 6 CR (4/17=24%) were obtained in refractory subset and 2 out 6 CR in relapsed subset (2/12=17%). Non-hematological toxicity grade >=3 was reported in 9 patients. Hematological toxicities were those usually reported with DHAP chemotherapy. In this first stage analysis the number of complete response pre-ASCT, 6 out 29 patients (21%), was lower than the level set for continuing the study (10 out 29 patients). The number of patients with non-haematological toxicities with grade>=3 during experimental therapy (9 out 29, 31%) was within the limits of the study design.
Summary/Conclusion: The results of the interim analysis, did not allow to reopen patients' accrual because we did not achieved 10 CR. Patients will be followed in the next years. Even if the study couldn't be completed after this interim analysis, the addition of Obinutuzumab to DHAP, led to a promising response rate in refractory patients (4 CR/17) moreover it was well tolerated and safe in this group of patients.

PS1035

COMPARISON OF R-CHOP, R-CVP, CHOP, AND CVP FOR PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA IN TAIWAN: A POPULATION-BASED STUDY, 2009-2013

H.-H. Huang[1,*], F.-Y. Hsiao[2,3,4], L.-J. Chen[1], H.-M. Chen[5], B.-S. Ko[1]

[1]Division of Hematology, Department of Internal Medicine, National Taiwan University Hospital, [2]Graduate Institute of Clinical Pharmacy, [3]School of Pharmacy, [4]Department of Pharmacy, [5]Health Data Research Center, National Taiwan University, Taipei City, Taiwan, Republic of China

Background: Diffuse large B cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma, and majority of the patients are older than 60 years. CHOP is the standard regimen for DLBCL patients, and additional rituximab improves the treatment responses and overall survival. However, elder DLBCL patients receive less anthracycline or omit it because of comorbidities, especially cardiac diseases.
Aims: To examine the clinical benefit of rituximab and anthracycline in different age groups of DLBCL patients, we used the Taiwan Cancer Registry Database (TCRD) and National Health Insurance Research Database (NHIRD) in our study.
Methods: We identified 4834 DLBCL patients with aged 20 years and older and known Ann Arbor stages from TCRD. According to the data from NHIRD, we excluded the patients receiving other chemotherapies or without any chemotherapy. 3989 DLBCL patients were included for further studies. 2859 DLBCL patients received R-CHOP, 750 received R-CVP, 252 received CHOP, and 128 received CVP.
Results: Compared to the anthracycline-containing groups (R-CHOP and CHOP groups), the median ages were older, the male-to-female ratio was lower, the Charlson comorbidity index was lower in the non-anthracycline groups (R-CVP and CVP groups). This observation showed that the clinicians tended to avoid anthracycline if the patients had older ages or more comorbidities. The overall survival (OS) and the time-to-treatment failure (TTF) were better if DLBCL patients received anthracycline (Figure 1 A and B; p value < 0.0001), and were dramatically improved with additional rituximab (Figure 1 A and B; p value < 0.0001). Multivariate analysis of OS and TTF demonstrated that the type of frontline chemotherapies (R-CHOP, R-CVP, CHOP, or CVP) was an independent prognostic factor. In the subgroup analysis, DLBCL patients were still benefit from the use of rituximab and anthracycline no matter in any gender (male, Figure1 C; female, Figure 1 D) or age groups (20-59 years, Figure 1 E; older than 60 years, Figure 1 F).

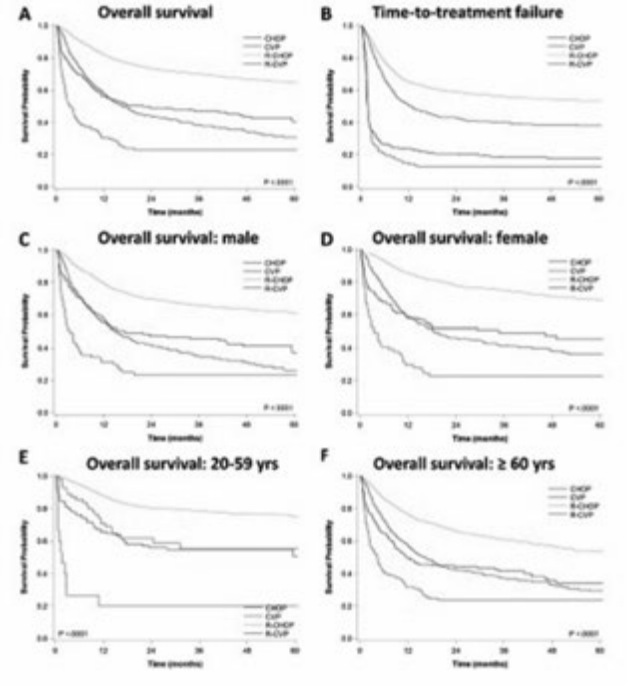


Figure 1.

Summary/Conclusion: From our population-based study, we illustrated that clinical benefit of rituximab and anthracycline in the patients with DLBCL. In the patients older than 60 years, additional anthracycline also improved their clinical outcomes.

PS1036

HIGH INCIDENCE OF CARDIOMYOPATHY IN PATIENTS RECEIVING CHOEP14 - POSSIBLE SYNERGISTIC CARDIOTOXICITY OF DOSE-DENSE DOXORUBICIN AND ETOPOSIDE

I. Zekanovic[1], S. Basic-Kinda[2], I. Radman[2], I. Hude[2], D. Dujmovic[3], I. Ilic[4], M. Kralik[5], M. Dobrenic[36], I. Aurer[2,3,*]

[1]Department of Internal Medicine, General Hospital Zadar, Zadar, [2]Division of Hematology, Department of Internal Medicine, University Hospital Centre Zagreb, [3]Medical School, University of Zagreb, [4]Deparment of Pathology and Cytology, [5]Department of Radiology, [6]Department of Nuclear Medicine, University Hospital Centre Zagreb, Zagreb, Croatia

Background: (R)CHOEP14 is a very effective front-line treatment of high-risk aggressive lymphomas. We observed an unusually high frequency of cardiotoxicity in patients treated with this regimen.
Aims: This study was performed to describe and analyze the frequency and risk factors for development of cardiac side-effects during and after (R)CHOEP treatment.
Methods: We retrospectively analyzed charts of all our patients treated with CHOEP14 or R-CHOEP14.
Results: Seventy-six patients were treated with 6-8 cycles of CHOEP14; 63, having B-lymphomas, in combination with rituximab and 13, having T-lymphomas, without it. Median age was 43, range 18 to 66 years; 46 were male and 30 female. All were fit prior to development of lymphoma and none had a history of cardiac disease. After a median follow-up of 29 months, 12 patients (16%) developed symptomatic cardiomyopathy with reduced systolic function, four during treatment and eight 3-60 (median 16) months after end of treatment. Five of the events were CTCAE grade 2, and 7 grade 3-5; one patient died of cardiac arrest. The incidence of cardiomyopathy did not correlate with age, sex, lymphoma type, hematological toxicity, mediastinal irradiation or use of salvage chemotherapy. Interestingly, 2 of the 12 patients with cardiotoxicity developed secondary tumors in comparison to only 1 of 66 without it.
Summary/Conclusion: The frequency of cardiomyopathy in our patients treated with (R)CHOEP was significantly higher than in patients of similar age receiving (R)CHOP, and even more so than in those receiving eBEACOPP or DA-(R)-EPOCH. The course of the cardiomyopathies was different than usually seen in doxorubicin-induced acute heart failure and they occurred earlier than usual for late-onset doxorubicin-induced heart failure. This indicates that the cardiotoxic effects of high peak concentrations of doxorubicin, due either to topoisomerase IIβ inhibition or to its intercalating action, are potentiated by additional topoisomerase IIβ inhibition by etoposide. Additional studies are needed to clarify involved molecular mechanisms which might be similar to those causing secondary cancers.

PS1037

PROGNOSTIC IMPACT OF SITES OF EXTRANODAL INVOLVEMENT IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA IN THE RITUXIMAB ERA

N. Inoue[1,*], M. Yokoyama[1], A. Nishihara[1], Y. Kusano[1], H. Yamauchi[1], Y. Mishima[1], N. Nishimura[1], S. Marshall[2], N. Tsuyama[3], K. Takeuchi[4], Y. Terui[1]

[1]Hematology Oncology, [2]Medical Oncology, [3]Pathology, The Cancer Institute Hospital Of JFCR, [4]Pathology Project for Molecular Targets Project, Cancer Institute, Tokyo, Japan

Background: Diffuse large B-cell lymphoma (DLBCL) sometimes involves extranodal lesion. The most common used prognostic score in patients with DLBCL is the revised international prognostic index (R-IPI) score. The score defines the more than two extranodal lesions of involvement is worse prognostic factor. However, R-IPI score doesn't consider the site of extranodal involvement.
Aims: This study was aimed at investigating the site of extranodal involvement associates with prognosis in patients with DLBCL.
Methods: We retrospectively analyzed the patients diagnosed DLBCL and treated with R-CHOP in our hospital from January 2005 to January 2017. We evaluated the 21 extranodal lesion by PET-CT, MRI or CT image. We categorized the extranodal to 18 classifications (the stomach or duodenum, bone, colon, muscle, skin, paranasal sinus, breast, lung, liver, thyroid, tongue or gingiva, eyes, testis, adrenal gland, kidney, uterus or ovary, pancreas, and the central nervous system). We analyzed overall survival (OS) and progression free survival (PFS).
Results: A total of 636 patients with DLBCL was collected data. Median follow-up time is 53 months and the 4-years OS is 80.7% (Median OS is not reached). All patients of complete remission rate is 92.3% after last R-

CHOP chemotherapy or radiation therapy. The 422 of 636 patients (66.4%) had extranodal lesion involvement. And the 297 of 422 patients (70.4%) had only one site of extranodal involvement. The extranodal site was the stomach or duodenum in 112 patients (25.2%), bone in 104 (23.0), colon in 49 (11.0%), muscle in 42 (9.5%) , skin in 41 (9.2%), paranasal sinus in 41 (9.2%),breast in 37 (8.3%), lung in 28 (6.3%), liver in 27 (6.1%), thyroid in 25 (5.6%), tongue or gingiva in 23 (5.2%), eyes in 20 (4.5%), testis in 16 (3.6%), adrenal gland in 11 (2.5%), pancreas in 10 (2.3%), kidney in 7 (1.6%), uterus or ovary in 6 (1.4%), the central nervous system (CNS) in 6 (1.4%). In the result of univariate analysis, muscle (p<0.01), skin (p<0.01), lung (p<0.01), liver (p<0.01), adrenal grand (p<0.01), pancreas (p<0.01), kidney (p<0.01) or CNS (p<0.01) of involvement is worse 4-years OS. However, in multivariate analysis result worse prognosis of OS in patients with liver (p=0.039), pancreas (p<0.01), lung (p<0.01) or skin (p<0.01) of involvement. Furthermore, we evaluated 4-years PFS. In the result of univariate analysis, bone (p=0.012), muscle (p<0.01), skin (p<0.01), lung (p<0.01), liver (p<0.01), adrenal grand (p<0.01), pancreas (p<0.01), uterus or ovary (p=0.021), kidney (p=0.045) or CNS (p<0.01) of involvement is worse 4-years PFS. In multivariate analysis result, bone, muscle, skin, lung, liver, adrenal grand, pancreas, uterus, ovary, kidney or CNS of involvement is also worse (p<0.01). The more than two extranodal lesions involvement is worse prognosis than 0 or 1 extranodal. (Four-years OS is 85.3% vs 62.4%, p<0.01. Four-years PFS is 77.7% vs 53.7%, p<0.01) (Table 1).

Table 1.

Site of involvement	Number(%)	4 years OS Rate	4 years OS Univariate analysis	4 years OS Multivariate analysis
stomach or duodenum	112 (25.2%)	75.6%	P=0.1	-
bone	104 (23.0%)	72.5%	p=0.073	-
Colon	49 (11.0%)	74.3%	P=0.768	-
Muscle	42 (9.5%)	61.1%	p<0.01	P=0.447
Skin	41 (9.2%)	57.8%	p<0.01	p<0.01
Paranasal sinus	41 (9.2%)	89.7%	P=0.761	-
Breat	37 (8.3%)	76.4%	P=0.786	-
Lung	28 (6.3%)	46.6%	p<0.01	p<0.01
Liver	27 (6.1%)	56.1%	p<0.01	P=0.039
Thyroid	25 (5.6%)	82.9%	P=0.539	-
Tongue or gingiva	23 (5.2%)	90.6%	P=0.089	-
Eye	20 (4.5%)	82.2%	P=0.583	-
Testis	16 (3.6%)	87.5%	P=0.902	-
Adrenal gland	11 (2.5%)	39.8%	p<0.01	P=0.132
Pancreas	10 (2.3%)	36.0%	p<0.01	p<0.01
Kidney	7 (1.6%)	42.9%	p<0.01	P=0.785
Uterus or ovary	6 (1.4%)	66.7%	P=0.358	-
Central nervous system	6 (1.4%)	33.3%	p<0.01	P=0.121

Summary/Conclusion: The patients with extranodal involvement DLBCL is not only the number of the lesion but also the site of extranodal involvement important.

PS1038

LIPOSOMAL DOXORUBICIN IN AGGRESSIVE B CELL LYMPHOMA SHOWS SIMILAR EFFICACY TO THE CONVENTIONAL FORMULATION: LONG TERM RESULTS FROM A RETROSPECTIVE COHORT STUDY

A. García-Noblejas[1,*], J. Cannata-Ortiz[1], E. Acuña[1], J. Loscertales[1], A. Alegre[1], R. Arranz[1]

[1]Hematology, Hospital La Princesa, Madrid, Spain

Background: Anthracyclines play a key role in the treatment of Diffuse Large B cell Lymphoma (DLBCL). Now that long term disease control can be achieved there is a raising concern about cardiotoxicity, mainly in the elderly population with associated morbidities. Liposomal doxorubicin (LD) has proven to be efficacious and less cardiotoxic in breast cancer through phase III studies. In DLBCL we are waiting the results of controlled trials.
Aims: To analyze the efficacy and toxicity of LD (Myocet^{R}) in DLBCL patients with cardiovascular risks (CVR), in comparison with a cohort who received the conventional formulation during the same time period.
Methods: This is a retrospective cohort study in patients with *de novo*DLBCL consecutively treated with immunochemotherapy from March 2006 to June 2012, with conventional doxorubicin (CD) or LD. Criteria to indicate LD were any of the following: left ventricular ejection fraction (LVEF) < 50%, CVR factors or prior cardiopathy. Selection of a 3.5 vs 1 ratio (treated with CD vs LD) was used to increase statistic power. We studied response rates, progression free survival (PFS) and overall survival (OS). Toxicity was evaluated with CTCAE v.4.0. Statistical package used was SPSS v15.0.
Results: 78 patients (pts) were retrieved: 61 pts in the control arm (group A) and 17 in the study arm (group B). Characteristics are shown in Table 1. Older age, LVEF < 50%, cardiopathy history and hypertension were significantly higher in group B but the other characteristics were balanced between both groups. Efficacy: 69 pts were evaluable for response and 9 were not as they received less than 3 cycles (4 due to infection /sepsis, 3 due to stomach/bowel perforation, 1 due to worsening of cardiopathy and 1 lost to follow-up). We observed similar OR (82% in group A and 83% in group B) and CR rates (70% in group A and 83% in group B). Median follow for surviving pts was up of 7.25 years (0.16 yrs – 11.2 yrs), 7.35 years in group A and 5.5 in group B. Considering all the patients, OS and PFS at 10 years in group A were 60% ±SD 9% and 46% ±SD 9%, respectively and 73% ±SD 11% and 50% ±SD 13%, respectively in group B without significant differences between both arms. Toxicity: grade 3-4 neutropenic events were in group A 17% vs 2% in group B (p=0.02), and febrile neutropenia in group A was 13% vs 5% (5%) in group B (p=0.033). Peg- GSF was systematically used in group B. During treatment, 1 patient (2%) in the control group presented atrial fibrillation and in the Myocet^{R} group 3 (18%) patients with previous cardiomyopathy had cardiac events (2 atrial fibrillation, 1 congestive heart failure). During follow-up, 11 patients had new cardiac events, 9 in group A and 2 in group B. Twenty three (29%) pts died: 12 due to lymphoma, 4 due to grade 5 infection during treatment, 3 due to infection while in CR after treatment, 1 due to allo-SCT after secondary AML, 2 due to stroke and 1 due to non filiated cardiac tamponade.

Table 1.

Characteristics	Group A N= 61	Group B N= 17	p
Age (range)	70 (41-88)	78 (59-89)	0.001
Male / female	21/40	9/8	0.165
ECOG >2	4 (7%)	2 (11%)	0.617
Ann Arbor III-IV	43 (70%)	10 (56%)	0.389
B symptoms	38 (63%)	8 (44%)	0.179
Comorbidities			
HBP	24 (39%)	13 (76%)	0.007
DM	3 (5%)	2 (12%)	0.308
Dyslipemia	11 (18%)	5 (29%)	0.304
Smoking	15 (25%)	2 (12%)	0.257
Cardiopathy			0.001
Atrial fibrillation	6 (10%)	3 (18%)	
Ischemic	-	3 (18%)	
cardiopathy	-	1 (6%)	
Other			
LVEF <50%	2 (4%)	5 (31%)	0.001

Summary/Conclusion: In this study, the association use of LD to inmunochemotherapy in fragile patients showed similar efficacy as conventional doxorubicin, without increased toxicity.

PS1039

CLINICAL CHARACTERISTICS AND PROGNOSTIC FACTORS OF HEMOPHAGOCYTIC SYNDROME IN ADULT PATIENTS: A RETROSPECTIVE SINGLE-CENTER STUDY FROM SINGAPORE

S.-Y. Ong[1,*], S. Y. Tan[2], Y. Chen[2], C. Nagarajan[2], N. Grigoropoulos[2], Y.S. Lee[2]

[1]Singapore General Hospital, [2]Singapore General Hospital, Singapore, Singapore

Background: Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening syndrome of excess immune activation. Acquired HLH has a different epidemiology in Asia.
Aims: Our retrospective study aims to review the clinical characteristics and outcomes of HLH in our tertiary institution to identify strategies to improve outcomes.
Methods: A retrospective review of bone marrow aspirate reports between 1 January 2007 and 31 December 2016 was performed to identify cases with presence of haemophagocytes on aspirate smears. Patients were included if they have HLH based on the HLH-2004 criteria. Analysis of parameters of potential prognostic significance included age ≥60, sex, race (Chinese vs Malay), etiology (NK/T lymphoma vs others), haematological parameters (hemoglobin (Hb) ≤9 g/dL, absolute neutrophil count (ANC) ≤ $0.5x10^9$/L, platelet ≤$40x10^9$/L), LDH ≥1500 U/L, bilirubin ≥100 µmol/L, AST ≥500 U/L, and Epstein-Barr Virus (EBV) titre above detectable range. Survival probabilities were calculated using the Kaplan–Meier estimator, with log-rank analysis used to compare between different groups. Univariate and multivariate analyses were performed to identify variables prognostic for survival. Forward stepwise variable selection at a 0.2 significance level was used to identify covariates to

build the multivariate model. All analyses were performed using Stata (Statacorp, College Station, TX, USA).
Results: 49 patients (33 male, 16 female) fulfilled the diagnostic criteria of HLH. The median age was 53 (range 17 - 78) years old. The median haematological parameters were Hb 8.3 (3.7-13.7) g/dL, ANC 1.16 (0 – 22)×10^9/L, and platelet 55 (3-129)×10^9/L. The underlying diagnosis was NK/T-lymphoproliferative disease (LPD) (n=32), B-LPD (n=8), infections (n=5) and others including autoimmune diseases, non-haematological malignancies, and acute myeloid leukemia (n=5). 82% of patients were not alive at analysis, and major causes of death include severe lactic acidosis, multi-organ failure syndrome, coagulopathy and bleeding, infection, and disease relapse. The median survival of those who died was 35 days (95% CI 17-68 days). In univariate analysis, older age ≥ 60, NK/T LPD, LDH ≥1500 U/L, bilirubin ≥ 100 µmol/L, ANC < 0.5x 10^9/L, triglyceride ≥ 5 mmol/L, platelet < 40x10^9/L, and presence of EBV DNA above detectable range (≥ 3.3 log) were associated with poor survival and included in the multivariate model. However, patient's sex, racial group, presence of splenomegaly, and laboratory values including Hb, AST, fibrinogen, and ferritin were not associated with survival (all p>0.2). In multivariate analysis, low platelet < 40 x10^9/L (HR 2.4, 95% CI 1.02-5.70, p=0.046), presence of EBV (HR 2.8, 95% CI 1.20-6.89, p=0.018), and high triglyceride ≥ 5 mmol (HR 5.90, 95% CI 1.98-17.01, p=0.001) were independently associated with poorer survival.
Summary/Conclusion: Consistent with published data, no cases of primary HLH were identified in our adult cohort. HLH related to malignancy have an aggressive course associated with high mortality rates despite treatment. Thrombocytopenia, positive EBV DNA, and high triglyceride levels were independently associated with worse survival. Increased bilirubin, AST, and LDH are frequently found in adult HLH and presence of these features should precipitate a workup. Novel therapies and needed to improve outcomes in adult HLH.

PS1040

THE IMPACT OF DIFFERENT IMMUNOCHEMOTHERAPY REGIMENS ON SURVIVAL OUTCOMES IN^{131} NEWLY DIAGNOSED PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA

G. Tumian[1,*], O. Trofimova[1], N. Kokosadze[1], I. Zavodnova[1], M. Kichigina[1], D. Tupitsyna[2], P. Zeynalova[1]

[1]24Kashirskoy sh., [2]N.N. Blokhin National Cancer Research Center, Moscow, Russian Federation

Background: Primary mediastinal large B-cell lymphoma (PMBCL) is recognized as distinct clinicopathologic subtype of diffuse large B-cell lymphoma, predominantly affects young female and characterized mainly by the bulky tumor mass within the upper mediastinum with frequent development of pleuritis\pericarditis and syndrome of the vena cava superior. There is not standard of first line therapy, in particular, discussed the efficacy of more intensive chemotherapy regimens and feasibility of radiation therapy.
Aims: The purpose of our study is evaluated outcomes of PMBCL patients, treated with three different rituximab-chemotherapy regimens (R-CT) with and without radiotherapy (RT).
Methods: We performed a retrospective analysis of 131 patients (pts) with PMBCL since 2005-2017 treated in N.N. Blokhin National Cancer Research Center. The median age was 30 years (16-70), 58% were female, 66% had I/IIE stages, B-symptoms was present in 55% pts. Historically, pts received three different R-CT regimens: R-MACOP-B 55 pts (42%), R-CHOP 40 pts (30,5%), DA-R-EPOCH 36 pts (27,5%). Radiation therapy to the mediastinum received 99 (76%) pts.
Results: With median follow-up of 37 months, the estimated 3-year progression-free survival (PFS) was 78,6% [95% confidence interval (CI) 78,0-96,2] and overall survival (OS) was 88,3% (95% CI 83,8-97,7). The pts that were not responded to first-line treatment, had very poor prognosis (median OS 17 months). There were significant differences in PFS and OS depending on the three CT regimens – PFS were 78% vs 65% vs 91% respectively (p=0.002), OS were 88% vs 62% vs 97% respectively (p=0,003). If we exclude from analysis 40 pts treated with R-CHOP, the differences in PFS and OS between R-MACOP-B and R-DA-EPOCH groups were not obtained (P>0.1) with less radiation used after DA-R-EPOCH (44% vs. 29%).
Summary/Conclusion: Immunochemotherapy combined with local irradiation has become the standard of care PMBCL. There was a significant benefit of intensive chemotherapy regimens before the R-CHOP. The patients not responded to first-line treatment have very inferior outcome

Bleeding disorders (congenital and acquired)

PS1041

TREATMENT OF TRAUMATIC BLEEDS WITH RECOMBINANT FACTOR XIII-A2 IN PATIENTS WITH CONGENITAL FXIII-A SUBUNIT DEFICIENCY: RESULTS FROM THE MENTOR™6 STUDY

L. Hvitfeldt Poulsen[1,*], B. Kerlin[2], D. Nugent[3], S. Kearney[4], J. Klovaite[5], M. Carcao[6]

[1]Centre for Haemophilia and Thrombosis, Aarhus University Hospital, Aarhus, Denmark, [2]Ohio State University College of Medicine, Nationwide Children's Hospital, Columbus, Ohio, [3]UC Irvine Medical School, Children's Hospital of Orange County, Orange, CA, [4]CHCMN Hemophilia and Thrombosis Center, Children's Hospital and Clinics of Minnesota, Minneapolis, MN, United States, [5]Novo Nordisk A/S, Bagsværd, Denmark, [6]Division of Haematology/Oncology and Child Health Evaluative Sciences, Research Institute, Hospital for Sick Children, University of Toronto, Canada

Background: Recombinant factor XIII-A_2 (rFXIII-A_2) has been developed for prophylaxis in patients with congenital FXIII-A subunit (FXIII-A) deficiency. Phase 3 clinical trials showed that rFXIII-A_2 provided effective prophylaxis (35 IU/kg every four weeks) with a good safety profile in pediatric and adolescent/adult patients with severe (<0.05 IU/mL) congenital FXIII-A deficiency. rFXIII-A_2 prophylaxis also allowed for effective hemostasis during minor surgery without the need for additional FXIII treatment. However, the data for the effectiveness of rFXIII-A_2 to treat traumatic bleeds are limited due to the low bleeding rate on prophylaxis.
Aims: Evaluate the treatment of traumatic bleeds in patients receiving rFXIII-A_2 prophylaxis in a planned interim analysis.
Methods: mentor™6 is a prospective, multinational, non-interventional, postauthorization safety study. The interim analysis study period was from May 17, 2013 to May 17, 2017. Patients of all ages received rFXIII-A_2 prophylaxis. Hemostatic response for the treatment of bleeds was assessed by the investigator using a four-point scale (excellent, good/effective, moderate/partly effective, and none).
Results: A total of 30 patients (median [range] age 21 [2–68] years) were receiving prophylaxis with rFXIII-A_2. Three traumatic bleeds were treated with a single dose of rFXIII-A_2 in three patients. All traumatic bleeds were mild/moderate in severity and occurred after falls. Patient 1 (13 year-old female), 6 days after the last rFXIII-A_2 prophylaxis dose, suffered a head injury that resulted in headache/dizziness but no loss of consciousness. FXIII activity at the time of injury was not measured; rFXIII-A_2 was given as preventive treatment. Patient 2 (21-year-old male) suffered a joint bleed and hematoma after a fall on to his knee 10 days after the last rFXIII-A_2 prophylaxis dose. Ultrasound of the knee was performed and 60 ml blood was drained from the knee; re-bleeding occurred 30 min later. FXIII activity prior to rFXIII-A_2 treatment was 0.25 IU/ml, rising to 0.96 IU/ml 1 hour after rFXIII-A_2 treatment. Patient 3 (12-year-old female) suffered a knee bleed after a fall 24 days after the last rFXIII-A_2 prophylaxis dose. Three days after the trauma, the patient was examined by the Investigator and, based on clinical signs, a bleed in the knee joint was diagnosed, which required treatment. No imaging was performed. FXIII activity prior to rFXIII-A_2 treatment was 0.08 IU/ml. The patient received a single dose of rFXIII-A_2 for treatment of the bleed, which was also considered as the regular prophylaxis dose (the trauma occurred 3 days before the next scheduled visit). Two patients (Patients 1 and 2) were given a single additional dose of rFXIII-A_2 to treat the traumatic bleed. Patient 3 received rFXIII-A_2 treatment as the regular prophylaxis dose. Patients 1 and 3 reported excellent hemostatic outcome to treatment with rFXIII-A_2 while Patient 2 reported a good outcome. No adverse events related to the additional rFXIII-A_2 treatment were observed.
Summary/Conclusion: These data suggest a single dose of rFXIII-A_2 after trauma, during prophylaxis with rFXIII-A_2, provides sufficient hemostatic management of traumatic bleeds, with a favorable safety profile, in patients with congenital FXIII-A deficiency.

PS1042

BEVACIZUMAB SIGNIFICANTLY IMPROVES SEVERE EPISTAXIS IN HEREDITARY HEMORRHAGIC TELANGIECTASIA

S. Zupancic Salek[1,*], G. Poje [2], D. Pulanić[1], S. Bašić Kinda[1], A. Boban[1], M. Vodanović[1]

[1]Haematology, [2]ENT Clinic , University Hospital Zagreb, Croatia

Background: Rendu-Osler-Weber disease or hereditary hemorrhagic telangiectasia (HHT) is a rare genetic disorder that affects blood vessels. Patients have abnormal blood vessels clusters like AVMs or telangiectasias which often rupture and bleed. Epistaxis is the most often manifestation of HHT which results in severe anaemia, iron infusions, and blood transfusion. QoL is very poor in these patients.
Aims: Aim of the work is to show effective results of treatment with bevacizumab in two patients with severe and recurrent epistaxis in HHT.
Methods: Methods: Prospective report of two patients (man and woman) treated with bevacizumab due to HHT and severe epistaxis with long follow up.
Results: Results: Two patients (man 61 yrs and woman 70 yrs old) with long lasting HHT started treatment with intravenous bevacizumab due to severe, recurrent epistaxis refractory to standard treatment. The man failed all medical and interventional approaches to stop the bleeding including ENT laser cauterisation procedures, septodermoplasty, etc. They have been both RBC transfusion dependent and he man received about 79 transfusions before starting treatment with bevacizumab and she had about 20 RBC transfusions. The initial dosing of bevacizumab consisted of 4 cycles each administered two weeks apart for both patients. The next several doses of bevacizumab were a month apart. The man experienced one severe epistaxis during the monthly infusions of the drug so we continue the treatment every two weeks for the next 7 months. He continued without bleeding and without side effects. They both have not got any RBC transfusion at all since the introduction of bevacizumab in the treatment. She is six months on the therapy every two weeks with very minimal epistaxis from time to time. ESS questionnaire was used to assess the severity of nose bleeds at the beginning and after starting the bevacizumab treatment.
Summary/Conclusion: Conclusion: Treatment of HHT and severe epistaxis with bevacizumab resulted in excellent efficacy and safety. Both patients showed a dramatic change in transfusion requirement and significant difference in ESS. Intravenous bevacizumab should be the considered as a standard first-line treatment option for HHT patients with severe bleeding and transfusion dependent anemia.

PS1043

AN AUDIT OF THE USE OF PROTHROMBIN COMPLEX CONCENTRATION

S. Bonney[1,*], J. Schofield[2], P. Nee[3]

[1]Haematolgy and Blood Transfusion Laboratory, [2]Haematology, [3]Accident and Emergecny, St Helens and Knowsley Teaching Hosptials NHS Trust, St Helens, United Kingdom

Background: Prothrombin complex concentrate (PCC) is licensed for emergency reversal of anticoagulation in severe and life-threatening bleeding, intracranial haemorrhage and prior to emergency surgery. It is an expensive resource costing approximately Eur2000 per adult dose. There is evidence of inappropriate use of PCC leading to delays to treatment, inappropriate dosing and use of adjunctive vitamin K. Much product, delivered to clinical settings, is wasted and we reported previously an annual wastage of approximately Eur 15,000 per annum, amounting to a UK-wide financial loss of nearly Eur3 millions. Guidelines governing the use of PCC exist in order to ensure the rapid and appropriate use of the product.
Aims: The aim of the present audit was to determine the appropriateness of use of PCC in a UK teaching hospital setting against published standards (BCSH 2011)
Methods: An audit of the emergency use of PCC was undertaken in a UK teaching hospital in 2014/15. Patients prescribed PCC were identified from the Laboratory Information System. Patients were followed up using the hospital's electronic patient management system and the following parameter were recorded: patient demographics, indications for oral anticoagulation, reason for reversal, coagulation metrics, use of vitamin K, date and time that PCC was prescribed and given and 30-day outcome. The audit was repeated in 2016/17 after an educational programme directed at hospital doctors.
Results: There were 103 prescriptions for PCC in 2014/15 and 113 in 2016/17. The clinical settings are listed in Table 1. The indications for PCC are listed in Table 2 and the delays from issue to administration are presented in Table 3. Table 4 shows the number of prescribed units administered, returned and wasted during the two audit periods. Vitamin K pre-treatment is recommended for bleeding on warfarin. Thirteen patients in the first audit and ten in the second did not receive vitamin K.
Pre-treatment with vitamin K is unnecessary in patients on direct-acting oral anticoagulants (DOAC). Eleven patients in the first audit and 24 in the second audit were on DOAC. Of these, seven patients in the first audit and nine in the second audit received vitamin K inappropriately.
Two patients in the second audit received PCC inappropriately for bleeding associated with use of dabigatran; a specific antidote, idarucizumab, is available for this agent.

Table 1. Time between PCC issue and administration.

Time between PCC issue and administration	2014-2015	2016-2017
<1 hour	29	30
1-2	13	21
2-3	8	9
3-4	4	7
>4	6	4
No administration time	15	35

Table 2. Reason for request.

Reason for request	2014-2015	2016-2017
Intracranial bleed	44	41
GI bleed	35	30
AAA	0	3
Emergency surgery	10	26
Haematoma/ mucosal bleeding	12	2
Sepsis	0	1

Table 3. Location of request.

Location of request	2014-2015	2016-2017
Accident and Emergency	61	70
Medical Assessment /GP Assessment	10	15
Theatre	0	5
Medical wards	3	5
Surgical ward	19	13
Stroke ward	8	5
ITU	2	0

Table 4.

	Prescribed	Administered	Returned	Wasted
2015/16	103	75	4	141
2016/17	113	106	42	2
Total	223	181	44	143

Summary/Conclusion: The audit shows that PCC is being used with increasing frequency in the acute hospital setting in the UK, with the emergency department the major user. Adherence to published standards on the use of this agent is not optimal and there is substantial wastage. An educational programme, directed at clinical doctors, was effective at improving standards and wastage was reduced significantly.

PS1044

HAEMOSTATIC ABNORMALITIES OR DEFECTS IN GREEK PATIENTS WITH GAUCHER DISEASE

V. Komninaka[1,*], K. Repa[1], T. Marinakis[2], A. Pouliakis[3], D. Korakakis[4], T. Kati [5], D. Tsokanas[6], M. Politou[4]

[1]Thalassaemia Center, Laiko Hospital of Athens, Greece, [2]Haematology, Georgios Gennimatas Hospital, [3]2nd Department of Pathology, Attiko University Hospital, [4]Haematology Laboratory - Blood Bank, [5]Heamatology Laboratory - Blood Bank, Aretaieio Hospital, [6]Haematology Department, Georgios Gennimatas Hospital, Athens, Greece

Background: Gaucher disease (GD) is the most common of the lysosomal storage disorders. Glucocerebrosidase deficiency results accumulation of, glucocerebroside, in macrophages.40–50% of Gaucher disease patients (GDPs) present with bleeding symptoms which have been attributed to

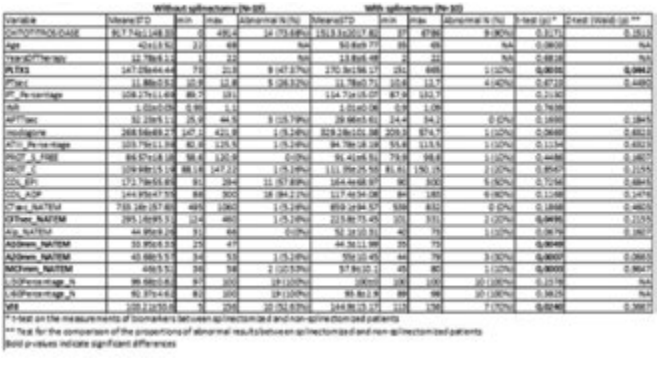
thrombocytopenia, clotting factor deficiencies and platelet disfunction. Enzyme replacement therapy (ERT) can reverse many of the systemic manifestations of (GD). However, little is known of the effects of ERT on the coagulation abnormalities and platelet function.

Aims: The aim of this study was to investigate parameters of coagulation as well as platelets function in (GDPs) in Greece and assess possible correlations with splenectomy and treatment

Methods: We studied 29 adult patients (14 males -15 females) with (GD), 27 with type I GD, and two patients with type III GD. 28 patients were under treatment with ERT. 10 of 29 patients had undergone splenectomy. All the patients were assessed with: Classic biochemistry and virilogy tests (HBV, HCV, HIV). Routine coagulation tests (PT, INR, aPTT) and fibrogen, D-Dimers, FVIII, vWF, Protein S, Protein C, ATIII, PAI (which were analysed with automatic coagulation analyser Sysmex Siemens). Platelets function (which was assessed with PFA-100 (COL, EPI, ADP). Global haemostatic potential (which was assessed with ROTEM® Tromboelastometry (NATEM) and the following parameters were recorded (CT, CFF, MCF, ML60 a-ANCKLE). Plasma chitotriosidase activity (CT) was also measured (as marker of disease activity) and ultrasound of the liver and spleen was performed, bleeding history and use of antiplatelets, NSAIDs history were recorded. The statistical analysis was performed by the SAS for Windows 9.4 software platform (SAS Institute Inc., NC, USA).

Results: All the patients presented normal hepatic biology. None of the patients took antiplatelets, NSAIDs. 10 of 29 patients had undergone splenectomy. 23 out of 29 patients had history of bleeding. 16 of 29 patients (55.2%) had abnormal PFA EPI. 22 of 29 patients (75.9%) had abnormal PFA ADP and 15 of 29 patients (51.7%) had both ADP and EPI abnormal but there was no difference between splenectomised and non splenectomised. The majority of the patients mentioned that there was improvement of the bleeding problems after splenectomy and treatment with ERT. 27 of 29 patients (93.1%) had normal values in routine clotting testing, fibrogen, protein S, protein C, AT III, with no significant differences between splenectomised and non splenectomised ones. Thromboelastometry parameters NATEM were within normal limits but patients with splenectomy had higher PLTS, p shorter CFT p, higher a angle p, higher MCF p and higher VIII p. The results of measurements are reported in Table 1. Chitotriosidase levels (as marker of disease activity) were elevated in both cohorts.

Table 1. Results of comparison between splenectomized and non-splenectomized patients.



Summary/Conclusion: Patients had normal rotem parameters while they had abnormal PFA 100 (COL_EPI, COL_ADP). Patients who have undergone splenectomy were more hypercoagulable in terms of Rotem parameters.Splenectomy does not seem to modify platelets activation.Patients bleeding diathesis has ameliorated after splenectomy and initiation of ERT, the patients after many years with ERT therapy, they still had abnormal platelet function tests. Platelet function tests should be performed on all Gaucher patients prior to surgical procedures, or with an unexplained bleeding disorder while thromboelastography does not seem to be able to detect such disfunctions limiting its value for prior to surgery assessments

PS1045

THALIDOMIDE USE IN HEREDITARY HEMORRHAGIC TELANGIECTASIA: A RETROSPECTIVE EVALUATION OF THE PATIENTS

M. Baysal[1,*], E. Umit[1], H.O. Kırkızlar[1], A.C. Özdöver[1], M. Demir[1]

[1]*Hematology, Trakya University Faculty of Medicine, Edirne, Turkey*

Background: Hereditary hemorrhagic telangiectasia (HHT) is a rare, autosomal dominant disorder characterized by telangiectasia and arteriovenous malformations (AVM) skin, mucosa and in internal organs such as liver and lungs. Its prevalence is estimated to be between 1/5000 and 1 / 10,000. Recurrent epistaxis due to nasal telangiectasia is the most common finding.HHT diagnosis is made clinically by Curaçao criteria. The presence of 3 of the 4 Curaçao criteria was enough for the diagnosis

Aims: Thalidomide is an immunmodulator drug commonly used in plasma cell disorders and especially in multiple myeloma. Angiogenesis and new vessel formation play a role in the pathophysiology of hereditary hemorrhagic telangiectasia It has been suggested that thalidomide may be effective in the treatment of HHT due to antiangiogenic effect.The efficacy of thalidomide is reported in small number of case series and phase two studies. Based on this knowledge we tried to present our experience and findings in this rare disorder

Methods: Data of six patients diagnosed with HHT according to the Curaçao criteria were recorded from their files in a retrospective manner. All patients were refractory to the local invasive procedures and cauterization.IBM SPSS V20 was used for statistical analysis. Descriptive analysis was used for all parameters.Patients were started to treat with thalidomide 50 mg daily. Thalidomide dose raised to the 100 mg according to the response.Epistaxis severity score which was interpreted and validated by Boag et.al. was used in the study. Besides these parameters and measurements; 36-Item Short Form Health Survey questionnaire (SF-36) was used for quality of life assessment before and after treatment.

Results: Six patients treated with thalidomide included in our study. Three patients were male and three were female. Mean age was 60.50±10,07 years (44-74). The mean ESS before treatment was 7,40±2,02 (4,31-9,66) and after treatment was 3,35±1,53 (0,92-4,94). One patient reported grade one dizziness and one patient reported grade one nausea which were resolved spontaneously. No other specific side effects were reported during treatment period. All SF-36 item scores were found to be increased after the treatment which may be regarded as improvement of quality of life with thalidomide treatment

Summary/Conclusion: HHT patients mostly suffer from epistaxis episodes. Epistaxis episodes brings a burden and reduces health related quality of life in HHT patients. Thus reducing bleeding symptoms and epistaxis episodes is an important goal and treatment evaluation tool. Hoag et. al. designed and validated. Epistaxis severity score (ESS) for HHT patients. ESS is an objective symptom assessment form for clinicians. Besides HTT patients frequently apply to emergency services with bleeding. Because of bleeding episodes, HHT patients are usually anemic and often transfused with erythrocyte suspensions or treated with iron supplements. Chronic nature of the disease may lead inability to search medical help for the symptoms of the patients. Thalidomide has promising results in HHT patients and there are many roads to go diagnosis and treatment of HTT and better edited, comprehensive studies are needed.

PS1046

DIAGNOSIS AND MANAGEMENT OF ACQUIRED VON WILLEBRAND DISEASE. A SINGLE CENTER EXPERIENCE

C. Santoro[1,*], A. Ferretti[1], E. Baldacci[1], R. Abbruzzese[1], A. Bruzzese[1], A. Serrao[1], F. Barone[1], R.A. Bochicchio[1], G. Maestrini[1], A. Chistolini[1], R. Foà[1], M.G. Mazzucconi[1]

[1]*Hematology, Department of Cellular Biotechnology and Hematology, Sapienza University, Rome, Italy*

Background: Acquired Von Willebrand syndrome (AVWS) is a rare acquired coagulopathy, often associated with an underlying disorder (lymphoproliferative, myeloproliferative, cardiovascular, neoplastic and autoimmune disorders). The diagnosis is not easy and relies on a negative familial and personal clinical hemorrhagic history and a late onset in life of bleeding symptoms, associated with a laboratory pattern for Von Willebrand Disease (VWD).

Aims: Aim of our study is to describe the experience in the diagnosis and management of AVWS patients (pts) at a single Italian hematologic center.

Methods: Between 2004-2017 we have diagnosed and managed 8 pts [5F, 3M; median age 62.4 years (50.4-81.6)] affected by AVWS. The diagnosis was made on the basis of clinical and laboratory features suggestive of AVWS. The screening for VWF:RCo inhibitor was made utilizing mixing studies.

Results: Reasons for diagnosis were: recent onset of bleeding symptoms in 5 pts, increased aPTT in 3. Five subjects received a diagnosis of AVWS type 2A, 1 of type 1 and 2 of type 3 [median VWF:Ag 15%, range 1.6-51% (n.v. blood group 0: 41-101%; non group 0: 50-130%); median VWF:RCo 5.65%, range <6.25-28% (n.v. blood group 0: 41-97%; non group 0: 52-124%); median FVIII:C 18.25%, range 2.1-53% (n.v. 58-130%)]. The pres-

ence of a VWF:RCo inhibitor was investigated in 4 pts: 3 were negative, 1 positive. All pts did not have a family or past personal history of bleeding disorders/symptoms. The spontaneous hemorrhagic events presented at diagnosis and during the follow-up were: GI and gum bleeding, muscle hematomas, ecchymoses, epistaxis. Moreover, 3 pts bled after dental extractions/procedures, although being prophylactically treated with VWF/FVIII concentrate. Desmopressin, tranexamic acid, VWF/FVIII concentrate and recombinant FVIII were used as prophylaxis or treatment of bleedings in all pts, with variable responses on clinical symptoms. Seven/8 cases showed a concomitant lymphoproliferative disorder: 1 gastric B-cell MALT lymphoma, 1 indolent B-cell lymphoma, 4 MGUS, 1 MGUS progressed to Waldenstrom's disease (WD). In one case, AVWS was idiopathic. The management of the lymphoproliferative disorders was as follows: the patient with gastric B cell MALT lymphoma was treated with rituximab, obtaining a complete remission (CR) of both the lymphoma and the AVWS. The patient with WD was treated first with R-CVP and then with Ibrutinib: she presents a stable lymphoproliferative disease and persistent AVWS. A watch and wait strategy was chosen for the patient with the indolent B-cell lymphoma. Strategies to manage AVWS in pts with a concomitant MGUS were: prednisone (PDN) + cyclophosphamide (CTX) and then high dose immunoglobulins (HDIg) in one patient, without obtaining a response to both therapies; infusion of HDIg in one other case with a transient CR of the VW disease laboratory parameters; no specific treatment in the other 2 MGUS cases. The idiopathic AVWS case was treated with PDN and obtained a CR of the VW disease laboratory parameters; he relapsed and was treated with CTX and PDN, obtaining a second CR.
Summary/Conclusion: AVWS is a rare syndrome, probably underdiagnosed because unrecognized. The rarity of the disease and the lack of specific assays are limiting factors. AVWS should be considered whenever a patient presents with a late onset in life of bleeding symptoms associated to laboratory characteristics compatible with VWD. It is mandatory to search for concomitant diseases.

PS1047

CONGENITAL FXIII DEFICIENCY IN PAKISTAN

M. Borhany[1,*], N. Fatima[2], M. Abid[2], T. Shamsi[2], V. Schroeder[3], H.-P. Kohler[3]

[1]Haematology and Blood Bank, [2]National Institute of Blood Disease and Bone Marrow Transplantation, Karachi, Pakistan, [3]Department for BioMedical Research,University of Bern, Bern, Switzerland

Background: Factor XIII (FXIII) deficiency is a rare bleeding disorder (RBD) with an incidence of about one in 1–2.5 million and its incidence is higher in populations with consanguineous marriages.
Aims: The aim of this study was to characterize patients and relatives from sixteen families with suspected FXIII deficiency from Pakistan and to identify the clinical characteristics and underlying mutations.
Methods: FXIII deficient patients, enrolled at National Institute of Blood diseases and Bone Marrow Transplantation were included in the study. The patients' medical histories were recorded in a questionnaire. As a first indicator of FXIII deficiency, a 5M urea clot solubility test was used. Plasma FXIII A- and B-subunit antigen levels were determined by ELISA. FXIII activity was measured with an incorporation assay. Sequencing of all exons and intron/exon boundaries of F13A was performed.
Results: We have analyzed 16 families in which 32 (female 18 and 14 males) were severe FXIII deficient with FXIII level <1%. 19 first-degree relatives with mean FXIII level 71.19±21.1 are asymptomatic. Each family had a history of consanguineous marriages except one. 50% had significant family history of bleeding. Age at first presentation ranged from birth to 18 years. In these patients; we identified 23 mutations which includes 19 missense mutations, 2 Splicing mutations and 2-nonsense mutations with 7 novel mutations. Main clinical manifestations were bleeding after injury (78%), umbilical cord bleeding (57%), intracranial bleed (43%), hematoma, bruises (39%), circumcision (35%), in female's abortions and menorrhagia (38%). Fresh frozen plasma / cryoprecipitate were used in the management of most patients and for weekly prophylaxis in 8 patients with grade III bleeding.
Summary/Conclusion: We have analyzed a cohort of 51 individuals from 16 families in which 32 were severe FXIII deficient (homozygous or compound heterozygous) and remaining were FXIII deficient carriers (heterozygous). We identified 23 mutations in these families leading to congenital FXIII deficiency. Diagnosis of FXIII deficiency should be made on time so that prophylaxis can be initiated immediately to prevent fatal bleeding and for genetic counseling.

PS1048

DISSEMINATED INTRAVASCULAR COAGULATION SCORING IN CHILDREN WITH ACUTE LEUKEMIA: IS THERE A SIMPLER AND ECONOMICAL ALTERNATIVE?

Y. El Chazli[1,*], H. Hassab[1], M. Salama[1], W. El Gendy[2]

[1]Pediatric Hematology/Oncology, Faculty of Medicine, Alexandria University, [2]Clinical and chemical pathology, Alexandria University, Alexandria, Egypt

Background: Coagulation abnormalities and disseminated intravascular coagulation (DIC) have been extensively studied in acute promyelocytic leukemia, but only a limited number of studies addressed this issue in children with other types of acute leukemia.
Aims: To detect coagulation abnormalities and DIC by using International Society on Thrombosis and Haemostasis (ISTH) scoring system in children with acute leukemia.
Methods: A diagnostic test accuracy study including children with acute leukemia (lymphoblastic and myeloid except the promyelocytic subtype) admitted to the Hematology-Oncology Unit at Alexandria University Children's Hospital, either at first diagnosis (before starting chemotherapy) or when presenting with systemic inflammatory response criteria (SIRS)/sepsis during chemotherapy. Patients were consecutively recruited between October 2016 and March 2017, after obtaining an informed consent. Prothrombin time (PT), D-dimer, fibrinogen, antithrombin III and protein C levels were assessed and the ISTH scoring system for overt and non-overt DIC was calculated. The patients were followed until disappearance of blasts from periphery or resolution of SIRS/sepsis.
Results: 25 patients were enrolled at first diagnosis, and 25 patients when presenting with SIRS/sepsis. Their median age was 47 months (range: 17 to 149 months). Forty-one patients had ALL and nine had AML. At baseline assessment, 32% of patients had a positive overt DIC score, with a score ranging from 0 to 7 and a median of 4, while 50% had a positive non-overt score (median of 4, range: -2 to 9). At baseline, the median platelet count, D-dimer, and protein C values were abnormal, while the median PT, fibrinogen and antithrombin III were within normal range; the same pattern was found when analyzing these parameters over the whole study period. A significantly higher percentage of patients had bleeding among the DIC positive group compared to DIC negative one (p=0.003), and the former group received significantly more transfusions (packed red cells, platelets, and plasma). By using receiver operating curve (ROC) analysis, platelet count, PT, D-dimer level, as well as fibrinogen level were found to be statistically significant discriminators of occurrence of overt DIC in children with acute leukemia. Interestingly, the PT diagnostic criterion using Youden index was 15 sec with a sensitivity of 47.62%, specificity of 82.22%, and ≤175 mg/dL for fibrinogen with a sensitivity of 67.21%, specificity of 71.21%. The platelet count & D-dimer were the best discriminators of overt DIC in newly diagnosed acute leukemia patients, while platelet count and protein C were best in patients with SIRS/sepsis. Looking at non-overt DIC score, there was a statistically significant moderate rate of specific agreement between non-overt DIC score calculated using major criteria only (the global coagulation tests and their trend) and non-overt DIC score using major and specific criteria (AT III and PC levels are added), with a Kappa of 0.436, p=0.000, and a high rate of negative agreement (83.2%).
Summary/Conclusion: These results indicate that using a lower cut-off for PT, a higher one for fibrinogen and adding more weight to a lower platelet count in the calculation of the overt DIC score might be indicated in children with acute leukemia. A revised non-overt DIC score using only the global coagulation tests and their trend over time would be a simpler and interesting alternative to the use of more expensive specific coagulation tests in resource-limited countries.

PS1049

EFFECT OF THROMBOELASTOGRAPHY DEFINED COAGULOPATHY IN TRAUMATIC BRAIN INJURY

D. De[1,*], D. Suvro Sankha[1], S. Das[2], S. Halder[2]

[1]Dept of Hematology, [2]the Mission Hospital, Kolkata India

Background: The presence of coagulopathy is common after severe trauma. It is associated with increased morbidities, increased transfusion requirement and mortality.
Aims: The aim of this study was to identify presence of coagulopathy in isolated traumatic brain injury, and its association with outcome.
Methods: This is a prospective observational study, with a cohort of patients admitted to neuro ICU with traumatic brain injury were evaluated. Conventional coagulation tests and Thomboelastography were done within first

24 hour of admission to identify coagulation status. The cohort is divided in 3 groups of hypercoaguable, normal, or hypocoaguable according to TEG report and the groups are compared. The SAPS II score and mortality rates were compared between the groups.
Results: Total 112 patients were included in the study during the period of December 2015 to November 2016. The overall mean age of the cohort was 58.65 years, and 80.4% were male. The overall mortality was 32.1%. by using TEG, about 44.64% patients showed hypercoaguability whereas hypocoaguable status was found in 4.46% patients. The mean age, sex ratio and other baseline parameters were comparable. The sensitivity of diagnosis of coagulopathy by conventional tests were 80.7%, but specificity was only 8.9%. Hypercoagulation status could not be picked up by conventional coagulation tests. However, the accuracy of diagnosing hypocoagulation status was 88.39% by conventional coagulation tests. The SAPS II score and mortality rates were comparable between hypercoaguable and normal hemostasis group (SAPS II score 43.66 and 42.46 respectively, p value >0.05, mortality rate were 32% and 28.1% respectively, p value >0.05) but hypocoaguable group showed significant higher SAPS II score and higher mortality compared to normal hemostasis (SAPS II score 60.0 and 42.46 respectively, p value 0.002, mortality rate were 80% and 28.1% respectively, p value 0.034) (Figure 1).

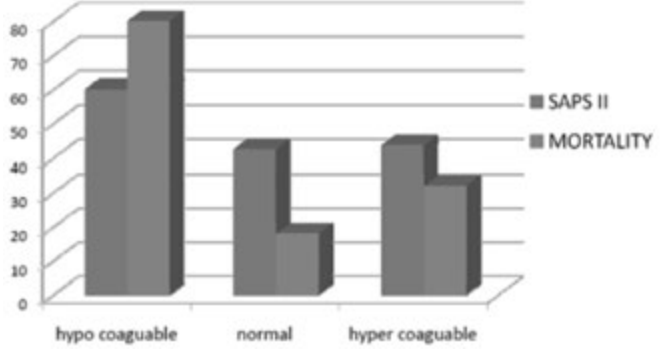


Figure 1.

Summary/Conclusion: Traumatic brain injury is often associated with coagulopathy. Thromboelastography is more reliable for diagnosing coagulopathy in these settings. The hypocoaguability is associated with higher SAPS II score and higher mortality rates.

Bone marrow failure syndromes incl. PNH – Biology & Translational Research

PS1050

ASSESSMENT AT DIAGNOSIS OF 200 PATIENTS WITH A PNH CLONE OR GPI-DEFICIENT CELLS ≥0.01% ENROLLED IN A NATION-WIDE MULTICENTRIC PROSPECTIVE OBSERVATIONAL STUDY

M. Le Garff-Tavernier[1,*], B. Drenou[2], A. Debliquis[2], R. Letestu[3], B. Chatelain[4], T. Boyer[5], J.-P. Vial[6], M. Peres[7], V. Harrivel[8], G. Socié[9], R. Peffault de Latour[9], O. Wagner-Ballon[10]

[1]Service d'Hématologie Biologique, APHP Groupe Hospitalier Pitié-Salpêtrière-Charles Foix, Paris, [2]Laboratoire d'Hématologie, Groupe Hospitalier de la Région Mulhouse Sud Alsace, Mulhouse, [3]Laboratoire d'Hématologie, APHP CHU Avicenne, Hôpitaux Universitaires de Seine-Saint-Denis, Bobigny, France, [4]Laboratoire d'Hématologie, CHU UCL Namur, Yvoir, Belgium, [5]Laboratoire d'Hématologie, CHU Lille, Lille, [6]Laboratoire d'Hématologie, CHU Bordeaux, Bordeaux, [7]Laboratoire d'Hématologie, CHU Toulouse, Toulouse, [8]Centre de Biologie Humaine - Laboratoire d'Hématologie, CHU Amiens Sud, Amiens, [9]Service d'Hématologie Clinique, APHP Hôpital universitaire Saint Louis, Paris, [10]Département d'hématologie et immunologie biologiques, APHP Hôpitaux universitaires Henri Mondor, Créteil, France

Background: The high frequency of minor PNH clones <1% highlights the use of high-resolution flow cytometry (FCM). Thus, the International Guidelines (Borowitz, 2010) were spread throughout French-speaking countries by a biological workshop, which allowed the detection of PNH cells with a 0.01% sensitivity in a multicenter approach. This diagnostic practice harmonization has been pursued through an inter-laboratory comparison program, involving more than 50 French-speaking centers since 2013 (Debliquis, 2015).
Aims: Our HPN^{AFC} group initiated a nation-wide multicenter prospective observational study on February 29th 2016, in order to collect all PNH cases ≥0.01% and notably to monitor the evolution of minor clones <1% during 5 years.
Methods: All patients with a PNH clone or GPI-deficient cells ≥0.01%, newly- or previously-diagnosed, detected from February 2016 until March 2021 in France are included in this Observatory, irrespective of age, provided that the center has validated a PNH FCM quality control. For each patient, the baseline assessment always corresponds to the initial PNH clone detection, even if it occurred before the Observatory opening. Thus, this strategy will allow the collection of all cases in the active French PNH file. Inclusions are made by the referent cytometrist of each center by filling in the e-CRF form available on the HPN^{AFC} website with clinical and biological data. This study was approved by the national research ethics board.
Results: As of January, 31th 2018, 40 cytometry laboratories have registered across France and 28 centers have submitted 265 inclusion points with a PNH clone or GPI-deficient cells ≥ 0.01%. All the cases have been reviewed by the 2 principal investigators, who carefully re-examined FCM data. This method enabled the validation of 200 cases, with 65 ongoing ones due to missing clinical or biological data. The patient median age at diagnosis was 46 years [10-87] with 14 pediatric cases (<18y) and a sex ratio of 0.90. Diagnoses were made between 1988 and 2018 with clinical information available in 93%. Out of these 186 patients, 28 (15%) had hemolytic anemia and more than half of them had aplastic anemia (n=119, 64%). Other clinical manifestations included 29 unexplained cytopenias (16%), 10 myelodysplastic syndromes (5%), 8 cases of hemoglobinuria (4%) and 6 rare cases of recurrent thrombosis (3%). Biological data showed a median hemoglobin value of 9.3 g/dL [4.1-16.5], 1.4 x10^9/L [0.1-11] neutrophils and 57 x10^9/L [1-360] platelets. The median clone size at diagnosis was 1.6% on neutrophils with a very wide range [0- 99.4], almost half of them being less than 1% and mainly composed of type III cells. The median clone size was slightly higher on monocytes (2.4% [0-99.6]) with a similar distribution of type III and type II cells. RBC analysis, performed for half of the patients, showed a majority of minor clones and no correlation was observed between PNH clone size on neutrophils and RBC. The median PNH clone size on neutrophils in case of aplastic anemia was 0.88%, yet some patients had very large clones ≥90%. Conversely, PNH clones from patients with hemolytic anemia were very large with a median size of 83.6%, but some patients had smaller clones of less than 20%.
Summary/Conclusion: Almost two years after its initiation, 200 confirmed patients have been yet enrolled in this National Observatory. A comprehensive follow-up of a foreseeable larger number of PNH clones (so far 115 out of the 200 already have at least 1 follow up point) will lead to better understand the minor PNH clone evolution.

PS1051

ASSOCIATION OF AUTOPHAGY-RELATED GENE 5 VARIANTS WITH ACQUIRED APLASTIC ANEMIA IN HAN-CHINESE POPULATION

Y. You[1,*], J. Huo[1]

[1]State Key Laboratory of Experimental Hematology, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Science & Peking Union Medical College, Tianjin, China

Background: Acquired aplastic anemia (AA) is a life-threatening bone marrow failure syndrome characterized by hypocellular bone marrow and pancytopenia peripheral blood. It is beyond controversy that immune-mediated quantitative and qualitative defects of hematopoietic stem/progenitor cells (HSPCs) plays an vital role in the pathophysiology of acquired AA. While, Autophagy is closely related to T-cell pathophysiology and the destiny of HSPCs, and besides, autophagy-related gene 5 (ATG5) is a crucial autophagy gene and has emerged as a spotlight in a multitude of immune functions.
Aims: Considering the essential role of autophagy,we hypothesized that genetic variants in ATG5 might contribute to acquired AA.
Methods: Via the approach of Sequenom Massarray system we have studied six ATG5 polymorphisms in a Chinese cohort of 176 patients with acquired AA and compared with 157 healthy controls.
Results: We observed a lower risk of acquired AA in the recessive model of rs510432 and rs803360 polymorphisms (adjusted OR[95% CI]=0.467[0.236-0.924], p=0.029 for ATG5: rs 510432; adjusted OR[95% CI]=0.499[0.255-0.975], p=0.042 for ATG5: rs 803360). Further more, the decreased risk was even more pronounced among non-severe AA (NSAA) compared with health controls (adjusted OR[95% CI]=0.356[0.141-0.901], p=0.029 for ATG5: rs 510432; adjusted OR[95% CI]=0.348[0.138-0.878], p=0.025 for ATG5: rs 803360; adjusted OR[95% CI]=0.352[0.139-0.891], p=0.027 for ATG5: rs 473543). Moreover, rs573775 can strongly predict the disease evolution and recurrence in patients with acquired AA (Figure 1).

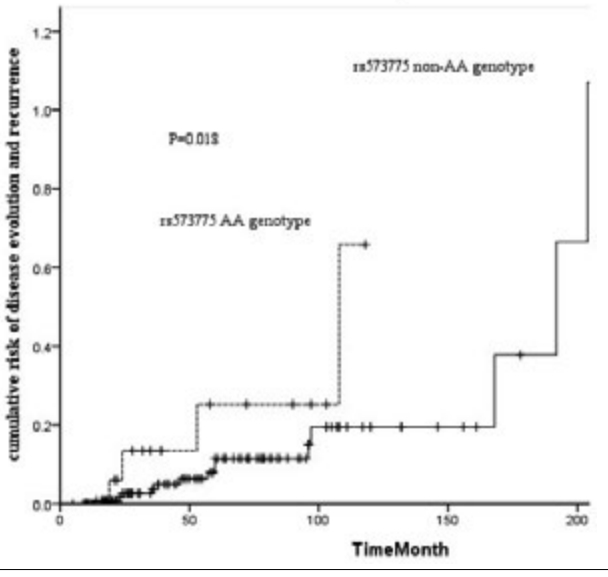


Figure 1.

Summary/Conclusion: Our results indicated that ATG5 variants are associated with acquired AA patients, which may facilitate further clarifying the autoimmune mechanisms and making the patients-tailored medical decisions.

Bone marrow failure syndromes incl. PNH – Clinical

PS1052

PHASE IB STUDY OF THE CXCR4 ANTAGONIST BL8040 COMBINED WITH IMMUNOSUPPRESSIVE THERAPY IN PATIENTS WITH APLASTIC ANEMIA

T. Kadia[1,*], G. Borthakur[1], N. Jain[1], N. Pemmaraju[1], K. Takahashi[1], K. Dwyer[2], J. Khoury[3], A. Vainstein[4], C. Bivins[1], S. Dara[1], L. Xiao[5], F. Ravandi[1], G. Garcia-Manero[1], H. Kantarjian[1]

[1]Leukemia, [2]Stem Cell Transplant Research, [3]Hematopathology, MD Anderson Cancer Center, Houston, United States, [4]BiolineRx, Modi'in, Israel, [5]Biostatistics, MD Anderson Cancer Center, Houston, United States

Background: Aplastic anemia (AA) is a bone marrow (BM) failure syndrome characterized by a severe depletion of hematopoietic stem and progenitor cells (HSPCs) in the BM leading to cytopenias. Most cases of acquired AA are the result of destruction of the HSPCs by autoreactive cytotoxic T-cells. This is mediated through immunosuppressive cytokines as well as perforin/granzyme-mediated apoptosis, requiring close proximity in the microenvironment. CXCR4 expression on pathogenic T-cells in AA facilitate their homing to the BM (Arieta Kuksin Blood 2015). Blocking the CXCR4 signaling axis in pathogenic T-cells can disrupt their homing to the BM may improve responses when combined with immunosuppressive therapy (IST). BL8040 is a CXCR4 antagonist that also has effects in mobilizing and increasing the numbers of HSPCs.
Aims: We designed a pilot phase Ib study evaluating the feasibility, safety, and efficacy of BL8040 combined with IST for pts with AA or hypoplastic myelodysplastic syndrome (hMDS).
Methods: Pts ≥ 18 years, with adequate organ function, with a diagnosis of severe AA or hMDS, including those with relapsed (not refractory) AA were eligible. Pts were treated with BL8040 0.75 mg/kg SQ on D1-10, hATG 40 mg/kg/day (or 35 mg/kg/day for age ≥ 55 yrs) IV on D11-14, methylprednisone 1mg/kg/day IV daily on D11-14, followed by PO prednisone tapered off over 1 month, and cyclosporine (5 mg/kg) PO daily for up to 6 months. BL8040 (0.75 mg/kg) was continued on D1-5 of every month start with month 2. BM was performed at baseline, day 10, month 3 and month 6. Blood (PB) and BM samples were collected during treatment to explore the dynamics of immune cell and HSPC mobilization.
Results: Eight pts have been treated, with a median age of 70 years (range, 23-75). Three pts had no prior IST, 4 pts had relapsed AA, and 1 pt had hypoplastic MDS. Table 1 summarizes baseline characteristics. Six pts (75%) had diploid karyotype, while 2 had insufficient metaphases. At baseline 1 pt had mutations in TET2 and JAK2, and 1 pt had a DNMT3a mutation by next-generation sequencing. 4 pts have completed at least 5 months of therapy, 1 is too early in the treatment course, and 3 pts came off study early due to allergic reactions: 2 pts had grade 2 allergic reactions to the first doses of BL8040 and came off study, while 1 pt was noted to have an allergic reaction to the hATG skin test. Steroid premedication in subsequent pts mitigated BL8040-associated allergic reaction. 4 pts are evaluable for response with 2 CRs. Besides allergic reactions, the regimen was well tolerated. There were no grade ≥ 3 related AEs. Most common AEs possibly related to therapy were injection site pain, headaches, chills, and hot flashes. Quantification of T-cell subsets in the PB and BM by flow cytometry was available in 5 pts so far at 2-4 time points during therapy. On treatment, 3 pts had a notable relative increase in T-regulatory cells in the BM; there was a general trend for decrease in CD4+/CD45+ central memory T-cells, and increase in CD4+/CD45+ effector memory and CD4+/CD45+ TEMRA T-cells in the BM and PB; and there was a trend towards increased CD45+/CD8+ T-cells in the PB. In almost all cases, there was a significant decline in CXCR4 expression across T-cell subsets in BM and PB.

Table 1.

Characteristic	Median (range) or N [%]
Age	70 (23 - 75)
WBC [x10^9/L]	2.9 (1.4 – 6.9)
Platelets [x10^9/L]	20 (1 - 37)
Total Bilirubin	0.95 (0.5 – 3)
Creatinine	0.79 (0.74 – 0.97)
PNH Clone Detected	2 [25%]
T-cell Receptor Clonality	6 [75%]

Summary/Conclusion: Adding BL8040 to IST appears to be safe and feasible in patients with AA. Encouraging responses are seen with good tolerability. Preliminary analysis shows increasing BM T-regs and decreased CXCR4 expression after treatment. Complete profiling of the immune repertoire and HSPCs of pts will be summarized.

PS1053

CLINICAL EFFICACY OF THERAPIES FOR TRANSFUSION-DEPENDENT NON-SEVERE APLASTIC ANEMIA: A RETROSPECTIVE COHORT STUDY IN MULTIPLE HOSPITALS

S. Lin[1], Y. Yang[2], H. Yin[3], C. Huang[1], J. Chen[1], L. Wu[1], J. Shen[1], B. Ye[1], Z. Hu[1], Y. Shen[1], Z. Zheng[1], W. Shen[3], X. Wu[3], X. Lu[3], G. He[3,*], J. Li[3]

[1]Hematology, The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, [2]Hematology, The First Hospital of Jilin University, Changchun, [3]Hematology, The First Affiliated Hospital of Nanjing Medical University, Jiangsu Provincial People's Hospital in Nanjing, Key Laboratory of Hematology of Nanjing Medical University, Collaborative Innovation Center for Cancer Personalize, Nanjing, China

Background: Aplastic anemia (AA) is a bone marrow hypoplasia disease caused by multiple reasons. Its symptoms include failure of hematopoiesis and decrease in white blood cells, hemoglobin and platelets, with bleeding and infection at the same time. Based on the results of peripheral blood and bone marrow test, AA could be classified as severe AA (SAA) and non-severe AA (NSAA). Some patients with NSAA have a lower level of hemoglobin or platelets, and require regular blood transfusion, which have influenced their daily life. This condition was defined as transfusion-dependent NSAA (TD-NSAA). These patients' long-term transfusions and decreased immunity make them susceptible to different infections. In addition, the side-effects of drugs can also cause heavy burden to patients physically, psychologically, and economically. Without treatment, TD-NSAA can easily progress to SAA which have little response to drug therapies. Therefore, it is clinically important to study the early interventions for TD-NSAA.
Aims: This study aims to evaluate the clinical efficacy and prognosis of currently popular treatments for patients with transfusion-dependent non-severe aplastic anemia (TD-NSAA)
Methods: Medical records of patients with TD-NSAA were collected from three hospitals and divided into four groups based on their treatment. Treatment efficacy was evaluated at 6 months after treatment, with subgroup analysis based on patients' disease courses. Adverse events and 3-year survival status were also reviewed.
Results: A total of 175 patients' records were collected in this study. The analysis of treatment efficacy indicated that the most effective treatment was allo-HSCT with the response rate of 84.62%, which was followed by ATG + CsA (68.52%), CsA + androgen (26.92%), and androgen (0%), respectively. The subgroup analysis showed that for patients with less than 6-month disease courses, ATG + CsA treatment could achieve similar response rate as allo-HSCT (84.00%). Besides, ATG + CsA showed better efficacy than CsA + androgen in all stratified arms ($p<0.05$). Three-year event-free survival rates were higher in patients treated with ATG + CsA (83.3%) than with CsA + androgen (64.4%) ($p=0.013$) (Figure 1).

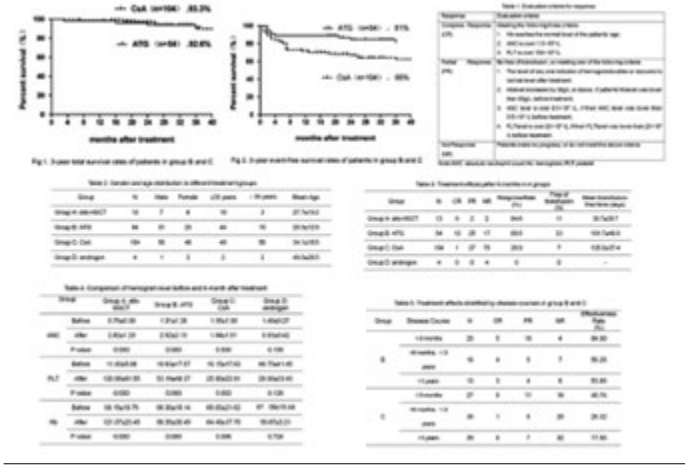


Figure 1.

Summary/Conclusion: In conclusion, TD-NSAA is clinically complicated, and its choice of treatment differs in considering the patients' age, disease courses, dependence on transfusion, socioeconomic status, and life expectation. At present, immunosuppression therapy has become the main choice of treatment. However, for patients with TD-NSAA for a long time, allo-HSCT may achieve better effect than combined ATG+CsA treatment. Allo-HSCT pre-treatment plan will be a new field to be explored for clinicians.

PS1054

IDIOPATHIC NEUTROPENIA OF INFANCY: DATA FROM THE ITALIAN NEUTROPENIA REGISTRY

P. Farruggia[1,*], G. Puccio[1], F. Fioredda[2], D. Onofrillo[3], G. Russo[4], A. Barone[5], S. Bonanomi[6], G. Boscarol[7], F. Di Marco[1], A. Finocchi[8], J. Serafinelli[8], R. Ghilardi[9], P. Giordano[10], G. Alessandro[10], S. Ladogana[11], L. Luti[12], R. Mandaglio[13], N. Marra[14], B. Martire[15], E. Mastrodicasa[16], C. Mosa[1], M. Motta[4], L. D. Notarangelo[17], M. Pillon[18], L. Porretti[19], A. Trizzino[1], M. Veltroni[20], F. Tucci[20], A. Guarina[21], F. Verzegnassi[22], U. Ramenghi[23], C. Dufour[2]

[1]Pediatric Hematology and Oncology Unit, A.R.N.A.S. Ospedale Civico, Palermo, [2]Clinical and Experimental Unit, G. Gaslini Children's Hospital, Genova, [3]Pediatric Hematology and Oncology Unit, Department of Hematol, Spirito Santo Hospital, Roma, [4]Unit of Pediatric Hematology and Oncology, Policlinico, University of Catania, Catania, [5]Department of Pediatric Onco-hematology, University Hospital of Parma, Parma, [6]Fondazione MBBM, Clinica Pediatrica, Università di Milano - Bicocca, A.O. San Gerardo, Monza, [7]Department of Pediatrics, Central Teaching Hospital Bolzano, Bolzano, [8]Universita degli Studi di Roma Tor Vergata, Roma, [9]Department of Pediatrics, Ospedale Maggiore Policlinico IRCCS, Milano, [10]Department of Biomedical Sciences and Human Oncology, Pediatric Section, University "A. Moro" of Bari, Bari, [11]Department of Hematology, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, [12]Pediatric Hematology Oncology, Bone Marrow Transplant, Azienda Ospedaliero Universitaria Pisana, Ospedale S. Chiara, Pisa, [13]Pugliese-Ciaccio Hospital, Catanzaro, [14]A.O.R.N. Santobono Pausillipon, Napoli, [15]Dipartimento di Scienze e Chirurgia Pediatriche, U. O. Oncoematologia Pediatrica, Ospedale Policlinico-Giovanni XXIII, Bari, [16]Pediatric Oncology Hematology Unit, S. Maria Della Misericordia Hospital, Perugia, [17]Oncology-Haematology and Bone Marrow Transplantation Unit, Spedali Civili, Brescia, [18]Dipartimento di Oncoematologia Pediatrica, Università di Padova, Padova, [19]Flow Cytometry Service, Laboratory of Clinical Chemistry and Microbiology, IRCCS "Ca' Granda" Foundation, Maggiore Hospital Policlinico, Milano, [20]Department of Pediatric Onco-Hematology, Meyer Children's Hospital, Firenze, [21]Department of Sciences for Health Promotion and Mother and Child Care, University of Palermo, Palermo, [22]Institute for Maternal and Child Health (I.R.C.C.S) Burlo Garofolo, Trieste, [23]Ematologia, Dipartimento di Scienze Pediatriche, Ospedale Infantile Regina Margherita, Torino, Italy

Background: Neutropenia is characterized by a reduced Absolute Neutrophil Count (ANC): it is defined as mild (ANC=1.0-1.5x10^9/L), moderate (ANC=0.5-1.0x10^9/L) and severe (ANC<0.5x10^9/L). Autoimmune Neutropenia of Infancy (AIN) is characterized by low risk of bacterial infection, tendency to spontaneously resolve and typically occurrence at ≤ 4-5 years of age; it is due to auto-antibodies, most frequently searched through GIFT in the patient sera, whose detection is often difficult (high frequency of false negatives). In case of negativity of the 1th test and with still a clinical picture consistent with AIN, the Italian guidelines recommend repeating the test up to 4 or more times and, if all tests are negative, no maturational arrest is assessed in bone marrow (BM) and neutropenia is clearly independent on drug exposure or infections, the patients will be defined as affected by "idiopathic neutropenia of infancy" (IN), even though the vast majority presumably present an AIN and the definitive diagnosis will be reached only "ex post," namely after the spontaneous recovery.
Aims: To describe the characteristics of the disease at presentation and the outcomes of IN patients and to compare them to children affected by AIN.
Methods: We analyzed the features of 78 IN patients enrolled in the Italian neutropenia registry over a 16-year time-span: they were compared to 311 children affected by AIN enrolled in the same period.
Results: Main characteristics of the study populations are shown in table I. Both the cohorts are the largest ever described. The main differences between AIN and IN (Table 1) are age at onset (later in IN) and length of disease (longer in IN). Earlier age at onset ($p=0.000031$), male sex ($p=0.00303$), absence of leucopenia ($p=0.00825$), higher number of lymphocytes ($p=0.0013$) and absence of monocytosis ($p=0.0142$) are associated with a significantly earlier recovery in the AIN group: a multivariate analysis by a Cox model showed that only age at onset and absence of monocytosis are independently significant ($p=0.00091$ and $p=0.00084$ respectively). In the IN group only age at onset was significant at bivariate analysis ($p=0.011$). None of the factors analyzed in the Cox model was statistically associated with a higher burden of infection.

Table 1.

	AIN	IN	p
Sex (Male%)	56.6%	50%	**0.029**
Age at onset (years, median)	0.8	1.4	**0.000066**
Neutropenia diagnosed by chance	32.6%	61.4%	**0.0000072**
ANC (median) at onset	0.44 x 10^9/L	0.42 x 10^9/L	0.78
Neutropenia (severe/moderate/mild)	55.8%/36.1%/8.8%	57.7%/30.8%/11.5%	0.49
Leucocytes (median) at onset	6.1 x 10^9/L	6.08 x 10^9/L	0.59
Leucopenia at onset	37.5%	40.3%	0.66
Monocytes (median) at onset	0.63 x 10^9/L	0.6 x 10^9/L	0.73
Monocytosis at onset	20.4%	29%	0.12
Lymphocytes (median) at onset	4.4 x 10^9/L	4.3 x 10^9/L	0.16
Severe infections	12.5%	11.5%	0.80
G-CSF "on demand" therapy	8.1%	7.1%	0.90
BM performed	32.6%	49.4%	**0.0061**
Recovery in patients with ≥ 5 years FUP	86.6%	85.4%	0.619
Analysis restricted to recovered patients			
Age at onset (years, median)	0.70	1.22	**0.0002017**
Age at recovery (year, median)	2.11	3.05	**0.00003534**
Median length of disease	1.19	1.60	**0.004062**
Patients with disease length ≤ 24 months	68.6%	58.1%	0.108
Patients recovered at less than 5 years of age	86.9%	76.8%	0.06222

Summary/Conclusion: Our report confirms that IN and AIN are similar, benign and self-limiting conditions but ages of appearance and remission were found to be later in IN and some factors affecting earlier recovery in AIN seems to be not relevant in IN.

PS1055

DYNAMICS OF CMV AND EBV LOADS AFTER IMMUNOSUPPRESSIVE TREATMENT WITH RABBIT ATG AND CYCLOSPORINE IN ADULT APLASTIC ANEMIA: A PROSPECTIVE OBSERVATIONAL STUDY

S.-S. Park[1,*], G.J. Min[1], Y.-W. Jeon[1], J.-H. Yoon[1], S.-E. Lee[1], B.-S. Cho[1], K.-S. Eom[1], Y.-J. Kim[1], S. Lee[1], H.-J. Kim[1], C.-K. Min[1], S.-G. Cho[1], D.-W. Kim[1], J. W. Lee[1]

[1]Division of Hematology, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic Of

Background: When the cellular immune response is compromised in patients receiving immunosuppressive treatment (IST), both Cytomegalovirus (CMV) and Ebstein-Barr virus (EBV) can reactivate and cause clinical disease.
Aims: We aimed to identify the natural courses of CMV and EBV after IST with antithymocyte globulin (ATG) and cyclosporine A (CsA) for aplastic anemia (AA).
Methods: In this prospective study, CMV and EBV viral load of 99 adult patients with acquired AA who received rabbit ATG and CsA were analyzed. Each viral cohort was classified into two groups by the presence of viral load at the time of IST as follows: no CMV viral load at baseline (CMV-G1, n=98) and the presence of CMV at baseline (CMV-G2, n=1); No EBV viral load at baseline (EBV-G1, n=88) and the presence of EBV load at baseline (EBV-G2, n=11). Each viral data were collected at baseline, 1, 3, 6, and 12 months after ATG treatment.
Results: Median age and follow-up periods of cohort were 41 (18-75) years and 17.9 (1.1-71.2) months, respectively. In CMV-G1, CMV reactivation and CMV disease occurred in 41 (42.2%) and 4 (4.1%) patients, respectively. Dynamics of CMV-load tended to increase until 3 months, but were completely resolved at 6 and 12 months. Four (4.1%) patients developed CMV diseases after median 79 (45-138) days from ATG. One patient of CMV-G2 who presented 4.08 log of load at the time of IST had resolved state at 1 and 3 months without CMV treatment. Median CMV-load at the time of CMV disease was 5.61 (negative - 5.87) log. However, highest CMV load of 95 patients without CMV disease were not reached to 5.0 (negative-4.87) log. In EBV-G1, EBV reactivation and disease occurred in 54 (61.4%) and 1 patients (4.1%), respectively. EBV load peaked at 1 month and gradually decreased throughout 12 months, but remained until 12 months. Dynamics of EBV-G2 revealed fluctuated EBV-load without time point of a specific peak. None of EBV-G2 presented EBV disease during the follow-up periods. Individual median peak EBV-load was 4.48 (2.76-6.00) log. One patient with EBV associated lymphoproliferative disease (EBV-LD) showed EBV-loads of 7.06 and 5.52 log at 1 and 3 months, respectively. Median highest of EBV-load in 98 patients without EBV-LD was 2.99 (negative-6.72) log.
Summary/Conclusion: Reactivation of CMV and EBV was common after IST with rabbit ATG and CsA and showed different dynamics pattern. To predict the onset of each viral disease, it should be recommended to monitor CMV load for at least 3 months and EBV load for at least 1 month after IST, respectively. Cut-off for preemptive therapy might suggest 5 log of CMV-load and 7 log of EBV-load, respectively.

PS1056

PHASE 2 STUDY OF X4P-001: A TARGETED ORAL THERAPY FOR PATIENTS WITH WHIM SYNDROME

D. Dale[1,*], A.A. Bolyard[1], E. Dick[1], M. Kelley[1], V. Makaryan[1], R. Johnson[2], T. Ebrahim[2], S. Parasuraman[2]

[1]Medicine, University of Washington, Seattle, [2]X[4] Pharmaceuticals, Inc., Cambridge, United States

Background: WHIM syndrome (Warts, Hypogammaglobulinemia, Infections and Myelokathexis) is a rare immunodeficiency disorder caused by mutations in the CXCR4 chemokine receptor. Patients present with severe chronic neutropenia and lymphopenia due to aberrant retention of leukocytes in the bone marrow as a result of CXCR4 hyperactivation. There is no approved therapy for the treatment of patients with WHIM and current therapeutic options do not address the primary pathophysiology. X4P-001 is an oral, selective, small molecule, allosteric antagonist of CXCR4 signaling that is being evaluated as a targeted therapy for WHIM. It is hypothesized that X4P-001 will stimulate immune response by increasing neutrophil and lymphocyte mobilization from the bone marrow into the circulation and lead to improvement in various clinical symptoms of the disease.
Aims: We report preliminary results from Phase 2 of a Phase 2/3 study examining X4P-001 therapy in patients with WHIM syndrome. The primary study objectives are to evaluate safety and tolerability of X4P-001 in WHIM patients and determine the dose required to achieve a consistent increase in circulating neutrophils and lymphocytes for the Phase 3 portion of the study.
Methods: Patients have received increasing doses of X4P-001 from 50 to 300 mg once daily with monitoring of serial blood counts at weeks 5, 13, and 21. Intra-patient dose escalation decisions are based on 24-hour serial area-under-the-curve (AUC) measurements of absolute neutrophil counts (ANC) and absolute lymphocyte counts (ALC), with dose escalation proceeding if both measures fell below prespecified thresholds. Subsequent to the abstract data cutoff date, some patients were escalated to 400 mg/day, updated data will be presented.
Results: As of 22 January 2018, 6 patients (4 females, 2 males; 4 with R334X mutations, one each with E343X and S365X; age range 19 to 57 years) have been treated with X4P-001. The study entry screening WBC (mean±SEM) was 0.73×10^9/L±0.13, ANC was 0.11×10^9/L±0.03, and ALC was 0.54×10^9/L±0.13. All patients had low normal or mild mean baseline hypogammaglobulinemia: IgG 738±105, IgA 67±21, IgM 66±13. Five patients have escalated to 300 mg/day with total duration of exposure ranging from 86 to 348 days. One patient was discontinued soon after beginning X4P-001 due to grade 1 drug-related rash. All patients have demonstrated a dose-dependent increase in ANC and ALC from screening values, with ALC increasing in greater proportion than ANC. At the 300 mg dose level, the mean pre-dose WBC was 1.33×10^9/L±0.29, with peak levels of 3.45×10^9/L±0.69 (2.7-fold increase). The pre-dose ANC was 0.37×10^9/L±0.10, with peak levels of 0.72×10^9/L±0.18 (2.1-fold increase) and pre-dose ALC was 0.83×10^9/L±0.18, with peak levels of 2.6×10^9/L±0.56 (3.4-fold increase). No changes in immunoglobulin levels have been observed. In all dose levels tested, X4P-001 was well-tolerated with no serious adverse events (SAEs) reported. One patient developed cholecystitis requiring cholecystectomy in an out-patient setting; was maintained on X4P-001 and received G-CSF during the preoperative period without any reported AEs and recovered without sequelae.
Summary/Conclusion: Preliminary data from this ongoing study suggests treatment with X4P-001 in patients with WHIM syndrome is generally safe and well tolerated with no SAEs reported. Dose escalation continues, and updated safety and efficacy results will be presented.

PS1057

SCREENING FOR TELOMERE DISEASE IS IMPORTANT TO IMPROVE THE OUTCOME OF HSCT IN PEDIATRIC PATIENTS SUFFERING FROM SEVERE APLASTIC ANEMIA

I. Nederlof[1,*], D. Bresters[2], C. Lindemans[1], M. Bartels[1]

[1]Department of Pediatric Hematology and Stem Cell Transplantation, University Medical Centre Utrecht, Utrecht, [2]Willem-Alexander Children's Hospital, Department of Stem Cell Transplantation, Leiden University Medical Center, Leiden, Netherlands

Background: Telomeropathies comprise a complex spectrum of diseases

caused by shortened telomeres, resulting in bone marrow failure, and other organ disease, including lung fibrosis and liver cirrhosis. Patients with telomeropathies, of which dyskeratosis congenita (DC) is the best-known example, are at increased risk of hematopoietic stem cell transplantation (HSCT)-associated complications, predominantly significant organ damage following myeloablative conditioning regimen. In patients suffering from severe aplastic anemia (SAA), the preexistence of underlying inherited bone marrow failure syndromes (IBMFS), including Fanconi anemia (FA) and DC, should be considered. Screening for telomeropathies is of particular importance in young patients as the disorders encompass a wide spectrum and clinical presentation can therefore be subtle. Determining short telomere length in patients with SAA can strongly suggest an underlying telomeropathy. For these patients, adherence to specific HSCT protocols, e.g. adjusted treatment regimen and directed testing of potential sibling donors, could be indicated. A better understanding of the clinical presentation of telomeropathies is necessary to identify these patients before HSCT.
Aims: To identify clinical characteristics for patients with a telomeropathy and investigate outcome of allogeneic HSCT in these patients.
Methods: We investigated patients aged 0-18 years undergoing allogeneic HSCT at two paediatric HSCT departments between January 2009 and September 2017. All patients with SAA (n=29), refractory cytopenias of childhood (n=14) and a proven telomeropathy (n=8) were included. A telomeropathy was diagnosed based on short telomere length, characterized as a length below the first percentile corrected for age, in lymphocytes and granulocytes determined by FlowFISH. A retrospective analysis on clinical characteristics, chemical parameters, liver- or pulmonary disorder, complications and toxicity was performed for all patients (n=51) versus the subgroup with a proven telomeropathy (n=8). Mann-Whitney and Fisher's exact tests were used to analyze the differences between groups.
Results: Congenital physical anomalies were reported more frequently in the telomeropathy patients compared to all included HSCT patients ($p<0.05$). The subgroup with telomeropathies had significantly higher ASAT and ALAT ($p<0.01$) and gGT ($p<0.05$) compared to all patients. In line with this, previously reported liver dysfunction was more common in patients with a telomeropathy ($p<0.05$). Bilirubin, LDH levels, severity of thrombocytopenia or leukocytopenia were similar in both groups. As the lungs may also be affected in telomeropathies, we compared pre-SCT radiological abnormalities by HR-CT of the lungs, but found no difference. Post-HSCT, a higher occurrence of toxicity was reported in the telomeropathy patients compared to the entire group ($p<0.005$). Occurrence of acute graft versus host disease was not increased in patients with telomeropathies and mortality rates did not differ between groups. Increased use of adjusted conditioning regimens in the telomeropathy group might explain these similarities.
Summary/Conclusion: Significant overall HSCT-toxicity can be a consequence of underlying telomere disease. Therefore, in patients with congenital physical anomalies and abnormal liver enzymes prior to HSCT, an underlying telomeropathy should be considered. Moreover, since clinical symptoms in patients with telomeropathies can be subtle, routinely testing of telomere length can help to identify patients that have an increased risk for HSCT-associated complications.

PS1058

RESULTS OF HEMATOPOIETIC STEM CELLS TRANSPLANTATION IN CONGENITAL BONE MARROW FAILURE SYNDROMES: SINGLE CENTER EXPERIENCE WITH FOCUS ON TCR AB+/CD19+ DEPLETION

Z. Shekhovtsova[1,*], L. Shelikhova[1], D. Balashov[1], D. Shasheleva[1], M. Persianceva[1], A. Maschan[1], M. Maschan[1]

[1]Dmitry Rogachev Federal Research Centre Of Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation

Background: To date allogeneic hematopoietic stem cells transplantation (HSCT) still remains the only curative option for patients (pts) with congenital bone marrow failure (BMF).
Aims: Safe and effective measures to prevent graft-versus-host disease (GvHD) are needed to diminish the impact of transplant-related complications.
Methods: A total of 35 pediatric pts with congenital BMF (13 f/22 m, median age 9 years, range (0,9-16) underwent allogeneic HSCT between May 2012 and December 2017. The cohort included 17 pts with Fanconi anaemia (FA), 8 with dyskeratosis congenita (DC), 4 with congenital amegakaryocytic thrombocytopenia (Amega), 3 with Diamond-Blackfan anaemia (DBA) and 3 cases of undefined constitutional form of BMF. Twenty-four pts received grafts from HLA-matched unrelated volunteer: 22 - after TCR αβ+/CD19+ depletion (abTCD) of PBSC, 2 – bone marrow (BM) without graft processing; 8 - a graft from HAL-matched sibling: 6 – received BM, one pt – UCB and in one case PBSC after abTCD was used; and in 3 cases transplants were from haploidentical parents with abTCD. Thus the majority of pts received transplants after abTCD – 27 out of 35 pts. All pts were given a pretransplant conditioning regimen that differed according to the original disorder: FA – ATG, radiotherapy, fludarabin (Flu), busulfan 4mg/kg and cyclophosphamide (Cy); DC - ATG, radiotherapy, Flu and Cy; Amega - ATG, radiotherapy, treosulfan 42 mg/m^2, Flu and Cy; and for DBA – ATG, Flu and treosulfan 42 mg/m^2. For all pts it was first HSCT. The median dose of CD34+ cells in the graft was $10x10^6$/kg (range 1,3-29). Median dose of TcR αβ+ cells after depletion was $12x10^3$/kg (range 1-345).
Results: Median time of follow up for survivors was 2,7 years (range, 0,2 – 5,6), median time from diagnosis to HSCT was 1 year (range, 0,3-12). Primary engraftment of WBC and platelets was achieved in 34 pts, with median time 14 days (range, 8-36 and 8-229, respectively). One pt with FA after haplo HSCT with abTCD failed to engraft and died 3 months later due to fungal infection. One pt with DC 30 days after MUD HSCT with abTCD rejected the graft. Patient received second HSCT from another MUD with abTCD, engrafted but died 106 days later due to infection. CI of acute GvHD (aGvHD) grade ≥II at 100days after HSCT was 34% (95% CI: 22-54), there was one case of aGvHD grade III after MUD HSCT with abTCD, involving skin and gut and progression to cGvHD. According to the proportion there were more cases of aGvHD≥II grade after HSCT without abTCD - in 50% (4 out of 8 pts) vs 30% (8 out of 27 pts). CI of clinically relevant chronic GvHD 2 years after HSCT was 7% (95% CI: 2-26). OS 2 years after HSCT was 84% (95% CI: 71-97). Seven pts died. Two patients died as described above primary due to graft failure. Two patients with DC died 3 and 5 years after HSCT due to progression of non-haematological complications of the main disease. The rest 3 cases of mortality were due to mixed bacterial, fungal and viral infections during the first 6 months after HSCT with a prolonged immunosuppression therapy background.
Summary/Conclusion: Allogenic HSCT in the cohort of pts in the research we report is effective in the view of correction of bone marrow failure, but the problem of TRM still remain. Although observed number of patients was relatively small, according to the results of the study abTCD seems effective option to prevent severe GvHD, one of the main complications of allogenic HSCT.

PS1059

REACTIVATION OF HEPATITIS B VIRUS INFECTION IN APLASTIC ANEMIA PATIENTS RECEIVING IMMUNOSUPPRESSIVE THERAPY

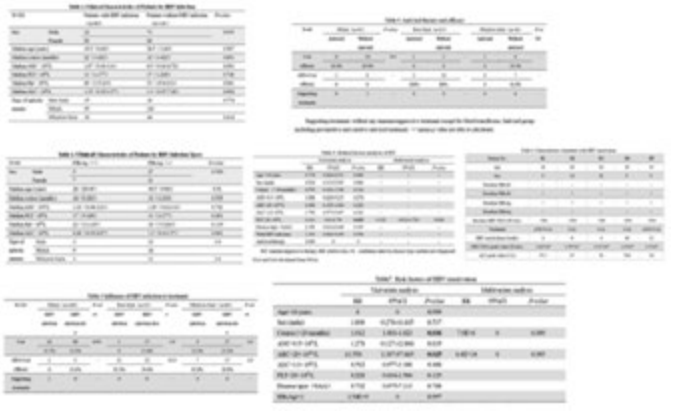
H. Yin[1], S. Wang[1], X. Lu[1], X. Wu[1], W. Shen[1], G. He[1,*], J. Li[1]

[1]Hematology, The First Affiliated Hospital of Nanjing Medical University, Jiangsu Provincial People's Hospital in Nanjing, Key Laboratory of Hematology of Nanjing Medical University, Collaborative Innovation Center for Cancer Personalize, Nanjing, China

Background: Aplastic anemia (AA) with hepatitis B virus (HBV) infection have already been reported in numerous studies, but the most focus was on clinical features rather than on virus reactivation, its assessment and prevention in immunosuppressive therapy (IST). Neither guidelines nor consensus from experts had recommendation for these issues [5, 6]. In this study, we analyzed HBV reactivation, prevention, treatment and IST results in aplastic anemia patients associated with HBV in our center over the past three years.
Aims: The aim of this study was to assess the therisk of hepatitis B virus (HBV) reactivation in hepatitis B surface antigen (HBsAg)-positive or negative, hepatitis B core antibody (HBcAb) -positive patients with aplastic anemia (AA) receiving immunosuppressive therapy of cyclosporin A (CsA) and/or anti-thymocyte globulin (ATG).
Methods: We analyzed clinical data of 60 AA patients with HBV infection out of 201 cases from our center during recent 3 years, laboratory test data such as levels of liver enzyme, HBV DNA, HBsAg, hepatitis B surface antibody (HBsAb), and HBcAb were also monitored. Entecavir ETV or lamivudine (LAM) therapy was initiated if HBV reactivation (defined as detectable HBV DNA) was encountered, or was used as antiviral prophylaxis regimen for some HBsAg-positive patients.
Results: Among 60 AA patients, 12 were chronically infected (HBsAg positive) and 48 were previously exposed (HBsAg negative/HBcAb positive). There was no difference in clinical features in AA patients with or without HBV infection, including gender, age, course of disease, as well as absolute neutrophil count, platelets and reticulocytes. The prevalence of non-severe AA (NSAA) progressed to severe AA (SAA) was similar in two groups *(35.6% vs 42.7%, p=1.0).* In the NSAA group, the response rate to CsA, response rate to ATG and CsA, and progression to SAA were similar in

patients with and without HBV infection (*35.7% vs 35.3%, p>0.05*; *42.8% vs 58.8%, p=1.0; 35.5% vs 42.7%, p=0.414)*. In the SAA group, the patients with or without HBV infection responded to ATG and CsA therapy similarly *(83.33% vs 59.0%, p=0.252)*. HBV reactivation was occurred in all 5 HBsAg positive patients without any antiviral therapy, while no HBV reactivation happened in other 7 patients received antiviral therapy. Disease course *(RR=1.012, p=0.036)* and absolute reticulocyte count *(RR=11.556, p=0.025)* were the risk factors for HBV reactivation by univariate analysis. Logistic regression indicated that HBsAg positivity without preventive therapy was the only strong factor for HBV reactivation (Tables).

Tables.



Summary/Conclusion: Antiviral prophylaxis is recommended for HBsAg-positive patients with AA who will receive IST because of high rate of HBV reactivation. HBV infection has no influence on the clinic course of AA, and antiviral therapy does not affect the efficacy of IST.

PS1060

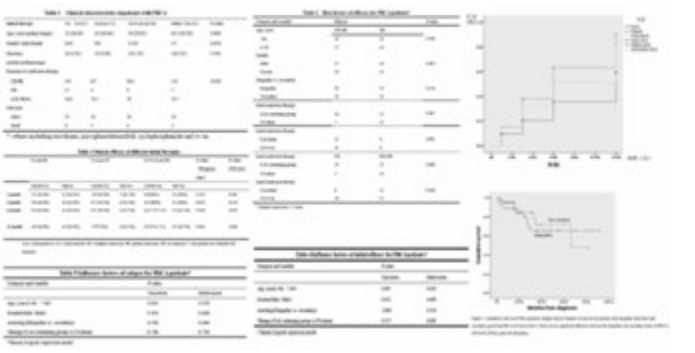
ADULT PATIENTS WITH ACQUIRED PURE RED CELL APLASIA: COMBINATION OF CYCLOSPORINE A AND CORTICOSTEROIDS PRODUCE BETTER CR

H. Yin[1], X. Wu[1], Y. Yang[2], W. Shen[1], G. He[1,*], J. Li[1]

[1]Hematology, The First Affiliated Hospital of Nanjing Medical University, Jiangsu Provincial People's Hospital in Nanjing, Key Laboratory of Hematology of Nanjing Medical University, Collaborative Innovation Center for Cancer Personalize, Nanjing, [2]Hematology, The First Hospital of Jilin University, Changchun, China

Background: Adult pure red cell aplasia (PRCA) is a syndrome characterized by a severe normocytic anemia, reticulocytopenia, and absence of erythroblasts from an otherwise normal bone marrow.
Aims: This article aims to evaluate whether the combination of cyclosporine A and corticosteroids produce better efficacy on PRCA than CsA alone or CS alone, and possible factors influencing it.
Methods: Clinical data of84 cases of PRCA were retrospectively analyzed from 2009 October.78 patients were evaluated in our two institutions (6 patients lost to follow-up).
Results: The remission induction therapy including CsA alone(n=17), CS alone(n=19), a simultaneous combination of CsA and CS (n=30), and other immunosuppressive agents (n=12), achieved remission in 13/17 (76.5%), 7/19 (36.8%), 21/30 (70%), 5/12 (41.7%) patients, respectively. The CR rate and the response rates of CsA-containing group (CsA alone and the combination of CS and CsA) was better than CS alone (51.1% *vs* 15.8%, p=*0.008*, 72.3% *vs* 36.8%, p=0.007). Combination of CS and CsA achieved better CR rate and response rate than CS alone (60% vs 15.8%, p=0.002, 70% *vs*36.8%, p=*0.022*). The response rate of CsA alone was better than CS alone (76.5% *vs* 36.8%, p=0.023*)*, While CsA alone did not show better CR rate than CS alone (35.3% vs 15.8%, p=0.255). Time of CsA-containing group to achieve remission was shorter than CS alone (p<0.05).
Summary/Conclusion: CS combined with CsA produced better CR than CS alone both in idiopathic and secondary PRCA. Combination of CS and CsA showed better CR rate and response rate than CS alone, and CsA-containing group produce remission faster than CS alone. It is recommended that CsA as the initial induction therapy for adult patients with acquired PRCA, and combination of CS and CsA could achieve better CR rate than CS alone.

Tables and Figures.



PS1061

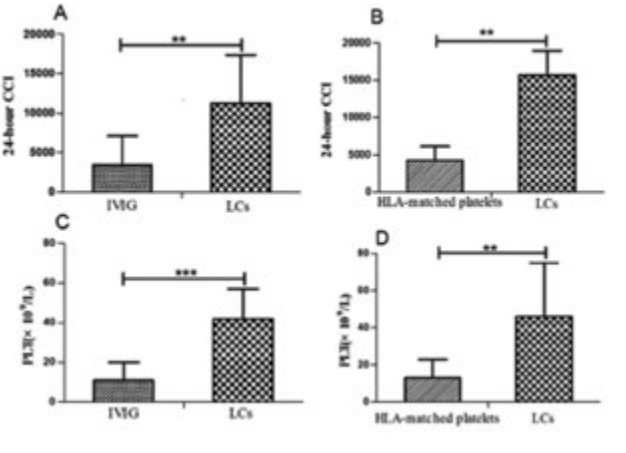
MANAGEMENT OF PATIENTS WITH SEVERE APLASTIC ANAEMIA WHO ARE REFRACTORY TO PLATELET TRANSFUSIONS USING LEUKOCYTE CONCENTRATES

J. Huo[1,*], Y. You[1]

[1]State Key Laboratory of Experimental Hematology, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Science & Peking Union Medical College, Tianjin, China

Background: British guideline recommended that platelet counts should be maintained at greater than 30×10^9/L during antithymocyte globulin (ATG) administration for severe aplastic anemia (SAA) patients. While, 5 to 27% of patients developed platelet transfusion refractoriness (PTR) produced by repeated transfusions of platelet concentrates. Intravenous immunoglobulin (IVIG) and HLA-matched platelets were used to manage PTR. Unfortunately, 40 to 60% of patients failed to achieve satisfactory recovery of platelet counts. HLA alloimmunization played a major causative role in PTR. 47 to 62% of patients with AA who had transfusions at multiple times acquired anti-HLA antibodies. Given this, we probed a unique strategy, namely using leukocyte concentrates (LCs) followed by random platelets, to "crush" the problem of PTR in SAA patients during ATG administration. LCs contain a very high number of white blood cells which bear substantial HLA antigens on the surface. Infusion of LCs could adsorb the anti-HLA antibodies and save random platelets from the antibody-directed destruction.
Aims: We conducted this retrospective study to assess the safety and efficacy of LCs treatment in patients with SAA who developed PTR.
Methods: A cohort of 253 patients with SAA who received ATG at our institute between December 2008 and November 2016 was reviewed. Of these patients, 18 patients who developed the complication of PTR caused by HLA alloimmunization with or without HPA alloimmunization were included in this study. All the 18 patients were given LCs therapy to improve platelet recovery. Corrected count increment (CCI) and post-transfusion platelet counts were used as indicators of post-transfusion platelet response.

Figure 1.



Results: Eighteen of 253 (7.1%) patients developing PTR prior to ATG therapy were managed with LCs. The mean 24-hour CCIs after LC transfusions

were 13,346, which was significantly higher than that of random platelets infusions without LC transfusions (1,327, p 0.001). Compared with the 24-hour CCIs of IVIG or HLA-matched platelets, those of LCs were significantly improved (Figure 1A,B). Additionally, significant improvements of post-transfusion platelet counts were also found after LC transfusions compared with IVIG or HLA-matched platelet infusions (Figure 1 C,D). Notably, sufficient platelet counts (mean, 53×10^9/L) were obtained and ATG therapy were completed without any serious bleedings after LC transfusions. The hematological response rate to IST in 18 patients was 44.4% at 3 months and 61.1% at 6 months. The 3-yr OS was 68.8% (95% CI 25.9-44.6%).
Summary/Conclusion: LCs transfusion can bridge between PTR and ATG administration and escort the SAA patients during the period of ATG administration.

PS1062

IRON OVERLOAD AND MANAGEMENT IN PATIENTS WITH DIAMOND BLACKFAN ANEMIA

S. Unal[1,*], G. Soltanova[2], N. Akarsu[1], M. Cetin[1], F. Gumruk[1]

[1]Center for Fanconi Anemia and Inherited Bone Marrow Failure Syndromes, [2]Department of Pediatrics, Hacettepe University, Ankara, Turkey

Background: Diamond Blackfan anemia (DBA) is a rare genetic ribosomopathy charactherized with severe anemia related to low erythroid precursors in the bone marrow. The patients are prone to transfusional iron loading, in addition to iron loading related to paucity of erythroid precursors in the bone marrow which interrupts the iron turn-over.
Aims: In this study, we aimed to define the severity of iron loading among DBA patients. There is limited data on iron loading in DBA patients.
Methods: The patients were evaluated cross-sectionally with serum ferritin and cardiac and hepatic T2* MRI. All of the patients were evaluated with serum ferritin measurments and 13 were evaluated with cardiac and hepatic T2* MRI.
Results: A total of 45 patients with DBA were included. The median age of patiens at evaluation was 9 years (1-35) and female:male ratio was 1.5. The age of diagnosis of DBA was median 3 months-old (0-72). All of the patients were found to have transfusion history. At time of evaluation for iron laoding 17 (37.8%) were on transfusion programme, 15 (33.3%) were in remission, 11 (24.4%) were on steroid treatment and 2 (4.4%) were transplanted. Mean serum ferritin levels were found 1103.86±1558.17 ng/ml (19.8-6839). The patients were further grouped according to number of transfusions as ≤ 5, ≤ 10, > 10, ≥ 20 times and the serum ferritin leveles were measured as 154.13±198.54 ng/ml (19.8-461), 149.64±149.07 ng/ml (23-421), 616.97±565.84 ng/ml (31.7-1658) and 2306.06±1947.76 ng/ml (455-6839), respectively. The difference between these 4 groups were found statistically significant. Out of 13 patients who were evaluated with MRI, 4 (30.8%) had nor iron laoding, 8 (61.5%) had hepatic iron overload (moderate degree in 4, moderate to severe in 2 and severe in 2 patients), one (7.7%) had minimal degree of cardiac iron laoding. The median hepatic and cardiac T2* values were 8.7 ms (1.36-30) and 28.4 ms (12-41), respectively. Of the 8 patients who had hepatic iron loading, 5 (62.5%) wer on chronic transfusion programme, wheras 3 (37.5%) were steroid dependent. Sixteen (35.6%) of the patients were under chelation with deferasirox treatment.
Summary/Conclusion: There is limited data on the iron status of patients with DBA. Iron overloading not only has negative impact on organ functions, but also affects hematopoietic stem cell transplantation outcome. The iron loading differs in various disorders such as in thalassemia major both cardiac and hepatic iron laoding is an issue, whereas in patients with thalassemia intermedia or sickle cell disease, most of the iron accumulates in liver. In DBA patients both cardiac and hepatic iron loading is a concern, however hepatic iron laoding is more common. Besides the patients who are on steroid treatment are also prone to hepatic iron laoding. This might be related to paucity of the eryhtroid precursors in bone marrow that impairs iron turn-over; besides most of the patients who are on steroid treatment also have received at least one-year course of transfusion in infancy prior to steroid initiation. Patients with DBA should be screened for iron loading as early as possible and chelation treatment should be initiated to prevent the related morbidity and mortalities.

PS1063

HIGHER RATE OF DYSMORPHIC FEATURES IN PATIENTS WITH DIAMOND BLACKFAN ANEMIA

G. Soltanova[1], O. P. Simsek Kiper[2], S. Unal[2,*], F. Gumruk[2], E. Taskiran[2], B. Karaosmanoglu[2], N. Akarsu[2], M. Cetin[2]

[1]Department of Pediatrics, [2]Center for Fanconi Anemia and Inherited Bone Marrow Failure Syndromes, Hacettepe University, Ankara, Turkey

Background: Diamond Blackfan anemia (DBA) is the inherited form of pure red cell aplasia which is accompanied with several dysmorphic features and propensity to malignancies. Of the dysmorphic features the most common ones are craniofascial findings.
Aims: In this study, we aimed to define dysmorphic features in our DBA patients.
Methods: The patients were examined by an experienced dysmorphologist and echocaridography, abdominal ultrasonography, ENT and eye examinations were also performed.
Results: A total of 45 patients with DBA were included. The median age of patiens at evaluation was 9 years (1-35) and female:male ratio was 1.5. Of the patients 86.7% were found to have at least one dysmorphic finding and of these with dysmorphic features 33% were craniofascial (microcephaly, mikrognathia, prognathia, retrognathia, pointed chin, microstomia, hipertelorism, malar hypoplasia, mid-fascial hypoplasia, synorfia, deep-set eyes, lower/upper slenting palpebral fissures, epichantal folds, broad nasal bridge, prominent columella, short/long philtrum, round/course face, low hair line, low-set ears, anteverted helix, high-arched palate, cleft palate/lip, thin lip, broad/high/narrow forehead), 26% were skeletal (pes planus, cubitus valgus, genu valgum, narrow shoulder, short metacarpals, short limbs, syndactylia / clinodactylia / brachydactylia, proximal oriented, rudimentary or hypoplastic thumb, flattening of thenar eminence), 10% were cardiovascular (ASD, VSD, PFO, MVP, Fallot tetralogy, bicuspit/dysplastik aorta, tricuspid valve anomaly, pulmonary stenosis, dilated cardiomyopathy), 9% were related to neck (short, webbed), 9% were neuromotor, 3% were ophtalmological (strabismus, congenital cataract) 2% were genitourinary (hypospadias, bilateral renal pelvis dilatation) and 12% were various other findings (including skin, hair, nail findings, inguinal sacral dimple, pilonidal sinus). Although, not included as a dysmorphic finding, 41% of the patients were found to have short stature.
Summary/Conclusion: The rate of congenital malformations in patients with DBA have been reported previously as 50%. However, in our series of patients we have found a higher rate of dysmorphic features. This might be related to detailed examination of our patients by a dysmorphologist. The most common sysmorphic finding was craniofascial abnormalities compatible with the reported patients. These symorphic findings may be the only finding in a subgroup of patients who never develop anemia, however they are usually non-specific or subtle findings. However the presence of any of these findings in a family member of an index DBA patient might be a clue for a silent DBA case in that person, since incomplete penetrance is an issue in DBA.

Chronic lymphocytic leukemia and related disorders – Biology & Translational Research

PS1064

OPTIMIZING MRD RESPONSE ASSESSMENT USING HIGH-SENSITIVITY PERIPHERAL BLOOD MONITORING DURING TREATMENT WITH IBRUTINIB COMBINED WITH OBINUTUZUMAB OR VENETOCLAX IN CLL

A. C. Rawstron[1,*], R.M. de Tute[1], N. Webster[2], D. Howard[3], K. Brock[4], S. Muñoz-Vicente[4], F. Yates[4], R. Bishop[4], T. Munir[1], P. Hillmen[2]

[1]St James's Institute of Oncology, Leeds Teaching Hospitals NHS Trust, [2]Leeds Institute of Cancer and Pathology, [3]CTRU, University of Leeds, Leeds, [4]Cancer Research UK Clinical Trials Unit, University of Birmingham, Birmingham, United Kingdom

Background: Minimal residual disease (MRD) in CLL is an independent predictor of outcome and an intermediate endpoint for licensure. Using MRD to guide treatment duration is under evaluation in several trials. However, different treatments vary in the extent of disease depletion in the peripheral blood (PB) compared to bone marrow (BM).
Aims: To assess MRD in PB vs. BM on ibrutinib (IBR) ±obinutuzumab (OBI) or venetoclax (VEN) & determine the duration/depth of PB MRD responses required to predict <0.01% BM MRD.
Methods: CLL cell percent of leucocytes (% MRD) was quantified using 8CLR ERIC-standard flow cytometry (detection limit 10-5/0.001%). PB (n=1481) & BM (n=478) samples were assessed in ADMIRE/ARCTIC (ISCRTN 42165735/16544962), IcICLLe (ISCRTN12695354) & CLARITY (ISCRTN13751862) trials.
Results: Rituximab (R) + fludaribine+cyclophosphamide (FC)±mitoxantrone (M) resulted in >0.88 log higher%MRD in BM compared to PB. There was no discernible compartment effect with IBR-monotherapy but no patients achieved <0.01% MRD. Patients receiving IBR+OBI or IBR+VEN showed higher BM than PB MRD levels (>0.36 and >0.44 log respectively) with a similar discrepancy rate at the IWCLL 0.01% threshold although most IBR+OBI discrepancies reflected >2 log higher BM vs. PB% MRD while all IBR+VEN discrepancies reflected <2 log difference. We next evaluated using PB MRD response to predict <0.01% BM MRD. No treatment-naïve (TN) patients (0/20) achieved <0.01% PB MRD with IBR-monotherapy; 3yr follow-up showed continuing PB CLL depletion in 10/14 evaluable patients but the rate of depletion from 24-36mth (median 0.27log, range -0.33 to 0.50) was markedly lower than the 1-12mth rate (median 1.5log, range 0.13 to 2.5). Relapsed/refractory (RR) patients (n=10) receiving OBI+IBR after >1yr prior IBR achieved <0.01% PB MRD in 7/8 evaluable patients of which 5/7 (50% of total) achieved <0.01% BM MRD; all 5/5 maintained <0.01% PB MRD for ≥6M prior to BM assessment. IBR-naïve RR patients (n=28) receiving IBR+OBI achieved <0.01% PB MRD in only 5/21 evaluable patients of which 3/5 achieved <0.01% BM MRD (11% of total). However, 2/5 subsequently became PB MRD-pos & overall 5/14 evaluable patients initially treated with IBR+OBI showed increasing PB% MRD 6-9mth after stopping OBI. In the CLARITY IBR+VEN trial, of 20 patients with >0.01% MRD at any time point 6mth prior to BM assessment, only 1/20 (5%) achieved <0.01% BM MRD. In contrast, of 12 patients with 6mth-sustained <0.01% PB MRD, 10/12 (83%) achieved <0.01% BM MRD of which all 10 had <0.001% PB MRD at the final assessment (Table 1).

Table 1.

Trial Name: timepoint/Rx for PB/BM assessment (n)	Median log difference	# discrepant at 0.01% MRD (%)	# with >2log difference (%)
ADMIRE/ARCTIC: 3mths post R-FCxM (n=272)	>0.88	59 (21.7%)	8 (2.9%)
IcICLLe: 1mth & 6mths of IBR (n=74)	0	none <0.01% MRD	0 (0%)
IcICLLe ext: 1mth & 9mths IBR+OBI (n=62)	>0.36	7 (11.3%)	6 (9.7%)
CLARITY: 8 or 14mths IBR+VEN (n=70)	>0.44	9 (12.9%)	0 (0%)

Summary/Conclusion: Ibrutinib monotherapy was associated with similar PB and BM MRD levels & long-term (3yr) monitoring was informative using PB only. A compartment effect was apparent during treatment with IBR+OBI & IBR+VEN and BM remains the optimal tissue for MRD response assessment. Sustaining a PB MRD response for 6mth, preferably using a 10-5/0.001% detection limit, can be used to predict a high probability of achieving undetectable BM MRD.

PS1065

DYNAMIC OF TELOMERIC PARAMETERS IN RELAPSING OR REFRACTORY (R/R) CHRONIC LYMPHOCYTIC LEUKEMIA (CLL), AN ANALYSIS OF THE FILO ICLL001 BOMP TRIAL

L. Bounaix[1,*], A. Tchirkov[2], F. Nguyen-Khac[3], P. Combes[4], P. Feugier[5], L. Sanhes[6], M.-S. Dilhuydy[7], H. Saad[8], L. Ysebaert[9], S. Choquet[10], J.-P. Vilque[11], A. Brion[12], C. Araujo[13], E. Ferrant[14], B. Dreyfus[15], A. J. Delmer[16], M. Truchan-Graczyk[17], S. Leprêtre[18], S. de Guibert[19], R. Guièze[20], L. Véronèse[20], R. Lemal[20], P. Vago[20], J.-O. Bay[20], M. Berger[20], A. Schuh[21], C. Ferrand[22], R. Delepine[23], V. Lebond[10], O. Tournilhac[20]

[1]Hematology, University Hospital, [2]Hematology, University Hospital of Estaing, Clermont-Ferrand, [3]Hematologic Cytogenetic, AP-HP, Paris, [4]Hematologic Cytogenetic, University Hospital of Estaing, Clermont-Ferrand, [5]Hematology, University Hospital, Nancy, [6]Hematology, University Hospital, Perpignan, [7]Hematology, University Hospital, Bordeaux, [8]University Hospital, Brest, [9]University Hospital, Toulouse, [10]University Hospital, Paris, [11]University Hospital, Caen, [12]University Hospital, Besancon, [13]Hospital, Bayonne, [14]University Hospital, Dijon, [15]University Hospital, Poitiers, [16]University Hospital, Reims, [17]Hospital, Saumur, [18]University Hospital, Rouen, [19]University Hospital, Rennes, [20]University Hospital, Clermont-Ferrand, France, [21]Oxford University, Oxford, United Kingdom, [22]EFS, Besancon, [23]University Hospital, Tours, France

Background: In patients (pts) with CLL, telomeric parameters have prognostic impact. In a retrospective study, we found that *TP53*-disrupted pts show severe telomere dysfunction associated with high genomic instability and that downregulation of shelterin complex genes is an adverse prognostic factor in *TP53* wild-type (wt) patients (Guièze, 2016). Recently, germline mutations in shelterin genes were found in familial CLL (Speedy, 2016) and a poor prognostic impact of somatic *POT1* mutations (Ramsey, 2013) was reported in a front-line prospective study (Herling, 2016). A key and potentially early role of telomere dysfunction in disease progression is clearly suggested.
Aims: The aim of this study was to assess telomeric parameters and their evolution in R/R CLL patients treated in a prospective trial.
Methods: From a cohort of 74 R/R CLL pts included in the FILO phase II BOMP trial (NCT0161298) we identified 19 pts with available samples, with ≥70% tumor cell purity, at 2 successive time points (TP) to study the evolution of telomeric parameters. TP#1 was inclusion and TP#2 the subsequent CLL relapse after treatment (Bendamustine, Ofatumumab and high-dose Methyl-Prednisolone). The median time interval between the two TP was 17.5 months (mo) (range 4.9 - 37.8). The median follow-up was 33.9 mo (range 15.7 – 48.5). Prior to inclusion, 19 pts received 1 to 3 lines of treatment including FCR combination for all pts. Six pts had *17p* deletion by FISH and *TP53* mutation by Sanger and 13 pts had wt *TP53*. Telomere length (TL) was assessed by qPCR and expression of telomerase (*hTERT)* and shelterin complex (*TRF1, TRF2, TIN2, POT1, RAP1, TPP1, TIN2 spliced variant*) genes by qRT-PCR at TP#1 and TP#2.
Results: Between TP#1 and TP#2, TL decreased significantly (p=0.0006) and the expression of all shelterin genes was also considerably reduced: *TRF1* (2 fold-change, p<0.0001), *TRF2* (2.7 fold-change, p<0.0001), *POT1* (1.8 fold-change, p=0.0002), *RAP1* (2 fold-change, p=0.0009), *TPP1* (1.8 fold-change, p=0.0003), *TIN2* (2.3 fold-change, p=0.0004), *TIN2 spliced variant* (2.2 fold-change, p=0.002). In contrast, *hTERT* expression remained unchanged (p=0.64). Telomere shortening kinetics (TSK) in individual patients was variable. We calculated the relative telomere shortening per month expressed as percentage of the initial TL and identified 3 groups of patients with slow (<0.69%, n=4), intermediate (n=11) and rapid TSK (>4.24%, n=4). One patient slightly extended TL. At TP#1, the median rate of *hTERT, POT1* and *TIN2* were 24.5, 36.7 and 45.1 respectively. A significantly higher TSK was found in patients presenting low expression (using median level as cut-off) of *hTERT* (3.2% *vs* 0.7%, respectively, p=0.002), *POT1* (3.140% *vs* 0.9560%, p=0.0054) and *TIN2* (2.870% *vs* 1.199%, p=0.04).
Summary/Conclusion: We found that following immunochemotherapy, telomere length and shelterin complex gene expression in CLL patients show variable evolution over time which may preclude a next step of genomic instability. To address this point, telomere parameters at inclusion and relapse will be confronted to other biological data including cytogenetics, FISH and mutational pattern.

PS1066

HIGH CD9 EXPRESSION IS ASSOCIATED WITH THE MYD88 L265P MUTATION AND REDUCED FAILURE-FREE SURVIVAL IN CHRONIC LYMPHOCYTIC LEUKEMIA

R. Improgo[1,2], B. Tesar[1,2], S. Kasar[1,2], J. L. Klitgaard[1,2], R. Magori-Cohen[3,4],

L. Yu[1,2], S.M. Fernandes[1], K. Hoang[1], H.T. Kim[3], J.R. Brown[1,2,*]

[1]Department of Medical Oncology, Dana-Farber Cancer Institute, [2]Department of Medicine, Harvard Medical School, [3]Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, [4]Department of Biostatistics, Harvard School of Public Health, Boston, United States

Background: The L265P somatic mutation in the Myeloid Differentiation Primary Response 88 (*MYD88*) gene is recurrent in chronic lymphocytic leukemia (CLL) but its role in CLL remains to be fully elucidated.
Aims: To understand the functional role of the MYD88 L265P mutation in CLL by examining gene expression profiles, clinical associations, and pathway inhibition.
Methods: Affymetrix U133 Plus 2.0 microarray data were analyzed using a published PAM-based approach. Since MYD88 L265P is associated with mutated *IGHV* (*IGHV*-M), which strongly influences gene expression in CLL, analysis was initially restricted to *IGHV*-M samples (n=10 MYD88 L265P, n=76 MYD88 WT). A composite gene signature score was derived from coefficient estimates of differentially expressed genes using a Cox model; this signature was then examined for associations with clinical outcome. RNA-Seq validation was performed using DESeq2 analysis. Cell TiterGlo was used to measure viability of CLL patient cells at baseline, upon CpG stimulation, and after drug treatment. Downstream pathway inhibition was examined using Western blot analysis.
Results: Microarray analysis identified 28 differentially expressed genes between *IGHV*-M MYD88 L265P and WT CLL. Gene Set Enrichment Analysis (GSEA) of these 28 genes in our RNA-Seq data demonstrated correlation with MYD88 L265P status ($q<0.05$). Analysis of a subset of the microarray samples by RNA-Seq identified 15 differentially expressed genes ($q<0.1$). Two of these genes, *CD9* and *RUNDC3B*, overlapped with the microarray analysis. Both genes showed higher expression in MYD88 L265P vs WT samples. Analysis for clinical association in a discovery cohort (n=150) showed that a higher 28-gene signature score predicted worse FFS ($p<0.0001$) and OS ($p<0.0001$). We then performed multivariable Cox regression analysis for FFS and OS, adjusting for established CLL risk factors. Since treatment status is highly correlated with *IGHV* and ZAP70 status, it was included with age and cytogenetics in a separate model for OS, and treated as a time-dependent variable. We found that a higher gene signature score predicted worse FFS ($p=0.003$) and OS ($p=0.0001$). To validate the 28-gene signature, we analyzed an independent cohort of CLL patients with available microarray data (n=87; n = 26 *IGHV*-UM, n=50 *IGHV*-M, n= 11 unknown *IGHV* status). The composite signature of the 28 genes again predicted worse FFS ($p<0.0001$) and OS ($p<0.001$). High *CD9* expression was associated with reduced failure-free survival (n=150, $p=0.007$); no clinical associations were found for *RUNDC3B*. To determine whether the MYD88 pathway can be therapeutically targeted in CLL, we sought to suppress MYD88 signaling by inhibiting its downstream effector, IRAK4, using ND-2158, a selective inhibitor of IRAK4 kinase activity (Nimbus Therapeutics). ND-2158 treatment led to a dose-dependent decrease in cell viability. CpG-stimulation sensitized *IGHV*-M MYD88 WT and L265P cells, but not *IGHV*-UM cells, to ND-2158 treatment ($p=0.001$ WT, $p=0.006$ L265P). Western blot analyses demonstrated that CpG stimulation led to a decrease in total IRAK1 and IkBa levels, which was inhibited by ND-2158 treatment in a dose-dependent manner ($p<0.0001$).
Summary/Conclusion: The MYD88 L265P mutation in CLL is associated with a 28-gene signature that predicted FFS and OS. Higher *CD9* expression was associated with MYD88 L265P and with reduced FFS. IRAK4 inhibition perturbs MYD88 pathway signaling, leading to CLL cell death.

PS1067

CERDULATINIB SYNERGISES WITH BCL-2 AND MCL-1 INHIBITORS TO INDUCE SUPERIOR CELL DEATH IN CHRONIC LYMPHOCYTIC LEUKEMIA

K.-R. Rogers Broadway[1], M.D. Blunt[1], L.D. Smith[1], L.I. Karydis[1], A. Minton[1], J.C. Strefford[1], F.K. Stevenson[1], M.S. Cragg[1], F. Forconi[1], G.P. Coffey[2], P. Conley[2], G. Packham[1], A. J. Steele[1,2,*]

[1]Cancer Sciences Unit, University of Southampton, Southampton, United Kingdom, [2]Portola Pharmaceuticals, South San Francisco, CA, United States

Background: B cell receptor (BCR) signaling is pivotal to chronic lymphocytic leukemia (CLL) pathogenesis. The recent CLARITY-TAP clinical trial update at ASH 2017, investigating the role of ibrutinib in combination with venetoclax provided compelling evidence that this was a useful strategy for the treatment of CLL. However Bojarczuk *et al.* recently demonstrated that complete inhibition of BCR-induced Mcl-1 expression was only achieved with SYK inhibitors such as entospletinib, whereas ibrutinib and idelalisib only had partial effects at the same concentrations. In our own studies entospletinib and PRT062607 also provided greater inhibition of BCR signaling compared to idelalisib and ibrutinib. Herein we compared our dual SYK/JAK inhibitor (cerdulatinib) with SYK (entospletinib, PRT062607), BTK (ibrutinib) or PI3K (idelalisib) kinase inhibition in combination with the Bcl-2 (venetoclax) or Mcl-1 (S63845) inhibitors and assessed downstream signaling and tumor viability.
Aims: Evaluate which BCR kinase inhibitors optimally synergies with Bcl-2 family inhibitors and determine whether dual SYK/JAK inhibition provide any additional anti-tumor activity.
Methods: Primary CLL cases were treated with and without IL-4/CD40 in the presence or absence of ibrutinib, idelalisib, entospletinib, PRT062607 or cerdulatinib (all 1μM). Combination studies were performed with 10 or 100nM Venetoclax or 300 and 1000nM S63846. Cell viability was assessed by annexin V/PI using flow cytometry and changes in protein expression by immunoblotting.
Results: CLL cells were treated with IL-4/CD40L or the vehicle control and immunoblotting performed for Bcl-2 family protein expression. Basal Mcl-1, Bcl-X_L and Bim protein was expressed at relatively low levels compared to Bcl-2. However treatment with IL-4/CD40L induced a substantial and significant increase in Mcl-1 and Bcl-X_L compared to the vehicle control, whilst Bcl-2 and Bim expression remained relatively stable. CLL cells pretreated with ibrutinib, idelalisib, entospletinib, PRT062607 or cerdulatinib significantly reduced IL-4/CD40L induced Mcl-1 and Bcl-X_L expression, however in all cases cerdulatinib produced a more robust inhibition of these proteins compared to the other kinase inhibitors. Interestingly treatment with all BCR kinase inhibitors resulted in an increase in Bim expression at both the RNA and protein levels. However at equivalent drug concentrations Bim was induced to greater levels following cerdulatinib treatment compared to idelalisib and ibrutinib. Next we evaluated Bim co-localization with Bcl-2 and Mcl-1 proteins using immunoprecipitation. Bim co-localized largely with Bcl-2 and a lesser extent with Mcl-1 in all our CLL cases *in vitro*. Consequently these cells were primed for death, therefore we hypothesized that venetoclax and/or S63845 would synergise with the BCR kinase inhibitors. Indeed, venetoclax and S63845 synergised with all BCR kinase inhibitors to induced greater levels of CLL cell death by displacing Bim. However in all cases the SYK/JAK inhibitor cerdulatinib synergized to a greater extent with the Bcl-2 family inhibitors compared to BTK, PI3K and SYK inhibitors alone.
Summary/Conclusion: *In vitro* SYK inhibitors offer superior tumor cell killing compared to BTK and PI3K inhibitors in combination with Bcl-2 family inhibitors. However cell death is further augmented by co-inhibition of SYK and JAK and consequently requires further investigation in CLL and other B cell malignancies.

PS1068

SELECTIVE CHK1 INHIBITOR MU380 EXHIBITS SINGLE-AGENT ACTIVITY AGAINST CHRONIC LYMPHOCYTIC LEUKEMIA CELLS WITH TP53 MUTATIONS

M. Boudny[1,*], J. Zemanova[1], P. Khirsariya[2], M. Borsky[1], J. Verner[1], J. Cerna[1], M. Mraz[1], A. Oltova[1], Z. Jaskova[1], M. Spunarova[1], V. Seda[1], Y. Brychtova[1], J. Mayer[1], K. Paruch[2], M. Trbusek[1]

[1]University Hospital Brno and Faculty of Medicine, Masaryk University, [2]Faculty of Science, Masaryk University, Brno, Czech Republic

Background: Significant therapeutic progress has been achieved in chronic lymphocytic leukemia (CLL) with development of small molecule inhibitors of the B-cell receptor (BCR) signaling and Bcl-2 protein. Majority of the published data concern BCR signaling inhibitor ibrutinib, and agree upon still inferior prognosis of patients with *TP53* defects; these patients constitute in the long term the most challenging group in CLL. Checkpoint kinase 1 (Chk1) is a protein operating in the intra-S and G_2/M cell cycle checkpoints and as such is essential for DNA replication. Various Chk1 inhibitors are tested for anti-cancer effects either alone or more frequently in combination with chemotherapy. Recently, we developed metabolically stable analog of a selective Chk1 inhibitor SCH900776 called MU380.
Aims: To analyze single-agent activity of our selective Chk1 inhibitor MU380 in (a) cell lines from lymphoid tumors, (b) dividing and non-dividing primary CLL cells, and (c) animal model.
Methods: Cell viability was measured by WST-1, apoptosis analyzed by western blot of PARP and caspase-3 proteins, and cell cycle determined by DNA content analysis. Pro-proliferative stimulation of primary CLL cells was done using co-culture with murine fibroblasts (CD32+), anti-CD40 antibody and IL-4. Genetic aberrations in CLL cells were assessed by FISH and NGS. In vivo effects were studied in mice strain NOD/SCID IL2Rγ-null with localized CLL tumors established by subcutaneous injection of MEC-1 cell line (5×10^6 cells). External measurement of tumors size and

volume was performed between days +14 and +29.
Results: After the 72 h treatment, MU380 reduced viability of all lymphoma (n=9) and leukemia (n=9) permanent cell lines (median IC_{50}=401 and 254 nM, respectively; P 0.001). CLL-derived cell lines were highly sensitive: IC_{50}=221 nM in *TP53*-wt OSU-CLL and 195 nM in *TP53*-mutated MEC-1. A pronounced cell accumulation in the S-phase (24 h) was followed by extensive apoptosis (at 48 h). Importantly, two cultures of healthy fibroblasts were substantially less affected (IC_{50}=3.7 and 8.1 μM). In primary CLL cells treated with the pro-proliferative stimuli (10 day co-culture), the 72 h treatment with MU380 reduced viability similarly in the *TP53*-mutated (n=7), *ATM*-mutated (n=3) and *ATM*-wt/*TP53*-wt (n=3) samples (all but one samples IC_{50} ~1 μM). Surprisingly, MU380 also reduced viability in the vast majority of non-stimulated CLL cultures (total n=96) with a low baseline Chk1 level. The effects were again similar among the tested genetic groups: median IC_{50}=0.34 μM in *TP53*-mutated samples (n=23), 0.38 μM in *ATM*-mutated samples (n=20), 0.35 μM in samples with sole 11q- (the other *ATM* allele intact) (n=17), and 0.41 μM in *ATM*-wt/*TP53*-wt samples (n=36). At the molecular level, MU380 triggered replication stress (γ-H2AX accumulation) and apoptosis, which was not, however, accompanied by the p53 protein accumulation (in p53-wt cells). Finally, MU380 significantly delayed growth of the localized *TP53*-mutated CLL tumors (subcutaneous MEC-1 injection) in mice. Seven doses of MU380 (each 20 mg/kg) were administered intraperitoneally (outside the tumor) between days +14 and +28 post-transplant. At day +29, the average tumor volume was 1072 mm^3 in the treated group vs. 1897 mm^3 in the control one (p=0.0015).
Summary/Conclusion: We present a remarkable single-agent anti-cancer activity of Chk1 inhibitor MU380 in CLL cells harboring *TP53* inactivation. Chk1 inhibition may represent novel therapeutic option for currently incurable *TP53*-mutated CLL.
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PS1069

PI3K INHIBITORS INHIBIT TUMOR MICROENVIRONMENT (TME) REGULATION OF PROGRAMMED DEATH-1 (PD1) AND PDL1/2 IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): A POTENTIAL APPROACH TO OVERCOME TUMOR-INDUCED TOLERANCE

A.G. Recchia[1], S. Bossio[1], L. De Stefano[1], A. Palummo[1], F. Storino[1], N. Caruso[2], M. Gentile[2], E. Vigna[2], D. Fenoglio[3], G. Filaci[3], F. Fais[4], A. Gulino[5], M. Manzoni[6], C. Tripodo[7], A. Neri[6], G. Cutrona[8], B. Martino[9], P. Tassone[10], M. Ferrarini[11], F. Morabito[1,*]

[1]Unità di Ricerca Biotecnologica, Fondazione Amelia Scorza-AIL, Aprigliano (CS), [2]Department of Onco-Hematology, Hematology Unit, A.O. of Cosenza, Cosenza, [3]Centre of Excellence for Biomedical Research and Department of Internal Medicine, University of Genoa, [4]Molecular Pathology Unit and Department of Experimental Medicine, IRCCS-A.O.U. San Martino-IST and University of Genova, Genoa, [5]Department of Health Science, Human Pathology Section, Tumor Immunology Unit, University of Palermo School of Medicine Palermo, Palermo, [6]Department of Oncology and Hemato-Oncology and Hematology Unit, University of Milano and Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milano, [7]Department of Oncology and Hemato-Oncology and Hematology Unit, University of Palermo School of Medicine Palermo, Palermo, [89]Molecular Pathology Unit, IRCCS-A.O.U. San Martino-IST, Genoa, [9]Hematology Unit, Azienda Ospedaliera "Bianchi Melacrino Morelli", Reggio Calabria, [10]Department of Experimental and Clinical Medicine, University of Catanzaro Magna Graecia, Catanzaro, [11]Dipartmento di Medicine Sperimentale, University of Genoa, Genoa, Italy

Background: BCR stimulation of membrane-proximal kinases such as PI3Kδ is implicated in the survival and growth of CLL B-cells. Interactions of malignant cells, T cells and macrophages, particularly through the PD1 pathway, play a crucial role in the CLL TME and ultimately leads to T cell exhaustion. The PI3Kδ inhibitor (PI3K-I) idelalisib (CAL101) is approved for patients with relapsed CLL. PI3K signaling is also involved in T-cell activation thus specific PI3K-Is that favor tumor cell intrinsic activity sparing T-cell functionality are required, such agents in preclinical development are AMG-319 and IPI-145.
Aims: To examine the expression the PD1 receptor and its ligands; the role of the TME in controlling PD1 axis expression; and the role of the PI3K-Is on the PD1 axis in the TME.
Methods: Early-stage CLL patients (n=110) were prospectively enrolled at diagnosis. Gene expression (GE) was evaluated by qPCR assays (Thermofisher) and flow-cytometry (FC, BD-Biosciences) was used to evaluate protein expression. Activated autologous T-cells (AAT), obtained by *in vitro* exposure of patient T-cells with anti CD3/CD28 Dynabeads (Thermofisher) and IL2 in co-culture with CLL cells, was used as *in vitro* model for mimicking the CLL TME. Cultures were monitored until substantial clumping was observed, and then tested for PD1 axis expression by FC. In selected experiments CAL101 (1uM/5uM), AMG319 (1uM), and IPI145 (1uM) (all from Selleck Biochemicals) were added to cell cultures.
Results: Baseline protein expression of PD1 and ligands from B-CLL samples (n=110) indicated that circulating CD19+CD5+ showed similar levels of PD1 and PDL2, but limited PDL1 expression, while no significant differences in PD1 or its ligands were observed in the GE (n=27). Conversely, PD1 and its ligands evaluated by FC (n=106) revealed higher expression of CD3+PD1+ cells, which was significantly higher than that of malignant B-cells. CD4+ and CD8+ expressed comparable levels of PD1, whereas CD16+PD1+ expression was lower. Expression of PD1 and PDLs on CD19+CD5+ cells (n=40) showed no significant correlations with prognostic markers. FC analysis (n=32) of CLL cells after AAT coculture indicated higher percentage expression of PD1 axis members and PDLs with respect to baseline B-CLL cells also confirmed by qPCR GE (n=15). Conversely, T-cell subpopulations had higher number of CD3+, CD4+ and CD8+ cells bearing both PD1 and PDL1, while no substantial increment for CD3+, CD4+ or CD8+/PDL2+ cells following AAT co-culture was observed. Only PDL1 in CD16+ cells was also upregulated. AAT in presence of PI3K-Is (48h) showed, an overall reduction of PDL1+ and PDL2+ B-cells by CAL101 (n=9) and AMG (n=5), and more strongly by IPI-145 (n=5). Similarly, reduction in mRNA transcripts for PDL1 and PDL2 (n=7), although with a strong decrease of PD-1, IPI also significantly decreased mRNA for both ligands. PD1 expression on CLL cells, CD4+ and CD8+ subsets induced by AAT was inhibited by CAL101. AMG had similar effects as IPI on the number of CD4+ and CD8+ cells also expressing PD1 and PDL1, but not for PDL2 for both B- and T-cells. CD16 cells showed the greatest reduction in PD1 expression by CAL101 5uM, AMG and IPI, however co-expression of PDLs were also reduced.
Summary/Conclusion: TME-derived signals regulate PD1 and PDL1/PDL2 expression in both CLL- and AAT-cells. PI3K-Is, CAL101, AMG319, and IPI145 may play a role in controlling immunescape mechanisms via regulation of PD1 axis expression on neoplastic B- and T-cells in CLL.
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PS1070

PEMBROLIZUMAB (PEM) ACTIVITY IN CLL IS IMMUNE TO TUMOR MICROENVIRONMENTAL (TME) STIMULI

L. De Stefano[1], A.G. Recchia[1], S. Bossio[1], A. Palummo[1], F. Storino[1], N. Caruso[2], M. Gentile[2], E. Vigna[2], D. Fenoglio[3], G. Filaci[3], F. Fais[4], A. Gulino[5], M. Manzoni[6], C. Tripodo[7], A. Neri[6], G. Cutrona[8], B. Martino[9], P. Tassone[10], M. Ferrarini[11], F. Morabito[1,*]

[1]Unità di Ricerca Biotecnologica, Fondazione Amelia Scorza-AIL, Aprigliano (CS), [2]Department of Onco-Hematology, Hematology Unit, A.O. of Cosenza, Cosenza, [3]Centre of Excellence for Biomedical Research and Department of Internal Medicine, University of Genoa, [4]Molecular Pathology Unit and Department of Experimental Medicine, IRCCS-A.O.U. San Martino-IST and University of Genova, Genoa, [5]Department of Health Science, Human Pathology Section, Tumor Immunology Unit, University of Palermo School of Medicine Palermo, Palermo, [6]Department of Oncology and Hemato-Oncology and Hematology Unit, University of Milano and Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milano, [7]Department of Oncology and Hemato-Oncology and Hematology Unit, University of Palermo School of Medicine Palermo, Palermo, [8]9Molecular Pathology Unit, IRCCS-A.O.U. San Martino-IST, Genoa, [9]Hematology Unit, Azienda Ospedaliera "Bianchi Melacrino Morelli", Reggio Calabria, [10]Department of Experimental and Clinical Medicine, University of Catanzaro Magna Graecia, Catanzaro, [11]Dipartmento di Medicine Sperimentale, University of Genoa, Genoa, Italy

Background: The programmed death receptor 1 (PD1) pathway is a general phenomenon involved in the neoplastic mechanism of immune escape. T cells from CLL patients exhibit defective immunity leading to T cell exhaustion. These functional defects coincide with higher PD1 expression on T cells. Antibodies blocking the PD1/PDLs axis are emerging for treatment of solid and hematological neoplasia. The applicability of these agents in CLL is promising.
Aims: To examine the 1) expression the PD1 receptor and its ligands on B-CLL and autologous T-cells; 2) role of the TME in controlling the expression of PD1 and its ligands; 3) role of the anti-PD1 inhibitor on the PD1 axis in the TME.
Methods: Baseline mRNA expression of PD1 and PDL1/2 in early-stage CLL patients, belonging to a prospective cohort (n=211, O-CLL1, clinicaltrial.govID:NCT00917540) was determined at diagnosis by GEP using the GeneChipVR Gene 1.0 STArray (Affymetrix) and was confirmed by QPCR. CLL clones derived from *ex vivo* exposure of autologous T-cells with anti-CD3/CD28 Dynabeads (Thermofisher) and IL2 (AAT) were used to mimic

the TME and model *in situ* T and B-cell interactions. Flow-cytometry (FC) was used to evaluate cellular phenotype (BD Biosciences). Cytokine levels were measured by FC (BD™Cytometric BeadArray, CBA). PEM was kindly provided by MERCK.
Results: CLL B-cells had higher mRNA transcripts of PD1 than PDL1 or PDL2m on GEP, which was confirmed by QRT-PCR performed in 20 samples of an independent cohort of 100 samples from B-CLL patients visiting our outpatient clinic. Baseline PD1/PDLs protein expression in these samples indicated that circulating CLL B-cells had similar levels of PD1 and PDL2, but limited PDL1 levels. Conversely, PD1 and its ligands in CD3+ cells showed higher expression of PD1, which was higher than that of malignant B-cells ($p<0.001$). CD4+ and CD8+ expressed comparable levels of PD1. CLL clones (n=15) exposed to TME stimuli by co-culture with autologous activated T-cells (AAT), monitored daily (range 2-7 days) for T-cell activation (identified as cell-clusters), showed a marked significant ($p<0.001$) up-regulation of PD1 ligand mRNA (PDL2>PDL1, 34.3±5.4-fold and 15.4±2.9-fold, respectively) in purified CD19+CD5+ compared to that of PD1 (4.7±0.9-fold). Similarly, cell surface protein expression (n=33) showed both ligands and PD1 were upregulated compared to baseline in B-cells with PDL2>>PDL1. T-cell subsets by FC detected stronger increase of CD3+, CD4+, and CD8+ cells bearing PDL1, and similar increment for either PD1 and PDL2+ in CD4+ or CD8+ cells following AAT co-cultures. In this context, PEM (0.37, 1.11, 3.3, 10.0 ug/mL) (n=8) did not influence the formation of AAT clusters on visual inspection nor PD1 /PDL1/2 protein and mRNA expression. PEM treatment (3.3-10 ug/mL) also failed affect IL-2, IFN-γ, or TNFα levels in supernatants, suggesting an attempt at reactivation of the T-mediated immune response was not possible in this strongly induced cellular context.
Summary/Conclusion: Our data suggest that TME-derived signals strongly induce gene and protein expression of PD1 and its ligands in both neoplastic B- and normal T-cells from CLL patients and PEM was unable to block PD1 axis protein and mRNA expression or cytokine secretion by T-cells in this context. Although, these unpromising results may be an artefact of the continuous superactivation of T-cells in our model, our data indicate that the presence of PEM failed to influence both the growth of CLL B-cells and the restoration of T-cell function. Drug combination studies are warranted.
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PS1071

DUAL MTOR KINASE INHIBITORS SYNERGIZE WITH IBRUTINIB TO INDUCE CLL APOPTOSIS, AND OVERCOME BCR- AND STROMAL-MEDIATED SURVIVAL ADVANTAGES CONFERRED IN THE MICROENVIRONMENT

M.W. Moles[1,*], E. Cosimo[1], H. Jorgensen[1], A. Michie[1]

[1]Paul O'Gorman Leukaemia Research Centre, University of Glasgow, Glasgow, United Kingdom

Background: Chronic lymphocytic leukemia (CLL) pathogenesis is inextricably linked to communication between CLL cells and non-malignant accessory cells of the tumor microenvironment (TME), conferring proliferative and pro-survival signals that mediate resistance to therapeutic agents and expansion of malignant clones. Therefore, inhibiting the signals orchestrating these events is key to disrupting disease progression, offering a potential cure for CLL. The Bruton's tyrosine kinase inhibitor, ibrutinib, has demonstrated excellent clinical activity in high-risk CLL patients through inhibition of B cell receptor-induced survival in the TME. However, resistance mutations in response to treatment have been demonstrated, underlining the importance of developing novel treatment strategies for this patient subgroup. The dual mTOR kinase inhibitor, AZD8055, selectively targets mTOR protein complexes, mTORC1 and mTORC2, which regulate essential cellular functions including cell growth, proliferation and survival. mTOR signalling is often deregulated in cancer, but little is known about its role in CLL pathogenesis. Interestingly, ibrutinib and AZD2014 (Vistusertib; a clinical analogue of AZD8055) synergize in diffuse large B cell lymphoma cell lines to enhance apoptosis.
Aims: We sought to examine whether combining ibrutinib with AZD8055/AZD2014 could represent a valid therapeutic approach for CLL under conditions mimicking the TME *in vitro*.
Methods: In the present study, we assessed the response of primary CLL cells to ibrutinib/AZD8055 treatment alone or in combination under culture conditions replicating TME signals: stromal cell cultures (NT-L); cultures mimicking T cell-CLL engagement (CD40L); and BCR ligation. We performed median-drug effect analysis to calculate combination indices (CI) to identify drug synergy between AZD2014 or AZD8055 and ibrutinib in CLL cells, and then established the impact of AZD8055 and/or ibrutinib on CLL cell viability under each TME condition. Finally, to delineate the molecular events underpinning the response to drug, substrate specificities of AZD8055 and/or ibrutinib downstream of mTORC1/2 were determined.
Results: The median drug-effect analysis revealed that both AZD2014 and AZD8055 synergize with ibrutinib ($CI < 1.0$) at clinically achievable doses in CLL cells. Treating BCR-ligated or NT-L-co-cultured CLL cells with AZD8055 or ibrutinib resulted in a significant reduction in cell viability, which was enhanced when combined. Although CLL cell proliferation was blocked, viability of CLL cells co-cultured on CD40L was not affected by AZD8055/ibrutinib alone or in combination. Analysis of drug selectivity for inhibition downstream of mTOR-mediated signalling revealed that AZD8055 reduced mTORC1 ($4EBP1^{T36/45}$, $S6^{S235/236}$) and mTORC2 (Akt^{S473}) activity in BCR-ligated or NT-L-co-cultured CLL cells and to a lesser extent on CD40L. Ibrutinib reduced phosphorylation of mTORC1/2 substrates in CLL cells upon BCR ligation, but to a lesser extent upon co-culture with NT-L or CD40L.
Summary/Conclusion: In conclusion, we report a synergistic interaction between ibrutinib and mTOR inhibitors in CLL cells, enabling enhanced apoptosis downstream of BCR- and stromal-signals. However, this could not be replicated in CD40L co-cultures. Analysis of drug selectivity downstream of mTORC1/2 suggests that AZD8055 inhibits molecular events required for cell proliferation. To this end, mTOR inhibitors may play a role in preventing CLL proliferation within the TME, and likely represents a promising therapy in combination.

PS1072

DEVELOPMENT OF SELECTIVE CASEIN KINASE 1 DELTA/EPSILON INHIBITORS FOR CANCER THERAPY

M. Gregorová[1,*], P. Janovská[1,2], V. Němec[3,4], M. Scholasterová[1], J. Verner[1,5,6], L. Poppová[5,6], Š. Pavlová[5,6], K. Plevová[5,6], Š. Pospíšilová[5,6], K. Paruch[3,7], V. Bryja[1,2]

[1]Institute of Experimental Biology, Faculty of Science, Masaryk University, [2]Institute of Biophysics, Academy of Sciences of the Czech Republic, [3]Department of Chemistry, CZ Openscreen, Faculty of Science, Masaryk University, [4], CZ Openscreen, Faculty of Science, St. Anne's University Hospital in Brno, [5]Center of Molecular Biology and Gene Therapy, Department of Internal Medicine–Hematology and Oncology, University Hospital Brno and Medical Faculty, Masaryk University, [6]CEITEC - Central European Institute of Technology, Masaryk University, [7]International Clinical Research Center, Center for Biomolecular and Cellular Engineering, St. Anne's University Hospital in Brno, Brno, Czech Republic

Background: Casein kinase 1 (CK1) δ/ε is a key component of both canonical and non-canonical Wnt pathways, which were shown to drive chronic lymphocytic leukemia (CLL) by several mechanisms (Wang *et al.*, 2014, Kaucka *et al.*, 2013). Also other types of cancer, such as breast, prostate cancer or others were previously shown to depend on Wnt/CK1 activity and the pathway inhibition was suggested as a potential form of therapy. In recent study, we showed that CK1 inhibition can block processes involved in CLL progression *in vitro* as well as in vivo in the Eμ-TCL1 mouse model of CLL (Janovska *et al.*, 2018). Importantly, CK1 inhibition showed synergistic effects with ibrutinib treatment in blocking chemotaxis of CLL cells, a process important for CLL pathogenesis and showed to be beneficial also in vivo.
Aims: Our previous *in vitro* and *in vivo* data showed that inhibition of CK1 activity is a valid approach to block key processes of CLL progression. Until now, our studies were based on commercially available inhibitors. However, these may often show low selectivity and may not be suitable for therapy of patients. Current work has focused on development of more active and selective compounds, which could be used in future in clinical practice.
Methods: Rational design, synthesis of novel compounds targeting CK1δ/ε, followed by *in vitro* and *in vivo* characterization of the novel inhibitors focused on toxicity towards cancer and normal cells, inhibition of cell migration and activity *in vivo* in mice.
Results: Rational design approach was used to design and synthesize novel inhibitors targeting CK1 δ/ε kinase. A set of ~70 compounds was characterized using a standardized set of assays focused on activity of CK1 in cells and cytotoxicity. Primarily, the experiments were focused on CLL and MCL, and showed that the tested compounds have significant anti-cancer activity with low toxicity towards normal healthy cells. *in vitro* testing showed high selectivity of the tested compounds towards CK1 δ/ε isoforms and very high activity, with IC50 values in nanomolar range. Selected compounds were tested *in vivo* in mice and were well tolerated, reaching plasma concentration in range effectively inhibiting CK1 kinase. Activity against other types of cancer (B-cell lymphomas, prostate, breast, pancreatic cancer) was also tested and showed that the rationale of targeting CK1 δ/ε may be applicable in several types of malignancies.

Summary/Conclusion: We have developed a set of highly active and selective CK1 δ/ε inhibitors, which show activity against multiple types of malignancies – e.g. CLL, B-cell lymphomas, prostate, breast and pancreatic cancer. With focus on CLL, we showed that these inhibitors have significantly higher toxicity towards CLL cells than to normal healthy B cells and efficiently block processes involved in CLL pathogenesis. *In vivo* testing of these compounds confirms their ability to efficiently inhibit CK1 activity.

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PS1073

Abstract withdrawn.

PS1074

NEXT-GENERATION SEQUENCING AND CRISPR/CAS9 APPLICATION TO ELUCIDATE THE IMPLICATIONS OF TP53 MUTATIONS IN DEL(13Q) CHRONIC LYMPHOCYTIC LEUKEMIA

M. Quijada-Álamo[1,2,*], M. Hernández-Sánchez[1,2], A.E. Rodríguez-Vicente[1,2], J.L. Ordóñez[1,2], S. González-Briones[1,2], A. Martín-Martín[1,2], I. Rodríguez-Iglesias[1,2], C. Pérez-Carretero[1,2], R. Benito[1,2], V. Alonso-Pérez[1,2], I. García-Tuñón[1,2], M. Martín-Izquierdo[1,2], J.M. Hernández-Sánchez[1,2], A. Montaño[1,2], C. Robledo[1,2], J.Á. Hernández[3], J.M. Hernández-Rivas[1,2]

[1]IBSAL, IBMCC, University of Salamanca, CSIC, Cancer Research Center, [2]Department of Hematology, University Hospital of Salamanca, Salamanca, [3]Department of Hematology, University Hospital Infanta Leonor, Complutense University of Madrid, Madrid, Spain

Background: *TP53* mutations can occur in up to 10% chronic lymphocytic leukemia (CLL) patients at diagnosis and 30-40% patients at relapse, being associated with short overall survival (OS), short progression free survival and chemotherapy resistance. However, the effects of *TP53* mutations in stable good prognostic CLLs have been less documented. Furthermore, the generation of *in vitro* models harboring the alterations detected by next-generation sequencing (NGS) in CLL patients would be useful to predict the response to treatments based on the patient mutational profile.
Aims: 1) To analyze the presence of *TP53* alterations by NGS and FISH in order to refine its impact in good prognosis del(13q) CLL cytogenetic subgroup. 2) To gain insight into the therapeutic implications of *TP53* inactivation in response to drugs currently used in CLL, generating *TP53*-mutated CLL cellular models by CRISPR/Cas9 technology.
Methods: The mutational status of *TP53* was analyzed by NGS in a cohort of 290 untreated CLLs, enriched for del(13q) cases (68.5%). In addition, the CRISPR/Cas9 system was used to generate *TP53* loss-of-function mutations in the CLL-derived HG3 cell line. Proliferation, apoptosis and cell cycle assays were performed to investigate the response of *TP53* wild-type (*TP53*wt) and *TP53*-mutated (*TP53*mut) cell lines to fludarabine and ibrutinib.
Results: 1) Mutations in *TP53* were observed in 7.2% of CLL patients. Overall, the frequency of alterations in this gene (either mutations or deletion) (*TP53*alt) was 8.3%. Within del(13q) subgroup, 7.6% (N=15) showed *TP53*alt. These patients presented significantly shorter OS than del(13q)-*TP53*wt cases (p=0.002). Additionally, 83.7% del(13q) patients harbored del(13q) as the sole cytogenetic aberration (del(13q)S) (N=164). Stratifying this CLL subgroup in terms of *TP53* mutational status, we observed that *TP53* mutations were associated with a reduced OS compared with del(13q)S-*TP53*wt patients (p=0.016). However, it is worth pointing out that a subset of patients (N=6) within del(13q)S-*TP53*mut group showed a high stable disease with no need of treatment and a similar OS compared to *TP53*wt cases (median not reached in both cases; p=0.575) . All of these cases carried *IGHV* mutated gene and their *TP53* mutations were predominantly subclonal. 2) Considering the NGS results, three HG3 cell lines (which harbors del(13q)) carrying different *TP53* frameshift mutations were generated using CRISPR/Cas9. Viability assays showed that HG3-*TP53*mut cell lines were more resistant to fludarabine than HG3-*TP53*wt cell lines (p<0.01). Annexin and cell cycle analyses revealed higher levels of apoptosis in HG3-*TP53*wt cells than in HG3-*TP53*mut cell lines (p<0.01) after 5 mM fludarabine exposure during 48 hours. By contrast, *TP53*mut cells responded to ibrutinib similarly as *TP53*wt cells, since no significant differences of viability were observed between HG3-*TP53*wt and HG3-*TP53*mut cells after ibrutinib treatment.
Summary/Conclusion: 1) *TP53* mutations detected by NGS allow us to better stratify the prognosis of del(13q) CLLs. 2) A subgroup of patients with del(13q)S-*TP53*mut-*IGHV*mut patients presents an stable disease despite the presence of *TP53* mutations. 3) CRISPR/Cas9 technology allow us to generate *in vitro* models with *TP53* mutations observed in patients in order to study the implications of these alterations in treatment response.

PS1075

ENCYCLOPEDIA OF CLL SUBSETS: AN ONLINE KNOWLEDGEBASE FOR SUBSETS OF CLL CASES WITH STEREOTYPED B CELL RECEPTORS

T. Reigl[1,*], K. Stranska[1,2], V. Bystry[1], A. Krejci[1], A. Grioni[1], S. Pospisilova[1,2], K. Plevova[1,2], N. Darzentas[1,3]

[1]CEITEC - Central European Institute of Technology, Masaryk University, [2]University Hospital Brno, Brno, Czech Republic, [3]Department of Internal Medicine II, University Hospital Schleswig-Holstein, Kiel, Germany

Background: Chronic lymphocytic leukemia (CLL) is documented as a biologically and clinically heterogeneous disease with varying clinical outcome. A related and remarkable phenomenon in CLL is the presence of subsets of patients with highly similar, or stereotyped, B cell receptors (BcR) – or 'CLL subsets' herein, which have been reported to involve one in three cases. Importantly, 'major' subsets (with >=20 cases in the studied cohort), involving one in eight cases, also exhibit homogeneity regarding both clinical presentation and outcome. Recently, assignment of new CLL cases to major subsets was included in the updated ERIC recommendations for immunoglobulin gene sequence analysis in CLL.
Aims: To construct and make publicly available a unique online knowledgebase, the 'Encyclopedia of CLL Subsets', for the interactive overview and breakdown of major CLL subsets in the context of up-to-date published information about their biological and clinical interpretation. To link this resource to ARResT/AssignSubsets, the online tool for the assignment of new CLL cases to those subsets.
Methods: We employ web technologies and appropriate bioinformatic and statistical methods to manage, analyse, and present data and results – e.g. R / Shiny, HTML / CSS / Javascript.
Results: The Encyclopedia of CLL Subsets [bat.infspire.org/arrest/subsets] is based on the currently available 19 major CLL subsets. Interactive visualisations provide access to immunogenetic and clinical data from published studies, with tooltips and links to relevant publications (Figure 1A). Literature collected as directly or indirectly related to CLL subsets currently comprises more than 240 entries and is available in interactive tables with highlighted mentions of subsets and of other useful, e.g. medical and immunogenetic, keywords (Figure 1B). Instructions and disclaimers assist users to understand the implications of using the resource, especially for clinical purposes. Finally, ARResT/AssignSubsets, the aforementioned online tool for assignment of new cases to these subsets, is bi-directionally linked to the Encyclopedia – this facilitates the primary usage scenario, i.e. the interpretation of the assignment of new CLL cases to the major CLL subsets.

Figure 1.

Summary/Conclusion: BcR stereotypy in CLL has important biomedical and clinical implications. The Encyclopedia of CLL Subsets is a unique online knowledgebase on these homogeneous groups of CLL patients in an otherwise heterogeneous entity. Around it, bioinformatic solutions enable researchers and clinicians alike to tap into this reference resource to get insights, link their data robustly, and collaborate productively.
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PS1076

ANALYSIS OF LOCUS-SPECIFIC LINE-1 AND ALU ELEMENT DNA METHYLATION REVEALS NOVEL EARLY EPIGENETIC CHANGES IN CHRONIC LYMPHOCYTIC LEUKAEMIA

T. Barrow[1,*], N. Wong Doo[2,3], R. Milne[3], G. Giles[3], E. Willmore[4], G. Strathdee[4], H.-M. Byun[5]

[1]Faculty of Health Sciences and Wellbeing, University of Sunderland, Sunderland, United Kingdom, [2]Concord Hospital, University of Sydney, Sydney, [3]Cancer Epidemiology Centre & Intelligence Division, Cancer Council Victoria, Melbourne, Australia, [4]Northern Institute for Cancer Research, [5]Human Nutrition Research Centre, Institute of Cellular Medicine, Newcastle University, Newcastle, United Kingdom

Background: Retrotransposons, such as LINE-1 (L1) and *Alu* elements, comprise more than 25% of the human genome. Their ability to retrotranspose throughout the genome is normally suppressed by epigenetic mechanisms. However, this repression is frequently lost in solid tumours through internal and external stimuli, and consequently somatic retrotransposition can be an initiating event in carcinogenesis. The epigenome in chronic lymphocytic leukaemia (CLL) is shaped by the maturation stage of the cell of origin, and its evolution during disease progression is correlated with the acquisition of genetic abnormalities associated with poor patient prognosis. Early work has demonstrated that L1 and *Alu* hypomethylation are associated with the acquisition of 17p deletions in CLL, but to date there has been no comprehensive or locus-specific analysis of retrotransposon DNA methylation.
Aims: To develop an approach to enable locus-specific analysis of L1 and *Alu* subfamily DNA methylation using the Illumina Infinium 450K microarray platform (H450K) and apply this to study aberrant methylation of L1 and *Alu* elements in CLL.
Methods: H450K probes mapping to retrotransposons were identified using RepeatMasker. The probeset was applied to a publicly-available dataset from a study of 138 CLL patients and 13 healthy individuals available from the International Cancer Genome Consortium. Leading hits were further analysed in Gene Expression Omnibus (GEO) datasets from 1,169 healthy individuals, 764 acute lymphoblastic leukaemia (ALL) patients, 174 acute myeloid leukaemia (AML) patients, and 31 diffuse large B-cell and Burkitt's lymphoma patients, and also prospective samples from 82 future CLL cases (<18 years from diagnosis) and 82 age-matched controls within the Melbourne Collaborative Cohort Study.
Results: We identified 9,549 probes mapping to 117 L1 subfamilies, and 12,806 mapping to 37 *Alu* subfamilies. In normal B-cells from healthy individuals, DNA methylation at these sites was routinely high (mean β: 0.75), with greater variation observed in older subfamilies (L1M and *Alu*J) in comparison to the youngest (L1H/L1PA and *Alu*Y), especially at CpGs within 200 bases of TSS. We identified 10,782 CpG sites within L1 and *Alu* sequences that were differentially methylated between CLL patients and healthy individuals (P_{fdr}<0.05), of which 55 were hypomethylated in >90% of CLL patients but never in healthy individuals. Hypomethylation of *Alu* elements was associated with evolutionary age, with older subfamilies (*Alu*J) displaying greater changes than younger ones (*Alu*Y). Hypomethylation of 17 leading hits was highly confined to CLL, never observed in healthy individuals and infrequently in ALL, AML and lymphoma. In prospective samples, methylation at each of the 17 loci, located across the genome, was highly correlated within individual patients. In contrast to diagnosed CLL patients, hypomethylation at the loci was observed in only 9 future CLL cases (11%). Notably, however, this was more commonly observed in samples taken <7 years before diagnosis (7 of 24, 29%) than in those taken more than 7 years before diagnosis (2 of 58, 3%).
Summary/Conclusion: We have identified locus-specific hypomethylation events of L1 and *Alu* elements that are highly frequent and specific to CLL, and which are present prior to diagnosis for some patients. Further work is required to establish how these epigenetic changes correspond to modulation of global DNA methylation patterns in leukaemogenesis.

PS1077

ILT3/DELTEX-1/SHIP-1 AS A NOVEL INHIBITORY AXIS CONTROLLING B CELL RECEPTOR SIGNALING IN CHRONIC LYMPHOCYTIC LEUKEMIA

V. Zurli[1], G. Wimmer[1], F. Cattaneo[1], V. Candi[2], E. Cencini[2], A. Gozzetti[2], D. Raspadori[2], G. Campoccia[2], F. Sanseviero[1], M. Bocchia[2], C.T. Baldari[1], A. Kabanova[1,*]

[1]University of Siena, [2]University Hospital of Siena, Siena, Italy

Background: The high proportion of long term non-progressors among CLL patients suggests the existence of a regulatory network which restrains the proliferation of tumor B cells.
Aims: In this work we undertook to identify determinants composing such network, which could be fundamental for our understanding of mechanisms that control CLL development.
Methods: We used transcriptomics, signaling and imaging studies to characterize ILT3, the immunoglobulin-like transcript 3, as an inhibitory receptor ectopically expressed on CLL cells and endowed with important regulatory function.
Results: The ectopic expression of ILT3 in CLL was a distinctive feature of neoplastic B cells and hematopoietic stem cells, thus identifying ILT3 as a selective marker of malignancy in CLL and the first example of phenotypic continuity between mature CLL cells and their progenitors in the bone marrow. ILT3 expression was found to be driven by Deltex1, a suppressor of antigen receptor signaling in lymphocytes. Triggering of ILT3 inhibited the activation of Akt kinase upon B cell receptor (BCR) stimulation. This effect was achieved through the dynamic coalescence of ILT3, BCRs, and phosphatase SHIP-1 into inhibitory clusters at the cell surface. Finally, our findings showed that in CLL cells ILT3 was co-expressed with other genes that might have tumour suppressing function.
Summary/Conclusion: Collectively, our findings define the mechanism which promotes the ectopic expression of ILT3 in CLL cells and indicate that ILT3 may functionally contribute to a regulatory network controlling tumour progression by suppressing the Akt pathway. Moreover, we hypothesize that ILT3 could be not only the marker of CLL cells but rather a marker of a tumour-suppressing gene expression program characterizing CLL cells. An in-depth analysis of such program will be provide insights into the mechanisms underlying the frequently observed indolent nature of CLL.

PS1078

DETIN: OVERCOMING TUMOR IN NORMAL CONTAMINATION

A. Taylor-Weiner[1,2,*], C. Stewart[1], T. Giordano[3], A. Macbeth[4], N. Lennon[4], E. Rheinbay[1], D.-A. Landau[5], C. Wu[6], G. Getz[17]

[1]Cancer Genomics, The Broad Institute, [2]Bioinformatics, Harvard University, Cambridge, [3]Department of Pathology, University of Michigan, Ann Arbor, [4]The Broad Institute, Cambridge, [5]Department of Medicine, Weill Cornell Medicine, New York, [6]Department of Medical Oncology, Dana-Farber Cancer Institute, [7]Department of Pathology, Mass General Hospital, Boston, United States

Background: A key step in sensitive and specific somatic mutation detection is comparison of the tumor sample to a matched germline control. Sensitivity to detect somatic variants is greatly reduced when the matched normal sample is contaminated with tumor cells. Procuring matched normal tissue for hematologic malignancies can be challenging. At times, the only available tissue may be contaminated with tumor cells. Tumor in normal (TiN) contamination can result from sampling techniques such as FACS sorting or skin tissue or saliva (that can be invaded by leukemic cells).
Aims: To overcome this limitation of somatic detection, we developed deTiN, a method that estimates TIN, and improves detection sensitivity when using a contaminated normal. We will demonstrate validation of deTiN's model as well as the effect of TiN contamination on genomic characterization of hematologic malignancies.
Methods: In order to experimentally validate deTiN, we performed an *in vitro* mixing experiment. We created 15 tumor and normal mixtures using a tumor (breast invasive ductal carcinoma, CRL-2321D) and a matched normal control (EBV-transformed lymphoblastoid, CRL-2362D) cell lines, with TiN ranging from 0.007 to 0.9. To examine the robustness and utility of deTiN in the analysis of hematological cancers, we applied deTiN to cohorts of CLL, AML and DLBCL patient derived sample pairs (N=1276).
Results: In the validation experiment, deTiN estimated the TiN mixture proportions with a mean absolute error of 0.019 (R^2=0.99, $p<10^{-4}$). TiN estimates were accurate across the full range of TiN values, including at the more common values of TiN<0.1 (mean absolute error = 0.005). We observed a significant decrease in mutation detection sensitivity when

using standard mutation calling protocols. Mutation detection sensitivity dropped below 50% for TiN values greater than 0.03. DeTiN was able to detect 96% of all known SSNVs using the ~0.03 contaminated normal compared to 52% without deTiN. Next we applied deTiN to cohorts of hematologic malignancies with matched normal tissues coming from a variety of sources including FACS sorted whole blood, saliva and skin biopsies. We detected varying levels of TiN across each of these sample collections. In CLL-normal sample pairs derived from patients (N=257), enrolled in a phase III clinical trial we found that TiN varied depending on patient disease status and control sample acquisition strategy. The mean fraction TiN observed in normals acquired by CD19- sorting was 0.028 (range= [0-0.17]). 78 of the 171 CD19- (46%) cases had TiN contamination greater than or equal to 0.02. In contrast, samples from patients who were MRD- exhibited no TiN contamination (mean=0;range=[0-0]). Finally, we show that deTiN is able to recover 20% more putative driver mutations in cohorts affected by TiN. deTiN recovered calls in both highly recurrent driver genes such as *SF3B1*, *ATM*, *TET2*, *ASXL1*, *JAK2* and *TP53*, as well as in significant genes mutated at lower frequencies such as *CHEK2*, *SAMHD1*, *XPO1*, *RUNX1*, and *FBXW7*. SSNVs and Indels identified by deTiN in these driver genes occurred in regions recurrently mutated in our cohort and in the COSMIC database, supporting their functional role in cancer.

Summary/Conclusion: We developed a novel method to detect TiN contamination and recover somatic events lost to this phenomenon. We validated the accuracy of deTiN's estimation of contamination using *in-vitro* mixing experiments and show that deTiN's model improves genomic characterization of hematologic malignancies.

PS1079

TRACKING THE MINOR CLONES IN OLIGOCLONAL CHRONIC LYMPHOCYTIC LEUKEMIA USING HIGH-THROUGHPUT IMMUNOPROFILING

K. Stranska[1,2,*], K. Plevova[1,2], H. Skuhrova Francova[1], H. Olbertova[1,2], V. Bikos[2], H. Jelinkova[1], E. Zapletalova[1], A. Mareckova[1], J. Kotaskova[1,2], B. Tichy[2], A. Komkov[3], G. Nugmanov[3], I. Mamedov[3], T. Reigl[2], N. Darzentas[2,4], H. Skabrahova[1], Y. Brychtova[1], A. Panovska[1], J. Mayer[1], M. Doubek[1,2], S. Pospisilova[1,2]

[1]University Hospital Brno, [2]CEITEC – Central European Institute of Technology, Masaryk University Brno, Brno, Czech Republic, [3]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Science, Moscow, Russian Federation, [4]University Hospital Schleswig-Holstein, Kiel, Germany

Background: In CLL, multiple productive IGH gene rearrangements are detected in 2–5% of cases by conventional low-throughput methods, rising to a quarter of cases using next-generation sequencing (NGS). We have shown at the single cell level in these cases that each productive IGH gene rearrangement denotes a separate B-cell clone. Typically, a major clone coexists with one or more minor ones. Shifts in clonal ratios and prevailing of initially minor clones in the course of the disease were shown repeatedly. It is, therefore, desirable to monitor them, although discrepancies exist among various methods used for their detection.

Aims: To test the capability of NGS immunoprofiling methods to detect minor clones in oligoclonal CLL, with focus on cases showing discrepancies using various methods, and cases with considerable clonal shifts during disease course.

Methods: Overall, 21 samples from 14 patients (7 patients were monitored in several time-points) were submitted to NGS immunoprofiling with DNA and RNA as a starting material: DNA IG NGS was performed by applying multiplex primers to IGHV and IGHJ genes, 20 ng of DNA per sample was used for library preparation; raw NGS reads were analyzed using ARResT/Interrogate. For RNA IG NGS, protocol based on template switching approach and using unique molecular barcodes was used; raw NGS reads were analyzed using MIGEC and in-house bioinformatics pipelines. The results from both methods were compared to those obtained using bulk sample Sanger sequencing and SCA.

Results: Only complete IGH rearrangements were analyzed as those were obtained by all methods applied. Overall, 19 samples from 12 patients and 9 samples from 9 patients were analyzed by DNA and RNA IG NGS, respectively, with 7 samples submitted to both methods. Median filtered-in read count for DNA IG NGS was 5,610 (1,885–13,461), median molecule count for RNA IG NGS was 4,521 (1,670–21,975).

All 9 minor clones that were detected discrepantly by either Sanger sequencing or SCA were confirmed with NGS immunoprofiling in both DNA and RNA. While DNA IG NGS clonal ratios largely corresponded to SCA results, we detected dramatic differences in 3/7 cases using RNA IG NGS, likely related to uneven IG RNA expression among clones. In 4 cases studied longitudinally, a clone disappeared in time as detected by Sanger sequencing. Using DNA IG NGS, we confirmed the clone presence in the initial sample in all cases (clonal ratios 0.06, 5.8, 4.8, and 27%), while it was undetectable in subsequent samples (obtained after 1, 3, 4, and 7 years). In other 4 longitudinally studied cases, a new clone emerged over time. We confirmed this observation by DNA IG NGS in two cases (changes in clonal ratios from 3.3% to 76.0%, and from 0% to 6.9% in 7 and 1 year, respectively). In the remaining two cases, DNA IG NGS did not detect the clone in both initial and subsequent sample, likely due to a technical error. Using RNA IG NGS, we detected additional clonotypes on top of those expected in 3 cases; DNA IG NGS performed in two of them did not confirm their presence. On the other hand, all 3 new clonotypes recorded in 3 cases using DNA IG NGS were confirmed, either by their detection in two subsequent samples, or in one case by RNA IG NGS, although in negligible fraction.

Summary/Conclusion: Detection of minor clones in oligoclonal CLL is problematic and its efficiency varies with different approaches. Their significance is still unclear and should be investigated in more detail.

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PS1080

SINGLE CELL ABNORMALITIES ON CLASSIC G-BANDING ANALYSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA: A NEW DIAGNOSTIC AND BIOLOGICAL CHALLENGE?

A. Athanasiadou[1,*], M. Gkaitatzi[1], G. Papaioannou[1], M. Iskas[1], A. Xochelli[2], O. Asteriou[1], Z. Lazarou[1], N. Stavroyianni[1], A. Hadzidimitriou[2], A. Anagnostopoulos[1]

[1]Department of Hematology & BMT Unit, George Papanicolaou Hospital, [2]Institute of Applied Biosciences, CERTH, Thessaloniki, Greece

Background: Chromosomal abnormalities have a well accepted clinical and prognostic value in chronic lymphocytic leukemia (CLL). Occasionally, conventional G-banding cytogenetics in CLL can detect chromosomal aberrations that do not fulfill clonality criteria.

Aims: Here we aim to assess single cell abnormalities (sc-abs) whose significance, frequency and type have not yet been identified.

Methods: We studied 725 primary CLL samples subjected to G-banding analysis by two parallel cultures with the addition of either Phytohemagglutinin and Interleukin 2 (PHA/IL-2) or an immunostimulatory CpG-oligonucleotide DSP30 and IL-2 (CpG/IL-2).

Results: Overall, 1450 cultures were set up. Sc-abs were detected in 284/725 (39%) samples. One sc abnormal karyotype was detected in 141 samples, 2 in 70 samples, >2 in 73 samples (10%) and >4 in 29 samples. Of 284 samples with sc-ab, 219 had sc-abs in PHA/IL-2 and 113 in CpG/IL-2 cultures. More than 2 sc-ab were revealed in 46 in PHA/IL-2 and 12 in CpG/IL-2 cultures. Overall, 616 sc-ab were detected and of these 224 (36.3%) were present in samples with abnormal karyotype while the remaining 392 (63.6%) in samples with normal karyotype. Amongst 616 sc-ab samples, 167 (27.1%) had a complex karyotype and 449 (72.8%) a non-complex one. Out of 616 sc-abs, 326 (52.9%) were structural, 125 (20.3%) were numerical and 165 (26.8%) were both structural and numerical. All chromosomes participated in these sc-abs. Fifty sc-abs concerned evolution of a detected abnormal clone. Since two culture types were used and PHA/IL-2 cultures have been already reported to be related with sc-ab detection, we further studied the 73 samples containing >2 abnormalities. Distribution of the number of sc-abs across the samples was as follows: 3 sc-abs were detected in 34 samples, 4 in 10, 5 in 11, 6 in 9, 7 in 3, 8 in 1, 9 in 3 and 12 in 2. Forty-eight of these 73 samples had normal karyotype: amongst these 17 had ≥ 5 abnormalities. The remaining 25 samples had abnormal karyotype: 12 had ≥5 sc-abs (p=0.29). In these 73 samples, sc abnormalities were revealed in 65 samples by PHA/IL-2 culture and 43 by CpG/IL-2 culture. Sc-abs were detected only in 7 samples cultured with CpG/IL-2 and in 30 samples cultured with PHA/IL2. Forty-eight samples were checked for 13q deletion:17 and 6 samples had single and double del(13q), respectively. Sequence analysis for IGHV somatic hypermutation status was available in 65/73 cases: 39 were IG-unmutated and 26 were IG-mutated. Clinical data was available in 59/73 cases. Time from diagnosis to testing ranged between 0-174 months (median: 44); 17/59 samples were diagnostic, while 31/59 samples involved treatment naïve patients; of these 13 subsequently progressed.

Summary/Conclusion: Sc-abs seem to be a common finding in CLL patients regardless of treatment status probably suggesting genomic heterogeneity and instability. They are distributed across all chromosomes and are mainly structural. Clearly the issue of sc-abs should be systematically addressed in larger patient cohorts and the combination of PHA/IL2 and CpG/IL-2 cultures will help to acquire more comprehensive genetic data by revealing sc-abs that are more frequent in PHA/IL2 cultures.

PS1081

HIGHER RESPONSIVENESS OF CLL CELLS TO B-CELL RECEPTOR STIMULATION IS ASSOCIATED WITH REDUCED EXPRESSION OF INHIBITORY MOLECULES OF THE NF-κB PATHWAY

R.W. Meijers[1,*], A.F. Muggen[1], L.G. Leon[1], O.B. Corneth[2], R.W. Hendriks[2], J.J. van Dongen[1], A.W. Langerak[1]

[1]Department of Immunology, Laboratory Medical Immunology, [2]Department of Pulmonary Medicine, Erasmus MC, Rotterdam, Netherlands

Background: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease based on both clinical (manifestation, disease course) and biological characteristics. We previously described (Muggen *et al*., Leukemia, 2015) that compared to normal B cells, human CLL cells have higher basal Ca^{2+} levels and respond poorly to B-cell receptor (BCR)-stimulation. In addition, we found differences in Ca^{2+} signaling among CLL cases, both in basal Ca^{2+} levels and levels upon B-cell receptor (BCR)-stimulation. Such differences in BCR responsiveness could reflect heterogeneity in CLL pathogenesis due to cell-intrinsic factors.
Aims: Here we aim to elucidate potential cell-intrinsic differences between CLL patients showing a good or a poor response upon BCR stimulation.
Methods: In peripheral blood CLL cells from 52 CLL patients the BCR responsiveness was determined *ex vivo* based on Ca^{2+} influx upon α-IgM stimulation. Phosphorylation levels of various signaling molecules downstream of the BCR, as well as the cell surface expression of markers associated with anergy and activation were assessed by flow cytometry. Transcription profiling of responsive CLL cases (n=6) and unresponsive CLL cases (n=6) was performed by RNA sequencing. RQ-PCR was used to validate transcriptional differences between these two CLL groups, as found by RNA sequencing.
Results: We observed that the increase in Ca^{2+} after α-IgM stimulation was accompanied by a higher phosphorylation of PLCγ2 and Akt molecules. Next to that, we found a significant positive correlation between the responsiveness to α-IgM and the expression levels of CD21, CD38, CD80, and CD27, which suggests that the α-IgM responsive CLL cells have a more activated cell signature. RNA sequencing revealed differences between the two groups of CLL patients, especially in the expression of NF-κB pathway genes. RQ-PCR validation of differentially expressed NF-κB pathway genes in an additional group of responsive and unresponsive CLL cases confirmed the significantly lower expression of the critical NF-κB inhibitors *NFKBIB* (p=0.021) and *NFKBIE* (p=0.009) genes in BCR-responsive CLL. Likewise, expression of the potential NF-κB inhibitors *NFKB1* (p=0.017) and *NFKB2* (p=0.009) was reduced in BCR-responsive CLL cases. Although no statistical differences were found in the expression of genes coding for the NF-κB subunits (*REL*, *RELA* and *RELB*), we did find strong correlations between the expression level of NF-κB inhibitors and the NF-κB subunits *REL and RELA*.
Summary/Conclusion: From our data we conclude that BCR-responsive CLL have a more activated cell surface phenotype and reduced expression of several components of the canonical NF-κB pathway that have been associated with inhibition of NF-κB signaling. These findings suggest that enhanced NF-κB activation may be critical for the responsive capacity of CLL cells to BCR stimulation, which would be in concordance with recent literature stating that progressive CLL cases display aberrant NF-κB signaling.

PS1082

COMPARISON OF CELLULAR AND CELL-FREE DNA FOR THE DETECTION AND MONITORING OF BTK MUTATED CLL CLONES IN PATIENTS WITH SUBSEQUENT THERAPY AFTER IBRUTINIB FAILURE

W. Schier[1,*], J. Christmann[2], H. Hannig[2], J. Krauter[1], A. Trummer[1]

[1]Hematology and Oncology, [2]Center for molecular diagnostics, Städtisches Klinikum Braunschweig, Braunschweig, Germany

Background: Ibrutinib is an orally available BTK inhibitor approved for the treatment in CLL patients. In patients with progression under ibrutinib, CLL clones with BTK and PLCG2 mutations, causing resistance towards ibrutinib, can be detected in up to 87 percent of cases. Only very few data are available regarding the monitoring of these resistant clones under subsequent therapies, e. g. venetoclax, and the sensitivity of cellular versus cell-free plasma DNA (cf-DNA) after reduction of peripheral lymphocyte counts.
Aims: To monitor BTK mutated CLL clones under the subsequent therapy with RT-PCR using DNA copy numbers and to compare the detection sensitivity of this method applying cellular or cf-DNA.
Methods: From July 2016 until February 2018, blood samples were taken simultaneously in EDTA and cf-DNA tubes from seven CLL patients at the time of progress under ibrutinib and at different time points thereafter during the subsequent therapy. DNA copy numbers were determined by using a modification of a published RT-PCR protocol with the aid of sequence specific primers for BTK C481S mutation or wildtype sequence and a fluorescence-labelled detection probe. Absolute copy number quantification was enabled by short control fragments (105nt) for mutated and wildtype DNA of which known amounts were included in a simultaneous RT-PCR reaction. Results are therefore given as a ratio of mutated to wildtype fragments (MUT/WT ratio). CLL samples without the BTK-C481S mutation (in Exon 14) were screened for BTK mutations in Exon 11, 15 and 16 as well as a PLCG2 mutation in Exon 19, 20 and 24 by Sanger sequencing.
Results: The seven patients received ibrutinib for a median of 26.4 (range: 16-33) months before progression. Mean follow-up after mutation analysis was 5.4 (range: 1-13.4) months. Three patients died under therapy with venetoclax, all due to Richter's transformation (RT). In five out of seven patients (71.4%), we were able to detect a mutation causing ibrutinib resistance: four showed a BTK-C481S mutation and one a PLCG2-G667E mutation, which has not been described so far and belongs to the SH2 domain. Within the four BTK mutated patients, three showed similar copy numbers and MUT/WT ratios for cellular and cf-DNA at time of progression, while in one, with a very low copy number, detection was only successful in cellular DNA. During the subsequent therapies (3x venetoclax, 1x R-CHOP for RT), MUT/WT ratios rapidly decreased but only one became undetectable. In 3 of 4 samples with a low copy number (<1000) using cellular DNA, cf-DNA showed no detectable mutation. So far, no increase of the MUT/WT ratio has been observed in the 4 patients remaining in clinical remission.
In the patient with the PLCG2-G667E mutation, ibrutinib was continued for further 5.7 months as progression was slow. Finally, the patient also had to be switched to venetoclax.
Summary/Conclusion: We were able to confirm the high incidence of BTK and PLCG2 mutations in CLL patients with progression under ibrutinib. In our setting, usage of cell-free DNA appears to be less sensitive compared to cellular DNA. Thus, for the monitoring of the BTK-C481S mutation during subsequent therapy, cellular DNA is still preferable, even when lymphocyte counts decline. Monitoring of ibrutinib resistant CLL clones during Venetoclax might be helpful in tailoring therapy in case of new progression.

PS1083

NOVEL STRATEGIES OF TARGETING P53 RELATED VULNERABILITIES IN T-PLL CELLS

J. Von Jan[1,*], E. Andersson[2], A. Schrader[1], B. Yadav[2], P. Mayer[1], S. Mustjoki[2], M. Herling[1]

[1]Department I of Internal Medicine, Center for Integrated Oncology (CIO) Köln-Bonn, University of Cologne (UoC), Köln, Germany, [2]Helsinki University Hospital Comprehensive Cancer Center, Helsinki, Finland

Background: T-cell prolymphocytic leukemia (T-PLL) is a mature T-cell neoplasm commonly presenting at an aggressive phase associated with a marked chemotherapy resistance. Nucleosides and alkylators show very limited clinical activity. Although the monoclonal antibody alemtuzumab induces high response rates, virtually all patients relapse and the mostly elderly individuals are often not eligible for allogeneic stem cell transplantation. Overall, the treatment options for T-PLL are scarce and its general prognosis with average survival times of <3 yrs. remains poor. We could show that although genomic *TP53* aberrations in T-PLL are rare (8% in our series), T-PLL cells fail to generate a distal pP53 response upon y-irradiation. Based on the confirmed expression of *wildtype* p53 protein in T-PLL, we conclude that this master regulator of a (therapeutic) damage response is retained in its inactive state by regulatory factors, *i.e.* by its suppressor MDM2.
Aims: We plan to expand the portfolio of p53 reactivators and other non-genotoxic substances to study those and their most synergistic combinations in rigorous pre-clinical test systems, including our T-PLL mouse models.
Methods: - CellTiter-Low® Luminescent Cell Viability Assay
- FACS analysis (7AAD/AnnV Staining)
- Immunoblotting
Results: In an *ex vivo* drug screen (306 substances, n=39 cases) we established a high efficacy of p53 re-activating agents from the class of p53 de-repressing MDM2 Inhibitors, e.g. Nutlin-3 or Serdemetan with an average sDSS of 7 and 9, respectively. The DSS is a drug sensitivity score based on the measured integrative and robust dose-response area under the curve. The sDSSs as the selective DSSs presents the leukemia-specific responses, comparing DSS from patient samples to the median DSS of healthy donors. We then tested in more detail the potential of such p53 reactivators as possible novel therapeutic approaches. Idasanutlin, an MDM2 inhibitor in clin-

ical testing for AML, activated the repressed p53 in T-PLL cells as shown by pSer15p53 immunoblots. This effect was enhanced by combination with the alkylating agent Bendamustine or the HDAC inhibitor Panobinostat. The latter contributed to p53 reactivation by conferring a higher net-acetylation state. Flow cytometry based evaluations of the pro-apoptotic potential identified Idasanutlin and Panobionstat as most effective single substances (LD50s Idasanutlin / Panobinostat: 0.6 μM/0.15 μM) out of our selected panel. In a small *in vivo* pilot study, we confirm the efficacy of Idasanutlin against murine T-PLL (WBC vehicle ctrl. vs Idasanutlin, p=0,003). A second *ex vivo* screen designed for synergy detection (n=13 T-PLL cases) identified Idasanutlin and Panobionstat as highly effective single agents. In agreement with the marked pSer15p53 induction induced by this combination (above), both agents combined showed a significant over-additive effect (ZIP score: 5.65, measured with the zero interaction potency (ZIP) reference model).
Summary/Conclusion: Reconstitution of a lost TP53 response in T-PLL cekks seems highly synergistic in a combination with inhibited deacetylation. Combination of a clinical-grade TP53 derepressor and HDACi appears as an attractive rationale for a future clinical trial in T-PLL.

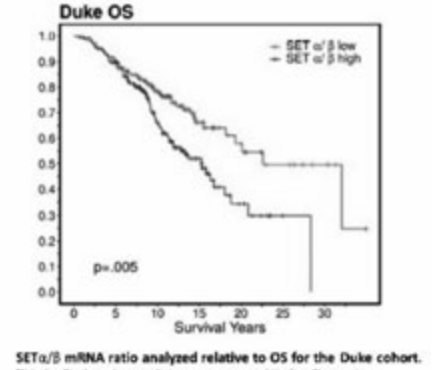
PS1084

HIGH SET ALPHA TO SET BETA ISOFORM EXPRESSION IS ASSOCIATED WITH INFERIOR OUTCOMES AND DISCRIMINATES FAVORABLE RISK IN TWO INDEPENDENT CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) COHORTS

D. Brander[1,*], A. Volkheimer[1], D. Christensen[1], L. Rassenti[2], T. Kipps[2], E. Guadalupe[1], Y. Chen[1], D. Zhang[3], X. Wang[3], K. Owzar[3], C. Davis[3], D. Friedman[1], J.B. Weinberg[1]

[1]Medicine, Duke University, Durham, [2]Medicine, UCSD, San Diego, [3]Duke University, Durham, United States

Background: SET oncoprotein, a physiological inhibitor of the most abundant serine-threonine phosphatase PP2A, has increased activity in malignancies. Investigators of SET have focused on total levels, without study of different SET isoforms. We previously demonstrated that the ratio of SET isoform (SETα and SETβ) protein levels by immunoblotting is significantly associated with overall survival (OS) and time to first treatment (TTFT) in CLL, and aimed to examine the importance of SET isoform mRNA expression by a novel assay in two large, independent cohorts.
Aims: 1. Examine the association of isoform expression of SET-α/β mRNA to clinical outcomes in two independent CLL patient cohorts
2. Test for interaction effect of SET-α/β mRNA ratio with established CLL risk biomarkers
Methods: We studied CLL patients at the Duke Univ/Durham VA (Duke) and the Univ California San Diego (UCSD) on IRB-approved protocols. Primers and probes for RT-qPCR were designed to amplify only the alpha or beta forms of SET. The SET α/β ratio was analyzed as a continuous variable for the primary analysis and as a binary variable (high *vs* low compared to the cohort median value; median SETα/β ratio=0.94 for Duke and 0.93 for UCSD) for visual display (Figure 1). Score test p-values and Wald test p-values were reported for univariate analysis and interaction analyses, respectively; Cox proportional hazard regression was used for the time to event analyses. Interactions of the dichotomous SET-α/β mRNA ratio with IGHV mutational status, FISH groupings (unfavorable FISH included any del17p, del11q, or 3 or more abnormalities), CD38 positivity and Rai staging were performed in the larger Duke cohort.



SETα/β mRNA ratio analyzed relative to OS for the Duke cohort. This is displayed as a dichotomous variable for illustrative purposes with the respective p-value. Duke patients with higher SET-α/β mRNA ratio as a continuous variable also had a significantly shorter OS.

Figure 1.

Results: The Duke combined population (n=307) was analyzed independently from the validation cohort of UCSD patients (n=166). The two cohorts were representative of other CLL populations, and there were no significant differences in median SET mRNA ratio or patient characteristics between cohorts. Duke patients with high SET-α/β mRNA ratio as continuous and dichotomous variable had significantly shorter TTFT [[HR 1.2 (1.2-1.3), p=0.001] and [HR 1.7 (1.3-2.3), p<0.001] for continuous and dichotomous, respectively]. As a dichotomous variable, Duke patients with high SET mRNA isoform ratio also had significantly shorter OS [HR 1.7 (1.2-2.5), p=0.005]. In the independent UCSD validation cohort, high SET-α/β mRNA ratio as a continuous and dichotomous variable was also significantly associated with shorter TTFT [[HR 1.3 (1.0-1.6), p=0.02] and [HR 1.8 (1.2-2.7), p=0.006 for continuous and dichotomous, respectively]. There was not a significant association of high SET mRNA ratio with shortened OS in the UCSD group; analysis for OS may have been limited due to a lower number of deaths at follow-up in this cohort. In Duke patients within the favorable risk mutated *IGHV (M-IGHV)* patients, those with high SET-α/β mRNA ratio had a shorter TTFT (significant interaction, p=.01). Similarly, in Duke patients with favorable FISH, high SET-α/β mRNA ratio predicted a shorter TTFT (significant interaction, p=.01).
Summary/Conclusion: SET-α/β mRNA isoform ratio expression modulates CLL aggressiveness and determines CLL outcomes of TTFT and OS in two large, independent CLL patient cohorts. High SET-α/β mRNA ratio adds valuable prognostic information and can discriminate within favorable risk groups those patients with poorer outcomes similar to the UM-*IGHV* and unfavorable FISH groups, respectively. SET is targetable, not just predictive, and could lead to new strategies in combination therapy.

PS1085

NOTCH1 MUTATIONS ARE AN INDEPENDENT PROGNOSTIC FACTOR IN IBRUTINIB- TREATED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS

G. Del Poeta[1,*], M. Postorino[1], M.I. Del Principe[1], F. Pozzo[2], E. Santinelli[1], R. Bomben[2], L. Pupo[1], F. Buccisano[1], M.F. Rossi[2], L. Maurillo[1], A. Biagi[1], A. Venditti[1], W. Arcese[1], P. de Fabritiis[1], M. Cantonetti[1], S. Amadori[1], A. Zucchetto[2], V. Gattei[2]

[1]Hematology, University Tor Vergata, Rome, [2]Clinical and Experimental Hematology Unit, CRO IRCCS, Aviano (PN), Italy

Background: Ibrutinib, an oral, selective inhibitor of Bruton's tyrosyne kinase (BTK), demonstrated exceptional activity in patients with relapsed/refractory CLL as well as in untreated patients with genetic abnormalities which predict chemoresistance (i.e. *TP53* deletion or mutation). In fact, overall response rate (ORR), progression-free survival (PFS), overall survival (OS) were far superior in comparison with chemoimmunotherapy (Byrd, 2014). However, CD49d+ CLL were characterized by inferior PFS than CD49d- CLL with a significant lower reduction in organomegaly and lymph nodes size (Tissino, 2018). Even more, CD49d expression was strongly correlated with *NOTCH1* mutations (M) cases that significantly overexpressed the NF-kB pathway genes as compared to *NOTCH1* wild type (WT) CLL (Benedetti, 2017) and thus may confer poor response to ibrutinib in *NOTCH1* M CLL.
Aims: The primary aims of our research were: 1) to verify the correlations of *NOTCH1 M* with other biological and clinical prognosticators in patients treated with ibrutinib; 2) to evaluate the impact of *NOTCH1 M* on ORR, PFS and OS; 3) to confirm *NOTCH1 M* as an independent prognostic factor.
Methods: *NOTCH1 M* were investigated with ARMS PCR for c.75447545delCT or by Sanger sequencing of *NOTCH1* exon 34 and all cases were confirmed with next generation sequencing (NGS).
Results: Therefore, we evaluated the efficacy of ibrutinib, in a real-life contest, on 82 patients, median age 59 years (38-85), median number of previous regimens 2 (0-4; 13.4% previously untreated). Patients received 420 mg oral ibrutinib once daily until progression or occurrence of unacceptable side effects. Median follow up on ibrutinib was 13 months. ORR was 94% (complete response (CR): 20%, partial response (PR): 42%, PR with lymphocytosis (PR-L): 31%). The estimate 1-year PFS and OS were 79% and 83%, respectively. Twenty patients (24%) discontinued ibrutinib therapy due to progression (n=12), adverse events (n=6) or due to other reasons (n=2). Five out of 6 patients with Richter's syndrome (RS) had baseline del(17p)/*TP53* M (p=0.020). PFS was significantly better for patients in first/second lines vs later lines of therapy (p=0.033). Twenty-six patients were *NOTCH1 M* (32%). *NOTCH1 M* were significantly correlated with trisomy 12 (9/13; p=0.008), with CD49d >30% (24/26; p=0.0004) and with unmutated *IGHV* (23/26; p=0.020). With regard to clinical outcome, PR was significantly correlated with *NOTCH1 M* (p=0.008). Interestingly, lym-

phocyte counts were significantly lower in *NOTCH1* M patients both before and after ibrutinib therapy (p=0.0018 and p=0.019, respectively). On the other hand, burden disease was significantly higher in *NOTCH1* M patients both before and after ibrutinib therapy (p=0.025 and p=0.001, respectively). Significant shorter PFS was observed in *NOTCH1 M* patients (52% vs 87% at 24 months, p=0.0001; Figure 1). On the contrary, del(17)p/*TP53* M patients did not show significant worse PFS (p=0.097). Moreover, patients with *NOTCH1* M showed significant shorter OS (56% vs 80% at 24 months, p=0.018; Figure 1). In multivariate analysis of PFS and OS including del(17)p/*TP53*, *IGHV* status and age, only *NOTCH1 M* were confirmed as an independent prognostic factor (p=0.011 and p=0.009, respectively).

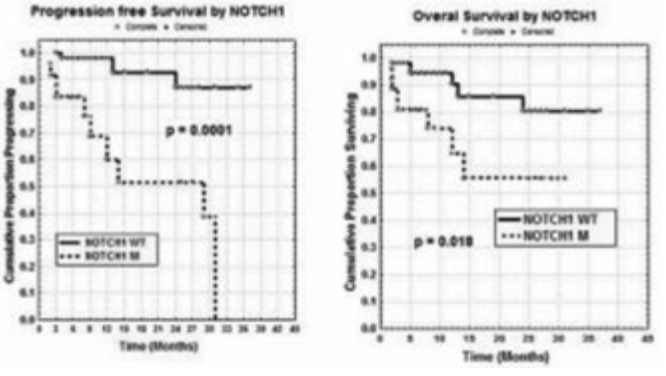


Figure 1.

Summary/Conclusion: Therefore, bcl-2 antagonists, such as venetoclax, working through a different pathway, could be combined with BTK inhibitors, in order to overcome resistance in some patients treated with ibrutinib, particularly within the CLL subset characterized by *NOTCH1 M.*

PS1086

INTEGRATED EXPRESSION PROFILING CHARACTERIZES BIOLOGICAL HETEROGENEITY IN REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA AND THE RISK OF RICHTER TRANSFORMATION

J. Bloehdorn[1,*], M. Sill[2], B.M.C. Jebaraj[1], K. Holzmann[1], A. Rouhi[1], F. Kuchenbauer[1], J. Bahlo[3], S. Robrecht[3], K. Fischer[3], B. Eichhorst[3], J. Edelmann[4], C. Schneider[1], L. Bullinger[5], M. Hallek[3], A. Benner[2], D. Mertens[1], H. Döhner[1], S. Stilgenbauer[1]

[1]Internal Medicine III, University Hospital Ulm, Ulm, [2]Division of Biostatistics, German Cancer Research Center (DKFZ), Heidelberg, [3]Department I of Internal Medicine and Center of Integrated Oncology Cologne Bonn, University Hospital Cologne, Cologne, Germany, [4]Barts Cancer Institute, Queen Mary University of London, London, United Kingdom, [5]Medical Clinic for Hematology, Oncology and Tumor Biology, Charite University Hospital, Berlin, Germany

Background: Impairment of TP53 is detected in up to 50% of refractory CLL cases and is associated with short survival after conventional chemoimmunotherapy. Resistance mechanisms also involve changes in microRNA expression, e.g. mir34a.
Aims: To investigate the transcriptional architecture of refractory CLL, we characterized a large multicenter treatment trial population by using combined mRNA and microRNA expression profiling.
Methods: The study was conducted on samples from refractory patients treated with alemtuzumab (CLL2H trial, multicenter, open-label, phase 2) and validated on treatment naïve samples (CLL8 trial, multicenter, open-label, phase 3). Profiling was performed on the Agilent Human microRNA Microarrays (miRBase Release v14) (n=58, refractory) and for the same refractory sample (n=51 with sufficient RNA available) or treatment naïve cases (n=337) with Affymetrix Human Exon 1.0 Arrays.
Results: Unsupervised clustering was used for class discovery on microRNA expression profiles and identified 3 independent clusters. Best response after alemtuzumab treatment with highest PR rate was observed for C1 (46%), while lowest PR rate was found in C3 (23%). Median progression-free survival (PFS) was 450 (C1) vs. 242 (C2) and 133 days (C3), median overall survival (OS) was 1319 (C1) vs. 629 (C2) and 466 days (C3). Inferior outcome in C2/C3 was not associated with genetic alterations. Richter transformation was observed for 11 of 58 cases (18.9%) of which 9 occurred in the C3 group, i.e. 30% of C3 cases transformed. Average time to transformation was 196 days (median: 96, range 0-824). Adverse prognostic impact of C3 classification was retained when cases with transformation were separated as single entity in the survival analysis. Investigating mRNA gene expression signatures using GSEA, we identified in C3 overexpression of genes coding for receptors or downstream effectors involved in transmembrane signaling, e.g. BCR components (BTK, CD79B, SYK), the TLR (TLR7, TLR10) and CD52. Genes overexpressed in C2 were functionally assigned to processes in the mitochondrial compartment and involved in the metabolism of hemoglobin (ALAS2, FECH, SLC25A39, BLVRB, HBG1, HBD, HBM, BCL2L1). Subsequent analysis on biologic features implies that C2 cases may be especially exposed to oxidative stress since corresponding transcriptional changes are involved in the antioxidative response and metabolic adaption including a deregulation of HIF1α. Using the cluster-defining genes identified in refractory CLL in a semi-supervised clustering of treatment naïve cases (n=337) from CLL8, showed identical expression patterns of clinical impact. With regard to respective clusters, OS was significantly different (p=0.04) with median OS in C1: not reached; C2: 77.6 months; C3: 86.4 months; Similar, PFS was different (p=0.01) with median PFS in C1: 59.1 months; C2: 36.1 months; C3: 35.9 months.
Summary/Conclusion: In conclusion, refractory CLL can be categorized in transcriptional subgroups with distinct biological features and predisposition to Richter transformation, independent of genetic variables. Being present both in refractory and untreated CLL, these signatures indicate the existence of biologic processes involved in the transformative process of CLL early in the disease course.

PS1087

DISTINCT DNA METHYLATION PROFILES IN DIFFERENT CYTOGENETIC SUBGROUPS OF CHRONIC LYMPHOCYTIC LEUKEMIA: THE INTRIGUING CASE OF TRISOMY 12

M. Tsagiopoulou[1,2,*], N. Papakonstantinou[1], M. Duran-Ferrer[3], V. Chapaprieta[3], T. Moysiadis[1], F. Psomopoulos[1], P. Kollia[2], E. Campo[3], K. Stamatopoulos[1], J.I. Martin-Subero[3]

[1]Institute of Applied Biosciences, Centre for Research and Technology Hellas (CERTH), Thessaloniki, Greece, [2]Department of Biology, National and Kapodistrian University of Athens, Athens, Greece, [3]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Universitat de Barcelona, Barcelona, Spain

Background: The pathogenesis of chronic lymphocytic leukemia (CLL) reflects an interaction between microenvironmental, genetics and epigenetics. CLLs show different cytogenetic alterations with clinical value, but it is unclear whether distinct CLL cytogenetic subgroups have different epigenetic landscapes.
Aims: We analyzed whether DNA methylation (DNAm) profiles can be linked to the pathophysiology of particular cytogenetic subgroups.
Methods: Using 450K methylation arrays, we investigated the poor prognosis del(11q) [n=29: 3 IG-mutated CLL (M-CLL)|26 IG-unmutated CLL (U-CLL)], the heterogeneous trisomy 12 [tris12, n=45: 17 M-CLL|28 U-CLL] and the favorable del(13q) [n=181: 131 M-CLL|50 U-CLL], as well as normal naïve (NBC, n=5) and memory (MBC, n=5) B cells. Moreover, methylation data were analyzed in the context of ChIP-seq data from MBC (Queiros *et al.*, Cancer Cell 2017) and expression arrays (69/250 CLLs).
Results: Principal Component Analysis (PCA) disclosed a distinct DNAm pattern for tris12 vs other cytogenetic subgroups, which was independent of IGHV gene somatic hypermutation status. We found 1760 CpGs contributing to the differences observed in the PCA; these findings were corroborated by hierarchical clustering analysis. We next performed differential methylation (DM) analysis (db=|0.4|, FDR<0.01) of each cytogenetic subgroup vs normal B cells. We observed 5409 DMCpGs in del(11q), 4497 in tris12 and 3412 in del(13q) cases, which frequently reflected a general CLL signature, as 2886 DMCpGs were shared in the three groups. The 685 hypomethylated CpGs unique to tris12, which were enriched in heterochromatin, enhancers, gene bodies and outside of CpG island (CGI), segregated tri12 cases from normal B cells and non-tris12 CLLs. The genes associated with these DMCpGs showed significant enrichment (FDR<0.001) in gene ontology (GO) terms, e.g. actin and kinase binding, glucuronosyltransferase activity, MHC protein binding. Additional DM analysis (db=|0.25|, FDR<0.05) of each cytogenetic subgroup vs all other CLLs revealed significant differences for tris12 vs non-tris12 (285 DMCpGs based on all CLLs, 646 DMCpGs based on U-CLL). In U-CLL, 523 hypo- and 123 hypermethylated CpGs were identified and targeted gene bodies outside CGIs. Hypermethylation was associated with polycomb-repressed regions and strong enhancers whereas hypomethylation was related to weak and strong enhancers. GO analysis showed that hypomethylated genes were enriched (FDR<0.001) in cytoskeletal protein, actin and kinase binding; and hypermethylated genes in transcription factor and histone deacetylase activity. DNA methylation correlated with gene expression (p<0.05) in only 3% of

the tris12-specific DMCpGs (compared to normal B cells and non-tris12 U-CLLs), in line with recent reports. Besides tris12, we also identified that del(11q) CLLs were also distinctive, albeit from a different perspective. In detail, as compared to normal B cells, this group had more DMCpGs than tris12 and del(13q) cases, although the signature was heterogeneous in different del(11q) cases. Comparing the epigenetic burden (EB, the number of DMCpGs per case) of each case vs MBC, del(11q) cases showed a higher burden than del(13q) (p=1.582e-06) and tris12 (p=0.0005) suggesting a more pronounced epigenetic evolution in these clinically agressive cases.
Summary/Conclusion: We highlight that tris12 CLLs have a distinct DNAm signature, further underscoring its unique biological background, and that del(11q) show a high EB which represents a potential predictive biomarker for CLL.

PS1088

GENE EXPRESSION PROFILING IDENTIFIES CHARACTERISTIC TRANSCRIPTIONAL SIGNATURES OF GENOMIC INSTABILITY IRRESPECTIVE OF TP53 IMPAIRMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA

J. Bloehdorn[1,*], J. Krzykalla[2], B.M.C. Jebaraj[1], A. Taylor-Weiner[3], K. Holzmann[1], J. Edelmann[4], S. Robrecht[5], J. Bahlo[5], K. Fischer[5], E. Tausch[1], B. Eichhorst[5], D.A. Landau[3], L. Bullinger[6], A. Benner[2], D.S. Neuberg[7], M. Hallek[5], C.J. Wu[3], D. Mertens[1], H. Döhner[1], S. Stilgenbauer[1]

[1]Internal Medicine III, University Hospital Ulm, Ulm, [2]Division of Biostatistics, German Cancer Research Center (DKFZ), Heidelberg, Germany, [3]Broad Institute , Harvard and MIT, Cambridge, United States, [4]Barts Cancer Institute, Queen Mary University of London, London, United Kingdom, [5]Department I of Internal Medicine and Center of Integrated Oncology Cologne Bonn, University Hospital Cologne, Cologne, [6]Medical Clinic for Hematology, Oncology and Tumor Biology, Charite University Hospital, Berlin, Germany, [7]Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, United States

Background: Cooperating pathogenic mechanism, other than alterations of TP53, ATM or NOTCH1 mutations, mediating the emergence of refractory disease, or decreased efficacy of chemoimmunotherapy, are incompletely understood in CLL. Research on genomic instability has frequently focused on functional loss of TP53 or ATM and subsequent downstream signaling with related impairment of DNA repair.
Aims: To elucidate other elements leading to genomic instability we used a multiplatform approach on specimen collected from patients registered on the CLL8 study (first-line, FC vs. FCR).
Methods: Data was generated for gene expression (n=337, Human Exon 1.0 ST arrays, Affymetrix), copy number aberrations (CNAs) (n=309, Human SNP Arrays 6.0, Affymetrix), mutation analyses combined with signature projections (n=171, whole exome sequencing, Illumina), telomere length (n=332) and protein data for confirmation in select cases (western blot n=11) using CD19 sorted samples. FISH, IGHV and TP53 mutation analysis were also available from trial enrolment.
Results: To explore tumor-heterogeneity we performed unsupervised clustering analysis using 2359 variably expressed genes (SD of > 0.5). Six distinct clusters (C1-C6) were identified that differed significantly in clinical and biologic characteristics. Variability (Fisher 's Exact Test) across all clusters was found for incidences of del(17p) (p<0.05), TP53 mutation (p<0.01), tri(12) (p<0.01), with enrichment of del(17p) and/or TP53 mutations in C2 (n=24/133, 18,5%), C3 (n=9/56, 16%) and tri(12) in C5 (n=8/11, 72.7%). With regard to mutation characteristics, TP53 frame shift mutations were enriched in C2, while splice site mutations were exclusively observed in C5. GSEA on hallmark gene sets highlighted DNA-repair amongst the processes specifically altered in C2 and C3. Notably, activation of DNA-damage response and repair was further confirmed by upregulation of TP53 and ATM mRNA (q<0.01), p53 and phospho-p53 in C2/C3. Further characteristics of genomic instability included increased telomere stress as indicated by significant variability of telomere length across clusters (p<0.01, Kruskal - Wallis rank sum test). Shortest telomeres were found in C2 cases (median 3.8 kb) with concurrent POT1 overexpression (q<0.01), indicating increased necessity of telomere protection. C2 and C3 showed the highest incidence of POT1 mutations (7.3%), which are reportedly associated with telomeric aberrations, chromosome breaks or fusions. Apart from POT1, distribution of driver mutations followed cluster specificity and showed a selective enrichment of alterations leading to genomic instability including e.g. CHEK2 or RPS15 in C2 and C3. When analyzing mutational signatures implicated in the pathogenic processes in cancer and documented in the COSMIC database, signatures for individual clusters indicated highest activation for signature 6 (defective MMR) and signature 9 (associated with the activity of AID during somatic hypermutation) in C2.
Summary/Conclusion: In conclusion, unsupervised clustering defined expression signatures that point to the pathogenic role of genomic instability in a considerable portion of patients with CLL in need of treatment. However, these signatures are not restricted to cases with TP53 impairment and rather integrate different biological mechanisms involved in genomic stability, such as alterations affecting telomere regulation and the proper execution of DNA-repair.

PS1089

BIALLELIC INACTIVATION OF ATM AND BIRC3 IN DEL(11Q) CELLS DRIVES GENOMIC INSTABILITY AND TUMOR EXPANSION IN CRISPR/CAS9-GENERATED MODELS OF CHRONIC LYMPHOCYTIC LEUKEMIA

M. Quijada-Álamo[1,2,*], M. Hernández-Sánchez[1,2,3,4], J.L. Ordóñez[1,2], A.E. Rodríguez-Vicente[1,2], V. Alonso-Pérez[1,2], I. García-Tuñón[1,2], A.B. Herrero[1,2], R. Benito[1,2], J.L. García[1,2], M. Gruber[3,4,5,6], C.J. Wu[3,4], J.M. Hernández-Rivas[1,2]

[1]IBSAL, IBMCC, University of Salamanca, CSIC, Cancer Research Center, [2]Department of Hematology, University Hospital of Salamanca, Salamanca, Spain, [3]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, [4]Broad Institute of Harvard and MIT, Cambridge, Massachusetts, United States, [5]CeMM Research Center for Molecular Medicine, [6]Department of Internal Medicine I, Division of Hematology and Hemostaseology, Medical University of Vienna, Vienna, Austria

Background: Chromosome 11q22.3 deletion (del(11q)) is one of the most common cytogenetic alterations in chronic lymphocytic leukemia (CLL) and usually involves both *ATM* and *BIRC3* genes. Concomitant mutations in *ATM* and/or *BIRC3* in the remaining allele have been associated with adverse prognosis in CLL. However, little is known about their functional impact as well as how multiple genetic alterations could synergistically act to drive tumor pathogenesis.
Aims: To assess the functional and clinical impact of del(11q) together with loss-of-function mutations in *ATM* and/or *BIRC3* through the analysis of isogenic CLL cell lines with del(11q), *ATM* and *BIRC3* mutations.
Methods: The CRISPR/Cas9 system was used to introduce del(11q), *ATM* and *BIRC3* mutations into CLL cell lines (HG3, MEC1). Proliferation, NF-kB2 activation, DNA damage response and *in vivo* xenograft studies were performed. In addition, the mutational status of untreated CLL patients (del(11q) N=27 and non-del(11q) N=174) was evaluated analyzing a panel of 42 CLL-related genes (including *ATM* and *BIRC3*) by NGS.
Results: By introducing two guide RNAs targeting 11q22.1 and 11q23.3 regions, an HG3-del(11q) cell line was generated. The presence of a monoallelic deletion was confirmed in 100% of the cells by FISH. Mutations in *ATM* and/or *BIRC3* were introduced in the remaining allele, generating HG3 del(11q) ATM^{KO}, del(11q) $BIRC3^{KO}$ and del(11q) $ATM^{KO}BIRC3^{KO}$ (three clones per condition). In parallel, single ATM^{KO} and $BIRC3^{KO}$ mutations, or the combination of both, were introduced in HG3 and MEC1 cells (three clones per condition). HG3-del(11q) clones presented cytoplasmic stabilization of NF-kB-inducing kinase (NIK), leading to NF-kB2 (p52) accumulation in the nucleus of CLL cells. In addition, biallelic inactivation of *BIRC3* in HG3-del(11q) cells resulted in higher p52 activation measured by ELISA assay, leading to a higher proliferation rate of HG3-del(11q) $BIRC3^{KO}$ clones compared to HG3-del(11q) $BIRC3^{WT}$ cells. Moreover, HG3-del(11q) ATM^{KO} cells displayed reduced p-H2AX levels and accumulation of unrepaired double strand breaks (DSB) after gamma-irradiation, as determined by immunofluorescence and neutral comet assays, exhibiting deficiencies in DSB repair. As expected, HG3-del(11q) $ATM^{KO}BIRC3^{KO}$ cells also presented NF-kB2 activation, enhanced proliferation and DNA damage accumulation, as a consequence of biallelic inactivation of *BIRC3* and *ATM*, respectively. These results were also confirmed in HG3 and MEC1 ATM^{KO} and $BIRC3^{KO}$ clones. *In vivo* subcutaneous xenograft models showed that HG3 $ATM^{KO}BIRC3^{KO}$ tumors presented proliferative advantage, higher p52 activation and greater genomic and mitotic instability than $HG3^{WT}$ tumors. In line with these results, NGS studies of CLL patients revealed that *BIRC3*-mutated patients showed shorter time to first treatment (TTFT) than $BIRC3^{WT}$ CLLs (p<0.001), whereas *ATM*-defective CLLs presented a higher number of mutations in CLL-related genes compared to ATM^{WT} cases (median 3 [1-6] *vs.* 1 [0-7]; p<0.001).
Summary/Conclusion: Del(11q) CLL cells with biallelic inactivation of *ATM* and *BIRC3* show enhanced proliferation through the activation of NF-kB2, and the accumulation of DNA damage contributing to genomic instability. These findings could be related with the shorter TTFT seen in *BIRC3*-mutated CLL patients and the high rates of genomic alterations observed in *ATM*-mutated CLLs. Thus, CRISPR/Cas9-generated models can be powerful tools to study the molecular mechanisms involved in the clinical impact of single and multiple CLL genetic alterations.

PS1090

MASS CYTOMETRY REVEALED LEUKEMIC MICROENVIRONMENT COMPLEXITY: IMPLICATIONS FOR IMMUNOTHERAPY IN A MOUSE MODEL OF CHRONIC LYMPHOCYTIC LEUKEMIA

M. Wierz[1,*], S. Pierson[1], L. Guyonnet[2], A. Oudin[1], G. Berchem[1,3], B. Janji[1], C. Guérin[2], J. Paggetti[1], E. Moussay[1]

[1]Department of Oncology, [2]Department of Infection and Immunity, Luxembourg Institute for Health, [3]Centre Hospitalier de Luxembourg, Luxembourg, Luxembourg

Background: Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of mature B lymphocytes in peripheral blood and primary lymphoid organs. The progression of CLL is highly dependent on complex interactions with immune cells and other non-malignant cells in the tumor microenvironment. Despite recent advances in CLL treatment, CLL remains an incurable disease.
Aims: We wanted to extensively characterize the immune cell populations in the murine CLL microenvironment to identify novel potential targets and consequently evaluate the efficacy of a dual antibody-based immunotherapy in CLL pre-clinical models.
Methods: We performed a mass cytometry analysis with a custom 35-marker panel to establish an extensive cartography of immune cell subsets populating the leukemic spleen of transgenic Eμ-TCL1 mice and of diseased C57BL/6 mice following adoptive transfer (AT-CLL). Following the identification of immune checkpoints, we injected antibodies against PD1 and LAG3 as dual therapy and monitored disease development by flow cytometry, blood cell count, and magnetic resonance imaging. The immune microenvironment was also analyzed after mice euthanasia.
Results: First, we observed that, compared to normal B cells, malignant CLL cells show a higher expression of immune checkpoints PD-L1 and LAG3 as well as several maturation and adhesion molecules such as CD44 and ICAM-1. Furthermore, PD-L1 expression on CLL cells was heterogeneous, a high expression being concomitant with a higher expression of maturation and adhesion molecules. Next, we identified a significant alteration in the immune cell composition in CLL reflecting strong immune suppression and dysfunctional anti-tumor immunity. More particularly, we noted an enrichment of CD8+ T cells displaying features of exhaustion such as the expression of the immune checkpoints PD1, LAG3 and TIM3 as well as an expansion of KLRG1+ CD69+ tumor-infiltrating Treg cells and Foxp3+ CD25low LAG3+ Treg cells, both showing enhanced suppressive capacities. In the myeloid compartment, we identified dysfunctional LAG3-expressing CD8+ dendritic cells and ICAM-1+ CLL-promoting patrolling monocytes as being more abundant in CLL.
As we observed a relevant upregulation of PD1 and LAG3 on the majority of immune cell populations, we tested a dual anti-PD1/anti-LAG3 immunotherapy in AT-CLL mice and transgenic Eμ-TCL1 mice. Indeed, dual PD1/LAG3 blockade efficiently limited CLL development in both murine models of CLL. More specifically, the percentage and number of CLL cells in blood and spleen significantly decreased in AT-CLL mice and an immuno-competent microenvironment was restored. In transgenic Eμ-TCL1 mice, PD1/LAG3 dual immunotherapy significantly reduced the spleen volume as determined by magnetic resonance imaging. Remarkably, the decrease in spleen volume strongly correlated with T cell phenotypes in peripheral blood before the beginning of the treatment.
Summary/Conclusion: In conclusion, we dissected and expanded the current knowledge of the phenotypic complexity of the CLL tumor microenvironment. We demonstrated that dual targeting of the immune checkpoints PD1 and LAG3 efficiently controlled CLL development in pre-clinical models and therefore could have potential benefits in CLL to restore a functional anti-tumor immunity.

PS1091

CD20 IS A DIRECT REGULATOR OF B-CELL RECEPTOR SIGNALING IN THE MICROENVIRONMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA

G. Pavlasova[1,2,*], V. Svobodova[1,2], M. Borský[2], J. Oppelt[13], J. Novotna[2], K. Cerna[1,2], K. Musilova[1,2], V. Seda[1,2], Y. Brychtova[2], M. Doubek[1,2], S. Pospisilova[1,2], J. Mayer[2], M. Mraz[1,2]

[1]Center of Molecular Medicine, CEITEC, Masaryk University, [2]Department of Internal Medicine - Hematology and Oncology, University Hospital Brno and Faculty of Medicine, Masaryk University, [3]National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czech Republic

Background: The biological function of CD20 and the reasons for the impressive activity of anti-CD20 antibodies remain elusive. We have previously shown that CLL cells which have recently exited the lymph node microenvironment ($CXCR4^{dim}CD5^{bright}$ cells) express higher CD20 levels. This is due to its direct up-regulation by CXCR4/SDF1 axis (Pavlasova *et al.*, Blood, 2016).
Aims: We hypothesized that CD20 expression is of direct functional importance for B-cell receptor (BCR) signaling as CLL cells with high CD20 express also higher cell surface IgM levels and this was coupled with higher responsiveness to BCR-crosslinking ($p<0.0001$).
Methods: CLL cells were obtained from untreated CLL patients or patients undergoing FCR therapy and analyzed for BCR signaling capacity and rituximab effect.
Results: CD20 silencing using CD20 siRNA in B cells revealed that low levels of CD20 impair the phosphorylation of BCR/PI3K-associated molecules (LYN, SYK, ERK, GAB1) after BCR-crosslinking and BCR-induced calcium flux ($p<0.05$). This also impaired LYN kinase phosphorylation, which is the first kinase phosphorylated in response to BCR ligation. This suggests that CD20 up-regulation in the microenvironment is required for proper activity of BCR and that CD20 is very "intimately" connected with BCR. Interestingly, the CD20 effect on phosphorylation of BCR-associated kinases was not entirely dependent on its known calcium-channel function (Bubien *et al.*, JCB, 1993), as the effect of CD20 silencing was present also in calcium-free media. This suggests that CD20 has also other functions, such as a putative docking site for BCR-associated molecules. To investigate the relevance of CD20 in primary CLL cells *in vivo* we also analyzed proteins involved in BCR signaling pathway and cell activation in CXCR4/CD5 CLL subpopulations. The $CD20^{bright}CXCR4^{dim}CD5^{bright}$ subpopulation (represents cells that recently exited the lymph nodes) had higher levels of pAKT/pERK/pCD79a ($p<0.001$) and a gene expression signature enriched for genes involved in BCR and MAPK signaling, migration and actin cytoskeleton organization ($p<0.0001$). The overall activated status of $CD20^{bright}CXCR4^{dim}CD5^{bright}$ cells is also evidenced by enrichment in proliferative Ki67-positive cells ($p<0.0001$). We further hypothesized that the higher CD20 levels on $CXCR4^{dim}CD5^{bright}$ cells make them the primary target of rituximab, since certain rituximab-mediated effects depend on CD20 levels. Indeed, rituximab was *in vivo* ~10-fold more efficient in eliminating BCR signaling proficient $CD20^{bright}CXCR4^{dim}CD5^{bright}$ CLL cells than $CD20^{dim}CXCR4^{bright}CD5^{dim}$ cells in FCR treated patients (day 0 vs 1 and 3; $p<0.0001$).
Summary/Conclusion: Altogether, we described for the first time a direct role of CD20 in BCR signaling and its contribution to the aggressiveness of an intra-clonal CLL cell subpopulation. Additionally, we showed that this BCR signaling proficient $CD20^{bright}CXCR4^{dim}CD5^{bright}$ intra-clonal CLL cell subpopulation is preferentially eliminated by rituximab *in vivo*, which might partially account for the success of anti-CD20 antibodies.
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PS1092

ROLE OF MTORC1 IN NORMAL HAEMOPOIESIS AND LEUKAEMOGENESIS

N. Malik[1,2,*], K. Dunn[1], C. Estell[1], O. Sansom[2], A.M. Michie[1]

[1]Institute of Cancer Sciences, University of Glasgow, [2]Beatson Institute for Cancer Research, Glasgow, United Kingdom

Background: Mammalian target of rapamycin (mTOR) is a protein kinase that mediates phosphoinositide-3-kinase (PI3K)/Akt signalling. This pathway is involved in a plethora of cellular functions including protein and lipid synthesis, cell migration, and apoptosis. Our laboratory has shown that mTOR substrates are activated in samples isolated from chronic lymphocytic leukaemia (CLL) patients, and in our kinase dead PKCα mouse model (PKCαKR) which exhibits a CLL-like disease.
Aims: We proposed to delineate the role of mTOR complex 1 (mTORC1) using conditional knock out (cKO) mouse models to determine its role in normal haemopoiesis and to determine whether it plays a role in CLL initiation and/or development.
Methods: We exploit the cre-loxP system in murine models where the gene of interest (*raptor*) is excised either, in a time-controlled manner (*Mx1*) or at the haemopoietic stem cell stage (HSC, *Vav*) to assess molecular events through q-PCR, western blotting and cell viability assays. We also retrovirally (RV) transduce BM cells with either a PKCα-KR (CLL-like) or MIEV

construct for transplantation into host mice.
Results: Upon cKO of raptor (mTORC1; *Mx1-cre*$^{+}$*raptor*$^{fl/fl}$) in adult mice, we observe splenomegaly, a disruption in normal haemopoiesis, along with a significant reduction in the B cell lineage in the BM. A block in B cell lineage commitment was indicated by the significant increase in Lin^{-}Sca-1^{+}CD117^{+} (LSKs) in the spleen, and a decrease in proB cells in the BM.
While assessing the role of mTORC1 during early developmental stages in *vav*-cre^{+}*raptor*$^{fl/fl}$ mice, we established that the embryos do not survive due to a disruption in erythropoiesis. A significant decrease in Ter119^{+} cells was noted in E15 foetal livers (FL) in KO mice, with aberrations at megakaryocyte-erythrocyte progenitor (MEP) stage. Thus, we analysed the ability of mTOR inhibitors to block the differentiation of K562 cells towards red blood cells (RBCs) in galactose-rich cultures *in vitro*. Treatment of K562 cells in galactose-rich cultures with either mTORC1 inhibitor rapamycin or the pan-mTOR inhibitor AZD8055 significantly reduces key molecular events of RBC differentiation: generation of CD71^{+}GlyA^{+} cells; expression of *HBB (*β-globin) and *GATA2*, thereby establishing the importance of mTORC1 signalling/function in the promotion of RBC development. To address the role of raptor in CLL initiation/maintenance *in vitro*, we RV-transduced *Mx1*-cre^{-} or *Mx1*-cre^{+}*raptor*$^{fl/fl}$ BM (post cKO) with a PKCα-KR or MIEV construct. RV-transduction from *raptor* cKO mice with PKCα-KR failed to rescue the B cell lineage block or generate a CLL-like disease. To test mTORC1 requirement for maintenance of disease *in vivo*, *Mx1*-cre^{-} or *Mx1*-cre^{+}*raptor*$^{fl/fl}$ BM cells were RV-transduced with PKCα-KR-and transplanted into host mice. Once disease was established, as detected by blood sampling and analysis of GFP^{+}CD19^{+}CD5^{+} cells, mice were inoculated with polyI:C to induce cKO. Our results indicate that cKO of raptor with established CLL-like disease results in increase in survival time and decrease in disease load.
Summary/Conclusion: Taken together, our data supports the work of others demonstrating a block in B cell lineage commitment. Elevation in the MEP population observed in the *vav*-cre^{+}-*raptor*$^{fl/fl}$ mice leads to neonatal lethality due to a block in erythropoiesis. Importantly, our results suggest a critical role for mTORC1 in CLL initiation and maintenance *in vivo*. These studies will pave the way towards testing the efficacy of clinically-relevant mTOR inhibitors in blocking/reversing CLL in pre-clinical mouse models.

PS1093

FIRST-IN-CLASS ORAL TYROSINE KINASE INHIBITOR (KAN0439834) TARGETING ROR1 INDUCED SIGNIFICANT APOPTOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

M. Hojjat-Farsangi[1,*], A. H. Daneshmanesh[1], F. Mozaffari[1], P. Kokhaei[1], L. Hansson[1], J. Vågberg[2], S. Byström[3], E. Olsson[3], C. Löfberg[3], C.C. Norström[3], J. Schultz[3], M. Norin[3], T. Olin[3], A. Österborg[1], A. Moshfegh[3], H. Mellstedt[1]

[1]Oncology-Pathology, Karolinska Institute, Stockholm, [2]Kancera AB, Solna, [3]Kancera AB, Stockholm, Sweden

Background: ROR1 is a transmembrane protein of the receptor tyrosine kinase (RTK) family expressed during embryogenesis, but repressed in adult tissues. In chronic lymphocytic leukemia (CLL), ROR1 is upregulated. Inhibition of ROR1 abrogated downstream kinase activities and induced apoptosis of CLL cells. KAN0439834, a small molecule inhibitor (ROR1-SMI) was synthesized with the aim to bind to the TK domain of ROR1 and inhibit phosphorylation.
Aims: In the present study we investigated the effects of KAN0439834, on survival of CLL cells, ROR1 dephosphorylation in CLL cells and signaling, and *in vivo* effects in NOD-SCID mice xenografted with human CLL cells.
Methods: MTT and Annexin V/PI assays were used to analyse cytotoxicity of KAN0439834. Fresh CLL cells were co-cultured with the stromal cell line HS-5 and incubated with KAN0439834 and apoptosis measured (Annexin V/PI). CLL cells were also cultured with KAN0439834 (50-250 nM) for 2 h and rWnt5a (25-50 μg/mL) was added. The cells were further incubated for 30 min before lysate preparation and cytotoxicity assayed after 24 h. Proximity ligation assay was used to investigate the effects of KAN0439834 on ROR1/LRP6 heterodimerization. Phosphorylated RTKs in CLL cells and the effects of KAN0439834 on phosphorylation of RTKs were analysed by the Human-Phospho-RTK Array and WB. Finally, in two mice experiments, fresh CLL cells were grafted into NOD-SCID mice and oral KAN0439834 treatment was started 7 days after transplantation and continued for 7 days in the first study and 14 days in the second study.
Results: KAN0439834 induced apoptosis of CLL cells (EC_{50}=250 nM) with a 60-fold selectivity for CLL cells compared to normal PBMC. KAN0439834 was significantly more effective than an anti-ROR1 mAb in inducing apoptosis of CLL cells. When CLL and HS-5 cells were co-cultured, HS-5 cells could prevent induction of apoptosis of CLL cells at low concentrations of KAN0439834, while at higher concentrations of KAN0439834 the presence of stromal cells had no effect. Wnt5a increased phosphorylation of ROR1. KAN0439834 dephosphorylated both ROR1 and SRC as well as inhibited Wnt5a-induced ROR1 phosphorylation in this system. KAN0439834 dephosphorylated ROR1, LRP6 and associated downstream signaling molecules as AKT, mTOR and CREB. Oral administration of KAN0439834 to NOD-SCID mice significantly inhibited growth of xenografted human CLL cells. Immunohistochemistry staining of spleens from the two studies showed a significant decrease of CLL cells in treated mice but not in controls.
Summary/Conclusion: KAN0439834 is the first generation of a novel class of ROR1-SMI developed by phenotypic screening using CLL cells. The small molecule was much more effective in inducing CLL apoptosis than the anti-ROR1 antibody. KAN0439834 inactivated various signaling pathways in the leukemic cells suggested to be associated with ROR1 signaling. The development of new anti-cancer drugs with other mechanisms of action than those clinically available when existing drugs fail is warranted to improve the prognosis of CLL. KAN0439834 may not only be potent in CLL but also in other ROR1 expressing tumors; such studies are in progress. Our results support the further development of ROR1 inhibitors as a new therapeutic principle.

Chronic lymphocytic leukemia and related disorders – Clinical

PS1094

PROGNOSTIC IMPACT OF POSITIVE MINIMAL RESIDUAL DISEASE (MRD) BELOW THE THRESHOLD OF 0.01% IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS AFTER IMMUNOCHEMOTHERAPY: STUDY OF A DATASET FROM 3 FILO TRIALS

R. Letestu[1,*], A. Dahmani[1], M. Boubaya[2], C. Arnoulet[3], L. Baseggio[4], L. Campos[5], B. Chatelain[6], A. Debliquis[7], B. Drénou[7], M.-C. Jacob[8], E. Legac[9], M. Le Garff-Tavernier[10], C. Quiney[10], N. Robillard[11], C. Aanei[5], F. Solly[5], M. Ticchioni[12], V. Lévy[2], S. Katsahian[13], R. Délépine[14], S. Vaudaux[15], V. Rouille[16], M.-C. Béné[11], C. Dartigeas[17], E. Van Den Neste[18], S. Leprêtre[19], P. Feugier[20], G. Cartron[21], V. Leblond[22], A.F. Cymbalista[1], O.B. O. FILO[23]

[1]Laboratoire d'Hématologie, [2]URC, APHP CHU Avicenne, Bobigny, [3]Laboratoire d'Hématologie, IPC, Marseille, [4]Laboratoire d'Hématologie, CHU Lyon, Lyon, [5]Laboratoire d'Hématologie , CHU Saint-Etienne, Saint-Etienne, France, [6]Laboratoire d'Hématologie, CHU UCL Namur, Yvoir, Belgium, [7]Laboratoire d'Hématologie, GHRMSA, Mulhouse, [8]Laboratoire EFS , CHU Grenoble, Grenoble, [9]Laboratoire d'Hématologie, CHR Orleans, Orleans, [10]Laboratoire d'Hématologie, APHP CHU Pitié-Salpêtrière, Paris, [11]Laboratoire d'Hématologie, CHU Nantes, Nantes, [12]Laboratoire d'Immunologie, CHU L'Archet, Nice, [13]URC, CHU Saint-Louis, Paris, [14]CHU Tours, Tours, [15]CLCC, Rouen, [16]CHU Montpellier, Montpellier, [17]Hématologie clinique, CHU Tours, Tours, France, [18]Hématologie clinique, UCLouvain, Louvain, Belgium, [19]Hématologie clinique, CLCC, Rouen, [20]Hématologie clinique, CHU Nancy, Nancy, [21]Hématologie clinique, CHU Montpellier, Montpellier, [22]Hématologie clinique, APHP CHU Pitié-Salpêtrière, Paris, [23]French Innovative Leukemia Organization, (FILO), France

Background: Currently, immunochemotherapy remains the standard of care in CLL for fit patients in first line. Clinical relevance of flow cytometry MRD at a 0.01% threshold has been widely demonstrated.
Aims: In this work, we assessed the prognostic value of high sensitivity MRD in a large cohort of Fludarabine Endoxan Rituximab (FCR) treated CLL patients within three FILO first line clinical trials.
Methods: We collected data from 859 patients randomized in 3 trials: FMP2007 (FCRx6 vsFCCampathx6), LLC2007SA (4FC+6R +/- R maintenance) and FMP2010 (FCRx6 vs high dose R FCx6). Only patients who received FCR based without maintenance were considered for this study (n=574). MRD was evaluated with high sensitivity flow cytometry techniques using 6 and 8 color panels through a paucicentric network. These procedures resulted in >1-Log improvement in sensitivity as compared to 4-color tests, allowing the detection of positive MRD down to 0.001%.
Results: Validated follow-up data were available for 540/574 patients with a median follow-up of 49,5 months. MRD was tested 3 months after the last FCR course in blood and bone marrow (BM). Completeness of data was good since results were available for 401 and 339 patients respectively with paired results in 329 patients. Cases with insufficient sensitivity (>10^{-4}) were excluded. When MRD was detectable in blood, it was positive in BM in 100% cases. In cases with undetectable blood MRD (und-MRD) residual disease was still detectable in BM in 64/197 patients (32.5%). We then studied the impact of MRD status on PFS using both blood and BM MRD. First, we applied the conventional threshold of 0.01% and as expected we observed that positive MRD was predictive of PFS. Then we considered separately patients who showed low level MRD positivity (<0.01%) from the patients with und-MRD. We observed that positive blood MRD, whether >0.01% or <0.01% (considered as negative with the conventional threshold) was associated with shorter PFS (Figure 1). When considering BM, the 0.01% threshold remained for prediction of adverse outcome. We considered the impact of sensitive MRD status on clinical response (CR+CRivs.PR+nPR). As expected und-MRD/CR group had the longest PFS (72.2 mo) whereas MRD+/PR group had the shortest PFS (35.5 mo). In the 2 other groups with discordant status (MRD+/CR and und-MRD/PR) the prognostic value of MRD appeared prevalent on that of clinical response. Finally, we correlated MRD and IGHV mutational status and examined the impact on outcome. In MRD+/Unmutated IGVH group we observed the shortest PFS (5-yPFS: 16%) while the longest PFS was noted in und-MRD/Mutated IGHV group (5-yPFS: 71%). The evolution of this latter group was not significantly different from that of the MRD+/Mutated IGHV group (5-yPFS: 60%, p=0.22). Interestingly, Unmutated IGHV patients reaching und-MRD had a similar PFS to that of patients with Mutated IGHV genes (5-yPFS: 60% and 71% respectively, p=0.34).

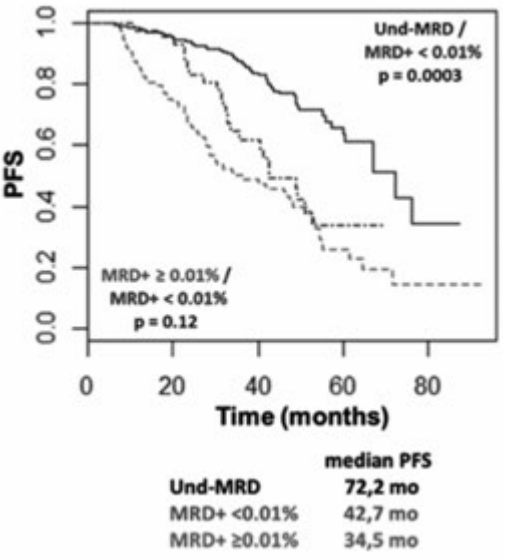


Figure 1.

Summary/Conclusion: In conclusion, this study shows the applicability and the usefulness of high-sensitivity MRD testing in a multicenter network. We show the respective value of blood and bone marrow sensitive MRD assessment, and the impact of MRD status on PFS, clinical response and IGHV mutational status. The prognostic importance of blood sensitive MRD suggests that it could be used as a routine tool and reduce the need for bone marrow assessment for MRD driven therapeutic strategies.

PS1095

EVALUATION OF THE INCIDENCE, RISK FACTORS, AND MANAGEMENT OF HYPERTENSION IN PATIENTS RECEIVING IBRUTINIB FOR HEMATOLOGIC MALIGNANCIES

T. Dickerson[1], T. Wiczer[1], A. Waller[1], J. Philippon[1], A. Guha[2], K. Rogers[1], J. Woyach[1], J. Byrd[1], D. Addison[2], F. Awan[1,*]

[1]The Ohio State University Comprehensive Cancer Center, [2]The Ohio State University, Columbus, United States

Background: The approval of Ibrutinib (IB) for various hematologic malignancies has significantly improved disease control rates, progression free, and overall survival. However, targeting of multiple kinases may result in a number of adverse effects and complications that can lead to discontinuation of the drug. New or worsening hypertension (HTN) has been reported in 10-29% of patients receiving IB on clinical trials. Recent observational data suggests that this rate may be as high as 40% in clinical practice and resistant to treatment with multiple agents. Management of patients who develop HTN on IB has not previously been well described. Additionally, the relationship between IB-associated HTN and cardiovascular complications is not known.
Aims: The aim of this study was to characterize the development of HTN in patients treated with IB; describe the management and complications of IB-associated HTN, and define risk factors for the development of HTN while on therapy.
Methods: In order to describe the incidence, management, complications, and risk factors for the development of HTN in patients treated with IB, a retrospective single-center cohort study was conducted of adult patients who started IB for hematologic malignancies over a 6 year period after obtaining appropriate IRB approval. HTN was defined as systolic blood pressure of at least 130 mmHg on 2 separate visits within 3 months. Severity of new or worsened HTN was defined according to the Common Terminology Criteria for Adverse Events (CTCAE) and was causality assessed using the Naranjo Scale. A composite cardiovascular event endpoint (MACE) that included incident myocardial infarction, unstable angina, heart failure, stroke, or need for percutaneous coronary intervention while excluding atrial fibrillation was assessed.
Results: Five hundred and sixty-two (562) patients treated with IB were identified. A majority of patients had CLL (73%) and were male (70%). Sixty-one percent had preexisting HTN at the time of starting IB therapy with 35% requiring at least one medication. With a median follow up of

>3 years, 26% of the patients developed new HTN. In patients who had preexisting HTN, 84% had worsening of their condition, which was defined as a worsening of the CTCAE grade of HTN in 93%, while 56% of these patients required a change in therapy. MACE occurred in 7% of patients and atrial fibrillation in 13% of patients. Incidence of HTN and comparative assessment of risk factors for HTN are being made using a competing risks regression model and survival analysis to evaluate the association between HTN and IB and will be reported in detail at the meeting.
Summary/Conclusion: In our retrospective study, patients receiving IB had a substantial increase in the incidence or severity of HTN. Assessment of pre-existing risk factors, close monitoring, and a team approach are required for optimal management of this complication.

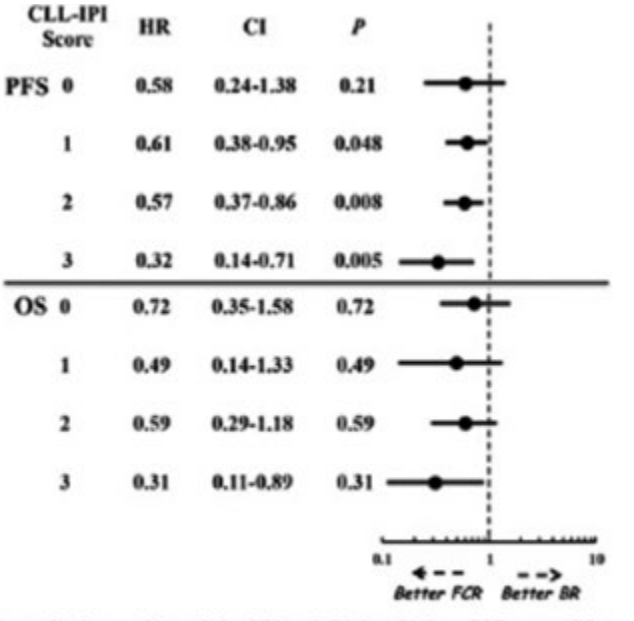
PS1096

EVALUATION OF THE CLL-IPI IN PREVIOUSLY UNTREATED CLL PATIENTS RECEIVING CHEMO-IMMUNOTHERAPY AS FIRST-LINE TREATMENT: ANALYSIS OF 845 CASES

M. Gentile[1,*], T. D. Shanafelt[2], G. Uccello[3], F. R. Mauro[4], G. Reda[5], D. Rossi[6], L. Laurenti[7], M. I. Del Prinicipe[8], G. Cutrona[9], I. Angeletti[10], M. Coscia[11], Y. Herishanu[12], A. Chiarenza[13], S. Molica[14], S. Ciolli[15], N. Goldschmidt[16], F. Angrilli[17], A. Giordano[18], A. Rago[19], O. Bairey[20], G. Tripepi[21], K. G. Chaffee[22], S. A. Parikh[23], E. Vigna[1], K. Zirlik[24], L. Shvidel[25], I. Innocenti[7], A. G. Recchia[26], F. Di Raimondo[13], G. Del Poeta[7], A. Cortelezzi[27], A. Neri[27], M. Ferrarini[28], G. Gaidano[29], N. Kay[30], A. Polliack[16], R. Foà[4], F. Morabito[26]

[1]Hematology, Ospedale Annunziata, Cosenza, Italy, [2]Hematology, Stanford University, California, United States, [3]Hematology, Hospital Annunziata, Cosenza, [4]Hematology, Università La Sapienza, ROMA, [5]Hematology, Fondazione Ca' Granda IRCCS, Ospedale Maggiore Policlinico, Milano, Italy, [6]Oncology, Oncology Institute of Southern Switzerland and Institute of Oncology Research, Bellinzona, Switzerland, [7]Hematology, Catholic University Hospital "A. Gemelli", [8]Hematology, Dipartimento di Biomedicina e Prevenzione, Università degli Studi di Roma "Tor Vergata", Roma, [9]Hematology, UOC Molecular Pathology IRCCS S. Martino-IST, Genova, [10]Hematology, Azienda Ospedaliera Santa Maria , Terni, [11]Hematology, University of Torino, A.O. Città della Salute e della Scienza di Torino, Torino, Italy, [12]Hematology, Tel-Aviv Sourasky Medical and Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv , Israel, [13]Hematology, Department of Biomedical Sciences, Division of Haematology, University of Catania and Ferrarotto Hospital, Catania, [14]Hematology, Azienda Ospedaliera Pugliese-Ciaccio, Catanzaro, [15]Hematology, Hematology University of Florence, Firenze, Italy, [16]Hematology, Hadassah Medical Center and Faculty of Medicine, Hebrew University, Jerusalem, Israel, [17]Hematology, Ospedale Santo Spirito, Pescara, [18]Hematology, Azienda Ospedaliero-Universitaria, Policlinico consorziale di Bari, Bari, [19]Hematology, Ospedale Santa Maria Goretti, Latina, [20]Hematology, Rabin Medical Center, Petah Tikva and Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, [21]Hematology, Consiglio Nazionale delle Ricerche, Istituto di Biomedicina ed Immunologia Molecolare, Reggio Calabria, Italy, [22]Hematology, Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, Minnesota, United States, [23]Hematology, [22]Department of Internal Medicine, Division of Hematology, Mayo Clinic, Rochester, Minnesota, Italy, [24]Hematology, University Medical Centre Freiburg, Freiburg, Germany, [25]Hematology, Kaplan Medical Center, Rehovot and Hadassah Medical Center and Faculty of Medicine, Hebrew University, Jerusalem, Israel, [26]Hematology, Biotechnology Research Unit, Azienda Sanitaria Provinciale di Cosenza, Aprigliano (CS), [27]Hematology, , Fondazione Ca' Granda IRCCS, Ospedale Maggiore Policlinico, Milano, [28]Hematology, Direzione Scientifica IRCCS, San Martino IST, Genova, [29]Hematology, Department of Translational Medicine, UPO, Novara, [30]Hematology, Department of Medicine, Division of Hematology, Stanford University, California, Italy

Background: An earlier international collaborative effort collected data from 3500 chronic lymphocytic leukemia (CLL) patients to develop a comprehensive tool for predicting overall survival (OS) based on the CLL-IPI (international prognostic index for CLL patients). This score, built on *TP53* deletion and/or mutation, *IGHV* mutational status, b2-microglobulin, clinical stage and age, represents a simple "globally applicable" model available in the daily clinical practice and able to improve risk stratification for all CLL patients.
Aims: The aim of our study was to evaluate the prediction power of the CLL-IPI on OS, assessed at the time of first treatment, in a cohort of CLL patients who received chemo-immunotherapy as first-line therapy. We also investigated whether CLL-IPI could predict progression free survival (PFS). Finally, we assessed the impact of this tool in a specific cohort of fludarabine-cyclophosphamide-rituximab (FCR) or bendamustine-rituximab (BR) treated cases.
Methods: This study included CLL patients who received a first-line treatment with chemo-immunotherapy [FCR, BR, pentostatin-cyclophosphamide-rituximab (PCR) and pentostatin-cyclophosphamide-ofatumumab (PCO)] and with data available for CLL-IPI at the time of progression. OS was estimated for low-, intermediate, high- and very high-risk patients. Additionally, risk-specific PFS was assessed. Methods included Kaplan-Meier curve, log-rank test and Cox regression analyses. The prognostic accuracy of predictive model was assessed by the C-index.
Results: A total of 845 CLL patients were included in this analysis. The majority of patients were Binet stage B and C (77.9%). The median age was 63 years (range 25-86) and 566 cases (67%) were male. Four hundred and two cases received the FCR schedule, 252 BR, 142 PCR and 49 PCO. After a median follow-up of 3.7 years (range, 3 months-15.7 years), 157 patients have died and 402 have experienced an event (death or progression). According to the CLL-IPI, 183 patients (21.7%) were classified as low-risk, 337 (39.9%) as intermediate-risk, 276 (32.7%) as high-risk and 49 (5.8%) as very high-risk. Stratification of patients according to the CLL-IPI predicted significant differences in terms of OS after first-line treatment. Low-risk patients had a 3-year OS probability of 96.6% (HR=1), intermediate-risk of 92.8% (HR=3.73,p<0.0001), high-risk of 81.4% (HR=7.35,p<0.0001) and very high-risk of 64.7% (HR=17.3,p<0.0001). The C-index was 0.69 (p<0.001) for predicting OS. PFS also differed between CLL-IPI risk groups. The 3-year PFS probability was 82.6% (HR=1) for low-risk, 63.6% (HR=2.27;p<0.0001) for intermediate-risk, 53.9% (HR=2.87,p<0.0001) for high-risk, and 32.8% (HR=5.01,p<0.0001) for very high-risk. The C-index for PFS was 0.61 (p<0.001). Comparing patients who received FCR to those who received BR, a significantly higher percentage of elderly cases and patients with a high-risk CLL-IPI score were evident in the BR group. A significant PFS benefit for FCR relative to BR was maintained across CLL-IPI intermediate, high- and very high-risk groups, while no significant difference was observed in the low-risk group (Figure 1). In contrast, an OS benefit for FCR relative to BR was only observed in the very-high risk group (Figure 1).



Cox univariate analyses of the PFS and OS benefit from FCR versus BR treatment as first-line therapy according to CLL-IPI score. Hazard ratios (HR) lower than indicates a lower risk of progression or survival for FCR-treated patients. The I bars represent 95% confidence intervals (CI).

Figure 1.

Summary/Conclusion: This is the first validation study of the CLL-IPI, assessed at the time of first treatment, in patients who received a variety of chemo-immunotherapy approaches. Results confirm the ability of the CLL-IPI to stratify patients' outcome in terms of both OS and PFS. Moreover, the CLL-IPI may help to select patients towards FCR or BR treatment.

PS1097

SPONTANEOUS CLINICAL REGRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA: CLINICAL AND BIOLOGIC FEATURES OF 27 NEW CASES FROM THE EUROPEAN RESEARCH INITIATIVE ON CLL (ERIC) INTERNATIONAL REGISTRY

I. Del Giudice[1,*], L.V. Cappelli[1], J. Delgado[2], A. Visentin[3], L. Trentin[3],

M. Mazzucchelli[4], A.M. Frustaci[4], C. Cavalloni[5], M. Gentile[6], L. Scarfò[7], M. Flogegard[8], M. Mattsson[9], S. Raponi[1], C. Ilari[1], L. Cafforio[1], M.S. De Propris[1], I. Della Starza[1], N. Peragine[1], P. Mariglia[1], P.P. Ghia[7], E. M. Orlandi[5], A. Tedeschi[4], G. Semenzato[3], A. Guarini[10], E. Montserrat[2], R. Foà[1]

[1]Department of Cellular Biotechnologies and Hematology, Hematology, Sapienza University, Rome, Italy, [2]Department of Hematology, Hospital Clinic, University of Barcelona, Barcelona, Spain, [3]Department of Medicine, Hematology and Clinical Immunology Unit, University of Padua, Padua, [4]Department of Hematology, Niguarda Cancer Center, ASST Grande Ospedale Metropolitano Niguarda, Milan, [5]IRCCS, Policlinico San Matteo, Pavia, [6]Department of Hemato-Oncology, Hematology Unit, Ospedale Annunziata, Cosenza, [7]Università Vita-Salute San Raffaele and IRCCS Istituto Scientifico San Raffaele, Milan, Italy, [8]Hematology, Medicine Clinic, Falun Hospital, Falun, [9]Department of Hematology, Uppsala University Hospital, Uppsala, Sweden, [10]Department of Molecular Medicine, Hematology, Sapienza University, Rome, Italy

Background: In chronic lymphocytic leukemia (CLL), spontaneous regression (SR) is a rare but possible event.
Aims: To identify the clinico-biological features of CLL patients (pts) experiencing a SR, ERIC (European Research Initiative on CLL) group has set up an international registry to collect and to analyze such cases from both the clinical and biologic standpoints.
Methods: Inclusion criteria are: (i) CLL diagnosis as per 2008 IWCLL criteria (i.e. clonal lymphocyte (Ly) count at diagnosis ≥5×10^9/L with a typical immunophenotype); (ii) subsequent spontaneous Ly count normalization to less than 5×10^9/L, persisting for ≥12 months; (iii) disappearance of disease-related signs/symptoms (i.e. splenomegaly or lymphadenopathy) if previously present; (iv) no prior therapy.

Table 1.

Clinical and biologic characteristics at diagnosis of 27 CLL patients with SR	
Sex	11 M (41%) / 16 F (59%)
Median age (range)	76.5 years (58-94)
Stage (Binet/Rai)	21 A/0 (78%); 2 A/I (7.4%); 1 A/II (3.6%); 3 B/II
Mean lymphocyte count (x10^9/L)	14.3 (5.6-51.9)
Beta 2-microglobulin (normal)	18/22 (81.8%)
LDH (normal)	21/22 (95.4%)
CD38 (<30%)	22/22 (100%)
CD49d (<30%)	8/11 (72.7%)
ZAP70 (<20%)	15/19 (78.9%)
FISH	
del 13q	10/24 (41.7%)
Normal	10/24 (41.7%)
Trisomy 12	3/24 (12.5%)
del 17p	1/24 (4.1%)
TP53 WT	13/13 (100%)
Mutated *IGHV*	24/25 (96%)
IGHV gene	1 VH1-2; 1 VH1-46
	1 VH3-7; 2 VH3-15; 1 VH3-21; 3 VH3-23; 3 VH3-30
	1 VH4-4; 1 VH4-31; 4 VH4-34; 2 VH4-59; 2 VH4-61
Events concurrent with SR	1 viral respiratory infections; 1 pneumonia
	1 intracerebral hemorrhage; 1 cerebral stroke
	1 pelvis fracture
	1 chronic hepatitis C with thrombocytopenia
	1 rheumatoid arthritis
	1 Richter syndrome
	3 concomitant neoplasia

Results: Of 32 registered pts from 2012 to 2018, 26 fulfilling the inclusion criteria and 1 outlier (see below) have been included: 14 cases were from Italy, 11 from Spain and 2 from Sweden (Table 1). The estimated frequency of SR was 0.33-1% of the total cases followed at each center. The median time from diagnosis to SR was 9 years (range, 1.4-25), the median follow-up was 12 years (3-27). The duration of SR was 48 months (12-151). The mean Ly count at diagnosis was 14.3×10^9/L (5.6-51.9). The mean Ly peak was 24.7×10^9/L (8.3-107). At SR, the mean Ly count was 3.4×10^9/L (1.3-5.6) and at the last observation, it was 3.4×10^9/L (1.3-5.6), with 0.9×10^9/L CLL cells (0-5.5) in 12 cases evaluated by flow cytometry, all staged A/0. No patient underwent treatment, except outlier pt #27. In 11/27 cases (41%), concurrently with the onset of SR, important clinical events were recorded (Table 1). No correlation with concomitant medication was observed. Two pts had a distinctive history: 1) Pt #26, 60 years old, female (mutated VH4-31, del13q). In 2013, diagnosis of stage A/I CLL, Ly 37.3×10^9/L. Nineteen months later, a Ly peak of 91.2×10^9/L was recorded. In 2015 she was restaged to A/0 and experienced a progressive decrease in her Ly count (39.6×10^9/L, 10.1×10^9/L, 5.6×10^9/L at the last observation in 2017). 2) Pt #13, 68 years old, male (mutated VH3-21, +12). In 2008, diagnosis of stage B/II CLL, Ly 51.9×10^9/L. Five months later, in 2009 a peak at 76.0×10^9/L was recorded; in 2011, the pt experienced several mild viral upper respiratory infections and CLL underwent SR that turn out to be complete in November 2011 and persists up to the last follow-up (Ly 1.9×10^9/L in April 2017), with stage A disease. Outlier pt #27 was the only who received treatment: 60 years old, male (mutated VH3-15, del13q), in 2009 was diagnosed with stage A/I CLL, Ly 14×10^9/L. In 2013, he received FCR for 6 cycles for disease progression (Ly 107×10^9/L) obtaining a PR but 18 months later he developed Richter's syndrome (DLBCL clonally unrelated to CLL) with the concomitant disappearance of the CLL clone from the peripheral blood and bone marrow, that has lasted up to August 2017 (Ly 2.2×10^9/L, CLL 0.037×10^9/L).
Summary/Conclusion: SR is a possibility, albeit infrequent, in the natural history of CLL, of which clinicians should be aware. In our large series, SR occurred preferably in pts in early clinical stage, low Ly count, negative CD38, favorable FISH, *TP53* WT, mutated *IGHV*, a preferential usage of the VH4-34, VH3-30 and 3-23 genes; SR was also observed in cases with trisomy 12. In most cases, SR was observed after a long observation period, usually in concurrence with infections or the appearance of second cancers, and were long-lasting, even in the presence of a persistent small clone of circulating leukemic cells.

PS1098

A PROGNOSTIC TOOL FOR THE IDENTIFICATION OF EARLY STAGE CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS AT IMMINENT RISK OF PROGRESSION

L. Terzi Di Bergamo[1,*], A. Condoluci[1,2], L. De Paoli[3], M. Gentile[4], G. Cutrona[5], C. Deambrogi[3], B. Gerber[2], V. Spina[1], A. Bruscaggin[1], E. Zucca[2], F. Cavalli[2], G. Stüssi[2], A. Neri[6], M. Ferrarini[7], F. Morabito[4], G. Gaidano[3], D. Rossi[1,2]

[1]Institute of Oncology Research, [2]Oncology Institute of Southern Switzerland, Bellinzona, Switzerland, [3]University of Eastern Piedmont, Novara, [4]Azienda Ospedaliera (AO) of Cosenza, Cosenza, [5]Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Azienda Ospedaliera Universitaria (AOU) San Martino-Istituto Nazionale per la Ricerca sul Cancro (IST), Genoa, [6]Department of Oncology and Hemato-Oncology, University of Milano, Milan, [7]Department of Experimental Medicine, University of Genova, Genoa, Italy

Background: Binet A CLL, that nowadays includes most of newly diagnosed cases, is usually managed with watch and wait. Binet A CLL has a considerable heterogeneous course. Some patients live for decades without therapy, while others require therapy within months from diagnosis. While current scoring systems anticipate overall survival among unselected CLL (i.e. MDACC, CLL-IPI, Barcelona-Brno scores), their performance in discriminating imminent risk of progression among Binet A patients is limited or unexplored.
Aims: Development of a model for prediction of treatment free survival (TFS) in Binet A CLL.
Methods: This is a multicentre, international, retrospective, observational study in which already existing and coded health-related personal data were further used. The adjusted association between exposure variables and TFS was estimated by Cox regression. Cox regression included exposure variables showing univariate association with TFS with Bonferroni corrected significant level <0.1. Backward elimination using likelihood ratio statistics with selection criterion p <.05 was used to derive the final model. An equally weighted combination of covariates was considered adequate if all pairwise ratios of regression coefficients ranged between 1:2 and 2:1. Discrimination capacity of the model was assessed through c-index.
Results: The study included a monoistitutional training cohort comprising 408 consecutive Binet A CLL patients initially managed with watch and wait, and followed for a median of 7 years. According to the CLL-IPI, 55% of patients scored low-risk, 37% intermediate-risk, 14% high-risk, and 4% very-high risk; according to the MDACC model, 32% scored low-risk, 67% intermediate-risk, and 1% high-risk; according to the Barcelona-Brno model, 67% scored low-risk, 27% intermediate-risk and 6% high-risk. Five independent predictors of TFS were identified: baseline lymphocyte count >15 G/L (HR 2.6), palpable lymph nodes (HR 2.3), palpable spleen (HR 2.7), unmutated IGHV genes (HR 2.7) and trisomy 12 (HR 2.4). Using weighted grading, a prognostic index was derived separating 4 different groups: low- (score 0), intermediate- (score 1), high- (score 2) and very high-risk (score 3-4) with significantly different probability of imminent need of therapy (2.8%, 6.9%, 24.6%, and 42.9% at 2 years for the low to very high-risk group, respectively) (Figure 1A). The ability of the model in discriminating treatment need (c-index 0.77) improved compared to the CLL-IPI (c-index 0.69), Barcelona-Brno score (c-index 0.66), and MDACC

score (c-index 0.61). The model was confirmed and the 4 risk groups were reproduced in a validation series comprising 287 Binet A CLL patients initially managed with watch and wait within the O-CLL1 (NCT00917540) prospective study (median follow-up 7 years; cumulative probability of treatment at 2 years: 0%, 10.4%, 17.0%, and 38.6% for the low to very high-risk group, respectively, c-index: 0.67) (Figure 1 B).

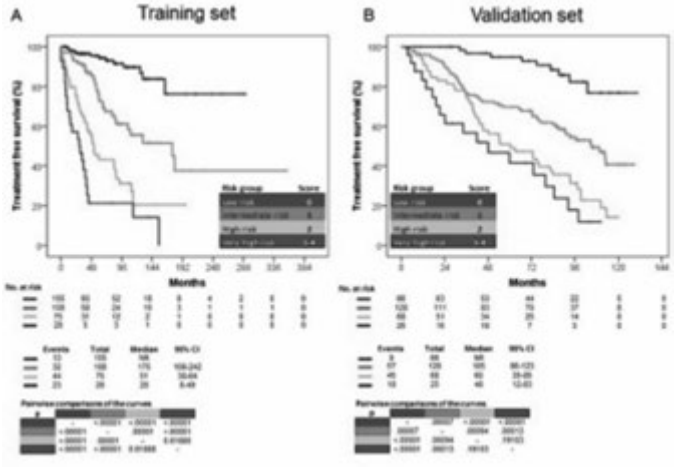


Figure 1.

Summary/Conclusion: The resulting "Binet A model" combines routine clinical and genetic variables into an easily applicable prognostic score for personalized estimate of the probability of imminent progression in early stage CLL. The Binet A model can help: i) design of clinical trials testing whether therapy initiation at an earlier stage than at manifestation of organ complications is beneficial for very high-risk patients; ii) precise prognostic counselling of patients regarding the implications of their disease.

PS1099

SURVIVAL CONTINUES TO INCREASE IN CHRONIC LYMPHOCYTIC LEUKEMIA: A POPULATION-BASED ANALYSIS AMONG 19,131 PATIENTS DIAGNOSED IN THE NETHERLANDS BETWEEN 1989 AND 2015

L. Van Der Straten[1,2,*], M.-D. Levin[2], O. Visser[3], E.F. Posthuma[4,5], J.K. Doorduijn[6], A.P. Kater[7], A.G. Dinmohamed[1,6,8]

[1]Research, Netherlands Comprehensive Cancer Organisation (IKNL), Utrecht, [2]Internal Medicine, Albert Schweitzer Hospital, Dordrecht, [3]Registration, Netherlands Comprehensive Cancer Organisation (IKNL), Utrecht, [4]Internal Medicine, Reinier The Graaf Hospital, Delft, [5]Hematology, Leiden University Medical Center, Leiden, [6]Hematology, Erasmus MC Cancer Institute , Rotterdam, [7]Hematology, Academic Medical Center, Amsterdam, [8]Public Health, Erasmus University Medical Center, Rotterdam, Netherlands

Background: The treatment landscape in chronic lymphocytic leukemia (CLL) rapidly evolved since the last decade with improvements in supportive care and the advent of anti-CD20 agents, kinase inhibitors, and anti-apoptotic agents. At present, it is largely unknown how these advances impacted survival of CLL patients at the population level.
Aims: The aim of this nationwide, population-study was to assess trends in short-term and long-term excess mortality (EM) among CLL patients diagnosed during a 27-year period in the Netherlands.
Methods: We selected all 19,131 CLL patients diagnosed between 1989-2015 (median age 69 years; range 21-101 years; 61% males; 14% with a prior malignancy) from the nationwide Netherlands Cancer Registry with follow-up through December 31, 2016. Patients were categorized into 4 periods (1989-1995, 1996-2002, 2003-2008, and 2009-2015) and 4 age groups (18-59, 60-69, 70-79, and ≥80). Age-standardized incidence rates (ASRs) were calculated per 100,000 person-years and standardized according to the European standard population. Relative survival (RS) was calculated as a measure of disease-specific survival. Multivariable evaluation of RS was performed using Poisson regression with adjustment for period of diagnosis, sex, age at diagnosis and a prior malignancy before CLL diagnosis. A $p<0.05$ indicates statistical significance.
Results: The annual ASR of CLL initially increased gradually since 1989; however, the overall incidence pattern subsequently stabilized at around 4.0 to 4.5 as from 2003. The overall ASR was consistently higher among males than females throughout the entire study period (5.0 *vs.* 2.6 in 1989-2015). Patients across the 4 age groups experienced continued EM as compared to the general population during all periods studies (Figure 1). Nevertheless, RS improved with each period for all 4 age groups. More specifically, 5-year RS (95% confidence intervals) was 80% (77%>83%), 71% (68%>75%), 64% (61%>68%), and 52% (46%>59%) in 1989-1995 for the 4 age groups, as compared with 94% (92%>96%), 90% (88%>92%), 84% (81%>87%), and 75% (69%>82%) in 2009-2015 ($p<0.001$ for all comparisons). Furthermore, 10-year RS was 58% (54%>62%), 51% (47%>55%), 42% (38%>47%), and 32% (23%>43%) in 1989-1995, as compared with 79% (76%>81%), 69% (65%>72%), 62% (58%>66%), and 43% (34%>53%) in 2003-2008 ($p<0.001$ for all comparisons). The multivariable analysis confirmed an improvement of survival over time, with an EM ratio (EMR) of 2.04 ($p<0.001$) in 1989-1995, 1.45 ($p<0.001$) in 1996-2002, and 0.65 ($p<0.001$) in 2009-2015, as compared with 2003-2008. Furthermore, females had lower EM, as compared to males (EMR, 0.66; $p<0.001$), whereas patients with a prior malignancy had higher EM, as compared with patients without a prior malignancy (EMR, 1.80; $p<0.001$). Subgroup analyses confirmed the consistency of the abovementioned associations across the 4 age groups, with the exception that females and males age ≥80 had comparable EM (EMR, 0.99; p=0.906).

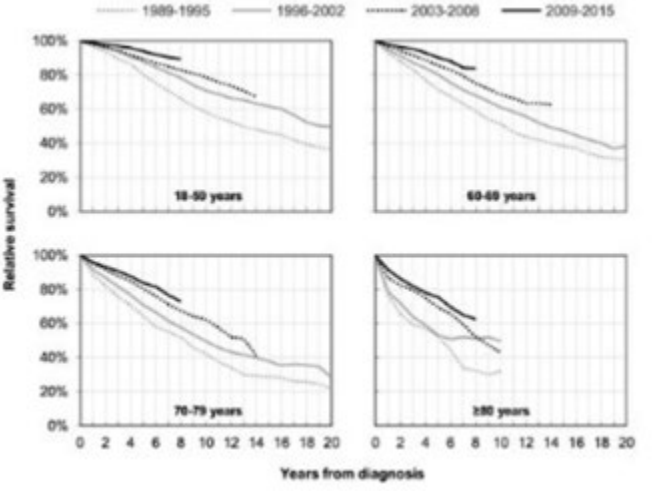


Figure 1.

Summary/Conclusion: In this large, nationwide, population-based study, 5- and 10-year RS improved over time among CLL patients across all age groups. Advances in supportive care, ameliorated management, and the advent of novel agents might have accounted for the improvement. Earlier detection of CLL might also have artificially increased survival estimates. However, this would have only marginally biased our results, as the overall ASR remained comparatively stable since 2003. As kinase inhibitors and anti-apoptotic agents have been introduced recently for routine use, EM will hopefully improve even more in the next decade.

PS1100

PROGNOSTICATION AND FIRST-LINE TREATMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA: A CONTEMPORARY, NATIONWIDE, POPULATION-BASED ANALYSIS AMONG 1,677 PATIENTS DIAGNOSED IN THE NETHERLANDS

L. Van Der Straten[1,2,*], M.-D. Levin[2], O. Visser[3], E. F. Posthuma[4,5], J.K. Doorduijn[6], A.P. Kater[7], A.G. Dinmohamed[1,6,8]

[1]Research, Netherlands Comprehensive Cancer Organisation (IKNL), Utrecht, [2]Internal Medicine, Albert Schweitzer Hospital, Dordrecht, [3]Registration, Netherlands Comprehensive Cancer Organisation (IKNL), Utrecht, [4]Internal Medicine, Reinier The Graaf Hospital, Delft, [5]Hematology, Leiden University Medical Center, Leiden, [6]Hematology, Erasmus MC Cancer Institute, Rotterdam, [7]Hematology, Academic Medical Center, Amsterdam, [8]Public Health, Erasmus University Medical Center, Rotterdam, Netherlands

Background: The natural history of chronic lymphocytic leukemia (CLL) is heterogeneous. Therefore, accurate prognostication is pivotal to predict outcome and plan risk-adapted therapy. At present, it is virtually unknown how prognostic tests and first-line treatment are applied among CLL patients at the population level.
Aims: The aim of this nationwide, population-study was to assess how prog-

nostic tests and first-line treatment were utilized among CLL patients diagnosed in a contemporary era in the Netherlands.
Methods: We selected all CLL patients diagnosed between 2014-2015 from the nationwide Netherlands Cancer Registry (NCR) with follow-up for survival until December 31, 2016. Information on cytogenetic (ie, karyotyping and/or FISH) and molecular testing, Rai stage, and first-line treatment started within one year after diagnosis is available in the NCR as from 2014 onward. Multivariable logistic regression (MLR) analysis was used to assess factors associated with cytogenetic testing and first-line treatment, adjusted for covariates presented in the Figure. One-year relative survival (RS) was calculated as a measure of early excess mortality (EEM). p<0.05 indicates statistical significance.
Results: A total of 1,667 CLL patients (median age 69 years; 63% males) was included in the study. Patient characteristics are presented in Table 1. The Rai stage was undeterminable in 139 (8%) patients because at least one of the parameters that compose the Rai stage was missing. Cytogenetic and molecular testing were performed in 435 (26%) and 106 (6%) of patients, respectively. Del(17p) and *TP53* mutations were detected in 12 of 435 (3%) and 6 of 106 (6%) patients, respectively. First-line therapy was started in 347 (21%) patients. R-Chl (36%; median age 77; interquartile range [IQR] 72-81) and FCR (30%; median age 61; IQR 56-65) were the most commonly applied therapies. Following MLR analysis, age per 10-year increase and female sex were associated with unperformed cytogenetics, whereas cytogenetic testing was more likely performed among patients diagnosed in academic centers, patients with Rai stage ≥1, and patients who started first-line treatment (Table 2). Now turning to variables associated with the start of first-line treatment (Table 3). Comorbidity per one condition increase and Rai stage 0-1 were factors associated with a lower odds to start first-line treatment. Conversely, patients with Rai stage 3-4 and patients in whom cytogenetic and molecular testing were performed were more likely to start with first-line therapy (Table 3). One-year RS (95% confidence intervals) was 100% (98%>101%), 99% (95%>101%), 97% (93%>100%), 87% (80%>92%), and 85% (76%>91%) for patients with Rai stage 0, 1, 2, 3, and 4, respectively.

Tables 1, 2, 3.

Table 1. Patient characteristics

Characteristics	No.	(%)
Total No. of patients	1677	
Female sex	620	(37)
Age, years		
Median (range)	69	(35-95)
18-59	300	(18)
60-69	542	(32)
70-79	527	(31)
≥80	302	(18)
Diagnosis in academic center	104	(6)
No. of comorbidities		
Median (range)	1	(0-9)
None	545	(32)
One	484	(29)
Two or more	648	(39)
Rai stage		
0	773	(46)
1	244	(15)
2	202	(12)
3	186	(11)
4	133	(8)
Unknown	139	(8)
Cytogenetics performed	435	(26)
Molecular genetics performed	106	(6)
First-line treatment	347	(21)
Type of first-line treatment		
Rituximab and chlorambucil (R-Chl)	124	(36)
Rituximab and FC (FCR)	104	(30)
Chlorambucil only	44	(13)
Rituximab and CVP (R-CVP)	32	(9)
Rituximab ± other	21	(6)
Bendamustine and rituximab	11	(3)
Ibrutinib	2	(1)
Rituximab and idelalisib	1	(0)
Other	8	(2)

Table 2. Results of the multivariable logistic regression analysis of covariates associated with cytogenetic testing

Covariate	OR	95% CI	P
Female sex	0.74	0.58 - 0.96	0.025
Age, years[a]	0.73	0.64 - 0.82	<0.001
Diagnosis in academic hospital	3.09	2.00 - 4.77	<0.001
Comorbidity[b]	0.97	0.86 - 1.06	0.456
Rai stage			
0	1	(reference)	
1	2.81	1.99 - 3.98	<0.001
2	2.17	1.48 - 3.16	<0.001
3	2.83	1.83 - 4.36	<0.001
4	2.37	1.47 - 3.83	<0.001
Unknown	1.01	0.59 - 1.71	0.984
Started first-line treatment	3.00	2.17 - 4.14	<0.001

Abbreviations: OR, odds ratio; CI, confidence interval.
[a]Per 10-year increase
[b]Per one condition increase

Table 3. Results of the multivariable logistic regression analysis of covariates associated with initiation of first-line treatment

Covariate	OR	95% CI	P
Female sex	0.94	0.67 - 1.30	0.705
Age, years[a]	1.15	0.98 - 1.35	0.084
Diagnosis in academic hospital	0.94	0.51 - 1.73	0.837
Comorbidity[b]	0.84	0.75 - 0.94	0.003
Rai stage			
0	0.05	0.02 - 0.09	<0.001
1	0.51	0.32 - 0.81	0.004
2	1	(reference)	
3	4.63	2.89 - 7.40	<0.001
4	5.03	3.04 - 8.30	<0.001
Unknown	0.35	0.18 - 0.66	0.001
Cytogenetic testing performed	2.70	1.94 - 3.76	<0.001
Molecular testing performed	2.44	1.42 - 4.21	0.001

Summary/Conclusion: This is the first population-based study showing how prognostic procedures and first-line treatment are utilized among CLL patients diagnosed in a contemporary era. Congruent with clinical practice guidelines, most, but not all, CLL patients with advanced disease and who started with first-line therapy underwent (cyto)genetic testing. A novel, although expected finding was that patients with Rai stage 0-2 do not experience EEM, whereas patients with Rai stage 3-4 experience considerable EEM, as compared with the general population. To date, no study has presented such outcomes corrected for the life expectancy in the general population. Collectively, population-based registries are useful instruments to assess guideline adherence and the prognostic value of staging systems at the population level.

PS1101

"IMMUNO-FLOW-FISH": HIGH THROUGHPUT FLUORESCENCE IN SITU HYBRIDISATION OF PHENOTYPED CHRONIC LYMPHOCYTIC LEUKAEMIA BY IMAGING FLOW CYTOMETRY FOR CLINICAL APPLICATION

W. Erber[1,2,*], H. Hui[3], K. Fuller[2,3]

[1]Faculty of Health and Medical Sciences, University of Western Australia, Crawley, [2]Translational Cancer Pathology Laboratory, PathWest Laboratory Medicine, Nedlands, [3]School of Biomedical Sciences, University of Western Australia, Crawley, Australia

Background: Imaging flow cytometry is emerging as a diagnostic tool for the assessment of leukaemia. It has the functionality of standard flow cytometry and generates high-resolution digital images of each cell and fluorescence signals with quantifiable numerical data. We have developed an automated high-throughput method for performing FISH on phenotyped whole cells and analysed by imaging flow cytometry.
Aims: Our aim was to investigate the capability and sensitivity of this "immuno-flowFISH" method to detect trisomy 12 and del(17p) in phenotyped chronic lymphocytic leukaemia (CLL) cells.
Methods: Blood from 55 *de novo* and treated CLL patients with lymphocyte counts ranging from 3.6-210 x10^9/L was collected into EDTA anticoagulant. After red cell lysis, cells were incubated with CD3, CD5 and CD19 antibodies conjugated to fluorochromes (BV480, AF647, BB515, V500c). Following fixation, cell membranes were permeabilised and double-stranded DNA denatured (73-74°C for 5 mins). FISH probes to chromosomes 12 (CEP12) or 17 (CEP17) and 17p12 (Spectrum Orange, Spectrum Green or Orange Red fluorochromes) were added and hybridised for 24-30 hours at 37°C. Nuclei were stained with SYTOX AADvanced. Data for up to 50,000 cells was collected on the Amnis ImageStream®^{X} Mark II imaging flow cytometer. Digital images (x60) and quantitative data (IDEAS software) were used to assess FISH signals overlying the SYTOX-stained nuclei of CD5/CD19-positive CLL cells for each probe. The number and percent FISH-positive CLL cells was determined. The ratio of FISH spot counts for CD5/CD19-positive CLL cells to CD3/CD5-positive T cells (FISH "mean spot ratio") was calculated. FISH results were compared with pre-treatment traditional FISH.
Results: A total of 10,000-50,000 cells (mean = 20,000 per case) was analysed per CLL case. Of the 55 CLL cases, there were 13 with trisomy 12 (+12) and 5 with del(17p) in the CD5/CD19-positive CLL cells. The FISH abnormalities were seen on the digital images and confirmed by quantitative mean channel fluorescence intensity of the probes. The CD3/CD5-positive T cells in all cases had diploid signals for each probe in all cells. In the +12 cases, 3 FISH spots for CEP12 were detected in 0.1-46.0% of the CLL cells (26-9,111 cells); the FISH "mean spot ratio" for CEP12 ranged from 1.09-1.36. There were 5 cases with del(17p) in the CLL cells; these all had normal diploid spots for the control CEP17 probe. The number of CLL cells with del(17p) ranged from 3.5-22.8% (363-3,382 cells) and the FISH "mean spot ratio" 0.86-0.96. The remaining 42/55 cases had normal 2-spot patterns for CEP12, 17p12 and CEP17 in CLL and T-cells. All CLL FISH results were concordant with pre-treatment FISH results.
Summary/Conclusion: Immuno-flowFISH is a powerful and highly sensitive automated high throughput method capable of detecting FISH abnormalities in intact phenotyped CLL cells. By analyzing CLL with 3 antibodies and probes, and a minimum of 10,000 cells per case, we have significantly increased both the sensitivity and specificity over traditional FISH (100-200 times more cells and restricted to the CLL population). Immuno-flowFISH is an exciting new automated method that significantly improves the accuracy and limit of detection of chromosomal abnormalities in CLL. This method is capable of detecting low-level abnormalities that are clinically significant in therapeutic decision-making and for disease monitoring.

PS1102

EXPRESSION OF ANTIGENS INVOLVED IN THE FORMATION OF AN IMMUNOLOGICAL SYNAPSE IN CHRONIC LYMPHOCYTIC LEUKEMIA

D. Badmazhapova[1,*], I. Galtseva[2], E. Zvonkov[1], Y. Davydova[2], N. Kapranov[2], E. Parovichnikova[2], V. Savchenko[2]

[1]Department of lymphoma chemotherapy, [2]National Research Center for Hematology, Moscow, Russian Federation

Background: The pathogenesis of Chronic lymphocytic leukemia (CLL) is very complex. Recently it was found that CLL cells are able to form immunological synapses with microenvironment cells, directly and indirectly affecting their function. Therefore, it became clear that the pathogenesis of

CLL includes not only inhibition of apoptosis but also the ability of CLL cells to cause T-lymphocyte anergy, thereby avoiding immune surveillance.
Aims: to study the expression of FAS, CD80 and CD86, PD-1, PD-L1 on CLL cells, and also to evaluate the main subpopulations of T-cells (naïve (CD95-CD28+), memory (CD95+CD28+), effector cells (CD95+CD28-)).
Methods: The study included 46 CLL patients: 16 patients were in progression of the disease after chemotherapy and 30 patients had newly diagnosed with CLL (primary). Primary CLL patients were categorized according to Binet stages. Stage A was established in 14 patients, B - 10, C - 6. The control group included 29 healthy donors. Peripheral blood samples were analysed on a 6-color flow cytometer BD FACS Canto II (BD Biosciences, USA). The panel of monoclonal antibodies included antibodies against: CD19 PE (SJ25C1), CD5 PerCP-Cy5.5 (L17F12), CD4 APC-Cy7 (SK3), CD8 PerCP (SK1), CD3 APC (SK7), CD279 (PD-1) FITC (MIH4), CD274 (PD-L1) PE-Cy7 (MIH1), CD80 FITC (L307.4), CD86 APC (2331), CD95 (FAS) PE-Cy7 (DX2), CD28 PE (CD28.2), CD25 FITC (2A3), CD45 APC-Cy7 (2D1) from BD Biosciences.
Results: In CLL patients, the% CD80+, CD86+, FAS+ B-cells were significantly lower than in donors. In primary CLL the% CD80+ B-cells was higher than in CLL progression. That means, CLL cells do not form a second co-stimulatory signal with T cells and promote their anergy. Low expression of FAS on CLL cells provides protection of FAS-mediated apoptosis. The data is presented in Table 1. Among primary CLL patients% CD80+ and CD86+ B-cells was lower in advanced stages of the disease (CD80: A – 5.94±1.61%, B – 3.49±1.95%, C – 0.43±1.67%; CD86: A – 8.95±2.49%, B – 4.04±3.11%, C – 1.45±0.75%, p<0.05). Probably, in advanced stages CLL the formation of the immune synapse is much more altered than in low grades CLL. PD-L1+ B-cells was lower in CLL patients than in donors.% PD-1+ B-cells was higher in CLL progression than in donors and primary CLL.% In primary CLL,% PD-L1+ B-cells was higher in stage A (A-10.02±5.75, B-0.25±0.03, C-2.05±1.32, p<0.05). Thus, in non-advanced stages CLL cells are resembling healthy B-cells. % PD-1+ T-helpers was higher in CLL patients than in donors, and in primary CLL it was higher in advanced stages (B vs C, 14.43±2.51 vs 27.43±4.75, p=0.046). This increase means that T-cells gain the "exhausted" phenotype.% PD-L1+ T-cells in CLL was lower than in donors.% naive T-cells were lower,% effector cells and% memory cells were higher in patients compared to donors.

Table 1.

Parameters, %	Primary CLL	CLL progression	Donors
B-cells			
CD19+CD80+	4.02±1.04	1.15±0.43**	16.89±1.55*
CD19+CD86+	5.81±1.62	2.46±0.66	9.77±2.32*
CD19+FAS+	6.14±1.21	11.34±2.60**	16.59±1.99*
CD19+PD-1+	12.31±2.64	19.57±3.75	1.27±0.23*
CD19+PD-L1+	5.17±2.78	4.14±2.99	35.76±3.13*
T-cells			
CD4+PD-1+	19.8±2.21	30.05±3.90**	10.78±0.93*
CD8+PD-1+	18.14±2.30	15.35±2.39	16.89±1.36
CD4+PD-L1+	0.93±0.15	0.59±0.12	1.21±0.16***
CD8+PD-L1+	0.43±0.08	0.25±0.05	1.09±0.26*
Naive CD4+	30.93±3.74	5.35±1.90**	40.20±3.83***
CD4+ memory	59.47±3.53	78.76±4.54**	54.36±3.27***
CD4+ effectors	9.32±2.17	15.75±4.68	5.24±1.85
Naive CD8+	16.92±3.39	3.76±1.61**	34.39±3.90*
CD8+ memory	26.75±2.49	33.74±4.60	27.97±2.03
CD8+ effectors	9.32±2.17	15.75±4.68	5.24±1.85*
* - differences between donors and both CLL, ** - differences between both CLL, *** - differences between donors and progression CLL			

Summary/Conclusion: Decline of CD80+ and CD86+ B-cells in CLL can cause ineffectiveness of an immunological synapse between CLL cells and T-cells, which leads to anergy of T-cells. Low expression of FAS allows CLL cells to avoid FAS-mediated apoptosis. A shift in the T-cell subpopulations toward memory cells and effectors, high PD-1 expression on T-cells, impairs antitumor immunity and, possibly, supports CLL progression.

PS1103

IDENTIFICATION OF NEWLY DIAGNOSED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA AT HIGH RISK OF INFECTION OR TREATMENT THROUGH MACHINE LEARNING ALGORITHMS

R. Agius[1], M.A. Andersen[2], B. Ledergerber[3], A. Pearson[4], A. Cozzi-Lepri[5], Y. Louzoun[6], C.L. Andersen[7], J. Bergstedt[8], J. Hjorth von Stemann[9], M. Jørgensen[10], C. MacPherson[10,11], M.-H. E. Tang[10], C.H. Grønbech[12], M. Fontes[10,13], J. Lundgren[9], J. Larsen[1], C.U. Niemann[2,*]

[1]Department of Mathematics and Computer Science, Technical University of Denmark, Lyngby, [2]Hematology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark, [3]University of Zurich, Zurich, Switzerland, [4]University of Chicago, Chicago, United States, [5]University College London, London, United Kingdom, [6]Department of Mathematics, Bar-Ilan University, Ramat Gan, Israel, [7]Department of Public Health, Copenhagen University, Copenhagen, Denmark, [8]Department for automatic control, Lund university, Lund, Sweden, [9]Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark, [10]Centre of Excellence for Health, Immunity and Infections (CHIP), Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark, [11] bioStone OU, Tallin, Estonia, [12]Technical University of Denmark, Lyngby, Denmark, [13]International Group for Data Analysis, Institut Pasteur, Paris, France

Background: For patients with Chronic Lymphocytic Leukemia (CLL), infections are a significant cause of morbidity and mortality despite improved supportive care. More than 30% of fatalities in CLL list infections as primary or contributory cause of death. Based on a prognostic index like CLL-IPI, patients at high risk of short treatment free survival can be identified. However, identification of patients at high risk of infection prior to treatment is warranted to allow for preemptive and prophylactic measures for those patients.
Aims: We set out to predict risk of early infection or treatment for patients newly diagnosed with CLL based on known prognostic factors and laboratory results. We systematically assessed the performance of various machine learning algorithms for this task.
Methods: By means of the nationwide Danish CLL registry, the Danish Microbiology Database, the Persimune data warehouse and health registries, data for 2772 CLL patients were assembled. The study was approved by the Danish Health Authorities and Data Protection Agency. The event of a blood culture was used as a proxy for severe infection, regardless of the result. Data on treatment for CLL and survival were registry based. Standard hematology and biochemistry results from general practitioners and hospitals, in addition to microbiology, cytogenetics, and IGHV mutational status, were included for the period 12 months prior to diagnosis until 3 months post diagnosis. We trained models to predict a composite outcome defined as either the development of an infection or starting CLL treatment within two years. Model development and data analysis were initiated during a one week international workshop with performance assessments of different approaches including simple linear-models, ensemble tree-based methods and deep learning approaches. Models were trained on a random split 80% of the data set and tested for generalizability on the remaining 20% test set. The here reported model, which performed best on the test set, was an extreme gradient boosting model using a combined set of features designed to capture information on the distribution of laboratory events and "Bag-of-Words" features akin to those used in early natural language processing models.

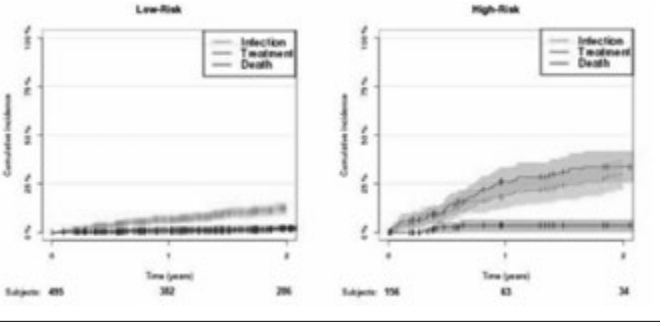


Figure 1.

Results: Within two years, 31% of newly diagnosed CLL patients had an event of severe infection or treatment, which our model could predict with a positive predictive value (PPV) of 70% and a true positive rate (TPR) of 58%. When restricting the analysis to the subset with full CLL-IPI variables available (cytogenetics, IGHV mutational status, beta2microglobuline, clinical stage and age), a PPV of 75% and a TPR of 58% was achieved. Thus, our model was capable of accounting for missing values with only a slight reduction in PPV. On the same subset, the CLL-IPI Index (score 0-3 vs. 4-10) achieved a PPV of 61% and a TPR of 28%. As seen from the cumulative incidence plots of infection, treatment and death (only first event counting), both infection and treatment events are predicted for the high risk population, which constituted 24% of the full population (Figure 1).
Summary/Conclusion: By applying machine learning models on baseline characteristics and laboratory values, previous prognostic approaches in

CLL were improved. We identified a sub-population (24%) of patients with a 70% risk of infection or CLL treatment within two years of diagnosis. A randomized, preemptive trial with targeted therapy for this patient population is being planned, by which we aim to change the natural history of immune dysfunction in CLL for this patient population.

PS1104

HEALTH-RELATED QUALITY OF LIFE (HRQOL) IN RELATION TO RESPONSE TO FIRSTLINE THERAPY AND SUBSEQUENT TREATMENTS – A METAANALYSIS OF HRQOL MEASURED IN PHASE III TRIALS OF THE GERMAN CLL STUDY GROUP

N. Kutsch[1,*], V. Goede[1], S. Robrecht[1], F. Kelma[1], J. Bahlo[1], K. Fischer[1], A.-M. Fink[1], R. Schlag[2], E. Lange[3], R. Weide[4], M. Dreyling[5], U. Jäger[6], S. Stilgenbauer[7], H. Döhner[7], M. Kneba[8], M. Ritgen[8], C.-M. Wendtner[9], M. Hallek[1], B. Eichhorst[1]

[1]Department I of Internal Medicine and Center of Integrated Oncology Cologne Bonn, University Hospital of Cologne, Cologne, [2]Onkologische Gemeinschaftspraxis Würzburg, Würzburg, [3]Evangelisches Krankenhaus Hamm, Hamm, [4]Praxisklinik für Hämatologie und Onkologie Koblenz, Koblenz, [5]Department of Internal Medicine III, Ludwig-Maximilians-Universität Munich, Munich, Germany, [6]Vienna General Hospital, Vienna, Austria, [7]Department of Internal Medicine III, University Hospital of Ulm, Ulm, [8]Department of Medicine II, University of Schleswig-Holstein, Kiel, [9]Department of Hematology, Oncology, Immunology, Infectious Diseases and Tropical Medicine, Klinikum Schwabing, Munich, Germany

Background: Treatment of advanced CLL with chemoimmunotherapy (CIT) has improved outcome with regards to progression-free survival and overall survival significantly. Besides these endpoints health-related quality of life (HRQOL) becomes increasingly relevant for physicians and patients (pts). The German CLL study group (GCLLSG) has performed a metaanalysis on HRQOL of pts receiving different chemotherapies (CT) and CIT within three phase III trials.
Aims: To evaluate HRQOL in relation to response to firstline therapy and subsequent treatments.
Methods: Data of 2159 pts from the CLL 8 (817 pts), CLL10 (561 pts) and CLL11 (781 pts) study protocol of the GCLLSG were pooled. In the CLL8 and CLL10 study, the questionnaires (ques) were sent to all patients at baseline and after 3, 6 or 8 and 12 months and then yearly as follow up (FU). In the CLL11 study, ques were sent out at screening, twice during therapy, at end of therapy and then every 3 months until 3 years, and later every 6 months until 5 years. The first three months of treatment are referred to treatment phase (TP) I, treatment beyond the first three months to TP II. HRQOL was evaluated using the EORTC C30 questionnaire. All scales were calculated according to the standard procedure as given by the EORTC-QLQ-C30 manual.
Results: 2065 pts receiving treatment filled out at least one ques. These pts were treated with the following frontline CT and CIT: 374 FC, 663 FCR, 268 BR, 116 Chlorambucil (Clb), 322 R-Clb and 322 G-Clb. 1334 pts filled out ques at baseline, 1139 pts in TP I and 575 pts in TP II. In the first 12 months of FU, 1773 pts filled out ques, while at later FU after 60 months only 291 pts completed ques. Comparing baseline global health status (GH) of responders versus non-responders, responders had a higher GH score than non-responders (mean 62.2 vs. 58.2, p= 0.028). Responders also had a higher GH than non-responders at FU until month 12 (mean 68.2 vs. 61.4, p<0.001). Similarly, physical functioning (PF) in responders was better at baseline (79.8 vs. 75.4, p=0.004) and in FU until month 12 (81.3 vs. 76.1, p<0.001). In contrast to that, role functioning (RF) and social functioning were worse in responders in TP I (73.9 vs. 79.6, p=0.017; 78.5 vs. 85.3, p=0.001). Responders had higher fatigue symptoms than non-responders in TP II only (mean 41.1 vs. 29.3, p=0.017), but not in TP I (mean 36.0 vs.31.9, p=0.060). Furthermore, a subgroup analysis of HRQOL according to progression after frontline treatment was performed. In patients with progression but without subsequent treatment (N=188) GH, PF, RF and fatigue were significantly impaired after progression occurred (before vs. after progression: GH: mean 69.5 vs. 65.9, p= 0.018; PF: 82.2 vs. 78.8, p=0.001; RF: 77.3 vs. 73.4, p= 0.029; fatigue: 30.1 vs. 33.1, p=0.025). In patients with progression and subsequent therapy (N=92) all HRQOL items were impaired after start of relapse therapy (Figure 1). Subsequent therapies consisted of CT in 20 pts and CIT in 68 pts with the exception of 2 stem cell transplants, 1 unknown treatment and 1 patient presumably receiving ibrutinib.
Summary/Conclusion: This meta-analysis shows that HRQOL in CLL pts is correlated to treatment interventions including outcome of therapy. HRQOL was impaired in pts with progression after frontline without immediate therapy, but was more profoundly negatively affected in all functioning and symptom scales when subsequent therapy was administered. The question remains if new oral inhibitors achieve a better tolerance and therefore an improved HRQOL.

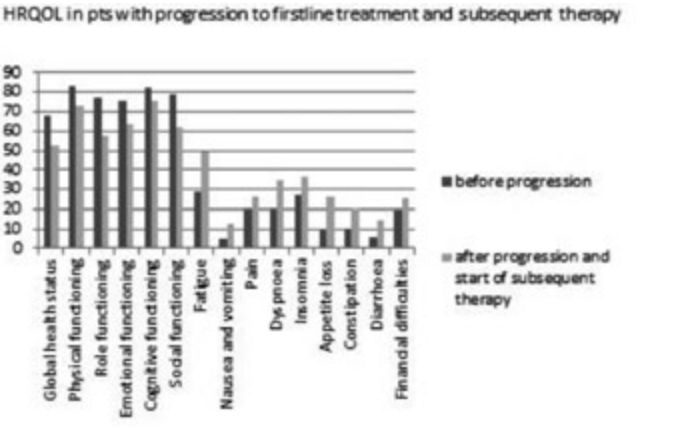


Figure 1.

PS1105

T-LARGE GRANULAR LYMPHOCYTIC (T-LGL) LEUKEMIA: A LONG-TERM STUDY ON 51 PATIENTS-COULD WE CONFINE THE USE OF THE TERM "LEUKEMIA"?

X. Yiakoumis[1,*], E. Koulieris[1], C. Kalpadakis[2], S. Sachanas[1], M. Moschogiannis[1], P. Tsirkinidis[1], E. Kyritsi[3], M. Angelopoulou[3], T. Vassilakopoulos[3], F. Kontopidou[4], P. Korkolopoulou[5], D. Rontogianni[6], H. Papadaki[2], G. Pangalis[1]

[1]Haematology, Athens Medical Center-Psychikon branch, Athens, [2]Haematology, University Hospital, University of Crete, Heraclion, [3]Haematology, University of Athens, Laikon General Hospital, [4]Second Department of Internal Medicine, University of Athens, "Hippocrateio" General Hospital, [5]Pathology, University of Athens, [6]Anatomic Pathology, Evangelismos General Hospital, Athens, Greece

Background: T-LGL leukemia is a clonal lymphoproliferative disorder with abnormal blood parameters and/or recurrent infections, as well as immunophenotypic confirmation of a clonal CD3+/CD8+/CD57+population, usually but not necessarily > $0.5x10^9$/L. The above criteria, through usage of sensitive techniques, help to identify even more frequently patients with clonal T-cell disorders, who seem to have a relatively heterogeneous clinical and prognostic features and their optimal long-term management is not defined.
Aims: To report a series of patients (pts) diagnosed with T-LGL leukemia, according to established criteria and to address the question regarding the universal use of the term "leukemia" for these patients.
Methods: Our pts were identified on the basis of an abnormal leucocyte number or differential, as having a T-LGL expansion by morphology and were further studied. hey underwent a complete clinical, laboratory, radiologic, and immunological screening, combined with a blood immunophenotype, a T-cell receptor clonality assay by PCR (TCR), as well as a bone marrow trephine biopsy, whenever possible, and then followed on a regular basis or treated accordingly.
Results: 51 pts (30 female) were included. 30 presented with a reverse differential, 13 with neutropenia ($0.4\text{-}1.5x10^9$/L, median 1.2) and 8 with lymphocytosis ($4.0\text{-}10.2x10^9$/L, median 5.6). Only 2 reported a concurrent autoimmune disease (1 rheumatoid arthritis and 1 systemic lupus erythematosus) and none mentioned serious/recurrent infections. Median age was 55 years (15-80) and median follow up from diagnosis 34 months (6-178). 20% have been followed for >5 years (60 months) and 10% > 10 years. Most pts reported the presence of the disorder for more than 3 years before diagnosis (range 0-21 years). All had a blood smear indicative of T-LGL lymphocytosis by morphology and underwent a blood immunophenotype which pointed out a T-LGL expansion with a median absolute number of CD8+/CD57+ of $0.545x10^9$/L (0.02-4.9). 44 were also found to have a clonal TCR by PCR while 7 were TCR (-). 31 underwent bone marrow core biopsy. 21/31 had a bone marrow infiltration (12-25%) and 10/31 had only reactive bone marrow findings. All patients with T-LGL absolute number >1.0 10^9/L (8/51) had a significant infiltration of bone marrow; the same was also observed in 6 pts with T-LGL <$0.5x10^9$/l; median T-LGL count in pts with bone marrow infiltration was $0.72x10^9$/L (0.02-4.95) (Table 1). Only 2 received supportive treatment (erythropoietin) due to symptomatic anemia, with good response and both remain asymptomatic

after 37 and 45 months respectively. All patients were alive, with no disease progression at last follow up. No evolution to frank leukemia, requiring additional studies or therapeutic management was observed in our series.

Table 1. Characteristics of T-LGL patients.

Number of pts	51
Male/female	21/30
Median age	55 (15-80)
Clinical presentation -Reverse differential -Neutropenia -Lymphocytosis -Anemia -Autoimmune diseases	 30 (59%) 13 (25%) 8 (16%) 2 (4%) 2 (4%)
Median T-LGL count	0.545 x 10^9/l (0.02-4.9)
TCR +/-	44/7
Bone marrow infiltration	21/31 (68%)
Median T-LGL count in pts with bone marrow infiltration	0.72 x 10^9/l (0.02-4.95)
Median follow-up duration	34mo (6-178)

Summary/Conclusion: We present a series of patients with clonal T-LGL lymphoproliferation who, with current use of international criteria, should bare the diagnosis of T-LGL leukemia. The prognosis was excellent for the entire series. The presence of serious symptoms and/or progression of the disease should be necessary for the diagnosis of and any therapeutic intervention for T-LGL leukemia. In their absence, the terminology of "monoclonal T-large granular cell lymphocytosis" could be more appropriate.

PS1106

FLOW CYTOMETRY AND PCR-BASED T-CELL CLONALITY TESTING FOR DISCRIMINATING BETWEEN REACTIVE AND NEOPLASTIC PROLIFERATION OF LARGE GRANULAR LYMPHOCYTES

N. Chernova[1,*], Y. Sidorova[2], E. Zakharko[3], N. Ryzhikova[2], E. Rybkina[3], V. Dvirnyk[3], A. Sudarikov[2], E. Zvonkov[1]

[1]Department Chemotherapy of Lymphomas, [2]Department of Molecular Hematology, [3]Clinical diagnostic laboratory, National Research Center for Hematology, Moscow, Russian Federation

Background: Large granular lymphocytes (LGL) lymphocytosis can be associated with viral infections, autoimmune disorders, chronic lymphoproliferative disorder of NK-cells (CLPD-NK) and T-cell large granular lymphocytic leukemia (T-LGL). Differential diagnosis between reactive and neoplastic LGL proliferation could be a challenge.
Aims: To evaluate the importance of morphological, immunophenotypic (flow cytometry) and molecular genetic (PCR-based T-cell clonality) studies in differential diagnosis for LGL proliferation.
Methods: 67 patients with increased peripheral blood LGL counts were enrolled in the study. Male/female ratio was 19/48; a median age was 53 (22–79) years. Morphology, immunophenotype by flow cytometry and PCR-detection of T-cell clonality (TCRG (Vγ-Jγ) and TCRB (Dβ-Jβ and Vβ-Jβ)) were performed in all cases.
Results: All patients were divided into 3 groups according to their diagnosis: CLPD-NK (n = 8), T-LGL (n = 42), reactive LGL lymphocytosis (n = 17). CLPD-NK was established based on LGL morphology, CD3-CD4-CD8+/-CD16+CD56+/- immunophenotype and germline configure uration of TCR genes. The heterogeneity of CD7 expression was identified in 2 of 8 cases. Persistent increase (> 6 mouths) in peripheral blood LGL counts was noted in 42 patients with T-LGL. 30 cases were TCRαβ-positive and 12 cases were TCRγδ-positive. Immunophenotypic variants of 30 TCRαβ-positive cases were: CD3+CD4-CD8+CD16-CD56- in 13, CD3+CD4-CD8+CD16+/-CD56+/- in 9, CD3+CD4+CD8-CD16+/-CD56+/- in 4, CD3+CD4+CD8+CD16+/-CD56+/- in 4. TCRγδ-positive cases were characterized by CD3+CD4- immunophenotype and variable expression of CD8, CD16 and CD56. CD3+CD4-CD8- variant of T-LGL was determined in 5 patients. The relative and absolute LGL counts were different depending on immunophenotypic variant of T-LGL. The heterogenic expression of at least one of CD2, CD5 and CD7 was noted for 85% of cases. TCRG (Vγ-Jγ) and/or TCRB (Vβ-Jβ, Dβ-Jβ) genes were clonally rearranged in 41 (97.6%) of 42 cases. Clonal rearrangements of δ-chain TCR genes only were observed in one TCRγδ-positive case of T-LGL.
Persistent increase (< 6 mouths) in peripheral blood LGL counts was observed in 17 cases of reactive lymphocytosis. Immunophenotype of LGL was CD3+CD4-CD8+CD16-CD56- in 11 of 17 cases, CD3+CD4-CD8+CD16+/-CD56+/- – in 6. The heterogenic expression of at least one of T-cell markers was observed in 47,1% of cases. Interestingly, LGL number exceeded 2x10^9/l twice in 5 of 17 cases. Notably, reactive LGL lymphocytosis was detected in 7 patients with other tumors of haematopoietic and lymphoid tissues: extranodal NK/T cell lymphoma, nasal type – in 1 case, T-cell/histiocyte-rich large B-cell lymphoma – in 1, myelodysplastic syndrome – in 5. Clonal rearrangements of TCRG (Vγ-Jγ) and incomplete TCRB (Dβ-Jβ) genes were determined in 3 and 1 cases respectively. TCRB (Vβ-Jβ) genes were not clonally rearranged in any case. The results of T-cell clonality testing are summarized in the Table 1.

Table 1.

Parameter	TCRG (Vγ-Jγ)	TCRB (Dβ-Jβ)	TCRB (Vβ-Jβ)	TCRB (Dβ-Jβ and/or Vβ-Jβ)
CLPD-NK, n=8	0	0	0	0
T-LGL, n=42	36 (85,7%)	22 (52,4%)	33 (78,6%)	37 (88,1%)
TCRαβ-positive T-LGL, n=30	25 (83,3%)	17 (56,7%)	29 (96,7%)	30 (100%)
TCRγδ-positive T-LGL, n=12	11 (91,7%)	5 (41,7%)	4 (33,3%)	7 (58,3%)
Reactive LGL lymphocytosis, n=17	3 (17,6%)	1 (5,9%)	0	1 (5,9%)

Summary/Conclusion: Discriminating between reactive LGL lymphocytosis and T-cell large granular lymphocytic leukemia still could be a challenge. Morphology, immunophenotype, relative and absolute LGL counts and the heterogenic expression of T-cell markers do not distinguish between neoplastic and reactive processes. Clonal TCRG (Vγ-Jγ) and TCRB (Dβ-Jβ) gene rearrangements were observed in reactive LGL lymphocytes and T-LGL. The complete rearrangements of TCRB (Vβ-Jβ) genes were specific for the tumor process and were not detected in either case of reactive lymphocytosis.

PS1107

A US BASED SURVEY: THE EXPERIENCES AND PERSPECTIVES OF 1147 CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS

B. Koffman[1,*], B. Dennison[2], K. Kennard[3], R. Furman[4], J. Byrd[5], J. Pagel[6], M. S. Davids[7], C. Nabhan[8], N. Kay[9], D. Brander[10], T. Siddiqi[11], B. Hill[12], J. Pinilla-Ibarz[13], B. Cheson[14], S. T. Rosen[15], W.G. Wierda[16], D.M. Stephens[17], F.T. Awan[18], M.Y. Choi[19], A. R. Mato[20], A.R. Mato[21]

[1]CLL Society Inc., Claremont, [2]CLL Society Inc., Pompton Lakes, [3]University of Pennsylvania, Abramson Cancer Center, Philadelphia, [4]Weill Cornell Medicine, New York City, [5]Ohio State University, Columbus, [6]Swedish Cancer Institute, Seattle, [7]Dana-Farber Cancer Institute, Boston, [8]Cardinal Health, Dublin, [9]Mayo Clinic, Rochester, [10]Duke University School of Medicine, Durham, [11]City of Hope National Medical, Duarte, [12]Cleveland Clinic Foundation, Cleveland, [13]H. Lee Moffitt Cancer Center and Research Institute, Tampa, [14]Georgetown University Hospital, Lombardi Comprehensive Cancer Center, Washington DC, [15]City of Hope Medical Center, Duarte, [16]MD Anderson Cancer Center, Houston, [17]University of Utah, Huntsman Cancer Institute, Salt Lake City, [18]The Ohio State University Comprehensive Cancer Center, Columbus, [19]Moores Cancer Center University of California San Diego, San Diego, [20]Memorial Sloan Kettering Cancer Center, New York City, United States, [21]CLL Program, Leukemia Service, Section of Hematological Malignancies, Memorial Sloan Kettering Cancer Center, New York City, United States

Background: The CLL literature focuses largely on the objective aspects of diagnosis (dx), active observation (AO), prognosis and management. While health care providers (HCPs) try to effectively educate and reassure patients, the literature on patients' subjective experience through the continuum of care is limited.
Aims: To better describe the patient experience from time of dx, through AO, prognostic testing and clinical trial consideration so as to identify unmet educational and psychological needs.
Methods: We utilized an online or paper 64 question survey directed to CLL patients to capture more information on their experience with CLL. The survey was IRB-approved and took place between October-December 2017. All analyses are descriptive in nature.

Results: 1147 patients from 48 American states completed the survey. Median age was 65 (range 28-86), 46% male, 96% Caucasian. 88% of patients cited conversations with their doctor as the most frequent educational format overall, followed by online sources (CLL specific 67%; general blood cancer 55%) and print materials (52%). Following education, 66% of patients reported a good understanding of sources of information on CLL, 64% of disease characteristics and 62% of therapy indications. 98% reported looking for more information themselves citing CLL-specific websites (90%), online CLL patient blogs or forums (69%) and general blood cancer websites (64%) as the main sources. At dx, 67% of patients reported receiving education by their HCP, however at disease progression, only 23% of patients report education from their HCPs. Effects of treatment and clinical trial opportunities were discussed by 43% and 35% respectively. At dx, 48% were told they had the "good" cancer, yet when AO was recommended, patients reported a mixed picture of anxiety (56%), relief (52%) and confusion (31%). During AO patients reported fatigue (51%), enlarged nodes (47%), anxiety (39%), depression (21%), and night sweats (21%). Also during AO, 653 patients (66%) utilized herbals and other non-traditional interventions for CLL management (Table 1). 34% and 16% of patients respectively reported that discussing prognostic indicators increased their anxiety and confusion. Only 1/3 of patients were offered clinical trial participation and those patients that declined participation or would have declined if asked stated their reasons as preference for a "proven" treatment (38%), distance from the trial site (29%), fear (20%), and frequent imaging (20%).

Table 1.

During AO	% used
Green tea or derivatives	60
Vitamin D	56
Prayer	36
Exercise	30
Curcumin	27
Other herbs/supplements	26

Summary/Conclusion: To our knowledge, this is the largest survey of CLL patients. Much can be learned by detailed surveying of CLL patients throughout their disease course, including previously unrecognized suboptimal interactions between CLL patients and their HCPs. AO is an emotional and active time. Prognostic testing is stressful for many patients. 1/3 of patients recall no HCP education at dx and re-education is infrequent. When patients decline clinical trials, it is due to preference for a "proven therapy", fear, distance and frequent imaging. Understanding how patients experience their disease is critical to improve communication between patients and their HCPs, which may ultimately advance CLL outcomes.

PS1108

CHRONIC LYMPHOCYTIC LEUKEMIA IN ICELAND: A NATION-WIDE EPIDEMIOLOGY STUDY FROM 2003 TO 2016

H. H. Thorvaldsson[1,*], B. Vidarsson[1,2], S.V. Sveinsdottir[1,2], A.M. Halldorsdottir[13]

[1]Faculty of Medicine, University of Iceland, [2]Department of Hematology, [3]Blood Bank, Landspitali - The National University Hospital of Iceland, Reykjavik, Iceland

Background: Chronic lymphocytic leukemia (CLL) is the most common leukemia in Western countries. Small lymphocytic lymphoma (SLL) is considered the same disease by the World Health Organization (WHO). CLL/SLL has not been extensively studied in Iceland with regards to epidemiology or outcome.
Aims: To assess the epidemiology, treatment and survival of CLL/SLL patients in Iceland.
Methods: This nation-wide study includes CLL/SLL patients diagnosed in Iceland during the years 2003-2016. Registries of patients with a CLL diagnosis were obtained from the Icelandic Cancer Registry, Landspitali National University Hospital and the Medical Center in Mjodd. Medical records were reviewed for information on symptoms, diagnosis and treatment. Survival data and causes of death were obtained from national registries. Time of data capture was March 2017.
Results: During the study period 213 patients were diagnosed with CLL/SLL in Iceland. The crude incidence rate was 4.8/100,000 person years (PY) and the WHO age standardized incidence rate was 3.4/100,000 PY. Symptoms were present at diagnosis in 98 (48.8%) patients (data available in 201 cases), but many patients (51.2%) were initially diagnosed due to incidental findings. The most common symptoms were palpably enlarged lymph nodes (n=51, 25.4%) and fatigue (n=48, 23.9%), while B-symptoms were present in 45 (22.4%). Of 77 (36.2%) patients that received chemotherapy, less than half (n=31, 40.3%) were treated within 5 years from diagnosis. The most frequently used first-line treatment was fludarabine, cyclophosphamide and rituximab (n=30, 39.0%). Both higher Rai stage ($p<0.01$) and the presence of symptoms at diagnosis ($p<0.01$) were significantly associated with shorter time to treat in univariate analysis. A total of 73 (34.3%) patients died during the study period. Five-year survival was 70% and median overall survival was 9.6 years. CLL/SLL was registered as the main cause of death in 31 (42.4%) cases, more frequently in patients that received chemotherapy ($p<0.01$). Higher lymphocyte counts and age at diagnosis, as well as earlier need for blood transfusion, were significantly related to poor survival in multivariate analysis.
Summary/Conclusion: The incidence rate of CLL/SLL in Iceland is similar to the incidence in other Western countries. Many patients were initially diagnosed with CLL/SLL after routine blood tests for other conditions. Most patients (85.4%) had not yet received any chemotherapy 5 years after diagnosis and the median overall survival was almost 10 years. The majority of patients died of other causes than CLL/SLL. Higher Rai stage at diagnosis predicted shorter time to treatment which is in accordance with the literature. However higher Rai stage was not significantly associated with shorter survival although there was a trend towards shorter survival for patients with Rai stage 3/4 compared to other Rai stages. That may be due to the small number of patients included, as well as the relatively short follow-up time.

Chronic myeloid leukemia – Biology & Translational Research

PS1109

ENRICHMENT OF ASXL1 PATHOGENIC VARIANTS AMONG PRIMARY REFRACTORY CML PATIENTS

K. Ayoola Gustafsson[1,*], U. Olsson-Strömberg[2], L. Arngården[2], M. Hermanson[3], P. Baliakas[1], L. Cavelier[4]

[1]Immunology,Genetics and Pathology, Science for Life Laboratory, Uppsala University, [2]Department of Medical Science, Division of Hematology, Uppsala University, [3]Immunology,Genetics and Pathology, Uppsala University Hospital, [4]Clinical Genomics, SciLife Laboratory, Uppsala, Sweden

Background: Little is known about the actual mechanism of resistance in patients with chronic myeloid leukemia (CML) with primary resistance to first and second-generation tyrosine kinase inhibitors (TKI), especially in the absence of mutations in the *ABL1* kinase domain and/or additional cytogenetic abnormalities. Preliminary evidence suggests that pathogenic variants in epigenetic regulator genes may be enriched among CML patients with suboptimal response. However, the studies published so far have included patients with various types of responses to TKI treatment, while in the great majority the presence of mutations within the *ABL1* kinase domain was evaluated with Sanger sequencing which does not allow the detection of small clones.
Aims: To evaluate whether pathogenic variants in genes are enriched at the time of diagnosis in primary refractory CML patients negative for mutations in the *ABL1* kinase domain evaluated with next generation sequencing.
Methods: The patient cohort consisted of 20 CML patients in chronic phase (male/female: 10/10, median age: 52) and primary failure, defined as the presence of >1% *BCR-ABL1* p210 IS (international standard) after one year of TKI treatment, excluding non-compliance. All patients were analyzed with (i) PacBio long-range sequencing, with a threshold of 1% for sensitive detection of *ABL1* kinase domain resistance mutations at the time of treatment failure, according to ELN guidelines and; (ii) NGS sequencing at the time of diagnosis using the TruSight myeloid panel (Illumina) with a threshold of 5%. The panel includes 54 genes recurrently mutated in myeloid malignancies. Longitudinal samples with a median follow-up of 21 months were available in 7 patients. The control group comprised 10 age and sex-matched CML patients with major molecular response within 6-12 months with first-line TKI therapy. Cytogenetic data was available for all patients.
Results: : 12 of 20 patients (60%) had a pathogenic truncating variant in *ASXL1* (n=7, 35%, median VAF=23%), *DNMT3A* (n=2, median VAF=51%), *IKZF1* (n=2, median VAF=9%), *GATA2* (n=1), *TP53* (n=1) or *NRAS* (n=1). All 12 patients carried only the t(9;22) translocation at diagnosis and did not acquire additional cytogenic abnormalities during the disease progression. Londitudinal samples of 3/7 patients with truncating *ASXL1* mutations and a median follow-up of 18 months, showed stable *ASXL1* VAF in parallel to *BCR-ABL1* level and no additional pathogenic variants. Interestingly, 5/7 patients carrying pathogenic variants in *ASXL1* were treated with third generation TKI, but showed no response and were eventually transplanted. Three of these patients with available clinical data remain in MMR after transplantation with a median follow up of 9 months. Within the 8 patients with no mutations in the TruSight myeloid panel, 3 developed clonal evolution with major route abnormalities (+8=2, der20=1), while 2/5 of the remaining patients carried variants of unknown significance (VUS) according to the ACMG criteria in the *CUX1* gene (median VAF=45%). No pathogenic variants or VUS were detected in the control group.
Summary/Conclusion: Detection of additional molecular abnormalities at the time of diagnosis especially in the *ASXL1* gene among patients with CML in chronic phase may be associated with primary resistance to the TKI treatment. Alternative therapeutic approaches should be considered for these patients.

PS1110

THE PIVOTAL ROLE OF INSULIN-LIKE GROWTH FACTOR PATHWAY AS A THERAPEUTIC TARGET IN ABL TYROSINE KINASE INHIBITOR RESISTANT LEUKEMIA CELLS

S. Okabe[1,*], T. Tauchi[1], Y. Tanaka[1], K. Ohyashiki[1]

[1]Department of Hematology, Tokio Medical University, Tokyo, Japan

Background: ABL tyrosine kinase inhibitors (TKIs) such as imatinib have demonstrated the potency against Philadelphia chromosome (Ph)-positive leukemia patients, however, some patients are still resistance to ABL TKIs. It has already reported that ABL kinase domain mutations have been implicated in the pathogenesis of ABL TKI resistance, however, mechanisms of intrinsic resistance without point mutation of ABL kinase domain are not fully understood.
Aims: Insulin-like growth factor (IGF) cause intracellular signaling that ultimately results in cellular growth and proliferation. Because IGF signaling pathways have crucial functions in hematological malignancies and solid tumors, IGF pathways may regulate ABL TKI sensitivity and drug resistant.
Methods: We established ABL TKI-resistant *in vitro* cell line models. The total expression profiles of four ABL TKI-resistant cell lines (K562 imatinib-R, K562 nilotinib-R, K562 dasatinib-R, K562 ponatinib-R) were examined using the Agilent Human Gene Expression Microarrays.
Results: We first investigated the relationship of IGF signaling pathways and ABL TKI sensitivity by microarray gene expression data from the online Gene Expression Omnibus (GEO). IGF is tightly regulated by six related IGF-binding proteins (IGFBPs). One of IGFBP, IGFBP5 is related to imatinib sensitivity and resistant in chronic myeloid leukemia (CML) patients from the public microarray datasets of GSE14671. We also found that gene expression of IGF2 and IGFBP5 were increased after imatinib treatment in CML patients (GSE12211). We next examined ABL TKI resistant cell lines (K562 imatinib-R, K562 nilotinib-R, K562 dasatinib-R, K562 ponatinib-R) in this study. BCR-ABL expression levels were not increased in ABL TKI resistant K562 cells compared to parental K562. We could not detect the BCR-ABL point mutation in ABL TKI resistant cells. Although cellular growth was reduced in ABL TKI resistant cells compared to parental cell line K562, these cells were highly resistant to ABL TKIs (K562 imatinib-R: imatinib 2 μM, nilotinib-R: nilotinib 2μM, dasatinib-R: dasatinib 100nM, ponatinib-R: ponatinib 50nM). Using the DNA microarray approach, we investigated gene expression profiles in cultured ABL TKI resistant K562 cells. We found gene expression of insulin-like growth factor 1 (IGF1) receptor (IGF1R) was increased ABL TKI resistant K562 cells. IGF1R gene amplification was confirmed by RT-PCR analysis. IGF1R is a transmembrane receptor implicated in the regulation of cell metabolism, growth, and survival. IGF signaling pathway may be a cause of resistance to ABL TKIs, we examined the IGF1R inhibitor in ABL TKI resistant cells. One of IGF1R inhibitor, linsitinib was not effective on parental K562 cells. In contrast, linsitinib inhibited ABL TKI resistant cells in a dose dependent manner. Combined treatment of ABL TKI resistant cells with imatinib and linsitinib caused more cytotoxicity than each drug alone. Caspase 3/7 activity and cellular cytotoxicity was also increased. We next blocked IGF1R function by small interfering RNA (siRNA). SiRNA transfected cells were reduced cellular proliferation. We also found drug sensitivity of the cells to the imatinib was increased compared to mock-transfected cells. Apoptotic cells and caspase 3/7 activity were increased after imatinib treatment in siRNA transfected cells.
Summary/Conclusion: The IGF signaling pathway is involved in ABL TKI sensitivity and drug resistant in CML cells. We also provide the promising clinical relevance as a candidate drug for treatment of ABL TKI resistant leukemia patients.

PS1111

ASXL1 MUTATIONS ARE FREQUENT IN CHRONIC MYELOID LEUKEMIA PATIENTS RESISTANT TO TYROSINE KINASE INHIBITORS BUT NOT IN PATIENTS WITH OPTIMAL RESPONSE

M. M. Machnicki[1,*], I. Solarska[2], M. Zawada[3], M. Pepek[1], I. Seferynska[4], T. Sacha[5], W. Sawicki[6], J. Niesiobedzka-Krezel[7], E. Wasilewska[8], K. Pruszczyk[1], M. Rydzanicz[9], P. Stawinski[9], J. Gora-Tybor[4], R. Ploski[9], T. Stoklosa[1]

[1]Department of Immunology, Medical University of Warsaw, [2]Department of Diagnostic Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, [3]Department of Hematology Diagnostics, The University Hospital, Cracow, [4]Department of Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, [5]Department of Hematology, Jagiellonian University, Cracow, [6]Department of Internal Diseases and Hematology, Military Institute of Medicine, [7]Department of Hematology, Oncology and Internal Diseases, Medical University of Warsaw, Warsaw, [8]Department of Hematology, Medical University of Bialystok, Bialystok, [9]Department of Medical Genetics, Medical University of Warsaw, Warsaw, Poland

Background: A subset of patients diagnosed with Chronic Myeloid Leukemia (CML) is at elevated risk of progression to CML blastic phase (CML-BP) due to primary or acquired resistance to treatment with tyrosine kinase inhibitors (TKI-res). While leukemic clones harboring BCR-ABL1 kinase domain mutations are responsible for majority of clinical resistance cases, predicting TKI response is still challenging.
Aims: We and others previously reported an incidence of somatic mutations

in myeloid-associated genes in CML patients progressing to CML-BP. Here, we aimed to assess prevalence of those mutations in a group of 23 patients with TKI-res (as confirmed by loss of molecular and/or cytogenetic response according to ELN criteria) and compare this group with our previously reported cohort of 36 CML patients, who reached major molecular response (MMR) within 6 months from the start of treatment and remained in MMR for at least 4 years.

Methods: We applied next-generation sequencing (Roche SeqCap EZ, Illumina HiSeq) to determine mutational status of ~1000 cancer-associated genes. We also analyzed disturbances in variant allele frequencies (VAF) of common SNPs along with coverage analysis to detect any major chromosomal aberrations.

Results: The most common detected genetic alterations in TKI-res patients were *ASXL1* truncating mutations (7/23 patients, 30,4%) and *BCR-ABL1* kinase domain mutations (5/23, 21,7%). In 3 patients (13%) we observed co-existence of *ASXL1* and *BCR-ABL1* mutations. All of *ASXL1*/*BCR-ABL1* mutations had VAFs ranging from 12 to 52%, suggesting that they were harbored by dominant clones, except for one patient (Res6) who had three different *ASXL1* mutations, two of which were subclonal (Table 1). We noted a significantly higher frequency of *ASXL1* mutations in TKI-res patients than in our cohort of MMR patients (7/23 vs 2/36, p=0.0213; Fisher exact test). We did not observe any additional chromosomal aberrations in any of the patients.

Table 1. BCR-ABL1 and ASXL1 mutations in patients from TKIres and MMR cohorts.

Patient	Mutation		VAF [%]	
	BCR-ABL1	ASXL1	BCR-ABL1	ASXL1
Res1	Gln252His	-	52.6	-
Res2	Ile418Val	Tyr591_Gln592fs	31.3	34.1
Res3	Met244Val	-	20.0	-
Res4	Thr315Ile	Ala640fs	19.0	12.2
Res5	Thr315Ile	Gly643_Gly644fs	28.5	31.2
Res6	-	Arg693*/Asp943_Leu944fs/Tyr591*	-	37.7/5.94/4.54
Res7	-	Glu797*	-	41.0
Res8	-	His630fs	-	40.4
Res9	-	Lys686fs	-	43.0
MMR1	-	Gly643_Gly644fs	-	21.6
MMR2	-	Gly643_Gly644fs	-	36.4

Summary/Conclusion: According to several reports, *ASXL1* is one of the most commonly mutated genes in CML besides *BCR/ABL1* itself. Our data indicates that inactivating mutations in *ASXL1* may precede clinical resistance or emerge with the TKI-resistant clone. This emphasizes the potential biological importance of somatic mutations in epigenetic regulators such as *ASXL1* in the resistance to TKI. Further studies in terms of patient cohorts extension as well as verification of detected mutations in diagnostic samples are warranted.

PS1112

THE UNCOVERING OF A BCR-ABL1 TYROSINE KINASE-INDEPENDENT SIGNATURE REVEALS NEW POTENTIAL THERAPEUTIC TARGETS IN CHRONIC MYELOID LEUKAEMIA STEM CELLS

E. Gomez-Castaneda[1,*], C. Munje[1], R. Hyde[1], F. Pellicano[1], D. Vetrie[2], H. Wheadon[1], M. Copland[1], T. Holyoake[1], L. Hopcroft[1], S. Rogers[3], H. Jørgensen[1]

[1]Paul O'Gorman Leukaemia Research Centre, [2]Wolfson Wohl Translational Cancer Research Centre, [3]School of Computer Science, University of Glasgow, Glasgow, United Kingdom

Background: Although the introduction of tyrosine kinase inhibitors (TKI) into clinical practice at the beginning of the century greatly increased life expectancy of patients with chronic myeloid leukaemia (CML), the majority of CML patients are not cured, and a small population of leukemia stem cells (LSCs) persist despite treatment and may reinitiate the disease upon treatment withdrawal. Thus, most patients require life-long therapy, meaning a higher risk of treatment side effects and the prolonged psychological burden of living with leukemia. It has already been demonstrated that CML LSCs are not addicted to BCR-ABL1 tyrosine kinase (TK) and that they survive under suppression of the kinase.

Aims: To uncover BCR-ABL1 TK independent mechanisms in CML LSCs and target these to eradicate CML LSCs.

Methods: Public microarray datasets were analysed with *limma/Bioconductor* to detect differential gene expression between CML LSCs and normal hematopoietic stem cells (HSC; GSE47927 and E-MTAB-2581) and to detect genes not affected by TKI treatment (E-MTAB-2594). Validation of gene expression was performed in CD34$^+$ cells from 5 CML patients and 2 healthy donors. For this, CML CD34$^+$ cells were cultivated in serum free media without growth factors for 7 days±5μM imatinib. Targeting of the BCR-ABL1 TK independent genes was performed by treating CD34$^+$ CML and normal cells with different concentrations of gemtuzumab-ozogamicin (GO) or cyclosporine A (CsA)±2 μM imatinib in serum free media with physiological growth factors (SCF, G-CSF, GM-CSF, IL6, LIF and MIPα). The effect of treatment was measured by cell counts; annexin V-DAPI, Ki-67-DRAQ7, and γH2AX staining; and colony forming cell (CFC) assay. IC_{50} were calculated using *drc* package for R.

Results: Microarray analyses revealed 527 consistently de-regulated genes in CML and 5,706 genes not affected by TKI treatment. The comparison of both lists revealed a 60 gene signature that is differentially expressed in CML LSC (as compared with normal HSC) and not affected by TKI treatment of CML CD34$^+$ cells. Of the 60, 4 genes (*CD33, CHST2, PPIF, ERG*) were validated in a set of independent patients and controls by qPCR. *CD33*, a myeloid cell surface marker, and *PPIF*, a key member of the mitochondria permeability transition pore that is an important regulator of both apoptosis and necrosis, were found to be upregulated in CML. Both can be targeted by clinical grade compounds GO and CsA, respectively. These drugs proved effective at targeting CML CD34$^+$ cells *in vitro*. GO had an IC_{50} of 136ng/mL, which is 19 times lower than the control after 72h of treatment and about 7 times lower than the FDA-approved dose for AML (≈1000 ng/mL). CFC counts were reduced by 95% after 72h 100 ng/mL GO + 2 μM imatinib in CML compared with 78% in normal controls, mirroring the apoptosis staining by flow cytometry (83% and 71% reduction in viability, respectively). Preliminary results shows that the IC_{50} of CsA was 4 μM when treating CML CD34$^+$ cells, 2-fold lower than in the healthy controls.

Summary/Conclusion: CML LSCs have de-regulated gene expression that is not corrected by TKI treatment, further supporting the hypothesis that CML LSCs have TKI-independent pathways contributing to TKI persistence. We have shown that targeting these proteins leads to a decrease in the number of CML CD34$^+$ cells and in the number of CFC. Taken together, our work confirms the existence of the BCR-ABL1 TK independent signature in CML LSCs and represents an avenue for novel therapy in CML.

PS1113

OPTIMAL TIME POINT FOR BCR-ABL1 KD MUTATION ANALYSIS IN CML PATIENTS; BASED ON 2013 ELN GUIDELINE

Y. R. Do[1,*], H.-L. Yoo[2], S.-H. Kim[2], S.-Y. Choi[2], M.-Y. Lee[2], H.-Y. Song[2], K.-H. Kang[2], K.-M. Ki[2], J.-H. Suh[2], S.-Y. Yang[2], S. Oh[3], S. H. Kim[4], D.Y. Zang[5], S.-E. Lee[6], D.-W. Kim[7]

[1]HematoOncology, Medicine, Keimyung University, Dongsan Medical Center, Daegu, [2]Leukemia Research Institute, [3]HematoOncology, Medicine, Sungkyunkwan University, Kangbuk Samsung Hospital, Seoul, [4]HematoOncology, Medicine, Dong-A University Hospital, Busan, [5]HematoOncology, Medicine, Hallym University Hospital, [6]HematoOncology, Medicine, [7]Department of Hematology, Seoul St. Mary's Hospital, The Catholic University , Seoul, Korea, Republic Of

Background: BCR-ABL1 kinase domain (KD) mutations are closely related to tyrosine kinase inhibitor (TKI) resistance in chronic myeloid leukemia (CML). Although mutation analysis has been suggested in CML patients with treatment failure, there is no standard guideline according to landmark responses at specific timepoints of European LeukemiaNet (ELN) recommendation.

Aims: To determine appropriate timepoints for mutation analysis, the frequency and type of BCR-ABL1 kinase domain mutation were analyzed using Sanger sequencing and were assessed by achieving landmark responses at specific timepoints.

Methods: From 605 newly diagnosed chronic phases CML patients, 961 peripheral blood samples were analyzed for BCR-ABL1 KD mutation and all of patients were treated with frontline imatinib for at least 3 months. The patients with atypical transcripts were excluded. Hematologic, cytogenetic, and molecular responses on 3, 6, and 12 months were also assessed by ELN criteria. To determine appropriate timepoint for mutation analysis, the frequency and type of BCR-ABL1 KD mutation were analyzed using Sanger sequencing and were assessed by achieving landmark responses at specific timepoints

Results: Of the 605 patients, BCR-ABL1 KD mutations were detected in 28 patients (4.6%) and a total of 33 mutations were detected. Of the 33 mutations, 23 mutations (69.7%) were highly resistant mutations of T315I and P-loop. In cytogenetic response criteria, the mutation frequencies of optimal, warning and failure group were 0.7% (5/671 samples), 1.8% (2/110 samples) and 16.0% (17/106 samples) respectively and were 0.7% (3/425 samples), 3.6% (13/359 samples) and 7.6% (13/172 samples) respectively in molecular response criteria. Under 18 different response criteria of

ELN recommendation, there was a higher incidence of mutations among patients with 12 month-cytogenetic treatment failure (21.8%; 12/55 patients), 3 month-cytogenetic treatment failure (20.0%; 3/15 patients) and 12 month-molecular treatment failure (9.3%; 10/107 patients). Interestingly, mutations were also detected in 5 optimal cytogenetic responders (2/280 patients at 6 month-criteria and 3/241 patients at 12 month-criteria; 1 G250E, 2 Y253H, 1 V280M and 1 M351T)

Summary/Conclusion: We conclude that some patients with treatment failure, especially with cytogenetic criteria, should be warranted for mutation analysis. In addition, the majority of patients with warning criteria may be enough only with a close monitoring without routine mutation analysis. However, as a few patients with optimal response had mutation, mutation analysis should not be totally excluded.

PS1114

CLONAL SELECTION DETERMINES RESULTANT DOMINANCE OF TYROSINE KINASE INHIBITOR-RESISTANT CELLS IN CHRONIC MYELOID LEUKAEMIA

B. Leow[1,2,*], L. Eadie[1,2], I. Pagani[1,2], D. Yeung[1,2], T. Hughes[1,2], D. White[1,2]

[1]Cancer Theme, South Australian Health and Medical Research Institute, [2]Health Sciences, University of Adelaide, Adelaide, Australia

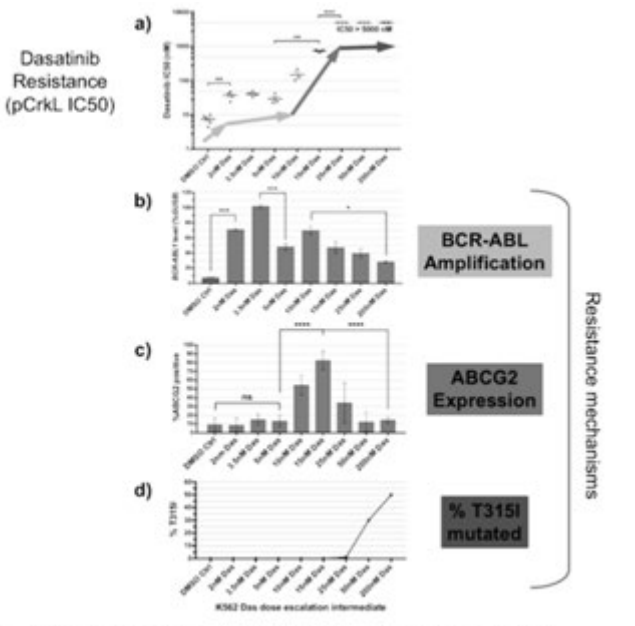
Background: Emergence of resistance to therapeutic drugs is detrimental to treatment effectiveness in CML. Modelling the clonal dynamics of accrued drug resistance can aid the design of therapeutic strategies. Excessive myeloproliferation is driven by the constitutively active tyrosine kinase, BCR-ABL, and has been treated effectively using tyrosine kinase inhibitor (TKI) therapy. However, TKI resistance is an issue in a significant subset of patients, whose prognosis remains poor. Mechanisms of TKI resistance include: the overexpression of BCR-ABL; perturbed TKI influx/efflux transporter activity; single nucleotide mutations in BCR-ABL which preclude TKI binding, and loss of dependence on BCR-ABL kinase activity. Several studies have observed a temporal order to the emergence of resistance mechanisms, with shifts in clonal architecture altering therapy sensitivity. Here, a model of natural selection describes accrual of drug resistance, informing the fitness and resultant clonal dominance of leukaemic cells during TKI treatment, as well as upon treatment withdrawal.

Aims: To determine the effect of various TKI resistance mechanisms on the survival of a BCR-ABL+ leukaemic cell population, over the course of increasing TKI exposure and treatment withdrawal. To examine TKI cross-resistance and find novel treatment strategies to target TKI resistant cells.

Methods: A dasatinib (Das) resistant K562 cell line was established by culture in increasing concentrations of Das over 11 months. Intermediate samples harvested over Das dose escalation were examined. Sensitivity of cell viability and BCR-ABL activity to gradient Das was determined by AnnV/7-AAD co-stain, and phospho-CrkL IC50. TKI resistance mechanisms were explored by transcriptome RNAseq, interrogating gene expression, structural rearrangement and nucleotide variant analysis. Results were validated by PCR, Sanger sequencing, flow cytometry (ABC membrane transporters ABCB1/ABCG2), and western blotting analysis (BCR-ABL and CrkL IC50 in the presence of ABCG2 inhibitor, Ko143). To determine the effect of resistance mechanisms on selection and clonal architecture, Das resistant cells were cultured for prolonged periods +/- Das, recording the effects on CrkL IC50 and continued expression of resistance mechanisms.

Results: Resistance to clinical doses of Das was attained during dose escalation, evidenced by increased Das IC50 and decreased Das induced cell death (Figure 1a, $p<0.01$). qPCR results implicated perturbed BCR-ABL signalling (Figure 1b), and expression of efflux pump, ABCG2 (Figure 1c), transiently overexpressed during acquired drug resistance. This finding was validated with functional studies: flow cytometry demonstrated increased membrane expression of ABCG2 ($p<0.001$), and inhibition of ABCG2 function using the small molecule inhibitor, Ko143, sensitised these cells to TKI-induced cell death. Additionally, variant analysis of resistant K562 lines identified the BCR-ABL kinase domain mutation, T315I, which completely abrogated Das binding (Figure 1d). Importantly, these resistance mechanisms also contributed to a significant decrease in sensitivity to the 3rd generation inhibitor, ponatinib, used for TKI-refractory disease. Withdrawal of TKI in culture resulted in lowered IC50, re-sensitising leukaemic cells to treatment.

Summary/Conclusion: In a therapy resistant context, flux in clonal cell populations leads to shifts in sensitivity to TKI therapy. Our model, recapitulating several relevant resistance mechanisms, enables an accurate description of the dynamics of drug resistance in future research.



Resistance to dasatinib in K562 cell line increases following dose escalation in dasatinib, via multiple, temporally overlapping resistance mechanisms.

Figure 1.

PS1115

WT1 ANALYSIS IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS

S. Coluzzi[1,*], T. Introcaso[1], F. Perutelli[2], S. P. Pascale[1], O. Villani[3], F. Guerrini[2], G. Bianchino[3], A. Matturro[1], I. Attolico[1], D. Vertone[1], N. Filardi[1], A. Amendola[1], R. Nuccorini[1], G. Pietrantuono[3], S. Galimberti[2], M. Pizzuti[1]

[1]U. O. di Ematologia, Azienda Ospedaliera Regionale San Carlo, Potenza, [2]Dipartimento di medicina clinica e sperimentale, Università di Pisa, Pisa, [3]IRCCS Crob, Rionero in Vulture (Pz), Italy

Background: Diagnosis and monitoring of CML patients is based on bcr/abl evaluation by RQ-PCR. ELN guidelines show that bcr/abl superior to 10% after 6 months of therapy and bcr/abl superior to 1% after 12 months of therapy are defined as failure of therapy. Different clinical parameters are used to predict patients' prognosis at onset in the three most commonly used score indexes. No other useful tools have been identified. WT1 gene is over expressed at onset in several diseases including 80% of Acute Myeloid Leukaemia cases (AML): gene's levels decrease during disease remission and are low in healthy people. WT1 is over expressed also in case of AML relapse. Few data are available on WT1 expression in CML patients.

Aims: The aim was to investigate the role of WT1 in CML outcome and prognosis.

Methods: We evaluated 58 patients with CML referred to our centres between 2009 and 2018. RT-PCR and RQ PCR on RNA were performed to quantify bcr/abl and WT1 in the peripheral blood of all patients at onset and after 3, 6, 9, 12 months of therapy and after (median follow up 39 months, range: 9-106). The type of transcript was p210 b2a2 in 10 pts, p210 b3a2 in 30, both in 4 pts, 14 were not analyzed. Sokal risk was low in 24 pts, intermediate in 19, high in 14, not evaluable in 1 patient. 34 patients received Imatinib, 7 Dasatinib and 17 Nilotinib as first line therapy.

Results: ROC analysis defIned a cut-off value of 156 (WT1/Abl*10e4) usefull to discriminate at onset patients who will have failure on ELN criteria basis from patients without failure (sensibility 69%, specificity 76%).
21/58 of patients (36%) had high levels of WT1 (WT1-H) at onset. Sokal risk was high in 8, intermediate in 6, low in 7. 37/58 of patients (64%) had low levels of WT1 (WT1-L): Sokal risk was high in 6, intermediate in 13, low in 17, not evaluable in 1. We observe that sokal risk is independent of WT1. 16/21 WT1-H patients were evaluable for bcr/abl at three months of therapy: 3 had bcr/abl > 10%, 7 between 1 and 10% and 6 lower than 1%. 32/37 WT1-L patients were evaluable at three months: 4 had bcr/abl > 10%, 9 between 1 and 10%, 19 lower than 1%. At sixth month the differences between the two cohorts seems more clear: 7/19 (36%) of WT1-H and only 6/31 (19%) of WT1-L patients showed bcr/abl levels>1%. Moreover, the outcome of the two groups seems different. During follow up, 11/21 (52%) WT1-H patients had a failure on ELN criteria basis, and needed to change

therapy with second line drug; 7 of them used Imatinib as first line therapy. 5/37 (13%) WT1-L patients had a failure and changed therapy. The EFS, intended as survival of patient until failure on ELN criteria basis, is rappresented in the Figure 1. Patient actual status is also different in the two groups. 14/21 (66%) WT1-H patients are in MR3 or better, 7/21 (33%) in MR2 or worse (among these 2 patients died). 29/37 (78%) WT1-L patients are in MR3 or better, 8 (22%) in MR2 or worse (1 patient died).

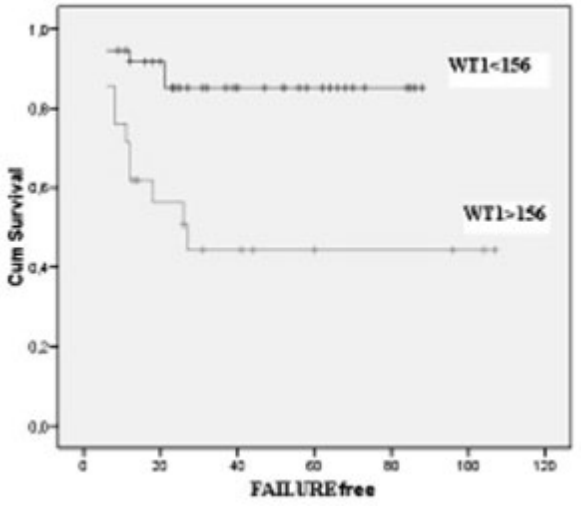


Figure 1.

Summary/Conclusion: In our study cohort, WT1-H patients seem to have a worse outcome than WT1-L, in terms of failure free survival and molecular response: 52% of WT1-H patients vs 13% of WT1-L patients had failure and needed to change therapy. So, WT1 could be a prognostic marker at onset. We suppose that WT1-H effect could be neutralized by correct use of second generation TKI. However, we need to study a larger number of patients to confirm this hypothesis.

PS1116

DIGITAL DROPLET PCR MAY BETTER IDENTIFY CANDIDATES FOR TREATMENT DISCONTINUATION IN CHRONIC MYELOID LEUKEMIA REGARDLESS OF MEDIAN TREATMENT DURATION

G. Colafigure Ii[1,*], M. Breccia[1], D. Diverio[1], M. Molica[1], M.G. Loglisci[1], A. Guarini[2], R. Foà[1]

[1]Department of Cellular Biotechnologies and Hematology, [2]Molecular Medicine, Hematology, Sapienza University, Rome, Rome, Italy

Background: Discontinuation of tyrosine kinase inhibitors (TKIs) in patients with chronic myeloid leukemia achieving a deep molecular response (DMR) would be an innovative cost-effective strategy in order to reduce long-term toxicity. Several studies have reported that about 40% of patients maintain the response after therapy discontinuation and reach a treatment free-remission. BCR-ABL1 transcript quantification is usually performed by RQ-PCR, but the lower limit of detection (LOD) or quantification (LOQ) may be insufficient to manage CML patients candidates for discontinuation. Digital droplet PCR (ddPCR) is a highly accurate molecular approach that could prove advantageous for the quantification of the BCR-ABL1 fusion transcript copy number.
Aims: To evaluate the feasibility of ddPCR evaluation in CML patients who have reached a DMR, in order to verify the advantage in terms of LOD and LOQ, to validate the test and to propose the quantification of residual transcript amounts as an alternative test prior to discontinuation.
Methods: We tested RNA samples from 50 patients who were in undetectable MR4.5 according to IS previously evaluated by RQ-PCR. The ddPCR assay was carried out in triplicate using the Biorad-QX100 platform. To test the BCR-ABL1 fusion transcript and ABL control gene, we used the same primers and probes used in the standard RQ-PCR reaction. Each reaction mixture was partitioned into approximately 20,000 droplets and then amplified. Cycled droplets were read in the QX200 droplet-reader and analysis of the ddPCR data was performed using the QuantaSoft analysis software. The threshold was manually set using the FAM channel and expressed as the absolute number of BCR-ABL1 copies. Samples yielding a minimum of 1 positive droplet scored as positive.
Results: We analyzed 50 patients in undetectable MR4.5 by RQ-PCR after a median time of treatment of 8.9 years [1.4-14.4]. There were 25 males and 25 females, median age was 63.4 years [37.5-85.5]. Forty-one patients had been treated with imatinib, while 9 patients had received second generation TKIs. In 29 (58%) patients, ddPCR confirmed the negativity of the samples, whereas in 21 (42%) a positivity was detected in at least 1 droplet with a median of 0.52 [0.22-2] BCR-ABL1 copies. We did not identify significant differences in baseline clinical features between ddPCR-positive and ddPCR-negative patients. No differences were revealed according to Sokal risk or in the type of TKI used as first-line treatment to reach a DMR between the two groups. Ten and 8 patients had been previously exposed to IFN-α in the ddPCR-positive and ddPCR-negative groups, respectively. The duration of stable DMR was 2.7 years [0.4-3] and 3.3 years [1.5-4.3] in ddPCR-positive and ddPCR-negative patients, respectively (p=ns). The median duration of treatment was 7.2 years in the ddPCR-positive group versus 9.6 years in the ddPCR-negative group (p=ns).
Summary/Conclusion: Our results suggest that ddPCR could be superior in terms of quantification of low levels of residual disease without the need of a calibration curve, as compared to classic RQ-PCR, in the same clinical conditions. In our cohort, considering any ddPCR positivity we could detect 42% of patients with a likely presence of minimal residual disease, suggesting that this tool increases the likelihood of identifying patients who could (or not) attempt TKI discontinuation within patients with an undetectable MR4.5, independently of the median time of treatment and of a stable DMR.

PS1117

FREQUENCY OF TYPICAL AND ATYPICAL FUSION TRANSCRIPTS IN PATIENTS WITH BCR-ABL1-POSITIVE LEUKEMIAS – A SINGLE INSTITUTION STUDY

G. Balatzenko[1,*], M. Guenova[2], K. Ignatova[3], S. Ivanova[4], J. Stoyanova[3], A. Lilova[3], V. Stoeva[3], Y. Petrov[3], E. Hadjiev[5], G. Tsvetkova[6], B. Spassov[3]

[1]Laboratory of Cytogenetics and Molecular Biology, National Specialised Hospital for Active Treatment of Hematological Diseases, [2]Laboratory of Hematopathology and Immunology, [3]Department of Clinical Hematology, [4]Laboratory of Cytogenetics and Molecular Biology, National Specialized Hospital for Active Treatment of Hematological Diseases, [5]Clinic of Hematology, Medical University Sofia, Faculty of Medicine, Department of Internal Diseases, UMHAT Alexandrovska, [6]Clinic of Hematology, Medical University Sofia, Faculty of Medicine, Department of Internal Diseases, UMHAT Alexandrovska, Sofia, Bulgaria

Background: At molecular level, Philadelphia (Ph)-chromosome results in a heterogeneous group of different *BCR-ABL1* fusion genes. The identification of the type of *BCR-ABL1* mRNA is highly recommended because: (i) some of these transcripts could be missed by the routinely used reverse-transcription polymerase chain reaction (RT-PCR) techniques; (ii) the conventional reagents and protocols used for molecular monitoring might yield false negative results; and (iii) clinical features, course of disease and response to treatment might be different depending on the type of transcripts.
Aims: To determine the incidence of typical and atypical transcripts in a large cohort of patients with *BCR-ABL1* leukemias.
Methods: Between 1998 and 2018, 950 patients (pts) were found to be *BCR-ABL1*-positive, as follows: 845 with chronic myeloid leukemia (CML) [402 females/443 males, mean age 51.8±17.1 years, including 25 (3.0%) children and 820 (97%) adults]; 87 with acute lymphoblastic leukemia (ALL) [39 females/48 males; mean age 39.4±23.1 years; 16 children (18.4%) and 71 (81.6%) adults]; 5 adults with acute biphenotypic leukemias (ABL) [2 females /3 males; mean age 43.5±11.5 years); 15 adults with AML [8 females/7 males; mean age 54.7±11.9 years]. *BCR-ABL1* (both p210 and p190) at diagnosis was determined by qualitative RT-PCR using BIOMED-1 protocol.
Results: In CML, typical p210 *BCR-ABL1* transcripts were found in 827 (97.9%) pts, including 460 (55.6%) b3a2, 340 (40.2%) b2a2 and 27 b3a2/b2a2 (3.2%). In 36 of these pts (4.4%), in addition to the p210 *BCR-ABL1* mRNA, e1a2 transcripts were found, most commonly in cases diagnosed in advanced stages of CML. In the remaining 18 (2.1%) cases, atypical *BCR-ABL1* transcripts were found, including e1a2 (n=9); e6a2 (n=3); e19a2 (n=2); b2a3 (n=2), b3a3 (n=1) and 1 case with transcripts amplified by m-BCR primer, not further specified. Besides, in one another case, despite of the presence of typical b2a2 transcripts at the diagnosis, a qualitative RT-PCR monitoring using 2 different commercially available kits for manual and automated testing showed false negative results suggesting the presence of additional rearrangements within the fusion gene. Among 12 pts with atypical transcripts treated with tyrosine kinase inhibitors, 8 (66.7%) fail to respond to therapy, while among the remaining 4 pts with molecular response, *BCR-ABL1* negativity was documented in 2 cases (16.7%) with

b3a2 and b2a2 transcripts, respectively. In ALL, p190 and p210 transcripts were detected respectively in 53 (60.2%) and 34 cases (38.6%)[i.e. 18 b2a2 and 16 b3a2; in 3 cases with co-expression of e1a2]. Atypical e1a3 (1.1%) was observed on one case. Pts with P190 transcripts were significantly younger compared to P210-positive pts – 36.6±21.8 years vs. 47.4±21.3 years (p=0.039). In ABL, 3 pts were positive for e1a2, 1 for b3a2 and 1 for b2a2. In AML, 7 pts were positive for p190 (e1a2) and 8 pts for p210 (6 b2a2; 2 b3a2, with co-expression of e1a2 in one of them).

Summary/Conclusion: Our study further confirms the extreme heterogeneity of *BCR-ABL1* positive leukemia patients, as detected by qualitative RT-PCR. In CML, atypical *BCR-ABL1* rearrangements, although rare, account for 2.1%, and raise several practical issues concerning their monitoring and frequently non-optimal response to the applied therapy.

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Chronic myeloid leukemia – Clinical

PS1118

PATIENT-SPECIFIC BCR-ABL1 GENOMIC FUSION ANALYSIS OF MINIMAL RESIDUAL DISEASE OF CML PATIENTS ELIGIBLE FOR TKI STOPPING SIGNIFICANTLY OUTPERFORMED MRNA DETECTION EITHER BY QPCR OR DIGITAL PCR

K. Machova Polakova[1,*], H. Zizkova[1], E. Motlova[1], P. Pecherkova[1], J. Zuna[2], L. Hovorkova[2], O. Zimmermannova[2], T. Jurcek[3], K. Vlcanova[1], H. Klamova[1], N.C. Cross[4,5], A. Hochhaus[6], T. Ernst[6]

[1]Institute of Hematology and Blood Transfusion, [2]CLIP, Dept. of Paediatric Haematology and Oncology, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, [3]Center of Molecular Biology and Gene Therapy, Internal Hematology and Oncology Clinic, Faculty Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic, [4]Wessex Regional Genetics Laboratory, Salisbury NHS Foundation Trust, Salisbury, [5]Faculty of Medicine, University of Southampton, Southampton, United Kingdom, [6]Abteilung Hämatologie/Onkologie, Klinik für Innere Medizin II, University of Jena, Jena, Germany

Background: Data from clinical studies on stopping tyrosine kinase inhibitor (TKI) treatment of eligible patients with chronic myeloid leukemia (CML) show that a TKI cessation treatment protocol is safe under strict conditions and 40-60% of patients may sustain treatment free remission. The critical issue of the protocol is the sensitive and precise monitoring of deep molecular response (DMR)/minimal residual disease (MRD). The ELN recommended the use of standardized qPCR of Major BCR-ABL1 transcripts for MRD assessment; a limitation of qPCR is accurate detection and quantification of rare targets. Published data indicates that digital PCR may enable more sensitive and accurate assessment of DMR. Furthermore, DNA assays detecting patient specific BCR-ABL1 genomic fusions may uncover the presence of residual CML cells in cases with undetectable BCR-ABL1 transcripts.
Aims: To assess differences of MRD detection of BCR-ABL1 by 4 approaches in CML patients who responded to TKI treatment with sustained DMR.
Methods: BCR-ABL1 DNA fusions were identified in 78 CML patients. TKI was stopped in 14/78 patients within the EURO-SKI study. So far, DNA specific assays for quantification by qPCR and droplet digital PCR (ddPCR) have been optimized for 42 patients. DNA and RNA were extracted from the same number of leukocytes per sample of peripheral blood (PB) from regularly scheduled monitoring in practice or EURO-SKI study. Bone marrow (BM) was collected at the time of sustained DMR in 7 patients to follow differences in MRD detection between BM and PB. For each approach applied, mRNA qPCR (1), DNA qPCR (2), mRNA ddPCR (3) and DNA ddPCR (4), we established stringent quality requirements to exclude poor quality samples from evaluations (Table 1). So far, we analyzed 829 paired PB samples with BCR-ABL1 transcripts at levels <0.1% BCR-ABL IS from 42 patients by (1) and (2) and 423 paired PB samples of 22 patients by (3) and (4). Two-proportion z-test was applied to compare the level of sensitivity.

Table 1. Condition for data evaluation.

Assay	Negative (score 3)	Positive unquantifiable (score 2)	Positive quantifiable (score 1)	Sensitivity
(1) *mRNA qPCR	Negative triplicates or averaged copies less than LoD	Averaged copies within the range LoD-LoQ	Averaged number of copies equal or higher than LoQ	At least 24000 copies of control gene GUSB ensuring sensitivity MR[4]
(2) mRNA ddPCR	Negative quadruplicates or averaged copies less than LoD	Averaged copies within the range LoD-LoQ	Averaged number of copies equal or higher than LoQ	
(3) **DNA qPCR	Negative triplicates or more than 4 Ct higher than the highest Ct of the individual calibration curve	Up to 4 Ct higher than the highest Ct of the individual calibration curve	Averaged Ct of triplicates within a quantification range specified by individual calibration curve	At least 20000 copies of control gene ALB (reflecting 10000 cells) ensuring sensitivity 10^{-4}
(4) DNA ddPCR	Negative quadruplicates (no copy detected in negative controls of 6 healthy donors)***	NA	Averaged copy number (no copy detected in negative controls of 6 healthy donors)***	

* Conditions differ from the evaluation of deep MR recommended by ELN (Cross et al. Leukemia 2016) to ensure reliable comparison with qPCR DNA analysis, which is patient specific.
** Conditions were applied as was previously described in Hovorkova et al. Blood 2017.
*** All droplets observed after ddPCR DNA analysis of patient-specific assays were negative when 6 healthy donors were analyzed.
Abbreviations: LoD = Limit of Detection; LoQ = Limit of Quantification; ALB = albumin (ALB is recommended control gene for analysis of patient specific Ig/TCR rearrangement; standardized primers and probes were applied from EURO-MRD consortia)

Results: DNA analysis showed better score for BCR-ABL1 detection and quantification by qPCR (for scoring see Table 1) in 338/829 (41%) paired samples (PS) compared to mRNA, while BCR-ABL1 at mRNA level was better in 42 (5%) cases (p<0.0001). When we adjusted the level of sensitivity for mRNA and DNA analysis of PS, we found, that at level $MR^{4-4.5}$ or 10^{-4}

(n=484), respectively, DNA analysis was more sensitive in 163 (34%) PS, while the mRNA BCR-ABL1 was more sensitive in 27 (6%) PS (p<0.0001). At level MR^5 and 10^{-5} (n=43), respectively, DNA was more sensitive in 14 (33%) PS and less sensitive than mRNA in 2 (5%) PS (p=0.0023). DNA analysis was more sensitive by ddPCR in 194/423 PS (46%), while mRNA was better in 6 PS (1.4%) (p<0.0001). DdPCR increased sensitivity of mRNA compared to qPCR in 96/614 PS (16%). We did not observe any differences in BCR-ABL1 detection between PB and BM using all 4 approaches in 7 patients with sustained DMR. In one patient BCR-ABL1 was positive by (3) and (4) in PB but not in BM. When we analyzed whole extracted DNA from BM by (4) we detected 2.2 BCR-ABL1 copies in $20x10^6$ copies of ALB.
Summary/Conclusion: BCR-ABL1 DNA-based approaches were more precise and sensitive than mRNA analyses in significant number of patient samples of peripheral blood. Whether this difference will be reflected also in better outcome prediction within TKI stopping protocols remains to be addressed in subsequent long-term analysis.
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PS1119

TREATMENT-FREE REMISSION AFTER SECOND-STOP OF IMATINIB THERAPY IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE

S. Oh[1,*], S.-E. Lee[2], S.-Y. Choi[3], S.-H. Kim[2], S.-H. Kim[4], D.Y. Zang[5], Y.R. Do[6], J.-Y. Kwak[7], J.-A. Kim[8], D.-W. Kim[9]

[1]Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University, [2]Hematology, Seoul St. Mary's Hospital, The Catholic University of Korea, [3]Hematology, Seoul St. Mary Hospital,The Catholic University of Korea, Seoul, [4]Internal Medicine, Dong-A University College of Medicine, Pusan, [5]Internal Medicine, Hallym University College of Medicine, Anyang, [6]Hematology-Oncology, Keimyung University Hospital, Daegu, [7]Hematology, Chonbuk National University Hospital, Jeonju, [8]Hematology, St. Vincent Hospital, Suwon, [9]Hematology, Seoul St. Mary Hospital, The Catholic University of Korea, Seoul, Korea, Republic Of

Background: Based on the accumulated results from clinical trials during the last 10 years, it is known that about 50% of chronic phase chronic myeloid leukemia (CP CML) patients with sustained deep molecular responses after enough tyrosine kinase inhibitors (TKIs) therapy may successfully stop their TKI therapy. Consequently, treatment-free remission (TFR) has been a new therapeutic goal. However, for the patients who relapsed after imatinib (IM) discontinuation, the questions on the best type of TKI for reintroducing and possibility of a second TKI discontinuation attempt are not clear.
Aims: We analyzed data from patients who regained durable deep molecular response after IM resumption for relapse and stopped IM therapy again in the Korean multicenter prospective study (Korean Imatinib Discontinuation Study; KID Study).
Methods: CP CML patients who were treated with IM for more than 3 years and had undetectable levels of BCR-ABL1 transcripts determined by quantitative reverse transcriptase polymerase chain reaction (PCR) for at least 2 years were eligible for KID study and in cases of MMR loss after 2 consecutive assessments, IM treatment was re-introduced. After IM resumption for MMR loss, the molecular response was evaluated every month until MMR was re-achieved and every 3 months thereafter. The second stop was permitted in the patients who were in second UMRD for at least 2 years.
Results: Among patients who lost MMR in 2 consecutive analyses and resumed IM in the KID study, 17 patients (9 men and 8 women) with a median age of 45 years (range, 18-63 years) entered into a second IM discontinuation after sustaining a second UMRD for a median of 25.9 months (range, 23.9-37.1 months). After a median follow-up of 17.1 months (range, 0.7-50.9 months) after second IM discontinuation, 13/17 patients (76%) and 11/17 patients (65%) lost UMRD and MMR, respectively. Among two patients who lost UMRD but not MMR, one patient showed fluctuation of *BCR-ABL1* transcript under the level of 0.1% on IS for 19.5 months and another patient have shown gradually increasing BCR-ABL1 transcripts under the level of 0.1%. Eleven patients who experienced second relapse (MMR loss) after a median 2.8 months (range, 1.8-30.7 months). All the patients who lost MMR were retreated with IM for a median of 14.6 months (range, 6.6-37.6 months); eleven patients re-achieved MMR at a median of 2.8 months (range, 1.0-10.2 months) and eight patients re-achieved UMRD at 7.4 months (range, 1.8-19.5 months). When we compared the molecular kinetics after the first and second IM discontinuation, there were no differences; median time to relapse was 3.7 months vs. 2.8 months after first and second IM discontinuation, respectively, and median time to re-achieved UMRD was 7.4 months vs. 7.4 months after first and second IM resumption, respectively.
Summary/Conclusion: Our data demonstrated that a second attempt might be possible and the median time to MMR loss after second discontinuation was similar to those of the first discontinuation. But the molecular kinetics after second IM resumption needs longer follow-up with more patients. Further studies on the predictors to select patients for a trial of second TFR and novel strategies such as intermittent therapy will be warranted.

PS1120

TWO YEARS OF THERAPY WITH MATINIB GENERICS IN CHRONIC MYELOID LEUKEMIA; A REPORT FROM THE POLISH ADULT LEUKEMIA GROUP (PALG) IMATINIB GENERICS REGISTRY

T. Sacha[1,*], J. Góra-Tybor[2], J. Niesiobędzka – Krężel[3], M. Szarejko[4], I. Pietkun[5], K. Myśliwiec[6], J. Gil[7], A. Pluta[7], M. Gniot[8], J. Wącław[9], A. Gołos[10], K. Giannopoulos[11]

[1]Department of Hematology, Jagiellonian University, Kraków, [2]Department of Hematology, Institute of Hematology and Transfusion Medicine, [3]Hematology, Oncology and Internal Medicine Department, Medical University of Warsaw, Warszawa, [4]Hematology and Transplantology, Medical University of Gdańsk, Gdańsk, [5]Hematology and Internal Medicine, Rydygier Specialistic Hospital, Kraków, [6]Hematology, Clinical Hospital in Zielona Góra, Zielona Góra, [7]Hematology, Markiewicz Memorial Oncology Center Brzozów, Brzozów, [8]Hematology, Medical University in Poznań, Poznań, [9]Hematology, Jagiellonian University Hospital, Kraków, [10]Hematology, Institute of Hematology and Transfusion Medicine, Warszawa, [11]Experimental Hematooncology, University of Lublin, Lublin, Poland

Background: Imatinib generics were introduced into Poland in 2014. An analysis of efficacy and safety after one-year therapy with imatinib generics of a large patient cohort (726) suffering from chronic myeloid leukemia in the chronic phase has suggested that they are not inferior to branded imatinib in terms of clinical efficacy and tolerability without any increased switching rate between imatinib generics and 2GTKI.
Aims: To evaluate the efficacy and safety of imatinib generics in the same cohort of patients after two years of therapy.
Methods: We report on 394 patients prospectively observed for two years within the Polish Adult Leukemia Group (PALG) Imatinib Generics Registry who completed a 24-month observation (all patients with available RQ-PCR results at the 24th month). The cohort consisted of previously untreated patients who started therapy with the imatinib generic, and patients switched to the generic from branded imatinib in 2014. All patients have been treated with at least two generics and a majority received three or more. All patients observed within the registry have been analyzed for: the frequency of molecular monitoring with RQ-PCR, the rate of sustained, improved and worsened molecular response, the rate of CCyR, MMR, MR4, and MR4,5 loss and for the switching rate between imatinib generics and 2GTKI during the first and the second year of therapy. The hematologic (3rd or 4th grade), and non-hematologic adverse events (all grades according to CTCAE criteria) in the first and second year of therapy were recorded .
Results: Four, three, two and one RQ-PCR tests were respectively performed in 53%, 25%, 16% and 6% of patients during the first year and in 64%, 9%, 12% and 15% of patients during the second year of observation. The molecular response under therapy with generics was respectively sustained, improved and worsened in 69.7%, 22.6%, and 7.7% of patients at 12 months and in 67.6%, 22.1% and 10% of patients at 24 months of therapy.During the first and the second year of therapy, MMR was respectively lost in 1.8% and 2.3%, CCyR in 1.5% and 0.8%, MR4.5 in 3.8% and 6.1% of patients. One patient lost MMR, CCyR and CHR at the 24th month of therapy. During the first and second year of observation, 4.5% and 3% of patients were respectively switched to 2GTKI, 3.8% and 1.3% for intolerance (non-hematologic toxicity only) and 0.8% and 1% for resistance during the first and second year. Hematologic toxicity (grade 3 or 4) during the first and second year was respectively observed in 2 and 3 patients, whereas non-hematologic toxicity (all grades)occurred in 17 patients during first year and in 36 patients during the second year of treatment.
Summary/Conclusion: This report on "real life" the effectiveness and safety of imatinib generics in a big cohort of CML patients after two years of observation suggests that they seem to be not less effective as Glivec in treating patients with CML CP; the responses being stable and safety profile is acceptable, without any increased switching rate between the 1st and 2GTKI during the first and second year of observation.

PS1121

MAINTENANCE THERAPY WITH DASATINIB ADMINISTRATED EVERY OTHER DAY IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

C. Loiseau[1,*], M. Spentchian[2], A. Charbonnier[3,4], M. Escoffre-Barbe[4,5], J. M. Cayuela[6], S. Rigaudeau[1], L. Roy[4,7], E. Cayssials[4,8], M. Gardembas[4,9], V. Dubruille[4,10], J.-C. Ianotto[11], F. Huguet[4,12], A. Guerci[4,13], V. Coiteux[4,14], P. Cony-Makhoul[4,15], F. X. Mahon[16], P. Rousselot[1,4,17]

[1]Service d'hématologie et oncologie, [2]Laboratoire d'hématologie, Centre Hospitalier de Versailles, Le Chesnay, [3]Service d'hématologie, Institut Paoli-Calmettes, Marseille, [4]French CML group, Bordeaux, [5]Service d'hématologie, CHU de Rennes, Rennes, [6]Laboratoire d'Hématologie, Hôpital Saint Louis APHP, Paris, [7]Service d'hématologie, Hôpital Henri Mondor APHP, Créteil, [8]Service d'hématologie, CHU de Poitiers, Poitiers, [9]Service d'hématologie, CHU d'Angers, Angers, [10]Service d'hématologie, CHU de Nantes, Nantes, [11]Service d'hématologie, CHU de Brest, Brest, [12]Service d'hématologie, Institut Universitaire du Cancer Toulouse - Oncopôle, Toulouse, [13]Service d'hématologie, CHU de Nancy, Nancy, [14]Service d'hématologie, Hôpital Claude Huriez, Lille, [15]Service d'hématologie, Centre Hospitalier Annecy Genevois, Pringy, [16]Institut Bergonié, Bordeaux, [17]INSERM U1173, Université Versailles-Saint-Quentin-en-Yvelines, Université Paris-Saclay, Saint-Quentin-en-Yvelines, France

Background: Dasatinib is a second-generation tyrosine kinase inhibitor (TKI) approved in first and subsequent lines for the treatment of patients (pts) with chronic phase-chronic myeloid leukemia (CP-CML). The standard dosing regimen is once daily resulting in a [C]max associated with a transient inhibition of BCR-ABL kinase activity. In order to optimize tolerability, we proposed a maintenance therapy with dasatinib once every 48h in CP-CML pts in deep molecular response (MR4; BCR-ABL^{IS}≤0.01%).
Aims: We first conducted a retrospective analysis on real-life CML pts allocated to maintenance therapy and then proposed a prospective maintenance study as part of the OPTIM dasatinib trial (EudraCT 2008-006854-17). The primary objective was to assess survival in maintenance without loss of MMR (BCR-ABL^{IS}>0.1%).
Methods: Kaplan-Meier estimates for maintenance without loss of MMR were calculated between two groups of pts in the retrospective cohort (pts in MR4 or pts matching the Euroski eligibility criteria (duration of TKIs ≥3y and duration of MR4≥1y). Pts with duration of TKIs ≥3y and duration of MR4≥1y were eligible for inclusion in the prospective maintenance study.
Results: Fifty-two CP-CML pts were eligible for the retrospective cohort, after one or two lines of therapy. Thirty-six pts were included in the prospective maintenance study after front line dasatinib. Median age at diagnosis was 47.8y (range 18.9-78) and 48% pts were male. Median duration of dasatinib in the retrospective cohort and in the prospective study was 40.5 months (range 6.2-116) and 47.7 months (range 32.7-88.4) respectively. Median daily dose before maintenance was 50 mg (range 40-100) and 100 mg (range 40-100) in both retrospective and prospective cohorts respectively. At baseline, 46% of the pts matched the Euroski criteria in the retrospective cohort.

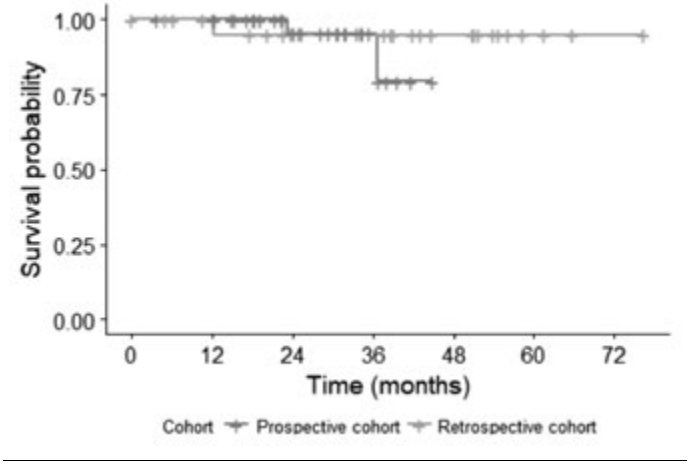

Figure 1. Survival in maintenance without MMR loss.

Median follow-up was 28.2 months and 30.7 months in both retrospective and prospective cohorts respectively. Kaplan-Meier estimate of survival without MMR loss in the retrospective cohort was 95.7% (95% CI, 89.9-100) at 12 months and 88.5% (95% CI, 79.4-98.6) at 24 months. We then focused on pts with duration of TKIs≥3y and duration of MR4≥1y. Only one patient lost MMR at 12.2 months. Survival in maintenance without MMR loss was 95% (95% CI, 85-100) at 12 and 24 months. As a comparison, 5 pts lost MMR if only MR4 was achieved at maintenance start. We then analyzed survival in maintenance without loss of MMR in the prospective study. Only 2 pts lost MMR after 23.3 and 36.6 months and maintenance without MMR loss was 100% at 12 months, 95.4% (95% CI, 71.8-99.2) at 24 months. These two pts regained a deep molecular response with dasatinib once daily without occurrence of BCR-ABL tyrosine kinase domain mutation. Focusing on pts included in the prospective study, 5 pts lost MR4 during maintenance at 3.3, 7.7, 8.9, 23.3 and 27.6 months including 2 pts who spontaneously regained MR4 during maintenance continuation and the 2 pts who lost MMR later. Tolerability was excellent with no withdrawal syndrome and no pleural effusion during maintenance. (Figure 1).
Summary/Conclusion: A maintenance therapy with dasatinib once every 48 hours after achievement of a deep molecular response is feasible. Pts with duration of TKIs≥3y and duration of MR4≥1y experienced very high rates of survival in maintenance without loss of MMR (>95%) and without dasatinib related toxicities. Our results suggest that maintenance with dasatinib is an attractive option for pts in sustained deep molecular response.

PS1122

PONATINIB 15 MG DAILY, COMBINING EFFICACY AND TOLERABILITY. A RETROSPECTIVE SURVEY IN ITALY

G. Binotto[1,*], F. Castagnetti[2], G. Gugliotta[2], E. Abruzzese[3], A. Iurlo[4], F. Stagno[5], A. Gozzini[6], P. Pregno[7], S. Galimberti[8], C. Fava[9], A. Isidori[10], G. La Barba[11], M. Tiribelli[12], M. Bonifacio[13], M. Annunziata[14], L. Luciano[15], I. Capodanno[16], M. Bocchia[17], F. Lunghi[18], L. Levato[19], M. M. Trawinska[3], F. Pane[15], G. Saglio[20], M. Baccarani[2], G. Semenzato[1], G. Rosti[2]

[1]Dept. of Medicine, Hematology and Clinical Immunology, Padua School of Medicine, Padova, [2]Institute of Hematology "L. and A. Seràgnoli", Department of Experimental, Diagnostic and Specialty Medicine , "S.Orsola-Malpighi" University Hospital, University of Bologna, Bologna, [3]Hematology Unit, "S. Eugenio" Hospital, Roma, [4]Hematology Division, IRCCS Ca' Granda - Maggiore Policlinico Hospital Foundation, University of Milan, Milan, [5]Chair of Hematology, University of Catania, Catania, [6]Haematology, AOU Careggi, University of Firenze, Florence, [7]Hematology Unit, Azienda Ospedaliero Universitaria Città della Salute e della Scienza, Turin, [8]Department of Clinical and Experimental Medicine, Section of Hematology, University of Pisa, Pisa, [9]Hematology Division, Ospedale Mauriziano, University of Turin, Turin, [10]Hematology and Stem Cell Transplant Center, AORMN Hospital, Pesaro, [11]Department of Hematology, "Spirito Santo" Hospital, Pescara, [12]Division of Hematology and BMT, Department of Medical Area, University of Udine, Udine, [13]Department of Medicine, Section of Hematology, University of Verona, Verona, [14]Hematology Unit, Cardarelli Hospital, [15]Hematology Unit, "Federico II" University of Naples, Naples, [16]Haematology Department, Arcispedale Santa Maria Nuova - IRCCS Reggio, Reggio emilia, [17]Chair of Hematology, University of Siena, Siena, [18]Hematology and Bone Marrow Transplantation Unit, IRCCS San Raffaele Scientific Institute, Milano, [19]Hematology Unit, "Pugliese-Ciaccio" Hospital, Catanzaro, [20]University of Turin, Orbassano, Italy

Background: The third generation tyrosine kinase inhibitor ponatinib is effective as salvage treatment for chronic phase (CP) Ph+ chronic myeloid leukemia (CML) patients resistant or intolerant to prior TKIs. Considering the demonstrated impact of ponatinib dose on the incidence of cardiovascular adverse events, a dose reduction to 15 mg daily is recommended in CP after the achievement of treatment milestones. Lower than 45 mg ponatinib dose strategies are currently under investigation in prospective clinical trials. There is a strong interest on independent data describing the efficacy and the safety of 15 mg daily ponatinib.
Aims: Describe and assess the efficacy and toxicity of low doses of ponatinib in CML-CP resistant or intolerant to previous TKIs.
Methods: A retrospective analysis of consecutive, CML-CP patients treated with ponatinib 15 mg as starting or de-escalated dose has been performed. No pre-specified criteria to switch to ponatinib or to reduce the dose were indicated.
Results: 62 patients are included, 53% males. Median age at ponatinib start was 57 (28-87) years. The median time from CML diagnosis to ponatinib treatment was 53.2 months (4.8-230). ABL1 mutations were detected in 29.3% patients (being 41% of them positive for the T315I mutation). Roughly half of the patients (45%) received ponatinib as 4th or 5th line of treatment. Ponatinib was started at 45, 30 and 15 mg, in 24, 13 and 25 patients, respectively. Resistance to prior TKIs was the reason for switch in all patients treated with higher than 15 mg dose, while 12/25 patients starting with 15 mg were purely intolerant or intolerant and resistant. The median treatment duration at any dose was 21.2 months, with 45/62 (72%) patients exposed to ponatinib for more than 12 months. Overall, 68% of patients improved response status. In patients treated with 45 or 30 mg the CCyR, MR3 and MR4/MR4.5 rates were 76%, 55% and 32% respectively. Responses were achieved after a median of 3.5, 4.3 and 6.7 months, respec-

tively. Dose reduction to 15 mg was decided after a median time of 10 months, mostly due to adverse events (73%) and less frequently, to prevent toxicity and/or following response attainment (27%). Median duration of treatment was 32.4 months, with a discontinuation rate of 5/37 (1 cerebrovascular accident, 2 severe hematologic toxicities, two cases of resistance/progression). Two deaths were registered (both in the 45 mg group), one after blast crisis and one for a fatal cardiovascular event. Focusing the 25 patients started with ponatinib 15 mg, median duration of treatment was 15 months (4.3-58.1). CCyR was obtained in 55% of patients lacking CCyR and MR3 in 50% without MMR with a median time of 3.8 and 3.4 months, respectively. During follow-up, MR3 or deeper responses were maintained or improved in 80% of cases, while 7 patients lost their best acquired response (4 MR4/4.5 to MR3, 1 MR3 to MR2, 1 CCyR to PHR and 1 progression to accelerated phase). Three patients of this group discontinued the treatment (1 coronary vasospasm-induced acute coronary syndrome, 2 muscle skeletal pain). After a median follow-up of 21 months, 35/54 (65%) patients on ponatinib mantain a MR3 or deeper response; 48 (89%) are taking 15 mg or lower.

Summary/Conclusion: This analysis confirms the efficacy of de-escalated ponatinib dose in CML patients resistant to prior TKIs, with acceptable toxicity profile. Promising data on 15 mg as a starting dose in selected patients (intolerant or with low level resistance) warrant further investigation in larger prospective trials.

PS1123

THE EUTOS LONG-TERM SURVIVAL SCORE PREDICTS RESPONSE AND SURVIVAL OF ELDERLY CHRONIC MYELOID LEUKEMIA PATIENTS BETTER THAN SOKAL SCORE

F. Castagnetti[1,*], G. Gugliotta[1], M. Breccia[2], F. Stagno[3], G. Specchia[4], L. Levato[5], B. Martino[6], M. D'Adda[7], E. Abruzzese[8], P. Pregno[9], M. Tiribelli[10], A. Iurlo[11], T. Intermesoli[12], F. Cavazzini[13], A. R. Scortechini[14], C. Fava[15], M. Bonifacio[16], M. Salvucci[17], M. Bocchia[18], E. Usala[19], A. Gozzini[20], M. Bergamaschi[21], S. Soverini[1], R. Foà[22], G. Martinelli[23], M. Cavo[1], G. Saglio[15], F. Pane[24], M. Baccarani[25], G. Rosti[1]

[1]Institute of Hematology "L. & A. Seràgnoli", Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, [2]Department of Cellular Biotechnologies and Hematology, "Sapienza" University, Rome, [3]Chair of Hematology, University of Catania, Catania, [4]Chair of Hematology, University of Bari, Bari, [5]Hematology Unit, "Pugliese-Ciaccio" Hospital, Catanzaro, [6]Hematology Unit, Azienda Ospedaliera "Bianchi-Melacrino-Morelli", Reggio Calabria, [7]Hematology Unit, Azienda Ospedaliera "Spedali Civili", Brescia, [8]Hematology Unit, "S. Eugenio" Hospital, Rome, [9]Hematology Unit, Azienda Ospedaliero Universitaria Città della Salute e della Scienza, Torino, [10]Division of Hematology and BMT, Department of Experimental and Clinical Medical Sciences, Azienda Ospedaliero-Universitaria di Udine, Udine, [11]Hematology Unit, Fondazione IRCCS "Ca' Granda", Ospedale Maggiore Policlinico, Milano, [12]Hematology Unit, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, [13]Chair of Hematology, "Arcispedale S. Anna" University Hospital, Ferrara, [14]Hematology Unit, Azienda Ospedaliero Universitaria "Ospedali Riuniti", Ancona, [15]Hematology Unit, Ospedale Mauriziano, Università di Torino, Torino, [16]Chair of Hematology, University of Verona, Verona, [17]Hematology Unit, Ospedale Santa Maria delle Croci, Ravenna, [18]Dipartimento di Scienze Mediche, Chirurgiche e Neuroscienze, University of Siena, Siena, [19]Hematology Unit, "Businco" Hospital, Cagliari, [20]Hematology Unit, AOU "Careggi", Firenze, [21]Hematology Unit, IRCCS AOU San Martino - IST, Genova, [22]Department of Cellular Biotechnologies and Hematology, "Sapienza" University , Rome, [23]Medical Oncology Unit, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, Meldola (FC), [24]Chair of Hematology, Department of Biochemistry and Medical Biotechnologies, "Federico II" University, Napoli, [25]Department of Hematology and Oncological Sciences "L. e A. Seràgnoli", University of Bologna, Bologna, Italy

Background: The EUTOS long-term survival (ELTS) score, based on a large cohort of chronic myeloid leukemia (CML) patients treated frontline with imatinib (IM), has been developed to discriminate the probability of dying of disease progression, but many clinicians still use the traditional Sokal score. Few comparisons between the two scores are available and the clinical usefulness of ELTS score has not been clearly demonstrated yet.

Aims: To compare the prognostic value of ELTS and Sokal scores in a cohort of CML patients treated in early chronic phase with tyrosine kinase inhibitors (TKIs) as first-line therapy. Given the different weight that the variable "age" has in the two score formulations, we hypothesized a different predictive value in specific age groups, so we compared the ELTS and Sokal scores in patients < 30 years, 30-64 years and > 65 years old.

Methods: Nine hundred and four adult patients were included, 559 treated with IM and 345 treated with nilotinib-based regimens (NIL). The patients were enrolled in six multicenter studies (NCT00481052, NCT00769327, NCT01535391, NCT00514488, NCT00510926, observational trial CML/023) conducted by the GIMEMA CML WP. The intention-to-treat population of each study was analyzed. Definitions: progression, transformation according to ELN criteria; leukemia related death (LRD): death after progression.

Results: Median age, 52 years (range 18-86); age distribution, 8% patients < 30 years old and 22% of patients ≥ 65 years old. The median follow-up was 6 years (range: 2-9 years). The patient distribution according to the different scoring systems was as follows: 57% low, 30% intermediate and 13% high ELTS score, 40% low, 39% intermediate and 21% high Sokal score, respectively. The risk distribution was comparable in patients treated with IM or NIL. Approximately 2/3 of patients with low (64%), intermediate (64%) or high (71%) ELTS score had the same risk classification according to the Sokal score. The concordance between the two scores, in particular for low and high risk category, was even better in patients < 30 (87% and 80%, respectively) or 30-64 years old (71% and 88% respectively); in contrast, in elderly patients only 8% of low ELTS patients had a low Sokal score, and only 48% of high ELTS score had a high Sokal score. Overall, both scores were able to predict significantly different probabilities of MR3, MR4, overall survival (OS) and LRD, but in elderly patients (≥ 65 years) only the ELTS score was able to predict the achievement of MR3 (99%, 87% and 75% in low, intermediate and high risk patients, respectively; p=0.001) and MR4 (82%, 61% and 50% in low, intermediate and high ELTS score patients, respectively; p=0.005). Interestingly, both scores predicted the OS, while only the ELTS score predicted a significantly different LRD probability (cumulative incidence 2%, 6% and 14% in low, intermediate and high risk patients, respectively; p=0.05). The results were similar considering patients ≥ 60 years old.

Summary/Conclusion: The risk distribution according to the ELTS and Sokal score and the concordance between the 2 scores was different in specific age groups (< 30, 30-64, ≥ 65 years old). In elderly CML patients treated with IM or NIL as frontline therapy the prognostic predictive ability of ELTS score resulted superior to the Sokal score.

PS1124

CARDIOVASCULAR TOXICITY IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH SECOND-GENERATION TYROSINE KINASE INHIBITORS IN REAL-LIFE PRACTICE. IDENTIFICATION OF RISK FACTORS AND ROLE OF PROPHYLAXIS

G. Caocci[1,*], O. Mulas[2], M. Annunziata[3], L. Luciano[4], M. Bonifacio[5], E. Orlandi[6], P. Pregno[7], S. Galimberti[8], A. Russo Rossi[9], E. Abruzzese[10], A. Iurlo[11], B. Martino[12], N. Sgherza[13], G. Binotto[14], F. Castagnetti[15], A. Gozzini[16], C. Fozza[17], M. Bocchia[18], A. Sicuranza[18], F. Stagno[19], F. Efficace[20], E. Usala[2], F. De Gregorio[4], L. Scaffidi[5], C. Elena[6], F. Pirillo[7], C. Baraté[8], M.M. Trawinska[10], D. Cattaneo[11], C. Labate[12], G. Gugliotta[15], M. Molica[21], G. Specchia[9], G. La Nasa[1], R. Foà[21], M. Breccia[21]

[1]Hematology, Department of Medical Sciences and Public Health, University of Cagliari, [2]Hematology and Transplant Center, Businco Hospital, Cagliari, [3]Hematology Unit, Cardarelli Hospital, [4]Hematology Unit "Federico II", University of Naples, Napoli, [5]Department of Medicine, Section of Hematology, University of Verona, Verona, [6]Hematology Unit, "Fondazione IRCCS Policlinico S. Matteo" University Hospital, Pavia, [7]Hematology Unit, Azienda Ospedaliero-Universitaria Città della Salute e della Scienza, Torino, [8]Department of Clinical and Experimental Medicine, Section of Hematology, University of Pisa, Pisa, [9]Hematology and Transplants Unit, University of Bari, Bari, [10]Hematology Unit, Sant'Eugenio Hospital Tor Vergata University, Roma, [11]Hematology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, [12]Hematology Unit, Azienda Ospedaliera "Bianchi-Melacrino-Morelli", Reggio Calabria, [13]Hematology and Transplant Center, Casa Sollievo della Sofferenza Hospital, S. Giovanni Rotondo, [14]Hematology Unit, University of Padova, Padova, [15]Department of Experimental, Diagnostic and Specialty Medicine, S. Orsola-Malpighi Hospital, University of Bologna, Bologna, [16]Hematology Unit, AOU Careggi, University of Florence, Firenze, [17]Department of Clinical and Experimental Medicine, Section of Hematology, University of Sassari, Sassari, [18]Hematology Unit, Azienda Ospedaliera Universitaria Senese, University of Siena, Siena, [19]Hematology Unit, Ferrarotto Hospital, Catania, [20]Data Center and Health Outcomes Research Unit, Italian Group for Adult Hematologic Diseases , GIMEMA, [21]Division of Hematology, Department of Cellular Biotechnologies and Hematology, Sapienza University, Roma, Italy

Background: Cardiovascular adverse events (CV-AE) represent emerging complications in chronic myeloid leukemia (CML) patients treated with second-generation tyrosine kinase inhibitors (2ndGTKIs). Current recommendations highlight the importance of a careful evaluation of CV risk factors but the role of a primary prophylaxis with aspirin is still a matter of debate.

Aims: We therefore analyzed a large real-life cohort of Italian CML patients

treated with a 2ndGTKIs as first or subsequent line of treatment. The primary objective was to evaluate the incidence of CV-AE and the association with the Systematic Coronary Risk Evaluation (SCORE) assessment and other baseline risk factors. Secondary objective were to evaluate the role of primary or secondary prophylaxis in preventing CV atherothrombotic events and to report the management of CV-AE complications in the clinical practice.

Methods: We evaluated 506 adult CML patients (mean age 52, range 18-87) who were treated with nilotinib (286) or dasatinib (220) as first or subsequent lines of treatment between January 2012 and December 2015. CV diseases (CVD) and risk factors, primary and secondary prophylaxis, and management of CV-AE were assessed at baseline and during treatment.

Results: Anamnesis for CVD was positive in 181 (35.8%) patients. The 60-month cumulative CV-AE incidence was 21.7%. Patients treated with nilotinib or dasatinib showed a CV-AE incidence of 24.7% and 16.4%, respectively (p=NS). A positive history for CVD (p=0.001) and a 2ndGTKI line of treatment >1 (p=0.002) were significantly associated to a higher incidence of CV-AE. Patients with both 2 risk factors (CML-CV high risk score) showed a CV-AE incidence significantly higher (45.9% vs 16.3% and 18.7%, p<0.001) (Figure 1). The atherothrombotic AE incidence was 13.1%. No significant difference in atherothrombotic AE incidence was found in patients who underwent aspirin primary prophylaxis before starting 2ndGTKIs. Considering only patients with age>60 years and CML-CV high risk score, atherothrombotic AE incidence was significantly lower in those treated with aspirin (0% vs 58.2%; p=0.01).

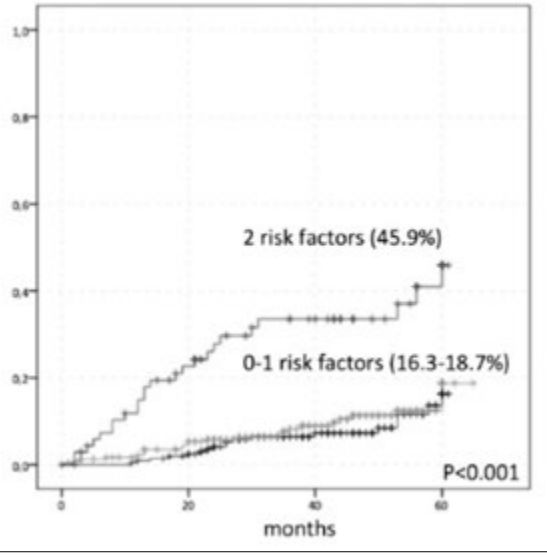

Figure 1.

Summary/Conclusion: This study confirmed the increased risk of CV-AE in CML patients treated with 2ndGTKIs in the real-life, particularly in those patients with positive anamnesis for CVD and 2ndGTKI line of treatment >1. Our findings emphasize the need of personalized prevention strategies based on CV risk factors; ideally, management and treatment of these patients should be performed in close collaboration with cardio-oncologists, angiologists and vascular surgeons. We suggest that patients with age >60 years and CV diseases undergoing a 2ndGTKI line treatment >1 are likely to be the best candidates to aspirin. Data on efficacy of primary prophylaxis in CV-CML high risk patients should be confirmed in prospective randomised trials.

PS1125

ULTRA-SENSITIVE DETECTION OF TYROSINE KINASE INHIBITOR RESISTANT MUTATIONS IN CHRONIC MYELOID LEUKEMIA PATIENTS USING MISEQ ILLUMINA NEXT GENERATION SEQUENCING PLATFORM

M. Romzova[1,*], D. Smitalova[1], N. Tom[1], T. Jurcek[2], J. Mayer[1,2], Z. Racil[1,2]

[1]Department of Molecular Medicine, Central European Institute of Technology, Masaryk University, [2]Department of Internal Medicine, Haematology and Oncology, University Hospital Brno, Masaryk University, Brno, Czech Republic

Background: The discovery of tyrosine kinase inhibitors (TKI) brought a revolution in the management of chronic myeloid leukemia (CML), increasing survival and life-quality of patients. However, a resistance caused by mutations in kinase domain (KD) of *BCR-ABL1* may occur, which can result in therapy failure. Although KD mutations screening is routinely done by Sanger sequencing (SS), its major disadvantage is detection limit above 20% VAF. Recently, numerous studies investigated next generation sequencing (NGS) of KD region, showing enhanced sensitivity below 15% and thus earlier mutation detection. These studies used a nested or semi-nested PCR for KD region amplification. Such approach is however hindered by possible errors introduced during reverse transcription (RT) and 2-round amplification steps (nested PCR), restricting the mutation detection limit to 1% and above. Majority of these studies used Roche platform for KD sequencing, and only one study employed Illumina platform, despite it is nowadays the most widely accessible sequencing platform.

Aims: Our aim was to develop a simple and efficient Illumina-based one-round PCR amplification protocol, which would reduce PCR-mediated errors and increase sensitivity of KD mutation detection ≤1%, with still acceptable specificity.

Methods: New Illumina-based NGS protocol for KD sequencing was established and tested on the set of 34 retrospective samples from 13 CML patients who developed TKI resistant mutations. We tested 15 follow-up (FUP) samples at the time of SS mutation detection, 11 FUP samples collected 1-18 month before SS mutation detection, 8 diagnostic samples and 6 healthy controls. High fidelity enzymes were used for RT and PCR amplification. The performance of protocol was assessed in comparison to routinely used SS. The bioinformatics (BI) pipeline was designed with respect to biological input (cDNA) and required very high sensitivity, allowing detection of insertions/deletions and single nucleotide variants with frequency down to 0.1%.

Results: No mutations were detected in *ABL1* KD of healthy controls, showing that employment of high-fidelity RT and PCR enzymes and BI filtering led to decreased background noise. NGS detected mutations in 15/15 (100%) FUP samples previously positive for mutation by SS (R^2_{VAF} =0.96). Further, NGS detected mutations in 7/11 (63%) SS negative FUP samples, collected before SS mutation detection and in 1/8 (12.5%) SS negative diagnostic samples (T315I mutation at 0.5% VAF). Together 8 different mutations were detected by SS multiple times (≥20% VAF), while NGS detected 15 different mutations multiple times, of which 19 times with ≥20% VAF, and 26 times at the level 0.1 – 11% VAF (Table 1). With exception of R307W and E450Q, all other mutations detected by NGS were previously described to be associated with TKI sensitivity. The mutation most frequently detected prior identification by SS was T315I (5 pts, 1-8 months earlier). Moreover, 3 other mutations were detected at earlier time point (3 pts; 12 – 20 months earlier).

Table 1.

	Diagnosis				Earlier in follow-up				At the time of Sanger sequencing detection			
Patient	SS	NGS >20%	NGS <20%	Month	SS	NGS >20%	NGS <20%	Month	SS	NGS >20%	NGS <2
1	0	0	0	M3	0	0	M351T 1.6% R307W 2.2%	M21	M351T 100%	M351T 91%	E450Q 5
2	0	0	0	M3	0	0	0	M9	F359V 80 %	F359V 95%	0
3	0	0	0	M4	0	0	0	M12	E279K 100 %	E279K 97%	F467S 1
4	0	0	0					M6	T315I 35% E255K 25% E255V 25%	T315I 31% E255K 25% E255V 26%	Y253F 1
								M9	T315I 100%	T315I 93 %	E255K 2 E255V 3 F359I 1.4
5	0	0	0					M3	E255K 85%	E255K 81%	0
6	0	0	0	M15	0	0	0	M21	0	0	E255K 1
7				M154	0	0	E255K 4.3%	M166 M169	E255K 90% E255K 100%	E255K 82% E255K 98%	0 0
8				M134	0	0	0	M137	0	0	T315I 11
9				M28	0	T315I 20%	0	M35 M40	T315I 20% T315I 42%	T315I 32 % T315I 50%	0 F467S 1
10				M5	0	0	T315I 1.6% F317L 0.4%	M6	T315I 95%	T315I 87 %	0
11	0	0	0					M18	F359I 100%	F359I 99 %	0
12	0	0	T315I 0.5%					M6	T315I 100%	T315I 99 %	Y253H 1
13				M206	0	0	T315I 1.4% F317L 4% F359V 6.4% G250E 1% M351T 0.7%	M214	T315I 18%	T315I 18%	V299L 0 F317L 3 F359V 0 G250E 0
								M234	T315I 65% V299L 35%	T315I 64% V299L 31%	F317L 0

Summary/Conclusion: Overall, we have confirmed that results from NGS analysis, using Illumina-based one-round PCR amplification protocol, highly correlated with SS when mutations with >20% VAF were analyzed. Due to high sensitivity (together with high specificity) Illumina-based NGS analysis was able to detect mutations in 24% more samples than SS and proved to be suitable for earlier detection of TKI resistant mutations at very low frequencies (≥0.1%).

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PS1126

PEG-INTERFERON ALPHA 2B CAN IMPROVE MOLECULAR RECURRENCES IN THE TREATMENT-FREE REMISSION; A PILOT STUDY WITH 18-MONTH FOLLOW-UP

M. Ayala[1,*], D. Moreno-Lorenzana[2], E. J. Cordova[3], X. Aquino[4], A. Chavez-Gonzalez[2]

[1]Hematology & BMT Unit, Specialties Hospital, Nacional Medical Center "La Raza", [2]Oncology Research Unit, Oncology Hospital, National Medical Center, Mexican Institute for Social Security, [3]Immunogenomics and Metabolics Disease Laboratory, Instituto Nacional de Medicina Genómica, SS , [4]Grupo Consulmed, Mexico

Background: In 2010, Mahon *et al.* reported the results of the STIM study in 100 patients with stable undetectable molecular response (uMR) for at least 2 years. Finding 6 months of relapses in 58% and year in 61% of patients, most of the losses of the uMR occur in the first 6 months of the suspension of the TKI. An update of the results at 65 months, found no relapse after 2 years of suspension.
Aims: Decrease molecular recurrence (MolRel) rate in patients with CML-CP in stable uRM with TKI suspension and maintenance for 6 months with P-IFNα2b.
Methods: A prospective, longitudinal, descriptive, single-cohort, open pilot study was conducted to evaluate the effectiveness of maintenance for 6 months with P-IFNα2b in reducing the rate of MolRel in patients with CML-CP in stable uRM. Treatment was initiated after elective discontinuation of TKI will receive 100 mg of P-IFNα2b subcutaneous weekly for 6 months. They will then suspend all treatment. MolRel surveillance will be initiated simultaneously with the TKI suspension, with peripheral blood simple taken for baseline quantitative q-PCR measurement and quarterly until 6 months of surveillance from the TKI suspension of or have molecular relapse. An analysis of lymphocyte subpopulations was performed by baseline flow cytometry and quarterly up to 6 months of suspended P-IFNα2b, as well as a blood analysis of basal and half-yearly microRNAs during the two years of suspension. In patients in whom molecular relapse is documented, TKI will be restarted at the dose prescribed prior to discontinuation of treatment.
Results: Thirty-one patients (16 male and 15 female) were included, with a median age at diagnosis of 44.5 years (25-77), the risks of Sokal to diagnosis were; high 20%, intermediate 23%, low 27%, N/A 30%. Twenty-four patients were suspended from imatinib, 6 of dasatinib, and one with nilotinib and the median time uRM was 49 (28-102). Twenty-seven patients completed 6 months of TKI discontinuation remaining at uRM 85%, the majority of MolRel occurred within 12 months of discontinuation; the molecular recurrence-free survival (MRFS) rate was 85%, 67% and 58% at 6, 12 and 24 months respectively. Patients who lost MMR (5/dasatinib and 8/imatinib) re-started the same TKI and all have now recovered MMR. The Adverse Events presented were grade 1-2 for myalgias 72% (17/55), headache 64% (30/34), hyperpigmentation 58% (41/17), asthenia 41% (17/24), adynamia 38%(14/24), arthralgias 35% (14/21), nausea 14/0%, alopecia 10/0%, fever 7/0%, diarrhea 3/0%, media time from TKI suspension is 19 (range 16-23) months (Figure 1).

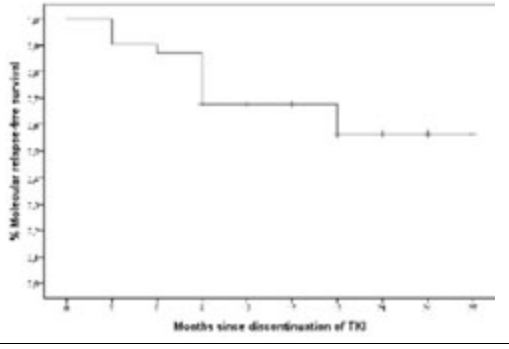

Figure 1. Molecular relapse-free survival.

Summary/Conclusion: Although these results are preliminary (since a sample of 50 patients is expected to be included), the trend suggests that P-IFNα2b may be useful as maintenance after TKI suspension to decrease the rate of molecular relapse at 6 months of the suspension of the TKI. It is expected to determine the involvement of the immunological behavior of lymphocyte subpopulations in the maintenance of uMR, using P-IFNα2b after TKI suspension, which will be reported later to complete the year of TKI suspension. To our knowledge this is the first prospective study in Latin America that reports this therapeutic strategy of TFR.

PS1127

CROSS-INTOLERANCE WITH BOSUTINIB AFTER PRIOR TYROSINE KINASE INHIBITORS IN PATIENTS WITH PHILADELPHIA CHROMOSOME–POSITIVE LEUKEMIA: PHASE 1/2 STUDY UPDATE

J. Cortes[1,*], J. Lipton[2], A. Hochhaus[3], V. Kota[4], M. Baccarani[5], S. Durrant[6], S. Assouline[7], D.-W. Kim[8], T. Brümmendorf[9], E. Leip[10], A. Viqueira[11], J. Aguiar[12], C. Gambacorti-Passerini[13]

[1]University of Texas MD Anderson Cancer Center, Houston, United States, [2]Princess Margaret Hospital, Toronto, Canada, [3]Klinik für Innere Medizin II, Universitätsklinikum Jena, Jena, Germany, [4]Winship Cancer Institute of Emory University, Atlanta, United States, [5]University of Bologna, Bologna, Italy, [6]Royal Brisbane Hospital, Herston, Australia, [7]McGill University, Montreal, Canada, [8]Seoul St. Mary's Hospital, Leukemia Research Institute, The Catholic University of Korea, Seoul, Korea, Republic Of, [9]Universitätsklinikum RWTH Aachen, Aachen, Germany, [10]Pfizer Inc, Cambridge, United States, [11]Pfizer Spain, Madrid, Spain, [12]Pfizer Inc, Groton, United States, [13]University of Milano-Bicocca, Monza, Italy

Background: Bosutinib has a distinct adverse event (AE) profile vs other tyrosine kinase inhibitors (TKIs) used to treat Philadelphia chromosome–positive (Ph+) leukemia.
Aims: Cross-intolerance between bosutinib and prior TKI therapy was evaluated after ≥4 years of follow-up of a phase 1/2 study (NCT00261846).
Methods: Patients with chronic phase (CP) chronic myeloid leukemia (CML; n=403) or accelerated/blast phase CML or Ph+ acute lymphoblastic leukemia (ADV; n=167) previously treated with imatinib±dasatinib and/or nilotinib received bosutinib (starting dose 500 mg QD) in a phase 1/2 study. Cross-intolerance (AEs leading to discontinuation of both prior TKI and bosutinib) and AEs causing prior TKI intolerance and recurring as grade 3/4 AEs with bosutinib were assessed.
Results: In imatinib-intolerant and dasatinib-intolerant patients, respectively, 18% and 24% in the CP CML group and 18% and 5% in the ADV group had cross-intolerance with bosutinib, which was most commonly due to hematologic AEs (Table 1). Cross-intolerance in imatinib-intolerant patients with CP CML due to AEs common with imatinib was low (rash 6%; diarrhea 10%; edema/fluid retention 0; myalgia 0); cross-intolerance due to pleural effusion was low in dasatinib-intolerant patients with CP CML (13%) and dasatinib-intolerant ADV patients (0). No deaths occurred due to cross-intolerance.

Table 1.

Cause of intolerance*	n	Bosutinib discontinuation due to same AE n (%)	Same grade 3/4 AE with bosutinib n (%)
CP CML – imatinib intolerance			
Any AE	120†	21 (18)	39 (33)
Thrombocytopenia	27	7 (26)	17 (63)
Neutropenia	21	2 (10)	7 (33)
Rash	18	1 (6)	2 (11)
Anemia	14	0	6 (43)
Edema	12	0	0
Diarrhea	10	1 (10)	3 (30)
Hematologic toxicity	7	4 (57)	5 (71)
Vomiting	7	1 (14)	1 (14)
Fatigue	7	1 (14)	0
Nausea	6	0	0
Fluid retention	5	0	0
Myalgia	5	0	0
CP CML – dasatinib intolerance			
Any AE	50	12 (24)	18 (36)
Pleural effusion	16	2 (13)	3 (19)
Thrombocytopenia	8	4 (50)	8 (100)
Pancytopenia	6	0	0
ADV – imatinib intolerance			
Any AE	22†	4 (18)	8 (36)
Thrombocytopenia	7	4 (57)	6 (86)
ADV – dasatinib intolerance			
Any AE	21†	1 (5)	10 (48)
Pleural effusion	7	0	2 (29)
Thrombocytopenia	5	1 (20)	5 (100)

* Causes in ≥5 patients shown; † Patients with known causes shown

Summary/Conclusion: Cross-intolerance with bosutinib was low and largely due to hematologic AEs, supporting bosutinib use in patients with Ph+ leukemia intolerant to prior TKIs, including those with intolerance due to rash or diarrhea.

PS1128

CHRONIC MYELOID LEUKEMIA ITALIAN MULTICENTER OBSERVATIONAL STUDY (CML-IT-MOS): ANALYSIS OF CLINICAL CHARACTERISTICS OF 1330 CML PATIENTS TREATED IN REAL-LIFE IN 66 ITALIAN CENTERS OF THE GIMEMA GROUP

G. Specchia[1,*], P. Pregno[2], F. Castagnetti[3], B. Martino[4], M. Breccia[5], M. Bonifacio[6], F. Stagno[7], A. Gozzini[8], M. Tiribelli[9], M. Bergamaschi[10], M. Pizzuti[11], E. Usala[12], L. Luciano[13], P. Leoni[14], A. Guella[15], F. Falzetti[16], R. Di Lorenzo[17], A. Russo Rossi[18], C. Monagheddu[19], M. Nicolosi[20], F. Saccona[21], F. Pane[22], G. Ciccone[21], M. Baccarani[23], G. Saglio[24]

[1]Hematology and BMT unit - University of Bari - Bari - Italy, Policlinic of Bari, Bari, [2]Hematol Dept - AOU Città della Salute e della Scienza of Turin, Turin, [3]Institute of Hematology and Medical Oncology, Policlinico Sant'Orsola Malpighi, Bologna, [4]Hematology Department, Bianchi-Melacrino-Morelli Hospital, Reggio Calabria, [5]Inst. of Hematology and Cellular Biotechnology, La Sapienza University, Umberto I Hospital, Rome, [6]Hematology Department, A.O.U. integrata Verona, Verona, [7]Hematology Department, A.O.U. Vittorio Emanuele Policlinic , Catania, [8]Hematology Department, Florence University, A.O.U. Careggi, Florence, [9]Hematology and BMT Dept, A.O.U. Santa Maria della Misericordia, Udine, [10]Hemato-Oncology, Hematol Dept, A.O.U. San Martino, Genua, [11]Hematology Department, San Carlo Hospital, Potenza, [12]Hematology Department, Businco Hospital, Cagliari, [13]Hematology Department, II University Federico II, Naples, [14]Hematology Department, Ospedali Riuniti, Università Politecnica of Marche, Ancona, [15]Medicine Department, Santa Chiara Hospital, Trento, [16]Hematology Department, A.O.U. S. Maria della Misericordia Hospital, Perugia, [17]Hematology Department, A.S.O. Spirito, University of Pescara, Pescara, [18]Hematology and BMT unit , Policlinico Consorziale Bari, Bari, [19]SSD Clinic Epidemiology, [20]Hematology Department, A.O.U. Città della Salute e della Scienza of Turin, [21]SSD Clinic Epidemiology, AOU Città della Salute e della Scienza of Turin, Turin, [22]Hematology Department, II University Federico II Naples, Naples, [23]Institute of Hematology and Medical Oncology , Policlinico S. Orsola Malpighi , Bologna, [24]Hematology Department, Mauriziano Hospital, University of Turin, Turin, Italy

Background: Chronic Myeloid Leukemia (CML) is a disease characterized by the presence of a BCR-ABL rearrangement, whose outcome, very poor until a few years ago, was totally changed by the advent of Tyrosine Kinase Inhibitors (TKI) therapy. Most of information about the clinical characteristics of the CML patients are based on data derived from clinical trials, that often exclude patients not fitting with strict inclusion criteria. In routine clinical practice may be important to know the characteristics of the entire CML patients' population and this is better reflected by registries studies including all consecutive patients.
Aims: To provide a robust and updated information on the clinical, hematologic characteristics and treatment response in non-selected Italian patients with chronic myeloid leukemia (CML) in each phase of the disease.
Methods: We retrospectively and prospectively recorder in a web-based database clinical, hematological, cytogenetic and biological information about all new diagnosis of CML patients referred to Hematology Centers of the GIMEMA Study Group from January 2012 to December 2017
Results: The study covered 1330 patients newly diagnosed with CML between January 2012 and December 2017 in 66 Italian centers. Median age at diagnosis was 59 years (range 17-71) and 59.7% were males. At diagnosis, 1075 (98.8%) patients were in chronic phase, 10 (1%) patients in accelerate phase and 3 (0.3%) in blastic crisis. Among 936 patients, 175 (13%), 421 (32%), 340 (25%) were high, intermediate and low risk by Sokal; 93 (7%), 480 (36%), 362 (27%) by EURO; 335 (25%), 77 (6%), 918 (69%) by EUTOS; 119 (9%), 246 (18%), 246 (18%) and 590 (44%) by ELTS, respectively. The median follow-up was 24.6 months and 26 patients died. At the cytogenetic analysis, among 533 patients there were: 443 (83,1%) without additional cytogenetic aberrations (ACA), 62 (11,6%) with major route and 28 (5,6%) with minor route ACA respectively. BCR-ABL transcripts among 1056 patients were: *b2a2* in 341 (32.3%), *b3a2* in 582 (55.1%), b2a2+b3a2 in 95 (9%), *e1a2* in 21 (2%), e19a2 in 3 (0.3%) *b3a2+e1a2* in 14 (1.3%) patients respectively. ECOG performance status was 0, 1, or ≥ 2 in 663 (52.3%), 227 (17.9%) and 53 (4.2%) cases respectively. According to co-morbidity, the Charlson comorbidity index was 0, 1, 2 or > 3 in 1013 (79%), 110 (8,6%), 75 (6%) and 71 (6%) patients respectively. Among 574 cases cardiovascular, lung, metabolic and oncologic diseases were observed in 423 (73%), 55 (9.6%), 112 (19.5%) and 73 (13%) patients respectively. Among 1143 cases 576 (50.4%) and 567 (49.6%) patients were treated in first-line with imatinib (IMA) and with II generation TKI respectively. One hundred fourty-two cases were pretreated with hydroxyurea
Summary/Conclusion: Our preliminary results of this observational epidemiologic study suggest that collection of clinical data of CML patients treated out of strictly clinical trials represent an essential tool for long/term treatment, able to observe setting strategies based on the clinical characteristics, the degree of response obtained and the toxicity related to the therapy in overall CML population. We are planning to analyze all these tools in order to observe the response according to ELN guidelines, toxicity and feasibility of treatment sequence in a cohort of patients treated in real-life. In conclusion, is needful to continue recruiting patients due to obtain a greater representativeness

PS1129

IMATINIB STOP STUDY FEASIBLE TO JAPANESE CLINICAL SETTING

K. Usuki[1,*], Y. Ueda[2], S. Fujisawa[3], H. Kobayashi[4], E. Kondo[5], N. Doki[6], T. Nakao[7], Y. Kanda[8], N. Kosugi[9], H. Kosugi[10], T. Kumagai[11], H. Harada[12], T. Nakao[7], M. Shikami[13], T. Sakura[14], K. Inokuchi[15], A. Saito[16], Y. Nawa[17], M. Ogasawara[18], J. Nishida[19], T. Kondo[20], T. Takahashi[21], C. Yoshida[22], H. Kuroda[23], S. Morita[24], S. Komukai[25], A. Kawaguchi[26], J. Sakamoto[27], S. Kimura[28]

[1]Department of Hematology, NTT Medical Center Tokyo, Tokyo, [2]Department of Hematology/Oncology, Kurashiki Central Hospital, Kurashiki, [3]Department of Hematology, Yokohama City University Medical Center, Yokohama, [4]Department of Hematology, Obihiro Kosei Hospital, Obihiro, [5]Department of General Medicine, Okayama University Graduate School of Medicine,Dentisty and Pharmaceutical Sciences, Okayama, [6]Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo, [7]Department of Hematology, Osaka City General Hospital, Osaka, [8]Department of Hematology, Jichi medical University, Saitama Medical Center, Saitama, [9]Department of Hematology, Numazu City Hospital, Numazu, [10]Department of Hematology, Ogaki Municipal Hospital, Ogaki, [11]Department of Hematology, Ome Municipal General Hospital, Ome, [12]Department of Medcine, Division of Hematology, Showa University Fujigaoka Hospital, Yokohama, [13]Department of Hematology, Daiyukai General Hospital, Ichinomiya, [14]Department of Hematology, Saiseikai Maebashi Hospital, Maebashi, [15]Department of Hematology, Nippon Medical School, Tokyo, [16]Department of Hematology, Fujioka General Hospital, Fujioka, [17]Department of Hematology, Ehime Prefectural Central Hospital, Matsuyama, [18]Department of Hematology, Sapporo Hokuyu Hospital, Sapporo, [19]Department of Intenal Medicine, Hasuda Hospital, Hasuda, [20]Department of Hematology, Hokkaido University Hospital, [21]Department of Hematology, Tenshi Hospital, Sapporo, [22]Department of Hematology, National Hospital Organization Mito Medical Center, Mito, [23]Dept.of Gastroenterology and Hematology/Clinical oncology, Steel Muroran Memorial Hospital,, Muroran, [24]Department of Biomedical Statistics and Bioinformatics, Kyoto University Graduate School of Medicine, Kyoto, [25]Clinical Research Center, Saga University Hospital, [26]Section of Clinical Cooperation System, Center for Comprehensive Community Medicine, Faculty of Medicine, Saga University, Saga, [27]Tokai Central Hospital, Kakamigahara, [28]Division of Hematology, Respiratory Medicine and Oncology, Department of Internal Medcine, Faculty of Medicine, Saga University, Saga, Japan

Background: Several studies have been reported to show about a half of CML patients who have continuously achieved adequate molecular response to BCR/ABL tyrosine kinase inhibitor (TKI) can achieve therapy-free remission (TFR). In Japan the real time quantitative polymerase chain reaction (PQ-PCR) of BCR/ABL had not been applicable to insurance until April 2015; the TFR has not been well investigated in Japanese clinical setting.
Aims: We conducted a multicenter phase II trial to test the safety and efficacy of discontinuing imatinib after at least 2 years of MR4.0-equivalent (UMIN Clinical Trials Registry UMIN000012472).
Methods: The patients who kept MR4.0 or MR4.0-equivalent (nested reverse transcriptase-polymerase chain reaction (RT-PCR) assay negative or a highly sensitive transcription-mediated amplification (TMA) method assay negative) for at least two years and confirmed MR4.0 at the beginning of the study were enrolled. Molecular recurrence was defined as loss of MR4.0 on 2 successive tests or loss of MR3.0 once. Recurrent patients were immediately treated with dasatinib or other TKIs including imatinib. In this study, rate of recurrence was assessed for patients with at least 12 months of follow up.
Results: Patients were enrolled at 25 participating institutions from January 2014 to May 2015. Among 110 enrolled patients, 99 patients (male 65, female 34, median age 62years) were evaluable. Median time from diagnosis to end of imatinib was 103(29-287) months, imatinib therapy duration 100(28-160) months and preceding MR4.0 or MR4.0 equivalent period 55(24-133) months. Recurrence-free survival rate was 68.6% at 12 months with Kaplan-Meier method. TFR rates of the patients whose time from diagnosis to the end of imatinib was longer than 103 months were significantly better than TFR rates of patients with shorter sick periods by univariate

analysis (p=0.0449).
Summary/Conclusion: This phase 2 study was contrived to be feasible in Japanese clinical setting. The outcome is comparable to other TFR studies.

PS1130

THE TARGET UK STUDY: AN EVALUATION OF TYROSINE KINASE INHIBITOR RESPONSE MONITORING PATTERNS AND REAL-WORLD MOLECULAR RESPONSE RATES IN UK PATIENTS WITH CHRONIC MYELOID LEUKAEMIA

D. Milojkovic[1], R. Clark[2], P. Neelakantan[3], J. Ryan[4], S. Collington[4], N.C. Cross[5], A. Mead[6,*]

[1]Imperial College Healthcare NHS Trust, London, [2]The Royal Liverpool and Broadgreen University Hospitals NHS Trust, Liverpool, [3]Royal Berkshire NHS Foundation Trust, Reading, [4]Novartis Pharmaceuticals UK Limited, Camberley, [5]University of Southampton, Southampton, [6]Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom

Background: Speed and depth of response to tyrosine kinase inhibitor (TKI) treatment are key predictors of patient outcomes in chronic myeloid leukaemia (CML), as reviewed in the latest European evidence-based recommendations for CML management (European LeukemiaNet [ELN] 2013; Baccarani *et al*., 2013). ELN2013 defines molecular responses as "optimal", "warning" and "failure" at specific milestones to guide TKI therapy switch. An early molecular response (EMR; BCR-ABL1IS <10%) at 3 months(m), BCR-ABL1IS <1% at 6m, and major molecular response (MMR; BCR-ABL1IS ≤0.1%) by 12m are key optimal responses to first line (1L) treatment. Furthermore, achievement of sustained deep molecular response (DMR; BCR-ABL1IS ≤0.01%) may give an advantage for successful treatment-free remission (Mahon FX ASH-educational, 2017). Real-world evidence on TKI management and outcomes is however currently lacking in the UK.
Aims: This study aims to evaluate TKI pathways, monitoring patterns and real-world response rates in UK patients with CML against ELN2013 to inform future clinical practice.
Methods: In a retrospective observational study at 21 UK centres; data on Pt characteristics, TKI choice, and molecular responses were collected by medical record review in 257 adult Pts (≥18 years) with chronic phase CML. Eligible Pts were first prescribed a TKI from January 2013 with ≥6m follow-up.

Table 1. Summary of molecular response.*

Molecular response	Overall responses* (n=257)		1st TKI† (n=257)		Switched to 2nd TKI ‡ (n=104)			
	Imatinib as 1L TKI (N=203)	2G TKI as 1L TKI (N=54)	Imatinib (N=203)	2G TKI (N=54)	2G TKI (N=62) ‡	Switched to 2nd TKI for intolerance/ other (N=42) ‡	Switched to 2nd TKI for resistance (N=62) ‡	All switched Pts (N=104) ‡
Available follow-up Median (range) months	26.1 (5.9 - 56.8)	24.9 (6.3 - 56.8)	14.4 (0.3- 56.0)	18.5 (0.5 - 55.3)	15.2 (1.1 - 51.4)	16.1 (2.4 - 49.6)	17.2 (1.1 - 51.4)	16.6 (1.1 - 51.4)
EMR at 3 months (+/- 1 month) in Pts with a BCR-ABL1 at 3m + [n(%)]	88/163 (54%)	29/41 (71%)	87/146 (60%)	28/37 (76%)	49/56 (88%)	25/26 (96%)	33/39 (85%)	58/65 (89%)
MMR (BCR-ABL1 ≤0.1%) by 12 month (+/- 1) in Pts with ≥13m follow-up ¶ [n(%)]	66/169 (39%)	27/47 (57%)	54/116 (47%)	24/35 (69%)	27/46 (59%)	15/22 (68%)	16/32 (50%)	31/54 (57%)
MMR at any time (BCR-ABL1 ≤0.1%) in Pts with ≥13m follow-up † [n(%)]	126/169 (75%)	39/47 (83%)	78/116 (67%)	32/35 (91%)	30/46 (65%)	16/22 (73%)	19/32 (59%)	35/54 (65%)
DMR at any time (BCR-ABL1 ≤0.01%) in Pts with ≥13m follow-up+ [n(%)]	78/169 (46%)	31/47 (66%)	50/116 (43%)	26/35 (74%)	20/46 (43%)	12/22 (55%)	10/32 (31%)	22/54 (41%)

*Where BCR-ABL1IS results were unavailable, comparison with ELN milestones (3/6/12m; with +/-1m window) was made using converted BCR-ABL1(ABL1% results.
† Overall response follow-up for all Pts =months from 1st TKI to data collection/death, 2G TKI, n=50 nilotinib, n=4 dasatinib. ‡ The 1L response follow up = months from 1st TKI to switch to 2nd TKI or data collection/death. ‡For Pts switching to 2nd TKI, follow-up= months from start of 2nd TKI to data collection/death (note outcomes include n=59 who received > 2 TKIs as a result of further TKI switch). ¶ EMR=Early Molecular Response. Patient had "optimal" response if BCR-ABL1 IS ≤10% at 3m (+/- 1m) post TKI start-date. Denominator shows number of Pts who had at least 4m follow-up and an RQPCR at 3m (+/- 1m) after starting that TKI line. +Denominator is number of Pts with at least 13m available follow-up from start of TKI line. Response could be achieved at any time (any line).‡ n=64 nilotinib, n=17 dasatinib, n=10 bosutinib, n=1 ponatinib. (n=12 imatinib 2L not included here). ‡ switched for intolerance (n=30)/switched for another reason (n=13)‡ n=53 on imatinib 1L; n=8 on 2G TKI 1L. ‡ n=64 nilotinib, n=17 dasatinib, n=10 bosutinib, n=1 ponatinib, n=12 imatinib.

Results: Median follow-up was 26m (range 6-57) and median age at start of 1st TKI was 53 years (144 [56%] Pts were male). Imatinib was used 1L in 203/257 Pts and a 2nd generation TKI (2G TKI) in 54/257 (n=50 nilotinib, n=4 dasatinib). In Pts with ≥13m follow-up post 1st TKI, 187/216 (87%) had ≥3 BCR-ABL1 RQ-PCR tests within 13m (208/216 [96%] had ≥1); 174/216 (81%) had a test at 3m, 151/216 (70%) at 6m, and 138/216 (64%) at 12m. In 2013, 2/14 centres used a lab reporting on the international scale versus 17/21 centres in 2017 (Table 1 summarises molecular responses).
During follow-up, 104/257 (40%) Pts switched TKI at least once, including 84/203 (41%) on 1L imatinib; documented reasons were resistance (61/104), intolerance (30/104) and other reasons (13/104). An ELN warning, >1 warning, or failure response was observed prior to switch in 22/104, 6/104 and 31/104 Pts respectively; 21/104 had a kinase domain mutation screen prior to switch. In the Pts switching to 2L following an ELN failure response, an optimal response of MMR was subsequently observed in 14/31 (7/14 with DMR), while 13 Pts remained on 1L following an ELN failure response (3/13 achieved MMR; 0/13 DMR).
Summary/Conclusion: Findings in this real-world UK cohort suggest a higher proportion of Pts treated with 2G TKI (1L or 2L) achieve optimal ELN responses than with imatinib. The observed proportion achieving an optimal response was greater in Pts who switched TKI following an ELN "failure" response than those who remained on 1L therapy, supporting the use of ELN2013 recommendations to guide TKI management. Results also highlight MMR and DMR can be achieved in real world practice even in Pts switching TKI following an ELN failure, warning, or documented resistance. However, the findings also demonstrate ELN2013 recommendations are not universally implemented in UK practice; the timing of PCR monitoring, the performance of mutational analysis, and management of Pts with an ELN warning/failure response frequently deviated from ELN recommendations highlighting areas for improvement for optimal patient management in the UK. Further follow-up is planned to investigate longer-term outcomes in this real-world cohort.

Enzymopathies, membranopathies and other anemias

PS1131

THE OCCURRENCE AND SURVIVAL OF COLD AGGLUTININ DISEASE IN DENMARK

L.C. Bylsma[1], A.G. Ording[2], T. Frøslev[2], F. Mehnert[2], J.P. Fryzek[1,2], J. Morales Arias[3], A. Röth[4], H.T. Sørensen[2], S. Berentsen[5,*]

[1]EpidStat Institute, Ann Arbor, MI, United States, [2]Department of Clinical Epidemiology, Aarhus University, Aarhus, Denmark, [3]Bioverativ, Boston, MA, United States, [4]Department of Haematology, University Hospital, University of Duisburg-Essen, Duisburg, Germany, [5]Department of Research and Innovation, Haugesund Hospital, Haugesund, Norway

Background: Cold agglutinin disease (CAD) is a rare subtype of complement-mediated autoimmune hemolytic anemia. CAD incidence, prevalence, and survival are poorly understood. CAD patients previously have been reported to be at increased risk of thromboembolic events (TE) compared to the general population. A better understanding of the basic epidemiology of this rare disease is needed.
Aims: Danish national population and medical registries were used to calculate the incidence and prevalence of CAD in Denmark, to characterize the CAD patient population, and to examine incidence of TE and survival with a matched comparison cohort from the general population.
Methods: CAD patients diagnosed during 1999-2013 were identified from the Danish National Patient Registry, using the first hospitalization with the ICD-10 discharge code D59.1A. The Registry covers all Danish hospitals. The Danish Civil Registry System was used to match each CAD patient on age, gender, and region of residence to 10 comparison cohort members from the general population who were alive at the time of the patient's diagnosis. Study participants were followed from the patient's diagnosis date through 2013. Incidence rate ratios for TE were calculated from a stratified Cox regression and survival was calculated using the Kaplan-Meier method. Both were adjusted for Charlson Comorbidity Index scores, which encompass cancer, heart disease, diabetes, and other comorbidities.
Results: In 2013, the prevalence of CAD was 1.26 per 100,000 persons in Denmark and the incidence rate was 0.18 per 100,000 person-years. We identified 72 CAD patients and 720 matched general population comparators. Median age at diagnosis was 68.5 years and 58% of the cohort was female. The probability of mortality at 1, 3, and 5 years from diagnosis was greater in the CAD patients when compared to the matched general population cohort. The Charlson comorbidity score-adjusted hazard ratio for mortality among CAD patients was 1.84 (95% CI: 1.10 – 3.06). The hazard ratios for patients with Charlson comorbidity scores 1-2 and 3+ were also increased for CAD patients when compared to the non-CAD cohort. The incidence rate of TE was 52.1 per 1000 person-years in CAD patients compared with 27 per 1000 person-years in the matched comparison cohort. The incidence rate ratio was 2.28 (95% CI: 1.20-4.34) in the crude analysis and became 1.43 (95% CI: 0.71-2.90) when we adjusted for Charlson Comorbidity Score. However, when stratified by Charlson comorbidity score (1-2 and ≥3), both models showed an increased incidence of TE in CAD patients (Charlson score 1-2 aIRR=2.22, 95% CI:1.32-3.72; Charlson score 3+ aIRR=3.43, 95% CI: 1.64-7.15). At 1 year, the cumulative incidence of TE was 4.6% in the CAD cohort and 0.9% in the matched comparisons; at 3 years, 8.4% and 4.8%, and at 5 years, 15.6% and 10.0%, respectively (Figure 1).

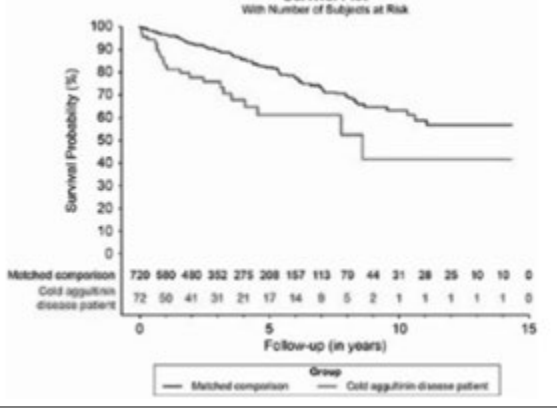

Figure 1. Kaplan-Meier survival curves for patients with cold agglutin disease and general population comparisons. Denmark. 1999-2013.

Summary/Conclusion: This population-based cohort study is the first to compare survival among CAD patients with survival in the general population. An increased rate of mortality and TE was observed among CAD patients, starting within the first year from diagnosis. These results indicate that CAD is a more severe disease than previously thought. Further studies should explore the potential of earlier instituted therapy in reducing mortality and risk of complications.

PS1132

DENSITY, HETEROGENEITY AND DEFORMABILITY OF RED CELLS AS MARKERS OF CLINICAL SEVERITY IN HEREDITARY SPHEROCYTOSIS

R. Huisjes[1], A. Makhro[2], E. Llaudet-Planas[3], L. Hertz[4], P. Petkova-Kirova[4], L.P. Verhagen[1], S. Pignatelli[1], M.A. Rab[1], R. M. Schiffelers[1], E. Seiler[2], W.W. van Solinge[1], J.L. Vives Corrons[3], M. Mañú-Pereira del[3], L. Kaestner[4,5], A. Bogdanova[2], R. van Wijk[1,*]

[1]Department for Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht, Netherlands, [2]Red Blood Cell Research Group, Institute of Veterinary Physiology, Vetsuisse Faculty and the Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland, [3]Red Blood Cell defects and Hematopoietic disorders unit, Josep Carreras Leukaemia Research Institute, Barcelona, Spain, [4]Research Centre for Molecular Imaging and Screening, Saarland University, Homburg, [5]Experimental Physics, Saarland University, Saarbrücken, Germany

Background: Hereditary spherocytosis (HS) originates from defective anchoring of the cytoskeletal network to the transmembrane protein complexes of the red blood cell (RBC). Most frequently HS is caused by mutations in the membrane proteins ankyrin (*ANK1)*, band 3 (*SLC4A1)*, α-spectrin (*SPTA1)* and β-spectrin (*SPTB*). In HS, RBCs are characterized by membrane instability and consequent reduced RBC deformability. Clinically, a large heterogeneity in disease severity is observed among patients. Predictors of clinical severity in HS are however currently lacking.
Aims: To address the large heterogeneity in disease-severity in HS, we explored the correlation between the genotype and specific RBC properties with clinical severity.
Methods: Blood samples from 22 well characterized HS patients were collected after informed consent. Patients were categorized according to their disease severity (mild/moderate/severe, Bolton-Maggs *et al.*, Br. J. Haematol 2012). RBC indices were determined by routine hemocytometry, EMA-binding was determined by flow cytometry and the osmotic fragility test was determined according to standard methods. RBC deformability and hydration status was measured by osmotic gradient ektacytometry on the Lorrca (Mechatronics, the Zwaag, NL). RBC density was further determined by Percoll density gradient separation, intracellular potassium levels and capillary-based measurements of MCV and MCHC. RBC vesiculation was measured using the CytoFLEX flow cytometer after staining of plasma with mouse anti-human CD235a-APC. In addition, we used reticulocyte counts, phosphatidylserine exposure, CD71 abundance, and HbA1c as markers of RBC turnover and clearance.
Results: We found that a more severe clinical picture was statistically overrepresented in patients with genetic defects in ankyrin *(ANK1)* and β-spectrin (*SPTB)*. Further detailed analysis of HS RBCs showed that RBCs from patients with moderate/severe HS have a lower maximum deformability (EI_{max}) and a higher concentration of circulating RBC vesicles when compared to patients with mild HS. In addition, we found that a lower mean RBC density is associated with lower hemoglobin levels in blood. Furthermore, moderate/severe HS is accompanied with increased RBC turn-over and increased clearance as reflected by increased reticulocyte count, increased CD71 and decreased HbA1c and abnormally high intercellular heterogeneity (increased RDW and more cell fractions on the Percoll density gradient).

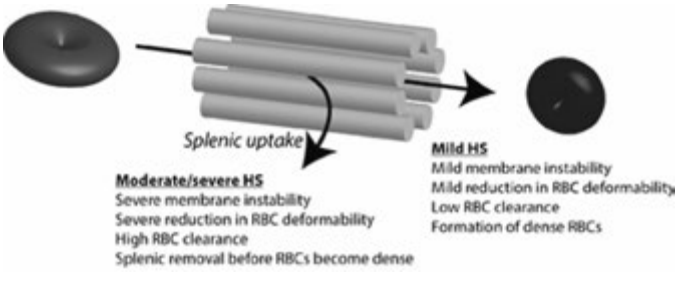

Figure 1.

Summary/Conclusion: In this study we show that disease severity in HS can not be solely attributed to the genotype. We found that RBCs patients with moderate/severe HS show a more prominent decrease in RBC deformability and show low RBC density when compared with patients with mild HS. We hypothesize that the severe RBC membrane instability in these patients facilitate early removal of these RBCs from the circulation by the spleen before their density increases (Figure 1). In contrast, in mild HS a less pronounced reduction in deformability is expected to result in prolonged RBC life-span and, hence, cells are subjected to progressive loss of membrane. RBCs from patients with mild HS thus become more dense before they are taken up by the spleen. Based on our findings, we conclude that RBC membrane loss, heterogeneity and cellular density are strong markers of clinical severity in HS.

PS1133

GAU-PED STUDY: UPDATE ON RESULTS AND LONG-TERM OUTCOME OF PATIENTS WITH GAUCHER DISEASE DIAGNOSED THROUGH A PAEDIATRIC ALGORITHM

W. Morello[1,*], M. Filocamo[2], M. Stroppiano[2], M. Di Rocco[3], N. Tovaglieri[4], G. Russo[5], F. Menni[6], G. Vergine[7], R. Burnelli[8], A. Pession[1]

[1]Pediatric Oncology and Hematology, Sant'Orsola-Malpighi University Hospital, Bologna, [2]Centro di Diagnostica Genetica e Biochimica delle Malattie Metaboliche Gaslini Hospital, [3]Pediatrics, Unit of Rare Diseases - Gaslini Hospital, Genoa, [4]Pediatrics, Niguarda Hospital, Milano, [55]Pediatric Hematology and Oncology Unit, Azienda Policlinico-Vittorio Emanuele Università di Catania, Catania, [6]Pediatrics, Fondazione IRCCS Ca Granda, Milano, [7]Pediatrcis, Azienda Ospedaliera di Rimini, Rimini, [8]Pediatrics, Ospedale di Ferrara, Ferrata, Italy

Background: Gaucher disease (GD) is an autosomal recessive lysosomal storage disease related to the deficient activity of beta-glucocerebrosidase (GBA). Typical features are spleno-hepatomegaly, thrombocytopenia, anemia, growth retardation, bone involvement, gammopathies, increased risk of malignancies and, in some patients, neurological manifestations. Symptoms are non-specific and the diagnosis can be delayed for years or missed. Enzyme replacement therapy (ERT) with recombinant GBA is safe and effective in preventing and reversing many clinical manifestations. However, if the diagnosis is delayed, major complications can be permanent.
Aims: We designed the GAU-PED study to evaluate the prevalence of GD among children referred to the haematology paediatric units and selected according a proposed diagnostic algorithm (*Di Rocco et al., Ped Blood & Cancer 2014*). Here, we report an update on results 30 months after the study start, focusing on the long-term outcome of identified GD patients.
Methods: The GAU-PED study, partially funded by Sanofi Genzyme, involves 53 centers of AIEOP Study Group, the Italian clinical research consortium in paediatric hematology. Patients (1-18 years old) referred for spleno-w/wo hepatomegaly and cytopenia (thrombocytopenia and/or anaemia), where other causes of splenomegaly were excluded, are tested for GBA activity on Dried Blood Spots (DBS). Patients whose GBA activity are below normal on DBS are recalled to confirm GBA deficiency using the gold standard assay. For every tested patient, clinical information are collected. The identified GD patients are followed-up.

Table 1.

Clinical characteristics of the 6 patients with Gaucher disease, identified during the first 30 months of enrolment (N/A, not applicable)

	CT_01	MN_01	GE_01	MP_01	FE_01	RN_01
Sex	F	F	M	M	M	F
Age At Dx (Years)	2	12	13	2	13	14
Symptoms To Dx (Months)	6	50	120	12	6	108
Enrolment To Dx (Months)	6	6	6	3	3	6
Splenomegaly	+	+	+	+	+	Splenectomy (Senegal)
Hepatomegaly	+	+	+	+	-	+
Thrombocytopenia	+	+	+	+/-	-	+/-
Anemia	+	-	+	+	-	+
Growth Retardation	+	-	-	+	-	-
DURATION OF ERT (Months)	22	20	19	14	6	-
Cytopenia after ERT	Resolved	Resolved	Improved	Resolved	N/A	-
Hepatosplenomegaly after ERT	Improved	Improved	Improved	Resolved	Improved	-
Growth improvement	No	N/A	N/A	YES	N/A	-
Adverse events	NO	NO	NO	NO	NO	-

Results: During the first 30 months 74 DBS have been collected from 22 centers. DBS values under 5 pmol/punch^{-1}/h^{-1} were found in 25/74 patients (34%). These patients have been recalled for the conventional enzymatic test. The diagnosis of GD has been confirmed in 6/25 (24%) DBS + patients. In all 6 patients the genetic analysis was consistent with GD. Overall, in the tested population, the prevalence of GD is 8.1% (95% CI, 3.34 - 17.4%) equal to 6/74 enrolled patients. The clinical characteristics of GD patients are summarized in Table 1. Three patients were females and 3 males. The mean age at diagnosis was 9 years (2 - 14 years). Mean time from clinical presentation to diagnosis was 50 months (6-120 months), while the mean time from enrolment to diagnosis was 5 months (3 - 6 months). ERT was started in all GD patients (60 U/kg every 2 weeks). Follow-up data are available for 5/6 patients, since the last patient has just been identified. After a median time from the beginning of ERT of 19 months (6-22 months), cytopenia improved in 4/4 pts, one patient had isolated splenomegaly. 5/5 patients showed a reduction of hepato-splenomegaly, with normalization in 1/5. 1/2 pts with growth delay showed an improvement of growth parameters. No patients had adverse events related to the ERT.
Summary/Conclusion: Our preliminary results support GD screening in a selected population of children with splenomegaly and/or cytopenia, at increased risk for the disease. The proposed algorithm is useful to increase awareness of GD among pediatric hematologists and to shorten the time to diagnosis. Even after a short follow-up, ERT has been able to reverse clinical manifestations and prevent major complications in the identified GD patients. Taking in consideration the long life expectancy of pediatric GD patients, the early diagnosis will have a strong impact on health and quality of life.

PS1134

Abstract withdrawn.

PS1135

LONG-TERM TREATMENT RESPONSE BASED ON SEVERITY OF GAUCHER DISEASE TYPE 1 AT BASELINE IN TREATMENT-NAÏVE ADULT PATIENTS AFTER 8 YEARS OF TREATMENT WITH ORAL ELIGLUSTAT

E. Lukina[1,*], N. Watman[2], M. Dragosky[3], H. Lau[4], E. Avila Arreguin[5], H. Rosenbaum[6], M. Foster[7], S. Gaemers[7], M.J. Peterschmitt[7]

[1]Orphan diseases, National Research Center for Hematology, Moscow, Russian Federation, [2]Hospital Ramos Mejia, [3]IMAI-Research, Buenos Aires, Argentina, [4]New York University School of Medicine, New York, United States, [5]Instituto Mexicano del Seguro Social Hospital de Especialidades, Col. La Raza, Mexico, [6]Rambam Medical Center, Haifa, Israel, [7]Sanofi Genzyme, Cambridge, United States

Background: Gaucher disease type 1 (GD1) is caused by deficient lysosomal enzyme acid β-glucosidase activity, leading to accumulation of glucosylceramide, primarily in macrophages (Gaucher cells). Thrombocytopenia, anemia, hepatosplenomegaly, and skeletal disease are common presenting symptoms. Hematologists frequently diagnose and manage GD1. Eliglustat, an oral substrate reduction therapy, reduces glucosylceramide accumulation by partially inhibiting glucosylceramide synthase and is approved as first-line treatment for adults with GD1 with poor, intermediate, or extensive CYP2D6-metabolizer phenotypes (>90% of patients).
Aims: We report 8-year results of a Phase 2 trial of oral eliglustat (NCT00358150, Sanofi Genzyme) in previously untreated GD1 adults by baseline disease severity (N=26).
Methods: Among the 19 patients who completed the trial, 8-year changes in hematologic and visceral outcomes were evaluated for those with and without moderate/severe baseline disease, defined as platelets <60 x10^9/L (n=8), hemoglobin <10 g/dL for women and <11 g/dL for men (n=7), spleen volume >15 multiples of normal (MN) (n=6), and liver volume >1.25 MN (n=15). We also report individual achievement of long-term Gaucher therapeutic goals (Pastores. *Semin Hematol* 2004), and individual changes in lumbar spine T-score categories.
Results: Trial withdrawals were due to pregnancy (n=3), unrelated adverse events (n=2), related adverse event (n=1), and administrative reasons (n=1). Final mean values for platelets, hemoglobin, spleen, and liver were similar for patients with and without severe baseline disease. All groups attained values within established long-term Gaucher therapeutic goals. Mean platelet counts (x10^9/L) increased by 164% (from 50 to 128) for patients with platelet counts <60 and by 75% (from 83 to 140) for patients with baseline platelet counts ≥60, with an overall increase of 113% (from 69 to 135) for all patients. Mean hemoglobin values in g/dL increased by 3.5

(from 9.6 to 13.1) among patients with moderate to severe baseline anemia and by 1.4 (from 12.3 to 13.7) in patients with no or mild anemia, with an overall increase of 2.2 (from 11.3 to 13.5). Mean spleen volume in MN for patients with severe splenomegaly decreased by 80% (from 26.8 to 5.7) and by 63% (from 12.2 to 4.5) for patents with baseline spleen volume ≤15, with an overall decrease of 69% (from 16.8 to 4.9). Mean liver volumes in MN for patients with baseline liver volume >1.25 decreased by 37% (from 1.81 to 1.12) and by 22% (from 1.08 to 0.85) for patients with baseline liver volumes ≤1.25, with an overall decrease of 34% (from 1.65 to 1.06). Individually, all patients met ≥3 of 4 therapeutic goals after 8 years of eliglustat; all patients showed clinical stability or improvement in all parameters. Baseline lumbar spine T-scores were normal in 5 patients, osteopenic in 8 patients, and osteoporotic in 6 patients. After 7–8 years, T-score categories remained normal or improved in 16 patients and were unchanged in 3 patients, 2 of whom were postmenopausal women. Overall, mean total lumbar spine T-score moved from the osteopenic to the normal range. Eliglustat was well-tolerated; 98% of adverse events were mild or moderate, and 94% were considered unrelated to treatment.

Summary/Conclusion: Treatment-naïve GD1 patients, many with severe baseline disease, experienced clinically meaningful and sustained improvements after 8 years of eliglustat by both aggregate and individual measures. The largest margins of improvement were seen among patients with the most severe baseline disease.

PS1136

COMBINED EMA-BINDING TEST AND OSMOTIC GRADIANT EKTACYTOMETRY SIGNIFICANTLY IMPROVE THE LABORATORY DIAGNOSIS OF HEREDITARY SPHEROCYTOSIS

A. Glenthøj[1,*], J. Petersen[1], A. Nardo-Marino[1], H. Birgens[1]

[1]Department of Hematology, Herlev University Hospital, Herlev, Denmark

Background: Hereditary spherocytosis (HS) is the most common genetic erythrocyte membrane disorder. The laboratory diagnosis of HS has traditionally been based on either osmotic fragility testing (OFT) or flowcytometric measurement of eosin-5-maleimide (EMA) binding to band 3 of the erythrocyte membrane. Osmotic gradiant ektacytometry analyses the erythrocyte deformability as a function of changing osmotic environment under constant shear stress. The test has only recently become widely available for clinical laboratory diagnosis of erythrocyte membrane disorders.
In this study, we combined the EMA-binding test with osmotic gradiant ektacytometry, comparing the results to a comprehensive NGS membranopathy panel.
Aims: To provide a more accurate and cost-effective approach to the laboratory diagnosis of HS and other erythrocyte membrane disorders.
Methods: We included a total of 52 consecutive patients (0 to 86 years old), all referred to our laboratory on the suspicion of a hereditary erythrocyte membrane disorder. In addition to a modified EMA-binding test using commercial rainbow beads as reference, we performed osmotic gradiant ektacytometry using the osmoscan module of the Laser-assisted Optical Rotational Deformability Cell Analyser (LoRRca MaxSis, RR Mechatronics, The Netherlands), as well as a comprehensive NGS membranopathy panel (including a-spectrin, b-spectrin, ankyrin, band 3, protein 4.1, and protein 4.2).
Results: 26 patients had pathogenic or likely pathogenic mutations in a cytoskeleton protein. Individually, osmotic gradiant ektacytometry was highly sensitive in identifying patients with pathogenic mutations and provided an overall accuracy of 0.83 (95% CI: 0.70-0.92), while EMA-binding test delivered a similar accuracy of 0.87 (95% CI: 0.74-0.94). Combination of EMA-binding test and ektacytometry (depicted in Figure 1) was sensitive to all pathogenic mutations and provided an excellent accuracy of 0.92 (95% CI: 0.81-0.98).

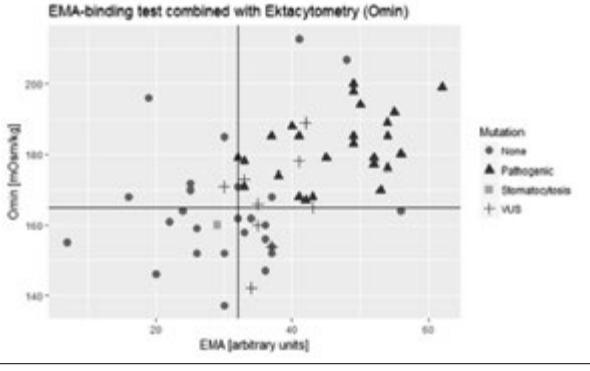

Figure 1.

Summary/Conclusion: Combination of osmotic gradiant ektacytometry and EMA-binding test provided superior precision in diagnostic workup of HS and related disorders.

PS1137

PREVALENCE AND PREDICTORS OF LIVER FIBROSIS EVALUATED BY VIBRATION CONTROLLED TRANSIENT ELASTOGRAPHY (FIBROSCAN®) IN ADULT PATIENTS WITH TYPE 1 GAUCHER DISEASE

F. Nascimbeni[1], E. Cassinerio[2], A. Dalla Salda[1], I. Motta[2], S. Bursi[1], S. Donatiello[3], V. Spina[3], M.D. Cappellini[2,4,*], F. Carubbi[1]

[1]Division of Internal Medicine and Metabolism, Regional Referral Centre for Lysosomal Storage Diseases, OCSAE, AOU Modena, University of Modena and Reggio Emilia, Modena, [2]Department of Medicine and Medical Specialities, Rare Disease Center, Ca' Granda Foundation IRCCS Ospedale Maggiore Policlinico, University of Milan, Milan, Italy, Milan, [3]Radiology Division, Radiology Division, OCSAE , AOU Modena, Modena, [4]Department of Clinical Sciences and Community Health, Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy, Milan, Italy

Background: Gaucher disease (GD), a rare lysosomal storage disorder, results from glucocerebrosidase (GBA) deficiency, which leads to glycosphingolipids accumulation in macrophages. Liver involvement in GD may range from hepatomegaly to cirrhosis, portal hypertension and hepatocellular carcinoma. Since the risk for liver-related complications is mainly driven by fibrosis, its assessment represents an important step in identifying patients at risk for manifestations of advanced liver disease. However, liver fibrosis has rarely been evaluated in previously published cohorts of GD patients. Vibration controlled transient elastography (VCTE) could be a useful tool for non-invasively screening GD patients for the presence of liver fibrosis.
Aims: We aimed at: 1) assessing the prevalence of significant liver fibrosis by measuring liver stiffness (LS) by VCTE (Fibroscan®) in a cohort of adult patients with type 1 GD; 2) identifying the predictors of LS and significant fibrosis among GD-related variables, enzyme replacement therapy (ERT) and metabolic features.
Methods: 37 adult patients with type 1 GD from two Italian referral centers (Modena and Milan) underwent assessment of GD severity (including Disease Severity scoring system (DS3) and Severity Scoring Index (SSI)), evaluation of metabolic features and VCTE for measuring LS. Significant fibrosis was defined as LS ≥7 kPa.
Results: 21 patients (57%) were males, median age was 46 [18-78] years. 33 (89%) carried at least one GBA N370S mutation. GD severity was mainly mild according to DS3 (1.1 [0-6.7]) and SSI (5 [1-16]). 10 patients (27%) were splenectomized. All but 2 patients were on ERT; median ERT length was 98 [0-294] months. 15 patients (41%) were overweight, 14 (38%) had arterial hypertension and 6 (16%) the metabolic syndrome (MetS). Median LS was 4.6 [3-15.1] kPa and 7 patients (19%) had significant fibrosis. LS was significantly correlated with scores (DS3: rho=0.499, p=0.002; SSI: rho=0.385, p=0.019) and biomarkers (ACE: rho=0.516, p=0.020; HDL cholesterol: rho=-0.423, p=0.009) of GD severity. LS was significantly higher in splenectomized patients (6.7 [3.3-11.3] vs. 4.4 [3-15.1] kPa, p=0.021) and in GD patients who had received ERT for less than 24 months (7.5 [4.4-15.1] vs. 4.3 [3.0-11.3] kPa, p=0.002). Consistently, patients with significant fibrosis presented statistically significant higher values of DS3 and SSI, higher levels of ACE and lower levels of HDL cholesterol, and were more often splenectomized than those without significant fibrosis. Length of ERT was significantly lower in GD patients with significant fibrosis than in those without. In the subgroup of 29 patients who were on stable ERT for at least 24 months, further to splenectomy status (p=0.009), moderate-marked GD according to DS3 (p=0.004) and SSI (p<0.001), non-N370S genotypes (p<0.001), also diastolic blood pressure (rho=0.424, p=0.022), BMI (rho=0.419, p=0.024) and the number of MetS components (rho=0.417, p=0.025) emerged as factors significantly associated with LS and/or significant fibrosis.
Summary/Conclusion: Significant liver fibrosis is present in a non-negligible proportion of adult type 1 GD patients. Splenectomy status, GD severity and GBA non-N370S genotypes were major GD-related predictors of liver fibrosis. Length of ERT was inversely correlated with liver disease in GD patients, suggesting a beneficial effect of ERT on liver fibrosis. However, GD patients on stable ERT should be monitored for metabolic complications, since MetS features may enhance liver disease progression despite optimal GD control.

PS1138

PREVALENCE AND CLINICAL SIGNIFICANCE OF SMALL PNH CLONES IN PATIENTS WITH PRIMARY AUTOIMMUNE HEMOLYTIC ANEMIA

B. Fattizzo[1,*], A. Zaninoni[2], R. Pasquale[1], L. Cro[3], A. Dunlop[4], A. Cortelezzi[5], A. Kulasekararaj[4], W. Barcellini[6]

[1]Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and University of Milan, [2]Hematology, [3]Cytofluorimetric Service, Central Laboratory, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan, Milan, Italy, [4]Hematology, King's College Hospital, London, United Kingdom, [5]Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and University of Milan, [6]Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan, Milan, Italy

Background: Autoimmune hemolytic anemia (AIHA) is caused by autoantibodies directed against autologous erythrocytes, diagnosed by the direct antiglobulin test (DAT), and classified as warm (IgG+ or IgG and C+), cold (C+), mixed (IgG+high titer C+), and atypical (DAT-, IgM warm, and IgA+). Recently, autoimmunity against marrow precursors, increased presence of fibrosis/dyserythropoiesis, and possible evolution to bone marrow failure (BMF) have been reported and correlated with poor outcome. PNH clone is a typical finding in up to 20-40% of patients with BMF and MDS, and its presence correlates with favorable prognosis and predicts response to immunesuppression.
Aims: To evaluate the prevalence of PNH clones in patients with primary AIHA, and assess its prognostic/predictive significance.
Methods: AIHA patients followed at two tertiary hematology centers in UK and Italy were followed-up from December 2007 until February 2018. The PNH clone was assessed at the time of AIHA diagnosis by FLAER flow-cytometry technique. Clinical characteristics, including therapy lines and marrow features, were also collected. Continuous and categorical variables were assessed by Student's t-test and chi-square or Fisher test, respectively. Kaplan Meyer method was used to evaluate and compare relapse free survival (RFS) and overall survival (OS).

Table 1.

Clinical characteristics	All patients N=35	PNH pos N=12	PNH neg N=23
Age yrs, median (range)	48 (5-78)	46 (5-77)	54 (21-78)
M(%) F(%)	18 (51) 17 (49)	6 (50) 6 (50)	12 (52) 11 (48)
WAIHA, N(%)	19 (54)	5 (41.9)	14 (61)
WAIHA IgG+C, N(%)	3 (8)	1 (8)	2 (9)
CAD, N(%)	6 (17)	2 (16.7)	4 (17)
Mixed, N(%)	5 (14)	2 (16.7)	3 (13)
Atypical, N(%)	2 (6)	2 (16.7)	0 (0)
Hb g/L, median (range)	75 (35-137)	74 (43-137)	83 (35-129)
LDH U/L, median (range)	489 (174-1958)	730 (476-1958)	372 (174-1867)
Ret x10^9/L, median (range)	137 (26-418)	133 (38-258)	137 (26-369)
BMRI, median (range)	72 (18-305)	85 (18-161)	70 (18-253)
Bone marrow evaluation	N=28	N=7	N=21
Cellularity %, median (range)	60 (15-100)	70 (15-70)	60 (20-100)
Lymphoid infiltrate%, median (range)	4 (0-29)	1 (0-6)	5 (0-29)*
Hypercellularity, N (%)	11 (39)	2 (28)	9 (43)
Fibrosis MF1, N (%)	19 (69)	4 (57)	15 (71)
Dyserythropoiesis, N (%)	13 (46)	1 (14)	12 (57)*
Treatment and outcome	N=35	N=12	N=23
First therapy line, N(%)	33 (94)	11 (92)	22 (96)
Second therapy line, N(%)	18 (51)	7 (58)	12 (52)
Third therapy line, N(%)	9 (26)	1 (8)	8 (35)
RFS months, median (range)	39.6 (0.8-194)	27.7 (2-112)	39.6 (2-194)
Evans, N (%)	7 (20)	4 (33)	3 (13)
Infections, N (%)	7 (20)	2 (17)	5 (21)
Thrombosis, N (%)	6 (17)	3 (25)	3 (13)
Acute renal failure, N (%)	5 (14)	0 (0)	5 (21)

*p≤0.05

Results: Table 1 shows clinical and morphologic characteristics of the 35 patients enrolled, altogether and divided according to PNH clone positivity. Twelve patients (34%) showed a positive clone, with a median size of 0.2% on granulocytes (range 0.1-16%). Considering AIHA type, 2 atypical forms (DAT negative) were PNH+, warm AIHA mainly PNH-, and cold and mixed forms equally distributed. PNH+ cases were more anemic, although not significantly, and displayed higher hemolytic pattern compared to PNH- ones, both considering absolute LDH levels and categorization (92% of cases versus 47% showing LDH levels above 1.5x ULN). As expected, Hb levels positively correlated with bone marrow responsiveness index (r=0.40, p=0.008) and negatively with LDH levels (r=-0.31, p=0.03). Considering marrow characteristics, PNH+ patients showed lower prevalence of hypercellularity (28 vs 43%), MF-1 fibrosis (57 vs 71%, p=0.07), dyserythropoiesis (14 vs 57%, p=0.01), and displayed a smaller lymphoid infiltrate (p=0.05), compared to PNH- ones. Lymphoid infiltrate was CD3+ in 1 PNH+ patient, CD20+ in 2 PNH- cases, and mixed in all the others. Regarding therapy, PNH+ and PNH- cases received 1st and 2nd lines (steroids, rituximab, immunesuppressors and splenectomy) with equal rate. However, the former less frequently required a 3rd treatment course (8% versus 35%, p=0.09) and displayed a significantly higher response to rituximab therapy (100% vs 70%, p=0.04). AIHA related complications occurred in 18 cases, with thrombosis observed more commonly in PNH+ patients (25% vs 13%), acute renal failure in PNH- only (21%), and infections equally in both groups (17 and 21%). Finally, 7 patients died during the observation period, mostly because of infections (N=5). The small number of events precluded any statistical analysis.
Summary/Conclusion: Here we firstly report the prevalence of a small PNH clone in about 30% of primary AIHA patients. PNH+ cases show an increased hemolytic pattern and a better response to first and second therapy lines, suggesting a prevalent autoimmune habitus.

PS1139

COMBINATION TREATMENT OF RITUXIMAB, CYCLOPHOSPHAMIDE, AND DEXAMETHASONE FOR WARM AUTOIMMUNE HEMOLYTIC ANEMIA

C. Piatek[1,*], H. Bocian[2], C. O'Connell[1], I. Weitz[1], H. Liebman[1]

[1]Hematology, [2]Medicine, University of Southern California, Los Angeles, United States

Background: Warm autoimmune hemolytic anemia (WAIHA) is an uncommon disorder of autoantibody-mediated destruction of red blood cells. Because of limited clinical trials, the management of WAIHA is largely based on expert opinion. The standard first and second line therapies are corticosteroids and rituximab, respectively. Although both have high initial response rates, low long-term response rates are often reported. Splenectomy may also be pursued as secondary line therapy; however, response rates are low in patients with secondary WAIHA. The combined regimen of rituximab, cyclophosphamide and dexamethasone (RCD) has been reported to be highly effective in the treatment of AIHA with chronic lymphocytic leukemia (CLL) in the upfront and relapsed setting.
Aims: To evaluate the efficacy of RCD therapy in patients with primary and secondary WAIHA without CLL.
Methods: Between 2009 and 2017, patients with primary and secondary WAIHA without CLL were treated with RCD at Los Angeles County-University of Southern California (LAC-USC) Medical Center and USC Norris Cancer Center. RCD was administered as follows: rituximab 375 mg/m2 IV day (D) 1, cyclophosphamide 750 mg/m^2 IV D1 or 2, and dexamethasone 12 mg orally or IV D1-7. Treatment cycles were repeated at 3-to-4-week intervals at the discretion of the treating physician. Maintenance therapy was defined as immunosuppressive therapy continued or started at the discretion of the treating physician ≤6 weeks after completion of RCD in the patients who achieved a PR or CR. The primary objective was to evaluate the efficacy of RCD therapy in terms of overall response (OR), combining CR and PR. A CR was defined as hemoglobin (Hb) ≥ 12 g/dL without transfusion. A PR was defined as Hb 10-11.9 g/dL or ≥ 2 g/dL increase in Hb without transfusion. Additional objectives were to evaluate the number of cycles until best response and the duration of a sustained response (SR). For patients not receiving maintenance therapy, a SR was defined as Hb ≥ 10 g/dL in the absence of any treatment. For patients receiving maintenance therapy, a SR was defined as Hb ≥ 10 g/dL in the absence of any treatment changes. The duration of SR was calculated from the date of last RCD cycle to the date that the patient no longer met the criteria for SR or date of last follow-up if the patient remained in a SR.
Results: 23 patients with WAIHA were treated with RCD (9 with primary WAIHA and 14 with secondary WAIHA) for a median number of 4 cycles (range 2-6). RCD was given for relapsed/refractory disease in 16 patients. The median pre-treatment Hb was 9.2 g/dL (4.3-12.2). The median best Hb achieved with RCD was 12.5 g/dL (10.6-15.1) with a median Hb

increase of 4.2 g/dL (0.4-9.8). The OR was 96% (16 with CR, 6 with PR) with a median number of 2 cycles given until best Hb response. Among the patients who achieved a CR, 11 had secondary WAIHA and 12 had received prior treatment. Among those who achieved a PR, 3 had secondary WAIHA and 2 had received prior treatment. 11 patients received maintenance immunosuppression (prednisone, azathioprine, mycophenolate, sirolimus, and cyclosporine) after completing RCD with a median duration of response of 6 months with responses ongoing in 7 patients. For the 12 patients not on maintenance immunosuppression, the median duration of response was 8 months with responses ongoing in 4 patients.
Summary/Conclusion: RCD had a high OR in the upfront and relapsed/refractory treatment of primary and secondary WAIHA with an acceptable toxicity profile.

PS1140

THE ESTABLISHMENT OF SD RAT MODEL AND HISTOPATHOLOGICAL STUDY OF IMMUNE HEMOLYTIC DISEASE INDUCED BY ANTIBODY

S. Yan[1,*], L. Li[1], Z. Li[1]

[1]Shanghai Jiao Tong University Affiliated Sixth People's Hospital, shanghai, China

Background: At present, the animal model of immune hemolytic preparation mainly in two ways, respectively for Playfair –MarshallClarke's autoimmune hemolytic anemia mouse model in 1973 and spontaneous immune hemolytic disease in NZB mice, The former model establishes need a longer period of preparation, with low success rate , the latter model is also expensive in price and not easy to get. Therefore, both of the two animal models were not widely used. In addition, subcutaneous injection of phenylhydrazine or other chemical agents can also establish the animal model of hemolytic anemia, but its further research is limited in Pathogenesis and pathological process and drug efficacy study of many immune factors inducing the clinical hemolytic disease.
Aims: To establish an immune hemolytic SD rat model, to provide an experimental platform for its pathogenesis, pathological process and the evaluation of drug effect.
Methods: The red blood cells of SD rats were isolated and intraperitoneally injected into balb/c mice to induce the production of anti SD rat red blood cell antibody serum.Twenty SD rats were randomly divided into two groups. The model group:were injected with 0.1 ml antiserum via tail vein ;the control group: were injected with 0.1 ml saline via tail vein.The clinical symptoms, hemolysis related indexes and histopathological changes of the main organs were observed in both two groups after injection.
Results: After the injection of antiserum,the model group of SD rat developed shortness of breath, fatigue and weakness, weakened ability of ingestion and water intake, skin and mucosal jaundice and hemoglobin urine visible to the naked eyes.Four days after the injection, the weight of SD rats in the model group was significantly lower than that in the control group ($p<0.01$), and the coefficients of liver and spleen increased significantly ($p<0.01$); The levels of blood WBC, MCV, MCH, DBIL, DBIL/TBIL and FHb all increased significantly, and RBC, Hb, HCT, MCHC and PLT levels decreased significantly, which were all statistically significant compared with the control group ($p<0.01$). In the model group of SD rat, hemolytic pathological changes were observed in liver, spleen, kidney, lung and small intestine, and erythroid proliferation was observed in bone marrow smears.
Summary/Conclusion: The SD rat model of immune hemolytic disease can be successfully established by using balb/c mouse anti-SD rat erythrocyte antibody serum, and the pathological changes of liver, spleen, kidney, intestine, bone marrow and other important organs can be observed.It has the characteristics of a high success rate, good repeatability and low preparation cost. Therefore, the establishment of the model is helpful to further study the pathogenesis of immune hemolytic disease and the efficacy of drugs, which have important clinical significance.

PS1141

ALPS AND GAUCHER DISEASE. A CLINICAL AND IMMUNOLOGICAL OVERLAP

M. Miano[1,*], E. Palmisani[1], F. Fioredda[1], P. Terranova[2], E. Cappelli[2], T. Lanza[2], R. Maggiore[1], A. Pezzulla[1], C. Dufour[1], C. Micalizzi[1], A. Beccaria[1], A. Madeo[1], M. Di Rocco[1]

[1]Haematology Unit, [2]Haematology Lab Unit, IRCCS Istituto Giannina Gaslini, Genova, Italy

Background: Gaucher Disease (GD) is anautosomal recessive lysosomal disorder secondary to a mutation in the GBA1 gene that causes a defective function of the catabolic enzyme β-glucocerebrosidase (GBA). The impaired function of this enzyme leadsto a progressive accumulation of its substrate-glucocerebroside (GC) -in various organs in particular in mononuclear phagocite system. Bone infiltration, hepatosplenomegaly and cytopenia represent the most common features of the disease. Since clinical manifestation can be subdolous, in some cases, phenotype can overlap with Autoimmune Lymphoproliferative Sindrome. Nonetheless, GD patients also show hyper-inflammatory features-secondary to machrophages engorgement and actviation-and animmune-dysregulation involving B , T and NK cells. However, few data are available on specific immunity pattern in these patients.
Aims: to evaluate immune-phenotype and other ALPS parameters in a cohort of patients with GD
Methods: We evaluated the test of apoptosis and the immunophenotypic and serological features usually typical of ALPS patients (Double Negative T-cells, TCR alfa/beta B220, B-memory cells, T-regs/HLA-DR ratio, IL-10, IL-18,) in a cohort of patients with GD followed-up at Istituto Giannina Gaslini.
Results: 35 patients (28 in treatment, 5 not) were studied. DNTs and TCR alfa/beta B220+ resulted to be >1.5% of T-lymphocytes and > 60% in 6/32 (19%) and in 7/32 (22%), respectively. B-memory cells and T-regs/HLA-DR ratio were <15% and <1 in 11/32 patients (34%). 3/32 evaluable (9%) had all these parameters concomitantly alterated. 4/19 (21%) evaluable patients were resistant to apoposis. IL-18 was pathological in 26/29 (89%) patients All patients had normal levels of IL-10 and sFAS.
Summary/Conclusion: This study shows that some patients with GD may present an immune-dysregulation pattern that can overlap with ALPS features. Therefore, the differential diagnosis of GD should be taken into consideration by clinicians during diagnostic work-up of patients with an ALPS-like phenotype

PS1142

ORGAN INVOLVEMENT IN RARE HEREDITARY HEMOLYTIC ANEMIA

S. Van Straaten[1,2,*], J. Verhoeven[1,2], S. Hagens[1,2], R. Schutgens[2], W. van Solinge[1], R. van Wijk[1], E. J. van Beers[2]

[1]Laboratory of Clinical Chemistry & Haematology, [2]Van Creveldkliniek, University Medical Center Utrecht, Utrecht, Netherlands

Background: In sickle cell anemia and β-thalassemia the occurrence of disease related organ damage is a well-known important determinant of morbidity and prognosis. However, for other forms of rare hereditary hemolytic anemia, not much is known about disease-related organ involvement, especially for non-transfusion dependent patients.
Aims: The goal of this study is to analyze occurrence and severity of organ involvement in several rare forms of hereditary hemolytic anemia.
Methods: This is a cross sectional, observational study on 97 patients with rare hereditary hemolytic anemia. It encompasses (early markers of future) organ damage, markers of altered organ function (e.g. endocrine changes) and other non-hematological symptoms known to influence morbidity and prognosis in chronically ill patients (e.g. inflammation). After informed consent patients were divided into four disease categories: sickle cell disease (SCD), other hemoglobin disorders (e.g. β-thalassemia), red cell enzyme disorders (e.g. pyruvate kinase deficiency) and red cell membrane disorders (e.g. hereditary spherocytosis). Data was collected through chart review. Clinical parameters of organ involvement were scored according to diagnosis by the treating physician. (Bio)marker based organ involvement was scored according to international standards. When the medical history of a patient was not available, parameters were regarded as missing. Only patients with more than 12 scorings items available were included in the analysis.
Results: At inclusion patients had a median age of 40 years (range 18-84) and a median Hb of 11,3 g/dL (range 6,4-16,4). 47% was female. Of the 90 patients with more than 12 scorings items available, 80 (88%) showed a form of organ involvement. Most reported organ involvement was liver iron overload (LIC>3 mg/g DW) as reported in 43/55 (78%) of patients. Most importantly, organ involvement was not limited to transfusion dependent patients: 29/34 (85%) patients who had never received a red cell transfusion did show some form of organ involvement. These patients did not have a significantly different median Hb or reticulocyte count compared to never transfused patients without organ involvement (SCD patients were excluded from this analysis) (Table 1).
Summary/Conclusion: This study shows that organ involvement is not limited to patients with SCD or β-thalassemia, but also occurs in other forms of hereditary hemolytic anemia. Importantly, organ involvement also occurs in patients who never received blood transfusion(s). This study also shows that often parameters indicative of organ involvement were not measured or not mentioned in patients' file. This indicates that screening for organ involvement does not structurally occur in patients with hereditary hemolytic anemia. The development of

better screening guidelines is therefore important to identify organ involvement in patients with hereditary hemolytic anemia at an early stage.

Table 1.

		All patients					Never transfused (SCD excluded)
		Sickle cell disease	Other hemoglobin disorders	Red cell enzyme disorders	Membrane/ hydration disorders	P value	
Cardio vascular	T2*<20ms	1/8(13%)	2/13(15%)	0/20(0%)	0/7(0%)	,304	0/13(0%)
	TRV≥2,5ms	0/12(0%)	5/14(36%)	2/12(17%)	0/4(0%)	,094	0/7(0%)
	BNP>30pmol/L	2/15(13%)	2/14(14%	0/22(0%)	2/11(18%)	,147	**1/17(6%)**
	Heart failure	0/15(0%)	1/17(6%)	0/29(0%)	0/28(0%)	,360	0/31(0%)
	Arrhythmia	1/15(7%)	2/17(12%)	0/29(0%)	2/28(7%)	,260	**2/31(7%)**
	Hypertension	1/15(7%)	1/17(6%)	2/29(7%)	2/28(7%)	1,000	**5/31(16%)**
	Priapism	4/15(27%)	0/17(0%)	0/29(0%)	0/28(0%)	,001*	0/31(0%)
	Thrombotic event	4/15(27%)	4/17(24%)	3/29(10%)	4/28(14%)	,439	**1/31(3,7%)**
Abdominal	LIC>3mgFE/gDW	6/8(75%)	10/12(83%)	17/24(71%)	10/11(91%)	,644	**11/17(65%)**
	Microalbumine/ Creat ratio>3,5	5/15(33%)	5/12(42%)	7/18(39%)	0/8(0%)	,169	**2/12(17%)**
	GFR<60 (CG)	1/13(8%)	0/14(0%)	0/26(0%)	0/23(0%)	,360	0/25(0%)
	GFR<60 (CKD-epi)	0/9(0%)	0/6(0%)	1/13(8%)	1/13(8%)	1,000	**2/27(7%)**
	Gallstones	8/14(57%)	3/17(18%)	16/27(59%)	11/28(39%)	,033*	**11/31(36%)**
	Cholecystectomy	4/15(27%)	5/17(29%)	23/32(72%)	8/29(28%)	,001*	**12/31(39%)**
	Liver cirrhosis	0/14(0%)	1/16(6%)	0/29(0%)	0/28(0%)	,345	0/31(0%)
Neurologic	CNS hemorrhage	1/15(7%)	1/17(6%)	0/29(0%)	0/28(0%)	,227	0/31(0%)
	Seizures	1/15(7%)	0/17(0%)	0/29(0%)	0/28(0%)	,169	0/31(0%)
Musculo-skeletal/ dermatologic	T score≤ -2,5SD	1/13(8%)	7/11(64%)	2/13(15%)	1/5(20%)	,015*	**2/10(20%)**
	Hip or vertebra fracture	0/15(0%)	1/16(6%)	0/29(0%)	1/28(4%)	,667	**1/31(3%)**
	Leg ulceration	2/15(13%)	2/17(12%)	2/29(7%)	0/28(0%)	,157	0/31(0%)
Endocrine	LH<1 in males	0/4(0%)	3/8(38%)	0/8(0%)	0/4(0%)	,178	0/6(0%)
	FSH<1 in males	0/3(0%)	2/8(25%)	0/7(0%)	0/2(0%)	,705	0/5(0%)
	Testosteron<0,8 in females /<11 in males	1/6(17%)	2/11(18%)	1/7(14%)	1/4(25%)	1,000	**1/6(17%)**
	Vitamin D<50	10/15(67%)	9/14(64%)	8/16(50%)	5/9(56%)	,796	**7/12(58)**
	Thyroid disease	1/13(8%)	0/15(0%)	0/18(0%)	0/17(0%)	,206	0/19(0%)
	Diabetes (all types)	1/15(7%)	1/17(16%)	0/29(0%)	1/28(4%)	,054	**1/31(3%)**
	IGF>2SD from normal	2/9(22%)	4/12(33%)	6/14(43%)	1/3(33%)	,789	**2/8(25%)**
Inflammatory	CRP>10mg/L	3/15(20%)	1/17(6%)	1/26(4%)	1/27(4%)	,238	**1/25(4%)**
Audiovisual	Hearing loss >15dB	0/2(0%)	1/8(13%)	0/2(0%)	0/0 (na)	1,000	0/0(na)
	Retinopathy	6/13(46%)	0/10(0%)	0/2(0%)	0/10(0%)	,044*	0/10(%)
Other	Fibromyalgia	0/15(0%)	0/17(0%)	1/29(3%)	2/28(7%)	,671	**1/31(3%)**
	Depression	2/15(13%)	2/17(12%)	2/29(7%)	0/28(0%)	,157	**1/31(3%)**

CG=Cockcroft& Gault. Test performed: Fisher Freeman Halton

PS1143

SUCCESSFUL TREATMENT OF MEGALOBLASTIC ANEMIA WITH BONE MARROW MYELODYSPLASIA IN LESCH-NYHAN SYNDROME

M. Machaczka[1,2,*], G. Kleinotiene[3], M. Klimkowska[4]

[1]Medical Faculty, University of Rzeszow, Rzeszow, Poland, [2]Hematology Center Karolinska, Karolinska University Hospital Huddinge, Stockholm, Sweden, [3]Cente of Hematology and Oncology, Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Clinics, Vilnius, Lithuania, [4]Department of Clinical Pathology and Cytology, Karolinska University Hospital Huddinge, Stockholm, Sweden

Background: Lesch-Nyhan syndrome (LNS) is a very rare (incidence 1-9 patients/1 million people), inherited disease of purine metabolism. Deficient activity of enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT) causes dysfunction of nervous system and kidneys. LNS is the most severe form of HPRT deficiency, with deeply decreased or complete lack of HPRT activity. Children with LNS usually present no symptoms at birth but 3-6 months later a delay in psychomotor development is revealed. Patients develop hyperuricemia, impaired kidney function, articular and nervous system symptoms, including cognitive impairment and behavioral problems such as self-mutilation. Megaloblastic anemia may also occur, which is not a constant feature of LNS but develops only in some patients. Anemia is cobalamin (vitamin B12) and folic acid (FA) resistant, however, it is believed that increased consumption of these vitamins occurs in LNS patients.

Aims: We present a case of a young man with LNS who developed severe megaloblastic anemia which has been successfully treated with adenine.

Methods: A 25-year-old man with LNS developed megaloblastic anemia (Hb 96 g/L, MCV 121 fL) refractory to 3 months' treatment with vitamin B12 and FA. Other lab values showed: WBC 6.3×10^9/L (ref.: 3.5–8.8), PLT 139×10^9/L (ref.: 145–348), cobalamin 560 pmol/L (ref.: 150–650), folic acid >1200 nmol/L (ref.: 330–870), S-methylmalonate 0,26 µmol/L (ref.: <0,4), P-homocysteine 6,1 µmol/L (ref.: 5–15). Bone marrow (BM) examination revealed a slightly reduced BM cellularity (hypocellular) with 1% of blasts, 5% of promyelocytes/promonocytes, and distinct maturation abnormalities in all hematopoietic cell lines, with pictures of multilineage dysplasia resembling a myelodysplastic syndrome (Figure 1 - BM smear: myeloid cell with ring-shaped nucleus, erythroid precursors with multiple or irregular nuclei, stippling).

Results: Thanks to pharmaceutical company Apoteket AB, capsules with adenine (formerly called as a vitamin B4) 300 mg per capsule were prepared for the patient and administered t.i.d. (900 mg/day). Oral adenine treatment rapidly cured the patient's megaloblastic anemia within one month (Hb concentration increased to 132 g/L and MCV decreased to 95 fL). Moreover, BM examination performed 6 months after onset of adenine therapy showed significantly improved cytomorphology of hematopoietic cells, primarily concerning erythropoiesis. Other hematopoietic cell lines still showed dysplastic features but to a significantly lesser extent. Megaloblastic anemia has been permanently cured in the patient thanks to long-term oral adenine administration. No adverse reactions have been observed. The effect of adenine treatment remains stable for 7 years now.

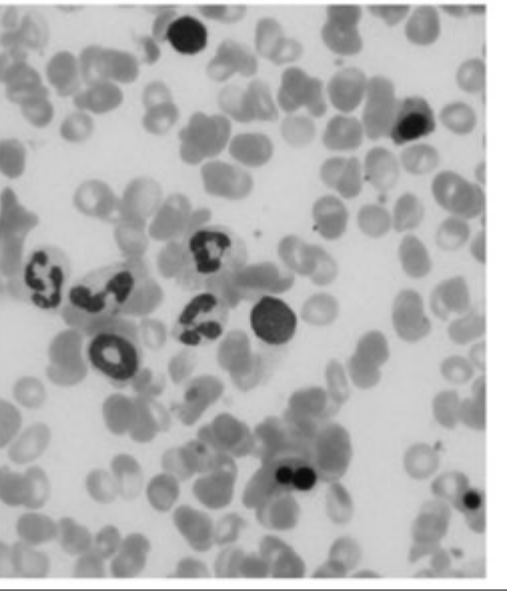

Figure 1.

Summary/Conclusion: Long-term oral administration of adenine, previously known as vitamin B4, has a potential to cure megaloblastic anemia in patients with LNS. Moreover, adenine significantly improves multilineage dysplasia in BM underlying the diagnosis of megaloblastic anemia. Protracted therapy with adenine is well tolerated, with no adverse events in the described patient under 7 years of treatment.

Gene therapy, cellular immunotherapy and vaccination – Biology & Translational Research

PS1144

CORD BLOOD DERIVED GAMMA-DELTA T CELLS CAN BE EXPONENTIALLY EXPANDED *IN VITRO* TO INDUCE POTENT CYTOTOXICITY AGAINST HUMAN ACUTE MYELOID LEUKEMIA CELLS

A. Cheung[1,2,*], K. W. Tan[3], D. Guo[1], A. Lau[3], X. Fan[3], Z.Y. Poon[1], Y.C. Linn[1], W. Hwang[1], S. Li[2], Y.T. Goh[1]

[1]Haematology, Singapore General Hospital, [2]Cancer and Stem Cell Biology, Duke-NUS Medical School, [3]Clinical Translational Research, Singapore General Hospital, Singapore, Singapore

Background: Despite the unsurpassed efficacy of standard chemotherapy for acute myeloid leukemia (AML) patients in the past decades, the overall survival (OS) remains dismal due to the high rate of disease relapse and treatment intolerance. There is thus an urging need for more effective treatment alternatives.
Aims: Early seminal studies had observed strong association of an increased number of donor derived γδ T cells (specifically the Vδ1+ subtype) in AML patients receiving allogeneic hematopoietic stem cell transplant (HSCT) with improved OS. As such, we hypothesize that these allogeneic γδ T cells may exhibit potent leukemia specific cytotoxicity and serve as an effective treatment for AML.
Methods: Given the rapid availability and widespread use of cord blood (CB) as an alternative for allogeneic HSCT, we had explored the potential of expanding CB derived γδ T cells *in vitro*. In our cohort of CB, 0.17±0.04% of mononuclear cells are γδ T cells. Contrary to adult mobilized peripheral blood, majority of the CB derived γδ T cells are of $V\delta1^+$ and $V\delta1^-\delta2^-$ subtype (93.6±2.4%). Importantly, our data showed that in keeping with the neonatal origin of CB, γδ T cells in CB display a predominately central memory (T_{cm}) and naïve (T_n) phenotype, making these cells favorable for extensive *in vitro* expansion. Using our in-house optimized *in vitro* expansion protocol, we were able to achieve up to 10^5-fold expansion of the starting γδ T cells over a period of 21 days.
Results: The expanded γδ T cells exhibit potent *in vitro* cytotoxicity against a range of human AML cell lines in a dose dependent manner, yet display minimal cytotoxicity against $CD34^+$ cells isolated from allogeneic CB samples. Notably, we found that NK receptors (NKR) such as NKG2D, NKp30 and NKp46, were minimally expressed (<10%) in the expanded γδ T cells. Consistent with this, when specific TCRγδ and NKR receptor-blocking antibodies were added to the mixture of γδ T cells and AML cell line in standard ^{51}Cr-release assays, no significant decrease in γδ T cell cytotoxicity were observed. These data suggest that the expanded γδ T cell cytotoxicity is likely to be mediated via TCRγδ and NKR independent signaling pathways. Intravenous infusion of the expanded γδ T cells into NOD/SCID/IL2R$\gamma^{-/-}$ (NSG) mice that had been xenografted with primary AML patient sample led to the detection of these cells across major hematopoietic tissues including the bone marrow (BM), spleen and liver, as well as in the circulation. Moreover, the homing potential of the infused γδ T cells significantly correlated with the amount of AML cells present in the tissues, demonstrating that these cells are chemotactic towards primary AML cells *in vivo*. Such *in vivo* γδ T cell treatment resulted in significant reduction of BM leukemic burden compared to untreated control. More importantly, BM cells from γδ T cells-treated mice had a significantly reduced ability to regenerate leukemia upon secondary transplantation, strongly suggesting that the infused γδ T cells also targets the primitive leukemia initiating cells.
Summary/Conclusion: In summary, our data demonstrates that *in vitro* expanded CB derived γδ T cells show potent AML-specific cytotoxicity both *in vitro* and *in vivo*, making it a promising alternative cell source for immunotherapy. Further investigations to enhance the mechanistic understanding would be needed to seed for future clinical translation.

PS1145

ENHANCED BONE MARROW HOMING OF NATURAL KILLER CELLS FOLLOWING MRNA TRANSFECTION WITH GAIN-OF-FUNCTION CXCR4

E. Levy[1,2,*], R. Reger[1], R. Childs*[1], M. Carlsten*[1,3]

[1]Hematology, National Institutes of Health, Bethesda MD, [2]Molecular Medicine, George Washington University, Washington DC, United States, [3]Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden

Background: Natural killer (NK) cells have proven to be powerful effectors against hematological tumor targets in pre-clinical studies. In subsets of patients with hematological malignancies, NK cell infusions can induce remission. However, the therapeutic potential of intravenously (IV) transferred NK cells has thus far been limited, perhaps in part the consequence of their suboptimal bone marrow (BM) homing capacity. As most hematological malignancies arise and reside in BM compartments, developing an NK cell product that has an improved ability to home to the BM may lead to improved clinical response. As hematopoietic stem cells (HSCs) are known to engraft in BM compartments via CXCR4/SDF-1a signaling, we explored the potential of NK cells genetically engineered to express a naturally occurring gain-of-function CXCR4 (CXCR4-R334X) receptor to enhance their BM homing capacity.
Aims: The aim of this study was to generate a modified NK cell product with improved BM homing capacity compared to unmodified NK cells.
Methods: Human NK cells were isolated from healthy donor buffy coats and expanded for 14 days with irradiated EBV-LCL feeder cells in IL-2 containing media. Electroporation of NK cells with mRNA coding for either the wild-type CXCR4 (CXCR4-WT) or the gain-of-function CXCR4-R334X receptor, was performed using the cGMP-compliant MaxCyte GT instrument. NK cell chemotaxis towards SDF-1a (0, 25, 50, 100, 200 ng/mL) was assessed *in vitro* 8 hours post transfection using transwell migration assays (n=10 donors). *In vivo* homing studies were conducted in NSG mice receiving either $10x10^6$ control NK cells, $10x10^6$ CXCR4-R334X NK cells, or as a BM homing control, $1x10^6$ plerixafor-mobilized CD34+ HSCs (n=9-10 animals per group). Blood, BM (femur), liver, lung, and spleen were harvested 24 hours post IV infusion and recovered cells were analyzed with flow cytometry.
Results: CXCR4-R334X transfected NK cells exhibited more prominent migration *in vitro* to SDF-1a at 25 and 50 ng/mL compared to control NK cells ($p<0.001$ for both concentrations). Overall, NK cell CXCR4 surface expression positively correlated with *in vitro* migration towards SDF-1a ($r=0.64$, $p=0.0002$). Consistent with the *in vitro* findings, significantly less circulating CXCR4-R334X transfected NK cells were found in blood of NSG mice ($t=3.45$, 95%CI=-0.1383 to -0.02828, $p<0.01$) and more CXCR4-R334X NK cells were found in the BM ($t=5.017$, 95%CI=0.002348 to 0.005756, $p=0.0001$) compared to control NK cells. The recovery of HSCs in BM compartments was significantly higher than that of non-transfected and transfected NK cells ($t=15.5$ and 14.2 respectively, and $p<0.0001$ for both). These findings demonstrate that low baseline homing of NK cells to the BM can be significantly improved by genetically modifying NK cells to express CXCR4-R334X (Figure 1).

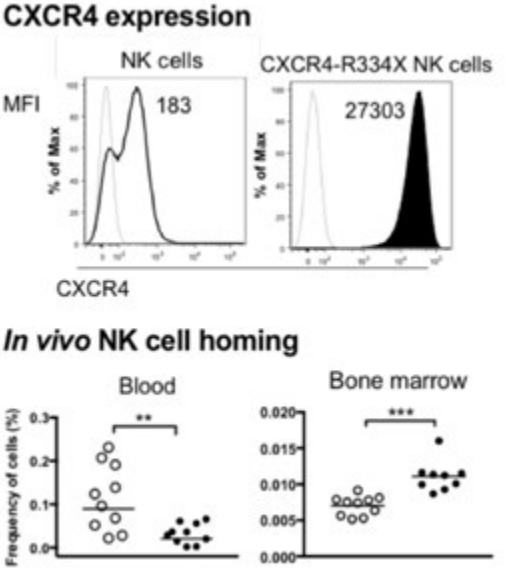

Figure 1.

Summary/Conclusion: This proof-of-concept study demonstrates that NK cell homing to BM compartments can be significantly enhanced as a result of transfection with CXCR4-R334X-coding mRNA. These data suggest CXCR4-R334X-expressing NK cells might be a more effective NK cell product than unmodified NK cells for the treatment of patients with BM-residing hematological malignancies. Efforts to modify ex vivo expanded NK cells to overexpress CXCR4 and other receptor/receptor ligands to establish BM homing capacity comparable to CD34+ HSCs are ongoing.

PS1146

INTERLEUKIN-15-CULTURED DENDRITIC CELLS ENHANCE ANTI-TUMOR GAMMA DELTA T CELL FUNCTIONS THROUGH IL-15 SECRETION

H. Van Acker[1,*], S. Anguille[2,3], H. De Reu[1], Z. Berneman[1,2,3], E. Smits[1,3,4], V. Van Tendeloo[1]

[1]Laboratory of Experimental Hematology, University of Antwerp, Antwerp, [2]Division of Hematology, [3]Center for Cell Therapy & Regenerative Medicine, Antwerp University Hospital, Edegem, [4]Center for Oncological Research (CORE), University of Antwerp, Antwerp, Belgium

Background: Dendritic cell (DC) vaccination can be an effective post-remission therapy for acute myeloid leukemia (AML). We recently showed that WT1 mRNA-electroporated conventional interleukin (IL)-4 DCs prevent or delay relapse in 43% of AML patients in remission after chemotherapy (Anguille *et al.* Blood 2017). Yet, these current IL-4 DC vaccines do not encompass the ideal stimulatory triggers for innate gamma delta (γδ) T cell anti-tumor activity. Promoting type 1 cytotoxic γδ T cells in patients with AML is, however, most interesting, considering these unconventional T cells are primed for rapid function and exert meaningful control over AML. Indeed, a recent meta-analysis of gene expression data from over 18000 cancers, including hematological malignancies, identified the presence of γδ T cells to be the most significant factor associated with favorable prognosis (Gentles *et al.* Nature Medicine 2015). We have recently shown that our IL-15 DCs are capable of recruiting various cytotoxic effector cells including γδ T cells suggesting the possibility of interaction (Van Acker *et al.* Oncotarget 2017).
Aims: In the current study, we characterized the IL-15 DC-mediated γδ T cell activation on both the phenotypic and functional level and investigated the potential mechanisms involved.
Methods: Human monocyte-derived DCs were generated according to our rapid DC culture protocol involving IL-15 for DC differentiation and a Toll-like receptor agonist for DC maturation. These IL-15 DCs were tested in terms of their capacity to boost type 1 cytotoxic γδ T cells.
Results: In this work we demonstrate that IL-15 DCs have the capacity to enhance the anti-tumoral functions of γδ T cells. IL-15 DCs of healthy donors and of AML patients in remission induce the upregulation of cytotoxicity-associated and co-stimulatory molecules on the γδ T cell surface, but not of co-inhibitory molecules, incite γδ T cell proliferation and stimulate their interferon-γ production in the presence of blood cancer cells and phosphoantigens. Moreover, the innate cytotoxic capacity of γδ T cells is significantly enhanced upon interaction with IL-15 DCs, both towards leukemic cell lines and allogeneic primary AML blasts. Finally, we address soluble IL-15 secreted by IL-15 DCs as the main mechanism behind the IL-15 DC-mediated γδ T cell activation.
Summary/Conclusion: These results indicate that the application of IL-15-secreting DC subsets could render DC-based anti-cancer vaccines more effective through, among others, the involvement of γδ T cells in the anti-leukemic immune response.

PS1147

SYNERGISTIC ANTITUMORAL EFFICACY OF A NOVEL REPLICATIVE ADENOVIRUS SG611-PDCD5 AND DAUNORUBICIN IN HUMAN LEUKEMIC CELLS

Y.-L. Zhou[1,*], Q.-M. Yao[1], J. Zhou[1], Y. Chang[1], J.-L. Li[1], Y.-Z. Wang[1], Q.-J. Qian[2], H.-P. Wu[2], L.-F. Li[2], Y.-H. Chen[1], Y.-R. Liu[1], X.-J. Huang[1], G.-R. Ruan[#1]

[1]Peking University People's Hospital, Beijing, [2]Laboratory of Viral and Gene Therapy, Eastern Hepatobiliary Surgical Hospital, Second Military Medical University, Shanghai, China

Background: Daunorubicin is a traditional chemotherapeutic agent and plays a pivotal role in leukemia therapy. However, the dose-related toxicity, especially cardiotoxicity, caused by daunorubicin limits its clinical use and remains a considerable challenge for clinicians. The apoptosis-regulating gene *PDCD5*, is downregulated in various tumors including leukemias, might provide a potential target for the diagnosis and treatment of leukemia.
Aims: The purpose of this study was to construct a triple-regulated oncolytic adenovirus carrying *PDCD5* gene expression cassette(SG611-PDCD5) and to explore the combined antitumor efficacy of SG611-PDCD5 in combination with low dose daunorubicin on leukemia cell lines and nude mouse xenograft models.
Methods: A variety of leukemic cell lines were cultured according to the providers instructions. The insertion and orientation of all recombined plasmids were confirmed by restriction enzyme digestion and polymerase chain reaction. The tumor-selective replication of the constructed conditionally replicating SG611-PDCD5 and its antitumor efficacy in combination with daunorubicin were characterized in several leukemic cell lines *in vitro* and in nude mouse xenograft model of K562 cells *in vivo*. Cell viability was detected by using Cell Counting Kit-8. Cell Apoptosis was detected in whole living cells using flow cytometry and in paraffin-embedded tumor tissues using the TUNEL kit.
Results: The triple-regulated CRAd carrying the *PDCD5* gene expression cassette, SG611-PDCD5 and the nude mouse xenograft models of K562 cells were successfully constructed. In SG611-PDCD5, the *E1a* gene with 24 nucleotides deleted from the CR2 region was controlled by the human telomerase reverse transcriptase (hTERT) promoter, *E1b* gene expression was directed by the hypoxia response element (HRE), and the *PDCD5* gene was controlled by the cytomegalovirus promoter. SG611-PDCD5 in combination with low-dose daunorubicin demonstrated more potent anti-proliferative and pro-apoptotic effects in leukemic cells in a dose-dependent manner *in vitro*. In xenograft nude mice models, tumor sizes in the control, daunorubicin, SG611-PDCD5, and combined treatment groups on day 10 were 170.1±47.8, 111.9±81.1, 60.7±12.3, 33.2±17.5 mm^3, respectively. TUNEL assay showed significantly more apoptotic cells in the SG611-PDCD5 plus daunorubicin group than in the SG611-PDCD5 or daunorubicin groups (25, 12.5, and 7.8 apoptotic cells/field, respectively; $p<0.05$). (Figure 1).

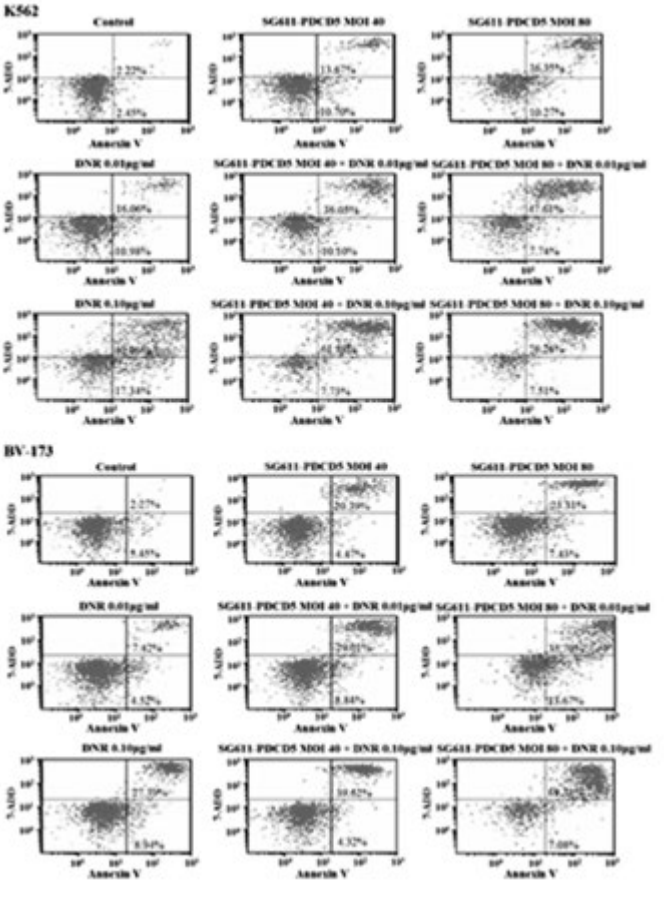

Figure 1. Apoptosis of K562 and BV-173 cells treated with SG611-PDCD5 followed by daunorubicin treatment. SG611-PDCD5 and low-dose daunorubicin synergistically showed pro-apoptosis effects that were significantly more potent than those of SG611-PDCD5 or daunorubicin alone.

Summary/Conclusion: Our findings suggested that combined treatment with SG611-PDCD5 and daunorubicin may be a promising strategy for enhancing chemosensitivity and thus lowering the dose-related toxicity of daunorubicin in leukemia therapy.

PS1148

NOVEL RNA CONSTRUCT INCREASES PERFORIN AND GRANZYME B IN NATURAL KILLER AND CYTOTOXIC T CELLS

A. Wagner[1,*], M. Chrobok[1,2], A. Hussain Baloch[1], C. Dahlberg[1], H. Nahi[1,3], H.-G. Ljunggren[1], E. Alici[1,2,3]

[1]Dept. of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden, [2]NSU Cell Therapy Institute, Nova Southeastern University, Fort Lauderdale, Florida, United States, [3]Haematology Centre, Karolinska University Hospital, Stockholm, Sweden

Background: Stimulation and activation of the cytosolic RNA recognition receptors RIG-I and MDA-5 are very attractive to boost the immune response of activated lymphocytes. Activation will stimulate the synthesis of a broad range of antiviral effector molecules, cytokines and chemokines that have a crucial role for priming, expansion and polarization of immune cells.
Aims: In this study, we aimed to identify optimal inducers of perforin and granzyme B production in cytotoxic lymphocytes.
Methods: We used NK cell and T cell *in vitro* response assays, measured granule content of Granzymes and Perforin, tested the compound in a mouse model for *in vivo* activity.
Results: Initially, we tested the hypothesis of RIG-I induction leading to better perforin and granzyme B expression by using previously published RIG-I agonists. While we can observe an increase in type I interferon secretion, we could not detect a significant increase in either of the cytotoxic granule components. In contrast, when we screened novel, much shorter and structurally different RNA constructs, a significant increase in expression of perforin and granzyme B could be detected in NK cells and T cells.
Introduction of the best construct to NK cells using transfection or extracellular vesicle-mediated delivery resulted in a rapid increase of both molecules, which could be further boosted by IL-2. To our knowledge, this is the first report of an RNA construct leading to such a drastic difference in the proteomic profile of cytotoxic lymphocytes. We administered the RNA construct to a syngeneic immunocompetent multiple myeloma (MM) mouse model in which activated NK cells are critical for MM rejection in a dose-dependent manner. When administered for a brief period shortly after establishment of minimal residual disease, the construct resulted in a significant delay in tumor development and increased survival compared to published RIG-I agonists. We have not observed any off-target effects or severe adverse events. Further studies are needed to elucidate the best platform and method of delivery for optimal anti-tumor activity.
Summary/Conclusion: Our novel RNA construct leads to an increase in cytotoxic granule content in Natural Killer and Cytotoxic T cells, and increased killing activity *in vitro*. When used *in vivo*, in a mouse model for multiple myeloma, the compound delayed tumor growth and increased survival of tumor-bearing mice.

Gene therapy, cellular immunotherapy and vaccination – Clinical

PS1149

THE REDUCTION OF GRAFT-VERSUS-HOST DISEASE AND TRANSFUSIONAL NEEDS AFTER THE APPLICATION OF MESENCHYMAL STEM CELL THERAPY FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

E. Molnar[1], A. Barta[2], A. Batai[2], Z. Csukly[2], L. Gopcsa[2], G. Tatai[2], L. Lengyel[2], T. Masszi[3], G. Mikala[2], M. Paksi[2], M. Réti[2], E. Torbagyi[2], H. Andrikovics[4,*], H. Bönig[5], P. Bader[5], P. Remenyi[2]

[1]Department of Serology, Hungarian National Blood Transfusion Service, [2]Department of Hematology and HSCT , Central Hospital of Sothern Pest, [33]rd Department Of Internal Medicine, Semmelweis University, [4]Laboratory of Molecular Diagnostics, Central Hospital of Sothern Pest, Budapest, Hungary, [5]Division for Stem Cell Transplantation and Immunology, University Hospital Frankfurt/Main, Frankfurt, Germany

Background: Steroid refractory *graft*-versus-host disease (GvHD) is a serious complication of allogeneic hematopoietic stem cell transplantation (HSCT). *More experience accumulates in* the immunomodulatory effect of mesenchymal stem cell (MSC) infusion in numerous *immunopathological* disorders – such as GvHD – and signals. *MSCs have a HLA-restrictive and non-immunogenic nature.*
Aims: *We have evaluated the efficacy of MSC transfusions in cases of GvHD refractory to conventional immunosuppressive treatment.*
Methods: *Patients with steroid-resistant GvHD* were treated with the licensed MSC product *(derived from bone marrow,* "MSC-FFM" /Kuci *et al.* Haematologica 2016/ submitted on a named-patient basis) *4 times per case weekly at a dose of 1 million cells/kg. This is a novel MSC product originating from 8 volunteer donors generated in the University of Frankfurt am Main, Germany. Clinical response was assessed 28 days after administering the first dose. Complete remission was defined as the complete disappearance of symptoms. Partial remission was assessed by the significant relief of symptoms and by the general improvement of the patient's condition. We have evaluated the red blood cell and platelet concentrate needs of the patients before and after the treatment.*
Results: Our 12 patients had received a total of 13 cycles of MSC-treatment (4 doses per cycle). The median age was 47 (19-56) years with a male/female ratio of 1:2. Distribution of the underlying malignancies (n): acute myeloid leukemia: 6; acute lymphoblastic leukemia: 2; myelofibrosis: 1; myelodysplastic syndrome: 1; multiple myeloma: 1; T-cell lymphoma: 1. Nine patients had undergone allogeneic HSCT with matched unrelated donors, the other three had stem cells derived from HLA-identic relatives. The first episode of GvHD after HSCT was observed on the median 63rd (7-455) day. The involved organs were skin (2), gut (4), skin and gut combined (7) and lung in 3 cases. We applied an average of 3 lines of treatment (it varies from 1-5 lines) before we started MSC treatment. The median time of MSC's first infusion was 274 (114-1981) days after stem cell transplantation (HSCT) and 165 (19-1974) days after the first episode of GvHD. Four of the 13 cycles of MSC-treatment led to complete remission (30.8%) and 7 resulted in partial remission (53.8%). All of the patients GvHD NIH stage score was 3 before MSC infusions, and it decreased to a median of 1 after treatment. The overall survival on the 180th day after the first GvHD was 75%. After the MSC treatment the need of red blood cell concentrate were reduced from an average of 12.83 to 8.92 (30.5% reduction) and the platelet concentrate need from an average of 107.17 to 50.75 (52.6% reduction). The average Corrected Count Increment indicating the effectivity of the platelet concentrates administered increased from 0.173 before MSC treatment to 0.511.
Summary/Conclusion: *According to our observation MSC-therapy with the novel and unique MSC product MSC-FFM is an effective treatment for GvHD in the majority of the observed cases with 83% overall cumulative response rate. The reduction of the transfusional needs of after the MSC treatment also shows promising results. The application of third-party MSCs offers a promising alternative in the therapy of GvHD and other GvHD-associated complications after HSCT. Further research is required to warrant the optimal start and dosage of the MSC treatment, along with the issue of long-term safety.*

PS1150

IMMUNOGENICITY OF TISAGENLECLEUCEL IN RELAPSED/REFRACTORY (R/R) B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL) AND DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) PATIENTS

K. Thudium Mueller[1], S.A. Grupp[2,3], S.L. Maude[2,3], J.E. Levine[4,5], M.A. Pulsipher[6], M. W. Boyer[7], K.J. August[8], G.D. Myers[8], R. Awasthi[1], E.R. Waldron[9], L.B. Pacaud[9], T. Taran[9], M. Cota[9], C.S. Tam[10], U. Jäger[11], S.R. Foley[12], P. Borchmann[13], S.J. Schuster[14], E.K. Waller[15], T.W. Laetsch[16,17,*]

[1]Novartis Institutes for BioMedical Research, East Hanover, [2]Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, [3]Division of Oncology, Center for Childhood Cancer Research and Cancer Immunotherapy Program, Children's Hospital of Philadelphia, Philadelphia, [4]University of Michigan, Ann Arbor, [5]Icahn School of Medicine at Mount Sinai, New York, [6]Division of Hematology, Oncology, and Blood and Marrow Transplantation, Children's Hospital Los Angeles, Keck School of Medicine of USC, Los Angeles, [7]Department of Pediatrics and Internal Medicine, University of Utah, Salt Lake City, [8]Children's Mercy Hospital Kansas City, Kansas City, [9]Novartis Pharmaceuticals Corporation, East Hanover, United States, [10]Peter MacCallum Cancer Centre, St Vincent's Hospital and University of Melbourne, Melbourne, Australia, [11]Dept. of Medicine I, Div. of Hematology and Hemostaseology, Medical University of Vienna, Vienna, Austria, [12]Juravinski Hospital and Cancer Centre, McMaster University, Hamilton, Canada, [13]Department of Haematology and Oncology, University Hospital of Cologne, Cologne, Germany, [14]Lymphoma Program, Abramson Cancer Center University of Pennsylvania, Philadelphia, [15]Winship Cancer Institute of Emory University, Bone Marrow and Stem Cell Transplant Center, Atlanta, [16]Department of Pediatrics, The University of Texas Southwestern Medical Center, [17]Pauline Allen Gill Center for Cancer and Blood Disorders, Children's Health, Dallas, United States

Background: Tisagenlecleucel, a chimeric antigen receptor (CAR) T-cell therapy, contains a murine single chain variable fragment (mCAR19) binding domain. Humoral immunity to anti-mCAR19 had no impact on safety or efficacy in pediatric r/r B-ALL patients (Mueller ASH 2017); immunogenicity in r/r DLBCL has not been studied.
Aims: Tisagenlecleucel immunogenicity was measured in r/r B-ALL (ELIANA [NCT02435849, n=75]; ENSIGN [NCT02228096, n=29]) and r/r DLBCL (JULIET [NCT02445248, n=99]) ≤12 months after infusion.
Methods: Cellular immunity was measured in PBMCs and tested for mCAR19 peptide-activated T cell responses by stimulated intracellular interferon-gamma production. Anti-mCAR19 antibodies (Ig) were measured by flow cytometry at baseline and after treatment. Treatment-induced Ig was defined as the ratio of postbaseline Ig levels to baseline. The impact of preexisting and treatment-induced Ig and T-cell activation on cellular kinetics, efficacy and safety were determined.

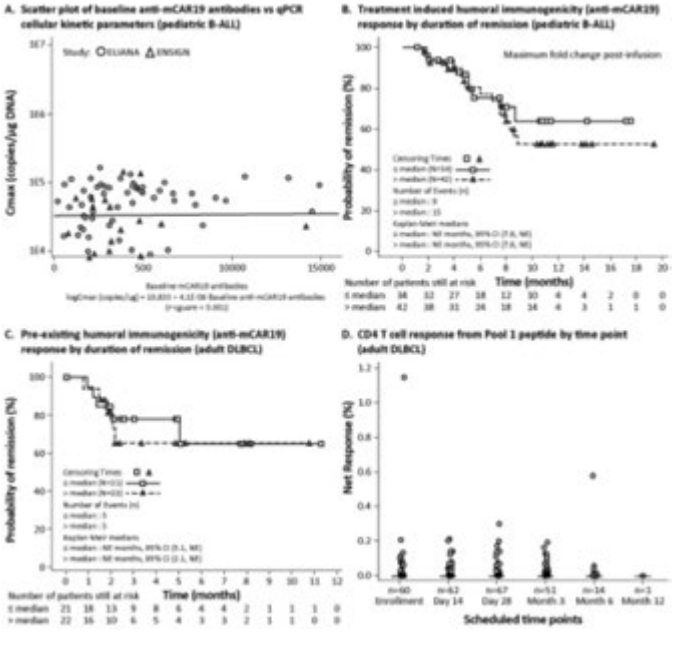

Figure 1.

Results: 84.6% of r/r B-ALL and 91.4% of r/r DLBCL patients had preexisting anti-mCAR19 antibodies. Treatment-induced rises in anti-mCAR19 antibodies occurred in 34.6% of r/r B-ALL and 5% of r/r DLBCL patients. No relationship was found between tisagenlecleucel expansion ($AUC_{0\text{-}28d}$) and preexisting humoral responses in r/r B-ALL (r^2=0.002) or r/r DLBCL (r^2=0.008), or treatment-induced humoral responses in r/r B-ALL (r^2=0.006). Results for C_{max} were similar (Figure 1 A, pediatric B-ALL). Treatment induced humoral responses did not impact expansion, persistence or duration of response (Figure 1 B, pediatric B-ALL) of CARs in B-ALL, but sample size prevented correlation analysis in DLBCL as only 5% of patients had treatment-induced Ig. Preexisting humoral immunity did not appear to impact transgene persistence, duration of response (Figure 1 C, DLBCL), event-free survival or safety in either indication. T-cell responses were consistent over time with net responses <1% at baseline and postinfusion for the majority of patients. T-cell responses did not appear to impact transgene expansion or persistence or patient outcomes (Figure 1 D, DLBCL).
Summary/Conclusion: Preexisting/treatment-induced humoral and antigen-specific cellular immunity did not impact tisagenlecleucel expansion, persistence, efficacy or safety.

PS1151

CONSIDERATIONS FOR TISAGENLECLEUCEL DOSING RATIONALE

R. Awasthi[1,*], K. Thudium Mueller[1], G.A. Yanik[2], C.S. Tam[3], S. Rives[4], J.P. McGuirk[5], M.W. Boyer[6], U. Jäger[7], A. Baruchel[8], G.D. Myers[9], P. Borchmann[10], S.M. Jaglowski[11], H.E. Stefanski[12], M.R. Bishop[13], E.R. Waldron[14], J. Hamilton[14], M. Cota[14], L.B. Pacaud[14], E.K. Waller[15]

[1]Novartis Institutes for BioMedical Research, East Hanover, [2]C.S. Mott Children's Hospital, Ann Arbor, United States, [3]Peter MacCallum Cancer Centre, St Vincent's Hospital and University of Melbourne, Melbourne, Australia, [4]Department of Pediatric Hematology and Oncology, Hospital Sant Joan de Déu de Barcelona, Barcelona, Spain, [5]Department of Blood and Bone Marrow Transplant, The University of Kansas Medical Center, Kansas City, [6]Department of Pediatrics and Internal Medicine, University of Utah, Salt Lake City, United States, [7]Dept. of Medicine I, Div. of Hematology and Hemostaseology, Medical University of Vienna, Vienna, Austria, [8]Pediatric Hematology-Immunology Department, University Hospital Robert Debré (APHP), Paris, France, [9]Children's Mercy Hospital Kansas City, Kansas City, United States, [10]Department of Haematology and Oncology, University Hospital of Cologne, Cologne, Germany, [11]The James Cancer Hospital and Solove Research Institute, The Ohio State University Comprehensive Cancer Center, Columbus, [12]Department of Pediatric Blood and Marrow Transplant, University of Minnesota, Minneapolis, [13]University of Chicago, Hematopoietic Stem Cell Transplantation Program, Chicago, [14]Novartis Pharmaceuticals Corporation, East Hanover, [15]Winship Cancer Institute of Emory University, Bone Marrow and Stem Cell Transplant Center, Atlanta, United States

Background: Analyses supporting a safe and efficacious dose for chimeric antigen receptor (CAR) therapies are not well defined and vary across CAR-T programs.
Aims: These analyses present key considerations to support tisagenlecleucel dose range in relapsed/refractory (r/r) pediatric and young adult acute lymphoblastic leukemia (B-ALL) and adult diffuse large B cell lymphoma (DLBCL) indications.
Methods: Data from pivotal phase II studies in B-ALL patients (pts) (ELIANA [NCT02435849, n=75]; ENSIGN [NCT02228096, n=29]) and DLBCL pts (JULIET [NCT02445248, n=99]) were used to investigate dose related impact on efficacy, safety, and exposure.

Table 1. Summary of dose-related analyses.

Endpoints	Analysis	Pediatric ALL (N=104)	Adult DLBCL (N=99)
Product attributes	Scatter plots/ regression	No impact	
Exposure		No impact	
Response	Logistic regression	For Day 28 response: Slight positive trend at lower end, while the probability plateaus for doses higher than 1.5×10^6 per kg for pts ≤ 50 kg and 1.0×10^6 cells for pts > 50 kg.	No impact on Month 3 response
		*OR: 1.56 (95% CI: 1.000, 2.424)	OR: 1.03 (95% CI: 0.624, 1.685)
	Quartile analysis	Similar response rate across dose quartiles	
CRS	Logistic regression	No impact (any G or G 3/4 CRS); slight positive trend (grade 4 CRS) for wt adjusted dose	Increased probability of CRS (G 3/4 or any G)
		*OR: 0.79 (95% CI: 0.327, 1.911)	†OR: 2.79 (95% CI: 1.394, 5.567)
Neurologic events (NE)		No impact	
		*OR (for G ≥2 NE): 1.12 (95% CI: 0.736, 1.700)	†OR: 0.99 (95% CI: 0.544, 1.801)

OR: Odds ratio for 2-fold increase in dose.
*for total dose; †any grade safety endpoint.

Results: Unlike conventional drugs, the dose in T cell therapy is a result of various factors: pt characteristics, manufacturing attributes, indication. Final product attributes (transduction efficiency,%T cells, cell viability, total cell count), exposure (maximal *in vivo* expansion), efficacy (Day 28 response in B-ALL, and Month 3 response in DLBCL), and safety endpoints (cytokine release syndrome, CRS; and neurological events) were evaluated against dose. The aforementioned final product attributes did not relate to the dose or *in vivo* expansion thus providing the clinical support for the product attributes specifications. Dose and exposure were independent. Clinically meaningful responses were observed across the dose range. The dose quartile analysis demonstrated similar response rate across all the quartiles in both the indications. Increased probability of any grade (G) or G 3/4 CRS was associated with increase in dose in DLBCL; no impact was observed in B-ALL.CRS is manageable with the CRS management algorithm. No impact of dose on neurological events was observed for both indications. Results are presented in Table 1. The proposed dose range, as CAR positive viable T cells, were based on totality of these analyses considering the benefit-risk ratio (B-ALL: body weight (wt) ≤50 kg, $0.2\text{-}5.0\times10^6$/kg, for >50 kg, $0.1\text{-}2.5\times10^8$; DLBCL: $0.6\text{-}6.0\times10^8$).
Summary/Conclusion: Product attributes, efficacy, safety, and exposure of CAR T, as key parameters supported the recommended dose range. These analyses serve as a recommendation for the dose-related relationships that needs to be investigated for justification of the proposed dose range for CAR therapies.

PS1152

MTL-CEBPA, A SMALL ACTIVATING RNA FOR INTRAVENOUS ADMINISTRATION TO ENHANCE C/EBPA EXPRESSION IN PATIENTS WITH LIVER CANCER SHOWS POTENTIAL USE IN NEUTROPENIA

V. Reebye[1], C. P. Tan[2,*], P. Andrikakou[1], J. Vasara[1], J. Voutila[2], R. Habib[2], D. Blakey[2], J. Rossi[3], N. Habib[1]

[1]Department of Surgery and Cancer, Imperial College London, [2]MiNA Therapeutics, London, United Kingdom, [3]Department of Molecular and Cellular Biology, Beckman Research Institute of City of Hope, Duarte, California, United States

Background: C/EBPα (CCAAT/enhancer-binding protein alpha) is a transcription factor and master regulator of cell differentiation, and cell cycle regulation. It plays an important role in a range of biological processes including lung development, hepatocyte function and maturation of blood cells in the myeloid lineage. We have developed MTL-CEBPA, a small activating RNA formulated inside liposomal nanoparticles for intravenous delivery to the liver. MTL-CEBPA induced activation of C/EBPα reverses liver diseases and improves liver function in preclinical diseased models. Patients currently being treated with MTL-CEBPA for advanced hepatocellular carcinoma (HCC) have shown a significant upregulation of C/EBPα expression in circulating white blood cells (WBCs). Consistent with this increase, total WBC and neutrophil counts also significantly increased dose dependently within 24 hours of MTL-CEBPA administration.
Aims: We evaluated whether, in addition to its benefits on liver disease, MTL-CEBPA could impact the bone marrow and lead to enhanced cells of the myeloid lineage particularly neutrophils to provide a rationale for its use in neutropenic patients.
Methods: Patients with advanced HCC and with acceptable hematologic liver and renal function were enrolled for a standard 3+3 dose escalation study (Clinical trial: NCT02716012). MTL-CEBPA was administered as a 1hour IV infusion on Day 1, 8 and 15 of a 28 day cycle. Correlative analysis of the blood was carried out to measure C/EBPα levels in PBMCs and haematological changes to WBC and neutrophil counts.
Results: Patients receiving a single dose of 28mg/m^2 MTL-CEBPA showed a significant increase of C/EBPα expression in the WBC by 1.4 fold. A dose escalation to 48 mg/m^2, 70 mg/m^2 and 90 mg/m^2 of MTL-CEBPA showed 2.2 fold, 1.8 fold and 2.4 fold increase in C/EBPα expression, respectively. Although C/EBPα expression in the WBCs appeared to have reached a saturation point in these patients, an increasing WBC count and neutrophil count correlated to each treatment points and at each dose increase. WBC and neutrophil counts increased by 40-50% within 24 hours of MTL-CEBPA infusion at a dose of 28 mg/m^2. Values returned back to the basal levels 24hours later but maintained this increase following each infusion at day 8 and 15. At subsequent dose increases of 48; 70 and 90mg/m^2, WBC counts increased by 50-60%; 60-80% and 90-100% above baseline values, respectively for each infusion at days 1, 8 and 15. In all instances, the values returned back to the basal range within 48hours of MTL-CEBPA treatment.
Summary/Conclusion: The data reported here demonstrates that MTL-CEBPA has a specific effect on the bone marrow by causing responsive changes in PBMCs, particularly in neutrophils. Since C/EBPα is a key transcription factor in maturation and differentiation of blood cells, we are the first to propose a clinically safe mode of increasing expression of C/EBPα in the bone marrow and PBMCs. Since MTL-CEBPA is already in a Phase 1 human trial for liver cancer the data presented here suggests this saRNA therapy may have utility in additional settings such as neutropenia.

PS1153

SIGNIFICANT ARI-0001 (A3B1:CD8:4-1BB:CD3Z CAR19) CELL EXPANSION IN A PATIENT WITH REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA THROUGH PRE-LYMPHOAPHERESIS IBRUTINIB ADMINISTRATION

V. Ortiz-Maldonado[1,*], M. Caballero-Baños[2], A. Bataller[1], A. Oliver[1], A. Rivero[1], T. Baumann[1], B. Marzal[2], M. Castella[2], A. Boronat[2], J. Cid[1], M. Diaz-Beya[1], J. Esteve[1], J. Yague[2], A. Urbano[1], M. Juan[2], J. Delgado[1]

[1]Hematology, [2]Immunology, Hospital Clínic Barcelona, Barcelona, Spain

Background: Patients with chronic lymphocytic leukemia (CLL) who do not respond or show refractoriness to available treatment options face a grim prognosis. Recently, several chimeric antigen receptor constructs targeting CD19 (CAR19), such as tisagenlecleucel, have been developed and showed promising results in patients with CD19+ B-cell malignancies including CLL. However, whilst excellent complete response rates (CRR) around 80% have been documented in acute lymphoblastic leukemia, much less impressive rates, around 20-35%, have been observed in CLL. This lower CRR may be secondary to the characteristic T cell dysfunction of CLL patients. Some studies have shown that ibrutinib not only has a direct antitumor effect but also reverses such T-cell dysfunctionality, thus optimizing CAR19 efficacy. Indeed, there is currently a clinical trial evaluating the role of ibrutinib in patients with CLL receiving tisagenlecleucel.
Aims: To report a case of exceptional ARI-0001 proliferation in a patient with CLL who received ibrutinib pre-apheresis.
Methods: A 53-year-old female diagnosed with CLL was referred to us after treatment with fludarabine, cyclophosphamide and rituximab, rituximab plus bendamustine, ibrutinib, idelalisib, obinutuzumab and venetoclax. The patient was either refractory or intolerant to all these agents. She was enrolled in the CART19-BE-01 clinical trial (clinicaltrials.gov NCT03144583) on the administration of ARI-0001 (A3B1:CD8:4-1BB:CD3z CART19) cells in patients with relapsed/refractory CD19+ B-cell malignancies. At screening, her bone marrow showed 29% of CLL cells and a CT scan revealed extensive lymphadenopathy in the neck, axillae and abdomen. Ibrutinib (420 mg/day) was restarted for two weeks before lymphoapheresis and then stopped. ARI-0001 production was accomplished using the CliniMACS Prodigy System (Miltenyi). A dose of $1x10^6$ ARI-0001 cells /kg was infused after lymphodepletion with fludarabine (90 mg/m^2) and cyclophosphamide (900 mg/m^2). ARI-0001 proliferation, CLL response and B-cell aplasia was later assessed by means of flow cytometry.
Results: A total of 2.8 x10^9 PBMCs were collected from the patient, with a CD4 / CD8 composition of 8.8% and 10.1%, respectively (19.7% CD3+). After aliquoting the apheresis product, a cell count of 100 x10^6 CD4/CD8 + cells was introduced to the CliniMACS Prodigy System. After 10 days of expansion, a total count of 350 x10^6 mononuclear cells was achieved (97.2% CD3+). The CD4 / CD8 composition of the final cell product was 69.5% and 29.2%, respectively, with a T cell transfection percentage of 23.3% (ARI-0001 cells). ARI-0001 cells were administered to the patient without complications. She soonly developed a transient grade I cytokine release syndrome (fever) that resolved with antipyretics plus antibiotics. No neurologic symptoms were observed. ARI-0001 cell expansion peaked at day +28, with 30% of total lymphocytes being ARI-0001 cells at that time (844 x10^9/L absolute ARI-0001 cell count). The patient achieved a CR with a negative MRD (<0.01%) measured in the marrow at week 4, with B cell aplasia and ARI-0001 cells detected in peripheral blood at last follow-up (month 3). Complete hematological recovery was seen at day +70, with no further complications.
Summary/Conclusion: This case of significant ARI-0001 cell proliferation is consistent with what has been observed with tisagenlecleucel and other emerging pre-clinical data. Preliminary data may suggest that ibrutinib, even when given in a short period of time (2 weeks), could improve ARI-0001 performance in this disease.

PS1154

HIGH BASELINE FERRITIN IS ASSOCIATED WITH GRADE 2 CRS REQUIRING TOCILIZUMAB OR GRADE ≥ 3 CRS IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS TREATED WITH BB2121 ANTI-BCMA CAR T CELLS

J. Berdeja[1,*], Y. Lin[2], J. N. Kochenderfer[3], N. Raje[4], N. Munshi[5], D. Siegel[6], M. Liedtke[7], D. Madduri[8], J. Rosenblatt[9], M. Maus[4], A. Turka[10], L. P. Lam[10],

K. Hege[11], T. Campbell[11], M. Massaro[10], F. Petrocca[10], S. Jagannath[8]

[1]Sarah Cannon Research Institute and Tennessee Oncology, Nashville, [2]Mayo Clinic, Rochester, [3]Experimental Transplantation and Immunology Branch, National Cancer Institute/National Institutes of Health, Bethesda, [4]Massachusetts General Hospital Cancer Center, [5]Dana-Farber Cancer Institute, Boston, [6]Hackensack University Medical Center, Hackensack, [7]Stanford University Medical Center, Palo Alto, [8]Mount Sinai Medical Center, New York, [9]Beth Israel Deaconess Medical Center, Boston, [10]Bluebird Bio Inc., Cambridge, [11]Celgene Corporation, San Francisco, United States

Background: Cytokine release syndrome (CRS) is the most common risk associated with chimeric antigen receptor (CAR) T cell therapies. Identification of predictors of severe CRS may enable earlier, more aggressive treatment of patients at high risk.
Aims: To report key parameters associated with grade 2 CRS requiring tocilizumab or grade 3 CRS (tCRS) in an ongoing phase I trial of anti-BCMA CAR T cell therapy bb2121 in relapsed/refractory multiple myeloma [CRB-401, NCT02658929].
Methods: Patients who received active doses in Part A (150 [6], 450 [9], or 800 [3] × 10^6 CAR T cells) were included. Standard criteria for grading CAR T-associated CRS were used (Lee 2015).
Results: Four of 18 (22%) patients developed tCRS, all within 30 days of bb2121 infusion. Pre-treatment serum ferritin > 1500 µg/L was seen in 5 patients (min, max: 1516, 4653 µg/L); 4 developed tCRS. None of 13 patients with ferritin ≤ 1500 (min, max: 17, 381 µg/L) experienced tCRS. Serial ferritin levels were measured in 11 evaluable patients; ferritin further increased 1-2 days prior to tCRS in 3 of 3 evaluable tCRS patients, but not in 7 of 8 (87.5%) evaluable patients with CRS not requiring tocilizumab (non-tCRS; median peak, 9902 vs 324 mg/L). Baseline C-reactive protein (CRP) and IL-6 levels were not associated with tCRS; both increased 1-2 days prior to tCRS and non-tCRS (median peak CRP, 163 vs 68 mg/L; median peak IL-6, 413 vs 17 pg/mL). Extensive bone marrow involvement (> 50% plasma cells) was seen in 3 of 4 tCRS patients. All 4 tCRS patients had CAR T cell expansion after infusion (peak vector copies min, max: 361,474, 959,041 copies/µg gDNA), but comparable expansion was observed in 8 of 12 evaluable non-tCRS patients. All 4 tCRS patients received ≥ 450×10^6 CAR T cells. The fifth patient with baseline ferritin >1500 µg/L had extramedullary disease with no bone marrow involvement and received 800×10^6 CAR T cells, but only developed grade 1 CRS. We found no association between tCRS and age, gender, number of prior lines of therapy, bridging therapy, immunoglobulin subtype, or high-risk cytogenetics.
Summary/Conclusion: The overall frequency of tCRS in this study was relatively low and associated with high baseline ferritin. High bone marrow involvement and CAR T cell dose may contribute to the risk.

PS1155

THE COMBINATION OF CD19/BCMA-DIRECTED CART CELLS INDUCED THERAPEUTIC RESPONSE AND REVERSIBLE COMPARTMENTAL CYTOKINE RELEASE SYNDROME CONFINED TO CEREBRAL REGION IN A PATIENT WITH CNS MULTIPLE MYELOMA

C. Li[1,*], J. Chen[1], Y. Zhang[1], J. Zhou[1], J. Zhang[1], D. Yang[1], P. Wang[1], C. Zhang[2], C. Fu[1], D. Wu[1]

[1]The First Affiliated Hospital of Soochow University,Jiangsu Institute of Hematology, Suzhou, [2]UniCar Therapy Ltd., Shanghai, China

Background: Patients with CNS diseases have increased risks of cerebral CRS after CAR-T therapy, but the machanism has been yet determined. Whether CAR-T cells could cross BBB and induce CRS directly is contraversial.
Aims: To provide an innovation to investigate the machanism of cerebral CRS after CAR-T treatment.
Methods: We report a case of a 56-year-old male diagnosed with multiple myeloma (κ-chain) with CNS involvement who received a combination of CD19/BCMA-directed CART and achieved response and reversible compartmental cytokine release syndrome confined to the cerebral region. The patient was first diagnosed with MM in 2014 and received induction treatment followed by ASCT. In January 2016 and June 2017 he experienced relapse twice, and in the second relapse involved the CNS (confirmed by MRI as shown in Figure 1A). The salvage therapy consisted of DECP regimen and pomalidomide but the CNS symptoms progressed along with both-side hemiparesis and incontinence sequentially. Intrathecal chemotherapy agents and daratumumab were given but had no response indicating a refractory disease. In November 2017, he was admitted in our hospital and was enrolled in our clinical trial of CAR-T therapy (NCT03196414). Autologous anti-CD19 and anti-BCMA CAR-T cells were manufactured and infused in consecutive three days with no preconditioning regimen. On Day 3 of the infusion, the patient developed CART-related toxicities including febrile, hypotension and hypoxia accompanied by escalations of cytokine levels indicating a grade 2 CRS. On Day 5, he developed somnolence and mental obtundation, and the neurological symptoms worsened gradually in the next 4 days. On Day 9 the patient developed pupil asymmetry. Methylprednisolone was then administered. Imaging showed decreased size of intracranial lesion while emerging demyelinating lesions. A dramatic increase of IL-6 was detected in CSF (peak value 6091.2 pg/ml) rather than in peripheral blood (peak value 240.5 pg/ml), while CAR-T cells existed both in CSF and peripheral blood (18,000 versus 251,000 cells/ml CSF) , indicating a compartmental CRS confined to the cerebral region.
Results: IL-6 level in CFS and peripheral blood significantly dropped (Figure 1B), and all CRS-related toxicities including CNS symptoms ameliorated gradually in 3 weeks. MRI performed on Day 46 revealed that the intracranial disease was stable though the demyelinating lesions remained (Figure 1C), and IL-6 level in CFS decreased to about 400 pg/ml with most CRS symptoms relieved.

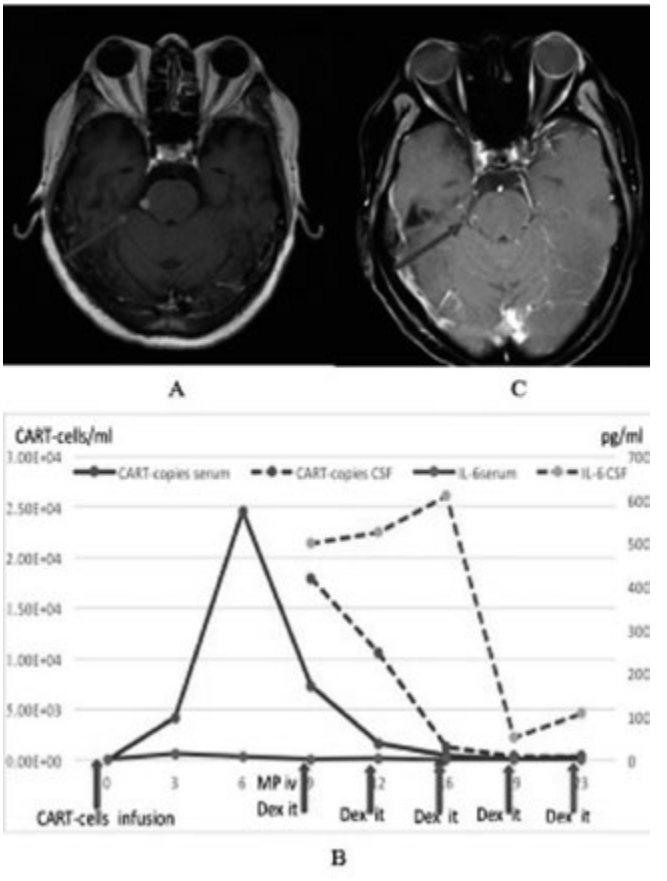

Figure 1.

Summary/Conclusion: This is the first description of a compartmental CRS confined to the cerebral region in a patient with CNS multiple myeloma which was then successfully reversed by appropriate medical interventions. The huge cytokine concentration gradient between peripheral blood and CSF in this case suggests the ability of CAR-T cells to cross the blood–brain barrier and induce cytokine increase and related toxicities in cerebral region. This case suggested that CART therapy is capable to treat diseases in CNS system with possibly severe but reversible CRS adverse events.

PS1156

INITIAL EXPERIENCE IN US COMMERCIAL MANUFACTURING OF TISAGENLECLEUCEL, A CHIMERIC ANTIGEN RECEPTOR (CAR)-T CELL THERAPY FOR PEDIATRIC RELAPSED/REFRACTORY B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

B. Majors[1,*], T. Spencer[2], S. Ericson[2], V. Romanov[2]

[1]Novartis Pharmaceuticals Corporation, Morris Plains, [2]Novartis Pharmaceuticals

Corporation, US Clinical Development and Medical Affairs, East Hanover, United States

Background: Tisagenlecleucel, a CD19-directed genetically modified autologous T cell immunotherapy, is approved for the treatment of patients up to 25 years of age with B-cell precursor acute lymphoblastic leukemia (B-ALL) that is refractory or in second or later relapse. The processing technology for tisagenlecleucel was transferred from an academic production facility to an industry-owned facility following current Good Manufacturing Practice for optimization, validation, and scale-up for use in global clinical trials and commercial manufacturing.
Aims: To present initial data on manufacturing process time and success for commercially manufactured tisagenlecleucel used to treat pediatric and young adult patients with relapsed/refractory B-ALL following approval by the United States Food and Drug Administration on Aug 30, 2017.
Methods: Key steps and timing targets for the commercial manufacturing process are shown (Figure 1). Leukapheresed mononuclear cells from patients are cryopreserved and shipped to a centralized manufacturing facility. The core manufacturing process consists of enrichment of T cells, activation *ex vivo*, transduction with a lentiviral vector containing the anti-CD19 CAR transgene, cellular expansion, formulation, and cryopreservation. The manufactured products then undergo extensive testing to assess product quality and safety. Once all quality release requirements are satisfied, the products are shipped to the treatment facilities for administration to patients.
Results: Of the initial37 sequential commercial patient orders placed for tisagenlecleucel that have been processed to completion and successfully supplied with final commercial tisagenlecleucel products, the median throughput time from receipt of leukapheresis material to return of manufactured product to treatment site was 23 days (range, 21–37 days). Consistent product quality was demonstrated by extensive testing. Rigorous sterility testing in the tisagenlecleucel manufacturing process is part of an overall sterility assurance strategy to ensure tisagenlecleucel is free of potential contaminants. Although cryopreservation of collected leukapheresis material prior to shipment for central manufacturing results in additional manufacturing time, it also has several advantages over shipping fresh leukapheresis material including timing flexibility for leukapheresis and shipping, no negative effects on cell function, enrichment of lymphocytes, and reliable and consistent manufacturing.

Figure 1.

Summary/Conclusion: There is an acute medical need of pediatric and young adult patients with relapsed/refractory B-ALL to receive tisagenlecleucel as promptly as possible. Continuous improvements in the tisagenlecleucel manufacturing process have resulted in the development and execution of high quality and safety standards. The current process, based on 37 initial commercial patients, highlights a median 23-day time period from receipt of leukapheresed material to return of manufactured tisagenlecleucel to the clinical site (including shipping time) with all 37 sequential patients receiving the final product.

PS1157

ALLOGENEIC NK CELLS AND THEIR USE IN CLINICAL TRIALS

L. Bielik[1,*], P. Lesny[1], J. Rychla[1], I. Kastankova[1], P. Otahal[1]

[1]Dept. of Immunoterapy, Institute of Hematology and Blood Transfusion, Prague, Czech Republic

Background: New trend in hematological malignancy therapies are haploidentical NK (natural killer) cells expanded *in vitro* in presence of growth and stimulation cytokines. These cells belong in the category of advanced therapy medicinal products (ATMP) and must be manufactured within the Good Manufacturing Practice (GMP) Quality Management System.
Aims: We aim for the production of clinically applicable NK cells for the experimental treatment of acute myeloid leukemia (AML) patients in our laboratoeries.
Methods: Patient apheresis was used as our starting material for production, from which 10^9 mononuclear cells were used further in process. Patient was not stimulated with growth factors prior to the apheresis. After immunomagnetic depletion of non-NK cells with commercially available kit (CD2, CD335), the NK cells were further stimulated with interleukins IL-2 and IL-15 during the 14 days expansion.
Results: After immunomagnetic depletion $79\pm23x10^6$ cells (8±2%) was acquired, from which 83±2% of cells were with NK phenotype ($CD45^+$, $CD16/CD56^+$, $CD3^-$). During the 14 days expansion we achieved a total yield of $1,1\pm0,2x10^9$ cells, comprised of 78±6% cells with NK phenotype. The NK cells had from the beginning of expansion high expression of perforin and the expression of activation marker CD69 rose from less than 5% to more than 80% of all cells in the suspension during the expansion. Furthermore, they were submitted to *in vitro* testing, in which they were highly cytotoxic against the K562 tumour cell line.
Summary/Conclusion: Produced amount of NK cells was in accord with the demand of clinicians, who planned to use the effective dose of 10^7 cells/kg of body mass. As a suitable primary packaging for expected treatment was chosen prefilled 50ml injection containing $20\pm2x10^6$ cells/ml. Production process was fully validated and we acquired authorization for production and subsequent clinical trial on AML within allogeneic hematopoietic transplantation from haploidentical donor after sequential preparation protocol from national regulatory authority.

PS1158

IMMUNO-T, A MOTION COMIC EDUCATING PATIENTS AND CAREGIVERS ON HOW IMMUNOTHERAPY WORKS

T. Kerre[1,2,*], C. Charakhani Nia[3], S. Calis[4], Y. Bertrand[4], L. Achten[5], J. Soetens[6], B. Casteels[7], D. Flamez[8,9], B. Bollaert[10]

[1]Cancer Research Institute Gent, Ghent University and Ghent University Hospital, [2]Hematology, Ghent University Hospital, [3]Hematology, UZ Gent, [4]Holofarm, [5]Telma, [6]Cancer Center, Ghent University Hopsital, [7]Cancer Center, Ghent University Hospital, [8]Cancer Research Institute Gent, [9]IOF Consortium, Ghent University, [10]WahWah vzw, Gent, Belgium

Background: The introduction of immunotherapy has been a major breakthrough in the treatment of cancer. In the general public and the average patient populations, the overall knowledge of the immune system and its role in cancer is very limited. Moreover, explaining the immune system and how immunotherapy works to patients and their caregivers, is challenging because of the extreme complexity.
Serious games and virtual aids have only been scarcely used for cancer patients or patients in general. However, the limited amount of literature evinces the benefit patients and caregivers experience when these services are employed.
Aims: We now have developed Immuno-T: a motion comic, explaining the genesis of cancer and the working mechanisms of three types of immunotherapy. A motion comic combines a digital comic with animation, sound effects, voice-over and/or a musical score. The aim was to develop a tool in order to inform patients and their caregivers on the complex subject of immunotherapy, create a feeling of hope, and increase adherence to the therapy with improved outcomes.
Methods: The scenario, story board and basic character design were developed by a hematologist and executed by game developers, and further refined in small focus groups consisting of hematologists-oncologists, psychologists, study coordinators, medical students, nurses and communication managers, into the first version. This version was evaluated by 40 people of different backgrounds, including a small number of patients, and further adapted into the final version.
Results: Immuno-T explains three types of immunotherapy in an engaging, easy-to-understand way. The majority of the evaluators (94.9%) considered the motion comic a good tool to explain immunotherapy to patients, in addition to the physician consultation. Almost 40% of the evaluators felt hopeful after watching the motion comic, 30% experienced other positive emotions (happy, well-informed, strengthened).
Summary/Conclusion: Although motion comics carry important advantages (a clear and graphic presentation of complex information and a more engaging way of delivering information), they have only rarely been used in healthcare settings. Immuno-T, while still evolving and growing, is ready to be rolled out in real hospital settings. We are currently preparing a clinical trial to evaluate the effectiveness of information transmission using Immuno-T: does it lead to better understanding of the given treatment? Increasing patients' knowledge of disease and treatment has a proven impact on hope, strength and empowerment, and on adherence to the treatment.

Hematopoiesis, stem cells and microenvironment

PS1159

PERTURBATION OF FETAL LIVER STROMAL CELL FUNCTION BY TRISOMY 21 PROMOTES INCREASED ERYTHRO-MEGAKARYOPOIESIS BY FETAL HSPC

N. Elliott[1,*], D. O'Connor[2], A. Roy[1], E.-M. Labbett[3], S. O'Byrne[1], S. Rice[4], N. Fordham[4], B. Liu[1], G. Cowan[2], A. Karadimitris[2], P. Vyas[4], I. Roberts[1]

[1]Paediatrics, University of Oxford, Weatherall Institute of Molecular Medicine, Oxford, [2]Centre for Haematology, Imperial College London, London, [3]University of Oxford, [4]MRC Molecular Haematology Unit, University of Oxford, Weatherall Institute of Molecular Medicine, Oxford, United Kingdom

Background: Children with Down syndrome (DS; trisomy 21- T21) have an increased risk of leukemia in early childhood. The reasons for this are unclear. We previously showed that T21 itself perturbs fetal liver (FL) hemopoiesis with increased erythro-megakaryopoiesis the most prominent finding. Although this is still evident in neonates, erythroid/megakaryocyte (MK) abnormalities largely resolve by age 12m. This, and the natural history of DS myeloid leukemia, which presents before or just after birth with liver involvement and spontaneously resolves in >80% of cases before 3m of age, implicates the FL microenvironment in T21-associated perturbation of hemopoiesis and myeloid leukemogenesis.
Aims: To investigate the impact of T21 on FL stromal cell function.
Methods: We performed transcriptomic (microarray, qRT-PCR), proteomic (mass spectrometry, ELISA) and functional analysis of T21 (n=3-7) and normal (n=3-9) disomic FL mesenchymal stromal cells (MSC), including their ability to support T21 and normal FL hemopoietic stem/progenitor cells (HSPC).
Results: While the morphological and immunophenotypic characteristics of T21 and normal FL MSC appeared identical, T21 MSC grew more slowly (doubling 3.0 vs 1.7 days; p=0.0044). Consistent with T21 effects on MSC function, co-culture of normal FL Lin-CD34+ cells on T21 or normal FL MSC showed markedly increased erythroid (5-fold) and MK (2-fold) output, recapitulating the T21 HSPC phenotype. Similarly, T21 HSPC had increased erythroid/MK output when co-cultured with T21 vs normal FL MSC although overall erythroid/MK output was greater from T21 HSPC consistent with both HSPC-intrinsic as well as microenvironment-induced effects of T21. Conditioned medium (CM) from T21 FL MSC stimulated erythroid/MK output from normal and T21 HSPC supporting a role for secreted proteins. To investigate the molecular basis for these effects, we first used microarray analysis to compare gene expression of T21 and normal FL MSC. Of 627 differentially expressed (DE) genes, most were overexpressed by T21 vs normal FL MSC; however, known cytokine genes were not increased in T21 MSC and neither *EPO* nor *TPO*, the principal erythroid and MK cytokine genes, were detectable. Although 28 of the top 50 DE genes were on Hsa21, none were known growth factors or predicted to encode secreted proteins. By contrast, of 25 DE upregulated genes of FC >2, 7 encoded secreted proteins of which only *IGFBP1*, previously implicated in increased erythropoiesis in primary myeloproliferative disorders, had known hemopoietic activity. Increased *IGFBP1* was confirmed by qPCR and IGFBP1 was present at high concentrations in T21 MSC CM but undetectable in normal FL MSC CM by ELISA. Addition of IGFBP1 directly to normal/T21 HSPC or MSC:HSPC co-cultures partially, but not completely, restored erythroid/MK output to that of T21 MSC:HSPC co-cultures. Proteomic analysis of normal and T21 supernatants to identify additional factors showed DE of ~40 secreted proteins including several with hemopoietic activity and 8 encoded by Hsa21 genes.
Summary/Conclusion: T21-mediated perturbation of gene expression in FL MSC fundamentally alters their secretome and function, promoting increased MK/erythropoiesis of FL HSPC.

PS1160

PROTEIN KINASE CK2 AS A NEW PLAYER IN GATA-1 REGULATION DURING ERYTHROPOIESIS

L. Quotti Tubi[1,2,*], S. Canovas Nunes[1,2], E. Mandato[1,2], S. Manni[1,2], M. Carrino[1,2], F. Piazza[1,2]

[1]Department of Medicine, Hematology and Clinical Immunology Branch, University of Padova, [2]Venetian Institute of Molecular Medicine, Padova, Italy

Background: Protein kinase CK2 is a Ser-Thr kinase usually represented as a holoenzyme, composed of two catalytic (α) and two regulatory (β) subunits, although these components can also work independently. CK2 promotes cell survival and proliferation and it is involved in hematological malignancies. Despite CK2 regulates key signaling pathways for hematopoiesis, its role in blood cell development is still unexplored. To fill this gap, we have generated a conditional knockout (KO) mouse model for CK2β in all the hematopoietic compartments (Vav1-CRE CK2β flox). CK2β KO resulted to be lethal, thus the analysis was done *in utero*. CK2 loss caused a depletion of all hematopoietic lineages, markedly of the erythroid population; moreover, CK2β KO led to down-modulation of some proteins downstream the Epo-receptor in particular of GATA-1 transcription factor.
Aims: Here, we wanted to understand at the molecular level, how CK2β could drive erythroid differentiation. We hypothesized a functional relationship between GATA-1 and CK2β. To demonstrate it, an *in vitro* model of erythroid differentiation was used based on G1E-ER mouse proerythroblast cell line that presents an inducible form of GATA-1 activated through β-estradiol.
Methods: G1E-ER cells were exposed to b-estradiol with or without CK2 inactivation with either inhibitor CX-4945 or siRNA against CK2β; Bortezomib was add to determine GATA-1 turnover. Through flow cytometry analysis we assessed erythroid differentiation, after staining with anti c-KIT, TER119 and CD71 antibodies, and we studied cell cycle, upon fixing and staining cells with propidium iodide. mRNA expression levels were evaluated by RT-PCR. WB was used to determine protein amount, immunofluorescence to investigate GATA-1 and CK2β cell localization.
Results: CK2 inhibition/silencing, in the presence of β-estradiol-induced differentiation, caused a reduction of differentiated TER119$^+$ cells and a down-regulation of CD71 expression; moreover, CK2 blockade prevented cell cycle arrest in G0/G1 phase, that is required for the onset of cell differentiation. Molecularly, we observed a down-modulation of STAT5, AKT and GATA-1 protein levels and an impairment of GATA-1 transcriptional activity. Nuclear GATA-1 resulted lowered and differently distributed. Treatment with Bortezomib restored GATA-1 levels. As CK2 influences HSP70, HSP90 and CDC37 ability to stabilize client proteins, including GATA1, we considered a possible indirect role of CK2 in GATA-1 protein turnover control. Indeed, we observed a decrease in these chaperone expression, upon CK2 inactivation, which could account for GATA1 instability. However, GATA-1 and CK2β also colocalized in the nucleus in presence of the β-estradiol suggesting a possible direct mechanism in CK2-mediated control of this transcription factor.
Summary/Conclusion: CK2β is essential for erythroid maturation, regulating STAT5, AKT and GATA-1. CK2β subunit could regulate these factors in two possible ways: an indirect mechanism, through the control of chaperones; a direct mechanism, through physical interaction. Further research will clarify the mechanism(s).

PS1161

TRANSCRIPTION FACTORS REGULATE DNA METHYLATION DYNAMICS IN HEMATOPOIETIC DEVELOPMENT

T. Suzuki[1,2,*], S. Maeda[2], E. Furuhata[2], Y. Shimizu[2], M. Kishima[2], H. Nishimura[2], H. Suzuki[2]

[1]Graduate School of Medical Life Science, Yokohama City University, [2]Center for Life Science Technologies, RIKEN, Yokohama, Japan

Background: DNA methylation of CpG dinucleotides at gene regulatory regions is a fundamental epigenetic modification for gene silencing. During the hematopoietic development, DNA methylation acts as a safeguard to prevent the expression of unnecessary genes, while regulatory regions of master transcription factors (TFs) must be demethylated before or during the differentiation. Thus, the dynamics of DNA methylation have to be strictly regulated to give rise to blood cells. However, while enzymatic mechanisms of DNA methylation and demethylation are well characterized, little is known about how DNA methylation dynamics are spatiotemporally regulated in hematopoietic development.
Aims: Here, we present that RUNX1 is capable of regulating DNA demethylation at the binsing site-directed manner.
Methods: ectopic expressin of RUNX1 followed by DNA methylome analysis, transcription factor binding motif enrichment analysis, co-immunoprecipitation analysis, and ChIP-seq.
Results: We have found that ectopic expressin of RUNX1 induces binding site-directed DNA demethylation. Coimmunoprecipitation and ChIP-seq analyses have showed that RUNX1 mediates DNA demethylation by recuiting DNA demethylation enzymes such as TET. Furthermore, comparing DNA methylome data of human iPS cells, hematopoietic progenotuore cells, and terminally differentiated blood cells, we have found that not only RUNX1 but also some hematopoietic TFs may be involved in the regulation of DNA methylation dynamics. Finally, methylome data and toranscriptome

data suggest that DNA demethylation is not represents the gene activation but rather the poised status for it.
Summary/Conclusion: Thus, our findings indicate a possibility that TF-mediated bindingsite-directed DNA methylation regulation plays an important role in hematopoietic development and functions.

PS1162

CANONICAL NOTCH SIGNALING IS DISPENSIBLE FOR ADULT STEADY-STATE AND STRESS MYELO-ERYTHROPOIESIS

S. Duarte[1,2,*], P.S. Woll[13], N. Buza-Vidas[1], D. Wai Loon Chin[3], H. Boukarabila[1], T. C. Luís[1], L. Stenson[1], T. Bouriez-Jones[1], H. Ferry[1], A.J. Mead[1], D. Atkinson[1], S. Jin[4], S.-A. Clark[1], B. Wu[1], E. Repapi[5], N. Gray[5], S. Taylor[5], A.P. Mutvei[4], Y. Long Tsoi[4], C. Nerlov[6], U. Lendahl[4], S.E.W. Jacobsen[1,3,4,6,7]

[1]Haematopoietic Stem Cell Biology Laboratory, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom, [2]Clinical Hematology Department, University Hospital Center of Coimbra, coimbra, Portugal, [3]Department of Medicine, Huddinge, Center for Hematology and Regenerative Medicine, Karolinska Institutet, [4]Department of Cell and Molecular Biology, Karolinska Institute, Stockholm, Sweden, [5]Computational Biology Research Group, [6]MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom, [7]sten.jacobsen@imm.ox.ac.uk, Stockholm, Sweden

Background: Canonical signaling through all Notch receptors converges on the Csl transcription factor recombination signal binding protein for immunoglobulin kappa J region (Rbpj). Canonical Notch signaling has been shown to be dispensable for hematopoietic stem cell (HSC) homeostasis in adult bone marrow (aBM). However, other studies have implicated an important role of canonical Notch signaling in regulation of myelopoiesis, erythropoiesis and megakaryopoiesis in aBM. However, these studies might also have targeted other pathways.
Aims: Investigate the role of canonical Notch signaling in steady-state and stress myelo-erythropoiesis through deletion of *Rbpj* in aBM.
Methods: *Rbpj*$^{fl/fl}$ mice were crossed with *Mx1-Cre* or *Vav-Cre* mice to effectively delete *Rbpj* in adult HSC/progenitor cells. HSCs and erythroid (E), megakaryocyte (Mk) and granulocyte-macrophage (GM) progenitors were enumerated and characterized phenotypically (by FACS), molecularly, and through functional Mk/E and GM *in vitro* colony forming assays, in steady state aBM as well as following transplantation and erythropoietic stress.
Results: FACS analysis of distinct stages of myelo-erythropoiesis revealed no defects, at any progenitor stage, in aBM of *Rbpj*-deficient mice. In addition, the number of GM, Mk and E colonies generated from unfractionated aBM cells as well as circulating platelets and red blood cells (RBC) counts were unaffected. No deficiencies were observed in the replenishment of HSCs and any stages of E as well as GM and Mk progenitors in mice competitively transplanted with *Rbpj*-deficient as compared to control WT BM cells. The transcript levels of genes encoding key Mk/E regulators were also unaffected in *Rbpj*-deficient Mk/E progenitors. Likewise, low expression of key Notch target genes detected in Mk/E progenitors in aBM was independent of canonical Notch signaling. We also assessed the impact of *Rbpj*-deficiency on mature circulating RBCs and the reactive regeneration of E progenitors after PHZ-induced haemolytic anemia. The reduction in RBC numbers was not aggravated by Rbpj-deficiency and E progenitors devoid of *Rbpj* were not affected in their ability to rapidly expand in response to PHZ-induced anemia, neither in BM nor spleen. In the previous study implicating a role of canonical Notch signaling in megakaryopoiesis, a dominant negative (dn) version of Mastermind-like protein 1 (MAML1) was used to block Notch signaling. While dnMAML1 has been demonstrated to block canonical Notch signaling, it has been less clear whether it may also affect other signaling pathways related to MAML1, such as MEF2C or p53. To address possible Rbpj-independent transcriptional alterations mediated by dnMAML1, we transfected *Rbpj*$^{-/-}$ embryonic stem (ES) with dnMAML1 and performed a gene expression array. Of a total of 13020 transcripts detected (CPM>1), only one gene (*Hsph1*) could be found to be significantly (but marginally) deregulated in *Rbpj*$^{-/-}$ dnMAML1 ES cells.
Summary/Conclusion: By specifically addressing canonical Notch signaling through deletion of Rbpj in aBM, we show that this pathway is dispensable for development and maintenance of Mk/E progenitors in steady-state and regenerative aBM. Moreover, the robust reactive erythropoiesis seen in response to PHZ-induced anemia occured independently of canonical Notch signaling. The explanation for the discrepancy between our findings and those based on other approaches to inhibit Notch signaling remains unclear, and we failed to obtain experimental support for Notch-independent effects of dnMAML1.

PS1163

ENHANCING BONE MARROW TREPHINE BIOPSY ANALYZES: DEFINING PEDIATRIC LYMPHOID SUBPOPULATION REFEERENCE RANGES BY AUTOMATED ENUMERATION

J. Malherbe[1,2,*], J. Liang[2,3], K. Fuller[2,4], B. Mirzai[2,4], T. Carter[4,5], C. Cole[1,2,4,5], W. Erber[1,2,4]

[1]School of Medicine, [2]School of Biomedical Sciences, Univeristy of Western Australia, Crawley, [3]Department of Haematology, Sir Charles Gairdner Hospital, [4]Path-West Laboratory Medicine, Nedlands, [5]Department of Haematology & Oncology, Princess Margaret Hospital, Subiaco, Australia

Background: Bone marrow examination involves the morphological assessment of aspirated material and the trephine (BMT) biopsy specimen. This includes cellularity, cytological detail and quantitative analyzes of cell populations of interest (*e.g.* percent composition). For aspirates, accurate quantitation is aided by flow cytometry. In contrast, manual "eye-ball" estimation of hematoxylin and eosin or immunophenotyped cells is performed for the BMT biopsy; this represents an estimate only and lacks reproducibility. Aspirate references tend to be applied to the BMT estimate. This is not valid as the aspirate data is skewed by peripheral blood contamination and hinders accurate diagnostic reporting. Accurate reference ranges for the number of cells in the bone marrow (uncontaminated by blood) are required and are presently not available.
Aims: We aimed to establish the number of lymphoid progenitor cells, T- and B-lymphocytes and plasma cells in normal BMT biopsies of infants, children and adolescents by performing combined immunophenotyping and automated digital enumeration. From this, we aimed to establish normal age-specific reference ranges for routine clinical application.
Methods: Archival, formalin-fixed, morphologically normal BMT biopsy specimens (N=238) were obtained from pediatric patients aged one month to 17 years of age. Cases were immunohistochemically stained to identify lymphoid progenitors (TdT), T-lymphocytes (CD3), B-lymphocytes (CD20) and plasma cells (CD138). Tissue Image Analysis enumeration software (Leica, Ireland) was used, with modifications, for the automated digital enumeration of these populations. Geometric mean (GM) values were generated for specific age groups and age-defined reference ranges (RR) were determined for each antigen using normalized data.
Results: A mean of 17,458 cells were analyzed per biopsy (range: 8,224-34,097 cells). Infants <2 years old showed high numbers of lymphoid progenitors (GM=3.5%, RR: 1.0-12.6%) and B-lymphocytes (GM=7.4%, RR: 0.25-22.8%) in comparison to older children. In contrast, T-cell numbers were low (GM=2.2%, RR: 0.37-12.8%) while plasma cells were virtually absent from the marrow (GM=0.08%, RR: 0.008-0.80%). With increasing age (*i.e.* 2-17 years), lymphoid progenitors declined to reach a steady state (GM range: 1.6-2.4%). B-cell numbers continued to decrease (2-5 years: GM=5.0%, RR: 0.48-13.4% versus 15-17 years: GM=2.7%, RR: 0.87-8.5%) while T-lymphocytes peaked during the age group of 6-9 years (GM=5.6%, RR: 1.9-16.6%). Following infancy, plasma cells significantly increased and remained constant, but still accounted for <1.0% of the total marrow population (GM range: 0.25-0.47%).
Summary/Conclusion: We have established normal pediatric reference ranges for lymphoid progenitor cells, B- and T-lymphoid cells and plasma cells using a novel, automated enumeration platform. The data is accurate and reproducible. Infant marrows are characterized by having high numbers of immature lymphoid precursors and B-lymphocytes. With increasing age, the marrow shifts towards a more mature profile with increased numbers of T-cells and plasma cells. These findings are in keeping with flow cytometry data. This demonstrates that automated digital enumeration of immunostained cells in BMT biopsies is valid and highly applicable to routine haematopathology practice. Moreover, this automated method overcomes laborious and inaccurate manual estimation of cell number.

PS1164

ROLE OF SPHINGOLIPID SIGNALING IN HEMATOPOIETIC STEM AND PROGENITOR CELL MOBILIZATION

E. Naser[1], J. R. Goethert[2], M. Hanoun[2], A. Carpinteiro[1,2,*]

[1]Department of Molecular Biology, University of Essen, [2]Department of Hematology, University Hospital of Essen, Essen, Germany

Background: Hematopoietic stem cell mobilisation is a prerequisite for high dose chemotherapy, which is a module common in the treatment of several hematopoietic and some oncological malignancies. In about 20% of the attempts stem cell mobilisation turns out to be diffcult or to be insufficient. Therefore, approaches to improve hematopoietic stem cell mobilisation are

a valuable aim.
Aims: The aim of this study is to investigate the role of sphingolipid signaling in hematopoietic stem and progenitor cell HSPC mobilization and improve the outcome of standard clinical mobilizing agents G-CSF and AMD3100 by targeting enzymes in the sphingolipid pathway.
Methods: C57BL/6 acid sphingomyelinase-knockout (Asm-/-) mice, transgenic mice overexpressing acid ceramidase (CAG-Asah1 tg), and control wild-type littermates (wt) were injected s.c. with rhG-CSF 250μg/kg/d for 5 days. On day 5, peripheral blood (PB), spleen and bone marrow were collected. PB was analyzed phenotypically, functionally *in vitro* (CFU-C) assay and *in vivo*. Wild-type mice were treated with Amitriptyline, an inhibitor of Asm. Amitriptyline was applied in the drinking water beginning 2 weeks before and during treatment with rhG-CSF.
Results: CAG-Asah1 tg but not Asm-/- mice showed significantly increased numbers of the lineage- Sca-1+ c-kit+ (LSK) population and CFU-C/mL PB compared with their wt littermates. PB from G-CSF-treated donors was transplanted into lethally irradiated CD45.1 mice. Recipients transplanted with PB from CAG-Asah1 tg but not Asm-/- donors showed significantly increased CD45.2 donor-derived PB chimerism compared with recipients transplanted with PB from CAG-Asah1 wt littermates. Amitriptyline synergizes with G-CSF and significantly increases LSK cells, CFU-C/mL PB and CD45.2 chimerism but, at least partially, via mechanisms independent of Asm inhibition.
Summary/Conclusion: Overexpression of acid ceramidase enhances G-CSF mediated HSPC mobilization via yet not defined mechanisms. Amitriptyline enhances G-CSF mobilization. Outlook: Utilization of Asm-/- model and pharmacological inhibition of Asm with Amitriptyline to enhance the rapid AMD3100-mediated HSPC mobilization.

PS1165

TELOMERASE TRANSCRIPTS FLOATING IN CANCER-DERIVED EXOSOMES REPROGRAM THE MICRORNA TRANSCRIPTOME PROFILE OF THE RECIPIENT CELLS

D. Likonen[1,*], E. Beery[1], P. Raanani[2], M. Lahav[1], U. Rozovski[2], O. Uziel[1]

[1]FMRC, Rabin Medical Center and Tel Aviv University, Petah-Tikva, Israel, [2]Hematological Institute, FMRC, Rabin Medical Center and Tel Aviv University, Petah-Tikva, Israel

Background: Previously, we showed that hTERT transcripts are exported in exosomes to various types of cancer derived cells. These transcripts, formed almost exclusively in neoplastic cells are subsequently taken up by non-neoplastic cells and alter their phenotype. How neoplastic cells modify recipient cells is currently unknown, but since hTERT has been shown to regulate microRNA expression, we hypothesized that hTERT(+) exosomes alter the microRNA profile of recipient cells.
Aims: 1) Verify that hTERT(+) exosomes are taken up by fibroblasts. 2) Determine whether exposure to hTERT(+) exosomes alter the microRNA profile recipient cells. 3) Determine whether the phenotype observed in recipient cells is driven by reprograming of the microRNA transcriptome.
Methods: We grew cells derived from T-cell leukemia cell line (Jurkat cells) in exosome free medium and isolated the exosomes after 72 hours by serial ultracentrifugation. Subsequently we verified that Jurkat cells shed exosomes at large quantities by NanoSight tracking device. We then stained the exosomes with FM-134, exposed foreskin derived fibroblasts to these exosomes and used flow cytometry to determine the time of maximal exosomal uptake. In addition we verified the uptake of the hTERT transcripts via exosomes by RT-PCR. We then extracted the RNA from recipient cells using RNeasy and performed high throughput analysis of the microRNA transcriptome (Rosetta Genomics, Rehovot Israel). We transfected the fibroblasts with a microRNA mimic harboring the sequence of microRNA 342 and studied proliferation (by the SRB method) and cells cycle rate (by PI) in the treated cells.
Results: As we previously showed Jurkat-cell derived exosomes, carry the hTERT transcripts. These exosomes were taken up by fibroblast in a time and dose dependent manner. By microarray we detected seven microRNAs that were more than 2 folds upregulated and two microRNAs that were more than 2 folds downregulated after exosomal exposure. The overexpression of these microRNAs was verified by RT-PCR. Furthermore, after transfection of naïve fibroblasts with miR342, we observed a marked increase in proliferation rate and significant decrease in apoptotic rates, recapitulating the phenotype of fibroblasts following exposure to hTERT(+) exosomes.
Summary/Conclusion: Our current study shows that hTERT(+) exosomes alter the microRNA transcription profile of recipient fibroblasts and that these changes contribute to the oncogenic phenotype of non-transformed fibroblasts.

PS1166

ALTERATIONS IN STROMAL PRECURSOR CELLS IN THE BONE MARROW OF PATIENTS WITH LEUKEMIA IN THE ONSET OF THE DISEASE AND AFTER TREATMENT

N. Petinati[1,*], I. Shipounova [1], N. Sats[1], A. Bigildeev[1], N. Drize[1], L. Kuzmina[1], E. Parovichnikova[1], V. Savchenko[1]

[1]National Research Center For Hematology, Moscow, Russian Federation

Background: Tumor cells and chemotherapy damage bone marrow (BM) stroma in patients with leukemia. Stromal precursor cells (multipotent mesenchymal stromal cells /MSC/ and colony forming unit fibroblasts /CFU-F/) take part in regulation and maintenance of hematopoiesis. The alterations in their characteristics can explain the changes in the hematopoietic system of patients.
Aims: The aim of the study was to compare the changes in MSC and CFU-F from the BM of patients with leukemia at the onset of the disease and at different stages of treatment.
Methods: The study included 33 AML, 20 ALL, 19 CML patients. In all patients, BM was taken at the time of diagnosis, in AML and ALL patients, BM was collected 37, 100 and 180 days after diagnosis, in patients with CML 125 and 225 days after initiation of treatment. From the BM, MSCs were isolated by the standard method and the concentration of CFU-F was determined. The relative expression level (REL) of various genes in CFU-F and MSCs was determined by real-time PCR. Control samples from the BM of healthy donors were selected for each group of patients according to age.

Table 1.

Leukemia stromal precursor cells characteristics compared to donors, fold

Diagnosis		AML		ALL		CML	
Time point		debut	therapy	debut	therapy	debut	therapy
CFU-F	Concentration per 10^9 BM cells	0.52±0.02	0.97±0.07	0.28±0.02	0.85±0.07	0.73±0.05	0.78±0.07
Relative expression level	*BGLAP*	9.5±0.04	2.21±0.05	5.30±0.01	3.36±0.12	2.90±0.01	5.85±0.25
	SPP1	3.4±0.01	0.84±0.04	2.20±0.01	1.20±0.08	2.30±0.01	0.61±0.03
	PPARG	8.5±0.03	2.09±0.04	8.8±0.03	2.98±0.13	3.80±0.02	1.74±0.07
	SOX9	12±0.03	41.67±1.1	23.0±0.12	28.00±1.05	13.00±0.05	27.50±1.02
	FGFR1	3.8±0.01	2.00±0.03	4.1±0.02	1.56±0.03	0.90±0.00	1.54±0.04
	FGFR2	3.5±0.02	2.33±0.07	2.0±0.01	1.40±0.08	1.00±0.01	1.75±0.011
	VEGF	7.8±0.02	1.70±0.03	0.9±0.00	1.37±0.03	1.00±0.00	2.36±0.07
	FGF2	1.4±0.01	1.03±0.03	0.7±0.00	0.81±0.04	1.30±0.01	1.37±0.06
	BMP4	16.8±0.08	5.67±0.16	10.0±0.05	5.92±0.18	3.00±0.02	10.33±0.47
MSC	MSCs cumulaive cell production	0.93±0.08	1.78±0.16	0.62±0.12	0.96±0.17	1.44±0.2	1.33±0.16
	Time to P0	1.25±0.03	1.16±0.03	1.55±0.06	1.17±0.04	1.28±0.04	1.28±0.04
Relative expression level	*IL6*	12.0±0.56	12.4±0.23	34.6±2.15	17.9±0.47	25.2±1.3	7.4±0.21
	IL1B	8.82±0.79	1.97±0.1	3.09±0.28	6.51±0.25	2.11±0.17	1.41±0.05
	IL1BR1	1.42±0.06	0.38±0.01	2.59±0.07	1.22±0.02	2.21±0.13	0.57±0.01
	CSF1	5.85±0.16	0.99±0.02	4.13±0.15	1.01±0.02	2.32±0.05	1.28±0.02
	SPP1	1.05±0.06	0.12±0.00	0.29±0.02	0.12±0.00	0.58±0.04	0.14±0.01
	FGF2	0.64±0.02	0.45±0.01	0.88±0.03	0.82±0.02	0.77±0.02	0.58±0.1
	SOX9	0.59±0.01	0.10±0.00	0.56±0.01	0.12±0.00	0.47±0.01	0.13±0.00
	FGFR1	1.71±0.02	0.35±0.00	1.20±0.03	0.49±0.01	1.17±0.02	0.41±0.00
	VEGF	0.69±0.01	0.08±0.00	0.43±0.01	0.07±0.00	0.65±0.02	0.07±0.00
	VCAM	1.45±0.03	0.54±0.01	3.84±0.22	0.65±0.02	1.62±0.04	0.56±0.01
	SDF1	1.02±0.02	0.09±0.00	1.18±0.03	0.09±0.00	2.43±0.13	0.08±0.00
	ICAM1	1.15±0.03	0.11±0.00	0.51±0.01	0.10±0.00	2.37±0.12	0.11±0.00

Results: The Table 1 shows the ratio of values MSCs and CFU-F characteristics of patients to donors. Concentration of CFU-F in BM of patients with acute leukemia is reduced several times in comparison with donors, and in ALL stronger than in AML. In patients CFU-F the REL of genes responsible for bone, fat and cartilage differentiation is increased both in the onset of the disease and at all stages of treatment. An exception is the REL of the *SPP1* gene associated with terminal bone differentiation, which is markedly reduced and becomes less than that of donors during treatment.
The total cell production of MSCs at the onset of the disease is not significantly different from donors in patients with AML, decreases in ALL and increases with CML. With the treatment, it significantly increases in patients with acute leukemia and continues to be elevated in patients with CML. In all patients, the time to P0, which characterizes the concentration of progenitor cells, is increased in comparison with donors, indicating that the number of MSCs decreases in the stromal microenvironment of leukemia patients. Unlike CFU-F, the differentiation potential in patients MSCs is lowered already in the debut, and then decreases further as decay. Adhesion molecules are increased at the onset of the disease and are greatly reduced with treatment. The REL of proinflammatory cytokines is increased at the onset of the disease and slowly normalize with treatment, with the exception of *IL6*, whose expression

remains elevated tenfold for all leukemia's. The most dramatic changes are associated with the expression of SDF1 and VEGF. The REL of their expression decreased very much as patients were treated.
Summary/Conclusion: The data obtained indicates that the recovery of the quantitative characteristics of stromal precursor cells occurs in the treatment; however, the qualitative characteristics suffer even more from the effects of therapy and are not restored in the observation periods. The function of hematopoiesis maintaining is disturbed in patients with leukemia at all stages of therapy.
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PS1167

LYMPHOPENIA AND RISK OF INFECTIONS AND INFECTION-RELATED DEATH IN DENMARK, 2003-2013: A PROSPECTIVE COHORT STUDY OF 98,344 INDIVIDUALS FROM THE GENERAL POPULATION

M. Warny[1,*], S. E. Bojesen[2,3], H. Birgens[1], J. Helby[2,3,4], B. Nordestgaard[2,3]

[1]Hematology, [2]Clinical Biochemistry, [3]Copenhagen General Population Study, Herlev and Gentofte Hospital, Copenhagen University Hospital, Denmark, Copenhagen, [4]Hematology, Zealand University Hospital, Roskilde, Denmark

Background: Neutropenia increases the risk of infections, but it is unknown if this also applies to lymphopenia.
Aims: To test the hypotheses that lymphopenia is associated with high risk of infections and infection-related death.
Methods: The study is an observational cohort study including 98,344 individuals from the Copenhagen General Population Study. The participants had 8,401 infections, and 1,042 infection-related deaths over a median of 6 years of follow-up. No participants were lost to follow-up. We measured lymphocyte count, and collected information on hospitalization due to any infection, specific infections, and registration of infection-related death.
Results: Individuals with lymphopenia ($<1.1\times10^9$/L) compared to those with lymphocytes in the reference range ($1.1–3.7\times10^9$/L) had hazard ratios of 1.42 (95% confidence interval 1.28-1.56) for any infection, 1.31 (1.13-1.51) for pneumonia, 1.44 (1.16-1.80) for skin infection, 1.26 (1.02-1.56) for urinary tract infection, 1.50 (1.20-1.87) for sepsis, 1.42 (1.04-1.94) for diarrhoeal disease, 2.08 (1.12-3.84) for endocarditis, and 2.18 (1.16-4.09) for other infections. The corresponding hazard ratio for infection-related death was 1.68 (1.36-2.08). Analyses were adjusted for age, sex, smoking status, cumulative smoking, alcohol intake, body mass index, plasma C-reactive protein, blood neutrophil count, recent infection, Charlson comorbidity index, medication use, and immunodeficiency/hematologic disease. The findings were robust in all stratified analyses and also only including events more than 2 years after date of examination.
Summary/Conclusion: Lymphopenia was associated with an increased risk of hospitalization with infections and an increased risk of infection-related death in the general population.

PS1168

HEMATOPOIETIC EFFECTS OF A COMPOUND: SXN AGAINST MYELOSUPPRESSION

L. Ding[1,*], F. Zhou[1], Y. Jin[1], Y. Liu[2], H. Liu[3], B. Wu[1]

[1]Department of Hematology, [2]Department of Clinical Oncology, Zhongnan Hospital of Wuhan University, Wuhan, [3]Department of Hematology, Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China

Background: Shengxuening (SXN), a compound extracted from silkworm excrement, is second grade national new traditional Chinese medicine. The main components of SXN are chlorophyll derivatives and sodium iron chlorophyllin(SIC). SIC, a water-soluble chlorophyll ferrous derivative, which has a quasi structure to heme, just the magnesium in the porphyrin ring has been replaced by iron, can be used as an iron fortificant and promote hematopoiesis.
Aims: The present study aimed to evaluate the efficacy and safety of SXN treatment for iron deficiency anemia(IDA) patients, investigating the hematopoietic effects of SXN on radiation induced myelosuppressed mice, exploring the underlying mechanisms.
Methods: Participants of Phase IV clinical trial were 2001 patients with IDA, randomly divided into three groups. Blood was tested before and after treatment. The mice were irradiated to induce myelosuppressed model. SXN was administered by gavage. The numbers of peripheral blood were counted and body weight, spleen indices were measured. The histology of femur was examined by hematoxylin and eosin staining. The levels of thrombopoietin (TPO), erythropoietin (EPO) and granulocyte-macrophage colony stimulating factor (GM-CSF) in serum were measured by ELISA. Hemopoiesis genes in liver was detected by quantitative real-time PCR array.
Results: The total effective rate in SXN treated group reached 84.8%, significantly increasing levels of RBC, HGB, reticulocyte counts, MCV, MCH, MCHC, SI, SF, reducing levels of TIBC. The incidence rate of AEs was 4.07%. In myelosuppressed model, SXN significantly increased the numbers of peripheral blood cells and improve the bone marrow morphology. The decreases of body weight and spleen indices can be reversed by SXN. Furthermore, SXN could increase the levels of EPO and GM-CSF in serum and enhance the expression of Blnk, Stat3 in liver.
Summary/Conclusion: SXN was proved to be safe and effective in IDA patient population, and confirmed to promote the recovery of hemopoietic function in myelosuppressed model, which attributed to improving bone marrow hematopoiesis; increasing the secretion of hematopoietic growth factors; stimulating the expression of hemopoiesis genes.

PS1169

PI3P MODULATES MEGAKARYOCYTE MATURATION AND PROPLATELET PRODUCTION *IN VITRO*

I. Bertović[1,*], R. Kurelić[1], M. Krauss[2], V. Haucke[2], A. Jurak Begonja[1]

[1]Department of biotechnology, University of Rijeka, Rijeka, Croatia, [2]Department Molecular Pharmacology and Cell Biology , Leibniz Institut for molecular pharmacology, Berlin, Germany

Background: Phosphoinositides (PIs) are short-lived membrane phospholipids that mediate crucial cellular functions inlcluding signalling, gating of ion channels, cytoskeleton regulation and motility. Phosphatidylinositol 3-monophosphate (PI3P), mainly produced by Vps34 kinase, is confined to endosomal compartment where it regulates intracellular membrane trafficking. We hypothesized that traffic of endolysosomal membranes regulated by PI3P could contribute to platelet formation. Platelets are produced within bone marrow from precursor cells, megakaryocytes (MK). MKs generate prolonged cytoplasmic protrusions called proplatelets that extend through the vascular sinusoids and release platelets into the bloodstream.
Aims: The aim of our study was to determine the role of PI3P and endolysosomal trafficking in megakaryocyte maturation and proplatelet formation.
Methods: We analyzed localization of PI3P, early (EE; EEA1) and late endosomes/lysosomes (LE/Lys; Rab7, LAMP1) by confocal microscopy using recombinant PI3P-binding probe (GFP-2xFYVE) in immature and mature primary mouse megakaryocytes (MKs). We found that in immature MKs, PI3P was confined to large vesicles, mostly colocalizing with EE, while in mature MKs PI3P resembled discrete vesicles localized to both EE and LE/Lys. Moreover, while in immature MKs PI3P and LE/Lys were mostly scattered or perinuclear, in mature MKs they translocated to the cell periphery. In mature MKs, PI3P (GFP-2xFYVE probe or expressed PX) colocalized with GPIbβ, or CD61 (MK plasma membrane markers). Also, PI3P partially colocalized with both $PI(4,5)P_2$ and LAMP1 at the same sites, and inhibition of PI3P production (PI3KCIII inhibitor) decreased LAMP1 localization on the plasma membrane. Importantly, PI3KCIII inhibitor significantly reduced the size of MKs when applied at earlier stages
Results: Overexpression of PI3P binding domains YFP-PX and GFP-2xFYVE, but not the expression of YFP/ GFP, diminished production of proplatelets, final stages of MK development, suggesting that they act as dominant-negative inhibitors. These results were further confirmed by inhibition of PI3P production (PI3KCIII inhibitor, overexpression of MTM1), or by inhibiting endosomal maturation to LE/Lys (concanamycin). Furthermore, overexpression of LE small GTPase Rab7 increased proplatelet formation from MKs.
Summary/Conclusion: Altogether, these results suggest that PI3P-positive LE/Lys contribute to membrane growth and proplatelet formation from MKs.

PS1170

IMPACT OF SPLENECTOMY ON CD34+ BLOOD COUNT

V. Savchenko[1], I. Galtseva[1,*], S. Kulikov[1], E. Parovichnikova[1], K. Danishyan[1], N. Kapranov[1], Y. Davydova[1], H. Julhakyan[1], A. Melikian[1], K. Sabirov[1], E. Mikhaylova[1], N. Tsvetaeva[1]

[1]National Research Center for Hematology, Moscow, Russian Federation

Background: Splenectomy may be carried out in the treatment of various hematological diseases such as idiopathic thrombocytopenic purpura (ITP), autoimmune hemolytic anemia (AIHA), lymphomas, primary myelofibrosis (PMF) etc. Spleen participates in the processes of blood cell recirculation,

so investigation of hematopoietic progenitor cells prior and after splenectomy is of great interest. The influence of splenectomy on the amount of CD34+ cells is unknown.
Aims: The aim of the study is to determine the alterations in the amount of CD34+ cells in peripheral blood of patients after splenectomy.
Methods: The study included 90 patients (23 males and 67 females, median age was 55 years) diagnosed with 20 different hematological and non-hematological diseases. Patients were divided into 4 groups: group I (AA, MDS, PNH), group IIa (Lymphomas, CML) and group IIb (AIHA, ITP), group III (PMF) and group IV (non-hematological diseases). The number of CD34+ cells was determined in peripheral blood prior to splenectomy and at day 1, 3, 5, 7 and 14 of post operational period. CD34+ count was determined by flow cytometry according to ISHAGE protocol. For measurement of absolute number of CD34+ cells we determined the absolute number of white blood cells (WBC). We analyze 379 samples of peripheral blood (PB): 90 prior splenectomy and 81, 68, 65, 67 and 20 on 1, 3, 5, 7 and 14 days after operation, respectively. Data is presented as mean±SEM. Wilcoxon criterion was used for paired comparisons (due to non-normal distribution of data) and $p<0.05$ was suggested as significant.
Results: The 3.1 times increase of WBC was shown immediately after splenectomy on +1 day ($p<0.0001$) which was followed by consisted 1,6-times decrease on +7d compared to WBC prior operation ($p=0.0003$).
CD34+ cells (%) in PB slightly decreased on +1d after splenectomy and was 0.9-times lower than initial ($p<0.0001$). This decrease was followed by consisted increase of CD34+ cells (%) up to 3-times in 2 weeks ($p=0.049$). PMF patients had higher CD34+ cells (%) than patients with other diseases before splenectomy. We observed significant decrease in CD34+% after splenectomy in PMF patients (2-times decrease on +1d and 9-times decrease on +3d, $p=0.0159$), but this decrease was followed by continuous increase on later dates. PMF patients had significantly higher CD34+ cells (%) in PB than other patients at any time point after operation.
Absolute number of CD34+ cells in all groups increased 2.6-times on +3d post operation ($p=0.002$), 3.4 ($p=0.0009$) and 3.7 ($p=0.0001$) times on +5d and +7d, respectively. Absolute number of CD34+ cells in peripheral blood samples varied significantly between patients with different diseases. PMF patients had higher absolute count of CD34+ cells before splenectomy and its decrease was observed on +3d after splenectomy. But absolute CD34+ count had increased on later date in PMF and was significantly higher than in patients with other diseases (Table 1).

Table 1.

Groups	n	prior	+1d	+3d	+5d	+7d	+14d
WBC, x10^3/ml							
Group I (AA, MDS, PNH)	12	2.80±0.42	4.92±1.03	4.28±1.24	2.76±0.32	3.66±0.34	3.62±0.36
Group IIa (Lymphomas, CML)	30	20.33±4.6	22.75±5.63	19.25±4.32	17.35±4.21	12.64±2.1	20.33±4.6
Group IIb (AIHA, ITP)	37	8.96±0.97	17.51±2.76	14.28±2.14	12.69±1.23	10.77±0.92	9.13±0.85
Group III (PMF)	5	9.18±2.2	22.74±6.48	17.25±5.51	14.13±3.94	15.77±3.66	17.41±1.85
Group IV (Non-hematological diseases)	6	4.18±1.47	11.63±1.70	12.90±3.16	9.48±1.94	9.08±2.46	6.85±3.36
Total	90	7.78±0.91	16.45±2.00	15.62±2.39	13.63±1.92	12.52±1.99	9.58±1.80
CD34, x10^3/ml							
Group I (AA, MDS, PNH)	12	0.41±0.34	0.56±0.45	1.54±1.26	0.09±0.05	0.11±0.05	0.09±0.05
Group IIa (Lymphomas, CML)	30	2.58±1.02	4.03±2.31	5.29±2.63	5.23±2.23	5.7±1.79	4.01±1.35
Group IIb (AIHA, ITP)	37	2.69±0.57	2.27±0.42	3.97±0.93	3.2±0.65	3.12±0.49	3.11±0.3
Group III (PMF)	5	125.43±39.29	184.70±62.27	33.66±7.82	52.19±6.89	118.24±47.27	113.30±23.49
Group IV (Non-hematological diseases)	6	0.22±0.07	0.77±0.48	0.91±0.40	3.04±1.61	2.59±1.66	1.22±0.86
Total	90	9.14±3.77	13.73±6.22	5.59±1.42	6.54±1.78	11.69±5.13	13.5±7.86

Summary/Conclusion: Alteration in CD34+ cells count after splenectomy is the evidence of great impact of spleen on functional activity of hematopoietic system. Constant increase of CD34+ cells in PMF could be explained by diminishing of hematopoiesis in bone marrow due to fibrosis and sporadic decrease on +3d reflects the extraction of spleen. PMF patients had the highest percentage and count of CD34+ cells in PB before and after splenectomy.

Hodgkin lymphoma – Clinical

PS1171

VERY LATE RELAPSES (VLRS) IN HODGKIN LYMPHOMA (HL) OCCURING ≥5 YEARS AFTER INITIAL TREATMENT WITH CHEMOTHERAPY±RADIOTHERAPY (CT±RT): PATTERN OF RISK OVER 35 YEARS AND THE SIGNIFICANCE OF HISTOLOGY

T. Vassilakopoulos[1,*], M. Angelopoulou[1], E. Kravariti[1], M. Dimopoulou[1], M. Siakantari[2], A. Liaskas[1], G. Gainaru[1], I. Assimakopoulos[1], M. Arapaki[1], P. Diamantopoulos[2], I. Konstantinou[1], G. Boutsikas[1], K. Petevi[1], K. Kanellopoulos[1], T. Giannikos[1], M. Dimou[3], M. Moschogiannis[4], X. Giakoumis[4], I. Pessach[4], S. Sachanas[4], T. Iliakis[3], D. Maltezas[3], C. Kalpadakis[1], V. Komninaka[1], P. Tsaftaridis[1], E. Plata[1], M. C. Kyrtsonis[3], P. Panagiotidis[3], E. Variami[2], N.A. Viniou[2], K. Konstantopoulos[1], G. Pangalis[4]

[1]Department of Haematology and Bone Morrow Transplantation, [2]1st Department of Internal Medicine, [3]1st Propaedeutic Department of Internal Medicine, Laikon General Hospital, National and Kapodistrian University of Athens, [4]Department of Haematology, Athens Medical Center, Psychikon Branch, Athens, Greece

Background: Despite significant improvements in the outcome of HL, VLRs (≥5 years after initial CT±RT) do occur. Data on VLRs after standard CT±RT are very limited, including our initial report and the recent large observational GHSG study with a 20-year follow-up (4935 patients; estimated 45% after BEACOPP). However, the incidence, risk factors and outcome of VLRs in HL require further evaluation, especially after ABVD-like CT. Very prolonged follow-up time is also of paramount importance.
Aims: To examine the incidence and prognostic factors for the development as well as the outcome of VLRs in patients with HL, who remain in first complete remission (CR1) for ≥5 years from treatment initiation.
Methods: Retrospective study of 931 adult patients, diagnosed with HL and remained in CR1 for ≥5 years after treatment initiation (maximum follow-up exceeding 35 years). Anthracycline-based chemotherapy was administered in 89% of patients [mainly A(E)BVD], whereas 11% received MOPP. Survival analysis was performed using competing-risk regression models.
Results: VLRs occurred in 52/931 patients (2/52 relapsed as composite lymphoma) and the most delayed relapse occurred after 35 years. The distribution and cumulative incidence (CI) of VLRs are presented in Table 1. CI rose linearly over time after 5 years and the annual incidence rate was roughly estimated at 0.40% per person-year. After treatment with ABVD CT±RT, the CI of VLR at 20 and 30 years, was estimated at 7.2% and 9.7% respectively. In multivariate analysis, independent protective prognostic factors were nodular sclerosing (NS) histology [hazard ratio (HR)=0.41, $p=0.007$], anthracycline-based (ABVD-type) CT±RT vs MOPP-type (HR=0.49, $p=0.04$) and CT+RT vs CT (HR=0.43, $p=0.03$). Among 830 patients treated with anthracycline-based CT, independent protective prognostic factor was the NS histology (HR=0.33, $p=0.006$); age ≤45 years was of borderline significance (HR=0.57, $p=0.14$). Most patients did not undergo autologous stem cell transplantation. The 5-year and 10-year overall survival after relapse was 67% and 46% respectively. Analysis of treatment and prognostic factors for the outcome of VLRs will be the subject of another study.

Table 1.

Patient subgroups	Patients/ Relapses	Cumulative incidence of relapse at n-years					Annual incidence (%)
		10y	15y	20y	25y	30y	
Total patients	931/52	3.4%	6.0%	8.2%	9.4%	11.1%	0.40
ABVD or equivalent	830/36	2.8%	5.3%	7.2%	8.4%	9.7%	0.32
MOPP or equivalent	101/16	8.0%	11.2%	14.1%	15.9%	17.7%	0.67
Nodular sclerosis	608/20	2.2%	2.8%	5.2%	5.2%	8.5%	0.23
Other histological subtypes	323/32	5.5%	11.5%	13.5%	16.3%	16.3%	0.71
Time	**Patients/ Relapses**	**Follow-up**					**Total**
		5-10y	**10-15y**	**15-20y**	**20-25y**	**25-35y**	
Relapses (#)	931/52	28	13	6	2	3	**52**

Summary/Conclusion: This study is the 2nd largest one aiming to determine the incidence of VLRs in HL, providing the longest follow-up and reporting on the prognostic factors for their development. We confirm the linear pattern of VLR up to 20 years after ABVD-like CT±RT, but we also highlight

that VLRs can occur up to at least 35 years after initial CT±RT. VLRs were more common after MOPP than after ABVD. Patients with non-NS histology had an increased risk of VLRs with mixed cellularity being the most adverse, pointing out to a potentially different biology among different histologic subtypes. The 10-year overall survival after VRLs was not satisfactory with conventional salvage therapy. These conclusions have clinical and biological significance and obvious implications for patient counseling.

PS1172

BRENTUXIMAB VEDOTIN WITH CHEMOTHERAPY FOR STAGE III OR IV HODGKIN LYMPHOMA: IMPACT OF CYCLE 2 PET RESULT ON MODIFIED PROGRESSION-FREE SURVIVAL

R. Chen[1,*], S. Ansell[2], A. Gallamini[3], J. Connors[4], K. Savage[4], G. Collins[5], A. Grigg[6], A. Sureda[7], N. Ghosh[8], T. Feldman[9], A. Fosså[10], E. Ozdemir[11], F. Offner[12], G. Engley[13], K. Fenton[13], C. Lee[14], H. Jolin[14], A. Gautam[14], M. Hutchings[15]

[1]Department of Hematology and Hematopoietic Cell Transplantation, City of Hope National Medical Center, Duarte, [2]Department of Internal Medicine, Division of Hematology, Mayo Clinic, Rochester, United States, [3]Research Innovation and Statistics, Antoine-Lacassagne Cancer Centre, Nice, France, [4]University of British Columbia and British Columbia Cancer Agency Centre for Lymphoid Cancer, Vancouver, Canada, [5]Oxford Cancer and Haematology Centre, Churchill Hospital, Oxford, United Kingdom, [6]Department of Clinical Haematology, Austin Hospital, Melbourne, Australia, [7]Clinical Hematology Department, Institut Català d'Oncologia-Hospitalet, Barcelona, Spain, [8]Department of Hematologic Oncology and Blood Disorders, Levine Cancer Institute/Carolinas HealthCare System, Charlotte, [9]John Theurer Cancer Centre, Hackensack University Medical Center, Hackensack, United States, [10]Department of Oncology, Oslo University Hospital-Norwegian Radium Hospital, Oslo, Norway, [11]Institute of Cancer, Hacettepe University, Ankara, Turkey, [12]Hematology, Department of Internal Medicine, Ghent University Hospital, Ghent, Belgium, [13]Seattle Genetics, Inc., Bothell, [14]Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, United States, [15]Department of Hematology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

Background: The ECHELON-1 trial demonstrated improved outcomes for patients with advanced Hodgkin lymphoma (HL) who received frontline A+AVD (brentuximab vedotin, doxorubicin, vinblastine, dacarbazine) vs ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine), with 2-year modified progression-free survival (mPFS) rates of 82% and 77%, respectively.
Aims: In this *post-hoc* analysis, we examine the mPFS outcomes and clinical characteristics by Cycle 2 positron-emission tomography (PET2) status, per independent review facility (IRF).
Methods: Patients were randomized 1:1 to A+AVD or ABVD on Days 1 and 15 for up to six 28-day cycles. PET scans were conducted at the end of Cycle 2 and end of treatment. PET2 results guided an optional switch to alternative therapy at the treating physician's discretion for patients with a Deauville score of 5. A switch to alternative therapy was not considered an event. The primary endpoint, mPFS, was defined as time to progression, death, or absence of a complete response with subsequent anticancer therapy, per IRF.

Table 1. Summary of mPFS by PET2 Status.

	2-year mPFS (per IRF), %			
	A+AVD	ABVD	HR	P-value
Overall	82.1	77.2	0.77	0.035
95% CI	78.8–85.0	73.7–80.4	0.603–0.983	
N	664	670		
PET2–	85.2	80.9	0.774	0.070
95% CI	81.9–88.0	77.3–84.0	0.586–1.022	
N	588	577		
PET2+	57.5	42.0	0.609	0.089
95% CI	41.0–70.9	28.6–54.8	0.341–1.088	
N	47	58		

Results: PET2 negativity rates (Deauville ≤3) were 89% (588/664 patients) in the A+AVD arm and 86% (577/670) with ABVD. Baseline characteristics were well-balanced across arms, with no significant differences in PET2– vs PET2+ patients in either arm. PET2 positivity rates (Deauville ≥4) were 7% (47/644) in the A+AVD arm and 9% (58/670) with ABVD; in total, 5 patients with a Deauville score of 5 switched to alternative frontline therapy. Subgroup analyses showed a favorable treatment effect for both subgroups in favor of A+AVD (Table 1), with 2-year mPFS (PET2– vs PET2+) of 85.2 vs 57.5% in the A+AVD arm, and 80.9 vs 42.0% in the ABVD arm. In both arms, outcomes for PET2+ patients were poor compared with PET– patients, consistent with findings from other studies.
Summary/Conclusion: Overall, ECHELON-1 demonstrated a treatment effect in favor of A+AVD over ABVD. This post-hoc analysis showed a similar treatment effect on mPFS consistently in favor of A+AVD regardless of PET2 status.

PS1173

NIVOLUMAB TREATMENT DISCONTINUATION IN RELAPSED OR REFRACTORY HODGKIN LYMPHOMA: PAVLOV FIRST SAINT PETERSBURG STATE MEDICAL UNIVERSITY EXPERIENCE

L. Fedorova[1,*], K. Lepik[1], N. Mikhailova[1], E. Kondakova[1], A. Kozlov[1], Y. Zalyalov[1], E. Borzenkova[1], V. Baykov[1], L. Stelmakh[1], L. Tsvetkova[1], B.V. Afanasyev[1]

[1]Raisa Gorbacheva Memorial Institute of Children Oncology Hematology and Transplantation, Pavlov First Saint Petersburg State Medical University, Saint Petersburg, Russian Federation

Background: Nivolumab has demonstrated high response rate and sustained remission in patients with relapsed or refractory Hodgkin lymphoma. However, information about the optimal duration of therapy with nivolumab after achieving the complete response (CR) and the duration of response after discontinuation of nivolumab therapy is lacking.
Aims: To assess the duration of remissions in patients with CR to nivolumab monotherapy after treatment discontinuation.
Methods: 19 adult patients (4 male/15 females, median age 31 years) with relapsed/refractory Hodgkin lymphoma who were treated with nivolumab (3 mg/kg every 2 weeks) and achieved CR were included in this analysis. During therapy, the response was assessed by positron-emission tomography/computed tomography (PET/CT) using LYRIC criteria every 6 cycles. Treatment was stopped because of toxicity or named patient program was closed. After nivolumab therapy had been stopped patients received no other treatment and disease was assessed every 3 months by PET/CT. Median follow-up time since cessation of therapy was 11,1 months.
Results: Before the nivolumab therapy initiation 11 (57,9%) patients had stage 4 disease, 12 (63,2%) patients had B-symptoms. 14 out of 19 patients had previously refractory disease (73,7%), 1 (5,3%) patient had early relapse. The median number of previous therapies was 5 (3-10). High dose chemotherapy with autologous stem cell transplantation (ASCT) was performed in 8 (42,1%), 7 (36,8%) patients received brentuximab vedotin (BV). The median number of nivolumab cycles was 20 (13-30). The median number of cycles before CR achievement was 6 (6-18). The median duration of therapy after achievement of CR was 6,9 months. At the time of analysis, all patients were alive and 17 (89,5%) out of 19 patients are free of relapse. Two patients relapsed after 6,4 months and 5,2 months after therapy discontinuation. In these patients the CR were achieved after 12 and 6 cycles and nivolumab monotherapy was continued for 20 and 25 cycles respectively. After disease relapse the first patient was retreated with nivolumab-containing regimen with achievement of CR and the second patient is also going to be retreated with nivolumab.
Summary/Conclusion: Our analysis demonstrate that in patients with complete response during nivolumab monotherapy, the sustained remissions without additional therapy is possible after cessation of nivolumab treatment but optimal time for discontinuation of therapy is still unknown. Future patients monitoring is planned with retreatment with nivolumab in case of relapse of the disease.

PS1174

BENDAMUSTINE IN COMBINATION WITH NIVOLUMAB IN PATIENTS WITH RELAPSED OR REFRACTORY HODGKIN LYMPHOMA AFTER FAILURE OR SUBOPTIMAL RESPONSES TO NIVOLUMAB THERAPY

K. Lepik[1,*], L. Tsvetkova[1], A. Kozlov[1], E. Kondakova[1], Y. Zalyalov[1], E. Borzenkova[1], V. Baykov[1], N. Mikhailova[1], B. Afanasyev[1]

[1]Research Institute of Pediatric Oncology, Hematology and Transplantology them. By R.M. Gorbachev, St. Petersburg, Russian Federation

Background: Immune checkpoints inhibitors therapy is associated with the high response rate in patients with relapsed and refractory Hodgkin's lymphoma (r/r HL) even after ASCT and brentuximab vedotin (BV). However, a fraction of patients fails to achieve an objective response or have disease progression with clinical manifestation after objective response achievement.

The lymphodepletion and tumor microenvironment shift induced by chemotherapeutic agents are considered as a perspective approach that may enhance the effect of immune checkpoint blockade. Bendamustine (benda) is a bifunctional alkylating agent, which is effective as monotherapy or in combination with BV in patients with r/r HL.
Aims: This report summarizes the preliminary results of First I. P. Pavlov State Medical University of St. Petersburg in the treatment of patients with r/r HL using nivolumab (nivo) in combination with bendamustine after the failure of initial nivolumab monotherapy.
Methods: The analysis included 30 (20 m/10 f) patients. Pts received 90 mg/m^2 benda on D1-2 and 3 mg/kg nivo on Day 1,14 of the 28-day cycle up to 3 cycles. After completion of the Cycle 3 the response evaluation was performed by total body PET-CT scan and assessed by investigators using LYRIC criteria. The adverse events profile was evaluated and scored according to NCI CTCAE v.4.03
Results: The median age was 30 years (21-60). The median number of prior therapy lines was 6 (range 3-11). All patients received nivolumab treatment with a median number of infusions - 18 (8-27). The reason for combined treatment initiation was disease progression in 20 (67%), and indeterminate response in 10 (33%) patients during nivolumab monotherapy according to LYRIC criteria. Prior to nivo-benda combination therapy, 20 (66%) pts had received benda containing regimens earlier during the course of the disease, 14 (46%) had received BV, and 6 (20%) had received nivo-BV combination. At the time of analysis median follow up was 9 months (3-15). Safety analysis showed that 27 (90)% of the pts had drug-related adverse events (AEs) of any grade. Grade 3-4 AEs was noted in 6 (23%) of treated patients. Fatigue (80%), nausea (73%) and pyrexia (37%) were the most common AEs. Grade 3-4 AE's included 4 cases of pneumonia, 1 uveitis, 1 colitis, 1 severe infusion reaction. One patient has discontinued treatment prematurely due consent withdrawal. All patients were evaluated for the response to treatment. The objective response was observed in 26 (86%) patients, a complete metabolic response was observed in 16 (52%) patients. One patient has disease stabilization, one disease progression and 2 have indeterminate response according to LYRIC criteria. Of patients achieved CR, 10 (63%) have received and failed the bendamustine containing regimen earlier, and 9 (56%) have received and failed brentuximab vedotin treatment. Allogeneic transplant was performed in 4 patients. At the median of 9 months, 29 (97%) of patients were alive, and 22 (73%) were free of disease progression. One patient died due to haploidentical transplantation complications.
Summary/Conclusion: Our results suggest that although an elevated incidence of AEs has been observed, toxicities with this regimen appear to be manageable and the combination of benda and nivo may be an effective salvage therapy in patients with r/r HL. Achievement of complete responses in patients that have failed either benda and nivo treatment with the combination of this drugs may suggest a synergistic effect. To draw more solid conclusions it is necessary to expand cohort of patients and increase the duration of follow-up.

PS1175

NIVOLUMAB RESTORES SENSITIVITY TO CHEMOTHERAPY IN CHEMOREFRACTORY CLASSICAL HODGKIN LYMPHOMA PATIENTS

C. Carlo-Stella[1,*], L. Castagna[1], M. Magagnoli[1], L. Morello[1], R. Mazza[1], F. Ricci[1], S. Viviani[2], S. Pileri[3], P. Corradini[4], A. Santoro[1]

[1]Hematology, Humanitas Clinical and Research Center, Rozzano, [2]Hematology, Fondazione IRCCS Istituto Nazionale Tumori, [3]Hematolymphopathology Unit, European Institute of Oncology, [4]Chair of Hematology, University of Milano, Milano, Italy

Background: Classical Hodgkin lymphoma (cHL) patients who relapse after autologous stem cell transplantation (Auto-SCT) and/or brentuximab vedotin (BV) experience unprecedented objective response rates (ORR) when treated with the anti-programmed death-1 (anti-PD-1) antibodies Nivolumab or Pembrolizumab. However, after an initial complete remission (CR) or partial remission (PR) in response to Nivolumab, a substantial proportion of patients will ultimately progress/relapse and have limited treatment options.
Aims: In order to investigate whether chemotherapy might represent a treatment option in this clinical setting, we retrospectively analyzed a series of relapsed/refractory cHL patients who received chemotherapy after experiencing progressive disease (PD) during Nivolumab therapy.
Methods: We analyzed 16 relapsed/refractory cHL patients who received Nivolumab in the context of the CA209-205 phase 2 trial and the CA209-254 Expanded Access Program. After an initial response, all these patients stopped Nivolumab due to progressive disease and were addressed to a variety of chemotherapy regimens. Response to chemotherapy and immunotherapy was assessed according to the Lugano Classification (JCO, 2014) and subsequent LYRIC modifications (Blood, 2016).
Results: The median age of study patients was 30 years (range, 19-57), 60% had Ann Arbor III/IV stage, 33% B-symptoms, 13% bulky disease, and 53% extranodal disease. Prior to Nivolumab therapy, they received a median of 4 (range, 3-6) chemotherapy regimens, 60% had primary chemorefractory disease, and 80% were refractory to last chemotherapy regimen. The best ORR to Nivolumab included CR (n =2), PR (n =9), SD (n =4), PD (n =1). After a median of 26 (range, 10-55) Nivolumab infusions, all patients experienced PD, defined according to LYRIC. In 8 patients, PD was histologically confirmed. Nivolumab was then stopped and the patients were addressed to a variety of chemotherapy regimens [BEGEV (n =5), BEACOPP (n =4), Bendamustine (n =1), BV (n =1), Auto-SCT (n =3), Gemcitabine (n =1), HD-VP-16 (n =1)]. After a median of 4 (range, 1-6) chemotherapy cycles, patients experienced CR (n =11), PR (n =4), and PD (n =1). Interestingly, in some patients, an objective response was achieved using regimens to whom they had resulted refractory prior to Nivolumab. No unexpected toxicity was observed during chemotherapy administration. Fourteen of 16 patients were addressed to Allo-SCT while in CR (n =10) or in PR (n =4), one patient in CR refused allo-SCT and remains in follow-up, and a patient in PD is still alive under Brentuximab Vedotin. At a median follow-up of 12 months (range, 1 – 31) after allo-SCT, 14 patients are alive in CR and 2 died (PD, interstitial pneumonia).
Summary/Conclusion: This retrospective analysis suggests that the great majority of patients who experience PD upon Nivolumab may be effectively rescued with chemotherapy. Notwithstanding their primary or acquired chemorefractoriness, cHL patients may achieve CR or PR using chemotherapy regimens to whom they were refractory prior to Nivolumab, suggesting that Nivolumab may restore chemosensitivity.

PS1176

EARLY DETECTION OF RECURRENCE IN HIGH-RISK HODGKIN LYMPHOMA IN FIRST COMPLETE REMISSION IMPROVES OUTCOME

N. Pugliese[1,*], M. Picardi[1], S. Luigia[1], R. Della Pepa[1], C. Giordano[1], F. Cacace[1], P. Zeppa[1], C. Salvatore[2], F. Pane[3]

[1]University of Naples Federico II, Naples, [2]University of Molise, Campobasso, [3]University Medical School, Salerno, Salerno, Italy

Background: Despite the high complete response (CR) rate to first-line therapy, approximately one-third of patients with advanced-stage Hodgkin lymphoma (HL) eventually relapses. Many relapses (30–50%) are clinically asymptomatic, without any physical and/or laboratory signs. For patients at high-risk of relapse, a close monitoring, based on imaging procedures is justified if an early detection of recurrence would allow a timely administration of salvage therapy and a survival improvement.
Aims: The primary objective of our study was to compare the event free survival (EFS) in three different surveillance cohorts, defined as the time period from the onset of first relapse after induction therapy up to the date of an event or the last follow-up visit after ASCT. Refractoriness to salvage therapy, second relapse or death from any cause were considered events. Secondary endpoints were: (i) the occurrence of relapse without clinical/lab findings in the whole imaging cohort and the relative EFS; (ii) the rate of complete response (CR) after salvage therapy in each group; (iii) clinical characteristic at first relapse as follow.
Methods: We analyzed in this study two cohorts of patients who were under follow-up after therapy for high-risk HL and had disease recurrence. Patients had received one of the following surveillance programs in use at our Institution between June 2004 and December 2016: i) follow-up based on the systematic use of either PET/CT (PET/CT cohort) or ii) ultrasonography (US) plus chest radiography (US/CXR cohort), as integrated part of the conventional follow-up strategy. The two study groups of HL patients were compared to the historical cohort of high-risk HL who received the conventional follow-up program including symptom assessment, blood tests and physical examination (Cilinical/Lab Cohort). All relapsed patients received a salvage therapy (DHAP or IGEV), followed by ASCT.
Results: After a median 33-months (range, 4–108 months), 123 patients experienced relapse. Of those, 43 patients had received the traditional follow-up (Clinical/Lab cohort), while the subsequent 80 were randomly assigned to the PET/CT and the US/CXR follow-up. The EFS after rescue therapy of the two study cohorts was much longer than that of patients who received the traditional Clinical/Lab surveillance (68% and 71% in the PET/CT cohort and UX/CXR cohort, respectively, vs 37.2%; p=0.004 Figure 1). This difference were mainly due to the earlier diagnosis of recurrence in patients with clinical and/or biochemistry markers negative. Indeed, the 2-years EFS was 75.1% in the 51 patients with not silent relapses while

42.2% in the 72 patients with silent relapse (p<0.0001). Furthermore, the CR rate after salvage therapy were higher in the PET/CT cohort (27/40, 67.5%) and US/CXR cohort (28/40, 70%), compared with the Clinical/Lab cohort (18/43, 41.9%), p=0.015. Lastly, advanced stages and bulky disease were more frequent within the Clinical/Lab cohort (67.4%/65.1%) than in the PET/CT (45%/22.5%) and US/CXR cohorts (40%/25%), p=0.028. No difference was found regarding the extranodal involvement and the involvement of three or more nodal areas.

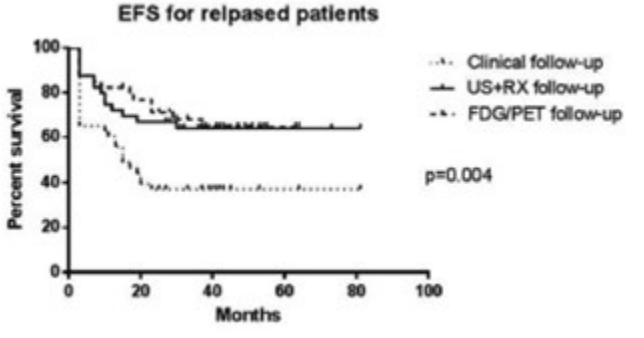

Figure 1.

Summary/Conclusion: Our data provided evidence that an imaging based follow-up strategy, independently if it was based on FDG PET/CT or US/CXR, ameliorated EFS for advanced stage HL patients in first relapse, by allowing us to detect HL recurrence at an earlier stage. We suggested that US plus CXR is a valuable adjunct to conventional follow-up in patients with high risk HL, to be preferred from FDG PET/CT, due to its low-cost and safety.

PS1177

THE RISK OF LUNG CANCER IN HODGKIN LYMPHOMA SURVIVORS; A US POPULATION-BASED STUDY

M.M. Moussa[1,*], A. Saad[2], M. Atia[2], M. Al-Husseini[2], S. Janoudi[3], L. Namous[3]

[1]Internal Medicine, Hematology and BMT unit, [2]Internal Medicine, Ain Shams University, [3]Internal Medicine, cairo university, Cairo, Egypt

Background: The advancements in treatment regiments of Hodgkin lymphoma (HL) have made it a curable disease, with a five-year survival of more than 80%. However, HL survivors are still at higher risk of morbidity and mortality compared to the general population. Second primary malignancies are a major issue with HL survivors, lung cancer is considered one of the most common and serious cancer to occur in this population.
Aims: We aim to estimate the risk of developing lung cancer in HL survivors.
Methods: We used the Surveillance Epidemiology and End Results 'SEER' program of the National Cancer Institute 'NCI' in the United States, to obtain data of HL patients. We selected all patients diagnosed with HL between 1973 and 2014. An event was defined as a diagnosis of a second primary lung cancer after a six-month latency period following HL diagnosis, and we followed patients to observe who experienced the event. We then calculated the Observed/Expected (O/E) ratio, and the excess risk per 10,000 person-years, to estimate the change of risk following the diagnosis when compared to the general US population.
Results: Our study included 26,589 HL patients, of which 594 patients were diagnosed with a second primary lung cancer. The risk of developing a primary lung cancer increased significantly after a diagnosis with HL with an O/E of 2.92 and an excess risk of 11.38 per 10,000. The highest increase in O/E was after 10 years or more following HL diagnosis (3.54), with 377 patients, and an excess risk of 16.83 per 10,000. When calculated according to sex, O/E of both males and females increased significantly; 2.82, and 3.10 respectively. Most patients who developed a second primary lung cancer were in the age groups of 20-44, and 44-65 (253 and 237 patients respectively), with a relatively less number of patients younger than 20 or older than 65. The number of patients younger than 20 who developed lung cancer was 19. However, this group's risk of primary lung cancer increased significantly with an O/E of 7.80. When we analyzed cases according to lymphoma's subtype, all subtypes were associated with an increase in the risk, with most cases having a nodular-sclerosis subtype before the subsequent primary lung cancer. The highest increase in lung cancer risk was following the mixed cellularity subtype with an O/E of 3.28.
Summary/Conclusion: The risk of lung cancer is higher in HL survivors than the general population. This increase in risk may be due to the interaction between different factors related to the cancer itself, radiation exposure, and unhealthy lifestyles. A strict screening program may help detect these cancers earlier, and therefore may decrease associated mortality. Further studies on survival of HL survivors with second primary lung cancers are needed.

PS1178

RELEVANCE OF PRE AND POST TRANSPLANT PET AND HASENCLEVER INDEX IN PATIENTS UNDERGOING AUTOLOGOUS TRANSPLANT FOR HODGKIN LYMPHOMA

S. Turkar[1,*], A. Gokarn[1], L. Nayak[1], S. Punatar[1], A. Bonda[1], L. Mathew[1], S. Kannan[1], B. Bagal[1], N. Khattry[1]

[1]Medical Oncology, Tata Memorial Hospital, Mumbai, India

Background: Autologous stem cell transplantation (ASCT) is the treatment of choice for patients with relapsed and refractory Hodgkin lymphoma (HL). PET response and Hasenclever index (HI) for patients with advanced-stage HL are considered to have prognostic significance in upfront setting. This retrospective analysis was done to evaluate the relevance of various prognostic factors including PET response at predefined time points and HI in patients with relapsed and refractory HL undergoing autologous stem cell transplant.
Aims: To evaluate prognostic factors including pre and post-transplant PET-CT and Hasenclever index in patients undergoing autologous transplant for Hodgkin lymphoma
Methods: All consecutive patients between 1st November 2007- 31st December 2015 who underwent autologous transplant for primary progressive, chemotherapy sensitive relapse or relapsed-refractory HL were included. PET-CT was performed in all patients after 2-3 cycles of salvage chemotherapy. Response assessment post salvage therapy was performed according to Cheson's criteria. PET-CT was done on day 100, day 360 post-transplant and then yearly for next 4 years. Prognostic factors evaluated for overall survival (OS) and relapse-free survival (RFS) included time between diagnosis – transplant; time between CR1 - first relapse; baseline, presalvage and pretransplant serum albumin, LDH and B2 microglobulin; PET status - pretransplant, at day 100 and day 360; remission status at time of transplant; HI at baseline and at relapse; stage at diagnosis and at relapse.
Probabilities of OS and RFS were estimated using the Kaplan–Meier method. Log-rank test and Cox regression analysis were used for univariate and multivariate analysis respectively.
Results: Consecutive 97 HL patients with median age at transplant of 22 years (range: 5-64) were included in the analysis. The median time from complete remission (CR) to first relapse and time from diagnosis to transplant were 1.2 years (range - 0-18years) and 2.56 years (range: 0.3-22 years) respectively. The median number of lines of chemotherapy pretransplant was 2 (range 2-4). At the time of transplant, 66 patients (68%) were in complete remission (CR), 28 patients(29%) in partial remission (PR) and 3 patients (3%) had the refractory state. Median follow up of the whole cohort was 4 years. The OS and PFS at 5 years was 67% and 60% for the whole cohort respectively. Five year OS according to PET status pretransplant (PET negative - 81% vs PET positive - 46%; p=0.001), day 100 (PET negative - 84% vs PET positive - 57%; p=0.033), day 360 (PET negative - 100% vs PET positive- 66%; p=0.007) showed significantly better OS with PET negativity. Five year RFS according to PET status pretransplant (PET negative – 71% vs PET positive - 46%; p<0.001), day 100 (PET negative – 77% vs PET positive - 60%; p=0.027), day 360 (PET negative - 82% vs PET positive- 56%; p=0.001) showed significantly better RFS with PET negativity. Similarly 5 year OS and RFS for patients with stage III and IV disease at relapse with HI ≥ 4 (OS- HI<4 – 71% vs HI ≥ 4 – 38%; p=0.033: RFS- HI<4 – 56% vs HI ≥ 4- 42%; p=0.02) had inferior outcomes. Multivariate analysis of HI at pre-salvage, PET status at pre-transplant, day 100 and at day 360 showed HI to be an independent prognostic marker for overall survival.
Summary/Conclusion: Hasenclever Index at the time of salvage therapy is the most important prognostic factor in our cohort.PET status is also an important prognostic factor with patients showing PET negativity at pre-transplant, day 100 and day 360 have better survival.

PS1179

A REAL WORLD STUDY OF CLINICAL CHARACTERISTICS AND TREATMENT OUTCOME OF HODGKIN LYMPHOMA IN MALAYSIA: A SINGLE CENTER EXPERIENCE IN A RESOURCE LIMITED SETTING

T.S. Leong[1,*], S.L. Hon[1], K.W. Ho[1], S.M. Tan[1], T.C. Ong[1], J.T.C. Tan[1], P.K. Liew[1], N.S. Lau[1], S.S. Abdul Kadir[1], V. Selvaratnam[1], J. Sathar[1]

[1]Department of Hematology, Ampang Hospital, Selangor, Malaysia

Background: Hodgkin's lymphoma (HL) is a rare hematological malignancy of the lymphatic system. It is one of the most curable cancers, with overall survival (OS) of approximately 80-90%. Demographic, treatment and survival outcome data for HL is scarce in the developing world, especially in the Southeast Asian region. Furthermore, a lot of the outcome data were extracted from large scale, phase III studies. Hence, it is still uncertain whether these results are reproducible in clinical practice.
Aims: This is the first 'real world' study from Malaysia, aiming to assess clinical characteristics, treatment and how it might affects survival outcomes in terms of overall survival (OS) and progression free survival (PFS) of HL patients in the context of resource limited setting in the developing world.
Methods: This is a 12-year -retrospective analysis of adult patients diagnosed with HL between 2006 to 2017 in Ampang Hospital, Malaysia. Ampang Hospital is the national hematology referral center with bone marrow transplant facility in Malaysia.
Results: There were 541 patients with a male to female ratio of 1.2 : 1. The median age of patients were 26 years old (range 12-86). They mostly consists of Malay (73.6%), followed by Indian (13.7%) and Chinese (11.5%). Most of the HL were of nodular sclerosing (n= 279, 51.6%) and mixed cellularity (n= 116, 21.4%) subtype. More than half (78.9%) of them had B symptoms at presentation. ABVD was the most common first line treatment (81%, n= 438) followed by escBEACOPP (9.1%, n=49). Approximately 23.7% of the patients have radiotherapy. One hundred and eighty two patients (33.6%) received salvage chemotherapy such as ICE (n= 152), DHAP (n=55) and gemcitabine (n=41). Twenty four percent of patients (n= 135) undergone bone marrow transplant (auto =122, allo= 13). There were 18 patients who were treated with brentuximab. More than half of the patients achieved complete remission (CR, n=298, 55.1%) while 18.5% achieved partial response (PR). The overall response rate (ORR) was 73.6%. Sixty six patients (12.2%) has progressive disease while seventy five patients (13.9%) has relapsed disease. With median follow up of 40 months, the 5 year overall survival (OS) and 5 year progression free survival (PFS) was 78.6% and 66.8% respectively. In multivariable analysis, only complete remission after first line treatment was of independent prognostic significance for both OS (p=0.001) and PFS (p= 0.002). In the subgroup analysis of the transplanted patient (n= 135), the overall survival at 5 years was 78.4%. Disease status at ASCT was also the best predictor of transplant outcome. Patients in CR before transplant had significantly better 5-years OS (86%, p <0.001) and PFS (82.4%, p<0.001).
Summary/Conclusion: In our cohort, the incidence peaked between 16 and 29 (n=346, 64), unlike the bimodal age distribution seen in Western countries. Patients at our center have a delayed presentation with advanced disease (n=459, 84.8%) and IPS score 3 or more (n=274, 59.7%).Default/refuse treatment rate was relatively high (n=54, 10%). . These are among the few reasons our cohort shows a comparatively lower OS and PFS compared to our global counterparts. Despite the emergence of expensive novel agent such as brentuximab and PD1 inhibitors, bone marrow transplant remained the choice of treatment for relapsed refractory HL in resource limited setting as it produces satisfactory OS. A properly designed registry involving all the centers in Malaysia with more patients and longer follow-up are still needed to examine the long-term survival of patients with HL in Malaysia.

PS1180

PROGNOSTIC VALUE OF FDG-PET QUANTITATIVE ASSESSMENT OF TUMOR REDUCTION BY ΔSUVMAX, ΔTMTV AND ΔTLG IN HODGKIN LYMPHOMA PATIENTS TREATED WITH AUTOLOGOUS HEMATOPOIEIC STEM CELL TRANSPLANTATION

P. Perlaza[1,*], V. Ortiz-Maldonado[2], S. Rodríguez[3], G. Gutiérrez[2], N. Sanchez[1], X. Setoain[1], C. Martinez[2]

[1]Deparment of Nuclear Medicine, [2]Deparment of Haematology, [3]Deparment of Radiology, Hospital Clinic, Barcelona, Spain

Background: Disease status of relapsed/refractory Hodgkin lymphoma (HL) after salvage therapy and previous to autologous hematopoietic stem cell transplantation (AHSCT) is a well-recognized prognostic factor. Although positron emission tomography using ^{18}F-fluordeoxyglucose (FDG-PET) is a standard assessment tool in HL, qualitative assessment is object of significant inter-observer variability. FDG-PET quantitative parameters, such as maximum standardized uptake value (SUVmax), total metabolic tumor volume (TMTV) and total lesion glycolysis (TLG), may be more reproducible and accurate to assess response and predict outcome of HL patients after AHSCT.
Aims: To determine the prognostic value of the SUVmax, TMTV and TLG index reduction in relapse/refractory HL patients treated with salvage chemotherapy and AHSCT.
Methods: We retrospectively analyzed 100 FDG-PET of 50 patients with relapse/refractory HL (from 2005 to 2015). FDG-PET was performed pre-salvage therapy (PET0) and pre-ASCT (PET-ASCT). SUVmax, TMTV and TLG reduction between PET0 and PET-ASCT (ΔSUVmax; ΔTMTV; ΔTLG, respectively) was computed. TMTV was measured with a semiautomatic method using 41% SUVmax threshold. Quantitative parameters were measured using MIM® Version 6.7. ROC analysis determined the cut-off to define good response (complete response, CR, plus partial response, PR) vs. bad response (disease progression) for survival outcomes. Kaplan-Meier estimation was used to analyze overall survival (OS) and progression free survival (PFS).
Results: In the analysis of OS, patients with a ΔSUVmax >43.7% (Area Under the Curve, AUC, 0.68), ΔTMTV >73.5% (AUC 0.75) and ΔTLG >82.7% (AUC 0.77) were considered good responders. The number of patients good responders according to SUVmax, TMTV and TLG index reduction, were 39 (78%), 27 (54%), and 32 (64%), respectively. The 5-year OS in the group of good responders vs. bad responders calculated with ΔSUVmax was 96% vs. 44% (p<0.001), with ΔTMTV 100% vs. 71% (p=0.012), and with ΔTLG 100% vs. 60% (p=0.001). In the analysis of PFS, patients with a ΔSUVmax >67.6% (AUC 0.68), ΔTMTV >18.2% (0.75) and ΔTLG >88.5% (0.77) were considered good responders. The number of patients good responders according to SUVmax, TMTV and TLG index reduction, were 24 (48%), 41 (82%), and 26 (52%), respectively. The 5-year PFS in the group for good responders vs. bad responders calculated with ΔSUVmax was 86% vs. 51% (p 0.008), with ΔTMTV 97% vs. 74% (p <0.001), and with ΔTLG 87% vs. 47% (p 0.002).
Summary/Conclusion: Our results suggest that in patients with relapsed/refractory HL, tumor reduction after salvage therapy and prior to AHSCT assessed by ΔSUVmax, ΔTMTV, and ΔTLG, is a good predictor for survival outcomes after transplant. Thus, patients with reductions higher than 86.1% of SUVmax, 18.2% of TMTV, or 88.5% of TLG have an excellent prognosis with PFS at 5-year over 85%. These results deserve confirmation in a large series of patients.

PS1181

DIFFERENTIAL DIAGNOSIS OF ENLARGED LYMPH NODE USING ULTRASOUND ELASTOSONOGRAPHY

N. Pugliese[1,*], S. Luigia[1], F. Cacace[1], R. Della Pepa[1], E. Vigliar[1], C. Giordano[1], D. De Novellis[1], D. Nappi[1], C. Frieri[1], F. Pane[1], M. Picardi[1]

[1]University of Naples Federico II, Naples, Italy

Background: In patients with suspected neoplastic diseases, lymph-node (LN) ultrasonography (US) with Doppler evaluation of hilar arterial vascularization, proved to be a powerful non-invasive tool to investigate lymphadenopathies. However, differential diagnosis could be difficult in some situations. The recent introduction of US elastography (ES) to measure tissue stiffness has increased the capability of conventional US for the early diagnosis of breast and thyroid cancer. In particular, quantitative ES, especially with strain ratio (SR) index, improves diagnostic accuracy for malignancy.
Aims: This prospective study aims to evaluate the diagnostic efficacy of real time ES in differentiating benign from malignant lymphadenopathies.
Methods: We evaluated enlarged LNs of 141 patients with suspect diagnosis of lymphoma using Power Doppler US scan followed by ES. The following LN features were considered for malignancy diagnosis: (a) long axis greater than or equal to 1.5 cm, (b) round shape, (c) hilus absent, (d) hypoechoic parenchyma, and (e) hypervascularization, (ie, neoangiogenesis with high resistive index value [≥ 0.6]). In all patients, we measured LN stiffness using a four points scale and the muscle-to-LN SR. The cutoff point of SR was calculated using the ROC curve analysis. After combined US+ES examination, each lymph node has undergone histological examination.
Results: The pathologic evaluation of lymph node biopsies provides the following diagnosis: 47 benign hyperplasia, 30 Hodgkin Lymphoma (HL), 25 DLBCL, 10 SLL/CLL, 12 follicular lymphomas, 4 acute lymphoblastic leukemia, 3 T-cell lymphomas, 2 marginal zone lymphoma, 2 mantle cell lymphoma and 6 metastatic carcinomas. There was a significant difference in median SR between benign (1.0; range 0.3-3.1) and malignant LN (1.87 range 0.57-3.86), p<0.0001. Among the numerous cutoff values tested, the

SR cutoff value of 1.41 enabled the best distinction between malignant and benign LN, with 92.1% specificity and 72.3% sensitivity. The AUC of the ROC curve was 88% (p<0.0001).
The overall diagnostic efficacy for malignancy of the Power Doppler US followed by ES indicated 97.4% specificity and 77.7% sensitivity, when considering HL.
Summary/Conclusion: ES is a simple, fast and non-invasive diagnostic method that may be a useful aid to US in the assessment of lymphadenopathies in patients suspected of having lymphoma. The high specificity of this test in all lymphomas subtypes could help to avoid unnecessary LN biopsy in patients with non-malignant lymphadenopathies. In the subset of HL it revealed higher sensitivity.

PS1182

FRONTLINE BRENTUXIMAB VEDOTIN PLUS CHEMOTHERAPY EXHIBITS SUPERIOR MODIFIED PROGRESSION-FREE SURVIVAL VS CHEMOTHERAPY ALONE IN PATIENTS WITH STAGE III OR IV HODGKIN LYMPHOMA: PHASE 3 ECHELON-1 STUDY

A. Gallamini[1,*], J. Radford[2], W. Jurczak[3], D. Straus[4], S. Ansell[5], W. S. Kim[6], A. Younes[4], S. Alekseev[7], Á. Illés[8], M. Picardi[9], E. Lech-Maranda[10], Y. Oki[11], T. Feldman[12], P. Smolewski[13], K. Savage[14], N. Bartlett[15], J. Walewski[16], R. Chen[17], R. Ramchandren[18], P.L. Zinzani[19], D. Cunningham[20], D.S. Heo[21], A. Rosta[22], N. Josephson[23], E. Song[23], J. Sachs[24], R. Liu[24], H. Jolin[24], D. Huebner[24], J. Connors[14]

[1]Research, Innovation and Statistics Department, A Lacassagne Cancer Centre, Nice, France, [2]University of Manchester and the Christie NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, United Kingdom, [3]Department of Hematology, Jagiellonian University, Kraków, Poland, [4]Memorial Sloan Kettering Cancer Center, New York, [5]Mayo Clinic, Rochester, United States, [6]Samsung Medical Center, Seoul, Korea, Republic Of, [7]Petrov Research Institute of Oncology, Saint Petersburg, Russian Federation, [8]University of Debrecen, Debrecen, Hungary, [9]Federico II University Hospital, Napoli, Italy, [10]Institute of Hematology and Transfusion Medicine, and Centre of Postgraduate Medical Education, Warsaw, Poland, [11]MD Anderson Cancer Center, Houston, [12]Hackensack University Medical Center, Hackensack, United States, [13]Medical University of Łódź, Łódź, Poland, [14]British Columbia Cancer Agency Centre for Lymphoid Cancer, Vancouver, Canada, [15]Washington University School of Medicine, St Louis, United States, [16]The Maria Sklodowska-Curie Memorial Institute and Oncology Centre, Warsaw, Poland, [17]City of Hope National Medical Center, Duarte, [18]Barbara Ann Karmanos Cancer Center, Detroit, United States, [19]Institute of Hematology 'Seràgnoli', University of Bologna, Bologna, Italy, [20]The Royal Marsden NHS Foundation Trust, Sutton, United Kingdom, [21]Seoul National University Hospital, Seoul, Korea, Republic Of, [22]National Institute of Oncology, Budapest, Hungary, [23]Seattle Genetics, Inc., Bothell, [24]Millennium Pharmaceuticals Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, United States

Background: Approximately 30% of patients (pts) with advanced-stage classical Hodgkin Lymphoma (cHL) have refractory disease or relapse after frontline treatment with doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD). Brentuximab vedotin is a CD30 directed antibody-drug conjugate approved for cHL after failure of autologous stem cell transplantation (ASCT) or ≥2 prior chemotherapy regimens and as consolidation post ASCT for increased risk cHL.
Aims: To compare effects of frontline brentuximab vedotin plus doxorubicin, vinblastine, and dacarbazine (A+AVD) vs frontline ABVD in pts with advanced cHL.
Methods: In this phase 3, unblinded, open-label, multicenter study, 1334 pts with Stage III (36%) or IV (64%) cHL were randomized 1:1 to receive intravenous A+AVD or ABVD on Days 1 and 15 for up to six 28-day cycles. Pts with a PET scan Deauville score of 5 after Cycle 2 could switch to alternative therapy at the treating physician's discretion. Towards end of enrolment, newly randomized pts receiving A+AVD were recommended G-CSF primary prophylaxis due to high incidence of febrile neutropenia (FN) vs ABVD. Primary endpoint was modified progression-free survival (mPFS; defined as time to progression, death, or evidence of incomplete response followed by subsequent anticancer therapy) per independent review facility (IRF) assessment. Overall survival (OS) was key secondary endpoint.
Results: Primary endpoint, mPFS per IRF, was met (hazard ratio [HR] 0.77 [95% CI 0.60–0.98]; p=0.04), with 117 and 146 events in A+AVD and ABVD arms, respectively (Figure 1), and was consistent with mPFS per investigator (INV) (HR 0.72 [95% CI 0.57–0.91]; p=0.006). Causes of mPFS events per IRF were progression (90 vs 102), death (18 vs 22), or receipt of additional anticancer therapy for incomplete response (9 vs 22) after A+AVD or ABVD, respectively. The 2-year mPFS per IRF was 82.1% (95% CI 78.8–85.0) with A+AVD vs 77.2% (95% CI 73.7–80.4) with ABVD, and per INV was 81.0% [95% CI 77.6–83.9) with A+AVD vs 74.4% [95% CI 70.7–77.7] with ABVD. There were 28 and 39 deaths in A+AVD and ABVD arms, respectively (interim OS HR 0.73 [95% CI 0.45–1.18]; p=0.20). Safety profiles were consistent with known toxicities of the single agents. Neutropenia was reported in 58% and 45% and FN in 19% and 8% of A+AVD and ABVD pts, respectively. Discontinuations due to neutropenia or FN were ≤1% in both arms. Grade ≥3 infections were more common with A+AVD (18%) than ABVD (10%). In A+AVD pts, G-CSF primary prophylaxis (n=83) reduced FN from 21% to 11% and Grade ≥3 infections and infestations from 18% to 11%. Peripheral neuropathy (PN) occurred in 67% of A+AVD pts and 43% of ABVD pts (Grade ≥3: 11% A+AVD [1 pt with Grade 4] vs 2% ABVD); 67% of pts experiencing PN in the A+AVD arm had resolution or improvement of PN at last follow-up. Pulmonary toxicity was more frequent and severe with ABVD (Grade ≥3: 3% ABVD vs <1% A+AVD). Of on-study deaths, 7/9 in the A+AVD arm were associated with neutropenia; these deaths occurred in pts who had not received G-CSF primary prophylaxis. Of 13 on-study deaths in the ABVD arm, 11 were due to, or associated with, pulmonary toxicity.

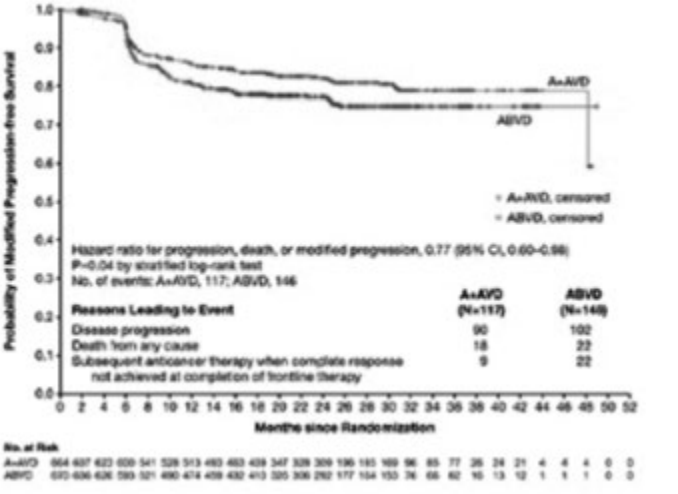

Figure 1.

Summary/Conclusion: Compared with ABVD, frontline A+AVD improved outcomes for pts with advanced cHL, including a 23% risk reduction in progression, death, or need for additional anticancer therapy. This supports the possible use of A+AVD as a new frontline option for pts with advanced-stage cHL.

PS1183

EARLY INTERIM POSITRON EMISSION TOMOGRAPHY (PET) EVALUATION OF RESPONSE AFTER 2 ABVD CYCLES IN ADVANCED HODGIN LYMPHOMA (HL): 8-YEAR EXPERIENCE IN HELLENIC DEPARTMENTS – CONCLUSIONS AND LIMITATIONS

T. Vassilakopoulos[1,*], P. Rontogianni [2], M. Angelopoulou[1], I. Assimakopoulos[1], G. Boutsikas[1], S. Chatziioannou[3], A. Liaskas[1], M. Moschogianni[4], M. Dimou[5], V. Prassopoulos[6], M. Dimopoulou[1], M. Siakantaris[7], F. Kontopidou[8], I. Poulakidas[9], P. Tsirkinidis[9], D. Boutsis[10], C. Poziopoulos[11], S. Sachanas[4], M. Kyrtsonis [5], E. Variami[7], N. Viniou[7], K. Petevi[1], A. Kanellopoulos[1], X. Giakoumi[4], G. Gainaru[1], M. Arapaki[1], I. Konstantinou[1], M. Belia[1], M. Eftasthopoulou[1], I. Pessach[5], T. Iliakis[5], D. Maltezas[5], V. Sotiropoulos[12], I. Datseris[2], J. Meletis[1], P. Panagiotidis[5], G. Pangaliis[4], K. Konstantopoulos[1]

[1]Department of Haematology and Bone Morrow Transplantation, Laikon General Hospital, National and Kapodistrian University of Athens, [2]Department of Nuclear Medicine, Evangelismos General Hospital, [3]Department of PET, The Biomedical Research Foundation (BRFAA) of the Academy of Athens, [4]Department of Haematology, Athens Medical Center Psychikon Branch, [5]1st Propaedeutic Department of Medicine, Laikon General Hospital, National and Kapodistrian University of Athens, [6]Department of Nuclear Medicine, HYGEIA Hospital, [7]1st Department of Internal Medicine, Laikon General Hospital, National and Kapodistrian University of Athens, [8]2nd Department of Internal Medicine, Ippokration General Hospital, National and Kapodistrian University of Athens, [9]Department of Haematology, 401 Military Hospital, [10]Department of Haematology, Navy Hospital, Athens, [11]Department of Haematology, Metropolitan Hospital, Piraeus, [12]PET Department, Athens Medical Center,

Athens, Greece

Background: A positive PET/Computed Tomography (PET/CT) after 2 cycles of ABVD (interim PET - iPET) has a strong adverse prognostic significance in patients with advanced HL.
Aims: The aim of this study was the description of treatment strategies according to iPET results in everyday practice and the evaluation of the outcome according to iPET and other potential prognostic factors in a multicenter setting in Athens, Greece
Methods: Retrospective analysis of 135 patients (134/135 ≤60 years old) with advanced HL according to GHSG (stages III/IV or IIB with mediastinal bulky disease and/or extranodal disease), who received ABVD treatment and underwent iPET. iPET was evaluated according to Deauville 5-point scale and was considered as positive in cases with scores 4 or 5 (residual uptake >liver). In case of iPET positivity, further treatment strategy (intensification to BEACOPP or not) was at the treating physician's discretion. However, consistent strategies were followed within the same Department, with 3/6 departments applying the intensification strategy and 1 continuing with ABVD. Two centers had the intention to intensify treatment but this was not done, due to lack of iPET(+) cases. Radiotherapy (RT) was administered at physician's discretion
Results: The median age was 32 years (15-73), 59% were males, 78% had nodular sclerosis, 11% had mixed cellularity and 4% nodular lymphocyte predominant histology, 42%, 43% and 16% had stage IV, III and II respectively and 66% of patients had B-symptoms. The median IPS was 3 (range: 0-6, ≥3 in 51%). Based on initial PET/CT, 54% of patients had stage IV. Overall, compared to published studies, the patient population described had more adverse characteristics. iPET was negative in 99 patients (73%) and positive in 36 (27%). Overall, the 5-year progression free survival (PFS) was 77%. In particular PFS was 87% in iPET(-) and 46% in iPET(+) patients (p<0.0001), despite that 58% of the latter received BEACOPP. *PET-2 (+) patients:* Out of 36 patients with PET-2(+), 21 received BEACOPP (19 escalated and 2 baseline), whereas 15 continued with ABVD. The 5-year PFS was 57% and 32% respectively (p=0.10). It was 61% vs 33% (p=0.16) for Deauville scores 4 and 5 respectively (68% vs 40%, p=0.31 for those who received BEACOPP and 53% vs 0%, p=0.003 for those who continued on ABVD). *PET2(-) patients:* According to conventional staging there was no difference in 5-year PFS between stages II-IIII or IV (90% and 83% respectively, p=0.46). However, when PET/CT was used for staging, there was a trend to inferior outcome for stage IV (5-year PFS 94% vs 80%, p=0.11).
Summary/Conclusion: This study confirms the prognostic significance of iPET in advanced HL, in spite of treatment escalation in many iPET(+) patients. Treatment intensification to BEACOPPesc in cases of iPET(+) seems to offer satisfactory disease control in real life. More research is need to identify prognostic factors for the outcome of iPET(-) patients.

Indolent Non–Hodgkin lymphoma – Clinical

PS1184

THE IMPACT OF CXCR4 MUTATION SUBTYPES IN THE RESPONSE AND PROGRESSION-FREE SURVIVAL OF PATIENTS WITH WALDENSTROM MACROGLOBULINEMIA TREATED WITH IBRUTINIB

J. Castillo[1,*], J. Gustine[1], K. Meid[1], T. Dubeau[1], L. Xu[1], G. Yang[1], Z. Hunter[1], S. Treon[1]

[1]Dana-Farber Cancer Institute, Boston, United States

Background: Ibrutinib is the only approved drug for the treatment of symptomatic Waldenstrom macroglobulinemia (WM), with a major response rate of 70% and a median progression-free survival (PFS) that has not yet been reached at 4 years of follow-up. Over 40 variants of CXCR4 mutations have been described in WM, and have been associated with lower response rates and shorter PFS in patients treated with ibrutinib.
Aims: To investigate the impact of CXCR4 mutation subtypes in clinical presentation, response and PFS in WM patients treated with ibrutinib.
Methods: We identified patients seen at our institution between May 2012 and December 2017 who met clinicopathological criteria for WM and received ibrutinib therapy. CXCR4 mutations were detected using Sanger sequencing. CXCR4 mutations were divided in 3 groups: wild-type (WT), frameshift (FS) and nonsense (NS). Response was defined based on IWWM6 criteria. PFS was defined as the time between ibrutinib initiation and disease progression or death from any cause. PFS curves were estimated using the Kaplan-Meier method, and compared using the log-rank test. p<0.05 was considered statistically significant.

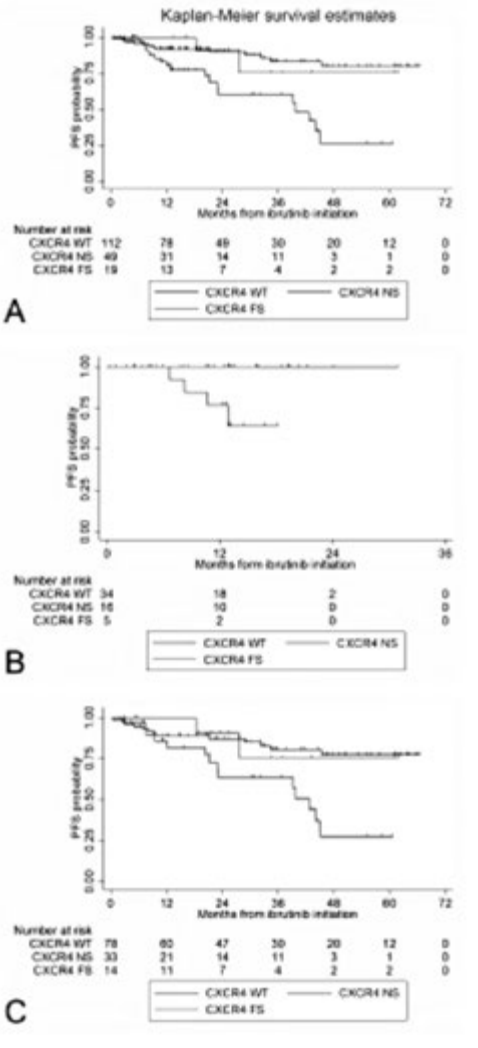

Figure 1.

Results: A total of 180 patients were included. The $MYD88^{L265P}$ mutation was detected in all patients. CXCR4 mutations were detected in 68 patients (38%), of which 49 (27%) had $CXCR4^{NS}$ and 19 (11%) had $CXCR4^{FS}$ mutations. Patients with $CXCR4^{NS}$ and $CXCR4^{FS}$ were more likely to have platelets ≤100 k/ul (17%, 32% and 5%, respectively; p=0.002), were more likely to have serum IgM >7,000 mg/dl (12%, 11% and 3%, respectively; p=0.04), were more likely to initiate therapy due to symptomatic hyperviscosity (27%, 21% and 9%, respectively; p=0.01), and were less likely to present with extramedullary disease (2%, 0% and 16%, respectively; p=0.008) than $CXCR4^{WT}$ patients. Patients with $CXCR4^{NS}$ had lower rate of major response than $CXCR4^{FS}$ and $CXCR4^{WT}$ patients (55%, 79% and 85%, respectively; p<0.001). When compared with $CXCR4^{WT}$ patients, $CXCR4^{NS}$ patients had lower odds of major response (OR 0.25, 95% CI 0.12-0.53; p<0.001) but major response rate was no different in $CXCR4^{FS}$ patients (OR 0.77, 95% CI 0.23-2.56; p=0.67). The median follow-up time is 23 months (95% CI 20-30 months), and 39 patients (18%) have progressed. The median PFS in $CXCR4^{NS}$ patients was 40 months (95% CI 21-45 months) and was not reached in $CXCR4^{FS}$ and $CXCR4^{WT}$ patients (log-rank p<0.001; Figure 1A). The 5-year PFS rate was lower for $CXCR4^{NS}$ patients (60%, 95% CI 40-76%) than for $CXCR4^{FS}$ (91%, 95% CI 51-99%) and $CXCR4^{WT}$ patients (90%, 95% CI 82-95%). In previously treated patients, the median follow-up time was 35 months (95% CI 31-41 months). The median PFS in $CXCR4^{NS}$ patients was 43 months (95% CI 23-NR) and was not reached in $CXCR4^{FS}$ and $CXCR4^{WT}$ patients (log-rank p=0.01; Figure 1B). The 3-year PFS rate was lower for $CXCR4^{NS}$ patients (64%, 95% CI 41-79%) than for $CXCR4^{FS}$ (76%, 95% CI 30-94%) and $CXCR4^{WT}$ patients (81%, 95% CI 68-89%). In previously untreated patients, the median follow-up time was 13 months (95% CI 12-16 months). The median PFS in $CXCR4^{NS}$ patients was not reached in any group, and the 1-year PFS rate was lower for $CXCR4^{NS}$ patients (77%, 95% CI 44-92%) than for $CXCR4^{FS}$ and $CXCR4^{WT}$ patients, in whom no progressions were seen (log-rank p=0.01; Figure 1C).

Summary/Conclusion: When treated with ibrutinib, WM patients with $CXCR4^{NS}$ were more likely to present with low platelets, high serum IgM level, were less likely to have extramedullary disease, and had lower major response rates and shorter PFS than $CXCR4^{FS}$ patients.

PS1185

IBRUTINIB SHOWS PROLONGED PROGRESSION-FREE SURVIVAL IN SYMPTOMATIC, PREVIOUSLY TREATED PATIENTS WITH MYD88 MUTATED WALDENSTROM'S MACROGLOBULINEMIA: LONG-TERM FOLLOW-UP OF PIVOTAL TRIAL (NCT01614821)

S. Treon[1,*], K. Meid[1], J. Gustine[1], T. Dubeau[1], M. L. Palomba[2], R. Advani[3], J. Castillo[1]

[1]Dana Farber Cancer Institute, Boston, [2]Memorial Sloan Kettering Cancer Center, New York, [3]Stanford University Medical Center, Palo Alto, United States

Background: Activating mutations in MYD88 mutations are present in ≥ 95% of WM patients and trigger malignant cell survival through activation of BTK and HCK, both targeted by ibrutinib. CXCR4 mutations are found in 30-40% of WM patients and confer *in vitro* resistance to ibrutinib.

Aims: We examined ibrutinib in previously treated WM patients based on the above findings (Treon *et al.* NEJM 2015), that provided support for the first ever FDA and EMA drug approval in WM. Herein, we provide a long-term follow-up of this study.

Methods: 63 symptomatic WM patients who received at least one prior therapy were enrolled. The median prior therapies were 2 (range 1-9), and 40% of patients were refractory to previous therapy. MYD88 and CXCR4 genotyping was performed for 63 and 62 patients, respectively. Ibrutinib was initiated at 420 mg/day, and dose de-escalation for toxicity permitted. Median time on ibrutinib was 47 (range 0.5-64 months).

Results: At best response, median bone marrow involvement declined from 60% to 20% (p<0.0001), and the median hemoglobin level rose from 10.5 to 14.2 g/dL (p<0.0001). The impact of MYD88 and CXCR4 mutation status on responses, and time to at least minor and major response attainment were as in Table 1. For patients with $MYD88^{Mut}CXCR4^{WT}$, the median PFS was not reached (5-year PFS was 73%; 95% CI 55-85%). For patients with $MYD88^{Mut}CXCR4^{Mut}$, the median PFS was 42 months (5-year PFS 46%; 95% CI 23-67%), and for those with $MYD88^{WT}$ disease it was 5 months (Log-rank p<0.001) The 5-year overall survival for all patients was 87%, and 93% and 80% for $MYD88^{Mut}CXCR4^{WT}$ and $MYD88^{Mut}CXCR4^{Mut}$ patients, respectively (Log-rank p=0.41). Adverse events (Grade ≥2) in ≥5% of patients during active follow-up were: neutropenia (22%); thrombocytopenia (14%), pneumonia (9%); GERD (8%); hypertension (8%); anemia (6%); and skin infection (5%). Seven patients (11%) had atrial arrhythmia, and 6 continued ibrutinib following medical management. Four patients came off protocol therapy for atrial fibrillation (n=1); infection unrelated to drug therapy (n=1), procedure related hematoma (n=1), and thrombocytopenia (n=1). Two others had disease transformation, both with prior nucleoside analogue exposure. One heavily pre-treated patient with pre-ibrutinib 5q- cytogenetics developed AML while a major responder on ibrutinib for WM.

Table 1.

	All Patients (n=63)	$MYD88^{MUT}$ $CXCR^{WT}$ (n=36)	$MYD88^{MUT}$ $CXCR4^{Mut}$ (n=22)	$MYD88^{WT}$ $CXCR4^{WT}$ (n=4)	P-value
Overall Responses (%)	90.4	100	86.4	50.0	<0.001
Major Responses (%)	77.7	97.2	63.6	0	<0.001
VGPR (%)	27.0	44.4	9.1	0	<0.001
Median Time to Minor Response or better (months)	1.0 (range 1.0-22.5)	1.0 (range 1.0-15)	1.0 (range 1.0-22.5)	1.0	0.10

Summary/Conclusion: The findings confirm that ibrutinib is highly active and produces long-term responses in symptomatic patients with relapsed and refractory WM. Prolonged ibrutinib therapy is associated with improvements in categorical responses, including attainment of VGPR. Response activity, time to major response, and PFS are impacted by MYD88 and CXCR4 mutation status in this patient population.

PS1186

IMPROVED DEPTH OF RESPONSE WITH INCREASED FOLLOW-UP FOR PATIENTS (PTS) WITH WALDENSTRÖM MACROGLOBULINEMIA (WM) TREATED WITH BRUTON'S TYROSINE KINASE (BTK) INHIBITOR ZANUBRUTINIB

J. Trotman[1,2,*], C. S. Tam[3,4,5], P. Marlton[6,7], D. Gottlieb[8], D. Simpson[9], G. Cull[10,11], D. Ritchie[3,4], E. Verner[1], J. Munoz[12], S. Ratnasingam[13], M.A. Anderson[2,4], P. Wood[6,7], E. Hedrick[14,15], J. Huang[14,15], S. Ro[14,15], J. Hilger[14,15], J. F. Seymour[3,4], A. W. Roberts[4,16], S. Opat[13,17]

[1]Concord Repatriation General Hospital, [2]University of Sydney, Concord, [3]Peter MacCallum Cancer Center, East Melbourne, Victoria, [4]University of Melbourne, Parkville, Victoria, [5]St Vincent's Hospital, Fitzroy, Victoria, [6]Princess Alexandra Hospital, [7]University of Queensland School of Medicine, Brisbane, [8]University of Sydney, Westmead Hospital, Westmead, Australia, [9]North Shore Hospital, Auckland, New Zealand, [10]Sir Charles Gairdner Hospital, Perth, Western Australia, [11]University of Western Australia, Perth, Australia, [12]Banner MD Anderson Cancer Center, Gilbert, AZ, United States, [13]Monash Health, Clayton, Victoria, Australia, [14]Beigene (Beijing) Co. Ltd., Beijing, China, [15]Beigene (Beijing) Co. Ltd., Emeryville, CA, United States, [16]Royal Melbourne Hospital, Parkville, Victoria, [17]Monash University, Clayton, Victoria, Australia

Background: Zanubrutinib (zanu) is a potent, specific and irreversible oral BTK inhibitor. In Phase 1 testing, we have demonstrated that high plasma concentrations can be safely achieved, resulting in complete and sustained BTK inhibition in blood and lymph nodes. Early clinical data from an ongoing Phase 1 trial has shown single-agent zanu to be safe and efficacious for the treatment of pts with chronic lymphocytic leukemia (Seymour 2017), WM (Trotman 2017), and other non-Hodgkin lymphomas (Tam 2017).

Aims: Report updated safety and efficacy data from WM pts with a median follow-up of >1 year in this Phase 1 trial.

Methods: This is an open-label, multi-center, dose-finding Phase I study of zanu in pts with B-cell malignancies, with indication-specific expansion cohorts: reported here are data from 67 WM pts enrolled as of 3 Nov 2017. Pts received doses of zanu ranging from 40 mg once daily to the final RP2D of 160 mg twice daily until disease progression (PD) or unacceptable toxicity. Pts were assessed by IgM monthly with full assessment of extramedullary disease every 3 months.

Results: 67 pts with WM were enrolled and evaluable for safety; 51 pts were evaluable for efficacy, excluding those with <12 weeks follow-up (n=13) or IgM <5 g/L at baseline (n=3). The 67 pts included 21 treatment-native (TN) and 46 relapsed/refractory (R/R; 1-8 prior therapies) and median follow-up 15.5 months (range, 0.1-37.6). The most frequent AEs (≥15%,

all Gr 1-2 but 1) were petechia/purpura/contusion (37%), upper respiratory tract infection (34%), constipation (18%) and diarrhea (18%). Gr 3-4 AEs included anemia (8%), headache (2%), and diarrhea (2%). Serious AEs (SAEs) were seen in 22 pts (33%) with 5 individual pts (8%) considered related to zanu; febrile neutropenia, colitis, atrial fibrillation, hemothorax (spontaneous), and headache. There was 1 fatal event from worsening pre-existing bronchiectasis in a pt with VGPR. Atrial fibrillation/flutter was experienced by 4 pts (6%), all Gr 1-2, and major hemorrhage seen in 2 pts (3%). Discontinuation of zanu due to adverse events was seen in 3 pts (5%): the previously mentioned fatal bronchiectasis, prostate adenocarcinoma, and gastric adenocarcinoma. Two pts (3%) discontinued study treatment due to PD as assessed by investigator, 1 pt remains on treatment post PD. The objective response rate was 92% (47/51), with a major response rate of 80% (41/51); rate of VGPR increased with increasing follow-up (Table 1). Median time to response was 88 days (range, 77-279). Of 22/51 (43%) efficacy evaluable pts with hemoglobin <10 g/dL at baseline, the median increased from 8.7 g/dL (range, 6.3-9.8) to 13.8 g/dL (range, 7.7-15.8). For all efficacy evaluable pts, median IgM decreased from 32.5 g/L (range, 5.3-88.5) at baseline to 4.9 g/L (range, 0.1-57). For the 21/51 pts with baseline extramedullary disease, median of maximum post-baseline SPD decrease was 46% (range, 18-81%). Median PFS has not been reached; 91% progression-free at 1 year (Figure 1).

Table 1. Efficacy per investigator assessment.

Best Response, n (%)	Best Overall Response (n=51)	Response Over Time for Pts Reaching 1 Year Follow-Up		
		Up to Week 12 (n=39)	Up to Week 24 (n=39)	Up to 1 year (n=39)
VGPR	22 (43.1)	3 (7.7)	6 (15.4)	14 (35.9)
PR	19 (37.3)	23 (59.0)	23 (59.0)	17 (43.6)
MR	6 (11.8)	10 (25.6)	8 (20.5)	6 (15.4)
SD	4 (7.8)	3 (7.7)	2 (5.1)	2 (5.1)

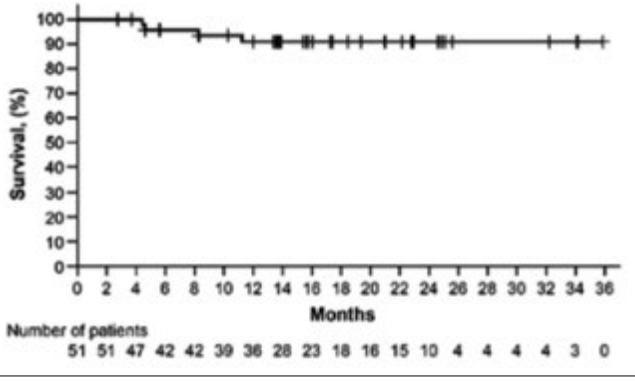

Figure 1. Progression-free survival, efficacy analysis set.

Summary/Conclusion: Zanu is generally well tolerated and highly active in WM with VGPR rates improving over time. A Phase 3 study comparing zanu with ibrutinib in WM is ongoing.

PS1187

CHARACTERIZATION OF MALT LYMPHOMA WITH T(11;18): LONG-TERM FOLLOW-UP

K. Toyoda[1,*], A. Miyagi Maeshima[2], J. Nomoto[1], T. Suzuki[1], S. Yuda[1], N. Yamauchi[1], H. Taniguchi[2], S. Makita[1], S. Fukuhara[1], W. Munakata[1], D. Maruyama[1], K. Tobinai[1], Y. Kobayashi[13]

[1]Department of Hematology, [2]Department of Pathology and Clinical Laboratory, National Cancer Center Hospital, [3]Department of Hematology, International University of Health and Welfare, Mita Hospital, Tokyo, Japan

Background: Translocation (11;18)(q21;q21) is one of the most common chromosomal abnormalities found in mucosa-associated lymphoid tissue (MALT) lymphoma, resulting in API2/MALT1 gene fusion which can activate both the canonical and non-canonical NF-κB pathway. It is well known that MALT lymphoma with t(11;18) shows a tendency to disseminate and be resistant to *Helicobacter pylori* eradication. However, the prognostic features including recurrence and histologic transformation (HT) are largely obscure.

Aims: To evaluate the impact of t(11;18) on MALT lymphoma, focusing on recurrence, survival and HT.

Methods: We conducted a single-institute retrospective analysis of 464 patients with newly diagnosed MALT lymphoma between 1997 and 2014. Among them, those who were screened for the translocation by FISH and/or RT-PCR were extracted. Whenever either serum IgM or IgG level was elevated, we examined *MYD88* gene status to rule out Waldenström macroglobulinemia using allele-specific PCR and Sanger sequencing. After adding information on t(11;18) to the archive data, all patients were categorized as positive, negative, and "not-tested" group. We first compared positive and negative group. Then, to explore a potential selection bias, the positive and negative groups were combined as a "tested" group and compared with the "not-tested" group. The comparisons were made regarding clinical characteristics as follows: sex, age, IPI, MALT-IPI, Ann Arbor Stage, number of extranodal lesion, primary site of lesion, monoclonal gammopathy, HT and initial therapy. To verify the impact of t(11;18) on MALT lymphoma circumspectly, we performed analyses of survival and cumulative incidence of HT only in patients who were tested for t(11;18) and were treated at our institute.

Results: There were 106 patients examined for t(11;18), consisting of 26 (25%) of the positive group and 80 (75%) of the negative group. In the positive group, 11 (42%) patients showed advanced stage III or IV, and primary sites were as follows: multiple (n = 10), lung (n = 6), stomach (n = 6), intestine (n = 2), orbit (n = 1) and salivary gland (n = 1). In addition, patients with t(11;18) had more extranodal lesions, compared to those without t(11;18) (≥2; positive *v* negative, 39% *v* 16% Fisher's exact test, p=0.027). Confirming previous studies reporting that MALT lymphoma with t(11;18) possessed characteristics of disseminated disease and refractoriness to *H. pylori* eradication, we also found that this entity had more frequent monoclonal gammopathy (31% *v* 8%; Fisher's exact test, p=0.008), especially of IgM subtype, some of which developed class switch from IgM.

The median follow-up period was 120 months. The positive group had a significantly shortened progression-free survival (PFS at 10 years; 26% *v* 57%; log-rank test, p=0.004). Additionally, this prognostic feature was present both in the localized and the disseminated disease presentation. The multivariate Cox regression analysis also demonstrated that the presence of t(11;18) significantly influences PFS (p=0.033). However, these findings did not translate into overall survival (OS at 10 years; 87% *v* 92%; log-rank test, p=0.58) or the incidence of HT (4% *v* 8%; Gray test, p=0.497).

Summary/Conclusion: MALT lymphoma with t(11;18) could be characterized by high incidence of monoclonal gammopathy, especially of the IgM subtype, a significantly higher recurrence rate, but with favorable overall outcome. Among the heterogeneities of MALT lymphoma with predictable benign prognosis, it is noteworthy to identify this distinct entity carrying API2/MALT1.

PS1188

CLONAL B-CELL LYMPHOCYTOSIS OF MARGINAL ZONE ORIGIN (CBL-MZ): DESCRIPTION OF MAIN DISEASE FEATURES, CLINICAL COURSE AND OUTCOME IN A SERIES OF 98 CASES

C. Kalpadakis[1,*], G. Pangalis[2], E. Konstantinou[3], T. Vassilakopoulos[3], V. Telonis[3], S. Sachanas[2], P. Tsirkinidis[2], M. Moschogiannis[2], X. Yiakoumis[2], M. Siakantaris[4], P. Tsaftaridis[3], T. Iliakis[3], M. Befani[3], M.-C. Kyrtsonis[5], A. Dimitrakopoulou[6], G. Boutsikas[3], P. Korkolopoulou[7], F. Kontopidou[8], E. Koulieris[2], C. Pontikoglou[1], M. Ximeri[1], M. Psylaki[1], M. Roumelioti[5], D. Rontogiannis[9], H. Papadaki[1], P. Panagiotidis[5], M. Angelopoulou[3]

[1]Department of Hematology, Department of Hematology, University Hospital, University of Crete, Heraklion, Crete, Greece, Heraklion, Crete, [2]Department of Haematology, Athens Medical Center – Psychiko Branch, Athens, Greece, Athens Medical Center, [3]Department of Hematology, [4]Department of Pathology, [51]st Department of Propedeutics, [6]Immunology Laboratory, University of Athens, Laikon General Hospital, [7]Department of Pathology, University of Athens, [8]Second Department of Internal Medicine, University of Athens, "Hippocrateio" General Hospital, [9]Department of Anatomic Pathology, Evangelismos General Hospital, Athens, Greece

Background: CBL-MZ has been recently recognized as a provisional entity with still many unresolved issues.

Aims: To describe disease characteristics and investigate possible prognostic factors for outcome in a large series of CBL-MZ cases with long follow-up.

Methods: CBL-MZ cases were selected based on the presence of circulating CD5-clonal B-cells with MZ features, without B-symptoms, no evidence of disease localization other than bone marrow (BM) and no cytopenias.

Results: 98 patients were analyzed. The median age was 70 years (range,33-90) without sex predilection. Reasons for referral were incidental finding of lymphocytosis (77%) or paraproteinemia (13%) or inverted differential

(10%). The median number of absolute lymphocyte counts (ALCs) and circulating CBL were 6780/μL (1000-150.000) and 3447/μl (185-145.000), respectively. Elevated LDH and paraproteinemia were found in 10% and 38%, respectively. All but one cases presented BM infiltration (median 30%) by small cells with an intrasinusoidal pattern in 1/3 of the cases. Immunophenotyping revealed positivity for CD23, CD11c and CD38 in 31%, 43% and 11% of the cases, respectively. MYD-88 was positive in 9/84 (11%). The presence of paraprotein was significantly associated with MYD-88 positivity (p<0.0001), lower ALCs (p=0.002) and CBL (p=0.001) counts, higher frequency of CD38 expression (p=0.03) and lymphoplasmacytic differentiation in the BM (p=0.001). The degree of BM infiltration was associated with the level of ALCs (p=0.003). At a median follow-up time of 42.3 months, 30 pts progressed but only 10 required treatment. Progression included: worsening lymphocytosis in 17%, cytopenias (4%), splenomegaly (5%), nodal MZL (1%) and increase in paraprotein levels in 3%. Treatment was delivered due to cytopenias in 7 cases, symptomatic paraprotein in 1 and bulky splenomegaly in 2.7 deaths have been recorded, one disease related. The median OS has not been reached; 5-year time to treatment (TTT) was 91%, and the median time to progression (TTP) 95.6 months. By univariate analysis ALC ≥median (6780/μL), p=0.014, CD38+, p=0.006 and elevated LDH, p<0.0001 were significantly associated with TTP. All three factors remained significant by multivariate analysis with a relative risk (RR) of 3.6, 9.0 and 12.9 for ALC, CD38 and LDH, respectively. Based on these parameters, we constructed a prognostic model for TTP that can stratify patients into 3 risk groups: low-risk - 0 risk factors; intermediate - ALC≥median; high-risk – any of the 2 remaining risk factors or ≥2 factors. Median TTP was 159, 96 and 49 months, for the low-, intermediate and high-risk groups, respectively (p<0.0001) Figure 1]. This model was prognostic of TTT, as well, though at a lower level of significance (p=0.03).

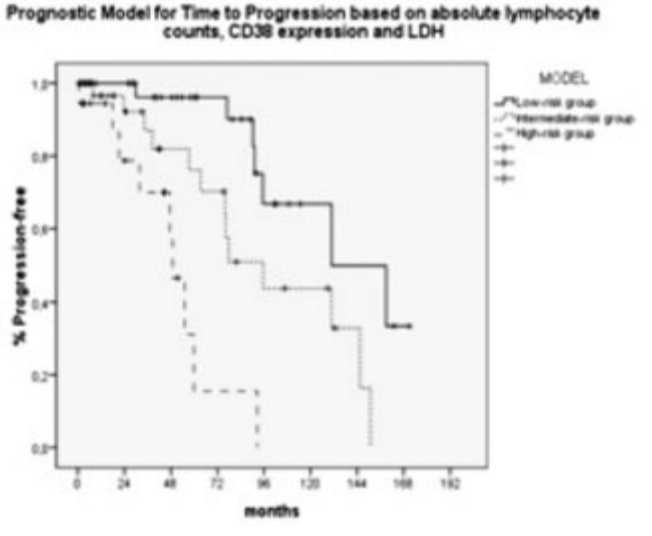

Figure 1.

Summary/Conclusion: CBL-MZ tends to remain stable in the majority of the cases. Progressive disease requiring treatment occurs rarely and it is usually associated with worsening cytopenias. Progression to SMZL was evident in 5%. A prognostic model for TTP using 3 factors was developed: ALCs, LDH and CD38 expression, requiring further validation.

PS1189

OVERALL SURVIVAL OF MANTLE CELL LYMPHOMA (MCL) PATIENTS HAS BEEN SIGNIFICANTLY IMPROVED DURING THE TIME, MAINLY DUE TO THE RITUXIMAB MAINTENANCE IN ELDERLY POPULATION, REAL WORLD DATA

M. Trneny[1,*], D. Salek[2], V. Campr[3], D. Belada[4], K. Steinerova[5], H. Mocikova[6], V. Prochazka[7], J. Duras[8], J. Pirnos[9], K. Kopeckova[10], A. Janikova[2], R. Pytlik[11], P. Blahovcova[12], P. Klener[13]

[1]1st Dept of Medicine, [1]st Faculty of Medicine, Charles University & General University Hospital Prague, 1st Faculty of Medicine, Charles University & General University Hospital Prague, Prague, [2]Dept. of Internal Medicine, Hematology and Oncology, University Hospital Brno, Brno, [3]Dept of Pathology, University Hospital Motol, Prague, [4]4th Department of Internal Medicine - Hematology, University Hospital, Hradec Kralove, [5]Dept. of Hematology/Oncology, University Hospital, Plzen, [6]Dept. of Clin. Hematology, 3rd Medical Faculty, Faculty Hospital Kralovske Vinohrady, Prague, [7]Department of Haemato-Oncology, University Hospital, Olomouc, [8]Department of Haematooncology, University Hospital, Ostrava, [9]Dept of Oncology, Hospital C. Budejovice, Ceske Budejovice, [10]Dept of Oncology, Faculty Hospital Motol, [11]Cell Therapy Department, Institute of Hematology and Blood Transfusion, [12]Czech Lymphoma Study Group, General University Hospital, [13]1st Dept of Medicine, 1st Faculty of Medicine, Charles University & General University Hospital Prague, Prague, Czech Republic

Background: The outcome of MCL patients improved during the time. The main contributors are implementation of HDT with ASCT, high dose Ara-C and rituximab, as part of induction as well as maintenance (RM).
Aims: There is however not clear which one contributes most, or if any has impact on the OS improvement of the whole group of MCL patients. To answer these questions, we have decided to perform analysis of MCL pts diagnosed in 2 period 1999-2005 (1999gr) and 2009-2015 (2009gr)
Methods: This analysis was part of NiHiL project (GovTrial No: NCT03199066), focused on prospective collection of diagnostic, epidemiologic and therapeutic data of NHL pts. All patients signed the informed consent. The eligibility criteria were: all consecutively diagnosed pts with confirmed MCL, availability of clinical characteristics- MIPI, therapy, follow-up. There were 232 pts in 1999gr (dg in 1999-2005) and 596 in 2009gr (dg in 2009-2015), altogether 828 pts. Pearson's Chi-square Test was used for group comparison, log rank test for survival analysis.
Results: The characteristics were comparable between 1999gr and 2009gr,except slightly higher median age in 2009gr (67vs 65y,p<0.001), lower proportion of low MIPI (16.6% vs 26.2%) and higher of high MIPI (50.3% vs 42.1%) in 2009gr vs 1999gr (p<0.01). Median follow-up 12.9y in 1999gr and 4.1y in 2009 gr meant signif. improvement- PFS med. 3.4y vs 1.9y (HR 0.68, CI 95% 0.53-0.77,p<0.0001) and OS median 6.4y vs 4.1y (HR 0.81,CI 95% 0.64-0.95 p<0.01).Pts ≤65y in 2009gr (n 258) have signif. improved PFS with median 7.6 vs 3.6 (HR 0.57, CI 95% 0.39-0.70, p<0.0001), but only trend to better OS with median not reached vs 8.4 (HR 0.81, CI 95% 0.66-1.06,p 0.16) compared to 1999gr (n 120). In contrary pts ≥66y in 2009gr have signif. improved PFS with median 2.3y vs 1.3y and HR 0.66. (CI 95% 0.48-0.80,p<0.0004) as well as OS with median 3.6y vs 2.3y and HR 0.71(CI95% 0.51-0.86,p<0.004). The differences in treatment strategies in 2009gr vs 1999gr consisted of signif. increase R use in induction Tx (93.3% vs 52.6%,p<0.0001),use of HD Ara-C (52.7% vs 29.7%,p< 0.0001),ASCT (25.2% vs 9.5%,p<0.0001),and RM in responded pts (59.0% vs 12.3% p<0.0001). The RM approach was centre based (range between 31.3% and 76.2% pts).We can distinguish 2 RM centre subgroups in 2009gr: RM maintenance policy centres (RM+) with 68.5% (66.4%>76.2%) pts on RM and no RM policy centres (RM-) with 34.6% (31.3%>38.7%) pts with RM. Other parameters were not different between RM+ and RM- centres (RM induction was slightly higher in RM+ 94.9% vs 90.7%,p 0.047).OS improvement was observed only in RM+ centres with HR 0.74(CI95%, 0.52-0.91, p<0.02) for all pts, HR 0.76 (CI95%, 0.44-1.14, p 0.189) for pts ≤65y and HR 0.63 (CI95%, 0.39-0.79, p<0.003) for pts ≥66y. No OS improvement was in RM- centres between 2009gr vs 1999gr,HR 0.91 (CI95%, 0.66-1.20, p ns) for all pts, HR 0.88 (CI95%, 0.53-1.36, p ns) for pts ≤65y and HR 0.82 (CI95%,0.54-1.88, p ns) for pts ≥66y.
Summary/Conclusion: Significant OS benefit was observed in MCL patients diagnosed in 2009-2015 compared to pts diagnosed 10years earlier with relative death risk reduction by 19%. This finding was based mainly on improvement in elderly population ≥66y with 29% death risk decrease. It was observed only in those centres who have adopted rituximab maintenance as a standard of care. Rituximab maintenance (together with R induction) seems to be the most important factors in OS improvement.
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PS1190

IBRUTINIB FOR THE TREATMENT OF BING-NEEL SYNDROME

J. Castillo[1,*], G. Itchaki[1], J. Paludo[2], S. Ansell[2], L. Palomba[3], S. Treon[1]

[1]Dana-Farber Cancer Institute, Boston, [2]Mayo Clinic, Rochester, [3]Memorial Sloan Kettering Cancer Center, New York, United States

Background: The oral BTK inhibitor ibrutinib is the only approved therapy for patients with symptomatic Waldenstrom macroglobulinemia (WM). Bing-Neel syndrome (BNS) is a rare complication of WM, in which WM cells gain access to the central nervous system (CNS) causing neurological deficits. Treatment options in patients with BNS are limited to agents with CNS penetration. Ibrutinib can penetrate into the CNS, but data on its efficacy in BNS is lacking.

Aims: To evalauate the efficacy of ibrutinib in patients with BNS.
Methods: We carried a retrospective study evaluating the efficacy of ibrutinib in patients with BNS. The diagnosis of BNS was established in patients with a clinicopathological diagnosis of WM by radiological and/or cytological evidence of CNS involvement by WM, and response was assessed based on recently published criteria. Ibrutinib was given orally at doses of 420-560 mg PO once daily until disease progression or intolerable toxicity.
Results: We present data on 11 patients with BNS treated with ibrutinib. The median age at diagnosis of WM was 60 years (range 48-73). 6 were men. The median lines of therapy for WM prior to BNS diagnosis was 2 (range 0-7). 4 patients (36%) were untreated for WM at the time of BNS diagnosis. Previous WM therapies included, alkylators (n=11), anti-CD20 antibodies (n=11), nucleoside analogues (n=4), proteasome inhibitors (n=2), immunomodulators (n=2), and autologous transplant (n=1). The median age at BNS diagnosis was 65 years (range 49-78). The median time from WM to BNS diagnosis was 6.6 years (range 0.1-15). In 2 patients, the diagnosis of BNS was made within 6 months of WM diagnosis. The median number of BNS lines prior to ibrutinib was 1 (range 0-4). Previous BNS therapies included high-dose methotrexate (n=5), intrathecal chemotherapy (n=2), bendamustine (n=1) and radiation therapy (n=2). In 5 patients, ibrutinib was the first line of treatment for BNS. The most common symptoms at BNS presentation were motor deficits (n=7), sensory deficits (n=5), cognitive deficits (n=4) and seizures (n=3). MRI findings included leptomeningeal enhancement (n=6) and brain masses (n=4). Cerebrospinal fluid (CSF) cytology showed presence of abnormal cells in 7 patients. CSF flow cytometry confirmed the presence of clonal CD19+ in all patients with abnormal CSF cytology. Biopsies were performed in 3 patients and confirmed presence of WM cells. 1 patient had normal MRI but had abnormal CSF cytology and flow cytometry. Tissue was not obtained in 1 patient but MRI showed leptomeningeal enhancement. The median serum IgM prior to ibrutinib initiation was 1218 mg/dl (range 616-3330 mg/dl), and the median hemoglobin level was 11.8 g/dl (9.2-13.5 g/dl). 7 patients received ibrutinib 560 mg PO once daily, and 4 received ibrutinib 420 mg PO once daily. At best response, median serum IgM and hemoglobin levels were 384 mg/dl (222-3330 mg/dl) and 13.3 g/dl (range 9.2-15 g/dl). MRI findings improved in 7 of 8 patients with available data and were stable in 1. CSF studies cleared in 2 patients and were stable in 2 patients. Symptoms improved in 8 of 9 patients with available data. To date, 4 patients have stopped ibrutinib for BNS, 2 progressed and received methotrexate-temozolomide and fludarabine-rituximab, 1 patient did not tolerate ibrutinib 560 mg and received bendamustine-rituximab, and 1 patient died of BNS progression. The 1-year and 2-year PFS rate were 69% (95% CI 30-89%) and 57% (22%>81%). The 2-year OS rate was 90% (95% CI 47-99%).
Summary/Conclusion: Ibrutinib is a safe and effective treatment option for patients with BNS.

PS1191

IBRUTINIB MONOTHERAPY IN SYMPTOMATIC, TREATMENT-NAIVE PATIENTS WITH WALDENSTROM'S MACROGLOBULINEMIA

S. Treon[1,*], J. Gustine[1], K. Meid[1], T. Dubeau[1], N. Raje[2], A. Yee[2], E. O'Donnell[2], J. Castillo[1]

[1]Dana Farber Cancer Institute, [2]Massachusetts General Hospital, Boston, United States

Background: Ibrutinib is active in previously-treated Waldenstrom's Macroglobulinemia (WM). MYD88 and CXCR4 mutations impact ibrutinib response. We report on the first prospective study of ibrutinib monotherapy in symptomatic, untreated WM patients, and impact of CXCR4 mutation status on outcome.
Aims: To evaluate ibrutinib monotherapy in symptomatic, treatment-naive WM patients. Primary study endpoint was assessment of overall and major response rates. Secondary endpoints included assessment of safety, and impact of MYD88 and CXCR4 tumor mutation status on response outcome.
Methods: Ibrutinib (420 mg) was administered daily until progression or unacceptable toxicity. Dose reduction was permitted for toxicity. All patient tumors were genotyped for MYD88 and CXCR4.
Results: 30 WM patients received ibrutinib. All carried MYD88 mutation, and 14 (47%) CXCR4 mutation. Following ibrutinib, median serum IgM levels declined from 4,370 to 1,513 mg/dL; bone marrow involvement declined from 65% to 20%; and hemoglobin rose from 10.3 to 13.9 g/dL ($p<0.0001$ for all comparisons). Overall (≥ minor) and major (≥ partial) responses for all patients were 100% and 83%, respectively. Major (94% vs. 71%) and very good partial (31 vs. 7%) responses were higher, and time to major responses more rapid (1.8 vs. 7.3 months; $p=0.01$) in wild-type versus mutated CXCR4 patients, respectively. With a median follow-up of 14.6 months, two patients (both CXCR4 mutated) progressed. The 18-month estimated progression-free survival for all patients is 92% (95% CI 73-98%). All patients are alive. Grade 2/3 treatment-related toxicities in >5% of patients included arthralgia (7%), bruising (7%), neutropenia (7%), URTI (7%), UTI (7%), atrial fibrillation (10%), and hypertension (13%). There was no grade 4, or unexpected toxicities.
Summary/Conclusion: Ibrutinib is highly active, produces durable responses, and is safe as primary therapy in symptomatic WM patients. CXCR4 mutation status impacts responses to ibrutinib.

PS1192

CLINICAL OUTCOME OF PATIENTS WITH LOCALIZED PRIMARY OCULAR ADNEXAL MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT) LYMPHOMA (OAL): LONG-TERM FOLLOW-UP RESULTS FROM A SINGLE-INSTITUTION OBSERVATIONAL STUDY

R. Nakai[1,*], D. Maruyama[1], A. Miyagi Maeshima[2], K. Tajima[1], Y. Ito[1], S. Hatta[1], S. Yuda[1], T. Suzuki[1], S. Makita[1], S. Fukuhara[1], W. Munakata[1], T. Suzuki[1], J. Itami[3], S. Suzuki[4], H. Taniguchi[2], Y. Kobayashi[1], K. Tobinai[1], K. Izutsu[1]

[1]Hematology, [2]Pathology, [3]Radiology, [4]Ophthalmology, National Cancer Center Hospital, Tokyo, Japan

Background: As the incidence of non-gastric MALT lymphoma, such as that derived from ocular adnexa, is relatively rare, several clinical questions, including optimal treatment strategy, especially the validity of watchful waiting (WW), incidence of systemic relapse, histologic transformation (HT) and survival, remain unclear. Although radiotherapy (RT) is regarded as the standard treatment for localized OAL, RT-related complications, such as dry eyes and cataracts, are not completely avoidable. We previously reported that WW may be an acceptable approach for OAL patients (pts) [Ann Oncol. 2006; 17: 135.]. Here, we conducted up-dated analysis for evaluating the clinical outcome of localized OAL pts in the recent era.
Aims: The objective of this study was to clarify the clinical outcomes of pts with localized OAL. Furthermore, we compared these outcomes with those of pts treated with WW or RT.
Methods: We retrospectively analyzed pts who were initially diagnosed with localized OAL and managed at our institution between 1997 and 2015. Time to progression was defined as the time from diagnosis to first progression regardless of the initial approach. Freedom from requiring systemic therapy was defined as the time from diagnosis to receiving systemic chemotherapy.

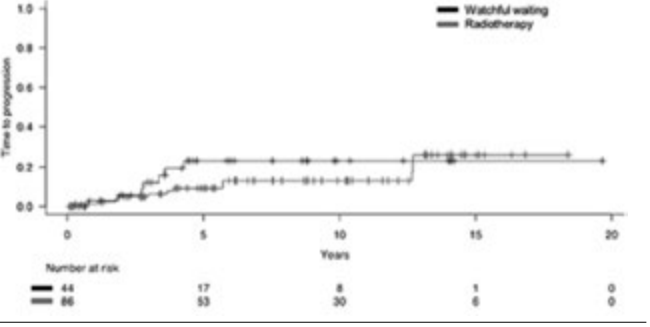

Figure 1.

Results: The subjects in this analysis were 130 localized OAL pts. The median age was 57 years (range 14-89), with 63 males (48%) and 67 females (52%). All 130 pts had stage I disease, consisting of 107 unilateral (82%) and 23 bilateral (18%) legions. The most frequently involved site was the conjunctiva (50%). Forty-four pts were initially managed with WW (WW group) and 86 pts received immediate RT (RT group) at the physician's discretion. During the median follow-up duration of 7.5 years (range 0.1-20), no patients died due to lymphoma progression or any other cause. Among the 44 pts in the WW group, 7 pts had lymphoma progression at their primary site (6 pts) or HT at a distant lymph node (1 pt). Thirty-seven pts (84%) in the WW group did not require any treatment during the follow-up period. In the RT group, all 86 pts achieved complete response after RT, but 12 pts relapsed at the opposite ocular adnexa (4 pts), other extranodal sites (3 pts), distant lymph nodes (3 pts), at the primary site within the radiation field (1 pt) or HT at submandibular gland (1 pt). Notably, 2 relapses at an extranodal site (pleura or stomach) were observed more than 15 years after RT. The time to progression estimated by the cumulative incidence at

5 and 10 years in both groups (WW vs RT) was 23% (95% CI 6-37%) and 9% (95% CI 2-16%), and 23% (95% CI 6-37%) and 13% (95% CI 5-21%), respectively, with no significant difference between the 2 groups (p=0.377) (Figure 1). Freedom from requiring systemic therapy at 10 years were 94% (95%CI 85-100%) in WW group and 96% (95%CI 91-100%) in RT group, respectively. There were no significant differences between the 2 groups (p=0.529). Regarding RT-related complications, 27 pts (31%) and 49 pts (57%) developed dry eyes and cataracts, respectively. Furthermore, nearly 30% of pts who developed cataracts after RT needed surgical intervention.

Summary/Conclusion: This updated observational analysis suggested that the WW strategy is an acceptable treatment option for selected pts with localized OAL because 84% of WW pts remained untreated at a median of 7.9 years, and there was no significant difference in time to progression, time to systemic therapy, incidence of systemic relapse, HT or survival between the WW and RT groups.

PS1193

CD5-NEGATIVE MANTLE CELL LYMPHOMA: A DIAGNOSTIC CHALLENGE IN LEUKEMIC NON-NODAL CASES EXCLUSIVELY DIAGNOSED IN PERIPHERAL BLOOD

S. Montesdeoca[1,*], X. Calvo[1], L. Arenillas[1], A. Puiggros[1], M. Salido[1], A. Salar[2], B. Sánchez-González[2], L. Colomo[3], N. Papaleo[3], R. Navarro[1], B. Costan[1], L. Florensa[1], B. Espinet[1], A. Ferrer[1]

[1]Laboratoris de Citologia Hematològica i Citogenètica, servei de Patologia. GRETNHE- Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), [2]Servei d'Hematologia, [3]Servei de Patologia. GRETNHE- Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Hospital del Mar, Barcelona, Spain

Background: Mantle cell lymphoma (MCL) is an aggressive mature B-cell neoplasm. Indolent variants, including leukemic non-nodal MCL (nnMCL), are now well recognized. One of the most characteristic features of MCL is the expression of CD5. Approximately 5-10% of cases are CD5 negative but precise data on this issue are scarce. Recent studies suggest that CD5 expression may be less common in leukemic nnMCL than in classic MCL (cMCL). Since the lack of expression of CD5 can hinder the diagnosis of MCL, it is important to contribute with useful data delineating this particular subset, specially in leukemic non-nodal cases, to avoid misdiagnosis with other diseases such as the recently recognized clonal B-cell lymphocytosis of marginal zone origin (CBL-MZ).

Aims: To determine the incidence of CD5-negative cases in a series of MCL patients diagnosed in a single institution, with special emphasis on cases diagnosed exclusively in peripheral blood (PB), and to analyze additional phenotypic features that could be useful to differentiate CD5-negative MCL from CBL-MZ.

Methods: 53 patients diagnosed with MCL according to the WHO classification between 1997 and 2017 (median age 68 years, range 42-88; 60% male; 44 cMCL, 9 clinically indolent leukemic nnMCL) were selected on the basis of PB flow cytometry (FC) availability. The diagnosis of MCL was first established in lymph node (24), spleen (3), tonsil (4), salivary gland (1), colon (1) and PB/bone marrow (BM) (20). All cases displayed the t(11;14)(q13;q32) by cytogenetics or FISH. Cyclin D1 expression was detected on histologic sections in all cases. FC analysis was performed according standard procedures. The expression of pan-B cell markers, CD5, CD10, CD11c, CD23, CD25, CD38, CD43, CD49d, CD200, kappa and lambda was assessed in most cases. Positivity was considered when at least 20% of cells expressed a single marker.

Results: CD5 expression was observed in 38/53 MCL patients (72%) by FC or immunohistochemistry (IHC). CD5 expression could be assessed by FC in PB and IHC on tissue samples (including BM) in 43/53 patients. In 7 cases, results were discordant (FC+/IHC-, 3 cases; FC-/IHC+, 4 cases). Overall, 9/44 (20%) cMCL and 6/9 (66%) clinically indolent leukemic nnMCL were CD5 negative. PB involvement by FC at diagnosis was detected in 48/53 (90%). Among cases with PB involvement, 15/48 (31%) corresponded to CD5-negative MCL by FC: 9/15 (60%) were cMCL and 6/15 (40%) leukemic nnMCL. All non-leukemic cases expressed CD5 in tissue samples. Regarding the immunophenotypical features of 20 MCL diagnosed exclusively in PB/BM, 10/20 (50%) were CD5 negative. None of these CD5-negative cases expressed CD43, all but one were CD11c negative, 1/10 expressed CD23, and 2/8 cases were CD25 positive.

Summary/Conclusion: In our series, lack of CD5 expression was observed with higher incidence (28%) than previously recognized. Of note, 40% of CD5-negative cases corresponded to leukemic nnMCL. Moreover, lack of CD5 was observed in 50% of cases diagnosed exclusively in PB/BM samples. To avoid MCL underdiagnosis and misdiagnosis with CBL-MZ, a high degree of suspicion is necessary in the diagnosis workup of the disease. Therefore, histologic and cytogenetic studies must be mandatory in all mature B-cell neoplasms with a non-specific phenotype. Additional immunophenotypical data can be potentially useful in the distinction of these entities, being especially helpful the absence of CD11c expression in MCL.

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PS1194

MARGINAL-ZONE LYMPHOMA WITH AND WITHOUT ASSOCIATION OF HEPATITIS C VIRUS INFECTION: CLINICAL CORRELATION AND TREATMENT RESULTS

G. Tumian[1,*], S. Lepkov[2], Y. Ribuchina[3], O. Kolomeytsev[3], Y. Kluchagina[3], P. Zeynalova[3], I. Subortseva[4], N. Kokosadze[3], S. Borisovckay [5], Y. Kemih [5], R. Ivashenko [5], O. Urvanceva [5], N. Kuprichina[3], T. Padjeva[5], O. Zaharov[6], I. Lazarev[6], V. Ivanova[6], O. Ettinger[7], I. Nikitin[7]

[1]N.N. Blokhin Cancer Research Center, [2]National Research Medical University named by N.I. Pirogov, [3]24Kashirskoy sh., N.N. Blokhin National Cancer Research Center, [4]National Medical Research Center for Hematology, [5]24Kashirskoy sh., City Clinical Hospital. V.M. Buyanova, [6]24Kashirskoy sh., Hematologic Moscow City Center at the State Clinical Hospital named by S. Botkin., [7]24Kashirskoy sh., National Research Medical University named by N.I. Pirogov, Moscow, Russian Federation

Background: The association between hepatitis C virus (HCV) and B-cell non-Hodgkin lymphomas (NHL) is now widely accepted, as a result of evidence from epidemiological and therapeutic studies carried out in the last decades. Among B-cell NHL indolent subtypes, HCV has been consistently associated with marginal-zone lymphomas (MZL). A large number of studies demonstrated that front-line antiviral therapy (AT) with interferon (IFN) and ribavirin is able to induce high response rate in HCV-associated indolent B-cell lymphomas.

Aims: The purpose of our study is evaluated clinical feature and outcomes of MZL patients with and without association HCV infection.

Methods: We performed a retrospective analysis of 56 pts with MZL (Group 1) and 27 pts with HCV+ MZL (Group 2) since 2005-2017 treated in N.N. Blokhin National Cancer Research Center. Median follow-up was 48 months and 52 months. There were statistically significant differences in the clinical manifestations of the MZL in patients in the Group1 and Group2 (p<0,05): the median age was 58 and 43 years, 21% and 59% were male, 41% vs 74% had IV stages. There were also differences in the distribution of MZL subtypes (p=0,05): Nodal 14% vs 7%, Extranodal 54% vs 30%, Splenic 14% vs 30%, Disseminated 18% vs 33% respectively of HCV-neg and HCV+MZL. Front-line systemic therapy for MZL without HCV was received 47 of 56 pts (84%): Rituximab monotherapy (13%), R-CHOP\CVP (53%), RB (25%), R-Chl (9%). All 27 pts with HCV+MZL as first-line has IFN-based AT.

Results: The overall response rate (ORR) and duration of response were comparable in the Group1 and Group2. ORR were 87% vs 83% (p=ns), the estimated 3-year progression-free survival (PFS) were 72% vs 67% respectively (p=0.6). In univariate analysis, there was a higher risk of not-response\relapse in HCV+MZL pts with low Hb and albumin levers and spleen involvement. In contrast, the favorable prognostic factors in this group were extranodal disease and serum mixed cryoglobulinemia (OR, 0.1;95% CI).

Summary/Conclusion: Antiviral therapy is a preferential first-line option in patients with HCV-associated marginal zone lymphoma. HCV+MZL patients on IFN-based AT without additional complications have the comparable high response rates and long-term progression-free survival, as patients with MZL on systemic antitumor therapy. Very preliminary data about the use of the new direct-acting antiviral agents (DAAs) suggest a similar activity in HCV+ NHL, but this data needs future investigation.

PS1195

STUDY OF THE DISSEMINATION PATTERNS AND CLONAL RELATIONSHIP IN DIFFERENT SITES IN MULTI-SITED MALT LYMPHOMAS. A SINGLE CENTRE ANALYSIS

M. Condom[1,*], D. Fernandez[2], F. Climent[2], M. Varela[3], J. Hernández Gañán[4], S. Mercadal[1], E. González-Barca[1], I. Carro[1], M. Oliva[1], M. Pané[2], C. Aguilera[1], A. Sureda[1], E. Domingo-Domènech[1]

[1]Hematology, Hospital Duran i Reynals, Institut Català d'Oncologia, [2]Pathology, Hospital Universitari de Bellvitge, [3]Pathology, Hospital Universitari de Bellvitge. Institut Català d'Oncologia, [4]Radiology, Hospital Universitari de Bellvitge, Barcelona, Spain

Background: Extranodal mucosa-associated lymphoid tissue (MALT) lymphomas account for 7-8% of all non Hodgkin lymphomas, being the stomach the most frequent location. It is a multiorganic disease in approximately 50% of extragastric MALT lymphomas, and 25% of gastric ones. In clinical practice, it is often assumed that simultaneous and subsequent lymphoid neoplasms in the same patient come all from one common origin, and so they are clonally related. However, the evidence supporting this fact in MALT lymphomas is scarce. To date, there is very few literature published about the clonal relationship of MALT lymphomas with multiorganic involvement.
Aims: To study clonality in multi-sited MALT lymphomas.
Methods: We reviewed all the cases of MALT lymphoma diagnosed at a single centre between 2009 and 2017. We selected the cases with multi-sited disease, including those with multifocal disease (multiple involvements within one organ, e.g. both side ocular lesions) and those with multiorganic disease, and we excluded those with exclusive skin involvement. Demographic and clinical data regarding different locations and treatment response were collected. Clonality was detected by polymerase chain reaction (PCR) for immunoglobulin heavy chain (IGH) FR3 and FR2 primers and for immunoglobulin kappa chain (IGK) at the locations with available specimens.
Results: Eighty patients were diagnosed with MALT lymphoma during the study period. 27 (34%) of those had a multi-sited disease. Thirteen patients were males and 14 females, with a median age of 60 years (range 38-84). In 19 patients the multi-sited involvement was synchronous and in 8 it was sequential. The most frequently involved primary locations were lung (9 specimens from 5 different patients) followed by stomach (7 specimens from 6 patients) and orbit (5 specimens from 3 patients). The most frequent dissemination patterns were: from primary site to lymph nodes (n=8), to the bone marrow (n=6), and bilateral involvement of paired organs (3 patients with bilateral lung involvement, 2 patients with dissemination within the gastrointestinal tract). Twenty-three patients received systemic treatment, 2 radiotherapy alone and 1 surgery alone. Clonality was analysed in 34 specimens from 20 different patients. In 9 patients it was detected in two or more of the specimens available. Regarding those nine patients, four had different clones in the different sites analysed. Two of these 4 had synchronous multi-sited involvement and in the other 2 cases it was sequential. Specific data regarding the patients' characteristics and the involved locations are depicted in Table 1.

Table 1. Clinical characteristics of the patients, involved locations and clonality.

Patient	Sex	Age at diagnosis	Primary location/s and clone	Treatment and cycles	Relapse	Secondary location/s and clone	Follow-up (time in months and status)
A	F	62	Endometrium (A) Stomach (A) Bone marrow (B)	R-FxS	No		20, ACR
B	M	55	Stomach (A) Loco-regional lymph node (B)	R-FxS	No		6, OT
C	F	68	Parotid gland (A)	RT	Yes	Right lung (D)	87, ACR
D	F	63	Salivary gland (A) Left breast (A)	R-FxS	No		24, ACR
E	F	58	Left parotid gland (A) Right breast (A)	RxA	No		23, ACR
F	F	70	Salivary gland/ Loco-regional lymph node (A)	RxA	Yes	Loco-regional lymph node (A)	27, AWD
G	M	40	Stomach (A) Right lung (A)				76, ACR
H	F	44	Left breast (A) Bone marrow* Left lung (A) Stomach (A)	R-BxS	No		70, AWDL
I	M	38	Duodenum (A) Esophagus (A)	R-FxS	Yes	Duodenum (B)	9, OT

*No biopsy. †Clonality non evaluable. R-F: Rituximab-Fludarabine; RT: Radiotherapy; R: Rituximab; R-Bendamustin; (A): same clone, (B): different clones; ACR: Alive, complete response, OT: ongoing treatment, AWD: Alive, with disease, AWDL: Alive, with high grade B lymphoma

Summary/Conclusion: MALT lymphoma was a multi-sited disease in 34% of our cases. Of the 9 cases in which clonality could be compared, almost half of them presented different clones, irrespective of having a synchronous or sequential multi-sited involvement. This is the largest series that compares clonality in multi-sited MALT lymphomas.

Infectious diseases, supportive care

PS1196

GENETIC POLYMORPHISMS OF DC-SIGN AND L-FICOLIN ARE ASSOCIATED WITH INFECTIOUS COMPLICATIONS AFTER INDUCTION CHEMOTHERAPY: A VALIDATION STUDY IN PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA

U. Schnetzke[1,*], C. Röllig[2], M. Fischer[1], H. Altmann[2], F. Stölzel[2], N. Alakel[2], G. Ehninger[2], M. Bornhäuser[2], A. Hochhaus[1], S. Scholl[1]

[1]Klinik für Innere Medizin II, Abteilung für Hämatologie und Internistische Onkologie, Universitätsklinikum Jena, Jena, [2]Medizinische Klinik I, Universitätsklinikum der Technischen Universität Dresden, Dresden, Germany

Background: Infectious events including invasive fungal disease (IFD) remain a significant cause of morbidity and mortality in neutropenic patients with acute myeloid leukemia (AML). Several studies propose a contribution of single nucleotide polymorphisms (SNPs) of the innate immune system to the individual risk of infections but the majority of those association studies are not validated in an independent cohort.
Aims: We sought to replicate the impact of 11 candidate SNPs in 6 genes (TLR2, TLR4, Dectin-1, DC-SIGN, PTX3, L-Ficolin). All SNPs were associated with infectious events such as sepsis, pneumonia or IFD in our own study cohort or as published by other groups.
Methods: Our initial cohort included 186 AML patients, all of them treated at Jena University Hospital using single induction chemotherapy according to the OSHO protocol. In contrast, our independent validation cohort consists of 138 adult patients with newly diagnosed AML who underwent "3 plus 7" induction chemotherapy at the Dresden University Hospital (SAL study group). Genotyping of TLR2, TLR4, Dectin-1, DC-SIGN, PTX3 and L-Ficolin SNPs was performed by TaqMan assay. Multiple logistic regression analyses were applied to evaluate the association between the polymorphisms and the occurrence of infectious events.
Results: We were able to validate the association between the DC-SIGN SNP rs4804800 and the development of sepsis (OR: 2.58; 95% CI: 1.1 – 6.2, p=0.042). A statistical trend for this SNP was seen for pneumonia including IFD. We could also replicate a trend towards a higher risk for developing pneumonia including IFD for patients carrying either the L-Ficolin SNP rs17549193 or rs17514136. Within the validation cohort two SNPs in the PTX3 (rs1840680) and Dectin-1 gene (rs16910526), respectively, were associated with the development of IFD: rs1840680: OR 7.4, (95% CI: 1.7 – 33.3, p=0.002) and rs16910526 OR 2.8, (95% CI: 1.2 – 7.0, p=0.027).
Summary/Conclusion: To our best knowledge, this study represents the first validation study of candidate genes that have been associated with infectious events in AML patients following induction chemotherapy. We are able to confirm an association between SNPs of DC-SIGN or L-Ficolin and the development of sepsis and pneumonia in AML patients.
The different impact of PTX3 (rs1840680) and Dectin-1 (rs16910526) polymorphisms on the occurrence of IFD in the validation cohort underlines the impact of case numbers but might at least in part also be due to slight differences between the induction regimens and the following duration of neutropenia.

PS1197

Abstract withdrawn.

PS1198

ONE-YEAR SURVIVAL AMONG PATIENTS WITH HEMATOLOGICAL DISEASE ADMITTED TO THE INTENSIVE CARE UNIT: A NATIONWIDE COHORT STUDY

P. Asdahl[1,2,*], S. Christensen[3], A. Kjaersgaard[4], C.F. Christiansen[4], P. Kamper[1]

[1]Hematology, Aarhus University Hospital, Aarhus, [2]Hematology, Regional Hospital West Denmark, Holstebro, [3]Intensive Care, [4]Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark

Background: Improved supportive care for critically ill patients has increased survival among patients with hematological diseases. Slowly, the reluctance to admit critically ill patients with hematological diseases to the intensive care unit is eroding. However, data on outcome after admission to the intensive care unit in comparison to non-hematological patients are limited and inconsistent.
Aims: The aim of the current study was to assess survival after admission to the intensive care unit among hematological patients treated according to contemporary treatment regimens in comparison to non-hematological patients.
Methods: This cohort study included all intensive care unit admissions from 2005 to 2015 registered in the Danish Intensive Care Database combined with information on hematological diagnoses from the Danish National Hematology Database. Information on survival was collected from the Civil Registration System. All data included was nationwide and population-based. Patients were followed until one year after admission to the intensive care unit. Survival was estimated using Kaplan-Meier survival function and compared using Cox proportional hazard regression with age, sex, Charlson co-morbidity index (CCI), and admission type as covariates.
Results: During follow-up 2,820 patients with hematological disease and 153,408 without hematological disease was admitted to an intensive care unit. Of the hematological patients, the majority had lymphoma (43%), myeloma (21%), or acute leukemia (16%). 30-day survival was 55% among hematological patients and 74% among non-hematological patients. One-year survival was 33% and 64%, respectively. Hematological patients had increased mortality across all age-categories, however, the relative rate was higher among younger individuals (Hazard ratio: <60 years: 2.6 (95% Confidence interval: 2.3–2.9); ≥60 years: 1.5 (1.4–1.6)). Similarly, hematological patients had increased mortality across all levels of co-morbidity (Hazard ratio: CCI 0: 2.8 (2.5–3.1); CCI 1–3: 1.7 (1.6–1.8); CCI >3: 1.3 (1.1–1.4).
Summary/Conclusion: Patients with hematological disease treated according to contemporary treatment regimens has poorer survival rates than non-hematological patients after admission to the intensive care unit. The differences in survival was most pronounced among younger individuals without co-morbidity.

PS1199

INTESTINAL COLONIZATION BY MULTIDRUG-RESISTANT GRAM-NEGATIVE PATHOGENS IN HEMATOLOGICAL PATIENTS: REAPPRAISAL OF SELECTIVE ORAL DECONTAMINATION

I. Stoma[1,*], I. Iskrov[2], A. Uss[3], N. Milanovich[4], I. Karpov[1], I. Lendina[5]

[1]Department of Infectious Diseases, Belarusian state medical university, [2]Department of cell transplants, [3]Republican center for hematology and bone marrow transplantation, [4]Department of Bone Marrow Transplantation, [5]Department of Hematology №[3], City clinical hospital №[9], Minsk, Belarus

Background: Intestinal colonization by MDR/XDR gram-negative bacteria leads to an increased risk of subsequent bloodstream infections in patients receiving chemotherapy as a treatment for hematologic malignancies. There is a lack of data on efficacy and safety of selective decontamination strategies in hematologic patients with neutropenia, especially in the setting of high-prevalence of MDR/XDR Gram-negative microorganisms.
Aims: The objective of this study was to evaluate the efficacy of oral colistin as an agent for selective decontamination of the intestinal carriage of MDR/XDR Gram-negative bacteria in patients with hematological malignancies.
Methods: In a tertiary national clinical and research hematology center adult patients with intestinal colonization by MDR/XDR Gram-negative bacteria were included in a randomized controlled trial (RCT) during a period of November 2016 to October 2017. Patients were treated with oral colistin for 14 days or observed while receiving treatment for the primary hematological diseases. The primary outcome was MDR/XDR intestinal decolonization on day 21 post-treatment assessed by an intention-to-treat analysis. Secondary outcomes included safety of treatment and changes in MICs of isolated microorganisms.
Results: In the primary outcome analysis on day 21 post-treatment 13 of 31 patients (41.9%) have shown a decolonization effect in treatment group and 12 of 31 (38.7%) in the control group, without any statistical difference. Although, a short-time positive microbiological effect of selective oral decontamination in the conducted study (61.3% vs 32.3%; OR 3.32; 95% CI 1.17–9.44; p=0.0241) was demonstrated on the last day of colistin treatment (day 14). The incidence of BSI (as shown on Figure 1) in decontamination group was lower in the first 30 days after the intervention (3.2% vs 12.9%), but overall in the 90-day observation period it did not show any advantages comparing to control group (log-rank test; p=0.4721). No serious adverse effects or increase in resistance to colistin was observed during the study.

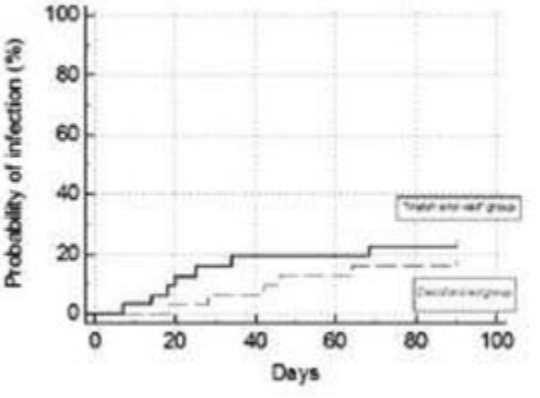

Figure 1.

Summary/Conclusion: This study suggests that in hematological patients with neutropenia the strategy of selective intestinal decontamination by colistin may be beneficial to decrease the rate of MDR/XDR Gram-negative intestinal colonization and the risk of bloodstream infections only in the short-term period, having no long-term sustainable effects.

PS1200

PREVALENCE AND RISK FACTORS OF RESISTANT GRAM-NEGATIVE BACILLI COLONIZATION AND INFECTION IN HOSPITALIZED HAEMATOLOGICAL PATIENTS

A. P. Gonzalez-Rodriguez[1,*], A.M. Fernandez-Verdugo[2], A.J. González-Huerta[1], P. Palomo[1], A. Alonso Cabrero[3], A. Sole[1], C. Valdes-Arias[1], T. Bernal[1], S. Gonzalez-Muñiz[1]

[1]Hematology- Stem cell transplantation Unit, [2]Microbiology, Hospital Universitario Central de Asturias, [3]Department of Medicine, University of Oviedo, Oviedo, Spain

Background: Hematological patients are frequently severely immunocompromised and there is a significant increased risk of mortality due to infection related with gram negative bacterial resistance (MRGN). Bacterial microflora of these patients changes from natural primary to secondary with higher proportion of mulirresistant bacteria.
Aims: To evaluate the prevalence and risk factors of MRGN colonization and infection among those colonized in hospitalized hematological patients.
Methods: Routine surveillance cultures and active screening (throat and rectal in parallel) in our hospital to know prevalence of MRGN colonization -extended spectrum **beta-lactamase** (ESBL) producing *Klebsiella pneumoniae (KB)*, multirresistant *Acinetobacter Baumanii* (MRAB) and multirresistant *Pseudomonas aeruginosa* (MRPA)- to indentificate risk factors for colonization and infection among those colonized. We collected samples in 130 hospitalized patients in Hematology Unit of Hospital Universitario Central de Asturias between 1st March 2016 and 30st October 2017. Main reason for admission was acute leukemia or myelodysplastic syndrome (n=74) followed by stem cell transplantation (12 allogeneic and 10 autologous).
Results: We screened 1843 samples: 306 rectal and 70 throats were positive for MRD. Sixty patients (46,2%) were colonized with a MRGN: 53 KB (6 KB and carbapenemasa) 8 MRPA and 6 MRAB. Seven of these were multiple MRGN carriers. Forty-two patients developed MRGN infection (32,3%): 26 KB, 4 MRPA and 3 MRAB (surveillance samples in 11,5% KB, 25% MRPA and 66,6% MRAB surveillance samples were negative). Risk factors for colonization and infection are showed in Table 1. Colonized patients

had increased risk for infection (80,95% vs 35,22%) and admission in intensive care unit (p< 0,001). High disease risk and poor performance status were risk factors for infection but not for colonization.

Table 1. Risk factors for colonization and infection.

	Infection Yes (42)	Infection No (88)	p	Colonization Yes(60)	Colonization No (70)	p
Days of hospitalization	35	27	0,01	35	24	<0.001
Prior quinolones prophylaxis	26 (61,9%)	67(76,1%)	ns	22 (36,7%)	15 (21,4%)	0.042
Prior exposure to beta lactam antibiotics	24 (57,1%)	25 (28,4%)	0,02	32 (53,3%)	17 (24,3%)	0,001
Recent hospitalization	10 (23,8%)	10 (11,5%)	ns	15 (25%)	5 (7,2%)	0,005
Prior mucositis	24 (57,1%)	16 (18,2%)	<0,001	29 (48,3%)	11 (15,7%)	<0,001
Catheter venous central	39 (92,8%)	49 (55,7%)	<0,001	51(85%)	37 (52,5%)	<0,001
Catheter urinary	10(23,8%)	12(14%)	ns	15(25,4%)	7(10,1%)	0,02
Disease Risk index high	22 (52,%)	28 (31,8%)	0,020	25(41,7%)	25 (35,7%)	ns
ECOG ≥ 2	28 (66,6%)	37 (42,0%)	0,007	34(56,7%)	31 (44,3%)	ns
Previous neutropenia febrile	15 (35,7%)	6 (6,9%)	<0,001	15(25%)	6(8,7%)	0,011

Summary/Conclusion: Rate of MRGN colonization is very high in our hospitalized patients and was associated with significant higher frequency of subsequent infection. Detection of carriers and the knowledge of local epidemiology can be useful to decide antibiotic therapy. In high risk hospitalized patients is mandatory the development of a screening programme for MDR strains associated by guidelines of contact isolation and antibacterial use. There is a growing problem of antimicrobial resistance among pathogens isolated in hematological patients and the paucity of new antibacterial drugs should limit use of drugs where there is no other alternative and to develop strategies to decrease these infections.

PS1201

PEDIATRIC INTENSIVE CARE ADMISSIONS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

M. Rompola[1,*], S. Rogosic[2], E. Kane[3], E. Roman[3], S. Kinsey[1]

[1]Leeds Institute of Cancer and Pathology, University of Leeds, [2]Pediatric Hematology, Leeds Children's Hospital, Leeds, [3]Epidemiology and Cancer Statistics Group, University of York, York, United Kingdom

Background: The outcomes for children with acute lymphoblastic leukemia (ALL) are steadily improving, with cure rates of over 93%. However, treatment-related mortality is still significant; infection being the leading cause of morbidity and mortality in these immunocompromised patients. Children with ALL who suffer from disease or treatment complications may require escalation of care to the pediatric intensive care unit (PICU).
Aims: To identify the main reasons for children with ALL to be admitted to PICU and review the impact on morbidity and mortality.
Methods: Using the PICANet national database, we reviewed the PICU admissions and chemotherapy records of patients diagnosed with ALL under the age of 16 years in Leeds Children's Hospital over a 13 year period (June 2004- May 2017), regardless of trial participation.
Results: Over the 13 year study period, approximately 450 under 16s were treated for ALL in the study centre, and 67 (15%) of these had at least one PICU admission (84 admissions in total). Fifty-three patients had one PICU admission and fourteen had more than one; over half of second admissions occurring within a month of the previous PICU discharge. Infection was associated with 61 (73%) PICU admissions. The most common causes for admission were sepsis (22/84), respiratory infection (16/84), and respiratory failure (13/84). The mean length of stay for patients with infection was 8.9 days compared to 3.9 days in patients without an infection. Complete treatment information was available for fifty-eight patients. Of these, nine patients had relapsed disease (11 admissions), three were infants (10 admissions), two had Philadelphia positive ALL (3 admissions), and two had had a haematopoietic stem cell transplantation (3 admissions). In the remaining forty-two patients, there were 49 PICU admissions: 27/49 in induction, 4/49 in consolidation, 7/49 in delayed intensification, and 11/49 in maintenance. Of the total 84 admissions, 49 admissions required ventilation, 73% of which were due to infection. In 45 of the admissions the patient required inotropic support; 80% of these admissions were secondary to infection. Twenty of the patients who died were ventilated, eleven of which were on inotropic support. There were twenty-two deaths within the first 30 days from discharge from PICU. Seven of those deaths were in patients with relapsed disease. Patients who died in PICU had shorter admissions with a mean duration of 5 days. The mortality rate for patients in PICU was 16 per 100 person-days at risk. Crude mortality rate ratio in patients at induction versus other phases was 3.5 p=0.05. The mortality rate was higher in those who were ventilated, however, only inotrope requirement reached statistical significance: crude rate ratio 3.5 p=0.03, increased to 4.6 p=0.02 controlling for ventilation status.
Summary/Conclusion: Most admissions to PICU in this cohort of patients occurred during induction, however, a significant number of admissions happened in maintenance treatment with 3 deaths in 11 PICU admissions during this phase. Children with ALL who are admitted to PICU have poor outcomes; nearly 1 in 3 children died. Patients with relapsed ALL are at particularly high risk of mortality in PICU (7/9). Overall, infection was the main underlying reason for the requirement of intensive care in children with ALL.

PS1202

OUTCOME OF HIGH RISK FEBRILE NEUTROPENIC PATIENTS WITH GUT COLONIZATION WITH CARBAPENEM RESISTANT ENTEROBACTERIACEAE(CRE) IN SURVEILLANCE CULTURES "

S. Lunkad[1,*], M. Mahapatra[1], T. Seth[1], S. Mahapatra[2], M. Aggarwal[1], R. Dhawan[1], R. Haldar[1], A. Kumar[2], R. Saxena[1], H.P. Pati[1], S. Tyagi[1]

[1]Hematology, [2]Microbiology, AIIMS, New Delhi, India

Background: Febrile neutropenia particularly with carbapenam resistant bacteria(CRE) is a leading cause of induction mortality in acute leukemia. Various studies reveal correlation between positive cultures and colonization with multiresistant bacteria. But does having CRE colonization would always result in a resistant blood stream infection and would this adversely affect the outcome of these patients is not known.
Aims: Primary Objectives: To study the mortality rate in those having CRE vs Non- CRE in surveillance culture. **Secondary Objectives:** To compare the duration of febrile period, antibiotic use and interruptions of chemotherapy in both CRE vs Non CRE groups.
Methods: It was a prospective observational study. Institute Ethics clearance was taken. Perianal surveillance cultures were taken on admission to hospital after taking an informed consent. Cultures were processed as per CDC guidelines and CRE was identified. These patients were divided in to two groups: CRE and Non CRE. These patients were followed prospectively during their induction course and above mentioned parameters were recorded
Results: A total of 100 patients were screened. 96 patients were included as 2 did not give consent, 2 were lost to follow up. 56 were CRE and 40 Non CRE. Their demographic data is as below in Table 1 and results in Table 2 Mortality in the CRE group was higher upto 42% and the difference in two groups was statistically significant. The duration of fever, use of antibiotics, interruptions of chemotherapy were also higher in CRE group but none reached statistically significant value.

Table 1. Demographic finding of the patients.

Parameters		CRE	Non CRE
Number		56	40
Median Age		28	28
Sex	Male	45	23
	Female	12	18
Disease	AML	27	18
	ALL	27	21
	MPAL	2	1

Table 2. Results of the study are as below.

Parameters	CRE	Non CRE	P Value
Organism most common in CRE screening	E. Coli (62%)	E. Coli (80%)	0.20
Death	24(42%)	8(20%)	0.019
Not received chemotherapy due to sepsis	8(14%)	2(5%)	0.14
Chemotherapy interruptions due to sepsis	18(32%)	6(15%)	0.57
Median duration of antibiotics	24 days	23 days	0.58
Median duration of fever	6 days	4 days	0.05

Summary/Conclusion: CRE colonization was seen in 58% of patients in our study. Preinduction screening of CRE colonization is an important tool as it is one of the important predictors of induction chemotherapy mortality.

PS1203

COLONIZATION WITH MULTIDRUG-RESISTANT BACTERIA (MDRB) IS ASSOCIATED WITH COMPLICATIONS AND POOR OUTCOME AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

A. Busca[1,*], R. Passera[2], C.M. Dellacasa[1], S. Manetta[3], G. Iovino[3], F.G. De Rosa[4], S. Corcione[4], L. Brunello[3], S. Aydin[3], L. Giaccone[1], R. Cavallo[5], B. Bruno[1]

[1]Transplant Unit, [2]Nuclear Medicine, [3]Hematology, [4]Infectious Diseases, [5]Microbiology, OU Città della Salute e della Scienza di Torino, Italy

Background: Composition of intestinal microbiota may influence early complications of allogeneic HSCT, including bacterial infections and acute GVHD.
Aims: To assess the impact of colonization with MDRB prior to HSCT on the outcomes of HSCT.
Methods: In the present study, rectal swabs were prospectively collected upon admission to the hospital and routinely repeated on a weekly basis from 157 patients with hematologic disorders who received HSCT between January 2013 and January 2018.
Results: Overall, 84 patients received HSCT from matched unrelated donors, 45 patients from a matched sibling donor and 28 from an haploidentical donor. The MDRB included extended-spectrum beta-lactamase (ESBL)-producing enterobacteriaceae and carbapenem-resistant enterobacteriaceae (CRE). Antimicrobial prophylaxis with fluoroquinolones (FQ) was administered to all patients until September 2016. Overall, 18 patients (12%) were colonized with MDRB at the time of HSCT; seven additional patients became colonized with MDRB during the post-transplant course. A significantly higher incidence of gram negative (GN) bacteremia occurred in patients colonized with MDRB at the time of HSCT: 9 out of 18 patients (50%) developed GN bacteremia, and in 7 cases (78%) the infection was due to MDRB (n=2 ESBL-producing *K. pneumoniae*; n=2 ESBL-producing *E. coli*; n=3 carbapenem-resistant *K. pneumoniae*); no gram positive (GP) bacteremia has been reported in this group of patients. By contrast, among the 139 patients who were not colonized upon admission to the transplant unit, 20 patients (14%) developed a GN bacteremia (p=0.01) and 9 of these (45%) were due to MDRB; 26 patients developed a GP bacteremia and 3 patients developed polymicrobial infections. Overall, 76 of 144 evaluable patients received FQ as antibacterial prophylaxis, and none of these was colonized with MDRB at the time of HSCT; by contrast, among the 68 patients who did not receive FQ, 13 were colonized with MDRB. The median duration of neutropenia was not statistically different between the colonized (13 days; range 11-22) and the noncolonized group (15 days; range 9-30). The incidence of grades II-IV acute GVHD showed a tendency to be higher in the colonized group than in the noncolonized group (56% vs 38%), however, the difference was not significant (p=0.182). Four of the 25 patients (16%) with pre- and post-HSCT MDRB colonization, died of sepsis or septic shock as compared to 5 of the 132 patients (4%) in the noncolonized group (p=0.37).
Summary/Conclusion: Our preliminary results suggest that patients colonized with MDRB are more susceptible to life-threatening infections and this may impact the overall outcome of HSCT recipients. Investigation regarding colonization with alert pathogens may have practical implications for the selection of prophylactic and infection-driven antibiotic strategies that may improve the outcomes of HSCT recipients.

PS1204

ANTIBIOTICS CAN SAFELY BE DISCONTINUED IN NEUTROPENIC HEMATOLOGY PATIENTS: A PROSPECTIVE COHORT

Z. Van De Wyngaert[1,*], C. Berthon[1,2], H. Debarri[1], C. Bories[1], S. Bonnet[1], M. Nudel[1], B. Carpentier[1], C. Legrand[1], S. Barbieux[1], P. Chauvet[1], A. Simonnet[1], A. Willaume[1], J.-B. Bossard[1], L. Renaud[1], K. Wattebled[1], G. Escure[1], N. Branche[1], I. Arib[1], M. Titecat[3], B. Quesnel[1,24], S. Alfandari[5]

[1]Maladies du Sang, CHU Lille, [2]INSERM UMR-S 1172 Institut pour la recherche sur le Cancer de Lille, [3]Service de bactériologie, Centre de biologie-pathologie, CHU de Lille, [4]Faculté de médecine, Université Lille, Lille, [5]Service de réanimation et maladies infectieuses, CH Dron, Tourcoing, France

Background: Antibiotics for febrile neutropenia in acute myeloid leukemia patients, undergoing intensive chemotherapy, are usually maintained until absolute neutrophil count >500/mm^3, because of the risk of uncontrolled sepsis in this vulnerable population. This can lead to unnecessarily prolonged antimicrobial therapy.
Aims: Based on recent European recommendations (ECIL-4), we modified our management strategy and introduced antibiotics discontinuation among patients receiving intensive chemotherapy, and treated for a first episode of febrile neutropenia.
Methods: The policy change concerned adult patients, newly diagnosed with AML, receiving intensive chemotherapy and presenting with a non-severe episode of febrile neutropenia, including fever of unknown origin, primary bacteremia and focal infections. Antimicrobial therapy was discontinued after ≥7 days of intravenous treatment, in hemodynamically stable patients, after resolution of clinical symptoms and ≥5 days of apyrexia, regardless of the absolute neutrophil count or the predicted duration of neutropenia.
Results: Antibiotics were stopped in 49 neutropenic patients. Median age was 51 years, 28 patients (57%) received induction and 21 (43%) consolidation chemotherapy. 32 patients (65%) were asymptomatic, 35 cases (71%) were undocumented. Median neutropenia length was 26 days [IQR 24–31]. Antibiotics were started at day 9 [5.75–14.25], and given for 10 days [7–16], most patients received piperacillin-tazobactam (n=23, 47%) or cefepime (n=13, 27%). After antibiotics discontinuation, 10 patients (20%) experienced fever recurrence, within a median 5.5 days [3–7.5]. None of these febrile episodes were severe. 39 patients (80%) remained afebrile, with neutrophil recovery occurring within 5 days [2–8.5]. Overall, 287 antibiotics days were spared; this represents 49% of all days with antibiotics. No patient had died at day 30 from intervention; 6 died during late follow-up, 2 from GVHD and 4 from relapsed or refractory leukemia.
Summary/Conclusion: Discontinuing antibiotics in neutropenic acute myeloid leukemia patients treated for a first episode of febrile neutropenia is safe, and results in significant antibiotic sparing.

PS1205

RISK OF INFECTIOUS COMPLICATIONS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA IN THE ERA OF NOVEL THERAPIES: A RETROSPECTIVE SINGLE INSTITUTION EXPERIENCE

F. Autore[1,*], I. Innocenti[1], F. Morelli[1], F. Sorà[1], A. Corbingi[1], D. Soldati[1], L. Fianchi[1], M. Criscuolo[1], L. Pagano[1], S. Sica[1], L. Laurenti[1]

[1]Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario A. Gemelli, Rome, Italy

Background: Recently, the development of novel therapeutic agents in the treatment of chronic lymphocytic leukemia (CLL) tends to decrease the rate of infections in spite of standard immuno-chemotherapy regimens. Ibrutinib and Idelalisib show a different profile due to the association of Idelalisib to monoclonal antibody. The few data about the infectious complications during the use of Obinutuzumab with Chlorambucil derive from German CLL Group.
Aims: The aim of this study is to evaluate incidence and type of infection in CLL patients treated with Ibrutinib, Idelalisib plus Rituximb and Obinutuzumab.
Methods: We present the retrospective analysis of CLL patients treated at our institution from 2015 to 2018. In the study 54 patients were evaluated: 37 patients treated with Ibrutinib, 9 patients treated with Idelalisib plus Rituximab and 8 patients treated with Obinutuzumab.
Results: We recorded 45 episodes of infections, of which 27 occurred in 12 patients treated with Ibrutinib, 15 in 6 patients treated with Idelalisib and 3 episodes of infections in 1 patient treated with Obinutuzumab. All the patients who presented infectious complications started these drugs as second or following line of treatment, except 1 patient who started Ibrutinib and 1 patient who was treated with Obinutuzumab as first line. The rate of infections was 0.73 episodes/patient for Ibrutinib and 1.66 episodes/patient for Idelalisib; each patient with infection showed a median of 2.25 and 2.5 episodes for Ibrutinib and Idelalisib, respectively. Only 1 patient treated with Obinutuzumab had 3 infectious events. In the group of Ibrutinib the most common infections were the respiratory tract infections (44%, 12 events out of 27), followed by urinary tract infections (22%, 6 events). In the group of Idelalisib 6 infections (40%) were viral (EBV or CMV) reactivations and 5 (33%) were respiratory tract infections. The patients treated with Obinutuzumab showed 2 bacterial urinary infections and 1 viral reactivation. Also the pathogens implicated in the infections were different between Ibrutinib and Idelalisib group: in the first 52% were bacterial, 26% fungal and 22% viral, on the contrary in the second group 20% were bacterial, 13% fungal and 67% viral (Table 1). The majority of the infections were grade I or II and no deaths due to infection were registered.

Table 1. Type of infection and pathogens in CLL patients.

	Infections with Ibrutinib (27 episodes)	Infections with Idelalisib plus Rituximab (15 episodes)	Infections with Obinutuzumab (3 episodes)
Respiratory tract infections	13 (8 bacterial, 5 fungal)	5 (2 viral, 2 fungal, 1 bacterial)	-
Urinary tract infections	6 (4 bacterial, 2 viral)	2 (1 bacterial, 1 viral)	2 (2 bacterial)
Blood infections or reactivactions	5 (3 viral, 1 bacterial, 1 fungal)	6 (6 viral)	-
Others	3	2	1

Summary/Conclusion: BCR signaling pathway inhibitors and new monoclonal antibodies showed a good safety profile even if the risk of infection in CLL patients remained high even with the use of less immunosuppressive therapies. We confirmed the higher prevalence of bacterial infections in patients treated with Ibrutinib and the higher prevalence of viral infections in patients treated with Idelalisib. A good safety profile is more evident in patients treated with these drugs as first line of treatment. In the future a multicenter study could collect data on infectious events in CLL patients treated with BCR signaling pathway inhibitors and new monoclonal antibodies exploring the risk factors due to clinical features of the patients, drugs and concomitant medications and prophylaxis, pathogens.

PS1206

EMERGING MICROBIAL RESISTANCE IN FEBRILE NEUTROPENIA- IS IT HIGH TIME TO EVALUATE QUALITY CONTROL MEASURES?

N. Anwar[1,*], N. Fatima[2], J. Hassan[1], M. Borhany[1], T. Shamsi[1]

[1]Hematology, [2]Research and Development, National Institute of Blood Disease and Bone Marrow Transplantation, Karachi, Pakistan

Background: Bacterial infections are one of the most common causes of morbidity and mortality among patients of febrile neutropenia. A considerable antimicrobial resistance has been observed in the susceptibility of the causative microorganism with the passage of time which poses a serious threat to public health especially in neutropenia setting.
Aims: A prospective study to observe the trend of infection by culture and susceptibility in patients of febrile neutropenia with back ground aim to review and re assess institutional policies and quality control measures for better surveillance in the treatment paradigm of hematological malignancies.
Methods: The study was conducted at National institute of blood diseases and bone marrow transplantation from January 2017 to December 2017. This study was approved by Institutional review board. Informed consent was taken by the patients enrolled in the study presenting with absolute neutrophil count (ANC) of less than 500/ml.
Results: A total of 403 bacterial isolates were obtained from 242 patients suffering from hematological disorders. Out of total, 25% were gram positive and 75% were gram negative. Staphylococcus aureus was the most common isolated gram positive organism i.e. 55%, followed by enterococcus species (36%). However, *E. coli* was the most common gram negative organism i.e. 40%, followed by Pseudomonas aeruginosa and klebsiella (28% and 27% respectively). The resistance patterns of various organisms were noted. Methicillin resistance was very high among staphylococcus aureus whereas vancomycin resistance was high among enterococcus species. There was higher level of resistance observed among isolates of the E. coli, pseudomononas and klebsiella to penicillin i.e. 57%, 40%, and 36% respectively and higher sensitivity to colistin i.e. 55%, 24% and 37% respectively. Isolates of staphylococcus aureus were resistant to penicillin (35%) and sensitive to fosfomycin(15%). Similar trend was observed in enterococcus. Resistance to piperacillin- tazobactam and meropenem was found in 32% and 29% of the isolates of all organisms respectively. E. coli, proteus and pseudomonas were found to be more prevalent in urine C/S, whereas staphylococcus aureus and salmonella were predominantly noted in blood C/S. High frequency of klebsiella and beta hemolytic streptococcus was found in throat C/S. However streptococcus epidermidis was frequently observed in pus as well as in blood C/S and it was found statistically significant (p value 0.000).
Summary/Conclusion: The study reveals an emergent need to reevaluate institutional policies and quality management. The emerging resistance could be hospital environmental related, bug resistance or lack of quality control. Further studies in future are needed in this aspect.

PS1207

THERAPEUTIC ADMINISTRATION OF IGM-ENRICHED IMMUNOGLOBULINS IMPROVES SURVIVAL IN HEMATOLOGICAL CANCER PATIENTS WITH SEVERE NEUTROPENIC SEPSIS

A. Forcina[1,*], A.M. Mazzone[2], F. Lorentino[1], G. Palazzo[2], F. Ciceri[1,3], G. Pisapia[2]

[1]Hematology and Bone Marrow Transplant Unit, IRCCS San Raffaele Scientific Institute, Milan, [2]Hematology and Bone Marrow Transplant Unit, San Giuseppe Moscati Hospital, Taranto, [3]University Vita-Salute, Milan, Italy

Background: Severe bacterial infections (SBI) occurring in neutropenic hematological cancer patients represent a major cause of mortality. Four-months overall survival (OS) in hematopoietic stem cell transplantation (HSCT) recipients developing Carbapemen-resistant *Klebsiella pneumoniae* (CR-Kp) or *Pseudomonas aeruginosa* (PA) SBI is particularly dismal, being as low as 40% (Girmenia *et al.*, Clin Infec Dis 2017).
Aims: The aim of the study was to explore infection-related mortality (IRM) and overall survival in consecutive neutropenic patients developing SBI, treated with IgM-enriched immunoglobulins (IgM-IGIV) in combination to antimicrobial therapy.
Methods: A total of 35 adult patients suffering from hematological malignancies and developing SBI according to standard criteria were retrospectively evaluated from April 2014 to February 2018. Sixteen out of 35 (48.5%) patients received autologous HSCT (auto-HSCT), 10/35 (28.5%) allogeneic HSCT (allo-HSCT) and 9/35 (22.8%) chemotherapy alone (CT). All patients were treated with IgM-IGIV 5 ml/kg/day for 3 consecutive days as adjunctive therapy to antimicrobial agents. Kaplan-Meier curves were used to estimate OS. Disease recurrence, graft-versus-host-disease and death from any other cause were competing risks for IRM. Univariate comparisons of survival curves were made using the Log-rank test, while the Gray's test was used for univariate comparisons of cumulative incidence functions
Results: Median time from SBI to IgM-IGIV administration was 2 days (range 0-8). Median follow-up after SBI was 186 days (range 1-938). The majority of patients was diagnosed with acute leukemia (54.2%), had active disease at onset on infection (65.7%) and was beyond first-line disease-specific treatment (64.2%). All patients had grade IV neutropenia. A microbiologically documented SBI was reported in 31/35 cases and sustained by Gram-negative bacteria in 20/35 (62.5%, 13 by CR-Kp, 4 by *Escherichia coli*, 2 by PA, 1 *Acinetobacter baumanii*) and by Gram-positive bacteria in 10/35 (31%). Three patients had polymicrobial infection, in one case with concomitant *Candida glabrata* bacteremia. In 4 patients, no isolates could be documented. Overall, 14-days crude mortality was 11.4% and only 13.3% in the case of SBI sustained by CR-Kp and PA. Despite first-line antimicrobial therapy was inadequate in 85.8% of cases, only 5/35 (14.2%) patients progressed to septic shock when IgM-IGIV were used. Four-months OS after transplant was not significantly different between auto- and allo-HSCT recipients (93.3% vs 71.4% p=0.16) and was of 70% overall after the BSI onset, being of 93.3% for auto-, 58.3% for allo-HSCT and 25% for patients receiving CT alone (p<0.001). According to etiology, 4-months OS was 77.1% when BSI were sustained by Gram-positive, 64.9% by Gram-negative and 75% in case of no isolates. Interestingly, 4-months OS in case of CR-Kp and PA SBI (n=15) was up to 70% (100% for auto-HSCT, 75% for allo-HSCT and 33% for CT patients, p=0.07), significantly higher compared to historical published data. Cumulative incidence of IRM in HSCT recipients was 0% at 4 months and of 5.4% at 1 year.
Summary/Conclusion: The addition of IgM-IGIV to antimicrobial targeted therapy in neutropenic cancer patients developing SBI may synergize with standard treatment to ameliorate survival and reduce infection-mortality, particularly when the infection is sustained by multidrug-resistant Gram-negative bacteria such as CR-Kp. This experience warrants further investigation in a prospective study in hematological patients.

PS1208

TETANUS, DIPHTHERIA AND POLIO IMMUNITY AFTER CHEMOTHERAPY AND RITUXIMAB IN PATIENTS WITH LEUKEMIA AND LYMPHOMA

S. Einarsdottir[1,*], P. Ljungman[2], T. Bergstrom[3], M. Brune[1]

[1]Department of Medicine, Section of Hematology, Sahlgrenska University hospital, Gothenburg, [2]Department of Cellular Therapy and Allogeneic Stem Cell Transplantation, Karolinska University hospital, Stockholm, [3]Department of Clinical Microbiology, Sahlgrenska University hospital, Gothenburg, Sweden

Background: After chemotherapy, children with acute lymphocytic leukemia lose immunity and need revaccination against tetanus, diphtheria and haemophilus influenza typ B (T Ek, Pediatr Blood Cancer, 2005).
Aims: In this study we aimed to assess immunity and possible need for revaccination in adult patients (pts) after conventional treatment (not BMT) for leukemia and lymphoma.
Methods: At a median of 17 (6-60) months after last chemotherapy 103 pts, age 59 (19-86) yrs, with acute leukemia (n=27) or high-grade lymphoma (n=76) were included. Of lymphoma pts, 47 (62%) had received rituximab as part of standard treatment. Pre-treatment sera were available in 69 cases enabling comparisons of immunity *pre*- versus *post*-treatment in individual pts. Healthy, age- and sex matched controls were available for 44 pts.
Tetanus antibodies were quantified using ELISA technique and antibody-levels ≥0,01 IU/ml were considered protective. Diphtheria antibodies were analyzed using neutralization test (n=73) and by ELISA (n=34), in both tests values ≥0,01 IU/ml were considered protective. Antibodies against poliovirus serotype -1 and -3 were assessed by a neutralizing test where a microneutralisation titer ≥2 was considered protective.
Results: *Diphtheria*: Post treatment antibody levels (IgG per IU/mL) were lower than pre-treatment levels (p=0.03) and controls (p=0.0005). Rituximab treatment did not have any impact on the *pre*- versus *post* comparison (p=0.35). Antibody levels were not correlated with gender or myeloid/lymphoid disease (p=1.0), but inversely correlated with age (p=0.02). There was no correlation between immunity (Ab-levels) and time since last chemo (p=0,18). *Tetanus*: Post treatment antibody-levels (IgG per IU/mL) were lower than pre-treatment levels (n=68; p=0.02), and pts were after treatment to a greater extent "not-immune" compared to "immune" (p=0.02). However, there was no difference in Ab-levels between pts and healthy controls (p=0.52). Tetanus immunity was similar in the myeloid and lymphoid groups (n=102; p=0.3). In the lymphoid group, there was a trend to a higher rate of "not-immune" versus "immune" in pts after previous rituximab treatment: 12/48 vs 3/31; p=0.07). Antibody levels were inversely correlated with age (p=0.02). There was no correlation between immunity (Ab-levels) and time since last chemo (p=0,4). *Polio:* No differences in antibody levels were seen between myeloid and lymphoid pts (p=0.18). Polio serotype 1 post-treatment Ab-levels were similar to pre-treatment levels (p=0.42,) and to levels in healthy controls (p=0.3). However, for serotype 3, post-treatment levels were lower than pre-treatment levels (p=0.006), but there was no difference in the comparison between patients and healthy controls (p=0.7). There was no impact of previous rituximab treatment in any polio serotype on the rates of "not-immune" vs "immune" (p=0.7).
Summary/Conclusion: We found that many leukemia and lymphoma patients lacked immunity to diphtheria (35%), tetanus (18%) or polio serotypes 1 or 3 (26%). There was a significant decrease in antibody-levels for both diphtheria and tetanus comparing samples at diagnosis with post-treatment samples. Rituximab treatment was associated with a trend in reduced immunity to diphtheria. There was no tendency of immune reconstitution after long-time follow-up.
We conclude that after standard chemotherapy, patients treated for leukemia and lymphoma with intensive chemotherapy may have an impaired humoral immunity to diphtheria, tetanus and polio. Measurement of antibody-levels and revaccination should be considered.

PS1209

THE DIAGNOSTIC UTILITY AND SENSITIVITY OF THE XPERT® MTB/RIF ASSAY IN DIAGNOSING MYCOBACTERIUM TUBERCULOSIS IN BONE MARROW ASPIRATE SPECIMENS

N. Subramony[1,*], L. Scott[1], J. Vaughan[1]

[1]Molecular Medicine and Haematology, National Health Laboratory Services/University of the Witwatersrand, Johannesburg, South Africa

Background: In South Africa, the World Health Organisation estimated 454 000 new cases of *Mycobacterium tuberculosis (M.tb)* infection (MTB) in 2015. Disseminated tuberculosis arises from the haematogenous spread and seeding of the bacilli in extrapulmonary sites. The gold standard for the detection of MTB in bone marrow is TB culture which has an average turnaround time of 6 weeks. Histological examinations of trephine biopsies to diagnose MTB also have a time delay owing mainly to the 5-7 day processing period prior to microscopic examination. Adding to the diagnostic delay is the non-specific nature of granulomatous inflammation which is the hallmark of MTB involvement of the bone marrow. A Ziehl-Neelson stain (which highlights acid-fast bacilli) is therefore mandatory to confirm the diagnosis but can take up to 3 days for processing and evaluation. Owing to this delay in diagnosis, many patients are lost to follow up or remain untreated whilst results are awaited, thus encouraging the spread of undiagnosed TB.
Aims: The Xpert® MTB/RIF (Cepheid, Sunnyvale, CA) is the molecular test used in the South African national TB program as the initial diagnostic test for pulmonary TB. This study investigates the optimisation and performance of the Xpert® MTB/RIF on bone marrow aspirate specimens (BMA), a first since the introduction of the assay in the diagnosis of extrapulmonary TB.
Methods: BMA received for immunophenotypic analysis as part of the investigation into disseminated MTB or in the evaluation of cytopenias in immunocompromised patients were used. Processing BMA on the Xpert® MTB/RIF was optimised to ensure bone marrow in EDTA and heparin did not inhibit the PCR reaction. Inactivated *M.tb* was spiked into the clinical bone marrow specimen and distilled water (as a control). A volume of 500mcl and an incubation time of 15 minutes with sample reagent were investigated as the processing protocol.
Results: 135 BMA specimens had sufficient residual volume for Xpert® MTB/RIF testing however 22 specimens (16.3%) were not included in the final statistical analysis as an adequate trephine biopsy and/or TB culture was not available. Xpert® MTB/RIF testing was not affected by BMA material in the presence of heparin or EDTA, but the overall detection of MTB in BMA was low compared to histology and culture. Sensitivity of the Xpert® MTB/RIF compared to histology and culture was 8.7% (95% confidence interval (CI): 1.07-28.04%) and sensitivity compared to histology only was 11.1% (95% CI: 1.38-34.7%). Specificity of the Xpert® MTB/RIF was 98.9% (95% CI: 93.9-99.7%).
Summary/Conclusion: Although the Xpert® MTB/RIF generates a faster result than histology and TB culture and is less expensive than culture and drug susceptibility testing, the low sensitivity of the Xpert® MTB/RIF precludes its use for the diagnosis of MTB in bone marrow aspirate specimens and warrants alternative/additional testing to optimise the assay.

PS1210

CLINICAL AND MICROBIOLOGICAL PROFILE OF BLOODSTREAM INFECTIONS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AND FEBRILE NEUTROPENIA

G. Mendez[1], C. Niveyro[1], H. Bernard[2*], S. Villamandos[1], J. Fernandez[1], P. Villalba Apestegui[1]

[1]Infectious Diseases, [2]hematology Department, Hospital Escuela de Agudos Dr Ramon Madariaga, Posadas, Argentina

Background: Patients with hematologic malignancies (HM) are at a high risk of infectious complications, and bacterial bloodstream infections (BSI) represent the most severe among these. The reported prevalence of BSI among HM patients ranges from 11% to 38%, and the crude mortality rate reaches up to 40%. Antimicrobial treatment depends on the local prevalence of pathogens causing infection and their antibiotic susceptibility, which may change over the time.
Aims: The aim of this study was to evaluate the clinical and epidemiologic characteristics and mortality rates of BSI that occurred in a large tertiary care university hospital.
Methods: A total of 244 patients with HM with 497 neutropenic episodes followed between june 2010 and august 2017 at Madariaga's Hospital in Misiones Argentina, were retrospectively reviewed. The microorganism isolation was performed using BACTEC 9240 with classical identification and antibiogram disk by diffusion. All statistical analysis were perfomed with Minitab 16 program.
Results: Throughout the study period, 497 febrile neutropenic episodes ocurred in 244 patients. The median age at the fever onset was 37 y (range 14-79 y) and 295 (59%) episodes ocurred in males. The predominant underlying diagnoses were acute leukemia (lymphoblastic and myeloid) *n*= 356 (71%), lymphoma *n*= 83 (16%) other diagnosis *n*= 58 (11%). One hundred and eleven (22%) patients had relapse of disease. Bloodstream infection: in 155 episodes (31%) 1 or more bacterial pathogens was detected in blood cultures. Gram-negative bacteria was detected in 82 of these episodes (58%), whereas in 58 (41%) only Gram-positive bacteria were found. Klebsiella pneu-

moniae and Escherichia coli were the most common GNB (21% and 31%), whereas the most common GPB were CoNS and *Staphylococcus aureus* (11% and 16%). Fifteen (10%) cases of polymicrobial bacteremia were recovered. In the group of BSI patients previous stay in hospital in the last 30 days (p 0.01), neutropenia >7 days (p 0.44), previous chemotherapy (p 0.01) and mortality (p 0.0001) were risk factors for the development of BSI (Table 1).

Table 1.

Data	No BSI Total (n=342)	BSI Total (n=155)
Age (years) Media Mediana	37 32	37 35
Sex Male	194 (56%)	101 (65%)
Type of tumor Acute leukemia Lymphoma	232 (67%) 65 (19%)	124 (80%) 18 (11%)
Relapse of disease Yes	272 (79%)	114 (73%)
Hospitalization 30 days Yes	197 (57%)	107 (69%) ***p 0.01***
ATB 30 days Si	103 (30%)	60 (38%)
Chemtherapy Yes	279 (81%)	138 (89%) ***p 0.01***
Central catheter Yes No	106 (30%) 236 (69%)	44 (28%) 111 (71%)
Neutropenia >7d Yes	247 (72%)	117 (75%) ***p 0.44***
Dead	13	***23 p 0.0001***

Summary/Conclusion: Appropriate empiric antibiotic treatment is an essential tool for managing infections in HM particularly during the neutropenic phases. Antibiotic choice should be based on the knowledge of epidemiological scenario, which is constantly changing because of the antibiotic pressure, causing the emergence of new and worrisome resistant strains. The frequency of severe complications including death was also significantly higher in bacteraemic patients (14%) compared to non-bacteraemic patients (3%).

PS1211

ROLE OF GRANULOCYTE TRANSFUSIONS IN COMBATING LIFE-THREATENING INFECTIONS IN PATIENTS WITH SEVERE NEUTROPENIA IN INDIA

S. Nityanand[1,*], A. Garg[1], A. Gupta[1], A. Mishra[1], M. Singh[1], S. Kumar[1]

[1]Hematology, SGPGIMS, Lucknow, Lucknow, India

Background: Bacterial and fungal infections still remain an important cause of mortality in patients with hematological malignancies and in recipients of hematopoietic stem cell transplants (HSCT) in developing countries like India. One of the most important contributing factors to this is the increase in the prevalence of multi- drug resistant organisms (MDROs) in the last decade. Granulocyte transfusions (GTX) from healthy donors may lead to early clearance of index infection and thus prevent mortality.
Aims: To evaluate the efficacy of GTX in combating life-threatening infections and preventing mortality in patients of hematological disorders/recipients of HSCT with severe neutropenia.
Methods: A prospective, observational analysis of patients with different hematological disorders/recipients of HSCT, who received GTX from January 2014 to December 2017, was carried out. All patients had an ANC < 0.5×10^9 /L and a life threatening sepsis defined by presence of hemodynamic instability/ impending septic shock/ continuous high fever despite the use of the highest line of antimicrobials. The primary outcome measures were resolution of the index infection and overall survival (OS) at 30 days. The secondary outcome measures were the frequency and nature of adverse reactions to GTX. Granulocytes were collected from blood group matched healthy donors who were mobilized with G-CSF (@10mcg/kg SC) and dexamethasone (8 mg IV), 12 hours prior to collection. Granulocytes were collected using Spectra Optia system. The target granulocyte collection was of 50×10^9 granulocytes/L.
Results: A total of 143 granulocyte collections were done for 66 infectious episodes (IEs) in 60 patients. The median age of the patients was 21yrs (interquartile range (IQR) 16-45yrs). MDROs were observed in 47/66 IEs (71.2%) and fungal infections were seen in 9/66 IEs (13.6%). For each IE, a median of 57.7×10^9 granulocytes/L (IQR- 47-68.4×10^9/L) were collected. Resolution of index infection after GTX was seen in 45/66 IEs (68.2%), and the 30 day OS was 67.7%. OS was significantly higher in patients who received GTX within 7 days of neutropenic sepsis in comparison to those who received after 7 days (81.8% vs 54.5%, p=0.01) (Figure 1). The mean durations of fever and neutropenia were shorter in patients who received early GTX within 7 days of the IE as compared to those who received after 7 days (8 days vs 19 days, p=0.001 and 20 days vs 32 days, p= 0.02, respectively). Patients with MDROs who received early GTX therapy had a better OS as compared to those who received late GTX (75.3% vs 56.4%, p= 0.02). Though the post GTX 6 hour increment in the TLC and ANC values were higher in the group which received a GTX dose > 50×10^9 granulocytes per IE in comparison to those who received ≤ 50×10^9, but the OS in the two groups was comparable (71.7% vs. 60%, p= 0.36). GTX were well tolerated and only 6 patients developed mild features of transfusion related acute lung injury (TRALI) which were managed conservatively, and 1 patient demonstrated hypocalcemic tetany.

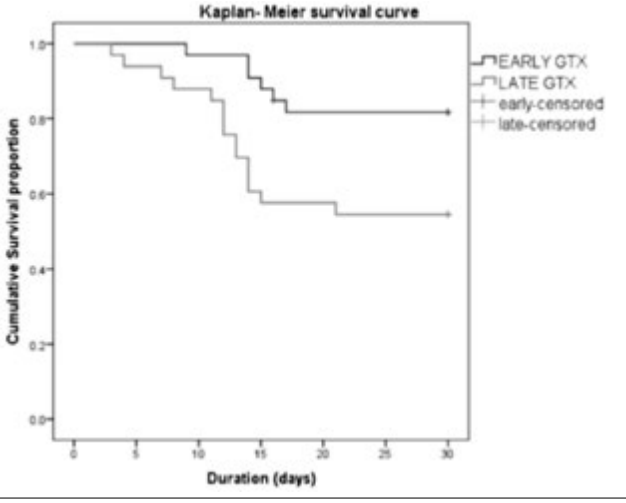

Figure 1.

Summary/Conclusion: GTX are an important modality of therapy in combating life threatening infections and preventing mortality in patients with severe neutropenia. Early institution of GTX before 7 days of neutropenic sepsis is associated with a lower mortality in comparison to when given after 7 days. GTX are well tolerated with no significant side effects. GTX may be of particular relevance in countries like India, where the incidence of infections is very high in neutropenic patients and there is an increasing emergence of MDROs.

PS1212

VACCINATION IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES OR SOLID TUMORS – GUIDELINE OF THE INFECTIOUS DISEASES WORKING PARTY (AGIHO) OF THE GERMAN SOCIETY FOR HEMATOLOGY AND MEDICAL ONCOLOGY (DGHO)

S. Mellinghoff[1,*], B. Liss[1], O. Cornely[1], C. Rieger[2]

[1]University Hospital of Cologne, Cologne, [2]Medizinische Klinik und Poliklinik III, Klinikum der Universität München, Munich, Germany

Background: Infections are a significant cause of morbidity and mortality in patients with haematological and solid organ malignancies. Prevention of infection by vaccination is hence an important aspect of clinical care of cancer patients. Immunocompromising effects of the underlying disease as well as specifics of antineoplastic therapies need to be considered when devising vaccination strategies.
Aims: To give evidence-based recommendations on vaccination in patients with haematologic and solid organ malignancies.
Methods: The AGIHO has reviewed currently available data to provide guidance on vaccination of cancer patients. It gives practical advice for clinicians in hematology and oncology.

Results: Examples are the recommendations on pneumococcal and influenza vaccination.
Summary/Conclusion: Even if there have been a variety of studies on the topic of vaccination of cancer patients in recent years, more information on vaccination strategies in cancer patients (e.g. with new immunomodulatory treatments) are warranted. In addition, coverage of vaccination is lacking in the general population as well as in patients at risk, in particular hematological and oncologic patients. Detailed information and clear recommendations by physicians have a high impact on acceptance of vaccination, while the main reasons for denial of vaccination are concerns about interaction with the malignant disease and potential side-effects. Increased awareness among physicians is of great importance to effectively improve vaccination in patients with malignant disease. To eliminate misperception and improve vaccination coverage in the population of cancer patients, educational programs for patients and for physicians focusing on safety and efficacy of vaccine are warranted.

PS1213

CHARACTERISTICS OF HEMATOLOGICAL PATIENTS WITH BLOOD CULTURES PROVEN SEPSIS – EXPERIENCE OF ONE CENTER

K. Misura Jakobac[1,*], N. Gredelj Simec[1], V. Zatezalo[1], I. Butic[2], A. Hanzek[3], D. Radic Kristo[1,4], S. Ostojic Kolonic[1,3]

[1]Department of Hematology, Merkur University Hospital, [2]Department of clinical microbiology and hospital infection, Clinic for Infectious Diseases "Dr. Fran Mihaljevic", [3]School of Medicine, University of Zagreb, Zagreb, [4]School of Medicine, University of Osijek, Osijek, Croatia

Background: Hematological patients are at higher risk for infections and their subsequent complications. Febrile neutropenia and sepsis are the main causes of non-selective mortality in patients with malignant hematological diseases. For adequate treatment, it is necessary to know the characteristics of the disease and the local microbiological situation. Patients with febrile neutropenia should be approached in accordance with the guidelines for management of sepsis with the aim of preventing the development of septic shock and possible death.
Aims: To determine the risk factors for sepsis development in hematological patients, the type of cause and the outcome of the treatment.
Methods: The available clinical, laboratory and microbiological data of hematological patients treated for sepsis at the Clinic for Internal Diseases, KB Merkur, were collected and analyzed from January 2013. until April 2017.
Results: We analyzed 239 patients and 319 episodes of fever with positive blood cultures. Of these, 38.55% patiens had acute leukemia, 31.32% had non Hodgkin's lymphoma, 15.26% had multiple myeloma and 6.014% patients had Hodgkin's lymphom. Other 8.835% of all patients were treated for some other diagnoses (amyloidosis, myeloproliferative neoplasm, myelodisplastic syndrom, aplastic anemia, Langerhans cell histiocytosis). 14% patients had undergone *autologous peripheral stem-cell transplantation.* A total of 80.8% of the patients had an active disease while having sepsis. Neutropenic were 70.5% of the patients. The most common cause of neutropenia was intensive chemotherapy (52.5%). Other causes of neutropenia were: lower intensity chemotherapy (19.2%), bone marrow transplantation (18.5%) or untreated hematological disease (9.8%). The average duration of neutropenia was 12.4 days (median 9). The average duration of the fever was 5.71 days (median 5) for not transplanted patients and 4.29 days (median 6) for transplanted patients. More than half of the patients (59.8%) had a central venous route (CVK) at the moment of febrile illness. A total of 36 different agents were isolated, out of which 53% were gram negative. The most commonly isolated agents were *E. coli* (17.1%), *K. pneumoniae* (16.2%), coagulase negative staphylococci (14.1%) and P. aeruginosa (8.6%). There was no statistically significant difference in the frequency of gram positive or negative pathogens between patients who had CVK and those who did not. Total mortality was 29%. Most of the deaths (84.5%) had prolonged neutropenia due to intensive chemotherapy. In 60% of the cases, the cause of death was gram negative sepsis. Among the patients with active disease who died in sepsis the most common diagnosis were acute myeloid leukemia (34.9%) and non Hodgkin's lymphoma (30.2%). We found a statistically significant correlation between the mortality rate and the couse of neutropenia (p=0.002). Also, statistically significant correlation was found between the mortality rate and disease activity in patients with sepsis (p=0.001). No statistically significant association of mortality with the type of causative agent or with the presence of CVK was found.
Summary/Conclusion: The analysis of our data showed a high mortality rate in hematological patients with sepsis, depending on the type and activity of the underlying disease which confirms the importance of an early and aggressive treatment of each infection in immunocompromised patients.

PS1214

CHARACTERISTICS AND OUTCOME OF POLYMICROBIAL BLOODSTREAM INFECTIONS (P-BSI) IN PATIENTS WITH HEMATOLOGICAL CANCER

M.E. Zannier[1,*], M.V. Dubbini[1], A. Candoni[1], C. Filì[1], D. Lazzarotto[1], N. Rabassi[1], C. Scarparo[2], A. Sartor[2], R. Fanin[1]

[1]Hematology, [2]microbiology, ASUIUD Udine Italy, udine, Italy

Background: Polymicrobial bloodstream infections (p-BSI) may complicate the course of hematological diseases. They may negatively impact on patients' survival, although they do not represent a frequent event.
Aims: Aim of our study was to characterize polymicrobial bloodstream infections occuring in patients with hematological cancer.
Methods: We analyze clinical characteristics and outcome of 109 p-BSI occurring in patients with hematologic diseases over a 9-year period (2008-2017).
Results: P-BSI were 25% (109/432) of total BSI observed at our center during the whole period of observation. Their incidence was 12.1 cases per year vs 37,5 cases per year of not-polymicrobial BSI. The most common underlying hematological disease was Acute Myeloid Leukemia (AML) (57%) and the median age at onset of p-BSI was 60 yrs (range 19-79). 51/109 (47%) patients had a refractory/relapsed underlying hematological disease, while 33% were in complete remission and 19% in first induction. The majority of cases had severe neutropenia (87%) and carried a central venous catheter (96%). In 58/109 (53%) cases there was another contemporary site of infection, including catheter-related BSI (24%), pneumonia (24%), abdominal infection (enteritis, cholangitis) (24%) followed by skin and soft tissue infection (16%). The most common bacterial association was Gram-negative plus Gram-positive bacteria (56%). The most common isolates were coagulase–neg *Staphylococcus* spp, *Enterococcus* spp, *E. Coli* and *P. aeruginosa*. Susceptibility and antibiotic treatment have been recorded. The resolution rate of p-BSI was 81%. The mortality p-BSI related was 16%. 25/109 (22%) p-BSI were complicated by acute renal failure, septic shock or acute heart failure. Septic shock occurred in 17% cases and the septic shock related mortality was 63%. Multidrug Resistant (MDR) bacteria were 10% of bacteria isolates. The mortality was significantly higher in MDR p-BSI (35% vs 12%), in patients with a contemporary other site of infection (24% vs 8%) and in patients with an active (not-complete remission) hematological disease (25% vs 0%) ($p<0.05$).
Summary/Conclusion: This observational study confirms that p-BSI is a rare infectious complication in hematological patients; it is more frequent during a severe neutropenia and in AML. The mortality p-BSI related is lower than 20% but if septic shock occurs the related-mortality is high (63%). A significantly higher mortality was also documented in p-BSI due to MDR bacteria and in those patients with active hematological disease.

PS1215

IS ANTIMICROBIAL PROPHYLAXIS NECESSARY FOR LYMPHOMA PATIENTS? A SINGLE CENTRE, REAL-LIFE EXPERIENCE

A. Yildiz[1,*], H. B. Afacan Öztürk[1], M. Albayrak[1], Ç. Pala Öztürk[1], O. Şahin[1], S. Maral[1], H. Okutan[2]

[1]Hematology, University of Health Sciences, Diskapi Yildirim Beyazit Training and Research Hospital, [2]Hematology, Lösante's Children and Adult Hospital, Ankara, Turkey

Background: Prophylaxis is strongly recommended in patients with hematological malignancy who are usually at higher risk for infection and neutropenic fever. It is still unclear whether or not there is a definite need for antimicrobial prophylaxis in intermediate-risk hematology patients such as those with lymphoma.
Aims: To assess the benefit of antimicrobial prophylaxis in lymphoma patients who were treated in our centre.
Methods: A retrospective analysis was made of patients admitted from January 2009 to December 2017 to the Hematology Department of Diskapi Yildirim Beyazit Training and Research Hospital, a tertiary referral hospital in Ankara, Turkey. The study included patients who were diagnosed with any type of lymphoma and given chemotherapy. Routine antimicrobial prophylaxis was administered to 127 lymphoma patients, and not to 65 lymphoma patients. These two groups were compared in respect of the incidence of total infection episodes (IE), febrile neutropenia (FN) episodes, and non-neutropenic clinically documented infection (CDI) episodes.
Results: For all patients with lymphoma and subtypes of NHL or HL, no significant difference was determined between the groups in respect of the total incidence of IE, FN and nonneutropenic CDI both during the first line

chemotherapy and throughout the total follow-up period (p>0.05). Patients with prophylaxis had a higher incidence of IE, which was treated with parenteral antibiotics both during the first line chemotherapy and throughout the total follow-up period (p<0.05) (Table 1).

Table 1. The comparison of patients with or without prophylaxis according to the incidence of infection types.

	Patients with prophylaxis (n:127)	Patients without prophylaxis (n:65)	RR	p
Events occuring in total follow-up period		No. of patients (%)		
Total infection episodes	38 (29.9%)	20 (30.8%)	0.972 (0.619-1.527)	0.904
FN episodes	21 (16.5%)	7 (10.8%)	1.535 (0.689-3.422)	0.284
Nonneutropenic CDI episodes	19 (15.0%)	12 (18.5%)	0.810 (0.420-1.565)	0.533
Events occuring during first line chemotherapy		No. of patients (%)		
Total infection episodes	37 (29.1%)	20 (30.8%)	0.947 (0.601-1.492)	0.814
FN episodes	21 (16.5%)	8 (12.3%)	1.344 (0.630-2.866)	0.439
Nonneutropenic CDI episodes	18 (14.2%)	12 (18.5%)	0.768 (0.394-1.495)	0.439

IE: Infection episodes, FN: Febrile neutropenia, CDI: Clinically documented infection, RR: Relative risk.

Summary/Conclusion: Antimicrobial prophylaxis was seen to have no effect on the total incidence of IE and FN. Therefore, the routine use of antimicrobial prophylaxis should not be recommended for patients with lymphoma.

PS1216

CASTLEMAN DISEASE WITH THE EXPERIENCE OVER 17 YEARS

F. D. Atilla[1], N. Soyer[2], M. Tombuloglu[1], F. Vural[2], F. Sahin[2], M. Tobu[2], D. Demir[3], N. Ozsan[3], M. Hekimgil[3], G. Saydam[1,*]

[1]Hematology, [2]EGE University, Izmir, Turkey, [3]Pathology, EGE University, Izmir, Turkey

Background: Castleman disease (CD) is a non-clonal lymphoproliferative disorder as a common cause of non-neoplastic lymphadenopathy. CD encompasses several distinct clinicopathological disorders at the intersection of haematology, immunology, oncology, rheumatology and virology that share a spectrum of histopathological features. An international collaborative working group has reached consensus definitions and classification, defining diagnostic criteria for CD which enables to clinicians reaching the proper diagnose.
Aims: The aim of this study is to review our CD patients from a single centre according to newly established diagnostic criterias.
Methods: All patients with a biopsy proven histopathological characteristics of CD diagnosed at Ege University Hospital between 2000-2017 years were reviewed for analysis. Clinical and laboratory datas were collected retrospectively. The patients were divided into two main groups based on the anatomical distribution of the disease: Unicentric CD (UCD) and multicentric CD (MCD). Also MCD were divided into two groups: HHV8 positive MCD and idiopathic MCD.
Results: A total of 64 patients were reviewed. Among the study group; 34 patients were excluded because two were diagnosed with synchronous Hodgkin lymphoma, one was diagnosed with POEMS, and the rest were unable to access all data. Detailed clinical and laboratory datas were summarized in Table 1. The mean age at diagnosis of 30 patients with adequate data was 48.8 (26-82). After histopathological evaluation, majority had hyaline vascular type (n=19), followed by mixed type (n=8) and plasma cell type (n=3). There were 16 patients in UCH group, none of them had HHV-8 and HIV positivity. After a median follow up of 54 months, the estimated 2-year OS was 87.7% [95% confidence interval (CI): 72.1–103.3]. There were 7 patients in the HHV-8+MCH group and two of them had Kaposi sarcoma. All of whom received chemotherapy and/or immunotherapy treatments. Clinical manifestations in the HHV-8+MCH group were; fever, splenomegaly, skin lesions, acute renal failure, oedema, effusion, respiratory symptoms, CRP/ferritin and LDH elevation. After a median follow up of 38.2 months, the estimated 2-year OS was 46% [95% confidence interval (CI): 17.1–74.9]. There are 7 patients in the iMCH group with HHV-8 negative and the clinical findings are very similar to the HHV-8+MCH group. Two-year overall survival in iMCH group was higher than HHV-8+MCH with 82.7% [95% confidence interval (CI): 58.6–106.8].

Table 1. CD Patient characteristics.

	UCD			MCD		
	Total	Single lesion	Single station	Total	HHV-8+MCD	iMCD
N	16	12	4	14	7	7
Sex male/female	7/9	6/6	1/3	10/4	5/2	5/2
Age (median), years	41.8	37.3	55.5	55.4	54.6	52.6
Lymph node localisation						
Mediastinum	3	2	1	12	7	5
Abdominal	3	2	1	12	5	7
Cervical	6	6	-	13	7	6
Axillary	3	1	2	13	7	6
Inguinal	-	-	-	11	5	6
Extranodal	1	1	-	1	1	-
Delay to diagnosis (median, months)	1.8	1.25	3.75	7.2	11.2	3.1
Fever	1	-	1	11	7	4
Complications						
Splenomegaly	-	-	-	12	6	6
Oedema effusion	-	-	-	4	3	1
Lung involvement	-	-	-	3	2	1
Skin lesions	1	-	1	10	6	4
Kidney involvement	1	-	1	5	2	3
Paraneoplastic pemfigus	-	-	-	-	-	-
Polyneuropathy	1	-	1	6	3	3
AIHA	-	-	-	1	1	-
AITP	-	-	-	1	1	-
Leucocyte count, $\times 10^9$ /L (min-max)	7760 (5650-11000)	7627 (5650-11000)	8162 (6170-9180)	9044 (3630-22800)	7960 (3630-16420)	10128 (5730-22800)
Haemoglobin, g/dL (min-max)	12.6 (7.9-16.2)	13.4 (12-16.2)	8162 (6170-9180)	10.7 (5.6-15.2)	11 (7.7-15.2)	10.5 (5.6-14.3)
Platelet count, 10^9/L (min-max)	318 (78-655)	301 (220-461)	370 (78-655)	229 (56-564)	186 (78-277)	272 (56-564)
CRP, mg/dl (min-max)	1.7 (0.1-14)	0.3 (0.1-0.6)	5.9 (0.3-14)	7.4 (0.1-22)	6 (0.3-12)	8.7 (0.1-22)
Serum albumin, g/L (min-max)	4 (2.1-5.1)	4.1 (3.3-5.1)	3.4 (2.1-4)	3.1 (2-4.5)	2.9 (2-4.5)	3.3 (2.1-4.5)
Gammaglobulin, g/L (min-max)	3 (2-4.4)	2.9 (2-3.8)	3.5 (2.9-4.4)	4 (2.6-5.8)	4.1 (3-5.7)	3.8 (2.6-5.9)
Monoclonal gammopathy	-	-	-	4	2	2
LDH > normal	2	-	2	11	6	5
Ferritin > 5 times normal	1	-	1	10	6	4
DAT	-	-	-	4	2	2
Follow-up (months)	54	55.5	49.7	37.2	38.2	36.1
Deaths	2	-	2	3	2	1

Summary/Conclusion: CH is a very rare lymphoproliferative disease which should be kept in mind in the differential diagnosis with asymptomatic and localized lymphadenopathies or widespread lymphadenopathies with severe systemic symptoms. Future studies should be multicentred and collaborative in order to evaluate significant numbers of patients and to establish up to date and effective treatment protocols for this rare but potentially life-threatening disorder.

Iron metabolism, deficiency and overload

PS1217

EFFECTIVENESS OF DIFFERENT IRON ORAL FORMULATION IN IRON DEFICIENCY ANEMIA DUE TO GASTROINTESTINAL BLEEDING: MULTICENTRIC RANDOMIZED STUDY

G. Giordano[1,*], D. Berardi[2], A. Parente[3], D. Castaldi[3], M.A. Cinotti[3], F. Vedruccio[3], V. Susca[4], L. Petrella[4], G. Berardi[5], M. Magri[6], G. Niro[6], R. Gigli[6]

[1]Medicine, Regional Hospital "A. Cardarelli", Campobasso, [2]Medicine, University la Sapienza, Rome, [3]Medicine, General Medicine, [4]Medicine, University of Molise, [5]Family Medicine, General Medicine, [6]Laboratory Medicine, Regional Hospital "A. Cardarelli", Campobasso, Italy

Background: Ther'are several different oral iron formulation with different mechanisms of uptake known or supposed (DMT1 for iron sulphate, microencapsulated iron and sunactive iron, transcellular and lymphatic way for sucrosomial iron, heme and peptones carrier for heminic chelated bisglycinated iron, peptones carrier for chelated bisglycinated iron).
Aims: Data regarding absorbtion and effectiveness for each kind of iron are lacking. Aim of this study is to see if ther'is some difference regarding effectiveness and tolerability among different oral iron formulation.
Methods: This study is a multicentric randomized study. 300 patients with iron deficiency anemia in gastrointestinal bleeding were randomized 1:1:1:1:1:1 to receive iron sulphate (65 mg of elemental iron oid), microencapsulated iron, sunactive iron, sucrosomial iron, heminic chelated bisglycinated iron (30 mg of elemental iron tid), chelated bisglycinated iron (15 mg of elemental iron tid). Patients charachteristics were similar in all six groups. Hemoglobin trend and side effects were recorded in general patients population and in C reactive protein (CRP) high level patients. Median Hb value at start of treatment was 8.2 g/dl. In group of patients with high CRP median Hb value was 7.8 g/dl. Median follow-up was 4.5 months (R 3-6).
Results: The Hb increase rate in the first two weeks of treatment is the same in the group of sucrosomial, chelated bisglycinated and heminic bisglycinated iron, but from the third to the sixth week Hb increase rate is higher in sucrosomial iron group. In this group from the sixth to the twelfth week the Hb increase rate is still higher, but shows a slight decrease. In all group Hb level achieves a plateau phase after three months and ferritin level starts to increase. At three monts higher levels of hemoglobin are present in sucrosomial iron (13.2 g/dl), heminic chelated bisglycinated iron (11.7 g/dl), chelated bisglycinated iron (11.3 g/dl). In group of patients with high CRP level (>30 ng/ml) the Hb increase is higher in sucrosomial iron group from the tenth week, is continuous until the sixth month (Hb 12.5 g/dl) and is linked to a marked decrease of CRP (5 ng/ml). All type and grade of side effects are higher in ferrous sulphate group (15/50) and in sunactive iron group (6/60)
Summary/Conclusion: Among different oral iron formulation in iron deficiency anemia sucrosomial iron shows a faster activity, an higher efficacy more evident in patients with high CRP value linked to a marked CRP level decrease after three month of treatment

PS1218

RENAL FUNCTION IN TRANSFUSION-DEPENDENT B-THALASSEMIA PATIENTS: A DECADE OF FOLLOW-UP AND COMPARISON BETWEEN CHELATION REGIMES

O. Tanous[1], Y. Azulai[2], T. Dujovny[3], N. Swartz[4], R. Colodner[2], A. Koren[5], C. Levin[3,*]

[1]Ward A, [2]Yetzhak Rabin 20, [3]Pediatric Hematology Unit, [4]Hematology Laboratory, [5]Hematology Unit, Emek Medical Center, Afula, Israel

Background: Glomerular and tubular dysfunctions in β-thalassemia major (β-TM) patients (pts) have been attributed to iron overload, chronic anemia and iron-chelation therapy (ICT) toxicity.
Aims: We studied glomerular and tubular function in β-TM pts treated with 2 different ICT regimes.
Methods: We studied 36 β-TM pts (18 females and 18 males). Mean age was 20.92±9.7 (range 5–45) years. 26 pts received deferasirox (DFX) for a mean period of 59 months; this was the first ICT for 6 of them; 10 pts were treated with deferoxamine (DFO) or DFO + deferiprone (DFP) (4 and 6 pts, respectively; hereafter DFO+/-DFP group). Clinical data were collected from pts' files.We evaluated serum urea, creatinine (sCr) and electrolytes, and estimated glomerular filtration rate (eGFR); fractional excretion of sodium (feNa), Urinary N-acetyl-β-D-glucosaminidase (uNAG) was also measured as a marker of tubular injury.
Results: eGFR was normal in all patients (mean 104.6±19 mL/min per 1.73 m^2); in the DFX group, eGFR was slightly lower than in the DFO+/-DFP group (100.9±17.09 vs. 114.0±22.31 mL/min per 1.73 m^2; p=0.0676). Increased uNAG was found in 9 pts (25%) – 30% in the DFX group vs. 10% in the DFO+/-DFP group, and was significantly higher in the DFX group (mean 10.4±6.1 vs. 5.3±2.7 IU/L, p=0.012). A moderate negative correlation was found between uNAG levels and mean serum ferritin for the prior 10 years (r = -0.35, p=0.03). In the DFO+/-DFP group, a strong positive correlation was found between uNAG levels and the amount of iron transfused over the prior 10 years (r = 0.7647, p=0.021); this correlation was not found in DFX pts. A moderate negative correlation was found for urinary Ca/Cr ratio with 10-year mean serum ferritin level and with 10-year amount of transfused iron (r = -0.35, p=0.34; r = -0.4, p=0.014, respectively). These correlations were stronger in the DFO+/-DFP group. Renal function had been previously evaluated in 20 pts treated with DFO (Smolkin *et al.*, 2008) and those results were compared with the current values. The eGFR significantly declined in pts switched to DFX (mean eGFR in first study 113.5±26 vs. 100.1±17 mL/min per 1.73 m^2, p=0.0093) but not in pts who continued DFO+/-DFP. A significant increase in sCr compared to the previous study was found in pts who switched to DFX (mean 0.51±0.9 vs. 0.67±0.1 mg/dl, p=0.0008), but not in pts who continued treatment with DFO+/-DFP. The same observation was made regarding urine Ca/Cr (mean 0.08±0.11 vs. 0.176±0.12, p=0.001).
Summary/Conclusion: A high prevalence of renal tubular abnormalities was observed in our pediatric and adult β-TM pts, particularly in the DFX group. The marker of tubular injury – uNAG – was negatively associated with mean 10-year serum ferritin, suggesting ICT's involvement in tubular injury. Moreover, uNAG was associated with transfusional iron burden in the DFO+/-DFP group, but not in the DFX group, proposing a mechanism other than iron overload for the pathogenesis of tubular injury in DFX-treated pts. Finally, glomerular function remained within the normal range in all pts; however a significant decline in glomerular function compared to a decade earlier was observed only in the pts currently treated with DFX. Strict follow-up of renal function in β-TM pts, especially children, is warranted.

PS1219

GENETIC STUDIES REVEAL NEW MUTATIONS IN THE CP GENE IN ACERULOPLASMINEMIA PATIENTS

G. Marchi[1,*], A. Barqué [2], M. Vila[2], C. Esteban[2], V. Chelban[3], H. Houlden[3], N.W. Wood[3], C. Piubelli[4], M. Dorigatti Borges[5], D. Martins de Albuquerque[5], K. Yotsumoto Fertrin[5], E. Jové Buxeda[6], J. Sanchez-Delgado[7], N. Baena-Díez[8], B. Burnyte[9], A. Utkus[10], P. Capelli[11], G. Kaubrys[10], E. Suku[12], K. Kowalczyk[13], B. Karaszewski[13], J. Porter[14], S. Pollard[15], P. Eleftheriou[14], P. Bignell[16], D. Girelli[4], M. Sanchez[2]

[1]Department of Medicine, University of Verona, Verona, Italy, [2]Iron Metabolism: Regulation and Diseases Group, Josep Carreras Leukaemia Research Institute (IJC), Badalona, Barcelona, Spain, [3]National Hospital for Neurology and Neurosurgery, London, United Kingdom, [4]University of Verona, Verona, Italy, [5]Hematology and HemotherapyCenter – Hemocentro Campinas, University of Campinas, Campinas, Brazil, [6]Internal Medicine, [7]Hepatology and Digestive Department, [8]Genetic Department, University Hospital ParcTaulí Sabadell, Sabadell, Spain, [9]Department of Human and Medical Genetics,, [10]Clinic of Neurology, Faculty of Medicine, Vilnius University, Vilnius, Lithuania, [11]Department of Diagnostics and Public Health, [12]Biotechnology, University of Verona, Verona, Italy, [13]Department of Adult Neurology, Medical University of Gdańsk, Gdańsk, Poland, [14]Joint Red CellUnit Unit, Haematology Department, University College London NHS Foundation Trust, Cancer Services, London, [15]Bradford Royal Infirmary, Bradford, [16]Oxford Molecular Diagnostic Centre, Molecular Haematology , John Radcliffe Hospital, Oxford, United Kingdom

Background: Aceruloplasminemia is a rare autosomal recessive genetic disease characterized by mild refractory anaemia, diabetes, retinopathy, liver disease and progressive neurological symptoms due to iron accumulation in brain, liver and retina. This disease is caused by mutations in *CP* gene that produce a strong reduction or absence of ceruloplasmin ferroxidase activity, leading to an impairment of iron metabolism. Up to date, around 60 families have been described worldwide, most of them in Japan. Prompt diagnosis and therapy are crucial to prevent neurological complications of the disease since, once established, they are usually irreversible.
Aims: The aim of our international collaborative study is to increase molecular and clinical knowledge of this rare disorder of iron metabolism.
Methods: We collected patients with an established diagnosis of aceruloplasminemia from multiple referral Centers. Patients were from several countries and had different origins (but none of them was from Japan). We sequenced the entire coding, intron-exon boundaries and 5' and 3' regulatory

regions of the *CP* gene by Sanger sequencing and next generation sequencing (NGS). The frequency of each variant and its possible pathogenetic role was evaluated by bioinformatical tools. In silico modeling provided a functional explanation of the pathogenic variants.
Results: In this study, we describe 11 families affected by aceruloplasminemia, and 13 mutations (12 of them being novel) in the *CP* gene. Functional characterization of a recurrent pathological splicing mutation was performed, along with computational modelling of missense mutations.
Summary/Conclusion: This series, the largest so far including non-Japanese patients, adds to the molecular and clinical heterogeneity of aceruloplasminemia, a disease expected to be increasingly diagnosed in the Next-Generation Sequencing era.

PS1220

DIAGNOSIS OF HEREDITARY HAEMATOLOGICAL DISEASES USING NEXT GENERATION SEQUENCING PANELS

L. Zalba-Jadraque[1], J. Fita-Torró[2], B. Cadenas-Sevilla[3], D. Beneitez-Pastor[4], J. L. Dapena[5], A. Blanco-Alvarez[4], E. Rivero-Arango[6], J.L. Fuster[7], M. Bermúdez-Cortés[8], L. Arenillas[9], S. Montesdeoca[10], L. Martínez[11], C. Pedro[11], S. Payan-Pernia[12], I. Badell-Serra[12], A. Remacha-Sevilla[13], S. Pérez-Montero[2], I. Hernández-Rodriguez[14], C. Tornador-Antolín[2], M. Sanchez[1,2,*]

[1]Iron Metabolism: Regulation and Diseases, Instituto Josep Carreras contra la Leucemia (IJC), Badalona, [2]BLOODGENETICS, esplugues de llobregat, [3]Iron Metabolism:Regulation and Diseases, Instituto Josep Carreras contra la Leucemia (IJC), Badalona, [4]Haematology Service, University Hospital Vall Hebrón, [5]Pediatric Onco-Haematology Service, University Hospital Vall Hebron, Barcelona, [6]Haematology Service, Hospital Arnau de Vilanova, Lleida, [7]Pediatric OncoHaematology Service, Clinic University Hospital Virgen de la Arricaca. Instituto Murciano de Investigación Biosanitaria (IMIB), Murcia, [8]Pediatric OncoHaematology Service, Clinic University Hospital Virgen de la Arricaca. Instituto Murciano de Investigación Biosanitaria (IMIB), Murcia, [9]Laboratory of Haematology Citology. Pathology Service, GRETNHE, IMIM-Hospital del Mar, [10]Haematology Service, Hospital del Mar, [11]9Haematology Service, Hospital del Mar, Barcelona, 9Haematology Service, Hospital del Mar, Barcelona, [12]Peadriatric Service, [13]Haematology Service, Hospital Sant Pau i la Santa Creu, Barcelona, [14]Haematology Service, University Hospital Germans Trias i Pujol (HGTiP), Institut Català d'Oncologia (ICO), Badalona, Spain

Background: Next Generation Sequencing (NGS) gene panels are currently used for clinical diagnosis of many hereditary diseases.
Aims: The objective of this work is the development and commercialization of gene panels for the diagnosis of hereditary haematological diseases.
Methods: All patients studied signed informed consent for genetic studies and for research. DNA extraction was done using FlexiGene DNA kit (Quiagen). Library preparation was done using a customized HaloPlex™ Target Enrichment System (Agilent), and was sequenced in Illumina platform. Data analysis was done using software SureCall software (Agilent) and subsequent processing with proprietary algorithms. The variants found as pathogenic or probably pathogenic are confirmed by Sanger sequencing.
Results: NGS gene panels have been developed for the diagnosis of inherited haematological diseases (v15). The panels were validated by including 27 cases with known mutations by Sanger methodology. In 26 of the 27 cases analyzed (96.3%) the mutation/s previously described was detected, in one case the mutation was present in a not covered region; subsequent re-design of the panel solved this problem.
Several cases will be exposed where a satisfactory diagnosis has been reached for different diseases. In particular, we will discuss a paediatric case of hypoferritinemia, 2 cases of Hemochromatosis (young and adult case), 2 cases of enzymopathies, one case of congenital dyserythropoietic anemia due to a mutation in KLF1 and a case of congenital sideroblastic anaemia with mutations in the YARS2 gene with a mild clinical presentation that has allowed the re-evaluation of the clinical symptoms in this type of patients.
Summary/Conclusion: The implementation of the new sequencing methodology in clinical practice for the diagnosis of hereditary haematological diseases allows the inclusion of the study of multiple genes and a rapid and effective diagnosis of these cases. In addition, the analysis of several cases has allowed us to extend the genetic diagnosis with research studies.

PS1221

HEMATOPOIETIC IMPROVEMENT AFTER DEFERASIROX TREATMENT IN A CHILD WITH APLASTIC ANEMIA

G. Ersoy[1,*], C. Bayram[1], B. Koç[1], E.P. Uysalol[1], A. Ayçiçek[1], N.N. Ozdemir[1]

[1]Pediatric Hematology Oncology, Kanuni Sultan Suleyman Education and Research Hospital, Istanbul, Turkey

Background: Aplastic anemia (AA) is a rare, life threatening disorder characterized by pancytopenia and hypocellular bone marrow in the absence of an abnormal infiltrate or marrow fibrosis. Bone marrow transplantation (BMT) if a human leukocyte antigen (HLA)-matched sibling donor is available or immunosupressive therapy (IST) for patients without an HLA-matched siblig donor are the recommended first-line treatments options for acquired severe AA.
Aims: Here we present a 7 5/12 year old male with acquired AA who lack an HLA-matched sibling donor, and failed IST but achieved a hematopoietic trilinear complete response and transfusion independence after deferasirox (DFX) treatment.
Methods: -
Results: A Syrian refugee presented with bleeding gums and bruises two years ago. His past medical history revealed that he had been diagnosed with severe AA 9 months ago, and was not responsive to IST at another center. Physical examination revealed multiple echimosis and petechia with no additional findings. Laboratory tests revealed pancytopenia and reticulocytes 0.5%. Bone marrow aspiration and biopsy was consistent with aplastic anemia, without blasts. Viral serology was negative, immunoglobulins were normal and DEB test for Fanconi anemia was normal. On follow-up, over a 6-month period, the patient received platelet transfusion every week due to oral mucosal bleeding, and required erythrocyte transfusion every 4 weeks. A second attemp of IST was recommended howeever the family rejected. At 5th month of follow-up, his ferritin level was 1487 ng/mL, and he was started on DFX treatment at a dose of 25 mg/kg/day. The patient achieved transfusion independence at the 6th month of DFX therapy, and complete hematopoietic recovery at the 22th month of DFX therapy with a ferritin level of 490 ng/mL. His last blood count is completely normal.
Summary/Conclusion: Deferasirox chelation therapy has been shown to inhibit nuclear factor-κB in patients with MDS, ALL and in leukemic cell lines, which is the current proposed mechanism for the improved erythropoiesis observed in such patients. Our case unexpectedly achieved total transfusion independence and hematopoietic complete improvement after DFX treatment and is presented to highlight the possible impact of DFX on hematopoietic recovery in patients with AA. Further prospective, controlled studies are mandatory for clinical use.

PS1222

TREATMENT WITH FERRIC CARBOXYMALTOSE FOR SEVERE IRON DEFICIENCY ANEMIA IN STABLE NOT ACTIVELY BLEEDING PATIENTS IN THE EMERGENCY DEPARTMENT

I. Motta[1,2,*], M. Migone De Amicis[1], A. Marcon[3], O. Milani[2], M. Porzio[2], A.M. Rusconi[2], A.M. Brambilla[2], N. Montano[2,3], M.D. Cappellini[13]

[1]Department of Internal Medicine, [2]Emergency Department, Fondazione IRCCS Cà Granda Policlinico, [3]Dipartimento di Scienze Cliniche e di Comunità, Università degli Studi di Milano, Milan, Italy

Background: Anemia affects a third of the world population and half of the cases are due to iron deficiency (ID). The current AABB red blood cell (RBC) transfusion guidelines recommend a restrictive strategy, with hemoglobin (Hb) thresholds <7 g/dL in hospitalized stable patients (pts) and <8g/dL in pts with cardiovascular disease. Despite these guidelines, variation in clinical practice exists. Moderate and severe anemia is a frequent cause of hospitalization; however data about the prevalence in the emergency department (ED) are scarce. Besides severity, the time of onset is crucial. Acute anemia may present with hemodynamic instability and requires prompt treatment including transfusion. Conversely, when anemia develops chronically, compensatory mechanisms arise and pts are often paucisymptomatic. No data are available about the management of stable pts who present to the ED with severe iron deficiency anemia (IDA). The AABB Choosing Wisely Campaign recommends not transfusing RBC for IDA without hemodynamic instability; however the management of these pts depends on physician experience. As a consequence, in many cases pts are transfused and then discharged from the ED. IDA treatment, beside the diagnostic work-up, is based on iron supplementation and oral iron is the gold standard. However, in presence of low levels of Hb, IV iron is more effective, bypassing intestinal absorption, and produces a faster increase in Hb. In the past the use of IV iron has been limited by hypersensitivity reactions. A new IV iron formulation, ferric carboxymaltose (FCM), allows the administration of high dose of iron with low immunogenic potential.
Aims: Aim of this study is to retrospectively analyze data from hemodynamically stable, not actively bleeding pts aged<60 years, who presented to the ED of Fondazione IRCCS Cà Granda Policlinico in Milan (Italy) in 2017 and 2018 with severe (<8 g/dL) IDA due to suspected or known source of chronic loss or malabsorption, and have been treated with FCM in the

ED and discharged afterwards.
Methods: Iron status (serum iron, transferrin, ferritin) performed within the last 3 months was accepted, while pts without iron status were tested in the ED. Once treated, pts were discharged and evaluated in a hematology out-patient clinic at T1 (after 10.1±3.7 days) and T2 (median 43, range 21-122 days). We collected hematological data from the ED (T0), T1 and T2.
Results: We treated 16 pts (14F/2M). Mean Hb value at T0 was 6.2±0.8 g/dL. Mean serum ferritin was 3.1±2.1 ng/ml, transferrin saturation 2.6±0.8%. 4/16 (25%) were transfused because symptomatic. 3 pts received 1 unit and 1 patient 2 units of RBC. The mean Hb after transfusions was 6.6±1.1 g/dL. 13/16 have been treated with 1000 mg of FCM and 3 with 500 mg. The net increase of Hb at T1 (excluding transfusions) was 2.6±1.1 g/dL with a robust reticulocytosis in most of the pts (mean 161785±95998, median 127000, range 67000-371000/mm^3). The net increase at T2 was 4.9±1.8 g/dL, with mean Hb 11.8±1.3 g/dL. Consistently MCV increased from 62.6 (T0) to 80.6±8.3 fl (T2). The cause was hemorrhoids in the 2 men, malabsorption in 1 woman and menstrual losses in 13/16. No adverse events have been observed.
Summary/Conclusion: Administration of FCM in the ED resulted to be safe and effective in stable not actively bleeding pts with severe IDA. All the pts have been discharged after treatment. Thus, we prevented inappropriate transfusions, reducing related risks, saving units for pts who need them and reducing costs of treatment. Further studies are needed to produce robust evidence for non-hospitalized pts with severe IDA.

PS1223

IRON DEFICIENCY ANAEMIA – ZINC PROTOPORPHYRIN, A SIMPLE, FAST, INEXPENSIVE DIAGNOSTIC METHOD

L. Relvas[1], J. Silva Gomes[1,*], T. Nascimento[1], A. Oliveira[1], E. Cunha[1], J. Pereira[1], C. Bento[1], T. Magalhães Maia[1], M.L. Ribeiro[1]

[1]Clinical Haematology, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

Background: Iron deficiency is the most common cause of anaemia worldwide. The ferritin level is the most reliable diagnostic test, however, in the presence of chronic inflammatory conditions, it can be difficult to interpret. The final reaction in haem biosynthesis is the incorporation of iron in a protoporphyrin ring. In the absence of iron, zinc is incorporated, leading to the formation of zinc protoporphyrin (ZPP). ZPP is quantifiable by fluorimetry in peripheral blood samples stored in EDTA or in capillary blood samples, making it a useful, fast and inexpensive method for the diagnosis of iron deficiency anaemia (IDA).
Aims: To determine a cut-off of ZPP measurement for the diagnosis of iron deficiency and to validate the use of this technique in a Haematology Laboratory.
Methods: The authors prospectively and anonymously analysed paediatric and adult blood samples with anaemia. Two groups were defined according to the following inclusion criteria: IDA group: anaemia, as defined by World Health Organisation (WHO) criteria by age and gender, ferritin <20 ng/mL and erythrocyte sedimentation rate (ESR) <22 mm/h; Control group: no anaemia according to WHO criteria, ferritin 20-120 ng/mL and ESR <22 mm/h. ZPP was measured by haematofluorimetry of EDTA samples (AVIV Biomedical).
Results: A total of 132 samples were included: 97 in the IDA group and 35 in the control group. IDA group: 74% female, median age 32.3 yo (2-79); 26% male, median age 26.4 yo (1-78); average haemoglobin (Hb) 10.5 g/dL (SD 1.29); average ferritin 8.3 ng/mL (SD 4.4); average ZPP 215.4 μmol/mol (SD 111.6); average ESR 17.7 mm/h (SD 8.1). Control group: 62.9% female, median age 40 yo (1-79); 37.1% male, median age 17 yo (2-69); average Hb 13.5 (SD 1.2); average ferritin 111.7 ng/mL (SD 117); average ZPP 77 μmol/mol (SD 21.3); average ESR 12.3 mm/h (SD 5.1). ROC curve analysis led to the definition of a ZPP cut-off of ≥ 98 μmol/mol for the diagnosis of iron deficiency, with a sensitivity of 90.8% and specificity of 86.67% when compared with the control group.
Summary/Conclusion: The results of this study allowed the authors to securely establish a cut-off of 98 μmol/mol of ZPP for the diagnosis of iron deficiency, with good sensitivity and specificity values. This diagnostic test does not require blood collection in a specific tube, is reliable when using capillary blood samples and may prove itself an excellent technique for the diagnosis of iron deficiency in paediatric populations and in the screening of blood donors. This test may also be an asset for the diagnosis of IDA in underdeveloped countries since it is fast, accurate, inexpensive and doesn't require elaborate machinery.

PS1224

STUDY OF A MEDICO-ECONOMIC ADVANTAGE IN USING NEW PARAMETERS OF CBC-DIFF-RETIC IN THE EARLY DETECTION OF IRON DEFICIENCY FOR DIALYSIS PATIENTS**

E. Sukhacheva[1,*], M. Hoffmann[2], D. Loze[3], G. Brochard[4]

[1]Beckman Coulter Eurocenter, Nyon, Switzerland, [2]Nephrology and dialysis department, Private Hospital La Louviere, [3]Cerballiance Haut de France Laboratory - La Digue, Lille, [4]Beckman Coulter France, Villepinte, France

Background: Cerballiance Haut de France laboratory is a part of Cerba-Healthcare Group which has an important place in specialized biology in France. The laboratory located in Lille analyses more than 2000 blood samples daily, including samples from the clinic La Louviere specialized for dialysis patients. Dialysis patients are monitored monthly for iron deficiency, beyond the standard of care, in order to quickly detect iron deficiency and to minimize Erythropoietin (EPO) dosing, providing less toxicity and economic advantages for the center. The method must follow the Kidney Disease Outcomes Quality Initiative (KDOQI) recommendations which includes iron, transferrin saturation and ferritin. The global recommendations and laboratory technologies are currently evolving, there are new parameters considered for the evaluation of iron deficiency whose application remains to be demonstrated. Low Hemoglobin Density (@LHD%), Red cell Size Factor (@RSF) and Microcytic Anemia Factor (@MAF) which are research use only parameters provided by Beckman Coulter DxH800 as part of routine CBC-DIFF and Reticulocytes count which are currently performed for patient monitoring.
Aims: We aimed to investigate if these parameters could potentially substitute the recommended parameters and present a medico-economic advantage by limiting the injection of expensive factors stimulating erythropoiesis and thereby also limiting the clinical risk to the patient.
Methods: The laboratory retrospectively studied 248 patients from the dialysis center of La Louviere. Normal ranges for @LHD%, @RSF and @MAF were defined using 318 normal patients. The recommended markers (@LHD%, @RSF and @MAF) were compared to: iron, transferrin, transferrin saturation and ferritin, in the group of patients with inflammation (defined as CRP>10mg/dL) and group without inflammation (defined as CRp<10mg/dL). At the next step among patients without inflammation, we identified responders and non-responders based on the dosage and frequency of EPO administration. All parameters were compared between groups of responders and non-responders. Finally we tried to define cut-offs for new parameters which can potentially be used to follow-up patients' treatment**.
Results: Normal ranges were analyzed for @LHD, @MAF and @RSF, which were used to interpret the results of different groups of dialyzed patients. The data demonstrated that these markers vary significantly for patients with or without inflammation: the group with inflammation has higher@ LHD, lower @MAF and lower @RSF values. @LHD demonstrated the best correlation with transferrin saturation. Among patients without inflammation non-responders have higher @RSF values and higher @LHD values than responders. In our cohort of patients, a criteria @LHD>15% and @MAF>10 seems to be acceptable to follow-up the treatment without risk of missing clinically important iron deficiency**.
Summary/Conclusion: In daily practice, the data suggests that @LHD and @MAF has potential in allowing for an early detection of iron deficiency, similar to transferrin saturation**. The monitoring of dialysis patients could be done with reduced cost, performing only CBC-DIFF-Retic and limiting the rhythm of transferrin saturation testing**.
@For research use only. Not for use in diagnostic procedures
***Clinical utility requires validation through a controlled clinical trial*

PS1225

ACERULOPLASMINAEMIA - THE EXTREMELY RARE ALSO EXISTS

J. Silva Gomes[1,*], A. Azevedo[1], T. Nascimento[1], D. Viegas[1], A. Roque[1], L. Relvas[1], J. Pereira[1], E. Cunha[1], A. Oliveira[1], C. Bento[1], T. Magalhães Maia[1], M.L. Ribeiro[1]

[1]Clinical Haematology, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

Background: Hereditary aceruloplasminaemia (HA) is a rare, autosomal recessive condition, characterized by iron overload affecting the liver, pancreas and brain, caused by mutations in the ceruloplasmin gene (*CP*), leading to an absence of the enzyme ceruloplasmin ferroxidase. Anaemia (usually normocytic or microcytic), associated with hyperferritinaemia and low transferrin saturation (TS), along with non-doseable serum ceruloplasmin and low serum copper are findings which may lead to a suspicion of HA. Some patients may develop symptoms associated with tissue iron overload, including diabetes mellitus and neurodegenerative symptoms. The incidence of

HA in western countries is unknown.
Aims: To report the first case of HA with a confirmed molecular diagnosis in a reference centre, diagnosed in the context of asymptomatic anaemia and hyperferritinaemia.
Methods: Patient Description: 38 year-old caucasian female, referred to Haematology consultation for normocytic normochromic anaemia known for at least several years. The patient complained of tiredness and mood changes, for which she had already been referred to Psychiatry consultations.
Results: Initial workup confirmed normocytic normochromic anaemia (Hb 10.8 g/dL) with normal reticulocyte count, as well as hyperferritinaemia (ferritin 825 ng/mL, TS <15%), with normal liver enzymes. During follow-up: ferritin levels progressively increased to a maximum of 1124 ng/mL, anaemia was stable and asymptomatic, TS <10% and liver enzymes were normal. *HFE* mutations associated with haemochromatosis were excluded. Liver iron overload was confirmed by magnetic resonance imaging (MRI), with hepatic iron levels of 270 µmol/g (N<36 µmol/g) and liver iron concentration (LIC) of 15.1 mg/g. Because of a positive result for anti-mitochondrial antibodies, liver biopsy was performed by recommendation of Hepatology, which excluded primary hepatic conditions, but revealed grade 4 haemosiderosis (scale of 0-4), with no fibrosis or copper deposits. A study of copper metabolism was performed which revealed non-doseable ceruloplasmin and low serum copper as well as decreased copper urinary excretion. Cerebral MRI revealed signs of iron overload in the basal ganglia, thalamus, cerebral cortex and cerebellar cortex. Sequencing of the *CP* gene revealed homozygosity for point mutation c.885C>G (p.His295Gln), a pathological variant not previously described in literature.
Summary/Conclusion: Although it is a very rare disease, the diagnosis of HA is particularly relevant because iron overload, when untreated, can lead to irreversible organ damage, including in the central nervous system. A high level of suspicion for HA is fundamental to achieve a diagnosis, and a few diagnostic clues may be useful: history of anaemia with hyperferritinaemia, low TS and confirmed organ iron overload, with or without accompanying neurodegenerative symptoms or mood disorders. In cases of hyperferritinaemia with low TS, when chronic illness has been excluded, a study of copper metabolism should be performed.

PS1226

IT TAKES A LOT OF IRON TO AVOID TRANSFUSION

M. Al-Obaidi[1,*], M. Chituku[1], C. Jardiel[1], R. Ingrey[2], A. Babb[1], N. Wiles[1]

[1]Haematology, [2]Medicine, West Middlesex Univesity Hospital, London, United Kingdom

Background: There is ample evidence of unnecessary transfusion in iron deficiency anaemia both in the literature and in our institute. In a concerted effort to optimise the safe and appropriate use of allogeneic red cell transfusion we established a weekly nurse led anaemia clinic with capacity to treat four to five patients weekly, and to which primary and secondary care physicians could refer patients for assessment, investigations and correction of iron deficiency with parenteral iron.
Aims: To assess effectiveness of the anaemia clinic in correcting iron deficiency and preventing transfusion.
Methods: Results for all patients seen in the clinic from January 2016 to December 2017 were analysed, blood parameters prior to and 8 weeks after iron infusions were reviewed, and any transfusions prior to or during the two year period were logged.
Results: One hundred and ninety two new patients were seen in clinic, 18 were not iron deficient. Of the remaining 173 patients, 40 were referred or treated repeatedly in the two years. Patient demographics and results are presented in Table 1. Females outnumbered males by 2.9:1 (138:36), and were younger (median age 50 years compared to 71 years). The commonest cause of iron deficiency was menorrhagia; other common aetiology themes included low iron diet, iron mal-absorption due to proton pump inhibitors, metformin and bariatric surgery. As well as possible gastrointestinal bleeding due to inflammatory bowel disease or malignancy or exposure to anticoagulant or antiplatelet agents. Most patients (87%) received iron infusion, at a dose calculated using the Ganzoni equation (using ideal weight where appropriate). Of those treated with intravenous iron the majority received total dose infusion of iron isomaltose Monofer® with a median dose of 800mg (500-1800), sixteen patients received iron sucrose Venofer® in divided doses (when only small dose needed, history of asthma (2), inflammatory arthritis (6), pregnancy (1) or reaction to single dose infusions (6) in the past). An additional three patients developed mild to moderate reaction such that the iron product was changed. Overall the treatment was effective with a median rise of 14g/L in haemoglobin, and similar overall improvement in ferritin and iron saturation; however a large proportion of patients required more than one iron infusion over the two year period; 44% of patients still had a haemoglobin <120 g/L on repeat blood testing. The rate of transfusion was low with only 12 patients receiving blood over the two years. Two patients required treatment on a regular (weekly / monthly) regimen due to the need for iron sucrose Venofer® divided doses and on-going epistaxis in one patient with hereditary haemorrhagic telangiectasia and gastrointestinal bleeding in a patient with large bowel angiodysplasia, these patients required a total of 17200mg and 5400 mg of Venofer® respectively, and 10% of clinic slots, they also received nine of the 37 units of blood transfused.

Table 1.

Patient demographics treatment and results		
Gender (female / male)	138/36	
Age	54 (18-91)	
Factors contributing to iron deficiency		
Menorrhagia	63 (45%)	
Proton pump inhibitor	54 (31%)	
Metformin	26 (15%)	
Bariatric surgery	8 (4.6%)	
Low iron diet	37 (21%)	
Antiplatelet / Anticoagulant	45 (26%	
Inflammatory / malignant bowel disease	6 (3.4%)	
Treatment		
Oral iron	23	
Monofer*	134 (89%)	
Venofer	16 (10%)	
Number of infusions over 24 months		
1	112	
2	21	
3 to 5	13	
>20	2	
Patients requiring transfusions	12(7%)	
Response to treatment	Prior to infusion	Following infusion
Mean Haemoglobin	104	118
Mean Ferritin	19	61
Mean Iron saturation	10%	36%

Summary/Conclusion: The dedicated iron deficiency clinic was well received by patients, primary and secondary care providers, and was able to correct iron deficiency in large numbers of patients. However, reducing the use of allogeneic blood transfusion in patients with on-going blood loss was only partially achieved by frequent hospital visits and very high doses of intravenous iron.

PS1227

PANCREATIC IRON LOADING IN EMOGLOBINOPATHIES

A. Meloni[1,*], L. Pistoia[1], M. C. Putti[2], F. Miciotto[3], D. De Marchi[1], F. Massei[4], V. Spadola[5], P. Bitti[6], R. Sarli[7], F. Sorrentino[8], N. Schicchi[9], P. Preziosi[10], M. Mangione[1], V. Positano[1], A. Pepe[1]

[1]Fondazione G. Monasterio CNR-Regione Toscana, Pisa, [2]Azienda Ospedaliero-Università di Padova, Padova, [3]"ARNAS" Civico, Di Cristina Benfratelli, Palermo, [4]Azienda Ospedaliero Universitaria Pisana – Stabilimento S.Chiara, Pisa, [5]Azienda Ospedaliera Civile - O.M.P.A. Ragusa, Ragusa, [6]Presidio Ospedaliero "San Francesco" ASL Nuoro, Nuoro, [7]Presidio Ospedaliero Orientale "M. Giannuzzi", Manduria (TA), [8]Ospedale "Sant'Eugenio", Roma, [9]Azienda Ospedaliero-Universitaria Ospedali Riuniti "Umberto I-Lancisi-Salesi", Ancona, [10]Ospedale "Sandro Pertini", Roma, Italy

Background: The MIOT (Myocardial Iron Overload in Thalassemia) project is a multicentric study aimed to validate the T2* Magnetic Resonance Imaging (MRI) technique as non invasive approach for the cardiac and hepatic iron overload assessment in patients with hemoglobinopathies and to correlate the T2* values with other clinical-instrumental parameters. More recently, the E-MIOT (Extension-MIOT) project has been approved, with the aim of adding the assessment of the pancreatic iron
Aims: More recently, the E-MIOT (Extension-MIOT) project has been approved, with the aim of adding the assessment of the pancreatic iron.
Methods: We report the baseline MRI findings at the end of the first year of recruitment in the E-MIOT study, outlying the differences among different emoglobinopathies.
Results: First, we selected all transfusion-dependent (TD) patients: 7 with sickle-cell disease-SCD or thalasso-drepanocytosis (42.9% F, 32.47±17.93 years), 16 with thalassemia intermedia-TI (56.3% F, 36.66±13.72 years), and 232 with thalassemia major-TM (55.6% F, 36.95±9.83 years). Sex, mean age, serum ferritin levels, MRI liver iron concentration (LIC) values,

and global heart T2* values were comparable among the three groups of patients. Pancreatic T2* values were significantly lower in TM patients versus both SCD and TI patients.
Second, we focused our analysis on all TI patients, divided in two subgroups: transfusion dependent and no-TD. Global pancreas T2* values and the number of patients with pancreatic iron (T2*<26 ms) were comparable between the two groups (see Table 1).

Table 1.

	TI-noTD (N=16)	TI-TD (N=16)	p
Sex (% of F)	33.3	56.3	0.300
Age (years)	42.78 ± 13.34	36.66 ± 13.72	0.427
Ferritin (ng/ml)	452 ± 251	750 ± 583	0.336
MRI LIC (mg/g dw)	5.37 ± 6.68	5.72 ± 8.58	0.270
MRI LIC < 3 mg/g dw, N (%)	9 (50)	5 (31.3)	0.268
Global heart T2* (ms)	43.94 ± 4.69	35.79 ± 9.58	0.008
Global heart T2*<20 ms, N(%)	0	1 (6.3)	0.471
Global pancreas T2* (ms)	26.57 ± 10.46	21.89 ± 13.37	0.317
Global pancreas T2*<26 ms, N(%)	8 (44.4)	9 (56.3)	0.492

Summary/Conclusion: SCD and TI TD patients have lower pancreatic iron loading than chronically-transfused TM patients. Much of this disparity can be explained by the larger transfusional burdens and durations observed in TM patients (years of regular transfusions: 34.18±10.75 in TM, 22.27±18.53 in SCD and 28.33±15.30 in TI, p=0.023). However, TI-TD and TI-noTD patients had comparable pancreatic T2* values. So, innate differences in iron handling and elimination among different diseases also could contribute to the differences in pancreatic iron loading.

PS1228

CHR AND%HYPO: BETTER METHODS FOR ASSESSING FUNCTIONAL IRON DEFICIENCY IN CHRONIC KIDNEY DISEASE?

L. Smith[1,*], F. Buckley[2], K. Gelly[2]

[1]*Hematological Malignancy Diagnostic Service (HMDS), NHS Lothian, Edinburgh,* [2]*Department of Hematology, NHS Tayside, Dundee, United Kingdom*

Background: Renal anemia is multifactorial but is associated with functional iron deficiency (FID). Serum ferritin alone is not a useful marker in identifying patients with FID, nor predicting response to iron replacement in patients with chronic kidney disease (CKD). The KDIGO guidelines 2012, which contain recommendations for assessment of iron status and prediction of response to iron therapy in CKD, were superseded by the NICE guidelines in 2015. The new guidelines recommended a shift from using% transferrin saturation (%TFsat) and ferritin in combination, to using either% hypochromic red cells (%HYPO) or reticulocyte hemoglobin concentration (CHr). Whilst analyzers are capable of generating these parameters, there are no existing external quality assessment (EQA) schemes available for them.
Aims: We aimed to assess whether: 1. The Siemens Advia 2120 generated reliable and accurate%HYPO and CHr results; 2. These parameters appear to be adequately predictive for FID in CKD patients on regular hemodialysis; and 3. There would be tangible cost implications in terms of testing and treating FID.
Methods: Samples were analyzed on one day, on a freshly calibrated and dedicated analyzer, within 4 hours. Data were captured for Hb,%TFsat, ferritin,%HYPO and CHr. Details of IV iron treatment were noted per patient. These results were applied to both existing and updated guidelines to determine the corresponding recommended treatment.
Results: Samples were collected from 37 patients on regular hemodialysis ('CKD group'), 3 patients with iron deficiency and 14 non-anemic non-iron deficient individuals. 18 patients in the CKD group were already on regular IV iron supplementation. Applying the NICE guidelines 2015 to the CKD group to identify patients who would benefit from iron supplementation: 8 patients were identified according to%TFsat and ferritin; 23 patients according to%HYPO and no patients according to CHr. Amongst the CKD group, withdrawal of iron supplementation was recommended in 7, 4 and 12 patients according to the above parameters respectively. CHr did appear to be predictive for iron deficiency in non-CKD patients, although this group was very small.
Summary/Conclusion: No clear trend was seen to suggest that%HYPO was more predictive for FID in CKD patients than%TFsat and ferritin.%HYPO did not reliably offer benefit over%TFsat and ferritin and could not be applied to delayed samples or patients with thalassemia. CHr was a particularly poor marker of FID in CKD patients on regular hemodialysis. It would also significantly increase the cost of iron status monitoring. It was not possible to draw conclusions regarding the interpretation of these assays in the context of IV iron therapy. On the basis of these results and in conjunction with our local Renal Department, we have chosen not to report%HYPO or CHr in our lab. We recommend that labs perform similar analyses locally prior to reporting these parameters. Development of EQA schemes will be necessary to maximize the utility of these results where a lab chooses to report them.

Myelodysplastic syndromes – Biology & Translational Research

PS1229

BONE MARROW FAILURE AND PREDISPOSITION TO LEUKEMIA IN GATA2 HAPLOINSUFFICIENT MICE

J. Weiss[1,*], I. Schmitt[1], C. Molnár[1,2,3], N. Fischer[1], M. Wlodarski[1], C. Niemeyer[1], M. Erlacher[1]

[1]Pediatric Oncology/Hematology, University Hospital Freiburg, [2]Faculty of Biology, [3]Spemann Graduate School of Biology and Medicine, University of Freiburg, Freiburg, Germany

Background: The transcription factor GATA2 is indispensable for the generation and maintenance of hematopoietic stem cells (HSCs). Heterozygous *GATA2* germline mutations have been associated with several clinical symptoms e.g. lymphedema, deficiencies of monocytes, B and/or NK cells. Strikingly, up to 75% of affected individuals develop myelodysplastic syndromes (MDS) with a risk of further progression to acute myeloid leukemia (AML).
Aims: Although GATA2 is considered to be a major predisposition factor for MDS, the underlying mechanisms leading to malignant transformation are largely unknown. The aim of this study is therefore to understand, how *GATA2* haploinsufficiency causes MDS/AML. Since murine HSCs lacking both *Gata2* alleles are not viable, we propose that *Gata2*$^{+/-}$ HSCs are able to live, but exhaust faster, live shorter and therefore lead to bone marrow failure. In such a situation, preleukemic cells would have the opportunity to emerge and proliferate, which would ultimately result in MDS and AML.
Methods: To verify this hypothesis, we put murine *Gata2*$^{+/-}$ stem and progenitor cells (=HSPCs) under different kind of stress conditions and analyzed them for signs of exhaustion and susceptibility to develop bone marrow failure. We performed competitive and serial transplantations and analyzed the HSPC compartment of aged *Gata2*$^{+/-}$ mice
Results: Analysis of competitively transplanted recipient mice showed that significantly fewer cells derived from *Gata2*$^{+/-}$ HSPCs than from WT HSPCs suggesting that *Gata2*$^{+/-}$ HSPCs were less competitive and less efficient than WT HSPCs. To further assess the reconstitution potential of *Gata2*$^{+/-}$ HSPCs under stress conditions, we performed serial transplantations. Decreased cell counts in spleen and lymph nodes after 1st and 2nd passages indicated an accelerated exhaustion of *Gata2*$^{+/-}$ HSPCs. Analysis of the HSPC pool of aged *Gata2*$^{+/-}$ mice revealed a reduced number of long-term HSCs and multipotent progenitors. When we performed a single transplantation of HSPCs and monitored recipient mice over time, we observed that 50% of them died within 6 months after transplantation. At time of death, recipient mice showed a strongly reduced bone marrow cellularity with complete lack of mature myeloid cells, disturbed B-cell differentiation and elevated T-cell counts in bone marrow and spleen. Histochemical analysis confirmed the presence of bone marrow failure. In addition, leukemic infiltrations were found in the spleens of some of the recipient mice. Predisposition to leukemia was also observed, when *Gata2*$^{+/-}$ mice were treated with the mutagen ENU. Both WT and *Gata2*$^{+/-}$ mice developed immature T-cell leukemia but *Gata2*$^{+/-}$ mice died significantly earlier than WT mice.
Summary/Conclusion: Our results demonstrate that *Gata2*$^{+/-}$ HSPCs are less competitive, exhaust faster and have a reduced lifespan compared to WT HSPCs. As a consequence, *Gata2*$^{+/-}$ mice are more prone to develop bone marrow follow and leukemia. Next, we will identify which second hits contributed to leukemogenesis in *Gata2*$^{+/-}$ mice. This will reveal cooperating pathways and give new insights into the oncogenic role of GATA2 in the hematopoietic compartment.

PS1230

GROWING IMPORTANCE OF FLOW CYTOMETRY (FCM) IN THE DIAGNOSTICS OF MYELODYSPLASTIC SYNDROMES: SENSITIVITY AND SPECIFICITY OF THE UP-TO-DATE SCORING SYSTEMS UNDER SPECIAL CONSIDERATION OF THE NEW IFSCORE

U. Oelschlaegel[1,*], L. Oelschlaeger[1], B. Mohr[1], K. Sockel[1], M. Bornhäuser[1], U. Platzbecker[1]

[1]Medical Clinic and Policlinic I, MK1-L06, University Hospital of Tu Dresden, Dresden, Germany

Background: Following the harmonization efforts of the international MDS-flow working group of the ELN, the value of flow cytometry (FCM) as one recommended part in MDS diagnostics is growing. Currently, different scores were applied, evaluating abnormal maturation pattern as expression of dyspoiesis in various hematopoietic cell lineages.
Aims: Aim of the recent study was to compare the different FCM scores concerning sensitivity and specificity referring to cytomorphology as standard diagnostic procedure.
Methods: 1076 FCM measurements were performed in bone marrow of 769 patients with MDS or cytopenias suspected for MDS. Additionally, 96 patients with AML, 45 with MPN, as well as 50 age matched hip surgery patients were utilized as controls. For measurement and analysis the FACS-CantoII cytometer including DiVa software was used, performing an 8-color panel and measuring 200,000 events per sample. The following FCM-scores were compared: FCSS (granulopoiesis/monopoiesis), Ogata-score (blasts), ELN-red-score and Mathis-score (both erythropoiesis), and the new iFScore (granulo-, mono-, erythropoiesis, and blasts).
Results: The sensitivity of the new iFS-score, evaluating granulo- ,mono-, erythropoiesis and blasts, is comparable with FCSS and much higher than that of Ogata- and the red-scores (81%, 82%, 59%, 31%, 40%). Of note, assigning the patients according to the WHO2016 classification a sensitivity of 63% was achieved even for the MDS-SLD subgroup (MDS-MLD=69%; del(5q)-syndrome=89%; EB1/2>90%; CMML=96%). Considering only patients with MDS-SLD or MLD with normal karyotype and without ring sideroblasts the sensitivity comprised 68% and 65%, respectively. Considering the very low cytogenetic risk and very low IPSS-R group sensitivity was even slightly better. Regarding specificity the new iFScore was also comparably high as the other scores with 98% (100%, 100%, 98%, 92%) in controls. In non-clonal cytopenias the new iFS showed a specificity of 81% compared to FCSS with 53% and to the only one cell lineage considering Ogata- and red-scores (95%, 96%, 89%). Considering sensitivity and specificity together the new iFScore seems to give the best results. Interestingly, despite inappropriate bone marrow aspirations (w/o spiculae) in 22 patients the new iFScore resulted in "consistent with MDS" in 72% of these cases. Finally, even being in cytogenetic CR 29% of the investigated MDS patients showed an iFScore consistent with MDS, having a borderline significant impact on overall survival (p=0.075).
Summary/Conclusion: Currently available and used FCM scores showed differences in sensitivity and specificity, with the new iFScore being the most comprehensive approach in MDS diagnostics by FCM evaluating granuo-, mono-, erythropoiesis and blasts. Especially in low risk MDS and cases with inappropriate bone marrow aspiration FCM can clearly contribute to MDS diagnostics. The value of FCM response monitoring with a special focus on evaluating the deepness of response remains to be studied further.

PS1231

EXPLORING THE LONG NON-CODING RNA TRANSCRIPTOME IN JUVENILE MYELOMONOCYTIC LEUKEMIA

M. Hofmans[1,*], T. Lammens[1], H. H. Helsmoortel[2], S. Bresolin[3], H. Cavé[4], C. Flotho[5], H. Hasle[6], M. M. van den Heuvel-Eibrink[7], C. Niemeyer[5], J. Stary[8], N. Van Roy[2], P. Van Vlierberghe[2], J. Philippé[9], B. De Moerloose[1]

[1]Department of Pediatric Hematology-Oncology and Stem Cell Transplantation, [2]Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium, [3]Department of Women and Child Health, University of Padova, Padua, Italy, [4]Department of Genetics, University Hospital of Robert Debré and Paris-Diderot University, Paris, France, [5]Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, University of Freiburg, Freiburg, Germany, [6]Department of Pediatrics, Aarhus University Hospital Skejby, Aarhus, Denmark, [7]Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands, [8]Department of Pediatric Hematology/ Oncology, Charles University and University Hospital Motol, Prague, Czech Republic, [9]Department of Clinical Chemistry, Microbiology and Immunology, Ghent University, Ghent, Belgium

Background: Juvenile myelomonocytic leukemia (JMML) is a rare and aggressive myelodysplastic/myeloproliferative disorder of early childhood. Hematopoietic stem cell transplantation results in long-term overall survival (OS) of only 50-60% and is fraught with frequent relapse and toxicity. Consequently, there is need to develop novel treatments. Recent work from our group identified *LIN28B* as key player in JMML. Moreover, we have illustrated that *LIN28B* overexpression deregulated expression of the long non-coding RNA (lncRNA) H19, the first lncRNA discovered in JMML biology. LncRNAs are a recently discovered class of RNAs, with a minimum length of 200 nucleotides, which play a crucial role in oncogenesis.
Aims: The major aim of this work was to document the lncRNA landscape in JMML patients and associate lncRNA expression with cytogenetic, mutational and clinical characteristics.
Methods: We studied a publically available RNA dataset (GEO number GSE71449) comprising of microarrays from a cohort of 44 JMML patients and 7 healthy age-matched controls. In total 23728 lncRNA probes (corresponding to 17845 unique lncRNAs) were identified. Differential gene

expression analysis between JMML patients and controls was performed with the samr package (R BioConductor).
Results: A total of 300 differentially expressed lncRNAs were identified (15 upregulated in JMML and 285 downregulated; Figure 1). Guilt-by-association analysis through pre-ranked gene set enrichment analysis discovered various gene sets (anti-)correlated with the top five up- and downregulated lncRNAs, and related to myeloid cell development, dendritic cell maturation, neutrophil function and different AML phenotypes. Furthermore, distinct lncRNA expression profiles could be attributed to specific patient characteristics. I.e., lncRNAs downregulated in patients with elevated HbF for age were anti-correlated with genes involved in HbF regulation. Moreover, the majority of these lncRNAs were also upregulated in patients with monosomy 7, confirming the inverse relation between monosomy 7 and elevated HbF. Sixty-one lncRNAs were differentially expressed between patients with and without *LIN28B* overexpression of which 60 were upregulated in *LIN28B* overexpression. Interestingly, both MEG3 and MEG8 were upregulated and are known to be involved in regulation of pluripotence and fetal development. Next, we hypothesized that lncRNA expression might influence clinical outcome. Multivariable cox-regression analysis with age, HbF, karyotype and molecular status identified 7 lncRNAs highly associated with OS ($p<0.001$). Interestingly, these lncRNAs did not associate with known prognostic characteristics and no common (anti-)correlated gene sets could be identified. A lncRNA score, derived as a linear combination of the expression of the significant lncRNAs, was used to dichotomize patients. Patients with a high lncRNA score had a significantly lower OS and EFS after HSCT (log rank test; $p<0.001$). Together, this suggested that lncRNAs might be of prognostic significance in JMML. However, studies in independent patient cohorts are necessary to validate the prognostic value of lncRNA expression in JMML.

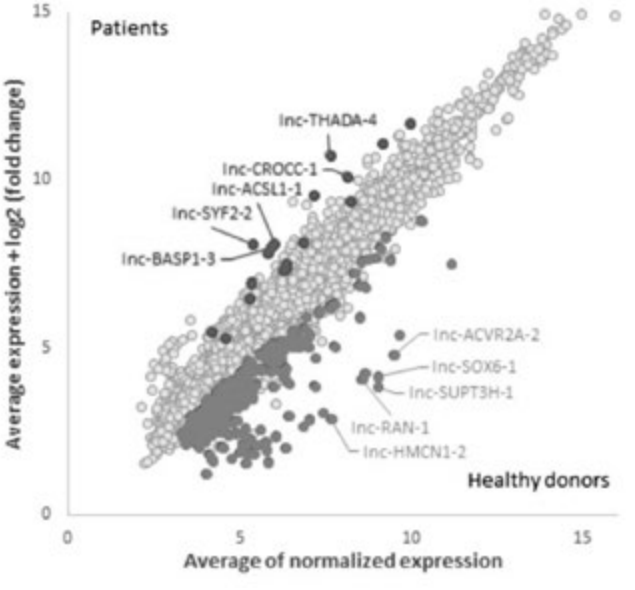

Figure 1.

Summary/Conclusion: We documented for the first time the lncRNA landscape in 44 JMML patients and identified deregulated lncRNAs associated with key processes in JMML pathogenesis and the biology of specific JMML subgroups. Follow-up studies are needed to confirm the prognostic value of lncRNAs. Altogether, this study paves the way for further functional research on the role of lncRNAs in JMML and their therapeutic application.

PS1232

A MIR-150 / TET3 PATHWAY REGULATES THE GENERATION OF MOUSE AND HUMAN NON-CLASSICAL MONOCYTE SUBSET

D. Selimoglu-Buet[1,*], J. Riviere[1], H. Ghamlouch[1], L. Bencheikh[1], M. Morabito[1], M. Diop[2], G. Meurice[2], M. Breckler[2], A. Chauveau[1], C. Debord[1], R. Itzykson[1], N. Chapuis[3], C. Willekens[1], O. Wagner-Ballon[4], O. Bernard[1], N. Droin[1], E. Solary[1]

[1]U1170 INSERM, [2]INSERM US23, CNRS UMS 3655, Gustave Roussy Institute, Villejuif, [3]Departement d Immuno-Hematologie, Institut Cochin, Paris, [4]Biological hematology and immunology department, Hôpital Henri-Mondor, Créteil, France

Background: Monocytes are heterogeneous leucocytes comprising two main subsets in mice and three in humans. The expression of CD14 and CD16 distinguishes $CD14^+/CD16^-$ classical from $CD14^+/CD16^+$ intermediate and $CD14^{low}/CD16^+$ nonclassical human monocytes. In mice, Ly6C expression distinguishes classical monocytes ($LY6C^{high}$) and nonclassical monocytes ($Ly6C^{low}$). A consensus model supports that nonclassical monocyte subset may derive from classical monocyte differentiation. Proportion of each subset is tightly controlled but the molecular mechanisms that regulate monocyte subset differentiation remain poorly understood.
Aims: Monocyte subset repartition can be deregulated in human diseases such as chronic myelomonocytic leukemia (CMML) in which the fraction of nonclassical monocytes typically decreases in the favour of classical monocytes. CMML samples were used as a starting model to identify some of the molecular mechanisms involved in the generation of nonclassical monocytes.
Methods: A microarray screen of miRNA expression was performed in peripheral blood monocytes of 33 CMML and 5 healthy donor blood samples and RT-PCR validation was performed in monocytes from a cohort of 139 CMML patients and 24 controls. We then combine analysis of knockout mouse models, CRISPR-CAS9 deletions, ChIP-Seq analyses, bisulfite sequencing and miRNA pulldown to explore the mechanisms by which the disease-associated deregulation of a specific miRNA leads to an abnormal generation of monocytes subsets in both mice and humans.
Results: The down-regulated expression of miR-150 is a characteristic feature of CMML monocytes. This down-regulation is explained by hypermethylation of a myeloid lineage specific promoter in *MIR150* gene, which is corrected in patients who respond to demethylating drugs and recapitulate a normal repartition of monocyte subsets. *miR150*$^{-/-}$ mice demonstrate an increase in $Ly6C^{high}$ monocyte fraction at the expense of $Ly6C^{low}$ monocytes. The number of myeloid progenitors is normal in *miR150*$^{-/-}$ mice and the remaining $Ly6C^{low}$ monocytes do not demonstrate an increased sensitivity to apoptosis. This abnormal repartition of monocyte populations in *miR150*$^{-/-}$ mice is a cell-autonomous phenotype that is rescued by re-expression of miR150 in bone marrow cells of *miR150*$^{-/-}$ mice before engraftment. Competitive reconstitution experiments combining WT and *miR150*$^{-/-}$ cells do not identify any significant fitness advantage to *miR150*$^{-/-}$ cells, but *miR150*$^{-/-}$ cells develop reduced numbers of $Ly6C^{low}$ monocytes than WT donors. In human cells, depletion or overexpression of miR-150 in $CD34^+$ cells alters the repartition of $CD14^+/CD16^-$ and $CD14^+/CD16^+$ cells generated in culture. In mice and humans, the expression of miR-150 is also lower in classical monocytes compared to nonclassical subsets. Pulldown experiments pointed to Ten-Eleven-Translocation-3 (TET3) mRNA as a miR-150 target in classical monocytes, with *Tet3*$^{-/-}$ mice generating an increased number of nonclassical monocytes.
Summary/Conclusion: Our results demonstrate that a miR-150 / TET3 pathway, which is conserved in mice and humans and is epigenetically deregulated in CMML cells, is involved in the generation of nonclassical monocyte subsets.

PS1233

IMPACT OF RED BLOOD CELL TRANSFUSION RATE, ADJUSTED BY THERAPEUTIC INTERVENTIONS, ON PROGRESSION-FREE SURVIVAL IN LOWER RISK MDS PATIENTS WITHIN THE EUROPEAN LEUKEMIANET MDS REGISTRY

L. de Swart[1,*], M. Hoeks[2], S. Crouch[3], A. Smith[3], S. Langemeijer[1], P. Fenaux[4], A. Symeonidis[5], J. Čermák[6], E. Hellström-Lindberg[7], R. Stauder[8], G. Sanz[9], M. Mittelman[10], M.S. Holm[11], L. Malcovati[12], K. Madry[13], U. Germing[14], A. Savic[15], A. M. Almeida[16], A. Guerci-Bresler[17], R. Itzykson[4], O. Beyne-Rauzy[18], D. Culligan[19], C. van Marrewijk[1], N. Blijlevens[1], D. Bowen[20], T. de Witte[21]

[1]Hematology, Radboudumc, Nijmegen, [2]Centre for Clinical Transfusion Research, Sanquin Research, Leiden, Netherlands, [3]Epidemiology and Cancer Statistics Group, Department of Health Sciences, University of York, York, United Kingdom, [4]Service d'Hématologie, Hôpital Saint-Louis, Paris, France, [5]Medicine, Div. Hematology, University of Patras Medical School, Patras, Greece, [6]Clinical Hematology, Inst. of Hematology & Blood Transfusion, Praha, Czech Republic, [7]Medicine, Div. Hematology, Karolinska Institutet, Stockholm, Sweden, [8]Internal Medicine V (Haematology and Oncology), Innsbruck Medical University, Innsbruck, Austria, [9]Haematology, Hospital Universitario La Fe, Valencia, Spain, [10]Medicine A, Tel Aviv Sourasky (Ichilov) Medical Center, Tel Aviv, Israel, [11]Haematology, Aarhus University Hospital, Aarhus, Denmark, [12]Hematology Oncology, Fondazione IRCCS Policlinico San Matteo, University of Pavia, Pavia, Italy, [13]Haematology, Oncology and Internal Medicine, Warszawa Medical University, Warszawa, Poland, [14]Haematology, Oncology and Clinical Immunology, Universitätsklinik Düsseldorf, Düsseldorf, Germany, [15]Clinic of Hematology - Clinical Center of Vojvodina, Faculty of Medicine, Novi Sad, Serbia, [16]Hematology, Instituto Português de Oncologia de Lisboa, Lisboa, Portugal, [17]Service d'Héma-

tologie, CHU Brabois Vandoeuvre, Nancy, [18]Service de Medecine Interne, CHU Toulouse, Toulouse, France, [19]Haematology, Aberdeen Royal Infirmary, Aberdeen, [20]St. James's Institute of Oncology, Leeds Teaching Hospitals, Leeds, United Kingdom, [21]Tumor Immunology, Radboudumc, Nijmegen, Netherlands

Background: Progression-free survival (PFS) of lower-risk (LR) MDS patients treated with red blood cell transfusions (transfusions) is usually reduced, but whether this effect is caused by the actual transfusion itself remains debatable. The EUMDS Registry contains data on 2,192 newly diagnosed LR-MDS patients from 16 European countries and Israel, collected at diagnosis and at 6 months intervals.
Aims: The aim of this analysis is to assess the effect of transfusion rate on PFS.
Methods: The cumulative dose received at the end of each interval was divided by the time since the start of the first RBCT to give an overall dose density in that period. Dose density rises over time when transfusion rate increases over time, but declines when a patient becomes RBCT independent after treatment. PFS was adjusted by relevant confounders, including age, gender, self-reported patient condition (proxied by EQ-5D index), and number of cytopenias at diagnosis, using Cox regression with time-varying covariates.
Results: We analysed a cohort of 1,273 patients with all relevant data available. Within this group 669 patients received transfusions after registration. Univariate analysis of the 669 transfused patients showed a strong association of age ($p<4\times10^{-4}$) with PFS. The EQ-5D index, baseline MDS diagnosis, bone marrow percentage and number of cytopenias were all associated with PFS in univariate regression. The dose density was associated with PFS ($p<1\times10^{-4}$) with a significant non-linear component ($p<1\times10^{-4}$). The following variables were entered into multivariate regression analysis: transfusion dose density, diagnostic age, number of transfusions received before diagnosis, EQ-5D index, bone marrow blast percentage and cytogenetic category. All variables entered in the regression retained statistical significance. The functional form of the dose density effect (p=0.009) is shown in Figure 1A. The dose density had an increasing effect until a dose density of about one unit/month. Thereafter, the effect leveled off. Treatment with ESAs, lenalidomide and iron chelators may improve erythropoiesis and reduce the need for RBCT, resulting in a gradual decrease of the subsequent RBCT dose densities in intervals during the response period. Patients, treated with ESA, lenalidomide, or iron chelators, showed a decrease of transfusion density in 22%, 53% and 40% of the patients, respectively. The observed patterns of dose density trajectories suggest that receipt of ESA, lenalidomide and iron chelation modulate the dose density and therefore, we included these variables as confounding variables in the regression model. This analysis showed that the impact of the dose density remained similar to the previous analyses, but in contrast the dose density effect continues to increase beyond 1 unit per month after correction for the three interventions (Figure 1B).

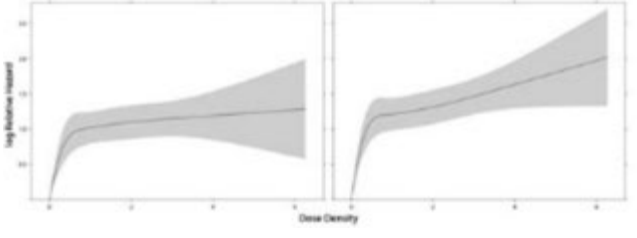

Figure 1.

Summary/Conclusion: The multivariate regression models showed that administration of transfusions is associated with decreased PFS when compared to non-transfused patients. The negative effect of transfusions on PFS already occurs at low transfusion densities below 1 unit per month. This indicates that the transfusion dependency, even at relatively low rate, may be considered as an indicator of poor prognosis.

PS1234

EXPRESSION PATTERN OF LONG-NONCODING RNAS ALTERS IN DIFFERENT FORMS OF MYELODYSPLASTIC SYNDROMES

K. Szikszai[1,*], Z. Krejcik[1], A. Hrustincova[1], M. Belickova[1], J. Cermak[2], A. Jonasova[3], M. Dostalova Merkerova[1]

[1]Department of Genomics, [2]Outpatient Department, Institute of Hematology and Blood Transfusion, [3]Faculty Hospital, Prague, Czech Republic

Background: Long non-coding RNAs (lncRNAs) regulate hematopoietic lineage differentiation at almost every stage and their abnormal expression contributes to various hematopoietic disorders. They may become useful molecular markers contributing to the detection of disease progression and increase of patients' survival. However, the information about influence of lncRNA expression on pathogenesis of myelodysplastic syndromes (MDS) is limited.
Aims: Genome-wide screening of lncRNA levels was employed to compare expression profiles between various subtypes and risk groups of MDS patients with the aim to find lncRNAs potentially relevant for MDS.
Methods: Microarray profiling (22,001 lncRNA transcripts and 17,535 protein-coding mRNAs) was performed on CD34+ bone marrow cells in a cohort of 54 patients with various subtypes of MDS, 14 patients with acute myeloid leukemia with myelodysplasia-related changes (AML-MRC) and 9 healthy donors. Reverse-transcription quantitative-PCR (RT-qPCR) was applied to validate the microarray results by quantification of expression level of selected individual lncRNAs in an independent cohort (106 samples).
Results: The most altered lncRNAs (adj. p<.05) in MDS patients across all the disease subtypes included e.g., H19, MEG3, DLX6-AS1, and PRKAR2A-AS1 (up-regulated compared to controls) and PRR7-AS1, MEF2C-AS1, TTN-AS1, and U3 (down-regulated). Interestingly, H19 lncRNA regulates early hematopoiesis and MEG3 was one of the first lncRNAs associated with MDS. Expression profiles of the patients with lower-risk subtypes (MDS-SLD, MDS-MLD, MDS with del(5q)) were more similar to the profiles of healthy controls, whereas those of the patients with higher-risk diagnoses (MDS-EB1/2) rather resembled to the profiles of AML-MRC. Concerning cytogenetic abnormalities, patients with del(5q) showed specific lncRNA profile with down-regulation of EPB41L4A-AS1, CTB-118N6, and MEF2C-AS1 that are located in 5q locus. Stratification of expression profiles according to IPSS-R confirmed significantly different levels of many lncRNAs between very low/low and very high/high risk categories, pointing out to the lncRNAs whose expression changes are related to the disease prognosis. The lncRNAs with decreased levels in very high/high patient group (compared to very low/low risk categories) were e.g., TCL6, LEF1-AS1, PCED1B-AS1, and CXADRP3. The opposite trend (increased in very high/high compared to very low/low patients) showed e.g., RBPMS-AS1, BAIAP2-AS1, and MCM3AP-AS41. Some of these lncRNAs have already been linked to hematopoiesis. For example, TCL6 has been previously reported as a potential player in T-cell leukemia and may be involved in leukemogenesis, and LEF1, protein-coding gene related to lncRNA LEF1-AS1, plays a critical role in hematopoietic cell differentiation.
Summary/Conclusion: The results of the present study identified a number of differentially expressed lncRNAs, providing one of the first insights into the involvement of lncRNAs in MDS. However, further characterization of those lncRNAs would contribute to better understanding of lncRNAs action in MDS pathogenesis. Besides, the work demonstrates a potential of non-coding RNAs to become, after additional experimental investigation, prognostic markers in myelodysplasia in future.
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PS1235

MDS WITH P53 DYSFUNCTION: A NEW PROGNOSTIC AND PREDICTIVE CATEGORY. A STUDY FROM THE ITALIAN MDS CLINICAL NETWORK

M. Rossi[1,*], G. Croci[2,3], E. Boveri[2], U. Gianelli[4], M. Ponzoni[5], L. Giordano[6], M. Ubezio[1], E. Travaglino[7], L. Morabito[1], C. MIlanesi[6], E. Saba[8], L. borin[9], M. Bernardi[5], V. Santini[10], M.T. Voso[11], S. Fenu[12], A. Orazi[13], A. Santoro[6], A. Bacigalupo[14], A. Rambaldi[15], M.G. Della Porta[6]

[1]Hematology, Humanitas Research Hospital, Milan, [2]IRCCS Policlinico S. Matteo, Pavia, [3]University of Insubria, Varese, [4]Pathology, University of Milan, IRCCS Ca' Granda-Maggiore Policlinico Hospital Foundation, [5]San Raffaele H. Scientific Institute, [6]Humanitas Research Hospital, Milan, [7]IRCCS Policlinico S. Matteo Foundation, Pavia, [8]Humanitas University, Milan, [9]San Gerardo Hospital, Monza, [10]Azienda Ospedaliero Universitaria Careggi, Florence, [11]Biomedicine and Prevention, University Tor Vergata, [12]Az. Osp. San Giovanni-Addolorata , Rome, Italy, [13]Weill Cornell Medical College, New York, United States, [14]Università Cattolica del Sacro Cuore - Policlinico A. Gemelli, Rome, [15]University of Milan, ASST Papa Giovanni XXIII, Bergamo, Italy

Background: TP53 mutations occurs in about 10% of patients with myelodysplastic syndromes (MDS), increasing up to 30-40% in those pts with therapy-related MDS. TP53 mutations frequently are loss of function and cause a stabilization of mutant p53 proteins, leading to their increased

accumulation at cellular level (detectable by immunohistochemistry). However, p53 hyperexpression in cancer can also be observed in the presence of wild-type TP53 genes

Aims: In this study we evaluated the prevalence and clinical implications of p53 dysfunction in a large MDS cohort

Methods: This is an observational retrospective analysis of 3 different groups of MDS pts: receiving best supportive care (BSC, n=495), hypomethylating agents (HMAs, n=198) or allogeneic transplantation (HSCT, n=305).Hystopathological analysis in order to evaluate p53 expression was done with a DO-1 antibody stain (Santa Cruz Biotechnology, Santa Cruz, CA). TP53 mutation status was carried out through massive parallel sequencing panel (JCO 2016;30:3627-3637). Recursive partitioning analysis (NEJM 2017;376:536-47) was used to generate a hierarchical model for overall survival integrating clinical and genetic characteristics

Results: Hyperexpression of p53 was defined with a 2% or more of positive cells (p53++/dark brown, nuclear staining).Bone marrow biopsy were reviewed by expert pathologists with a concordance >95% (p<.001) for recognition of percentage of p53++ cells.Globally TP53 mutations were detected in 11% of pts, these pts had a paucity of other gene mutations; TP53 mutations were infrequent in pts with splicing mutations vs pts with wt splicing factor genes (p=.002). Known poor prognostic factors, such as int-2/high and poor/very poor cytogenetic risk were associated to TP53 mutations (p=.02 and p<.001 respectively). Pts with TP53 mutation had a shorter survival than wt pts (15 vs 66 months, p<.001), data has been confirmed within a multivariable model. Mutations of TP53 were stable over time in serial samples showing no disappearance and no late appearance. A reduction in mutant clone correlated with response to HMAs and HSCT, however clones increased in non-responders and persisted at disease relapse. Pts with high p53 expression (17% of all) had a same survival independently by the presence or absence of TP53 mutations. (15 vs 17 months, p=ns). Than we focused on the MDS with p53 dysfunction category,which includes pts with TP53 mutations and/or p53 hyperexpression.Through the recursive partitioning analysis we compared clinical and genomic variables. In pts receiving BSC, p53 dysfunction had a bad prognostic impact on disease progression and survival (p=0.001)(Figure 1A). In pts treated with HMAs, p53 dysfunction was predictive for a lower efficacy of the treatment(CR 21% vs 25%; p=.038) and higher% of relapse (p=.01). Eventually pts treated with HSCT with the p53 dysfunction had a higher risk of relapse after transplant when compared with pts without p53 disfunction. (p=0.002) (Figure 1B).

Figure 1.

Summary/Conclusion: MDS with p53 dysfunction is a well defined clinical category characterized by poor prognosis due to high risk of disease evolution and low probability of response to disease modifying treatments. Hystopathological and molecular assessments which can define p53 status should be widely adopted for a better prognostic definition of MDS pts.

PS1236

A FLOW-CYTOMETRY SCORING SYSTEM OF ABNORMAL GRANULOCYTES AND MONOCYTES MATURATION PROFILES FOR DIAGNOSIS OF MYELODYSPLASTIC SYNDROMES

S. Barreau[1], A. Green[1], O. Kosmider[1], L. Willems[2], V. Bardet[3], M. Fontenay[1], N. Chapuis[1,*]

[1]Service d'hematologie biologique, [2]Service d'hematologie clinique, APHP-Hôpitaux Universitaires Paris Centre, Paris, [3]Service d'hematologie biologique, APHP-Hopital Ambroise Paré, Boulogne Billancourt, France

Background: Diagnosis of myelodysplastic syndromes (MDS) mainly relies, on evaluation of dysplasia, quantification of blast cells and detection of cytogenetic abnormalities. Multiparametric flow cytometry (MFC) was recently reported to be a helpful additional tool for MDS diagnosis. However, the number of phenotypic abnormalities recommended to be analyzed is high and their quantification is difficult. Simplified approaches were developed and clearly demonstrated their utility but also showed a relative low sensibility possibly due to the restricted number of phenotypic abnormalities analyzed.

Aims: We aimed at developing a simple useful MFC tool based on the evaluation of granulocyte and monocyte maturation profiles in order to allow reproducible MDS diagnosis with high specificity and sensitivity.

Methods: We apply a 1-tube panel strategy allowing detection of abnormal expression of CD11b, CD56, CD10, CD13, CD14, CD33, CD34, CD64, CD16 and CD45 on both granulocytes and monocytes and the calculation of Ogata score (Navios, Beckman Coulter). We tested 283 samples including 20 control samples for the creation of a reference database. For each case, granulocytes and monocytes maturation profiles were compared to the reference maturation database (Infinicyt, Cytognos). The addition of the standard deviations (SD) outside the normalized database maturation (2 SD) for each stage of maturation (5 and 3 for granulocytes and monocytes respectively) for all tested parameters defined the Diff-Score.

		Ogata Score >2	Diff Score >17.2	Both
Learning Cohort				
Specificity (%)		93.4 (n=61)	92.1 (n=63)	85.2 (n=61)
Sensitivity (%)	All	52.6 (n=97)	60.6 (n=99)	73.2 (n=97)
	MDS-Low	37.1 (n=62)	46.0 (n=63)	61.3 (n=62)
	MDS-High	81.5 (n=27)	92.9 (n=28)	96.3 (n=27)
	MDS/MPN	75 (n=8)	62.5 (n=8)	87.5 (n=8)
Validation Cohort				
Specificity (%)		87.9 (n=33)	97 (n=33)	84.8 (n=33)
Sensitivity (%)	All	50 (n=52)	69.2 (n=52)	80.8 (n=52)
	MDS-Low	28.6 (n=35)	60 (n=35)	71.4 (n=35)
	MDS-High	90.9 (n=11)	81.8 (n=11)	100 (n=11)
	MDS/MPN	100 (n=6)	100 (n=6)	100 (n=6)

Figure 1.

Results: Among 178 consecutive patients referred for MDS suspicion, 99 patients were classified with proven MDS by morphological analysis of bone marrow and/or by cytogenetic analysis (MDS-SLD n=1; MDS-RS-SLD n=3; MDS 5q- n=6; MDS-MLD n=41; MDS-RS-MLD n=12; MDS-EB-1 n=19; MDS-EB-2 n=9; CMML-0/1 n=6 and CMML-2 n=2), 16 patients with ICUS and 63 patients with non-hematologic causes of cytopenia. We observed significantly more differences of both monocyte and granulocyte maturation profiles compared to the normal reference database in patients with MDS than in patients without MDS (p<0.0001). A similar result was observed after the comparison of cumulative differences detected in both granulocyte and monocyte maturation profiles (p<0.0001) for all types of MDS. A ROC analysis was performed to test the capacity of Diff-score to diagnose MDS. The area under the ROC curve (AUC) was 0.781 (95% CI, 0.711-0.850). A cutoff value of 17,2 SD outside the threshold (2 SD) established on the normalized reference database was calculated with a specificity of 94.9% and a sensitivity of 60.6% (46%, 92.9% and 62.5% for MDS-low (MDS

without excess blasts), MDS-high (MDS-EB-1/2) and MDS/MPN respectively). Interestingly, combination with the Ogata Score allowed MDS diagnosis with a specificity of 85.2% and a sensitivity of 73.2% (61.3%, 96.3% and 87.5% for MDS-low, MDS-high and MDS/MPN respectively). These results were validated in a second independent cohort of patients with MDS (n=52; with 35 MDS-low, 11 MDS-high and 6 MDS/MPN) or with non-clonal cytopenia(s) (n=33) with specificity and sensitivity values of the "Diff Score of 17.2 SD outside the threshold" criterion of 97% and 69.2%, respectively. Again, combination with the Ogata Score increased the sensitivity to 80.8% and to 71.4% for MDS-low samples (Figure 1).
Summary/Conclusion: We report here a simple useful MFC tool based on evaluation of granulocyte and monocyte maturation profiles which in combination to the Ogata-score allows reproducible MDS diagnosis with better sensitivity and higher specificity than the Ogata score alone.

PS1237

ALTERED CARBONYLATION PATTERN IN MYELODISPLASTIC SYNDROMES

A. Rodríguez García[1,*], M. Linares[1], K. Quiroz[1], T. Cedena[1], A. Arenas[1], M.L. Morales[1], R. Ayala[1], J.M. Bautista[1], J. Martínez López[1]

[1]Fundación Investigación Hospital Universitario Doce de Octubre, Madrid, Spain
**ARG and ML authors have contributed equally.*

Background: Myelodysplastic syndrome (MDS) is an aging associated clonal hematopoietic disorder characterized by a bone marrow failure in which immature blood cells do not mature properly. Oxidative stress seems to play a role in its pathogenesis. Interestingly, high ROS levels have been observed in MDS, which has been correlated with a diminished survival. However, the oxidation of proteins is of particular concern since it leads to aggregation, unfolding, or conformational changes that may confer a loss of structural or functional activity.
Aims: As an altered proteome has also been described during MDS, in this study we have analyzed the pattern of oxidized protein associated to MDS through detection of protein carbonylation. Moreover, we analyzed whether MDS patients could be beneficed by Deferasirox as antioxidant therapy.
Methods: Protein carbonylation was analyzed in bone marrow (n=14; 8 MDS and 6 controls) by immunohistochemistry after derivatization of carbonyl compounds with 2,4-dinitrophenylhydrazine (DNPH) and specific antibody detection of the 2,4-dinitrophenyl hydrazones. Protein carbonylation was also studied in expanded erythroblasts during *in vitro* erythropoiesis (n=9; 5 MDS and 4 controls) by 1-D and 2-D immunoproteomics. p21 mRNA levels were analysed by qRT-PCR.

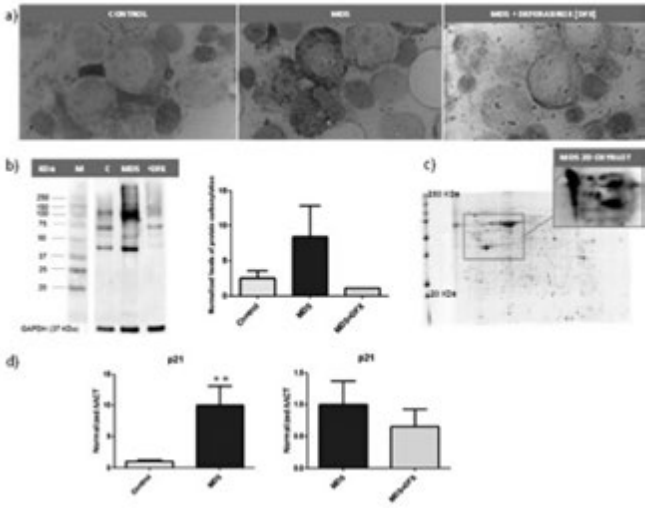

Figure 1.

Results: Immunohistochemistry assays showed a higher level of carbonylation in MDS samples (Figure 1a). We observed carbonylation in the cytoplasm of myeloid cells, whereas erythroid cells remained negative in both, patients and controls. Interestingly, MDS patients treated with Deferasirox presented lower levels than non-treated patients. Further, to assess carbonylation in the erythroid precursors, we analyzed the pattern of carbonylated proteins in expanded erythroblasts. Again, a higher level was observed in MDS vs. control group, which was reverted after Deferasirox treatment (Figure 1b). Most carbonylated proteins ranged in a size between 40-250 KDa. A highly carbonylated 40 KDa band was detected in all analyzed SMD samples. As seen in the 2D immunoblots, these differences in carbonylation were caused by a limited number of proteins or aggregates, rather than to a large spectrum of proteins (Figure 1c). Finally, as oxidative stress signaling pathways could regulate the cell cycle by p53, we analyzed the expression of p21, a p53 target during stress response. Upregulation of p21 was significantly observed in MDS samples. Remarkably, this effect was reverted upon Deferasirox treatment (Figure 1d).
Summary/Conclusion: An altered protein carbonylation pattern of myeloid and erythroid lineages was observed in MDS patients. This boosted oxidative cellular environment seems to activate signaling pathways involving p21. Deferaxirox treatment partially reverted these oxidative lesions at cellular level.

PS1238

EXPRESSION LEVEL OF SPECIFIC MIRNAS BEFORE AZACITIDINE THERAPY INITIATION MAY PREDICT FUTURE EFFICACY OF THE TREATMENT IN MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA

M. Dostalova Merkerova[1,*], Z. Krejcik[1], A. Hrustincova[1], K. Szikszai[1], A. Jonasova[2], J. Cermak[3], M. Belickova[1]

[1]Department of Genomics, Institute of Hematology and Blood Transfusion, [2]Faculty Hospital, [3]Outpatient Department, Institute of Hematology and Blood Transfusion, Prague, Czech Republic

Background: Azacitidine (AZA) is a hypomethylating agent that can improve the clinical outcome in approx. 50% of patients with myelodysplasia, and the prediction of AZA responsiveness is important for the therapy management.
Aims: We searched for specific microRNA (miRNA) profiles associated with AZA treatment in patients with higher-risk myelodysplastic syndromes (MDS) or acute myeloid leukemia with myelodysplasia-related changes (AML-MRC) with the aim to identify potential molecular markers of the response.
Methods: Microarray profiling (Human miRNA Microarrays, Agilent Technologies) was performed on CD34+ bone marrow cells in a discovery cohort (28 samples from 12 patients at baseline and during AZA therapy and four healthy donors) to preselect miRNAs with altered expression between responders and non-responders. To validate the microarray results and find clinically significant molecules, reverse-transcription quantitative-PCR (RT-qPCR) was applied to measure expression levels of individual preselected miRNAs in a validation cohort (50 samples from 27 patients at baseline and during AZA therapy and 11 controls).
Results: At baseline, the miRNA profiles stratified patients according to their future response status. The complete responders showed the most distinct miRNA expression pattern, whereas the patients with partial response had miRNA profiles that were more similar to those of the non-responders. Expression analyses of particular miRNAs between responders and non-responders found that the overall response rate was significantly higher in the patients with upregulated miR-17-3p and downregulated miR-100-5p and miR-133b at baseline. Importantly, the high level of miR-100-5p at baseline was associated with shorter overall survival (HR = 4.066, p=.008). To determine the miRNAs involved in AZA response, we compared miRNA expression of responders after AZA treatment with paired pretreatment samples and observed significant deregulation (p=.05) of 30 miRNAs (including decreased levels of miR-10b-5p, miR-15a-5p/b-5p, miR-24-3p, miR-148b-3p, and miR-199a-3p and increased levels of miR-1202 or miR-1260a), while the level of these miRNAs remained unchanged in the non-responders.
Summary/Conclusion: Our study demonstrates that responders and non-responders have distinct miRNA expression patterns before and during AZA therapy and that the level of specific miRNAs before therapy initiation may predict the future efficacy of AZA treatment.
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PS1239

MULTICENTRIC VALIDATION OF THE "MONOCYTE ASSAY" FOR CHRONIC MYELOMONOCYTIC LEUKEMIA DIAGNOSIS BY FLOW CYTOMETRY

S. Tarfi-Bekkour[1], V. Harrivel[2], F. Dumezy[3], J. Guy[4], M. Roussel[5], A. Mimoun[6], P. Fenaux[7], N. Chapuis[8], E. Solary[9], D. Selimoglu-Buet[9], O. Wagner-Ballon[1,*]

[1]Département d'Hématologie et Immunologie biologiques, APHP Hôpitaux universitaires Henri Mondor, Créteil, [2]Centre de Biologie Humaine - Laboratoire d'Hématologie, CHU Amiens Sud, Amiens, [3]Laboratoire d'hématologie, Centre Hospitalier Régional Universitaire de Lille, Lille, [4]Laboratoire d'hématologie, Centre Hospitalo-universitaire Dijon, Dijon, [5]Laboratoire Hématologie, Centre Hospitalo-universitaire de Rennes, Rennes, [6]Laboratoire d'hématologie, Centre Hospitalier de la Côte Basque, Bayonne, [7]Service d'Hématologie clinique, Hôpital Universitaire Saint-Louis, [8]Laboratoire Hématologie, Hôpital Universitaire Cochin, Paris, [9]INSERM U1170, Institut Gustave Roussy, Villejuif, France

Background: Accumulation of classical monocytes (cMO) $CD14^{++}CD16^{-}$ ≥94% of total peripheral blood monocytes analyzed by flow cytometry (FCM) was demonstrated to be a specific and sensitive tool that distinguishes chronic myelomonocytic leukemia (CMML) from reactive monocytosis (Selimoglu-Buet, 2015). The 94% threshold was further validated in two independent studies (Talati, 2017; Patnaik, 2017) and is increasingly used as a diagnosis marker of CMML.
Aims: Since this "monocyte assay" has been largely adopted by French diagnosis laboratories, we evaluated its performance in a multicentric evaluation.
Methods: A short survey was sent by personalized emails between July and September 2016 to 48 French laboratories performing routinely FCM diagnosis of myeloid neoplasms. Afterwards, a few selected centers were asked to send their raw data files as well as the corresponding cMO percentage evaluation in order to perform a double centralized blind analysis. Biological and clinical data were also provided when available.
Results: We obtained a comprehensive reply to the survey with a 100% rate of response. Thirty of 48 centers (63%) use routinely the "monocyte assay": apart from the three centers that participated to the initial study and keep using it, 27 centers spontaneously implemented it, most often following the initial publication and/or a meeting presentation. All the surveyed cytometrists consider the "monocyte assay" useful for CMML diagnosis.
In a second step, five centers (named A to E) provided 329 FCM raw data files with the corresponding cMO percentage. All these files were reanalyzed in a blind fashion by two different operators (skilled and trainee). These congruent centralized analyses compared to the cMO percentage determined by the centers showed an excellent correlation (r=0.93; p<0.0001, Figure 1A) without any major bias. As the cut-off value of 94% cMO is the most relevant parameter in the "monocyte assay", we compared the patients with cMO≥94% by centralized analysis (i.e. suspected of being diagnosed as CMML) and found an excellent agreement between both analyses (matching for 110 analyses/ 115 (95.7%), Figure 1B). Conversely, of the 214 patients with a fraction of cMO<94% by the centralized analysis, 201 (93.5%) displayed cMO<94% in the original center. In most of the 18 divergent cases, the difficulty in drawing cMO gate was related to an insufficient number of cMO analyzed. Hence, we decided to exclude all the files showing cMO event number below 10,000 (Figure 1C), thereby improving the correlation (r=0.94) and the agreement for the 245 remaining files (total matching files: 95%).

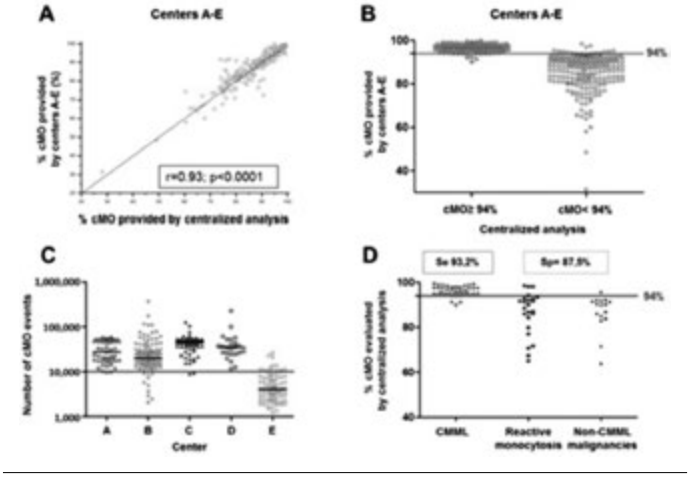


Figure 1.

Among these latter files, we collected clinical data for 84 patients for whom an overt diagnosis was made, namely 46 CMML, 24 reactive monocytosis and 14 non-CMML malignancies. cMO accumulation ≥94% determined by centralized analysis was observed in 43 of the 46 CMML (Figure 1D), indicating a sensitivity of 93.5% in accordance with previous results. A specificity of 86.8% was calculated as 5 of the 38 "non-CMML" patients displayed cMO<94% determined by centralized analysis. Interestingly, using these data we established a new Receiver Operator Curve (ROC) and obtained yet again a 94% cut-off value.
Summary/Conclusion: This study demonstrates a strong adhesion of the French laboratories to the "monocyte assay" for CMML diagnosis set-up in 2015, with 30 user centers so far. We observed a low variability of the cMO percentage quantification between centers and validated the published 94% cut-off value with a 93.5% sensitivity and a 86.8% specificity. Besides, we propose conclusion models for result reporting.

PS1240

CHARACTERIZATION AND DIFFERENTIAL EFFECTS OF MESENCHYMAL STROMAL CELL-DERIVED EXTRACELLULAR VESICLES IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

M. Wobus[1,*], A. Mies[1], U. Oelschlägel[1], C. Kahlert[2], F. Stölzel[1], M. Bornhäuser[1], U. Platzbecker[1]

[1]Hematology/Oncology, [2]Department of Gastrointestinal, Thoracic and Vascular Surgery, Medical Faculty, University Hospital TU Dresden, Dresden, Germany

Background: Beyond the genetically altered hematopoietic compartment, the pathogenesis of myelodysplastic syndromes (MDS) relies on a defective bone marrow niche, a highly specialized microenvironment ensuring stem cell self-renewal and differentiation during hematopoiesis. Within this niche, intercellular communication e.g. between hematopoietic stem and progenitor cells (HSPCs) and mesenchymal stromal cells (MSCs), is facilitated either by direct cell-cell contact or by soluble factors. Recently, non-contact communication processes were described on the basis of secretion of extracellular vesicles (EVs) that were able to modify functional properties of recipient cells by the transfer of bioactive molecules including RNAs and proteins. However, detailed mechanisms and the relevance for MDS pathogenesis as well as EV's potential as diagnostic and/or therapeutic targets remain to be defined.
Aims: Here, we investigated distinct characteristics of MDS MSC-derived EVs and their impact on HSPC phenotype and function.
Methods: MSCs were isolated from bone marrow of MDS patients (n=10) and age-adjusted healthy donors (HD, n=10)). After FCS-depleted culture for 48h, EVs were purified by using the Total Exosomes Isolation Kit (Thermo Fisher Scientific). Concentration and size of the EV population were analyzed with a ZetaView instrument (Particle Metrix GmbH) with multi-parameter nanoparticle tracking analyses (NTA) as well as transmission electron microscopy (TEM). Moreover, EVs were characterized by flow cytometry and Western blot. The functional impact of incorporated EVs on HSPCs derived from HD was evaluated by colony (CFU) and differentiation assays, proliferation and survival assays, phenotyping as well as trans-well migration experiments.
Results: NTA and TEM revealed a heterogeneous morphology of the EV populations with an average size of 148 nm for HD MSC-derived EVs and 156 nm for MDS MSC-derived EVs. EV concentration was higher in HD than in MDS samples (5.9E+08 vs. 2.1E+08 particles/ml). All EV preparations expressed typical exosomal markers including CD9, CD63 and CD81. Flow cytometry analysis of isolated EVs and their corresponding MSCs revealed that EVs expressed common MSC markers such as CD90 (MDS only), CD105 and CD146 (HD + MDS).
Next, we demonstrated the incorporation of Vybrant DiI-labeled EVs from both HD and MDS MSCs into CD34+ HSPCs by confocal imaging. The uptake of EVs had no influence on HSPC proliferation and survival. However, the *in vitro* differentiation of HSPCs was modulated in the presence of EVs. Of note, the presence of MDS MSC-derived vesicles resulted in increased numbers of CFU-G (81±15 with MDS EVs vs. 15.5±4.5 without EVs), CFU-M (79.5±16.5 vs. 26±10) and CFU-GM (51.5±9.5 vs. 18.5±4.5). The priming into the myeloid lineage of HSPCs by MDS EVs showed an increased expression of CD69 and CD11b as well as down-regulation of HLA-DR after granulocytic/monocytic differentiation in the presence of EVs. One possible underlying mechanism could be the transfer of miRNA-29a, which was detected at significant higher levels in HSPCs after incubation with MDS EVs. Moreover, we observed a higher migratory capacity towards a SDF-1 gradient of HSPCs which were pre-incubated with MDS EVs in comparison to HD EVs (33.3% vs. 12.2%) which was associated with higher CXCR4 expression levels.
Summary/Conclusion: In summary, we describe new mechanisms for the

intercellular communication between MSCs and HSPCs in the MDS microenvironment. The specific modulation of EVs from MDS patients offers new therapeutic and/or prognostic targets.

PS1241

MYELODISPLASTIC SYNDROMES: MECHANISMS INVOLVED IN THE TRANSFORMATION TO ACUTE MYELOID LEUKEMIA (AML)

F. Hernández Mohedo[1,*], P. Montes Ramos[2], M. Bernal Sánchez[2], P. Jiménez Gámiz[2], M. Corzo Corbera[2], A. R. González Ramirez[3], M. Jurado Chacón[1], F. Ruiz-Cabello Osuna[2]

[1]UGC Hematología Y Hemoterapia, [2]Análisis Clínicos e Inmunología, Hospital Universitario Virgen de Las Nieves, [3]Fundación de Investigación Biosanitaria Alejandro Otero (Fibao), Granada

Background: Myelodysplastic Syndromes (MDS) are a heterogeneous group of clonal neoplasms with variable risk of progression to Acute Myeloid Leukemia (AML) (1). Recently, several authors indicated that the progression may be mediated by a selective growth of a leukemic clone influenced by the mutational status. In this line, Next Generation Sequencing (NGS) studies have identified recurrent somatic mutations where mutations in high molecular risk (HMR) genes (*TP53, ETV6, ASXL1, RUNX1, EZH2*) have a relevant impact on survival and risk of progression to AML (2,3). However, independently of these factors, clinical evidence supports the existence of a dysregulation of the immune system (4).
Aims: To study both the mutational status and the role of the tumor microenvironment in the progression of the Myelodysplastic Syndrome
Methods: The study of the tumor microenvironment was carried out in 56 patients with primary MDS according to WHO-2008: RARS (n=2), RCMD (n=21), RCUD (n=2), Isolated 5q (n=7), RAEB-1,-2 (n=13), U.MDS (n=2) and LMA secondary to MDS (sAML) (n=9), using multiparametric flow-cytometry. 40 peripheral blood samples were used as controls. The mutational study was divided into: early state, pre-leukemic state (RAEB-1,-2) and sAML. Samples were sequenced by NGS techniques using a panel of 54 genes associated with myeloid neoplasms (TruSight, Illumina) and the MiSeq platform (Illumina, San Diego).
Results: In relation to the tumor microenvironment, we observed a significant increase of NK cells with $CD56^{-}CD16^{bright}$ ($p<0.01$) and $CD56^{bright}$ phenotypes ($p<0.01$) in patients with MDS compared to controls. In AML patients, we observed a decrease in the latter NK cell subset ($p<0.05$) together with a reduced cytotoxic effector NK cell numbers ($CD56^{dim}$ $CD16^{bright}$) ($p<0.01$) as compared to controls. Based on the expression of activating receptors on CD56+ NK cells, we observed a significant decrease of NKG2D both in patients with MDS ($p<0.05$) and AML ($p<0.01$) when compared to controls. We also observed that the increase of the NKG2D expression correlates with the low risk of progression according to IPSS/IPSS-R score ($p<0.01$). In contrast, the NKp46 and CD161 receptors were normally expressed in the MDS group, but not in patients with AML, where the expression of the CD161 receptor was significantly decreased ($p<0.01$). On the other hand, we detected higher numbers of granulocytic-myeloid derived suppressor cells (Gr-MDSC) in patients with MDS ($p<0.001$) and AML ($p<0.001$), with the increase in the monocytic-MDSC (Mo-MDSCs) in the latter group ($p<0.05$). In addition, we observed an increase in MDSC cells (Gr- and Mo-MDSCs) in patients with a high risk of progression according to the IPSS and IPSS-R score ($p<0.001$). Regarding the mutational profile, we observed that the percentage of patients with mutations in HMR genes was higher in the RAEB group (n=8, 53%) than in the early MDS group (n=9, 25.7%). In the latter, however, we detected a higher percentage of patients with mutations in NO-HMR (n=17, 47%), mainly related to DNA methylation and the RNA splicing compared to RAEB group (n=3; 20%). In addition, the variant allele frequency (VAF) in HMR genes was more than 40% in 6 of 8 patients in the RAEB group, whereas in the early stage group the VAF was less than 30% in 6 of 10 patients. Finally, AML group presented ≥3 mutated genes compared to the MDS group ($p<0.05$).
Summary/Conclusion: Both immune escape and acquired genetic changes can satisfactorily explain the progression of MDS

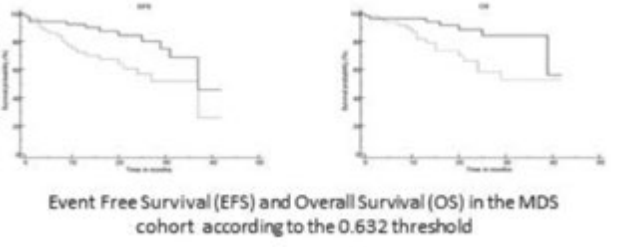
PS1242

COMPLETE BLOOD COUNT- BASED SCORE FOR THE DIAGNOSIS AND PROGNOSIS OF MYELODYSPLASTIC SYNDROMES IN CYTOPENIC PATIENTS

R. Boutault[11], P. Peterlin[2], M. Boubaya[3], K. Sockel[4], C. Godon[1], O. Theisen[1], Y. Le Bris[1], F. Kroschinsky[4], M. C. Béné[1], U. Platzbecker[4], M. Eveillard[1,*]

[1]Hematology Biology, [2]Hematology Clinic, CHU Nantes, Nantes, [3]Statistical Department, CHU Avicennes, Bobigny, France, [4]Hematology Clinic, Medizinische Klinik und Poliklinik I, Dresden, Germany

Background: A prospective study was performed in order to investigate whether suspected myelodysplastic syndromes (MDS) could be detected on a complete blood count (CBC) using the recently developed XN-10® analyzer (Sysmex, Kobe, Japan).
Aims: The principal objective of the study was to discriminate MDS patients from a cohort of 399 cytopenic controls by developing a scoring system. The secondary objective was to explore whether this new score, at diagnosis, had a value to predict disease evolution.
Methods: The study enrolled 109 untreated patients over 50 years old (61 men/48 women, median age 78) for whom a diagnosis of MDS was concluded based on CBC, bone marrow (BM) smears examination and karyotype. CBC included both quantitative parameters (hemoglobin [Hb] level, Mean Corpuscular Volume [MCV], reticulocytes, platelets, absolute neutrophil counts [ANC]) and positioning and dispersion parameters including Ne-WX, a new parameter which characterizes the dispersion of neutrophils. According to WHO criteria, patients were diagnosed with MDS-EB (n=33), MDS-MLD-RS (n=35), MDS-SLD-RS (n=25), MDS-SLD (n=12) without RS and MDS with isolated del(5q)(n=4). A diagnosis of MDS was excluded from the control cohort by BM examination, clinical features and biochemical data.
Results: Each WHO-MDS subgroup was significantly different from controls for MCV, ANC, platelets and Ne-WX ($p<0,0001$). Within MDS-SLD, platelet counts were similar to those of controls, yet significantly higher than for MDS-EB ($p=0.004$) or MDS-MLD ($p=0,029$). No other parameter discriminated between MDS subgroups.
Multiparametric analysis highlighted three parameters significantly different between the MDS and cytopenic control cohorts: median MCV (100.3±10.5fL; $p<0.0001$), median ANC (2.41±2.50x10^9/L; $p<0.001$) and median Ne-WX (384±70; $p<0.0001$). The latter, by itself, allowed to suspect MDS with a sensitivity of 71% and a specificity of 86%. Logistic regression and multivariate analysis were used to define a multiparametric screening MDS-CBC score with the combination of the three parameters. This score allowed to detect MDS with 86% sensitivity and 88% specificity at a threshold of 0.2. Moreover, high score values (>0.632) at diagnosis significantly correlated with decreased survival in MDS patients. The median EFS was 37 months in both high and low MDS-CBC score groups, while at 24 months the EFS was significantly higher for low scores at 84.6±5 vs 57.1±8 ($p=0.02$) Two year OS was significantly different at 88.5±5 months for low MDS-CBC scores vs 58.5±9 for high MDS-CBC scores ($p=0.008$; Figure 1). The 15 false-negative MDS patients were all IPSS low risk and five of them reached a positive score during follow-up. MDS could be excluded in 89% of cytopenic controls. False positive patients had one or several of the MDS-CBC score altered for other reasons (i.e. liver disease, vitamin deficiency, immunosuppressive treatment). A validation cohort tested in another center confirmed the power of the MDS-CBC score.



Event Free Survival (EFS) and Overall Survival (OS) in the MDS cohort according to the 0.632 threshold

Figure 1.

Summary/Conclusion: Overall, this study demonstrates that a simple CBC allows to screen for MDS using a multiparameter score including the newly available parameter Ne-WX. Blood smear examination should be performed in case of a positive score, in patients older than 50 with cytopenia, taking into account the clinical context. This score may improve the efficiency of laboratory practice and could be a valuable help in early screening, diagnosis and management of this hematological disease.

PS1243

AUTOPHAGY IN MYELODYSPLASTIC SYNDROMES: THE ROLE OF HIF-1A/REDD1 MOLECULAR PATHWAY

I. Stergiou[1,*], K. Kambas[1], S. Giannouli[2], D. Aglaia[3], K. Ritis[4], A. Tzioufas[1], M. Voulgarelis[1]

[1]Department of Pathophysiology, School of Medicine, [2]2nd Department of Internal Medicine, National and Kapodistrian University of Athens, [3]Immunology and histocompatibility departement, Laiko General Hospital, Athens, [4]Department of internal Medicine, Democritus University of Thrace, Alexandroupolis, Greece

Background: Hypoxia is a prominent feature of the bone marrow (BM) microenvironment influencing both normal and malignant hemopoiesis. It has been proposed that differential Hypoxia Inducible Factor 1α (HIF-1α) protein expression and programming finely regulate normal haemopoiesis by promoting both quiescence and survival of hemopoietic stem cells (HSCs) within hypoxic BM endosteal niche, as well as proliferation and differentiation of hemopoietic progenitor cells (HPCs) in less hypoxic BM vascular niche. DNA damage response 1 gene (REDD1) is a direct transcriptional target of HIF-1α linking hypoxia to energy regulation and autophagy. Evidence suggests that metabolism and autophagy are developmentally programmed and essential for effective haemopoiesis. A severely altered topobiology of the BM niche in myelodysplastic syndromes (MDS) is indicated by the presence of abnormal localization of immature precursors (ALIP), which is characterized by HPCs displacement towards the more hypoxic intertrabecular space. This abnormal localization could lead to aberrations of the HIF-1α/REDD1 axis and programming of HSPCs, thus compromising haemopoiesis in the context of MDS.
Aims: To study the implication of HIF-1α/REDD1/autophagy axis in differentiation/maturation defects of haematopoietic BM cells of MDS patients.
Methods: BM aspiration samples were collected from 15 newly diagnosed previously untreated MDS patients and 7 age matched controls with non-malignant hematologic disorder. We included patients with all MDS subtypes (9 MDS with multilineage dysplasia, 1 MDS with unilineage dysplasia, 2 RAEB I, 2 RAEB II, 1 MDS dep5q) excluding patients with MDS-RARS subtype. CD34 and myeloid lineage cells were collected with the use of magnetic beads and ficoll bilayer protocol respectively. BM cell populations were determined by FACS analysis using standard gating strategies. HIF1a and REDD1, gene expression was quantified with qRT-PCR and protein expression with FACS analysis. Determination of autophagy was performed using immunofluorescence and immunoblotting for LC3B, with concurrent definition of cell populations with CD34 and MPO staining.
Results: We demonstrated significant *REDD1* overexpression in mRNA level both in CD34 (p=0,005) and myeloid cells (p<0,0001) from MDS patients compared to controls, using qRT-PCR. Expectedly, no difference in *HIF1A* expression in mRNA was observed in these cells. However, both HIF-1α and REDD1 protein expression were significantly higher in MDS patients compared to controls (CD34: p<0,05 for HIF-1α, p<0,01 for REDD1 / myeloid cells: p<0,05 for HIF-1α, p<0,05 for REDD1), as assessed by flow cytometry. Higher REDD1 protein expression was shown in patients with high grade dysplasia as assessed by the Ogata classification system. Moreover, HIF-1α and REDD1 protein coexpression in these cells verified HIF-1α/REDD1 signaling. Finally, increased autophagy was present in CD34 and myeloid cells from MDS patients compared to controls by immunofluorescence study for LC3B with concurrent staining for CD34 and MPO (Figure 1).

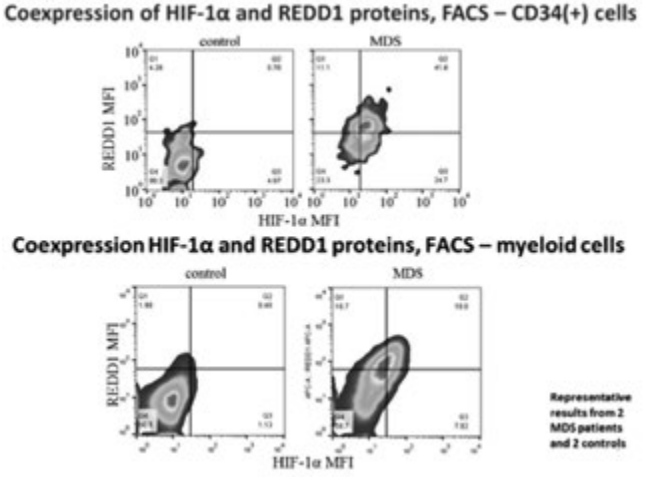


Figure 1.

Summary/Conclusion: Our current results prove activation of the HIF-1α/REDD1 pathway both in CD34 and myeloid cells from patients with MDS compared to controls, leading to increased autophagy in these cell populations. This pathway may lead to dysregulation of differentiation potential for the myeloid cells, unraveling dysregulated autophagy as a new pathogenetic mechanism for the MDS. The exact type of autophagy remains to be clarified.

Myelodysplastic syndromes – Clinical

PS1244

AN OPEN-LABEL PHASE 1 STUDY TO EVALUATE THE SAFETY AND TOLERABILITY OF DURVALUMAB IN PATIENTS WITH MYELODYSPLASTIC SYNDROME AFTER TREATMENT WITH HYPOMETHYLATING AGENTS

G. Garcia-Manero[1,*], M. Gaddh[2], U. Platzbecker[3], R. C. Lindsley[4], S. Larson[5], L. Godley[6], J. Palmer[7], F. Quddus[8], T. Chevassut[9], P. Fenaux[10], R. Komrokji[11], R. Lyons[12], G. Roboz[13], C. Schiffer[14], A. Al-Kali[15], A. Zeidan[16], N.S. Yao[17], B. Chao[17], M. Tang[17], C. Black[17], J. Karakunnel[17], J. Bothos[17], L. R. Silverman[18]

[1]MD Anderson Cancer Center, Houston, [2]Winship Cancer Institute of Emory University, Atlanta, United States, [3]University Hospital Carl Gustav Carus, Dresden, Germany, [4]Dana-Farber Cancer Institute, Boston, [5]David Geffen School of Medicine at UCLA, Los Angeles, [6]University of Chicago, Chicago, [7]Mayo Clinic, Phoenix, [8]Bon Secors Hematology & Oncology, Greenville, United States, [9]Brighton and Sussex Medical School, University of Sussex, Brighton, United Kingdom, [10]Hôpital St Louis/Université Paris, Paris, France, [11]H Lee Moffitt Cancer Center, Tampa, [12]Texas Oncology and USONcology Research, San Antonio, [13]Weill Cornell University, New York, [14]Karmanos Cancer Center, Detroit, [15]Mayo Clinic, Rochester, [16]Yale University, New Haven, [17]MedImmune, Gaithersburg, [18]Icahn School of Medicine at Mount Sinai, Tisch Cancer Institute, New York, United States

Background: Upregulation of programmed death ligand-1 (PD-L1) has been observed in patients with myelodysplastic syndrome (MDS). PD-L1 expression on myeloblasts has been associated with MDS transformation to acute myeloid leukemia (AML) and with reduced antileukemic effect of CD8+ cytotoxic T lymphocytes in AML. Anti–PD-L1 antibodies, such as durvalumab, block the interaction of PD-L1 with programmed death-1 (PD-1) and CD80, which reduces the suppressive effects of PD-1 on immune cells. This trial evaluated durvalumab in patients with MDS with disease progression after treatment with hypomethylating agents (HMAs); it began as a durvalumab monotherapy study (described here) and was expanded to include combinations with tremelimumab with/without azacitidine.
Aims: This study evaluated the safety of durvalumab in patients with MDS. Efficacy and pharmacokinetic (PK) end points were also evaluated.
Methods: Adults with pathologically confirmed MDS who were immunotherapy-naive and previously treated with HMAs (with no response, relapse, or inability to tolerate) were included; all patients provided written informed consent. Patients received durvalumab 10 mg/kg intravenously every 2 weeks. Safety evaluations included dose-limiting toxicities (DLTs) and adverse events (AEs). Response was defined by the International Working Group 2006 MDS response criteria. Serum concentrations of durvalumab were evaluated to determine drug exposure and PK parameters.
Results: Forty patients were treated (median age, 73 years [48-96]; 29 men, 11 women; 18 low/intermediate-1 risk; 22 intermediate-2/high risk). Median duration of follow-up was 10.8 months (range 1.2–41.2). All patients had ≥1 AE; 26 (65%) had durvalumab-related AEs; 12 (30%) had grade ≥3 durvalumab-related AEs. The most common treatment-related AEs were diarrhea (n=6; 15%), fatigue (n=6; 15%), increased aspartate aminotransferase (AST) (n=4, 10%) and increased alanine aminotransferase (ALT) (n=4, 10%). The most common grade 3 or greater treatment-related AEs were decreased platelet count (n=2, 5%), pleural effusion (n=2, 5%), and pneumonitis (n=2, 5%). The best overall response was marrow complete remission (mCR) in 7 patients (18%) all of whom had >5% baseline blasts (including 4 in the intermediate-2/high-risk group who had blast decreases from 10% to 5%, 6.8% to 1.8%, 10% to 4%, and 11% to 3%). There were no complete (CR) or partial responses (PR); 8 patients (20%) achieved stable disease (SD; 3 in the low/intermediate-1 risk group; 5 in the intermediate-2/high-risk group). Disease control (CR/PR, mCR and SD) rates were 33% in the low/intermediate-1 risk group and 41% in the intermediate-2/high-risk group. Median overall survival was 23.8 months for the low/intermediate-1 risk group and 8.0 months for the intermediate-2/high-risk group. Median time on treatment was 8.7 months (range 1.2–29.2); 45% discontinued treatment due to progressive disease and 15% had 1 or more related AE leading to treatment discontinuation (1 each of systemic immune activation, increased ALT, increased AST, increased transaminases, nephritis, pleural effusion, pneumonitis, maculo-papular rash). Durvalumab PK results will be presented.
Summary/Conclusion: Durvalumab demonstrated a safety profile consistent with other PD-1/PD-L1 targeting agents with minimal clinical activity in patients with MDS previously treated with HMAs. Based on these results and preclinical studies the trial was expanded to include treatment combi-

nations with tremelimumab with/without azacitidine; this portion of the study is currently ongoing.

PS1245

SINGLE AGENT TALACOTUZUMAB IN ELDERLY HIGH-RISK MDS OR AML PATIENTS FAILING HYPOMETHYLATING AGENTS – RESULTS OF THE SAMBA STUDY BY THE EMSCO NETWORK

U. Platzbecker[1,*], K. Götze[2], J. Krönke[3], U. Oelschlägel[1], F. Schulze[1], K. Sockel[1], J. M. Middeke[1], F. Chermat[4], S. Gloaguen[5], M. Puttrich[6], C. Weigt[6], K. Jersemann[6], P. Fenaux[7], R. Schlenk[8], A. Giagounidis[9], L. Adès[7]

[1]Department of Medicine I, University Hospital Dresden, Dresden, [2]Department of Medicine III, Klinikum rechts der Isar, TUM, Munich, [3]Department of Internal Medicine III, University Hospital Ulm, Ulm, Germany, [4]Groupe Francophone des Myélodysplasies, Paris, France, [5]EMSCO, [6]GWT-TUD GmbH, Dresden, Germany, [7]Service d'Hématologie seniors, Hôpital Saint-Louis, Paris, France, [8]Nationales Centrum für Tumorerkrankungen (NCT), Heidelberg, [9]Dept. for Oncology, Hematology and Palliative Care, Marien Hospital Düsseldorf, Düsseldorf, Germany

Background: AML and MDS patients failing hypomethylating agents (HMA) based therapies have a dismal prognosis highlighting an unmet medical need for these patients. CD123 (Interleukin-3 receptor alpha chain [IL3RA]) is overexpressed in MDS and AML progenitors and seems to be a promising target for immunotherapy. Talacotuzumab (TAL, JNJ-56022473) is an IgG1 monoclonal antibody targeting CD123 preferentially via antibody-dependent cellular cytotoxicity (ADCC). In addition the antibody inhibits IL-3 signaling to impede proliferation of leukemic progenitor cells.
Aims: The SAMBA trial of the German and French MDS study groups within the EMSCO network assessed the safety and efficacy of single agent TAL treatment in patients with higher-risk MDS or AML failing first line treatment with HMAs.
Methods: TAL was given i.v. at a dose of 9 mg/kg once every two weeks (14d ± 2d) for a total of 6 infusions and could be continued in responding patients. The study was accompanied by an immune monitoring via flow-cytometric analysis to investigate the dispersion of T- and NK cells in blood (PB) and bone marrow (BM) at the time of screening and during therapy in comparison with healthy, age matched controls. Of special importance were NK cells, as they are activated by high-affinity binding by the Fc-region of TAL inducing ADCC at targeted cells.

Table 1. Patient characteristics within the SAMBA trial.

Patient characteristics	n = 24
Age (years), median (IQR)	77 (71-90)
Women, n (%)	7 (29)
AML, n (%)	19 (79)
MDS, n (%)	5 (21)
Cytogenetics	
Normal karyotype, n (%)	12 (50)
Aberrant karyotype, n (%)	12 (50)
Complex aberrant (≥3), n (%)	5 (21)
ELN2017 AML risk classification (n=19)	
fav, n (%)	2 (10)
int1/2, n (%)	8 (42.5)
adv, n (%)	9 (47.5)
MDS, n (%)	5 (9)
Haematological parameters at screening	
WBC in /nl, median (range)	2.51 (0.37-12.64)
Hemoglobin in g/dl, median (range)	9.25 (8.05-11.7)
PLT in /nl, median (range)	28 (4-1018)
Neutrophils in /nl, median (range)	0.59 (0.0-5.59)
Lymphocytes in /nl, median (range)	0.80 (0.024-4)
marrow blasts %, median (range)	27 (7-89)
Years from diagnosis to treatment start, median (range)	1.76 (0.61-7.82)
Therapy outcome	
Days on treatment, median (range)	56 (1-282)
Dosage IMP in mg median (range)	653.5 (59-828)
Applied cycles median (range)	3 (0-17)

Results: A total of 24 AML/MDS patients having failed HMA with a median age of 77 years were included (Table 1). Therapy resulted in grade 3/4 infusion related side effects (pneumonia, n=1; infusion-related reaction, n=3; septic shock, n=1) in 4 patients, which led to early study discontinuation. Most frequent severe adverse events observed were infections (38%), cytopenias (9%), cardiac and gastrointestinal (7.5%, each) as well as nervous systems disorders (7.5%) in this older HMA-refractory MDS/AML population. Fourteen patients were assessed for response after 4 infusions and 8 after 6 infusions, respectively. Overall, 5 responders were identified; four AML patients with a marrow partial remission and one with a marrow complete remission; none of the responding patients achieved formal hematological improvement. Duration of response in these patients was short (median 1 month, range 1-6). Three patients are still on treatment based on subjective benefit in two of them despite losing objective response and one MDS patient with disease stabilization. Median survival for the entire cohort of patients is 4.8 months (95%>CI, 2.7–8.7). Prior to therapy, patients already displayed a disturbed immune profile compared to healthy controls. In fact, patients had significantly less mature NK-cells (75% CD56 dim NK-cells in patients vs. 87.9% in controls; $p<0.01$) including a higher expression of inhibiting (NK-KIR) and less activating (NKG2D and DNAM1) NK-cell receptors ($p<0.01$).
Summary/Conclusion: Even though ADCC-mediated targeting of CD123 seems to be a promising treatment option, patients with advanced MDS/AML displayed significant alterations in their immune cell repertoire which may have contributed to the moderate clinical benefit with single agent TAL treatment.

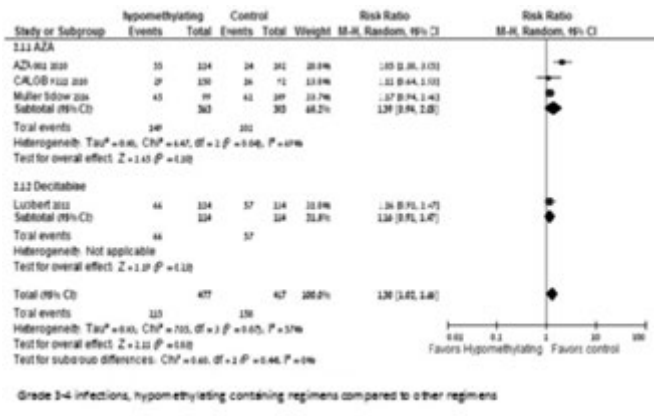
PS1246

HYPOMETHYLATING AGENTS ASSOCIATED INFECTIONS - SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

L. Shargian-Alon[1,2,*], R. Gurion[1,2], P. Raanani[1,2], D. Yahav[2,3], A. Gafter-Gvili[2,4]

[1]Hematology, Rabin Medical Center, Petah Tikva, [2]Sackler School of Medicine, Tel-Aviv, [3]Infectious disease unit, [4]Medicine A, Rabin Medical Center, Petah Tikva, Israel

Background: Hypomethylating agents (HMA) are considered the standard of care for elderly patients with higher-risk myelodysplastic syndrome (MDS), who are not candidates for allogeneic stem cell transplantation, due to survival benefit. HMA are also considered as an emerging treatment option for acute myeloid leukemia (AML) patients who are not eligible for intensive chemotherapy. Although HMA are regarded as safe and are usually administrated in the outpatient setting, infectious complications remain a main concern. Data in the literature regarding the effects of HMA on the risk of infection seem to be poorly documented and heterogeneous.
Aims: To assess the risk of infectious complications among MDS and AML patients treated with HMA.
Methods: We conducted a systematic review and meta analysis of all randomized controlled trials (RCTs) comparing HMA containing regimens (alone or combined with other chemotherapeutic agents) to any other regimen administered to patients with MDS or AML.
A comprehensive search encompassing *the Cochrane Library*, MEDLINE, conference proceedings, and databases of ongoing trials was conducted until February 2018. Two reviewers appraised the quality of trials and extracted data. Primary outcome was grade 3 to 4 infections; Secondary outcomes included: febrile neutropenia, fever of unknown origin (FUO), neutropenia grade 3-4, infection related mortality and all-cause mortality. Relative risks (RRs) with 95% confidence intervals (CIs) were estimated and pooled. A fixed effect model was used to pool data unless there was significant heterogeneity, in which case a random effects model was used.
Results: We identified 9 trials published between the years 2002 to 2016 and randomizing 2184 patients that fulfilled the inclusion criteria and reported relevant outcomes. Three trials included patients with MDS, four trials included AML patients and two included both. The HMA arm consisted of azacitidine in five trials and decitabine in four trials. Six trials evaluated patients in the first line setting, and HMA was given as a standalone treatment. The control arm consisted of best supportive care (BSC) in three trials. In additional three trials control arm consisted of either BSC, low dose cytarabine or intensive chemotherapy. HMA were associated with an increase in grade 3-4 infections, compared to the comparator [RR 1.30 (95% CI 1.02-1.66), Figure 1]. This was true for the whole cohort and for the subgroup of patients older than 60 years [RR 1.19 (95% CI 1.01-1.39)]. In addition, there was an increase in the rate of FUO events and neutropenia [RR 1.48 (95% CI 1.15-1.92) and RR 1.48 (95% CI 1.22-1.78), respectively]. There was no difference in fatal infections [RR 1.44, 95% CI 0.72 to 2.89]. Yet, treatment with HMA reduced all-cause mortality [RR 0.74, 95% CI 0.66-0.88].



Grade 3-4 infections, hypomethylating containing regimens compared to other regimens

Figure 1.

Summary/Conclusion: Treatment with HMA was associated with an increase in grade 3-4 infections rate. Although there was no difference in infection related mortality, all-cause mortality at 6 months was significantly lower among patients treated with HMA. Thus, the overall survival advantage of HMA is achieved despite the higher toxicity profile of these drugs, and probably due to a better effect on disease control.

PS1247

RESPONSE TO HYPOMETHYLATING AGENTS IMPROVES LONG-TERM OUTCOMES FOR LOWER-RISK PATIENTS WITH MYELODYSPLASTIC SYNDROME IN CASE-MATCHED COHORTS

D. Baek[1,*], Y.J. Lee[1], S.K. Sohn[1], J. S. Ahn[2], H.J. Kim[2], H.J. Shin[3], W.S. Lee[4], S.M. Lee[4], I. C. Song[5], Y. Choi[6], H. S. Lee[7], S.W. Park[8], Y. Choi[9], Y. Y. Cho[10], S.H. Bae[10], J.H. Moon[1]

[1]Hematology/Oncology, Kyungpook National University Hospital, Daegu, [2]Hematology/Oncology, Chonnam National University Hwasun Hospital, Chonnam, [3]Hematology/Oncology, Busan National University Hospital, [4]Hematology/Oncology, Inje University Busan Baik Hospital, Busan, [5]Hematology/Oncology, Chunnam National University Hospital, Chonnam, [6]Hematology/Oncology, Chungnam National University Hospital, Chungnam, [7]Hematology/Oncology, Kosin University College of Medicine, Busan, [8]Hematology/Oncology, Gyeongsang National University Hospital, Jinju, [9]Hematology/Oncology, Ulsan University Hospital, Ulsan, [10]Hematology/Oncology, Daegu Catholic University Hospital, Daegu, Korea, Republic Of

Background: Hypomethylating agents (HMAs) are also used to treat patient with lower-risk myelodysplastic syndrome (LR-MDS) relapsing after best supportive care (BSC). However, little is known about the predictive factors using HMAs and the survival benefit of HMAs for LR-MDS.
Aims: This study evaluated the factors affecting the use of HMAs and compared the long-term outcomes after matching baseline clinical factors between BSC and HMA groups
Methods: The data of 353 patients diagnosed with International Prognostic Scoring System (IPSS) lower risk (low and intermediate-1) between Oct 1992 and Jul 2013 were retrospectively analyzed. HMAs were given continuously until progression. The response was assessed using the International Working Group response criteria for MDS. The cumulative incidence of HMA treatment was analyzed using Gray's test considering non-relapse death as competing event.
Results: HMAs were administered in 243 patients, while 110 patients were treated with BSC. Among 243 patients treated with HMAs, azacitidine and decitabine were administered in 191 patients (78.6%) and 52 (21.4%), respectively. HMAs were administered median 5 cycles (range 1-26 cycles) and overall response was achieved in 104 patients (42.8%) including 30 complete response (12.4%), 15 partial response (6.2%), and 59 hematologic improvement (24.3%). The cumulative incidence of HMA treatment was significantly lower in very low risk groups by revised IPSS (IPSS-R) or WHO prognostic scoring system (WPSS), and category 1 by Low Risk Prognostic Scoring System (LR-PSS). Factors using HMA treatment were intermediate or poor cytogenetic risk (OR 0.53, p=0.001), intermediate/high risk group by WPSS (OR 1.55, p=0.001), and category 3 by LR-PSS (OR 1.95, p<0.001) in multivariate analysis. The 3-year overall survival (OS) rate in 243 patients was 69.1±6.5%, 47.4±6.1%, and 46.3±4.7% in BSC group, HMA responder, and HMA non-responder, respectively (p=0.023). Among 162 case-matched cohorts, 3-year OS rate was comparable between BSC group (67.1±7.4%) and HMA responder (58.1±10.8%), while that of HMA non-responder was low (32.2±7.2%, p<0.001). In the IPSS lower risk patients, following factors adversely affected OS in the multivariate analysis: age (HR 1.03, p=0.001), ECOG performance status ≥ 2 (HR 2.65, p<0.001), higher risk group by IPSS-R (HR 2.69, p<0.001) or WPSS (HR 1.63, p=0.022), and HMA non-responder (HR 3.79, p=0.001). In the case-matched cohorts, age (HR 1.03, p=0.018), ECOG performance status ≥ 2 (HR 2.47, p=0.024), low platelet < 50×10^3/L (HR 2.30, p=0.009), higher risk group by IPSS-R (HR3.10, p<0.001), and HMA non-responder (HR 3.01, p=0.001) were associated with inferior OS rate.
Summary/Conclusion: Our study demonstrated that the higher risk groups by WPSS or LR-PSS among IPSS lower risk patients showed an increased risk of using HMAs. Although long-term outcomes for the patients treated with HMAs shows an inferior OS rate, the OS rate of HMA responders among case-matched cohorts showed an improved OS rate similar to BSC group.

PS1248

A SYSTEMATIC REVIEW PROVIDES LIMITED CLINICAL EVIDENCE FOR ADDITIONAL EFFICACY OF G-CSF + ESA COMPARED TO FULL-DOSE ESA ALONE IN THE TREATMENT OF ANEMIA IN PATIENTS WITH LOW-RISK MDS

L. Affentranger[1,*], J. Bohlius[2], N. Bonadies[1]

[1]Department of Haematology and Central Haematology Laboratory, Inselspital, Bern University Hospital, University of Bern, [2]Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland

Background: Myelodysplastic syndromes (MDS) are a group of heterogeneous hematopoietic stem cell disorders characterized by dysplasia and peripheral cytopenia with a propensity to evolve towards acute myeloid leukemia (AML). The treatment of patients with low-risk MDS includes the administration of hematopoietic growth factors like erythropoiesis stimulating agents (ESA) eventually in combination with granulocyte colony stimulating factor (G-CSF). However, the evidence on the additional effect of G-CSF on erythropoiesis remains controversial.
Aims: The aim of this systematic review was to identify the level of evidence for treatment of anemia with the combination of G-CSF and ESA in low-risk MDS patients.

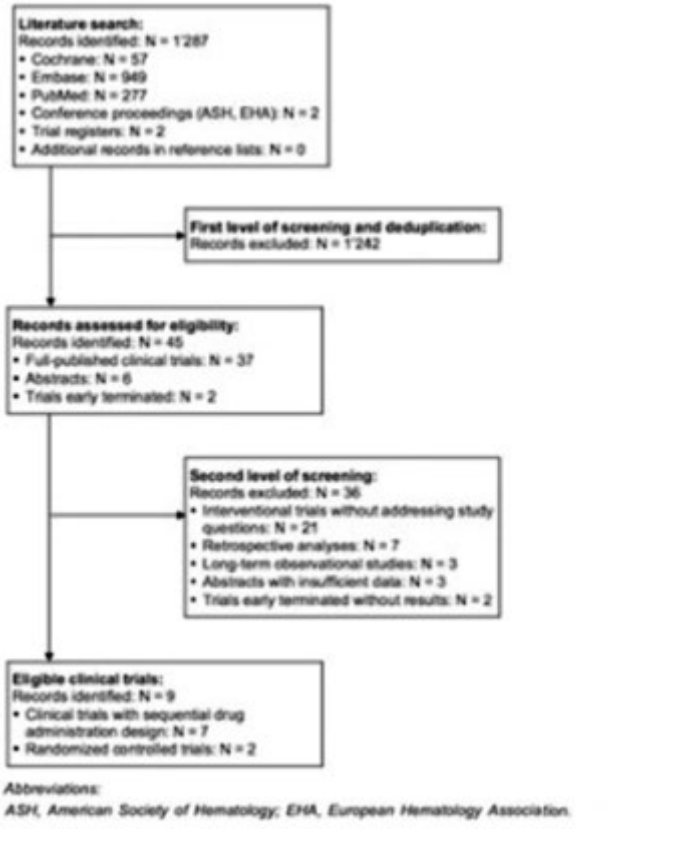


Figure 1. Flow chart of the systematic literature search and the study selection process.

Methods: We performed a systematic literature review by examining the

Cochrane Library, *Embase* and *PubMed* databases and checked additionally ongoing trials as well as conference abstracts (Figure 1). Randomized controlled trials (RCTs) investigating ESA + G-CSF versus ESA alone as well as prospective trials investigating sequential administration of ESA + G-CSF after ESA alone were considered. The primary endpoint of interest was erythroid response according to the *International Working Group* (IWG) criteria and secondary endpoints included duration of response, quality of life (QoL), progression and safety.
Results: We could identify nine eligible publications. Only two small RCTs showed erythroid response rates (ERR) ranging from 65-73% in patients receiving ESA + G-CSF compared to 33-40% in patients treated with standard doses of ESA only (up to 40'000 IU recombinant human erythropoietin (rhEPO) per week). In seven studies with a sequential drug administration design, ERR ranged from 13-71% after treatment with standard- or full-dose ESA (60'000-80'000 IU rhEPO per week) alone and from 39-74% after the combination therapy with G-CSF. Thus overall ERR increased by 0-42% after the administration of combination therapy in these non-randomized clinical trials. In all publications, secondary endpoints were not evaluable due to missing data.
Summary/Conclusion: Two small RCTs showed a clinical efficacy for the addition of G-CSF to standard-dose ESA in low-risk MDS patients. However, evidence for the addition of G-CSF to full-dose ESA is only available from non-randomized clinical trials with sequential drug administration design. We conclude that the number of clinical trials is low, the sizes of study populations small and the study designs not fully appropriate to provide undisputable evidence that G-CSF+ESA provides additional efficacy compared to full-dose ESA alone in the treatment of anemia in low-risk MDS patients. Evidence from properly designed RCTs on the efficacy of G-CSF added to full-dose ESA as well as information on duration of response, QoL, progression and safety are currently lacking and desirable for the future.

PS1249

LOW RISK MDS WITHOUT RING SIDEROBLASTS. CLINICAL AND BIOLOGICAL CHARACTERIZATION FROM THE SPANISH GROUP OF MDS

F. López Cadenas[1,*], M. Sánchez Barba[2], T. Bernal [3], F. Ramos[4], M. Tormo[5], M. Arnan[6], G. Sanz[7], C. Pedro[8], A. Vicente[9], V. Marco Betes[10], L. Hermosín Ramos[11], M. Pedreño[12], B. González[13], A. Fernández González[14], J.C. Caballero Berrocal[15], F. Prosper[16], D. Valcarcel[17], M. Díez Campelo[1]

[1]Hematology department, HOSPITAL CLÍNICO UNIVERSITARIO DE SALAMANCA (SANIDAD CASTILLA Y LEÓN), [2]Statistics department, Universidad de Salamanca, Salamanca, [3]Hematology department, Hospital Universitario Central de Asturias, Oviedo, [4]Hematology department, Complejo Asistencial Universitario de León (CAULE), León, [5]Hematology department, Hospital Clínico de Valencia, Valencia, [6]Hematology department, ICO-Hospital Duran i Reynals, Hospitalet de Llobregat, [7]Hematology department, Hospital Universitario La Fe, Valencia, [8]Hematology department, Hospital del Mar Medical Research Institute (IMIM), Barcelona, [9]Hematology department, Hospital La Ribera, Alzira (Valencia), [10]Hematology department, Hospital Arnau de Vilanova, Lleida, [11]Hematology department, Hospital Universitario de Jerez, Jerez de la Frontera (Cadiz), [12]Hematology department, Hospital Doctor Peset, Valencia, [13]Hematology department, Hospital Universitario de Canarias, Tenerife, [14]Hematology department, H de Cabueñes, Gijon, [15]Hematology department, Hospital Clínico Universitario de Valladolid, Valladolid, [16]Hematology department, Clínica Universidad de Navarra, Pamplona, [17]Hematology department, Hospital Universitario Vall d'Hebron, Barcelona, Spain

Background: Low risk myelodysplastic syndromes (LR-MDS) are a heterogeneous group of patients poorly characterized. The subgroup of patients with ring sideroblasts (RS) shows a homogeneous clinical and genetic profile and novel therapeutic approaches had been recently incorporated (Luspatercept). Nevertheless, in non-RS patients the clinical-biological characteristics and outcome are heterogeneous. Accurate clinical and biological knowledge of this large group of patients is mandatory to improve the therapeutic approaches in the near future.
Aims: Our Aim was to analyze baseline characteristics of clinical and biological parameters involved in overall survival (OS) among LR-MDS patients without RS.
Methods: We performed a multicenter research from the Spanish MDS registry. Data from 2215 (low and int risk according to IPSS and very low, low and Int risk according to R-IPSS) LR-MDS patients without RS were retrospectively analyzed. Funding PI17/01741; GRS 1349/A/16; GRS 1179/A/15; and CM17/00171.
Results: Median age at diagnosis was 73.5 years (p25 – p75 67-81) and 59% of patients were male. MDS was secondary in 7,8%. According to WHO2008 classification: 55.9% were RCUD/RCMD, 7,3% 5q- syndrome, 2% RAEB, 19.7% MDS/MPS and 0.8% unclassifiable MDS + other subtypes, 13.4% of patients are under morphological review. At presentation, median and range (p25-p75) of Hemoglobin, neutrophils and platelets at diagnosis was 10.6 g/dL (9.3-12.1), 2,35x10^9/L (1,4-3,97) and 151x10^9/L (92-235), respectively. Only 4.2% of patients presented peripheral blasts at diagnosis. Regarding bone marrow (BM) blast, a median of 1.6% was detected (p25-p75 0,5-3,0). Bone marrow cellularity was hypercellular in 50.6% of patients. Median ferritine level was 180ng/mL (p25-p75 76-382). According IPSS 64,7% and 35,3% of patients were low and intermediate-1 risk respectively. In addition, most of the patients were in the low risk R-IPSS (89,2% very low/low) and only 10,8% of patients were intermediate R-IPSS risk. Among 1943 patients with data available and with a median follow up of 32 months, median overall survival was 5,7 years (p25-p75 2,5-11,4). Regarding OS, older age at diagnosis(p<0.001), male gender (p<0.001), secondary MDS (p=0.024), hypercellular BM (p<0.001), Hb <10g/dL (p<0.001), Plt<100x10^9/L (p=0.002), the presence of PB blasts (<0.001), >5% of BM blasts (p=0.009), ferritine level >1000 mg/dL (p=0.015), int IPSS(<0.001), int R-IPSS (<0.001), poor cytogenetics by IPSS (<0.001) and poor and very poor cytogenetics according to R-IPSS (0.012) adversely impact on survival in this subset of patients. Nevertheless, in the multivariate analysis (Table 1), only male gender, secondary MDS, Hypercellular BM, Hb<10g/dL, Plt<100x10^9/L and PB Blast retained statistical significance.

Table 1.

	Sig.	HR	95,0% CI	
			Inf.	Sup.
Male gender	**0,000**	**1,971**	**1,645**	**2,361**
Secondary MDS	**0,017**	**1,489**	**1,073**	**2,066**
Hypercellular BM	**0,027**	**1,215**	**1,023**	**1,444**
Hb<10g/dL	**0,000**	**1,691**	**1,346**	**2,126**
Plts<100x10^9/L	**0,045**	**1,508**	**1,009**	**2,253**
PB Blast>2%	**0,000**	**2,823**	**1,874**	**4,254**
Ferritine level (>1000ng/mL)	0,46	1,138	0,807	1,605
BM Blast>5%	0,985	1,002	0,794	1,265
IPSS_Category	0,178	1,143	0,941	1,387
IPSSr_Category	0,26	1,09	0,938	1,267

Summary/Conclusion: Clinical and biological characteristics of LR-MDS patients without RS are necessary in order to improve our knowledge in this subset of patients. A better understanding could help us to personalize therapeutic options in this heterogeneous subset of patients.

PS1250

SEROTONIN RECEPTOR TYPE 1B CONSTITUTES A THERAPEUTIC TARGET FOR MDS AND CMML

A. Banus Mulet[1,2,*], A. Etxabe[1,2], J.M. Cornet Masana[3,4], M.C. Lara Castillo[1], M.A. Torrente Marchante[5], J. Esteve[1,4,5,6], R.M. Risueño[1]

[1]Institut de Recerca Contra la Leucèmia Josep Carreras, [2]Faculty of Pharmacy, Barcelona university, Barcelona, [3]Institut Germans Trias i Pujol, Badalona, [4]Faculty of Medicine, Barcelona university, [5]Department of Hematology, Hospital Clínic , [6]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

Background: Myelodysplastic syndromes (MDS) and chronic myelomonocytic leukemia (CMML) are chronic myeloid clonal neoplasms. To date, the only potentially curative therapy for these disorders remains allogeneic hematopoietic progenitor cell transplantation (HCT), although patient eligibility is limited due to high morbimortality associated with this procedure coupled with advanced age of most patients. Moreover, HCT is followed by a high relapse incidence. Thus, new therapeutic approaches are urgently

needed. Previously, dopamine receptors (DRs) and serotonin receptors type 1 (HTR1s) were identified as a cancer stem cell therapeutic target in acute myeloid leukemia (AML). Here we interrogated the function of DRs and HTR1s in MDS and CMML.

Aims: Our main goal was to determine the function of DRs and HTR1s in the survival of both MDS and CMML cells. By studying the expression of these receptors and evaluating the effect of specific antagonist at different levels. The expression of HTR1s and DRs was interrogated in MDS (n=72) and CMML (n=19) primary samples by flow cytometry using specific surface antibodies for DRs and HTRs simultaneously with the live-dead discriminator dye 7AAD and an anti-human CD45 antibody for blast identification. The cytotoxicity induced by specific antagonists was also assessed by flow cytometry. Clonogenic assays were performed in CMML primary samples cells after 18-hour treatment with antagonists followed by 14-day culture in complete Methocult medium.

Methods: The expression of HTR1s and DRs was interrogated in MDS (n=72) and CMML (n=19) primary samples by flow cytometry using specific surface antibodies for DRs and HTRs simultaneously with the live-dead discriminator dye 7AAD and an anti-human CD45 antibody for blast identification. The cytotoxicity induced by specific antagonists was also assessed by flow cytometry. Clonogenic assays were performed in CMML primary samples cells after 18-hour treatment with antagonists followed by 14-day culture in complete Methocult medium.

Results: HTR1s and DRs were differentially expressed in MDS and CMML (>3 and >4 fold of change, respectively) in patient bone marrow samples compared to healthy donors. Moreover, treatment with HTR1B antagonists significantly reduced cell viability in primary samples from both MDS and CMML. In addition, HTR1B antagonists showed a significant effect in clonogenicity capability of CMML cells. Moreover, HTR1B antagonist showed a synergistic cytotoxic effect with currently approved hypomethylating agents.

Summary/Conclusion: These results suggest that HTR1B might constitute a novel therapeutic target for MDS and CMML. Due to its druggability, the clinical development of new regimens based on HTR1B antagonists is promising.

PS1251

INTENSIVE CHEMOTHERAPY (IC) IS MORE EFFECTIVE THAN HYPOMETHYLATING AGENTS (HMA) FOR THE TREATMENT OF YOUNGER PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS) AND ELEVATED BONE MARROW BLASTS

P. Strati[1,*], G. Garcia-Manero[1], T. Kadia[1], G. Borthakur[1], M. Konopleva[1], N. Daver[1], C. DiNardo[1], N. Short[1], M. Yilmaz[1], K. Naqvi[1], S. Pierce[1], J. Cortes[1], H. Kantarjian[1], F. Ravandi[1]

[1]MD Anderson Cancer Center, Houston, United States

Background: Younger patients with high risk MDS are commonly treated with IC or enrolled in clinical trials investigating IC. The AZA-001 trial has shown a survival advantage for patients with high-risk MDS treated with HMA as compared to IC. However, this study was conducted mainly in an older population.

Aims: We retrospectively analyzed the characteristics and outcomes of patients with MDS or MDS/myeloproliferative neoplasms (MPN), younger than 60 years of age, with ≥ 10% bone marrow blasts who received frontline treatment with HMA or anthracycline-cytarabine-based IC at our institution between 10/1993 and 12/2017.

Methods: IC was defined as a regimen including both anthracyclines and > 1 g/m^2/daily of cytarabine. Response categories were assessed using the modified International Working Group (IWG) criteria. Response duration (RD) was defined as time from achievement of response until progression or relapse. Patients who did not progress or relapse were censored at the date of death or last follow-up.

Results: One-hundred and six patients were included in the study, 57 treated with HMA and 49 with IC. Patients treated with IC were significantly younger (45 vs 55 years, p=0.03), had a higher percentage of bone marrow blasts (15% vs 13%, p<0.001), and were less likely to have unfavorable cytogenetics (27% vs 46%, p=0.05), a gene mutation (31% vs 60%, p=0.003) or a diagnosis of MDS/MPN (2% vs 21%, p=0.003). Use of IC was associated with shorter time to response (1 months vs 2 months, p<0.001) and higher overall response rate (ORR)(82% vs 60%, p=0.02). On univariate analysis (UA) age younger than 50 (p=0.03) and use of IC (p=0.02) were associated with ORR, but on multivariate analysis (MVA) only use of IC maintained its association (OR 4.3, 95% CI 2-9.1; p<0.001). Forty-seven (96%) patients treated with IC and 56 (98%) treated with HMA discontinued treatment (p=0.60), after a median time of 4 and 5 months, respectively (p=0.74). Patients receiving HMA tended to discontinue treatment as a consequence of progression (58% vs 39%, p=0.07), whereas rates of discontinuation secondary to early death (4% vs 10%, p=0.25) or toxicity (2% vs 8%, p=0.18) were comparable; 31% of patients on HMA and 33% on IC proceeded to stem cell transplant (SCT). After a median follow-up of 15 months (range, 1-178 months), 38 (51%) out of 74 patients lost their response, and median response duration (RD) was 19 months (range, 1-166 months). Factors associated with longer RD on UA were lack of unfavorable cytogenetics (p=0.04), consolidation with SCT in first remission (p=0.009) and use of IC (26 months vs 11 months, p=0.03) (Figure 1); on MVA, use of IC maintained its association (HR 2.9, 95% CI 1.4-5.8, p=0.03). Eight (8%) patients transformed to acute myeloid leukemia during frontline treatment, 7 (12%) on HMA and 1 (2%) on IC (p=0.07). At most recent follow-up, 65 (61%) patients died, median overall survival was 21 months (range, 1-178 months) and was significantly longer for patients treated with IC (43 months vs 15 months; p=0.05) (Figure 1).

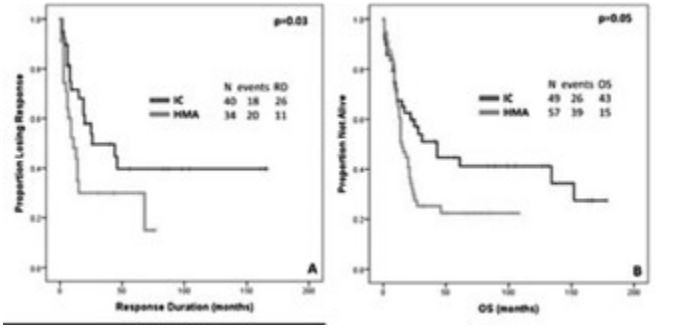


Figure 1.

Summary/Conclusion: IC is more effective than HMA for younger patients with MDS and bone marrow blasts ≥ 10%, irrespective of other baseline characteristics. Strategies combining targeted agents with either HMA or IC should be investigated, to determine whether this advantage will be maintained.

PS1252

FETAL HEMOGLOBIN INDUCTION DURING DECITABINE TREATMENT OF ELDERLY MDS/AML PATIENTS: A POTENTIAL DYNAMIC BIOMARKER FOR OUTCOME

J. Stomper[1,*], G. Ihorst[2], S. Suciu[3], P. Sander[1], H. Becker[1], P. Wijermans[4], E. Bissé[5], R. Claus[6], M. Lübbert[1]

[1]Department of Hematology, Oncology, and Stem-Cell Transplantation, [2]Clinical Trials Unit, Faculty of Medicine and Medical Center - University of Freiburg, Freiburg, Germany, [3]EORTC Headquarters, Brussels, Belgium, [4]Department of Hematology, Haga Hospital, The Hague, Netherlands, [5]Institute for Clinical Chemistry and Laboratory Medicine, Faculty of Medicine and Medical Center - University of Freiburg, Freiburg, [6]Department of Internal Medicine, Medical Center Augsburg, Augsburg, Germany

Background: Hematologic responses to DNA-hypomethylating agents (HMA) in MDS/AML are often delayed and not attained by all patients. Fetal hemoglobin (HbF) is among the potential novel outcome predictors for epigenetic treatments, being induced *in vitro* and *in vivo* by HMAs and HDAC inhibitors (Press *et al*., Leuk. Lymph. 2017); recently, we could demonstrate that HbF elevation already prior to decitabine (DAC) treatment was associated with superior outcome of MDS/AML patients (Lübbert *et al*., Br. J. Haematol. 2017).

Aims: We now asked whether early HbF induction during DAC treatment also had predictive value. Additionally, we sought to investigate the potential of DAC for *in vitro* induction of erythroid differentiation and HbF expression.

Methods: 16 MDS and 37 AML patients enrolled on two clinical trials, the 06011 EORTC-GMDSSG phase III trial of higher-risk MDS, and the 00331 phase II trial of older, non-fit AML patients, received DAC (15 mg/m^2 TID×3 days / 42 day cycle). After 4 courses, AML patients attaining CR, PR, or an antileukemic effect continued with DAC maintenance treatment at 20 mg/m^2. HbF levels were measured in peripheral blood erythrocytes by HPLC before treatment and sequentially, i.e. after the end of each treatment course. HbF > 1.0% was considered induced. *In vitro* effects of DAC were investigated in two myeloid leukemia cell line models with bilineage differentiation potential (K562, HEL).

Results: Compared to the median pre-treatment HbF of 0.8%, we observed overall induction to 1.1% after course 2 (the first on-treatment time point

with robust HbF induction), and, in patients achieving CR or PR as best overall response, a median HbF of 1.9% at this time point compared to 0.8% in patients not attaining CR/PR (p=0.015). Correlation analyses revealed an association between% HbF after DAC course 2 and responses of the different hematopoietic lineages after course 4. For platelet counts, Spearman's correlation coefficient r_s was 0.49 (p=0.01); for neutrophils, r_s=0.35 (p=0.08); for Hb, r_s=0.36 (p=0.08); for% bone marrow blasts, r_s=-0.48 (p=0.03). In addition, HbF induction after 2 courses was associated with early platelet-doubling (p=0.006). In MDS patients, induced HbF after course 2 was associated with longer median overall survival compared to non-induced HbF: 22.9 vs. 7.3 months (hazard ratio [HR] 0.21 [95% CI 0.05 – 0.87], p=0.03), progression-free survival was 7.7 vs. 2.4 months (HR 0.32 [95% CI 0.10 – 1.10], p=0.07), AML-free survival was 13.1 vs. 7.6 months (HR 0.42 [95% CI 0.13 – 1.38], p=0.15). For AML patients, median OS from end of course 2 was 20.0 vs. 10.4 months (HR 0.61 [95% CI 0.21 – 1.75], p=0.35), PFS was not determined. Linear regression analyses, in conjunction with FISH analyses, strongly suggested that HbF induction observed after 4 treatment courses occurred preferentially in the emerging non-clonal erythroid cells. *In vitro* DAC treatment of K562 and HEL cells resulted in induction of an erythroid (but not megakaryocytic) differentiation program, of gamma globin expression, and of HbF tetramer formation.

Summary/Conclusion: HbF induction during DAC treatment was successfully modelled in myeloid cell lines, was associated with early platelet doubling, was predictive for subsequent objective responses and, in MDS patients, for longer survival. Thus HbF may provide a dynamic biomarker during HMA therapy of MDS/AML. This is addressed prospectively within the EORTC trial AML21 ("inDACtion vs. induction", NCT0217287).

PS1253

CLINICAL IMPACT ON THE THERAPEUTIC STRATEGY ADAPTED TO THE MUTATIONAL PROFILE IN PATIENTS WITH MYELODYSPLASTIC SYNDROME ASSOCIATED WITH DEL(5Q)

C. Alarcón-Payer[1,*], F. Hernández Mohedo[2], A. Jiménez Morales[1], M. Bernal Sánchez[3], Z. Mesa Morales[2], J. Badiola González[2], F. Ruiz Cabello[3], A. Romero Aguilar[2], M. Jurado Chacón[2]

[1]Servicio de Farmacia, Hospital Universitario Virgen de las Nieves, [2]UGC Intercentro Hematología y Hemoterapia, Hospitales HUVN-PTS, [3]Servicio de Análisis Clínicos e Inmunología, Hospital Universitario Virgen de las Nieves, Granada, Spain

Background: According to OMS-2016, myelodysplastic syndrome (MDS) associated with del(5q) is manifested by a transfusion-dependent progressive bone marrow failure, with Lenalidomide acting as the intended drug to treat this syndrome. Recent attention has received the mutational profile analysis using massive parallel sequencing also called as new generation sequencing (NGS) to predict lenalidomide response in function of the presence or absence of a high-risk mutational profile.

Aims: To analyze the clinical impact of the directed risk-stratification therapy and to evaluate the clinical benefit associated to the discontinuation of the Lenalidomide treatment due to side effects or intolerance.

Methods: Three-year prospective observational study on 69 cases of MDS, 17 of them with del(5q). Mutational profile analysis using a NGS prior to Lenalidomide treatment decision-making, with TP53 mutation as ultra-high risk profile for discouraging its use. Treatment discontinuation was studied in those candidates with side effects or intolerance. The variables considered in this study were: beginning of treatment, Lenalidomide mean dose, ending of treatment and beginning of discontinuation, side effects, time after discontinuation, evaluation of the drug withdrawal response according to IWG-MDS (Chenson *et al.* 2006), and cost savings.

Results: 69 MDS cases were analyzed by NGS (58% male and 42% female). According to the myeloid panel, the mutational profile was classified as: high-risk (3,6%), low-risk (12,7%), intermediate risk (10,9%), very high-risk (4,2%) and very low-risk (24,6%). 17 cases were detected as MDS associated to del(5q) (24,6%) and 17,6% of them showed positive TP53 mutation and were treated with hypomethylating agents instead of Lenalidomide meanwhile 11,7% of them showed DNMT3A, ASXL1, SF3B1 and TET2 mutations. 64,7% of the cases were treated with Lenalidomide, the treatment were discontinued in 54,5% of them due to side effects and the dose reduced in 18% due to intolerance. The reported side effects were: Grade 4 neutropenia, rhabdomyolysis, erythematous reactions and haemolytic crisis. 100% of patients in which Lenalidomide was discontinued due to side effects, maintained complete haematological and cytogenetic response, reaching a mean monitoring time of 12 months since the withdrawal of Lenalidomide. The cost saving associated to the discontinuation of Lenalidomide 10mg was 48.000 euros per patient per year.

Summary/Conclusion: The use of NGS allows selecting the mutational profile of each patient, resulting in a change in the therapeutic decision-making, the selection of more cost-effective drugs and a directed and personalized treatment. Discontinuation of Lenalidomide, due to side effects or intolerance, involves a clinical benefit to those patients who maintain a complete haematological response after interruption of the treatment.

PS1254

CHRONIC MYELOMONOCYTIC LEUKEMIA TREATED WITH 5-AZACYTIDINE. RESULTS FROM THE HELLENIC 5-AZACYTIDINE REGISTRY

P. Diamantopoulos[1,*], I. Kotsianidis[2], A. Symeonidis[3], V. Pappa[4], A. Galanopoulos[5], D. Gogos[6], S. Karakatsanis[7], E. Papadaki[8], A. Palla[9], E. Hatzimichael[10], M. Dimou[1], S. Papageorgiou[4], S. Delibasi[11], M. Papaioannou[12], M. Papoutselis[13], K. Tsirou[14], A. Kourakli[3], D. Tsokanas[5], A. Anagnostopoulos[14], P. Panayiotidis[1], N.-A. Viniou[1]

[1]Laikon General Hospital, National And Kapodistrian University of Athens, Athens, [2]University Hospital of Alexandroupolis, Alexandroupoli, [3]University Hospital of Patras, Rio, [4]Attikon General Hospital, National and Kapodistrian University of Athens, [5]G. Gennimatas District General Hospital, Athens, [6]Vostaneio General Hospital, Mytilene, [7]Sotiria General Hospital of Thoracic Diseases, Athens, [8]University of Crete, Herakleion, [9]St. George General Hospital, Chania, [10]University of Ioannina, Ioannina, [11]Evangelismos Hospital, Athens, [12]University General Hospital of Thessaloniki AHEPA, Thessaloniki, [13]Democritus University of Thrace, Alexandroupoli, [14]Papanikolaou General Hospital, Thessaloniki, Greece

Background: Chronic myelomonocytic leukemia (CMML) has been classified as a myelodysplastic syndrome/myeloproliferative neoplasm since 2002. Treating CMML remains challenging, partly due to the lack of large CMML-specific clinical trials. Supportive treatment is usually offered to lower-risk patients while hypomethylating agents (HMA) have been approved for higher-risk patients, but their effectiveness and safety have not been thoroughly studied. Approval studies for the use of HMAs in MDS included only a few patients with CMML, and very few and small-sized phase I/II CMML-specific studies with HMAs are available, showing overall response rates varying from 35% to 69% and overall survival (OS) from 12 to 37 months. Moreover, the literature lacks real-world data on the use of HMAs in patients with CMML.

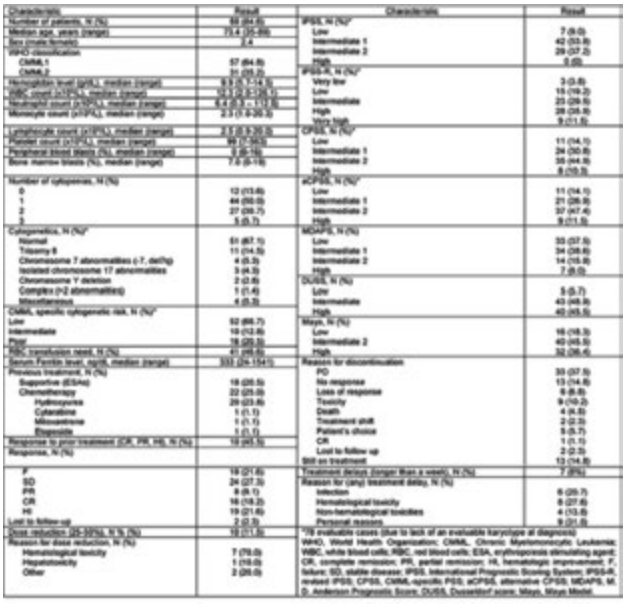
Aims: To provide real-world data on treatment response and survival in a cohort of patients with CMML treated with 5-azacytidine (5AZA) and to evaluate the implementation of the currently available risk stratification systems to our population.

Methods: We retrospectively recorded the characteristics of patients with CMML treated with 5AZA through the Hellenic 5-azacytidine registry. The International Prognostic Scoring System (IPSS), the revised IPSS (IPSS-R), the CMML-specific PSS (CPSS), the alternative CPSS (aCPSS), the M. D. Anderson Prognostic Score (MDAPS), the Dusseldorf score for CMML (DUSS), and the Mayo Model were used for risk stratification. 5AZA was administered as monotherapy subcutaneously at 75 mg/m^2/day for 7 days every 28 days. Responses were assessed per the modified 2006 International Working Group response criteria for MDS. Complete remission (CR), partial remission (PR), and hematological improvement (HI) were defined as response and stable disease (SD) or failure (F) as no response. IBM SPSS statistics, v23.0 (IBM Corporation, N. Castle, NY, USA) was used for statistical analysis.

Results: The baseline demographic and hematologic characteristics of 88 patients with CMML are listed in Table 1. Sixty-six (75%) patients were treatment-naïve, the rest being previously treated mostly with hydroxyurea with a variable response. The median number of 5AZA cycles was 8 (1-40), and most patients experienced no treatment delays. Infections, hematological and non-hematological toxicity were the major causes of treatment delays and dose reductions. Response to 5AZA is shown in Table 1. The overall response rate was 48.9%. The median duration of response was 12.1 months (95% CI, 9.0-15.2). Response did not differ between patients with CMML1 and CMML2 (X^2, p=0.82).

The median OS was 29.7 months and the median survival after 5AZA failure was 4.5 months. CMML2, anemia (<10 g/dL), circulating blasts, high serum ferritin level and thrombocytopenia were correlated to lower OS, but circulating blasts and ferritin level were the only factors retaining their statistical significance in multivariate analysis (HR, 3.47, p=0.014 and 2.84, p=0.022 respectively). All risk stratification systems could predict OS, but DUSS and aCPSS were better implemented to our group of patients.

Table 1. Patient characteristics and results.



Summary/Conclusion: Patients with CMML are underrepresented in large clinical trials on HMAs in MDS. Several CMML-specific prognostic models have been developed but none has been universally adopted. Our study supports the use of 5AZA in patients with CMML providing real-world data in the largest – to our knowledge – cohort of patients homogeneously treated with 5AZA, since response rates were rather high (48.9%).

PS1255

OUTCOME OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND STABLE DISEASE DURING TREATMENT WITH AZACITIDINE: A REPORT FROM GRUPPO ROMANO MIELODISPLASIE (GROM) AND RETE EMATOLOGICA LOMBARDA (REL)

L. Maurillo[1,*], M. Riva[2], P. Niscola[3], P. Anna Maria[4], F. Buccisano[5], F. Luana[6], L. Borin[7], M. Criscuolo[6], G. Reda[8], M. Breccia[9], M. Turrini[10], S. Gumenyuk[11], P. Ferrando[12], C. Tatarelli[13], M. Carabba[14], A. Astasia[15], D. Lambertenghi[16], A. Molteni[17], M.T. Voso[1]

[1]Ematologia, Dipartimento Biomedicina e Prevenzione, Policlinico Tor Vergata, Roma, [2]ASST Ematologia, Grande Ospedale Metropolitano Niguarda, Milano, [3]Ematologia, Ospedale S. Eugenio, Roma, [4]Ematologia, ASST degli Spedali Civili di Brescia, Brescia, [5]Ematologia, Dipartimento Biomedicina e Prevenzione, Università Tor Vergata, [6]Ematologia, Università Cattolica Sacro Cuore, Roma, [7]Ematologia, ASST Monza, Monza, [8]Ematologia, IRCCS Ospedale Maggiore Policlinico, Milano, [9]Ematologia, Università La Sapienza, Roma, [10]Ematologia, Ospedale Valduce , Como, [11]Ematologia e unità Trapianto, Istituto Nazionale Tumori Regina Elena IRCCS, Roma, [12]Ematologia, ASST Lecco, Lecco, [13]Ematologia, Ospedale S. Andrea, Università La Sapienza, Roma, [14]Ematologia, Ospedale San Raffaele, Milano, [15]Ematologia, ASST Sette Laghi Varese, Varese, [16]Ematologia, ASST Santi Paolo e Carlo, Milano, [17]Ematologia, ASST Cremona, Cremona, Italy

Background: Treatment with azacitidine (AZA) in MDS may improve overall survival (OS) even in patients who do not necessarily achieve complete or partial remission (CR or PR), raising the question whether bone marrow (BM) response should be considered the only therapeutic goal. In this regard, it is therefore not clear whether patients who achieve stable disease (SD) after 4-6 cycles of therapy should stop or continue the treatment.
Aims: We retrospectively evaluated the outcome of 100 high risk MDS patients with SD according to International Working Group (IWG) 2006 criteria, after 4 cycles of therapy.
Methods: Patients from the two Italian regional networks GROM and REL were included. Patients were diagnosed with MDS according to 2008 WHO criteria: 65% had Refractory anemia with excess blasts-2 (RAEB-2), 21% Refractory anemia with excess blasts-1 (RAEB-1), 9% Refractory cytopenia with multilineage dysplasia (RCMD), 2% Refractory anemia with ring sideroblasts (RARS), 3% Refractory cytopenia with unilineage dysplasia (RCUD). Median age was 70 years (range 33-85), 59% were male. Median follow up was 36 months (range 6-217) and 40% of patients progressed in acute myeloid leukemia. Karyotype was evaluable in 97 patients and, according to the revised International Prognostic Scoring System (IPSS-R) cytogenetic scoring system, 58% had good risk, 16% intermediate-risk, 12.5% poor risk and 13.5% very poor risk karyotype, corresponding to higher-risk disease by IPSS-R (9.5% intermediate, 51.5% high, and 39% very high risk). AZA was administered at the conventional dose of 75 mg/m^2 daily for 7 days per month. The median number of cycles delivered was 11 (range 4-78). After 4 cycles of therapy, 16 patients did not continue treatment for medical decision or patient's wishes.
Results: Thirty-one (37%) out of 84 patients who continued AZA treatment, improved response (IR) after a median number of 8 cycles (range 5-13). IR were: 6 CR, 1 marrow CR (mCR), 8 PR and 16 hematological improvement (HI). IR rate was significantly higher in patients who continued treatment after 4 cycles and received 10 or more cycles of therapy (p=0.002 and 0.001, respectively). Evolution in AML was significantly more frequent in patients in SD compared with those who obtained IR (82.5% vs 17.5% respectively, p=0.02). Median OS was 19.7 months. In univariate analysis, patients who received more than 10 cycles of AZA, those who went on treatment with AZA after 4 cycles and those who achieved IR had significantly longer OS duration (p=0.00002 Figure 1, 0.02 and p=0.005, respectively). In multivariate analysis 10 or more cycles of AZA and IR were significantly associated with prolonged OS (p<0.0001 and 0.05, respectively).

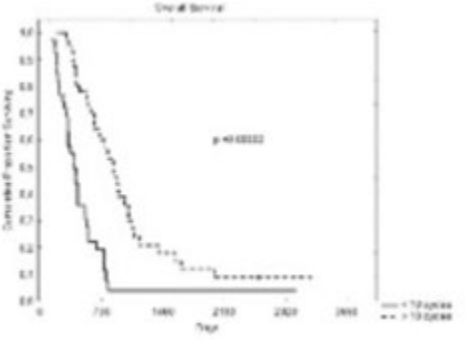


Figure 1.

Summary/Conclusion: In conclusion: 1) a substantial fraction of patients (37%) classified as SD after 4 cycles of AZA will improve response with additional treatment; 2) patients keeping on treatment with AZA (≥ 10 cycles) and patients who achieve a IR had a longer survival. Our analysis suggests that, in the absence of therapeutic alternatives, patients in SD should continue treatment with AZA until disease progression or unacceptable toxicity.

PS1256

PREDICTIVE FACTORS OF OVERALL SURVIVAL AND TREATMENT RESPONSE IN HIGH-RISK OLDER PATIENTS WITH MDS AND CMML UNDER HYPOMETHILATING AGENTS. LATIN-AMERICAN MDS GROUP - GLAM

M. Iastrebner[1,*], C. Lazzarino[2], S. Grillé[3], M. Di Stefano[4], A. Enrico[5], G. Flores[6], I. Fernandez[7], G. Alfonso[8], M. Pimentel[9], E. Nucifora[10], R. Ovilla[11], J. Ramírez[12], R. Tavares[13], M. Sarmiento[14], S. Araujo Schuster[15], V. Abello Polo[16], J.C. serrano[17], F. Huaman Garaicoa[18], C. Wellington[19], I. Reyes[20], C. Belli[21]

[1]Hematology, Sanatorio Sagrado Corazón, Ciudad de Buenos Aires, [2]Hematology, Hospital Paroissien, Buenos Aires, Argentina, [3]Hematology, Hospital de Clinicas, Montevideo, Uruguay, [4]Hematología, Hospital Domingo Luciani IVSS, Caracas, Venezuela, Bolivarian Republic Of, [5]Hematology, Hospital Italiano La Plata, La Plata, [6]Hematology, Hospital Durand, Buenos Aires, [7]Hematology, Fundaleu, Ciudad de Buenos Aires, [8]Hematology, Hospital Posadas, Haedo Pcia Buenos Aires, Argentina, [9]Hematology, INCART, Santo Domingo, Dominican Republic, [10]Hematology, Hospital Italiano de Buenos Aires, Ciudad de Buenos Aires, Argentina, [11]Hematology, Hospital Angeles Lomas, México, México, Mexico, [12]Hematology, IESS. HTMC, Guayaquil, Ecuador, [13]Hematology, HC-UFG, Goiania, Brazil, [14]Hematology, IIHEMA,Academia Nacional de Medicina, Ciudad de Buenos Aires, Argentina, [15]Hematology, HC-UFMG, Belo Horizonte, Brazil, [16]Hematology, Hospital san José, Bogotá, [17]Hematology, Unidad Hematología Especializada, Cucuta, Colombia, [18]Pathology, Instituto Oncológico Nacional SOLCA, [19]Hematology Department, HTMC, [20]Hematology Department, Clínica Kennedy, Guayaquil, Ecuador, [21]Genetic Laboratory, IIHEMA, Academia Nacional de Medicina, Ciudad de Buenos Aires, Argentina

Background: Older patients with a severe disease are always a dilemma for physicians. A more detailed screening of candidates for hypomethylating

treatment by using predictive factors may maximize clinical success and minimize the exposure of inappropriate patients to excessive toxicity.
Aims: To evaluate overall survival and clinical predictive factors in an older high risk (HR) MDS and CMML cohort under HMA treatment as well as identify patients more likely to reach response in the real world.
Methods: This retrospective and multinational study was focused on older patients (>65 years old) with HR (IPSS-R>3.5 or CPSS>1). The population was selected from a Latin-American Ad-Hoc database of 340 patients who received hypomethylating agents (HMAs) between Jan-2007 and Jan-2018. OS was evaluated as from HMA initiation, either censoring or not, other treatments such as chemotherapy, other HMA or HSCT, up to time to death or last follow-up. Kaplan Meier, Log-rank test and Cox-regression were applied to evaluate survival. Chi2/Fisher Exact test and logistic regression were used to analyze categorical variables regarding response. Predictive factors included age, gender, comorbidities by Charlson Comorbidity Index (CCI), physical performance (ECOG), transfusion dependence, different cut-offs for hemoglobin level, platelet and neutrophil counts, BM and PB blasts, and karyotype.
Results: We evaluated 138 patients (MDS 92 [67%], CMML 32 [23%], sMDS 8 [6%], and oligoblastic AML 6 [4%]) with median follow-up of 12.4 months and median number of HMA cycles of 7 (range 1-37). Median age was 75 (range 66-89) with a male predominance of 57%, 34% CCI≥3, 23% ECOG ≥2 and 66% were transfusion dependent before treatment. Predictive factors of shorter OS at treatment initiation included CCI≥3 (p=0.041), HR karyotypes (p=0.002), Hb gender-adjusted (M<9/F<8 g/dL, p=0.009), platelet count ≤30,000/µL (p=0.010) with a borderline significant of transfusion dependency (p=0.076) with similar results to last follow-up. After Cox's regression analyses, high risk karyotypes (HR3.0, 95%IC 1.6-5.7, p<0.001) sustained its impact with a statistical tendency of Hb gender-adjusted (HR1.6, 95%IC 0.9-2.8, p=0.081) and platelet count <30,000/µL (HR1.7, 95%IC 1.0-2.9, p=0.067). When patients were evaluated to last follow-up, previous transfusion dependency (HR2.1, p=0.009) and blast in PB (HR1.8, p=0.026) were also relevant. A total of 119 patients were evaluated for response to treatment with an overall response rate of 61% (CR/mCR/PR: 34%, HI: 15%, SD: 12%) during a median follow-up period of 12.8 months. Non-responders, 22 (47%) received an optimum treatment (>4=DAC or >6=AZA cycles), showed a dismal outcome with a median OS of 7.6 m (vs responders: 25.9 months, p<0.001). Not achieving response was related to ECOG≥2 (34% vs17%, p=0.026), Hb level <10g/dL (87% vs 69% p=0.020), platelets count <30000/µL (47% vs 27%, p=0.021), presence of PB blast (35% vs16%, p=0.029), with a statistical tendency for CCI≥3 (40% vs 25%, p=0.084) and other Hb cut-offs. The logistic regression analyses confirmed the importance of ECOG≥2 (OR 4.3, p=0.009), platelets count <30,000/µL (OR2.6, p=0.049) and presence of PB blast (OR3.4, p=0.031) as the main parameter associated with treatment failure.
Summary/Conclusion: In our cohort, short OS was mainly influenced by high-risk karyotype and, failure to achieve response was significantly associated with a PS≥2, platelets count <30,000/µL and presence of PB blast. These results identified predictive factors that might be useful to tailor treatment.

PS1257

IRON SUPPORT ENHANCES ERYTHROPOIETIN EFFECTIVENESS IN REFRACTORY ANEMIA WITH HIGH RETICULOCYTE COUNT, TRANSFERRIN SATURATION BELOW 30% AND HIGH LEVEL OF ERYPTOSIS

G. Giordano[1,*], A. Parente[2], D. Berardi[3], D. Castaldi[2], M.A. Cinotti[2], F. Vedruccio[2], V. Susca[4], L. Petrella[4], G. Berardi[5], M. Magri[6], G. Niro[6], R. Gigli[6]

[1]medicine, Regional Hospital "A. Cardarelli", [2]medicine, general medicine, campobasso, [3]medicine, university la sapienza, rome, [4]medicine, university of molise, [5]family medicine, general medicine, [6]laboratory medicine, Regional Hospital "A. Cardarelli", Campobasso, Italy

Background: Eryptosis (erythrocyte apoptosis) is an enhanced phenomenon in myelodysplastic syndromes(MDS) positively related to reticulocyte count and triggered by iron deficiency. Iron support is not recommended in MDS,also at low risk, in which iron chelation is a pivot of treatment
Aims: Aim of this study is to see if iron support enhances erythropoietin effectiveness in refractory anemia with high reticulocyte count, transferrin saturation below 30%, regardless of ferritin level
Methods: Between july 2015 and december 2018, 40 patients affected by refractory anemia with IPSS low-risk were studied. Median follow-up was 16 months (R12-28). Patients were randomized 1:1 to receive in group A (with corrected reticulocyte count <2.5%, transferrin saturation >30%) sucrosomial iron 30 mg 2 tablets orally/day +erythropoietin 40000 IU sc/t.i.w + calcium levofolinate 7.5 mg/day orally + Vitamin B12: 400 mg/day orally. In group B (with corrected reticulocyte count >2.5%, transferrin saturation <30%)patient received sucrosomial iron 30 mg 2 tablets orally/day + alpha erythropoietin 40000 IU sc/t.i.w. + calcium levofolinate 7.5 mg/day orally + Vitamin B12: 400 mg/day orally. In group A median age was 75 years (R70-81), M/F: 8/12. In group B median age was 72 years (R68-77), M/F: 10/10. Caryotype was normal in group A and B patients. Median level of haemoglobin was 9.5 g/dl in group A (R9-10) and 8.9 g/dl (R8.5-10) in group B. Median ferritin level was 900 ng/ml in group A (R890-1100) and 800 ng/ml g/dl (R750-950) in group B. Eryptosis was measured with cytosolic Ca2+, utilizing Fluo3 fluorescence, after cells staining with the Fluo-3 AM dye (Biotium, Hayward, USA). Levels of eryptosis at start of treatment were higher in group B.
Results: Group A patients increased Hb level of 1 g/dl after a median time of 6weeks (R4-7), 11 g/dl after 3 month. Group B patients increased Hb level of 1 g/dl after a median time of 4weeks (R3-5), 12g/dl after 3 month. Median ferritin level was 1100 ng/ml in group A (R950-1300) and 850 ng/ml (R800-900) in group B. After treatment levels of eryptosis were higher in group A.
Summary/Conclusion: Iron support enhances erythropoietin effectiveness in refractory anemia with high reticulocyte count, transferrin saturation below 30% probably reducing level of eryptosis involved in ineffective erythropoiesis.

PS1258

Abstract withdrawn.

PS1259

SEVERE THROMBOCYTOPENIA AS A PREDICTOR OF SURVIVAL AND RESPONSE TO HYPOMETHYLATING AGENTS: DATA FROM A LATIN-AMERICAN COHORT

C. Lazzarino[1,*], M. Iastrebner[2], J. Gonzalez[3], J. Arbelbide[4], A. Basquiera[4], R. Crisp[5], D. Espinosa[6], L. Kornblihtt[7], E. Macchiavello[8], R. Peressin[9], N. Pintos[10], R. Bengió[11], M. Rivas[12], M. Rosenhain[13], B. Gusmao[14], V. Prates[15], C. Belli[11]

[1]HIGA Dr Diego Paroissien, [2]Sanatorio Sagrado Corazón, [3]Hospital Durand, [4]Hospital Italiano , [5]Hospital Nacional Posadas, Buenos Aires, Argentina, [6]Hospital San José, Bogotá, Colombia, [7]Hospital de Clínicas, Buenos Aires, [8]Clínica La Pequeña Familia, Junín, [9]HR Dr Ramón Carrillo , Santiago del Estero, [10]Sanatorio Municipal Julio Méndez, [11]IIHEMA/Academia Nacional de Medicina, [12]Hospital Universitario Austral, [13]Hospital Tornú, Buenos Aires, Argentina, [14]Hospital Einstein, Sao Paulo, Brazil, [15]Hospital Italiano de La Plata, La Plata, Argentina

Background: Hypomethylating agents (HMA) are the first line option for high-risk patient and lower-risk patient with transfusion dependence, or, during the follow-up according to worsening clinical features. Thrombocytopenia is a frequent finding in myelodysplastic syndromes (MDS) during the course of the disease and, recently, severe thrombocytopenia (<30,000/µL) has been proposed as an independent adverse prognostic factor for survival.
Aims: To examine the influence of severe thrombocytopenia <30,000/µL in the outcome and response to HMA therapy in a MDS cohort from Latin-America (LA).
Methods: We identified 215 eligible MDS patients (excluding CMML, secondary MDS and AML) from a multicentric retrospective LA database of 340 patients who were treated between January-07/January-18. Statistical analysis included Kaplan-Meier survival analysis, Cox proportional hazard and Logistic regression.
Results: The median age of patients was 71 years (range 20-89) being 74.9% >60 years old and 54% were males. At treatment initiation, 79.0% showed hemoglobin <10g/dL, 32.7% platelet counts <30,000/µL, 14.7% poor karyotypes and 67.4% at IPSS-R>3.5. Among the low-risk group, 75.7% patients were resistant to erythropoietin and a remaining 8.6% were transfusion dependent. Regarding HMA therapy, 92.1% patients received AZA and 7.9% DAC, with a median number of cycles of 7 (1-58) during a median period of 8.1 months (m). The median overall survival (OS) of the cohort was 21.9m, after cessation 6.1m and since last treatment 4.0m. The rate of AML transformation was 29.8% and 52.6% died. In this sub-analysis, we investigate the prognostic parameters that were useful to predict outcome from treatment to last follow up. Cox regression analysis revealed that male sex (HR 2.0, 95% CI 1.3-3.1, p=0.002), poor karyotypes (HR 1.7, 95% CI

1.0-2.9, p=0.044), lower hemoglobin level (ref. ≥10g/dL, <10g/dL-≥8g/dL, HR 2.6, 95% CI 1.4-5.1, p=0.003; <8g/dL, HR 2.4, 95% CI 1.3-4.6, p=0.006), platelet count <30.000/µL (HR 3.2, 95% CI 2.1-5.0, p<0.001) and older than 60 years (HR 1.8, 95% CI 1.1-3.0, p=0.022) were independently associated with a reduced OS. All this parameters sustained their independency when other treatments were censored (chemotherapy, other HMA or HSCT). A total of 176 patients were evaluated for response to treatment with an overall response rate of 67% (CR/mCR/PR: 30.7%, HI: 20.4%, SD: 15.7%) evaluated at optimum treatment (6-AZA/4-DAC). The median survival in responders was 30.0 months compared with 7.9 months in non responders (p<0.001), with a median follow-up period of 12.4 months. The outcome of patients with SD was similar to those with CR/mCR/HI (p=0.760). Among those parameters that were predictive for survival, failure to achieve response (Logistic regression) was associated with lower hemoglobin level (ref.≥10g/dL, <10g/dL-≥8g/dL, OR 5.6, 95% CI 1.5-21.1, p=0.011; <8 g/dL OR 5.9 95% CI 1.6-21.9, p=0.008) and platelet count <30,000/µL (OR 2.9, 95% CI 1.4-6.0, p=0.005).

Moreover, a severe thrombocytopenia <30,000/µL adversely impacts on the OS of responders (15.1 months vs 39.0 months, HR 3.8, 95% CI 2.1-6.7, p<0.001, Figure 1).

Summary/Conclusion: Our results highlight the adverse impact of the severe thrombocytopenia <30,000/µL on the outcome of patients under HMA influencing the response rate and the survival, even in responders.

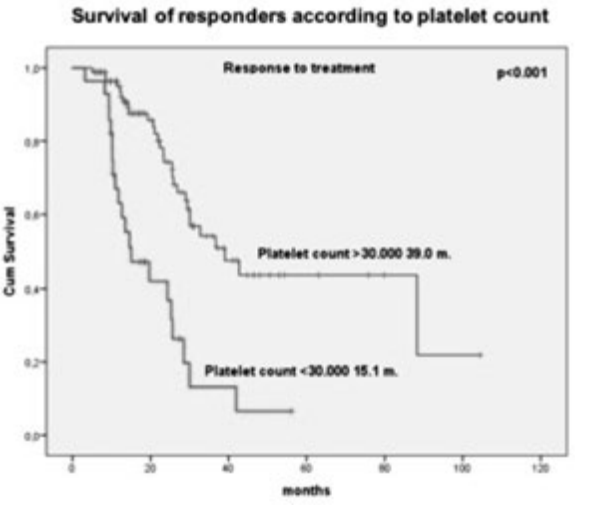


Figure 1.

Myeloma and other monoclonal gammopathies – Biology & Translational Research

PS1260

TLR4 SIGNALING DRIVES MESENCHYMAL STEM CELLS (MSC) COMMITMENT TO PROMOTE TUMOR MICROENVIRONMENT TRANSFORMATION IN MULTIPLE MYELOMA

C. Giallongo[1,*], D. Tibullo[1], G. Camiolo[1], N. L. Parrinello[2], P. La Cava[2], A. Romano[2], F. Puglisi[2], E. Martino[2], G. Sapienza[2], C. Conticello[2], G.A. Palumbo[2], F. Di Raimondo[2]

[1]Division of Hematology, [2]University of Catania, Catania, Italy

Background: MSC, through a complex crosstalk with neighboring cells/factors, can inhibit many effector functions of immune cells, thereby promoting an immunosuppressive state in the tumor microenvironment that allows tumor cells to overcome the immune surveillance.
Aims: Since it has been demonstrated a connection between the stimulation of specific Toll-like receptors (TLR) and MSC activation status, including two distinct phenotypes defined MSC1 (TLR4-dependent) or MSC2 (TLR3-dependent), we hypothesized that MSC derived from Multple Myeloma patients (MM-MSC) could be activated to better 'serve' the cancer cells.
Methods: Healthy Peripheral blood mononucleated cells (PBMC) were cultured with healthy controls (HC-), MGUS-, SMM- or MM-MSC. After 6 days, educated neutrophils (ed-N) were isolated using magnetic microbeads. Immunocompetent adult Zebrafish was used as *in vivo* model for myeloma cells engraftment.
Results: Only SMM- and MM-MSC induced a neutrophils N2 phenotype able to promote suppressive effects on T cell proliferation ($p<0.001$) and to exert a pro-angiogenic activity ($p<0.05$). No N2 activation was observed in neutrophils co-cultured with HC- or MGUS-MSC, demonstrating that tumor-associated MSC were functionally different from HC-MSC. By adding Bortezomib, Lenalidomide or Pomalidomide during co-culture, ed-N lost the pro-angiogenic activity but not the immunosuppressive ability.Using specific agonists for TLR4 (LPS) or TLR3 (poly(I:C)), we observed that healthy MSC acquired the same immunological alteration of SMM- and MM-MSC after a pre-treatment with LPS. Moreover, wester blotting analysis confirmed the activation of TLR4/MyD88 pathway in MM-MSC but not in HC-MSC.To examine if myeloma plasma cells (PC) play a role in MSC polarization, before performing co-cultures with PBMC, we pre-treated HC-MSC with MM cell lines. Plasmacell pre-treatment drove healthy MSC to activate neutrophils in immunosuppressive and pro-angiogenic cells. Therefore, we investigated if PC activated TLR4 pathway in healthy MSC and we found that co-culture with PC induced IRF3 nuclear translocation, indicating the involvement of a TLR4-MyD88-independent pathway in MSC commitment. Next, we explored the effects of the "activated" status of MM-MSC investigating their pro-tumor role *in vivo*. Six days after implanting a mixtures of fluorescently labeled MM cells plus HC- or MM-MSC, zebrafish co-injected with PC and MM-MSC showed enhanced tumor colonization and growth (calculated as tumor volume and fluorescence intensity) compared with animals injected with PC and HC-MSC (control) ($p<0.05$). Flow cytometry detection of hCD138+ cells confirmed less MM cells in zebrafish injected with PC and HC-MSC ($p<0.001$). Therefore, we analyzed the expression of the master regulator transcription factors for Th1/Th2 (tbx21 and gata3) and Th1- and Th2-type cytokines to better assess *in vivo* the involvement of the immune escape mechanisms promoted by co-injection of PC with MM-MSC. As compared to control animals, gata3, IL-4 and IL-13 were significantly up-regulated in zebrafish injected with PC plus MM-MSC, revealing that MM-MSC and PC mixture promoted a Th2 responce. To investigate TLR4 role, we used TAK-242 to inhibit the signaling in MM-MSC before injection in zebrafish. Animals co-injected with PC and MM-MSC pre-treated with TAK-242 showed 48% less tumor engraftment compared to zebrafish injected with PC and MM-MSC.
Summary/Conclusion: TLR4 signaling plays a pivotal role in MSC commitment towards an inflammatory phenotype which is associated with a tumor permissive microenvironment.

PS1261

WHOLE EXOME SEQUENCING OF SYSTEMIC LIGHT CHAIN (AL) AMYLOIDOSIS

I. Cuenca[1,*], B. Sanchez-Vega[1], B. Paiva[2], D. Gomez-Sanchez[1], S. Barrio[1], D. Alignani[2], M. Lasa[2], N. Puig[3], J.J. Perez[3], R. Lecumberri[4], F. Prosper[4], E. Ocio[3], M. E. Gonzalez[5], A. Garcia de Coca[6], J. de la Rubia[7], M. Gironella[8], A. Perez[9], L. Palomera[10], A. Oriol[11], M. Casanova[12], J. E. de la Puerta[13], R. Payer[14], F. de Arriba[15], J. Alonso[16], F. Taboada[14], V. Cabañas[17], J.-J. Lahuerta[1], M.-V. Mateos[3], J. San Miguel[2], J. Martinez-Lopez[1]

[1]Hospital Universitario [12] de Octubre, Madrid, [2]Centro de Investigación Médica Aplicada, Pamplona, [3]Hospital Universitario de Salamanca, Instituto de investigación biomédica de Salamanca (IBSAL), Centro de investigación del cáncer (IBMCC CSIC-USAL), Salamanca, [4]Clínica Universidad de Navarra, Pamplona, [5]Hospital de Cabueñes, Gijón, [6]Hospital Clínico Universitario de Valladolid, Valladolid, [7]Hospital Universitario y Politécnico La Fe, Valencia, [8]Hospital Universitari Vall d'Hebron, Barcelona, [9]Hospital Universitario Son Espases, Palma, [10]Hospital Clínico Universitario Lozano Blesa, Zaragoza, [11]Hospital German Trias i Pujol, Badalona, [12]Hospital Público Costa del Sol, Marbella, [13]Hospital de Galdakao, Vizcaya, [14]Hospital Universitario Central de Asturias (HUCA), Oviedo, [15]Hospital Morales Meseguer, Murcia, [16]Hospital Río Carrión, Palencia, [17]Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain

Background: High-throughput sequencing studies have rendered seminal knowledge in monoclonal gammopathies such as multiple myeloma (MM) and Waldenström's macroglobulinemia (WM). Unfortunately, the low incidence of AL amyloidosis and its typically low tumor burden, often masked by a polyclonal plasma cell (PC) background, account for the limited information on its tumor cell biology. Thus, it remains unknown if AL amyloidosis harbors a unifying mutation as occurs in WM or if, in its absence, there are recurrent mutations and if these overlap with those observed in MM.
Aims: To perform whole exome sequencing (WES) in a series of patients with AL amyloidosis and to compare mutational profiles in AL amyloidosis vs MM.
Methods: A total of 28 patients with confirmed diagnosis of AL were included. WES was performed in 56 paired samples of FACSorted bone marrow tumor plasma cells and peripheral blood mononucleated cells. Each tumor sample was captured in triplicate using Agilent's SureSelect Human All Exon V6 + UTR kit and sequenced on the Illumina NextSeq 500 platform. Data was analyzed with Strelka software to discard germinal mutations, ANNOVAR for functional annotation, and a data reduction strategy to identify candidate variants. The mutational signature was analyzed with DeconstructSigs software. We used the MMRF CoMMpass dataset (895 patients) to compare the mutational landscape of MM vs AL amyloidosis. We also determined immunoglobulin gene rearrangements in AL amyloidosis by next generation sequencing.
Results: The mean depth coverage for control and tumor samples was 64x and 186x, respectively. A total of 1983 somatic SNV and 133 INDEL were identified, with an average of 71 (20-281) SNV and 5 (0-25) INDEL per patient. When compared to MM (average of 66 SNV and 2,5 INDEL), patients with AL showed similar mutational load. None of the most frequently mutated genes in MM (i.e. *KRAS, NRAS, FAM46C, BRAF, TP53, DIS3, PRDM1, SP140, RGR1, TRAF3, ATM, CCND1, HISTH1E, LTB, IRF4, FGFR3, RB1, ACTG1, CYLD, MAX, ATR*) were recurrently mutated in patients with AL. The only genes commonly mutated in AL amyloidosis and MM were MUC16 (recurrence of 17% and 8%, respectively) and IGLL5 (recurrence of 17% each). Overall, the most frequently mutated genes in this series were IGLL5, MUC16 and DHFR2 (recurrence of 17% each). Most patients with AL harbored between 1 and 8 mutational signatures (Alexandrov, Nature 2013), implying that multiple mutational processes are operative. The most frequent mutational signatures were 1 (spontaneous deamination of methylated cytosines that to C>T transition at CpG sites), 3 (failure of DNA double-strand break-repair by homologous recombination) and 9 (T>G transversions at ApTpN and TpTpN trinucleotides), present in 96%, 54% and 46% of patients, respectively. Regarding the immunoglobulin gene repertoire, we noted that 26% of patients with AL amyloidosis harbored more than one clone; this extent in clonal heterogeneity being similar to that found in MM (23%). The most frequent IGH gene involved was *IGHV3-30* in both AL (recurrence of 10%) and MM (recurrence of 12%).
Summary/Conclusion: This is the first WES study performed in a series of patients with AL. We demonstrated the lack of a common driver mutation in this disease and unveiled that recurrently mutated genes in AL amyloidosis do not overlap with those observed in MM. Overall, these results may have significant impact in our understanding of the pathogenesis of AL amyloidosis and its differential diagnosis vs other monoclonal gammopathies.

PS1262

BI-ALLELIC LOSS OF FAM46C BY CRISPR/CAS9 SYSTEM ENHANCES MYELOMA CELL GROWTH

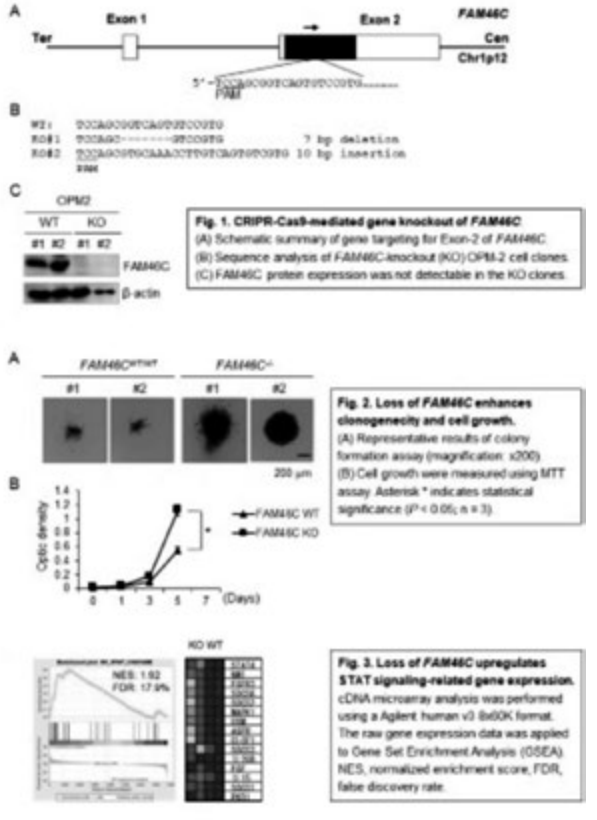
J. Kanasugi[1,*], I. Hanamura[1], A. Ota[2], S. Karnan[2], S. Mizuno[1], K. Uchino[1], S. Suzuki[3], Y. Hosokawa[2], A. Takami[1]

[1]Division of Hematology, Department of Internal Medicine, [2]Department of Biochem-

istry, [3]Department of Tumor Immunology, Aichi Medical University, Nagakute, Japan

Background: Multiple myeloma is a plasma cell malignancy which develops by acquiring a series of genomic alterations. Translocations involving the immunoglobulin loci are related with myelomagenesis. The sequential acquisition of additional genetic aberrations such as del(1p), del(17p) and 1q21 gain lead to the progression and drug resistance. Del(1p) is detected in around 20% of newly diagnosed myeloma. *FAM46C* is a candidate tumor suppressor gene at chromosome 1p12 and has been reported to be associated with a non-canonical poly (A) polymerase. However, pathogenic role of FAM46C in myeloma cells has not been well characterized.
Aims: The aim of this work was to elucidate the *FAM46C* gene function associated with myeloma cell growth and drug resistance *in vitro* and *in vivo* using CRISPR/Cas9 system.
Methods: We generated transfectants disrupting *FAM46C* using a CRISPR/Cas9 system with the human myeloma cell line OPM2 and explored its biological characteristics. A plasmid purchased from Addgene, containing *EGFP*, *Cas9* and a 20bp single guide RNA sequence for *FAM46C exon14* (Figure 1A) was constructed. After transfection using Nucleofection™, and GFP sorting, we performed single cell cloning, and then screened for *FAM46C* expression by western blotting. We obtained 2 clones with bi-allelic loss of *FAM46C* as determined by genomic sequencing (Figure 1B and 1C). Using these clones, cell proliferation, clonogenicity and drug sensitivity were examined by MTT and colony formation assay. OPM2 and OPM2 with bi-allelic disruption of *FAM46C* were xenografted into immunodeficient SCID mice (n=6, each), and the tumor sizes were measured twice a week. The gene expression changes by *FAM46C* disruption were analyzed by cDNA array analysis (Whole Human Genome 4×44k Oligomicroarray Chip, Agilent Technologies). A gene set enrichment analysis (GSEA) was performed to investigate a specific set of genes associated with FAM46C disruption.
Results: The *FAM46C* KO cells showed the higher cell proliferation compared with parent cells both *in vitro* (Figure 2) and *in vivo*. The sensitivity to lenalidomide and pomalidomide was similar between *FAM46C* KO and parent cells by MTT assay. GSEA revealed that loss of *FAM46C* upregulated expression of genes related with STAT signaling (Figure 3).

Figures 1, 2, 3.



Summary/Conclusion: The findings indicate that bi-allelic loss of *FAM46C* enhances myeloma cell proliferation but did not affect the sensitivity to IMiDs. This suggests that bi-allelic loss of *FAM46C* may be associated with disease progression. The growth advantage was due to upregulation of STAT signaling. Our genome-editing approach using CRISPR/Cas9 system is useful for exploration of a molecular pathology and therapeutic implication for oncogenic gene mutations in myeloma.

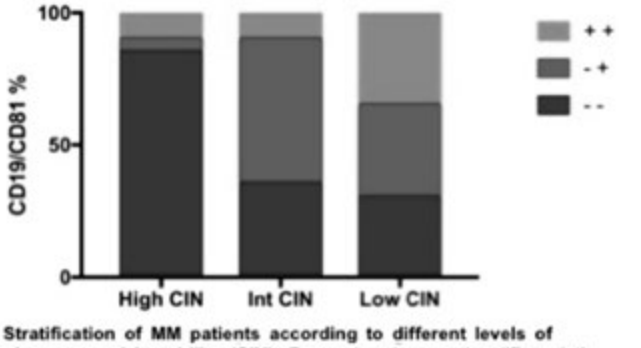
PS1263

A HIGH LEVEL OF GENOMIC COMPLEXITY CORRELATES WITH ADVANCED PLASMA CELL DIFFERENTIATION STAGES IN NEWLY DIAGNOSED MM PATIENTS

M. Martello[1,*], R. Termini[1], B. Santacroce[1], E. Borsi[1], V. Solli[1], C. Benni[1], A. Poletti[1], L. Pantani[1], B. A. Zannetti[1], S. Rocchi[1], E. Zamagni[1], P. Tacchetti[1], F. Ulbar[1], M. Arpinati[1], G. Chirumbolo[1], N. Testoni[1], G. Martinelli[2], M. Cavo[1], C. Terragna[1]

[1]DIMES-Department of Experimental Diagnostic and Specialty Medicine, L. e A. Seràgnoli, bologna, [2]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) Srl, IRCCS, Meldola, Italy

Background: Chromosomal instability (CIN) is a driving force of myelomagenesis: the continuous modification of plasma cell (PC) genomes favours the acquisition of progressively more DNA alterations, clonal evolution and heterogeneity, thereby promoting tumour development. Similarly, the pliancy of Multiple Myeloma (MM) plasma cells differentiation status acts as adaptive strategy to exogenous stress (e.g. in response to therapy). However, the genomic background that supports any diverse plasma cell differentiation phenotype has not yet been inferred.
Aims: To correlate the genomic background with the phenotypic plasticity of MM clone(s) at diagnosis, in order to stratify patients (pts) according to both the level of CIN and their PC differentiation stages, and ultimately to evaluate the impact of this stratification on the disease outcome.
Methods: A total of 78 newly diagnosed pts were included in the present study. Most pts (68/78) received a PI-based treatment as a front-line therapy. In each patient, both the BM CD138+/CD38+ PC and CD19+ B cell compartments were characterized by 6-color multiparametric flow citometry analysis, combining CD138-PE, CD38-PE-Cy7, CD20-APC, CD19-APC-Cy7, CD27-FITC, CD45-FITC, CD28-APC, CD44-FITC, CD54-APC, CD81-PerCP-Cy5.5, CD56-APC and SHH-PE, as a functional marker of Hedgehog pathway activation (Miltenyi Biotech). Whole copy number abnormalities (CNAs) characterization of CD138+ purified BM PCs was carried out by SNP array hybridization with Cytoscan HD array (Affymetrix).
Results: According to the detected CIN, as described both by total CNAs and portion of genome changed (GC), three major subgroups were identified: the first one, including 21 pts with high CIN (medium tot. CNAs = 550,% GC ≥ 25%); the second one including 25 pts with a intermediate CIN (medium tot. CNAs = 315,% GC = 10-25%) and the third one including 18 pts with low CIN (medium tot. CNAs = 105,% GC ≤ 10%). As expected, in pts with high CIN, more than a quarter of unstable genome was due to the hyperdiploidy; however, they were also characterized by a higher prevalence of high-risk features. Indeed, 1p deletion (*FAF1*), 16q deletion (*WWOX*, *FANCA*) and 17p deletion (*TP53*) were the most recurrent abnormalities, and almost exclusively associated to high CIN pts (p<.05). A detailed immunophenotypic analysis of the three subgroups of pts showed that high CIN background mainly characterized mature PCs (17/21 = 81%) (Figure 1), as described by: a) a significant deregulation of both CD19 and CD81 markers; b) a higher expression of CD28 and CD44, which usually characterized advanced disease stages; c) a reduced expression of CD20, CD27 and CD45, commonly associated to preceding PC differentiation stages (p<.05).



Stratification of MM patients according to different levels of chromosomal instability (CIN). For each subgroup the differentiation stages distribution of the is indicated: CD19-/CD81- (blue), CD19-/CD81+ (red) and CD19+/CD81+ (green).

Figure 1.

Finally, the presence of more mature PCs with high CIN characterizes pts carrying baseline clinical features associated to bad prognosis (e.g. n. PET lesions, k/l ratio, ISS III, β2-microglobulin; p<.05). In addition, these pts tend to obtain high quality response rates (≥CR) to PI induction therapy.
Summary/Conclusion: A high level of genomic complexity correlates with an advanced PCs differentiation stages in newly diagnosed MM patients, and this is lastly associated with a prevalence of bad prognosis features. Chromosomal instability, together with cellular phenotypic pliancy, represents an important, yet poorly defined, mechanism by which MM clone(s) accelerate their own evolution and survival. *Acknowledgements: AIRC IG2014, Fondazione Berlucchi.*

PS1264

MOLECULAR SEGMENTATION OF MULTIPLE MYELOMA BY INTEGRATIVE MULTI-OMICS ANALYSIS

M. Ortiz Estevez[1,*], M. Samur[2], F. Towfic[3], K. Mavrommatis[4], E. Flynt[5], B.A. Walker[6], M. Trotter[1], G. Morgan[6], N. Munshi[2], A. Thakurta[5]

[1]Reasearch Analytics, CITRE, Celgene Institute Translational Research Europe, Sevilla, Spain, [2]Dana-Farber Cancer Institute, Harvard Medical School, Boston, [3]Reasearch Analytics, Celgene Corporation, Summit, [4]Reasearch Analytics, Celgene Corporation, San Francisco, [5]Translational Development, Celgene Corporation, Summit, [6]Myeloma Institute, University of Arkansas for Medical Sciences, Little Rock, United States

Background: There is currently no comprehensive classification of newly diagnosed Multiple Myeloma (ndMM) tumor biology that integrates heterogeneous molecular information including gene expression (GEP), mutation (SNV), copy number alteration (CNA), and structural variant (SV) profiling. A robust classification of ndMM at this level of resolution would yield the potential to improve diagnosis, prognosis and treatment of patients with MM by describing the etymology of the disease.
Aims: As part of the Multiple Myeloma Genome Project (MGP) collaborative initiative, we aimed to develop an integrative molecular segmentation strategy with the overall goal to provide actionable insights into the heterogeneity of ndMM biology.
Methods: In order to investigate molecular segments of ndMM, we assembled a high quality clinical (ISS, Age, Sex) and uniformly processed genomics dataset and defined progression-free survival (PFS) and overall survival (OS) across all studies involved. To overcome FISH and cytogenetic data variability between studies, we re-derived 18 common structural variants including del17p, t(4;14), t(6;14), t(11;14), t(14;16) and t(14;20) using whole genome and whole exome sequencing data to ensure high confidence calls across all studies. SNVs were aggregated into pathways to improve interpretability and assign functionality to their effect. Two multi-omics integrative clustering methods were run multiple times with number of clusters ranging from K= 2 to 20 and patient resampling: 1-Cluster of Clusters (CoCA) and 2-iClusterPlus. The number of clusters was selected based on feature and sample resampling and convergence of the Bayesian Information Criterion (BIC) and Normalized Mutual Information (NMI). Consensus matrices generated by iClusterPlus and CoCA were compared using BIC and NMI to select more robust patient segmentation.
Results: We discovered that copy-number, gene expression, and SVs account for highest proportion of variance across segments when compared to single nucleotide variants (as defined by contribution to overall variability in Joint and individual variation explained (JIVE), coherence of clusters in iClusterPlus and CoCA).
Multi-dimensional unsupervised analysis identified 12 stable patient segments (no cluster contained less than 5% of samples) including patient tumors enriched in t(4;14) and t(11;14) and various subsets of del17p patients stratified by CNA and distinct mRNA expression patterns. The highest risk group (11% of samples, C8), as measured by PFS and OS, is defined by a combination of biomarkers including del13q/14q/17p and amp1q and a down-regulation of related gene expression profiles; while the lowest risk group (5% of samples, C2) is significantly associated with low mutation rate. (Figure 1).
Summary/Conclusion: This is the first analysis of a large dataset in ndMM to perform an unsupervised biomarker analysis to stratify patients into 12 segments based primarily on SVs, CNAs and mRNA differences across tumors. The segments are driven by features such as SVs involving 14q, CNAs (del17p, del13q, amp1q), and specific GE profiles. In contrast, SNVs contributed less to the variance across MM segments. This finding suggests that wider DNA alterations and potentially related transcriptomic changes drives the MM segments. To understand the biological consequences of alterations, genomic and transcriptomic pathway analysis are being performed. These analyses will likely reveal underlying biological drivers of the disease, lead to potential new targets and foster mechanism-based approaches for MM therapeutics development in specific molecular segments.

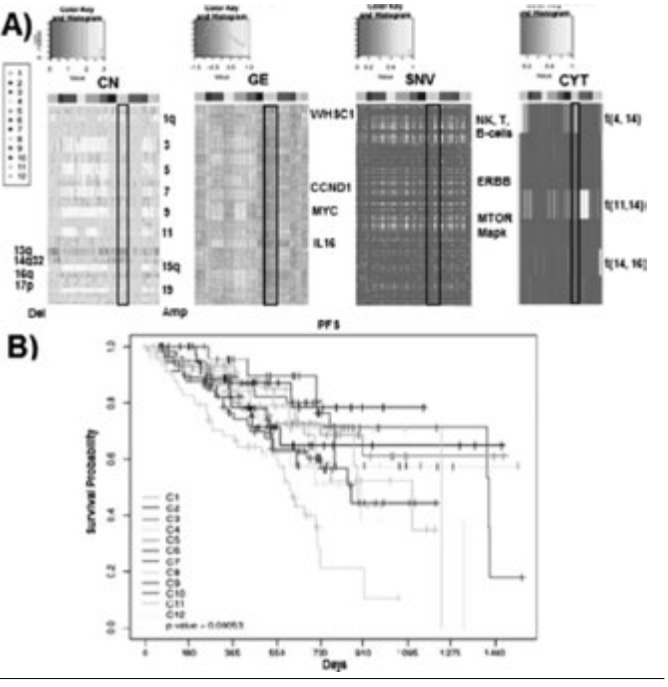


Figure 1.

PS1265

SYSTEMATIC IDENTIFICATION OF PHARMACOGENOMICS INTERACTIONS MODULATING DRUG RESPONSES IN MULTIPLE MYELOMA

M. M. Majumder[1,*], M. Ammad-ud-din[2], D. Tamborero[3], P. Anttila[4], R. Silvennoinen[5], S. Eldfors[1], J. Lievonen[4], A. Parsons[1], M. Suvela[1], N.L. Bigas[3], P. Martinen[2], E. Jantunen[5], S. Kaski[2], K. Porkka[4,6], C. Heckman[1]

[1]Translational and Personalized Medicine, Institute for Molecular Medicine Finland(FIMM), HiLIFE, University of Helsinki, Helsinki, [2]Helsinki Institute for Information Technology (HIIT), Aalto University, Espoo, Finland, [3]Dept. of Experimental and Health Sciences, University Pompeu Fabra, Barcelona, Spain, [4]Department of Hematology, Helsinki University Hospital and Comprehensive Cancer Center, Helsinki, [5]Department of Medicine, Kuopio University Hospital, Kuopio, [6]Hematology Research Unit, University of Helsinki, Helsinki, Finland

Background: Multiple myeloma (MM) displays enormous genetic complexity and heterogeneity, which may ascribe to diversity in response observed in patients sharing identical prognostic markers or disease stage. Precision medicine holds the promise of selectively and efficiently eradicating malignant cells by targeting the mechanisms driving their key phenotype. However, the success critically relies on relevant biomarkers that can be used to identify responding patients.
Aims: To decipher complex pharmacogenomics interactions, we comprehensively assessed *ex vivo* responses of 100 MM samples to 142 drugs and compared to matching genomic, transcriptomic and clinical profiles of the patients. Utilizing a unique data resource, we aimed to i) stratify myeloma patients based on their *ex vivo* drug response, ii) understand drug response and resistance mechanisms, ii) identify molecular indicators of response, and iii) evaluate drug repositioning possibilities
Methods: 100 bone marrow (BM) aspirates were collected from 32 newly diagnosed and 51 relapsed myeloma patients. CD138+ cells were isolated and tested against a 142 oncology compounds. DNA and RNA were sequenced for 98 and 78 samples, respectively. Pairwise and multivariate statistical approaches were applied to provide integrative analyses on the datasets.
Results: Based on overall drug response profiles, the patients clustered into four drug sensitivity subgroups (Group-I-IV). A higher mutation load in patients was associated with higher *ex vivo* sensitivity to signal transduction inhibitors (PI3K-AKT-mTOR, HDAC and CDK inhibitors) and associated with poor survival. 14% of the samples exhibited a resistant phenotype and showed elevated expression multidrug resistance genes *ABCB1* (*MDR1*) and *ABCC3* (*MRP3*). Integrative analysis allowed assimilation of multiple datasets and revealed putative molecular signatures associated to individual

drugs. For example, a hyperdiploid karyotype was associated to *ex vivo* response to glucocorticoids and bromodomain inhibitors. *CCND1* and *BMI1* expression correlated with sensitivity to BCL2 inhibitor venetoclax. MEK inhibitor response was detected in 55% of samples with *NRAS*, *KRAS*, *NF1*, and *BRAF* mutations. 20% of the patient samples harbored mutations in genes involved in DNA damage repair signaling, namely *ATM*, *ATR*, *MSH* and *TP73*, in a mutually exclusive pattern. Patients with these mutations had a high relapse rate, poor overall survival (HR=3.2,95%CI 1.1-9.02), and displayed acquired sensitivity to PI3K-mTOR and HDAC inhibitors in longitudinal samples. Sensitivity to midostaurin (median IC50, 211 nM), identical to FLT3 mutated AML, was detected in 43% of relapsed patient specimens enriched for mutations to *TP53*, *NRAS* and *FAM46C*.
Summary/Conclusion: We identified molecular subgroups of myeloma patients with distinct drug response profiles, which are driven by underlying molecular alterations. Acquisition of DNA damage repair signaling alterations conferred poor prognosis and corresponded to PI3K-mTOR and HDAC inhibitor sensitivity. Integrative analyses leveraging on a multi-omics dataset uncovered biomarkers, which could facilitate identification of responding patients to individual drugs. We identified drug candidates that could potentially be repurposed to treat myeloma patients. Our study provides a valuable resource and framework to identify novel pharmacogenomics interactions and facilitate precision therapies, especially for relapsed/refractory myeloma patients.

PS1266

ADAR1-MEDIATED-NEIL1 EDITING IS OF BIOLOGICAL SIGNIFICANCE IN MULTIPLE MYELOMA

P.J. Teoh[1,*], O. An[1], T.-H. Chung[1], H. Yang[1], W.J. Chng[1,2,3,4]

[1]Cancer Science Institute of Singapore, [2]National University Cancer Institute, [3]Medicine, National University of Singapore, [4]Haematology-Oncology, National University Health System, Singapore, Singapore

Background: The physiological and biological roles of RNA editing have been actively studied in recent years and emerging evidence revealed a close association between an aberrant ADAR1-mediated-A-to-I editing and tumorigenesis in various cancer types. As compared to solid tumors, this topic has not been comprehensively studied in hematological malignancies, and only until recently that a deregulated A-to-I editing was critically reported in multiple myeloma (MM), whereby, *GLI1* (Hedgehog signaling gene) editing was of prognostic significance in MM patients. As RNA editing is a pervasive mechanism constituting 85% of human transcriptome, we hypothesize that an aberrant editing can occur in other genes in MM.
Aims: We aim to delineate other biologically important genes that are abnormally edited. Although ADAR1 can promiscuously edit mRNA, the occurence at the coding sequences (CDS) can potentially modify the protein structure and function, thus, our focus was on the genes that are hyperedited at the CDS.
Methods: To study the A-to-I editing, we performed whole transcriptome sequencing of the normal and malignant plasma cells obtained from healthy volunteers and MM patients, respectively. We also analysed the publicly available RNA-sequencing dataset from MMRF (CoMMpass). For the identification of ADAR1-specific-editing events, we performed ADAR1 knockdown and overexpression on human myeloma cell lines (HMCLs) and genes showing non-synonymous editing were selected for further validation. Site-directed mutagenesis was done to generate stable HMCL overexpressing the edited variant of the candidate gene. We performed functional assays on these cells to look at its phenotype comparatively to cells overexpressing the WT gene.
Results: There was a global increase of exonic editing events along the disease progression route, underscoring its potential biological importance. Based on the list of differentially edited genes on ADAR1-knockdown and -overexpressed HMCLs and a thorough literature search, we selected NEIL1 (DNA base-excision repair gene) editing (A726G, Lys242Arg) for downstream analyses. RNA-immunoprecipitation and Sanger sequencing confirmed NEIL1 as a direct ADAR1 target. Analysis of the CoMMpass dataset revealed that NEIL1 editing was ubiquitious, whereby, 69% of the patients had A726G editing, of which the highest number of events was found in the relapsed patients. Importantly, the recoded NEIL1 exhibited gain-of-function properties and an inefficient oxidative damage repair capability. Although unrepaired DNA damage is known to lead to apoptosis, we observed that the recoded NEIL1 ironically caused increased resistance to melphalan. NEIL1-edited cells displayed an enrichment of double stranded DNA breaks (DSB) proteins (p53, p-H2Ax, p-CHK1, Ku80), implying that they could be more sensitive to DSB-inducing drugs. Indeed, treatment of the cells with etoposide (DSB inducer) alone and in combination with melphalan confers a higher cellular inhibition to NEIL1-edited cells than to the NEIL1-WT cells.
Summary/Conclusion: A defective DNA damage repair can predispose the genome to increased random mutagenesis, thus, it is plausible that an aberrant NEIL1 mRNA (A726G) and its protein product (Lys242Arg) may be one of the mechanisms by which RNA editing affects the genome integrity and how it wires the MM cells into acquiring undesirable mutations to further trigger disease pathogenesis. For the first time, our study collectively shows that ADAR1-mediated-NEIL1-editing is biologically and functionally relevant in MM and should be further explored as a potential predictive biomarker.

PS1267

DETECTION OF MYD88 AND CXCR4 MUTATIONS IN CELL-FREE DNA OF PATIENTS WITH IGM MONOCLONAL GAMMOPATHIES

T. Bagratuni[1,*], I. Ntanasis-Stathopoulos[1], M. Gavriatopoulou[1], N. Mavrianou-Koutsoukou[1], C. Liacos[1], D. Patseas[1], N. Kanellias[1], M. Migkou[1], D. Ziogas[1], E. Eleutherakis-Papaiakovou[1], M. Roussou[1], D. Fotiou[1], E. Terpos[1], K. Efstathios[1], M. Dimopoulos[1]

[1]National And Kapodistrian University Of Athens, Athens, Greece

Background: Mutational characterization of Waldenström's macroglobulinemia (WM) currently relies on DNA from CD19-selected cells derived from bone marrow (BM) aspirates, which is an invasive technique, associated with significant patient discomfort. Liquid biopsy is being integrated into cancer diagnostics with profound therapeutic implications. Peripheral blood cell-free tumour DNA (cfDNA), also known as liquid biopsy, has been recently shown to be a powerful non-invasive molecular biomarker in monitoring tumor status in several cancers.
Aims: In this study, we aimed to characterize the mutational status of WM/IgM MGUS patients by using peripheral blood plasma-derived cfDNA and using matched tumor DNA (tDNA) from BM-CD19$^+$ selected cells, in order to determine whether cfDNA, can be used as an additional diagnostic tool in identifying the mutational profile of WM.
Methods: A total of 68 consecutive patients with IgM monoclonal gammopathies and 10 controls were included in this study. Peripheral blood (10-12mL) was collected in EDTA tubes and DNA was extracted using the MagMax cell free DNA isolation kit. BM aspirates were collected at the same time with peripheral blood, and were processed for CD19 enrichment, using CD19 magnetic beads, followed by DNA extraction. tDNA and cfDNA samples were analyzed for the MYD88 L265P and for CXCR4 mutations. The presence of L265P mutation was initially assessed by Allele-Specific PCR and then confirmed with direct sequencing. The presence of CXCR4 mutations was assessed with direct sequencing.
Results: Among the 68 patients, 54 patients had both tDNA and cfDNA informative samples. $MYD88^{L265P}$ mutation was detected in 40 out of 54 patients (74%) in both tDNA and cfDNA; in 4 out of 54 (7%) the mutation was seen in tDNA but not in cfDNA and 10 out of 54 (19%) patients harbored the $MYD88^{WT}$ genotype both in tDNA and cfDNA. Thus, the overall concordance between tDNA and cfDNA for *MYD88* genotype was 93% (50 out of 54 patients). Among patients with IgM MGUS, WM in remission and sWM /NDWM/relapsed (RR) WM, the concordance rates were 100% (3 out of 3 patients), 96% (25 out 26 patients) and 88% (22 out of 25 patients), respectively. The assessment of *CXCR4* mutations in both tDNA and cfDNA was feasible in 47 out of 68 patients(69%). In seven patients (7 out of 47, 19%), mutations were detected in both paired samples. *CXCR4* mutations by either cfDNA or tDNA were present in 2 out of 5 MGUS (40%) patients, 7 out of 35 (20%) patients with disease in remission and in 5 out of 26 (19%) patients with sWM/NDWM/RRWM. The pathogenic mutation *S338X* was present in one patient with sWM and one with NDWM. The *F29L*, *P27T*, *P31T* and *I53L* mutations were also found 2-4% of patients. In 3 out of 47 patients the *E343D, H228Q* and *L50X* truncating mutations were detected in tDNA but not in cfDNA. Overall, the concordance rate between tDNA and cfDNA was 92% (43 out of 47 patients).
Summary/Conclusion: In conclusion, PB cfDNA is a useful, minimally invasive, cost-effective tool for the detection of *MYD88* and *CXCR4* mutations in patients with IgM monoclonal gammopathies avoiding unnecessary BM assessment.

PS1268

NEXT-GENERATION SEQUENCING ALTERNATIVES FOR THE DETECTION AND QUANTIFICATION OF MINIMAL RESIDUAL DISEASE IN MULTIPLE MYELOMA PATIENTS

A. Medina[1,*], C. Jiménez[1], N. Puig[1], B. Sánchez-Vega[2], M. González[1], R.M. Ayala[2], M. E. Sarasquete[1], B. Paiva[3], T. Cedena[2], I. Rapado[2], L. Rosiñol[4], E. Ocio[1], A. Oriol[5], J. Miller[6], F. Martínez-Rucobo[6], M.T. Hernández[7], R. Martínez[8], M.V. Mateos[1], J.J. Lahuerta[2], J. Blade[4],

J.F. San Miguel[3], J. Martínez-López[2], R. García-Sanz[1]

[1]Departamento de Hematología, Hospital Universitario de Salamanca (HUSAL), IBSAL, IBMCC (USAL-CSIC), CIBERONC, Salamanca, [2]Departamento de Hematología, Hospital [12] de Octubre, CIBERONC, Madrid, [3]Clínica Universidad de Navarra, Centro de Investigación Médica Aplicada (CIMA), IDISNA, CIBERONC, Pamplona, [4]Hospital Clinic, Barcelona, [5]ICO Hospital Germans Trias i Pujol, Badalona, Barcelona, Spain, [6]Invivoscribe Technologies, Inc, San Diego, United States, [7]Departamento de Hematología, Hospital Universitario de Canarias, Santa Cruz de Tenerife, [8]Departamento de Hematología, Hospital Clínico San Carlos, Madrid, Spain

Background: Minimal residual disease (MRD) has emerged as a key parameter in the evaluation of multiple myeloma (MM) response to therapy. Next-generation sequencing (NGS) detection of the IGH clonal rearrangement has demonstrated some advantages over allele-specific oligonucleotide PCR (ASO-PCR) to detect low MRD levels in MM. However, only the LymphoSight® strategy, (Sequenta, San Francisco, CA) has demonstrated utility in clinical practice; accordingly, such strategy is the only one accepted by the International Myeloma Working Group (IMWG) criteria (Kumar *et al.*, Lancet Oncol 2016). Hence, additional commercial or academic efforts to evaluate MRD by NGS in MM would be very welcome.
Aims: Here, we have evaluated the LymphoTrack® commercial kit (Invivoscribe, San Diego, CA) to validate its potential applicability and usefulness in the MRD detection of MM. Results were compared with those obtained with Multiparametric Flow Cytometry (MFC).
Methods: 244 bone marrow samples from a cohort of 122 patients were evaluated at two different time-points: diagnosis and follow-up after response achievement (median, 3 months after transplant, or at the end of the induction treatment). All patients had been enrolled in clinical trials from the Spanish MM group: GEM2005menos65 (n=24), GEM2010 (n=17) and GEM2012 (n=81). We first tried to identify tumor clonal VDJH rearrangements in diagnostic samples through the amplification of IGH-FR1/FR2 regions followed by conventional Sanger sequencing. NGS analysis of the diagnostic sample was also performed in 81 cases. We then assessed MRD levels in follow-up samples using the LymphoTrack® NGS assay. More than 650 ng DNA (>10^5 cells) per reaction were used to warrant a sensitivity of 10^{-5} or better. 1 uL spike-in DNA corresponding to 100 clonal cells was added to the reaction to allow the absolute quantification of tumor plasma cells. Amplicons were then sequenced in a MiSeq® platform (Illumina, San Diego, CA), processed using the LymphoTrack® software, and results were compared with those obtained by MFC.
Results: Myeloma-specific clonal rearrangements and CDR3 regions were obtained in 121/122 patients (99.2%). Therefore, we tested MRD in their corresponding follow-up samples. Two samples (1.6%) were excluded from the final MRD analysis due to low read counts in the post-therapy sample. Of the remaining 119 cases, 66 were MRD positive and 53 were MRD negative by sequencing. A high correlation between MFC and NGS MRD results was observed (R=0.943) although we found 24 discordant cases: 9 cases were negative by MFC but positive by NGS, and 15 cases were positive by MFC but negative by NGS. Time to Tumor Progression (TTP) was significantly longer in the MRD negative than in the MRD positive subsets by NGS, providing a projected 40-month Time to Tumor Progression of 94.4% vs. 65.4%, respectively (p=0.001). This was translated into a better Overall Survival (OS) for patients with a negative MRD, who had a 40-month projected OS of 97.8% compared with 71% for patients with a positive MRD (p=0.01) (Figure 1A and 1B).

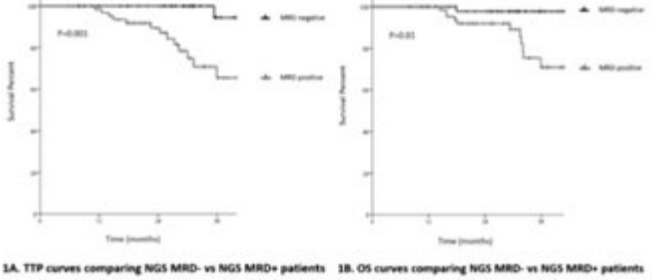


Figure 1.

Summary/Conclusion: LymphoTrack® applicability was very high (98.8%), like that reported for MFC and other NGS approaches. There was a high correlation between MRD levels by NGS and MFC, with an excellent capacity to predict the outcome. These results reinforce the usefulness of the MRD assessment by NGS for patient risk stratification in MM and provides a new approach easily available for most laboratories.

PS1269

IRON INCREASES THE EFFICACY OF STANDARD MULTIPLE MYELOMA THERAPY IN TRANSGENIC VK*MYC MODEL

J. Bordini[1,*], M. Ponzoni[2], M. Marcatti[3], C. Camaschella[4], A. Campanella[4]

[1]Division of Genetics and Cell Biology, Vita-Salute San Raffele University, [2]Division of Experimental oncology, [3]Department of onco-hematology, [4]Division of Genetics and Cell Biology, IRCSS Ospedale San Raffaele, Milan, Italy

Background: Multiple Myeloma (MM) cells rearrange iron trafficking proteins to promote iron retention and favor proliferation. However, iron balance is essential even in cancer cells. We have previously demonstrated that iron excess delays disease development in MM pre-clinical models and that the proteasome inhibitor bortezomib (BTZ) interferes with the cell defensive response against iron excess, maximizing iron toxicity and oxidative damage within MM cells. Accordingly, high iron dose increases BTZ efficacy *in vivo* (Bordini *et al.* Leukemia 2017). Searching for the mechanisms of iron toxicity we reasoned that, since MM cells stem from non-malignant professional secretory precursors, iron excess might interfere with proteasome and autophagy degradation pathways. In addition iron may affect also the microenvironment: it has been reported that iron loaded tumor associated macrophages (TAM) shift from pro-survival to pro-inflammatory phenotype that negatively affects tumor expansion in implanted mammary tumors and in liver and lung metastasis models.
Aims: To demonstrate that iron supplementation increases the efficacy of proteasome inhibitors-based protocols in transgenic Vk*MYC MM murine model and to analyze the molecular mechanisms underlying iron toxicity with the final aim of providing novel, even iron-independent, targetable pathways in MM.
Methods: Vk*MYC mice were treated with three consecutive cycles of BTZ, melphalan and prednisone (VMP regimen). In every cycle, a pool of mice was additionally treated with iron dextran (100 mg/Kg). Treatment efficacy was determined by serum monoclonal component reduction. To analyze protein homeostasis, we assayed poly-ubiquitinated (poly-Ub) proteins level and of the autophagic marker LC3 in MM cell lines treated with increasing concentration of Ferric Ammonium Citrate (FAC), either alone or in combination with BTZ. To evaluate macrophage polarization, bone marrow derived macrophages (BMDM) were cultured 72h in medium conditioned by MM cell lines and supplemented or not with FAC.
Results: At the end of the first VMP cycle mice treated with iron (VMP-Iron) showed stronger disease reduction than VMP-Saline mice (p <0,01). Subsequent VMP-Iron cycles successfully controlled the disease while VMP-Saline mice became refractory. No alteration of liver and kidney function was observed in VMP-Iron mice at the end of treatment. Within tumor tissue, iron accumulated preferentially in bone marrow macrophages. *in vitro*, we observed a dose dependent accumulation of poly-Ub proteins after iron exposure, suggesting that iron burden might impair proteasome functionality. Addition of iron further increased poly-Ub proteins levels upon BTZ. Moreover, BTZ increased LC3 levels and consumption rate, suggesting increased autophagic flux as compensatory mechanism after proteasome inhibition. Iron addition to BTZ reduced LC3 consumption, suggesting a decrease of autophagic response and a mechanism of reversing BTZ resistance. As for macrophage polarization, BMDM exposed to medium conditioned by MM cells rearranged iron proteins expression toward the iron recycling phenotype as observed in M2-like polarization. Addition of FAC stimulated TNF-alpha expression, suggesting a switch to M1-like phenotype associated to anti-tumor activity.
Summary/Conclusion: High iron dose negatively affects MM cells proliferation by interfering with multiple cellular pathways and tumor microenvironment. Since iron increases VMP regimen efficacy, our data suggest that iron administration might impact on MM therapy.

PS1270

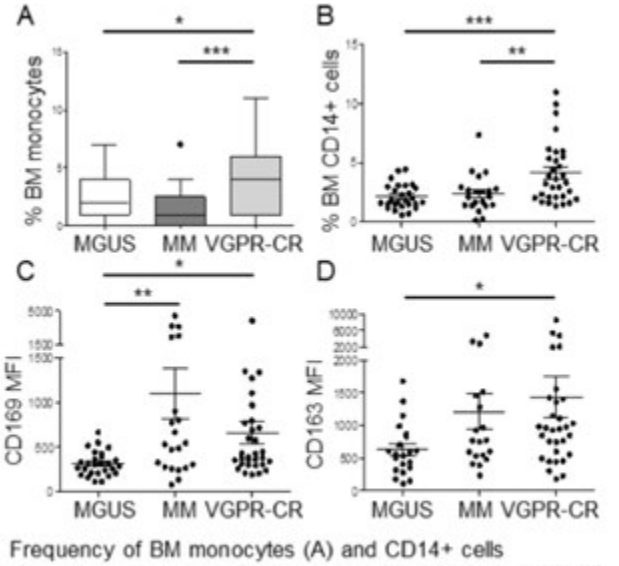
BONE MARROW MACROPHAGES IN PATIENTS WITH MULTIPLE MYELOMA IN COMPLETE REMISSION RETAIN PRO-TUMOR M2 PHENOTYPE

E. Lozano[1,*], T. Diaz[1], M.-P. Mena[1], M.T. Cibeira[1], I. Isola[1], L. Rosinol[1], J. Blade[1], C. Fernandez de Larrea[1]

[1]Amyloidosis and Myeloma Unit. Hematology Department, Hospital Clinic-IDIBAPS, Barcelona, Spain

Background: Macrophages may play pro-tumor or anti-tumor roles depending on the hematological malignancy and the stage of the disease. In this sense, immune regulation control proliferation of aberrant plasma cells (PCs) in the asymptomatic monoclonal gammopathy of undetermined sig-

nificance (MGUS) that precedes multiple myeloma (MM) and in patients with MM in sustained complete remission (CR) after treatment. Thus, a better understanding of the roles of macrophages at different stages of MM pathogenesis may provide new opportunities for therapeutic intervention.
Aims: The aim of this study was to investigate macrophage contribution to immune regulation in MGUS, symptomatic MM and patients with MM who have achieved CR or very good partial response (VGPR).
Methods: Frequency of monocyte-macrophage in bone marrow (BM) was analyzed by May-Grünwald-Giemsa staining of BM smears. We quantified BM CD45+CD3-CD4^{med}CD14+ cells and expression of CD169 (also known as Siglec1) and the M2-associated marker CD163 by 8-color flow cytometry. Concentrations of soluble CD169 and CD163 in BM plasma were measured by ELISA.
Results: Statistical analysis of frequency of BM monocyte-macrophages comparing MGUS (n=33), symptomatic MM (n=45) and MM in VGPR/CR (n=59) showed a significant increase of monocyte-macrophages in VGPR/CR status versus MM (ANOVA test ***p<0.0001) (Figure 1A). However, more than 4% of BM monocytes in patients with CR (n=67) was associated with a trend toward shorter PFS (2.5 vs. 4.9 years, p=0.092). To better understand the role of macrophages in patients in VGPR/CR, we next quantified frequency and changes in macrophage phenotype by flow cytometry. Indeed, we found that patients with MM in VGPR/CR (n=31) showed significantly higher frequency of CD14+ cells compared to patients with symptomatic MM (n=21) and MGUS (n=27) (ANOVA test ***p=0.0003) (Figure 1B). Moreover, expression of CD169 was significantly higher in symptomatic MM and VGPR/CR compared to MGUS (ANOVA test **p=0.0051), without differences between MM and VGPR/CR. Similarly, expression of the M2-associated marker CD163 was higher in MM and patients in VGPR/CR compared to MGUS (ANOVA test *p=0.03) (Figure 1 C-D) indicating no significant differences in macrophage phenotype in VGPR-CR compared to symptomatic MM.
Since CD169 and scavenger receptor CD163 can be found as soluble form after shedding, we next quantified their levels in BM plasma. No significant differences were found in sCD169 and sCD163 between MGUS (n=16) and symptomatic MM (n=31). We then measured sCD169 and sCD163 in paired samples from patients with active MM and in CR after treatment (n=14). Values of sCD169 were significantly increased in CR (Mean±SEM 3.214 ng/mL±0.422) compared to the previous corresponding values when active MM (0.8571±0.231; paired t test ***p=0.0001). Moreover, levels of sCD163 were also higher in CR (573.2 ng/mL±58.65) compared to active MM (388.4±37.24, paired t test **p=0.0085).



Frequency of BM monocytes (A) and CD14+ cells (B) by flow cytometry. Expression of CD169 (C) and CD163 (D) in BM CD14+ cells. *p<0.05, **p<0.01, ***p<0.001.

Figure 1.

Summary/Conclusion: The frequency of macrophages in BM from patients with MM in VGPR/CR is increased compared to patients with symptomatic MM. However, the high expression of M2-marker CD163 both soluble and on cell surface suggests that BM macrophages from patients with MM in VGPR/CR may retain the pro-tumor M2-phenotype found in patients with active myeloma. M2-macrophages may collaborate to support malignant cell survival in symptomatic MM and during relapses, suggesting that targeting macrophage could offer new opportunities of therapeutic approach in myeloma.

PS1271

PTC-028 DEMONSTRATES POTENT PRE-CLINICAL ACTIVITY AND PROVIDES A NOVEL OPPORTUNITY TO MODULATE BMI1 IN MULTIPLE MYELOMA

A. Bolomsky[1], J. Muller[2], K. Stangelberger[1], R. Heusschen[2], M. Weetall[3], A. Branstrom[3], N. Zojer[1], J. Caers[2], H. Ludwig[1,*]

[1]Wilhelminen Cancer Research Institute, Vienna, Austria, [2]Laboratory of Hematology, University of Liège, Liège, Belgium, [3]PTC Therapeutics Inc., South Plainfield, NJ, United States

Background: The polycomb group protein BMI1 was linked to the pathogenesis of MM more than a decade ago, and represents a prominent intrinsic driver of MM with close associations to high-risk genes such as MYC and FOXM1. However, similar to other promising drug targets in MM biology (e.g. IRF4), direct modulation of these factors is impeded by the lack of clinically effective inhibitors.
Aims: In the current study, we explored the anti-myeloma efficacy of PTC-028, the first in class BMI1 protein modulator and confirmed its applicability to target a so far not drug-able key molecule in MM.
Methods: The anti-MM efficacy of PTC-028 (kindly provided by PTC Therapeutics Inc.) was studied *in vitro* using human MM cell lines (HMCLs) and primary MM cells. Candidate gene expression levels were analyzed by quantitative PCR (qPCR), Western Blot and/or flow cytometry (FC). Mechanistic studies were conducted via FC, phospho-specific ELISAs and immunoblot experiments. Confirmatory studies are employed using lentiviral transduction of BMI1 overexpression or knockout plasmids, respectively.
Results: PTC-028 induced a rapid decrease of BMI1 protein levels and demonstrated potent anti-MM activity in all primary MM cells and HMCLs tested (median IC50: 39 nM, range: 11-102 nM). Anti-myeloma activity persisted in the presence of bone marrow stromal cells and in proteasome inhibitor (PI) resistant HMCLs. Importantly, median IC50 values were significantly reduced compared to a transcriptional repressor of BMI1 - PTC-209 (median IC50 39 vs. 680 nM, p<0.05). We also observed a significant correlation between PTC-028 IC50 values and the reduction of BMI1 protein levels in HMCLs (R=0.86, p=0.01), suggesting that alterations of BMI1 levels could serve as predictive marker for treatment efficacy.
Mechanistic analyses demonstrated an accumulation of MM cells in the G2/M phase of the cell cycle as well as the induction of apoptosis accompanied by the presence of cleaved caspases 8 and 9, cleaved PARP, and depolarization of the mitochondrial membrane potential. Time course experiments with U266 and KMS-12-BM cells revealed rapid induction of G2/M cell cycle arrest (evident 6h post treatment) in line with an accumulation of CDK1 and Cyclin B1 at the protein level. This was followed by a significant reduction of MCL1 protein levels during mitotic arrest. Further validation of these results was obtained by qPCR analysis demonstrating upregulation of mitosis associated genes (*CCNB1, AURKA, BIRC5*) and downregulation of *MCL1* 6h and 24h post treatment.
BMI1 was reported to interact with several signaling axes important for the survival of MM. We therefore examined the impact of PTC-028 on MYC, AKT, ERK, and GSK3b activity. These analyses demonstrated a significant reduction of MYC and AKT activity 24h post treatment, while ERK and GSK3b activation remained unaffected. In addition, MYC protein expression was found to be downregulated together with FOXM1 expression suggesting that PTC-028 might provide a novel strategy to target a so far not drug-able high-risk network.
Summary/Conclusion: This study demonstrates potent *in vitro* activity of the first in class BMI1 modulator PTC-028 in a panel of HMCLs, primary MM cells, and PI-resistant HMCLs. Moreover, our data point to significant impact on a currently not targetable high-risk network consisting of BMI1, MYC and FOXM1. Based on these potent *in vitro* results further pre-clinical evaluation of PTC-028 in murine models of MM is in progress.

PS1272

ARGININE DEPRIVATION SUSTAINS PLASMA CELL FITNESS AND BIOENERGETICS IN MULTIPLE MYELOMA

A. Romano[1,*], L. Oliva[2], N.L. Parrinello[1], J. Manteiga[2], P. La Cava[1], K. Todoerti[3], C. Giallongo[1], D. Tibullo[4], G. Camiolo[4], F. Puglisi[1], C. Bellofiore[1], U. Orfanelli[1], M. Trudu[2], C. Conticello[1], G.A. Palumbo[4], F. Ciceri[5], G. Tonon[5], R. Sitia[5], S. Cenci[2], F. Di Raimondo[1]

[1]CHIRMED- Dipartimento Chirurgia generale e specialità medico-chirurgiche-Hema-

tology Section, Università degli Studi di Catania, CATANIA, [2]Age and disease related Unit, Fondazione Centro San Raffaele, Milano, [3]Laboratory of Pre-Clinical and Translational Research, Reference Cancer Center of Basilicata, IRCCS CROB, Rionero in Vulture, [4]Università degli Studi di Catania, CATANIA, [5]Fondazione Centro San Raffaele, Milano, Italy

Background: Multiple myeloma (MM) originates from a clone of transformed plasma cells (PCs) that establish vicious interactions with the multicellular bone marrow (BM) environment. Disease progression depends on the ability of malignant PCs to subvert such environment to reshape host immunity and support tumor growth.
Aims: To evaluate how environmental signals, such as arginine deprivation, can modulate p62 and IRF4 in MM.
Methods: Little being known on metabolic changes during MM progression, we first deployed a comprehensive metabolic analysis at different stages of disease. We adopted ultra-high performance liquid and gas chromatography followed by mass spectrometry (UHPLC/GC-MS) on an independent series of 167 samples of BM and peripheral plasma collected *ad hoc* from 125 individuals, and age-matched healthy volunteers (n=29). Upon exclusion of drug metabolites and xenobiotic compounds, 284 endogenous metabolites were processed by PCA (unsupervised) and OPLS-DA (supervised) multivariate analyses to identify myeloma-associated metabolites. Next, we explored the expression levels of key metabolic genes in a large series of highly purified BM PC samples from healthy donors (n=4) and patients with MM (n=129), primary PC leukemia (pPCL, n=24), and secondary PC leukemia (sPCL, n=12) from a proprietary dataset (GSE66293).
Results: Metabolomics revealed the presence of robust metabolic differences in the progression from MGUS to MM, sustained by reduced amount of lysolipids and selected amino acids. In particular, we found MM progression to correlate with shortage of arginine, a well-established tolerogenic signal. These findings were validated by HPLC and ELISA in an independent cohort confirming the association of arginine deprivation with progression from MGUS through MM. Moreover, enolase-1 (ENO-1), phosphoglycerate kinase 1 (PGK-1), and dihydrolipoamide dehydrogenase (DLD) were increased upon progression, suggesting that branched chain amino acids, alpha-ketoglutarate, and glycine cleavage products are used in MM to sustain NADH availability and energy production. Using arginine deprivation and pharmacological inhibitors of glucose metabolism (2-deoxyglucose) and GCN2 signalling (ISRIB) we collected evidence that MM cells generally deploy TCA and oxidative phosphorylation, with glucose catabolism as a major source of ATP. Next, we sought to determine whether the adaptation to amino acid depletion through autophagy altered cellular glucose dependence in three MM cell lines chosen for their cytogenetic alterations: U266 (t 11;14); H929 (t 4;14) and MM.1s (t 14;16). Progressive arginine deprivation (1 μM-10 nM) did not affect proliferation *in vitro*, even in low-glucose media. However, long-term arginine deprivation, or treatment with human recombinant arginase-1 (which reduced extra-cellular arginine availability within 12 hours of exposure), altered the cellular dependence on mitochondrial ATP generation via oxidative phosphorylation, increased glutamine anaplerosis, and induced increased expression of p62 and IRF4 in a time-dependent manner. In primary MGUS and MM samples, we identified $CD11b^+CD33^+CD15^+CD14^-HLA\text{-}DR^-$ myeloid cells as the chief source of Arg-1, which was not expressed by T-cells, $CD11b^+CD33^+CD15^-CD14^+HLA\text{-}DR^-$ myeloid cells or PCs.
Summary/Conclusion: Our study implicates arginine shortage, likely accounted for by increased Arg-1 in myeloid cells, in MM progression. Reduced arginine availability in the BM may promote a large-scale metabolic adaptation of MM to the microenvironment, sustaining tumor growth and offering new potential targets for synthetic lethality.

PS1273

DEMETHYLATION SENSITISES T(4;14) MULTIPLE MYELOMA TO RAS-MAPK PATHWAY INHIBITION

J. Hocking[1,2,*], A. Spencer[1,2], S. Mithraprabhu[2], T. Khong[2]

[1]Malignant Haematology and Stem Cell Transplant, Alfred Hospital, [2]Australian Centre for Blood Disease, Monash University, Melbourne, Australia

Background: The t(4;14) translocation is present in 15% of multiple myelomas. It juxtaposes the MMSET/FGFR3 locus on chromosome 4 with the IgH gene on chromosome 14 resulting in dysregulation of both MMSET and FGFR3. It defines a high risk subset of multiple myeloma with worse outcomes and shortened overall survival. FGFR3 is a receptor tyrosine kinase that inputs via the RAS-MAPK, and is considered mutually exclusive with activating mutations of RAS (RAS^M). MMSET is a histone methylator and thought to act predominantly through epigenetic modification.
Aims: We investigated the impact of trametinib (MEK inhibitor) with and without azacitidine (AZA) pre-treatment (hypomethylating agent) on human myeloma cell lines (HMCLs) harbouring t(4;14) or neither t(4;14) nor RAS^M (WT). We aimed to identify mechanisms of action of AZA treatment that may sensitise HMCLs to MEK inhibtion.
Finally we evaluated the combination of AZA and trametinib in our murine xenograft model to determine effects on disease kinetics and survival.
Methods: Three t(4;14) HMCLs with FGFR3 overexpression (19-500 fold overexpression by RT-PCR c/w WT HMCLs) and 2 WT HMCLs were pre-treated with AZA (200nM) daily for 7 days followed by trametinib (10nM, 100 nM, 1 μM) for 72 hours. Proliferation, cell viability and cell cycle were evaluated by propidium iodide based flow cytometry. RNA was isolated pre- and post-AZA treatment for RNA sequencing using the HiSeq2500 platform to identify gene epxression changes that may impact on MEK inhibitor sensitivity. A second set of HMCLs harbouring the t(4;14) was tested to validate the initial results. Mechanistic studies were undertaken to evaluate the effects on histone methylation (targets of MMSET). The effects on known targets of AZA - DNMT3a and DNMT3b were also evaluated. DNMT3b siRNA knockdown was undertaken in one t(4;14) HMCL. Murine xenograft studies of a t(4;14) and WT were performed to evaluate the combination of azacitidine and trametinib *in vivo*.
Results: Neither the AZA (200 nM) pre-treatment alone nor single agent trametinib resulted in any significant effect on either group of HMCLs in all outcomes measured. Conversely trametinib exposure following AZA pre-treatment resulted in a significant reduction in proliferation ($p<0.002$) in all t(4;14) HMCLs at all doses. The most profound reduction in proliferation of 55-92% ($p<0.002$) was seen at 1 μM. Similarly following trametinib (1 μM) a relative increase of 25-55% of cells in the pre-apoptotic phase of the cell cycle was observed in 2 of 3 t(4;14) HMCLs. These results were recapitulated in the validation set of t(4;14) HMCLs. Interestingly, no significant changes in all outcomes measured were observered in the WT HMCLs despite pre-treatment with AZA. We found that t(4;14) HMCLs overexpress DNMT3b compared with WT HMCLs ($p<0.0001$). Whilst no difference was observed in expression of DNMT3a. No consistent change or effect was observed on the histone methylation marks at H3K27 or H3K36. Finally, in our murine xenograft pre-treatment with AZA then trametinib resulted in slower disease growth kinetics and an increase in overall survival in mice with t(4;14) treated with the combination compared with either single agent and in all outcomes compared with WT.
Summary/Conclusion: The combination of AZA and trametinib is effective against high risk t(4;14) MM representing a potential novel targeted therapeutic approach that warrants further investigation.

PS1274

CD96 (TACTILE) NEGATIVELY REGULATES THE CYTOTOXIC FUNCTIONS OF NATURAL KILLER CELLS AGAINST MULTIPLE MYELOMA

T. Duggan[1], S. Sarkar[1,2], S. Chauhan[1,2], J. Daly[1], M. O'Dwyer[1,2,*]

[1]Apoptosis Research Centre, National University of Ireland, [2]Onkimmune Ltd., Galway, Ireland

Background: Cellular immunotherapeutic approaches, including Natural Killer (NK) cell therapy, have recently shown very promising results in the treatment of blood cancers, including Multiple Myeloma (MM). CD96 (TACTILE) is a newly identified inhibitory checkpoint receptor expressed on the surface of NK cells. Ligand binding studies have shown that CD96 competes with CD226 (DNAM-1), a co-activating receptor on the surface of NK cells. This competitive interaction between CD96 and CD226 for binding to tumour bound CD155 could influence the cytotoxic activity of NK cells. Although the precise role of CD96 in regulating NK cells is not clearly understood, recent studies have shown that blocking CD96 with a monoclonal antibody can reduce tumour cell metastasis in murine models of solid tumours. In contrast, blocking CD96 receptors on human NK cells *in vitro* have shown conflicting results, with CD96 acting as an activating or inhibitory receptor depending on the tumour subtype.
Aims: In this study, our aim was to explore for the first time the immunomodulatory functioning of CD96 in mediating human NK cell cytotoxicity against MM.
Methods: CD155 expression was determined on a panel of cell lines MM1S, RPMI-8226, JJN3, H929, and U266. CD96 surface expression of KHYG1 NK cell line was knocked-down by nucleofection with either scrambled control (SC) siRNA or CD96 siRNA (GE, cat: L-020045-02) using AMAXA Nucleofector II. 72 hours post-nucleofection, the cells were analysed for surface CD96 expression and subsequently co-cultured with MM cell lines. Functional assays were performed with MM cell lines at E:T ratios of 0.125:1, 0.25:1, 0.5:1, 1:1. 2:1 and 4:1.NK cell-induced cytotoxicity was measured by FACS-based methods. KHYG1 NK cells were cultured either

at normoxia (21% O_2) or hypoxia (1% O_2) for 72 hours to determine CD96 expression.
Results: Immunophenotyping revealed that MM cell lines have a broad spectrum cell surface expression of CD155, the ligand for CD96, and therefore we classified them as CD155-*high* (JJN3), CD155-*mod* (U266, MM1S, RPMI-8226), and CD155-*low* (H929). Thereafter cytotoxicity assays were performed against a panel of MM cell lines (JJN3, U266, and H929) with control SC siRNA KHYG1 or CD96 siRNA KHYG1. CD96 siRNA KHYG1 was significantly more cytotoxic towards the moderately expressing CD155 cell line U266 at E:T 1:1, 2:1, and 4:1 as compared to SC siRNA KHYG1. Furthermore, this increase in cytotoxicity was also observed against the CD155-*high* cell line JJN3 at E:T 0.5:1 and 4:1. Interestingly, no significant increase in cytotoxicity was observed for the CD155-*low* cell line H929, suggesting the presence of the CD155 ligand on the tumour cell surface is necessary for CD96 mediated impairment of NK cell cytotoxic function. Furthermore, CD96 expression was significantly up-regulated under hypoxia as compared to normoxic culture conditions.
Summary/Conclusion: This study demonstrates the immunosuppressive function of the CD96 receptor during human NK cell-mediated cytotoxicity against MM *in vitro*. The contribution of this inhibitory signalling could be of paramount significance considering its upregulation under hypoxia. The enhanced cytotoxicity observed upon knockdown of CD96 could be of further therapeutic benefit during treatment with Bortezomib, as this is known to upregulate DNAM-1 ligands (CD155). Finally, we propose that siRNA based targeting of CD96 using clinical grade electroporation systems (e.g. MaxCyte GT) on therapeutically administered NK cells could significantly improve the clinical efficacy of contemporary NK cell-based treatments.

PS1275

GLUTAMINE-DEPENDENCE TARGETING BY ASPARAGINASE SIGNIFICANTLY INCREASES ANTI-MM ACTIVITY OF PROTEASOME INHIBITION

P. Minetto[1,*], D. Soncini[1], A. Cagnetta[1], F. Guolo[1], V. Retali[1], S. Ruberti[1], G. Rivoli[1], N. Bisso[1], N. Di Felice[1], M. Miglino[1], L. Canepa[1], M. Gobbi[1], R.M. Lemoli[1], M. Cea[1]

[1]Clinic of Hematology, Department of Internal Medicine (DiMI), University of Genoa, Policlinico San Martino, IRCCS per l'Oncologia, Genoa, Italy

Background: Human tumors exhibit high dependence on Glutamine (Gln) for their metabolic processes and macromolecule synthesis, condition that has been defined as "Gln-addiction". The role of such vulnerability has been recently investigated also in Multiple Myeloma (MM) cells, with Gln uptake exploited as novel therapeutic target. Indeed, MM cells by exhibiting significant lack of Gln synthetase become highly sensitive to Gln depletion as reported with L-asparaginase (ASNase), a bacteric enzyme that by degradating circulating asparagine and glutamine leads to intracellular aminoacids depletion.
Aims: Based on these data, here we explored the anti-MM activity of *Erwinia chrysantemi*-derived L. Asparaginase (ASNase; 10-folder higher glutaminase activity than its native formulation) in combination with carfilzomib (KAR).
Methods: The IC_{50} value of both E. chrysantemi ASNase and KAR was evaluated in a panel of HMCL carrying a different genetic background. Next, we found that low doses of KAR enhances significantly the anti-MM activity of ASNase. The effect of such combination was also tested in presence of IL6 and IGF-1, which mimics BMSCs milieu. Cell death analysis was measured with Annexin V/Propidium Iodide (AV/PI) staining followed by flow-cytometric analysis. The efficacy of co-treatment was confirmed by treating freshly isolated CD138 positive cells obtained from newly diagnosed MM patients (NDMM). Finally, western blot analysis was employed to fully elucidate mechanisms causing the observed synergism.
Results: ASNase treatment showed potent cytotoxic activity in all HMCL lines tested with an IC_{50} value ranging from 0,03 U/mL to 0.3 U/mL. High sensitivity was also observed on primary MM cells obtained from NDMM (IC_{50} =0.3 U/mL). Importantly, anti-MM activity of ASNase was further increased by adding low doses of the irreversible proteasome inhibitor KAR with a Combination Index value less than 1 in almost all tested drug-concentrations. Similar data were observed in tumors cells obtained from NDMM patients. Of note, the combination ASNase plus KAR resulted more potent than single agent treatment also in culture condition of Gln deprivation, suggesting that ASNase toxicity is not only related to its ASN-ase glutaminase activity. IL6 or IGF-1 addition did not reduce anti-MM activity of ASNase plus KAR, indicating that BMSCs microenvironment does not reduce its activity. A time and dose-dependent activity of co-treatment was also observed, with higher efficacy measured at 48 h compared with 24h of drugs exposure. Caspase-3 and PARP cleavage was observed in co-treated cells and the addition of the pan-caspase inhibitor Z-VAD-FMK reverted the cytotoxic effects of the combination revealing the role of apoptosis in observed synergism. Authophagic features, including LC3II cleavage, were observed in tumor cells treated with ASNase; the addition of authophagy inhibitor 3-Methyladenine was not able to revert the cytotoxic effect of the combination.
Summary/Conclusion: Gln dependence represents an attractive therapeutic target to be exploited in plasma cells disorders. Our preliminary data show that *E. chrysantemy* derived asparaginase exerts a potent anti-MM activity by affecting neoplastic cells metabolism. This activity is further increased by the addition of the irreversible proteasome inhibitor carfilzomib mainly through an apoptotic-mediated mechanism while autophagy plays a cytoprotective role in L-asp-treated plasmacells.

PS1276

MITOCHONDRIAL ACTIVITY PLAYS A CRITICAL ROLE IN MULTIPLE MYELOMA RESISTANCE

A. Ortiz[1,*], Y. Ruiz-Heredia[2], M. L. Morales[2], C. Barcenas[2], R. M. García-Martin[2], V. Garrido[2], I. Baquero[2], R. Alonso[2], J. Martínez-López[2], M. Linares[1], M. Gallardo[1]

[1]CNIO, [2]Hospital 12 de octubre, Madrid, Spain
**MG and ML contribute equally to this manuscript*

Background: Mitochondria control crucial biological pathways such as proliferation, apoptosis and cell growth. Some studies evaluated the role of mitochondria in cancer, demonstrating its relation to cancer formation and growth. However, the implication of mitochondrial activity in the pathogenesis of Multiple Myeloma (MM) stills remains unknown and only a few studies connect the mitochondria status and MM.
Aims: We have studied the impact of mitochondrial genes, protein expression, and activity in MM progression and treatment resistance. Furthermore, we have studied the potential exploitation of mitochondrial activity as a functional target in the MM therapy.
Methods: We have performed gene expression studies by RT-PCR of known factors that regulate and are involved in the mitochondrial function such as: c-Myc, transcription factor A mitochondrial (TFAM), elongation factor thermo unstable (EF-Tu), nuclear respiratory factor 1 (NRF1) and hnRNPK. We have studied a total of 34 cDNA sample patients (12 MGUS, 15 MM at Diagnosis, 7 MM at relapse). In order to validate gene expression results, we developed an immunohistochemistry (IHC) assay of COX II, representative protein of mitochondrial burden. We evaluate a total of 49 patients (9 MGUS, 20 MM at Diagnosis, 20 MM at relapse). Next we analyzed the mitochondrial activity with the study of COX2 histoenzymatic reaction in 15 patients (2 MGUS, 9 MM at Diagnosis and 4 MM at relapse). Finally, we have tested the effect in plasma cells of Metformin and Tigecycline, two drugs that were demonstrated to inhibit the mitochondrial activity, in monotherapy and in combination with Bortezomib over four MM cell lines (JJN3, L363, NCI-H929 and NCI-H929 R20). Besides, we analyze the mitochondrial DNA (mtDNA) burden in these cell lines. Our further studies include *in vivo* validation in NSG mice models of results obtained *in vitro*.

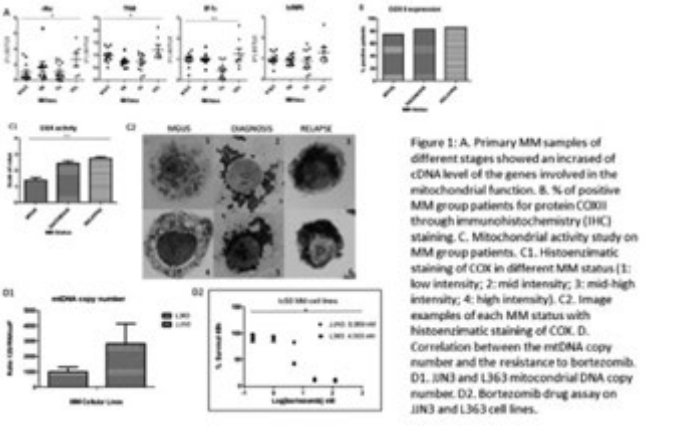


Figure 1: A. Primary MM samples of different stages showed an incrased of cDNA level of the genes involved in the mitochondrial function. B. % of positive MM group patients for protein COXII through immunohistochemistry (IHC) staining. C. Mitochondrial activity study on MM group patients. C1. Histoenzimatic staining of COX in different MM status (1: low intensity; 2: mid intensity; 3: mid-high intensity; 4: high intensity). C2. Image examples of each MM status with histoenzimatic staining of COX. D. Correlation between the mtDNA copy number and the resistance to bortezomib. D1. JJN3 and L363 mitocondrial DNA copy number. D2. Bortezomib drug assay on JJN3 and L363 cell lines.

Figure 1.

Results: We have observed a significant overexpression of genes C-Myc, TFAM, EF-Tu, and a higher expression trend of hnRNPK in MM relapsed patients compared with MGUS and newly diagnose MM groups (p-value *<0.05; p-value **<0.001) (Figure 1A). Moreover, IHC reveals overexpression of mitochondrial COXII protein in newly diagnose MM and relapsed

groups compared with MGUS (Figure 1B). By a functional assay we have demonstrated that gene and protein overexpression drives to an increase of activity, comparing MGUS and MM at diagnosis versus MM at relapse (p-value ***<0.0001) (Figure 1C). We confirmed the correlation between higher mitochondrial burden and resistance to bortezomib in JJN3 and L363, and NCI-H929 and its resistant, NCI-H929 R20 (p-value*<0.05) (Figure 1D). *In vitro* drug assays showed a synergistic effect of tigecycline with bortezomib, suggesting that could be used as a potential therapy in combination for MM patients.
Summary/Conclusion: Mitochondrial machinery plays a critical role in the development, progression and resistance of MM patients. Mitochondrial protein components that generates the activity could be prospective targets for MM treatment. Tigecycline demonstrates synergistic effect with Bortezomib suggesting potential use as novel drug combination therapy in MM patients.

PS1277

ELOTUZUMAB PROMOTES SELF-ENGAGEMENT OF SLAMF7 BETWEEN NATURAL KILLER AND MULTIPLE MYELOMA CELLS TO ENHANCE CYTOTOXICITY

T. Pazina[1], A.M. James[2], A. Jhatakia[3], R.F. Graziano[3], N.A. Bezman[3], M. D. Robbins[3,*], A.D. Cohen[4], K. S. Campbell[1]

[1]Fox Chase Cancer Center, [2]American Association for Cancer Research, Philadelphia, [3]Bristol-Myers Squibb, Princeton, [4]University of Pennsylvania, Philadelphia, United States

Background: Elotuzumab is an immunoglobulin G1 monoclonal antibody targeting signaling lymphocytic activation molecule family member 7 (SLAMF7), which is highly expressed on multiple myeloma (MM) cells, natural killer (NK) cells, and, to varying degrees, other immune cells. Preclinical reports show that elotuzumab promotes potent NK cell–mediated antibody-dependent cellular cytotoxicity via Fc interaction with FcgRIIIA (CD16), resulting in killing of SLAMF7+ MM cells, which is further enhanced in combination with lenalidomide. Previous preclinical studies also suggest that elotuzumab can enhance NK cell activity via a costimulation mechanism independent of CD16 binding. In patients with relapsed/refractory MM, elotuzumab combined with lenalidomide and low-dose dexamethasone improves progression-free survival.
Aims: To characterize how elotuzumab affects interactions between NK and MM cells to enhance NK cell–mediated cytotoxicity.
Methods: We generated 2 MM cell lines (MM.1R and RPMI8226) modified to express low or high levels of SLAMF7, as well as the SKOV3 ovarian adenocarcinoma cell line transduced to express SLAMF7. CRISPR/Cas9 technology was used to generate a SLAMF7-deficient human NK-92 cell line. Variants of this SLAMF7-deficient line and an NK-92 line expressing high levels of SLAMF7 that expressed or lacked CD16 were also generated. Cytotoxicity was measured with CytoTox 96® (Promega) and xCELLigence® real-time cell analysis (ACEA Biosciences) platforms. A human SLAMF7 extracellular/T-cell receptor [TCR]-zeta intracellular fusion construct was transfected into a 3A9 mouse T-cell line and used as a reporter of SLAMF7 engagement by measuring interleukin-2 production using an enzyme-linked immunosorbent assay.
Results: Consistent with previous reports, addition of elotuzumab strongly enhanced cytotoxicity of CD16-expressing SLAMF7+ NK-92 cells against SLAMF7+ MM and SKOV3 cells. Elotuzumab also substantially boosted cytotoxicity by CD16-deficient SLAMF7+ parental NK-92 cells toward SLAMF7+ RPMI8226 and SKOV3 cells. Knockout of SLAMF7 on parental NK-92 cells, however, abrogated elotuzumab-mediated cytotoxicity toward SLAMF7+ SKOV3 targets. Additionally, by using a SLAMF7/TCR-zeta–expressing reporter cell line and plate-bound recombinant SLAMF7, we found that elotuzumab promoted reporter activity, suggesting it may facilitate or enhance SLAMF7–SLAMF7 interactions. Interestingly, other anti-SLAMF7 antibodies were ineffective in stimulating reporter activity.
Summary/Conclusion: We conclude that elotuzumab has an additional function as an NK cell–activating antibody. SLAMF7 naturally engages with itself in homotypic interactions. Elotuzumab uniquely promoted NK cell–mediated cytotoxicity in a CD16-independent manner, but only if both NK and target cells expressed SLAMF7. This suggests that elotuzumab can facilitate or enhance SLAMF7–SLAMF7 interactions between NK cells and MM targets. As elotuzumab is clinically used in combination with lenalidomide, additional studies are needed to understand the impact of combination treatment on elotuzumab-mediated SLAMF7–SLAMF7 interactions. Based on these preclinical observations, SLAMF7 expression on NK cells warrants further investigation as a potential biomarker for elotuzumab efficacy.

PS1278

CARFILZOMIB-INDUCED CARDIOTOXICITY: MOLECULAR MECHANISMS AND THE EMERGING ROLE OF METFORMIN AS A PROPHYLACTIC THERAPY

G. Kremastiotis[1], P. Efentakis[1], A. Varela[2], C. H. Davos[2], E.-D. Papanagnou[3], I. P. Trougakos[3], E. Kastritis[4], Z. Kanaki[2], E. Iliodromitis[5], A. Klinakis[2], M.A. Dimopoulos[4], I. Andreadou[1], E. Terpos[4,*]

[1]Laboratory of Pharmacology, Faculty of Pharmacy, National and Kapodistrian University of Athens, [2]Biomedical Research Foundation Academy of Athens, [3]Department of Cell Biology and Biophysics, Faculty of Biology, National and Kapodistrian University of Athens, [4]Department of Clinical Therapeutics, [5]Second Department of Cardiology, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece

Background: Carfilzomib (Cfz) is an irreversible proteasome inhibitor, which is used for the treatment of relapsed/refractory multiple myeloma (RRMM). In phase III trials, Cfz has been associated with higher cardiotoxicity and heart failure rates compared to the control treatment. Due to the severity of these adverse events and the lacking data regarding the induced cardiotoxicity, there is an imperative need for the elucidation and abrogation of the underlying mechanisms of Cfz-induced cardiotoxicity.
Aims: The aim of this study was to investigate the molecular mechanisms of Cfz-induced cardiotoxicity and to evaluate possible cardioprotective effects of concomitant medications based on our initial results.
Methods: Protocol 1: Male C57BL/6 mice, were randomized into: Control (/S 0.9%, n=7) and Cfz group (n=8). Based on the results showing below we also developed a second protocol using metformin (met); Protocol 2: Male C57BL/6 mice were randomized into: Control (/S 0.9%, n=8); Cfz (n=8) and Cfz+Met (n=10). Cfz (8 mg/kg ip) was administered every 48 hours in both protocols and Met (140 mg/kg po) every 24 hours for 6 days. Fastening glucose levels were monitored. At baseline and at the end of treatments mice underwent echocardiographic assessment. Animals were sacrificed and blood and myocardial tissue samples were obtained for the analysis of proteasome peptidases activity, protein phosphatase 2A (PP2A) activity and molecular signaling mechanisms. Protein kinase Akt, along with its downstream NO synthases; endothelial (eNOS) and inducible (iNOS), were identified as targets of possible endothelial dysfunction and inflammation. Moreover, the transcription factor FOXO1, downstream target of Akt and AMPKα, was identified in order to investigate possible changes in the expression of apoptotic factors. Finally, AMPKα was identified since – besides phosphorylating eNOS and FOXO1 – it functions as a regulator of autophagy.
Results: Administration of Cfz resulted in significant reduction of the chymotrypsin-like (CT-L) proteasome activity in myocardial tissue and peripheral blood mononuclear cells of Cfz-treated mice vs controls (p<0.01). Protocol 1: Reduction in fractional shortening (FS%) was observed in the Cfz group vs Control at Day 6 (39.87±0.47% vs 42.05±0.64% respectively, p<0.05). Cfz increased PP2A activity vs Control (p<0.05), without altering PP2A expression. A decrease in pAkt/tAkt (p<0.05), peNOS/teNOS (p<0.05), pAMPKα/tAMPKα (p<0.001) and an increase in the expression of iNOS (p<0.01) was observed in the Cfz group vs Control. Protocol 2: Met did not reduce fastening glucose levels at day 6 in Cfz+Met compared to Control and Cfz groups. Echocardiographic assessment at day 6 revealed that Met reversed Cfz-induced reduction in the FS% in Cfz+Met vs Cfz group (43.4±0.5% vs 41.5±0.4% respectively, p<0.05). AMPKα phosphorylation was significantly increased in the same group compared to Cfz group (p<0.01).
Summary/Conclusion: The present study demonstrates that Cfz induces cardiac dysfunction via increasing PP2A activity, leading to decreased phosphorylation of Akt, eNOS and AMPKα. The disturbance of Akt/AMPKα/eNOS axis and the increase of iNOS, suggests that Cfz might intervene with oxidative stress, apoptosis and myocardial energetic pathways. Thus, Cfz-induced increase in PP2A activity seems to be essential in the mechanism of cardiotoxicity. Met restored AMPKα phosphorylation and reversed Cfz-induced contractile dysfunction, emerging to be a potent pharmacological intervention for the management of Cfz-induced cardiotoxicity.

PS1279

JAGGED1/2 INHIBITION PROMOTES TUMOR CELLS RESPONSE TO BORTEZOMIB IN A ZEBRAFISH MODEL OF MULTIPLE MYELOMA

M. Colombo[1,*], M. Mazzola[2], R. Colella[1], S. Garavelli[1], M. T. Palano[1], D. Giannandrea[1], N. Platonova[1], A. Neri[3], A. Pistocchi[2], R. Chiaramonte[1]

[1]Health Sciences, [2]Medical Biotechnology and Translational Medicine, [3]Oncology

and Hemato-oncology, Università degli Studi di Milano, Milano, Italy

Background: Multiple myeloma (MM) is the second most frequently diagnosed hematological malignancy and today is still incurable, mainly due to the development of drug resistance that causes relapse and contributes to the fatal outcome of this disease. MM cells accumulate in the bone marrow (BM) and establish complex interactions with the surrounding normal cells, forcing them to assume a pro-tumor behavior. In this process, a key role is played by the two Notch ligands Jagged1 and 2, whose dysregulated expression causes an aberrant activation of the Notch pathway both in MM cells and in the BM niche cells.
Aims: We aimed to validate the effect of Jagged1/2 silencing on MM cells resistance to the standard-of-care drug Bortezomib by: i) *in vitro*, on co-culture of MM cell lines and BM stromal cells (BNSC); ii) *ex vivo*, on co-culture of primary cells from MM patients and BMSC; iii) *in vivo*, using a zebrafish xenograft model of MM that allows a rapid and reliable screening of MM cells response to chemotherapics.
Methods: Jagged1/2 expression was inhibited transiently in MM cell lines using specific siRNAs and constitutively in primary MM cells using a lentiviral vector that encodes specific shRNAs. Cells were cultured alone or co-cultured with BMSC and treated with 8nM Bortezomib. Apoptosis was evaluated by Annexin V staining and flow cytometry. For *in vivo* experiments cells were stained with the CM-Dil vital dye, resuspended in PBS + 3% polyvinyl pyrrolidone and injected in the yolk area of 2dpf (days post fertilization) zebrafish embryos. Injected embryos were treated with 10μM Bortezomib or DMSO for 48h. MM cells growth in zebrafish was evaluated by fluorescence microscopy and tumor area were calculated using ImageJ and normalized on tumor volume at the time of injection.
Results: Jagged1/2 blockade reduces MM cells ability to induce Notch activation in BMSC, causing a decrease in their capacity to sustain MM resistance to Bortezomib. Results obtained *in vitro* on MM cells lines were further validated on co-culture of primary CD138+ cells and BMSC from newly diagnosed MM patients. The analysis performed on xenotransplanted embryos showed that the treatment with 10μM Bortezomib caused a decrease of about the 50% in tumor growth in comparison to DMSO-treated controls, with no effect on embryos viability. Jagged1/2 knockdown alone has a comparable effect to Bortezomib, while the combination of Bortezomib and Jagged1/2 inhibition results in a stronger decrease in tumor growth of about the 75% in comparison to the vehicle-controls.
Summary/Conclusion: Our findings demonstrate that Jagged1/2 inhibition represents a suitable strategy to promote MM response to the standard of care drug Bortezomib, contrasting BM-induced drug resistance.

PS1280

TGFB INHIBITION IN COMBINATION WITH CHEMOTHERAPY REPAIRS EXISTING LYTIC BONE LESIONS IN A NOVEL PLATEAU PHASE MODEL OF MULTIPLE MYELOMA

A. Chantry[1,*], G. Alanna[1], D. Jenny[1], E. Holly[1], J. Paton-Hough[1], S. John[1], L. Michelle[1]

[1]Oncology and Metabolism, University of Sheffield, Sheffield, United Kingdom

Background: Multiple myeloma (MM) causes a destructive bone disease in >85% of patients and current therapies do little to repair existing bone damage. We previously identified that combined bone anabolic and anti-resorptive therapy repairs osteolytic lesions in mice with high tumour load. In patients, if bone repair agents were given, they would be administered in combination with chemotherapy.
Aims: This study aimed to determine if bone recovers after chemotherapy and if this is enhanced by bone anabolic therapy.
Methods: Human U266-GFP-luc MM cells were i.v. injected into NSG mice (n=5-7/group). After tumour and lytic bone lesion development, mice were administered first-line chemotherapeutics (bortezomib±lenalidomide)±a bone anabolic (SD208; transforming growth factor β receptor 1 inhibitor) or vehicles for 2 weeks. Tumour and bone lesions were monitored *in vivo* by bioluminescence imaging (BLI), serum paraprotein ELISAs and μCT. Flow cytometry, histomorphometry, μCT, TRAP and P1NP ELISAs and QPCR were performed for endpoint analyses.
Results: Chemotherapy significantly reduced total body tumour burden and paraprotein, and increased survival. Combined chemotherapy was more effective than either given alone, reducing tumour to levels undetectable by BLI and paraprotein. However, flow cytometry revealed low tumour levels of 100MM cells/10^6 bone marrow cells. Lytic bone lesions developed ~8 weeks after tumour inoculation. Vehicle treated mice exhibited progressive bone lesion development and virtually no trabecular bone at endpoint. Lesions in mice administered bortezomib±lenalidomide were unchanged after 1 week but began to repair after 2 weeks, with significantly reduced TRAP+ osteoclasts and increased osteoblasts, indicating recovery of bone. Mice treated with chemotherapy + anabolic SD-208 exhibited enhanced repair of bone lesions, with partial repair of perforating cortical lesions on all tibial surfaces within 1 week and complete repair of lesions within 2 weeks. SD-208 also significantly increased trabecular bone volume after 2 weeks.
Summary/Conclusion: This study identified SD-208 enhances MM bone lesion repair when combined with first-line chemotherapeutics. Future studies combining SD-208 and chemotherapy with anti-resorptive therapy will identify optimum treatment regimens for translation of bone anabolic therapy into MM clinical trials.

PS1281

MULTIPLE MYELOMA: SINGLE PLARFORM ABSOLUTE COUNT OF CIRCULATING PLASMA CELLS AT DIAGNOSIS CORRELATE WITH POOR PROGNOSIS PARAMETERS

V. E. Muccio[1,*], M. Gilestro[2], E. Saraci[1], S. Spada[1], M. Ruggeri[1], S. Caltagirone[1], D. Oddolo[1], M. Cavo[3], V. Pavone[3], S. Ronconi[3], D. Vincelli[3], A.M. Cafro[3], C. Cellini[3], C. Musolino[3], S. Molica[3], A. Bernardini[1], F. Gay[1], P. Musto[3], M. Boccadoro[1], P. Omedé[2]

[1]Myeloma Unit, Division of Hematology, University of Torino, [2]Myeloma Unit, Division of Hematology, Città della Salute e della Scienza di Torino, Torino, [3]Italian Multiple Myeloma Network, GIMEMA, Italy, Italy

Background: Risk stratification of newly diagnosed multiple myeloma (NDMM) patients is based on clinical and laboratory parameters. In previous studies, circulating plasma cells (CPC) showed a significant correlation with more aggressive disease: CPC were detected by flow cytometry after separation by ficoll gradient, which can reduce the recovery of plasma cells, or by acquiring a defined total number of events, and the absolute CPC number was obtained using an automated hematology analyzer.
Aims: This is the first study to perform a single platform absolute count of CPC. We compared CPC with patients' baseline characteristics.
Methods: We collected 413 peripheral blood (PB) samples of NDMM patients enrolled in the UNITO-MM-01/FORTE. For the single platform tube, the antibody combination CD38PC7/CD138PC5.5/CD45KO/CD56PE/CD19PB was mixed with 100 μL of EDTA PB dispensed with reverse pipetting, added with 500 μL of lysing solution. After 15 min, 100μL of flow count were dispensed with reverse pipetting and acquired with Navios flow cytometer. In order to reduce the acquisition of cellular debris, a "live gate" was set on CD45/CD38 dot plot and all the events CD38 and CD45 negative were excluded. The CPC clonality was confirmed, in a second tube, by the determination of kappa and lambda light chains of intracytoplasmic immunoglobulins.
Results: CPC were detected in 390 of 413 samples (94.4%): median values were 0.03% (range 0%>51%), 2.37/mm^3 (range 0/mm^3-6272/mm^3), number of absolute CPC was 58 (range 0-441000); cellular events acquired 190000 (range 4428-1300000). Statistically significant higher values of CPC were found in samples from patients with poor prognosis features: Hb <10 g/dL, ISS stage III, R-ISS stage III, Albumin <3.5 g/dL, b2-microglobulin >5.4 mg/dL, LDH >upper limit, PC in biopsy ≥60%, presence of del13, ECOG 3 (all with a p value <0.001); ampl1, High Fonseca cytogenetic risk, High Morgan cytogenetic risk all with a p value <0.05.
A linear correlation was found between CPC and hemoglobin (r=-0.46 p<0.001), bone marrow aspirate plasma cells (r=0.36 p<0.001), plasma cells in biopsy (r=0.38 p<0.001), b2-microglobulin (r=0.25 p<0.001). CPC absolute values were sorted in quartiles (0/mm^3-0.86/mm^3, 0.86/mm^3-2.37/mm^3, 2.37/mm^3-11.2/mm^3, 11.2/mm^3-6272/mm^3) and associated with poor prognosis features. Significant differences expressed by Cramer's V >0.2 were observed between CPC and hemoglobin (V= 0.41 p<0.001), ISS (V= 0.26 p<0.001), R-ISS (V= 0.24 p<0.001), ≥60% of plasma cells in biopsy (V= 0.23 p<0.001), bone marrow aspirate plasma cells sorted in quartiles (V= 0.21 p<0.001), LDH upper the high limit (V= 0.24 p<0.001), del13q14 (V= 0.21 p=0.002), 1q gain (V=0.21 p=0.001).
Summary/Conclusion: The single platform cytometric method quantified CPC in 94.4% of PB samples from NDMM patients. Higher CPC number significantly correlated with poor clinical and laboratory features, confirming that CPC are an indicator of more aggressive disease, as showed by other studies. This method allows a high recovery of CPC, needs a small amount of PB sample, is a fast procedure, does not need cell separation, and is accurate. Moreover, it can be performed in all patients and can be particularly useful when cytogenetic score cannot be defined.

PS1282

MULTIPLE MYELOMA PATHOGENESIS: THE ROLE OF JUNB IN BONE MARROW ANGIOGENESIS

S. Malvestiti[1,*], F. Fan[1], H. M. Bashari[1], E. Morelli[2], A. Seckinger[3], D. Hose[3], H. Goldschmidt[3], I. Zoernig[1], L. Bakiri[4], M. Sattler[5], S. Vallet[1,6], M. Pecherstorfer[6], E.F. Wagner[4], P. Tassone[2], D. Jaeger[1], K. Podar[1,6]

[1]Medical Oncology, National Center for Tumor Diseases, University of Heidelberg, Heidelberg, Germany, [2]University "Magna Graecia" of Catanzaro, Catanzaro, Italy, [3]Section Multiple Myeloma, Department of Internal Medicine V, National Center for Tumor Diseases, University of Heidelberg, Heidelberg, Germany, [4]Genes Development and Disease Group, Spanish National Cancer Research Centre (CNIO), Madrid, Spain, [5]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, United States, [6]Department of Internal Medicine II, University Hospital Krems, Karl Landsteiner University of Health Science, Krems, Austria

Background: Angiogenesis is a hallmark of cancer. It importantly affects disease progression in multiple myeloma (MM) patients and correlates with adverse prognosis. Particularly, a significant rise in vascular density (VD) in the bone marrow (BM) is fundamental for the progression of MGUS to clinical manifest MM. The increase in vascularization is induced by oncogene-mediated expression of various angiogenic growth factors, most prominently VEGF. Over the past years, members of the AP-1 family of transcription factors (TFs) have been proposed as new potential therapeutic targets. Our previous work demonstrated a pivotal role for the AP-1 family member JunB in MM cell proliferation, survival and drug resistance (Fan *et al.*, 2017). However, whether JunB also affects MM BM angiogenesis still needs to be elucidated.
Aims: The present study aims to clarify the role of JunB in BM angiogenesis both *in vitro* and *in vivo*. One major objective is to evaluate whether production and secretion of angiogenic factors in MM cells within the BM microenvironment is JunB-dependent. Moreover, this project wants to assess the functional interrelation of JunB, IL-6 and other cytokines and growth factors. Further topics that have been addressed are the functional effect of genetic JunB downregulation and overexpression on BM angiogenesis and the specific role of JunB in BM angiogenesis in *in vivo* models of human MM in the BM microenvironment.
Methods: Expression patterns of JunB and angiogenic factors were evaluated in 2 independent Oncomine data sets. Co-cultures (CoCs) consisting of bone marrow stromal cells (BMSC) or osteoblasts (OB) and TetR-shJunB/MM.1S cells were performed in the presence and absence of doxycycline to investigate whether JunB knockdown interferes with paracrine induction of angiogenic factors and BM angiogenesis. Effects were assessed with RTqPCR, ELISA and *in vitro* angiogenesis assays. Obtained results were verified using JunB-ER/MM1.s cells, in which JunB activity is induced by 4-OHT. Data obtained in MM1.s models were confirmed using IL-6-mediated JunB stimulation or traditional JunB knockdown in other MM cell lines (e.g. MR20, RPMI8226, U266). Effects were assessed with RTqPCR. The functional role of JunB on BM angiogenesis was studied *in vivo* using MM xenograft mouse model. NSG mice were inoculated with TetR-SCR/MM.1S or TetR-shJunB/MM.1S and fed with doxycycline. IHC was then performed on tumor samples for Ki-67, anti-CD31 and anti-JunB.
Results: Similar to JunB Oncomine analysis revealed significant induction of VEGF, VEGFB, IGF-1 and PlGF expression progressing from healthy donors to MGUS and MM. We investigated next whether BMSC- and OB-induced production and secretion of angiogenic factors in MM is mediated via JunB. Knockdown of BMSC-mediated JunB upregulation in TetR-shJunB/MM.1S cells significantly reduces expression of VEGF, VEGFB, IGF-1 and PlGF. Subsequently, conditioned media obtained from doxycycline-treated BMSC: and OB:TetR-shJunB/MM.1S CoCs significantly inhibited angiogenesis. Conversely, 4-OHT-mediated induction of JunB activity potently enhanced the expression and secretion of angiogenic factors and angiogenesis. Data were confirmed in other MM cell lines (MR20, RPMI8226, U266). Furthermore, JunB inhibition in *in vivo* models induced a significant reduction in growth and VD in tumors formed by Tet-shJUNB/MM.1S versus control.
Summary/Conclusion: In conclusion, our findings show for the first time a pivotal role of JunB in MM bone marrow angiogenesis and thereby strongly support this transcription factor as a novel promising therapeutic target in MM treatment.

PS1283

TARGETED LIPOSOMAL MULTI-DRUG DELIVERY TO TUMOR-ASSOCIATED ENDOTHELIUM AS A NOVEL STRATEGY TO REVERSE MICROENVIRONMENT-INDUCED DRUG RESISTANCE IN MULTIPLE MYELOMA

C. Federico[1,*], B. Muz[1], F. Azab[1], P. de la Puente[1], J. Sun[1], K. Alhallak[1], J. King[2], V. Kapoor[1], A. Roccaro[3], D. Thotala[1], D. E. Hallahan[1], S. Achilefu[4], R. Vij[2], A.K. Azab[1]

[1]Department of Radiation Oncology, [2]Department of Medicine, Washington University in St. Louis School of Medicine, Saint Louis, United States, [3]Clinical Research Development and Phase I Unit, ASST Spedali Civili di Brescia, Brescia, Italy, [4]Department of Radiology, Washington University in St. Louis School of Medicine, Saint Louis, United States

Background: The bone marrow microenvironment (BMME) induces drug-resistance in multiple myeloma (MM). We have previously shown that the inhibition of CXCR4 signaling attenuates the interaction between MM cells and BMME, re-sensitizing MM cells to bortezomib (BTZ). Moreover, we found that inhibition of ROCK kinase blocked the interaction of MM with the BMME. Therefore, we propose to inhibit ROCK kinase as a novel strategy to sensitize MM cells to BTZ. However, the limitations of this strategy are: the kinetic synchronization of the delivery of both drugs and the specific delivery to the MM-BMME for limiting side effects. We found that the MM-associated endothelium significantly expresses high levels of P-selectin, which provides an opportunity for selective delivery to the BMME-associated MM.
Aims: To reverse BMME-induced BTZ resistance we developed P-selectin targeted liposomes towards MM-associated endothelium by decorating their surface with P-selectin glycoprotein ligand 1 (PSGL-1), the physiological ligand of P-selectin. To ensure synchronized delivery, liposomes were loaded with BTZ and ROCK inhibitor (ROCKI). Based on their solubility, BTZ and ROCKI were encapsulated in the lipid membrane and in the aqueous core, respectively.
Methods: We assessed the expression of P-selectin in endothelial cells (ECs) by flow cytometry analysis. Liposomes were prepared by thin layer evaporation method, followed by extrusion. Coupling of PSGL-1 to the liposomes was performed by carbodiimide chemistry. Liposomes were characterized by dynamic light scattering analysis and the entrapment efficiency (EE) of both drugs was quantified by HPLC analysis. The binding of PSGL-1-targeted liposomes to P-selectin was assessed by Biacore assay. Moreover, the *in vitro* binding to ECs and to ECs in the BM from MM-bearing mice was investigated by flow cytometry analysis. The effect of free or liposomal-ROCKI on migration and adhesion *in vitro* was tested by trans-endothelial migration assay and fluorescence spectroscopy. Cytotoxicity, proliferation, cell cycle, apoptosis and cytoskeletal signaling of the drugs in free or liposomal forms were evaluated by MTT and western blot. The effect of free or liposomal drugs on tumor progression *in vivo* was tested by live imaging.
Results: Expression of P-selectin in ECs isolated from MM patients was higher than healthy donors (3-fold), from MM-bearing mice compared to naïve mice (7-fold), and in MM-activated ECs compared to naïve ECs *in vitro* (7-fold). PSGL-1-targeted liposomes showed specific binding to immobilized P-selectin and to MM-activated ECs *in vitro* and *in vivo*. Loading of the liposomes with BTZ and/or ROCKI did not affect the structure and the physical parameters of the liposomes. The EE of the drugs was around 54% and 66%, respectively. MM cell adhesion to ECs, trans-endothelial migration and SRC, cofilin and FAK signaling were decreased by liposomal-ROCKI compared to free drug. Liposomal-BTZ induced significant reduction of cell viability, MAPK, PI3K, and cell cycle signaling; and induced pro-apoptotic signaling in MM cells compared to free BTZ. The combination of both drugs in the liposomal form reversed the BMME-induced resistance to BTZ *in vitro*, and induced a remarkable decrease in tumor growth, improved overall survival, and reduction of side effects compared to the free drugs.
Summary/Conclusion: Our data indicate that the synchronized and PSGL-1-targeted liposomal delivery of BTZ and ROCKI reversed the BMME-induced resistance and sensitized the MM tumors to BTZ better than the free non-synchronized non-targeted drugs, *in vitro* and *in vivo*.

PS1284

WNT/B-CATENIN AND HEDGEHOG INHIBITORS AS THERAPEUTIC APPROACHES IN B-CELL NEOPLASMS

C. Ferreira[1], J. Jorge[2,3], R. Alves[2,3], A.C. Gonçalves[2,3], A.B. Sarmento Ribeiro[2,3,4,5,*]

[1]Chemistry Department, University of Aveiro, Aveiro, [2]Laboratory of Oncobiology and Hematology and University Clinic of Hematology, Faculty of Medicine, University of Coimbra (FMUC), [3]Coimbra Institute for Clinical and Biomedical Research (iCBR) - Group of Environment Genetics and Oncobiology (CIMAGO), FMUC, [4]Clinical Hematology Department, Centro Hospitalar e Universitário de Coimbra (CHUC), [5]Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra, Portugal

Background: B-cell neoplasms are a heterogeneous group of diseases that

include B-cell lymphomas and plasma cell disorders. Multiple myeloma (MM) is a malignant neoplasm originated by the proliferation of monoclonal plasma cells that remains incurable. Diffuse large B-cell lymphoma (DLBCL) is an aggressive lymphoma, with a fast growing that is rapidly fatal if untreated. Inappropriate activation of conserved embryonic signaling pathways critical for stem cell self-renewal and differentiation in hematopoiesis, such as WNT/β-catenin and Hedgehog (Hh), has been implicated in a number of cancers. Deregulation of components involved in WNT/β-catenin signaling had been implicated in a wide spectrum of diseases including B-cell neoplasms. The key mediator of WNT signaling, β-catenin, serves several cellular functions and its inhibition showed successful results in some cancers. On the other hand, abnormal Hh signaling triggered by mutations in Hh pathway, and paracrine or autocrine activation by Hh ligands, may induce cancer development or progression. Hedgehog pathway inhibitors have shown clinical benefit and have been approved to treat patients with advanced basal cell carcinoma.

Aims: The main goal of this study was to evaluate the therapeutic potential of WNT/β-catenin and Hedgehog inhibitors, respectively IWR-1 and vismodegib, alone and in combination in *in vitro* models of MM and DLBCL.

Methods: The therapeutic potential of IWR-1 and vismodegib, in monotherapy and in therapeutic combination, was evaluated in two *in vitro* models of MM and DLBC, the H929 and FARAGE cell lines, respectively. The metabolic activity was evaluated by resazurin assay and cell death by optical microscopy (May-Grunwald staining) and flow cytometry (FC; Annexin V/7-AAD double staining). Cell cycle analysis was evaluated by FC, using a PI/RNase solution. Proteins related to apoptosis (BAX, BCL2, caspases) and some molecules related to WNT and Hh signaling pathways [β-catenin and phosphorylated (p-)ERK] were quantified by FC. The results were statistically analyzed by uni- and multivariate tests, considering a level of significance of 95% ($p<0.05$).

Results: The results showed that IWR-1 and vismodegib reduced metabolic activity in a time-, dose- and cell type-dependent manner. H929 cells were more sensitive to IWR-1 (IC_{50} 40μM) than FARAGE cells (IC_{50} 75 μM), while FARAGE cells were more sensitive to vismodegib (IC_{50} 57 μM) compared to H929 (IC_{50} 70 μM). The reduction of metabolic activity was more pronounced in the combination regimens as well as in fractional administration of drugs compared to single-dose regimens, especially in MM cells. DLBCL cells were more sensitive to therapeutic combinations than MM cells. IWR-1 and vismodegib induced cell death mainly by apoptosis, associated with increased activated-caspases, and an anti-proliferative effect with cell cycle arrest in G_0/G_1 phase. In addition, both drugs induced a decrease in β-catenin and p-ERK expression levels, more pronounced in MM cells (H929).

Summary/Conclusion: The results suggest that MM cells are more sensitive to single inhibitor regimes and LDGCB cells to combination regimes. However, both drugs, IWR-1 and vismodegib, may represent potential therapeutic approaches in these lymphoid neoplasms.

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PS1285

MULTIPLE MYELOMA INDUCES CHANGES IN BONE MARROW ADIPOCYTES *IN VITRO* AND *IN VIVO*

M. Reagan[1,*], C. Falank[1], M. Farrell[1], M. McDonald[2], P. Croucher[2], H. Fairfield[1]

[1]Center for Molecular Medicine, Maine Medical Center Research Institute, Scarborough, United States, [2]Bone Biology Division, Garvan Institute of Medical Research, Sydney, Australia

Background: Multiple myeloma (MM) is a hematological malignancy that is characterized by clonal proliferation of transformed plasma cells within the bone marrow (BM) and severe bone disease. Although MM cells are initially sensitive to many therapies, patients eventually relapse with refractory disease. Because MM cells show a dependency on the BM microenvironment for survival and proliferation, and BM adipocytes (BMAs) demonstrate a unique, endocrine signaling capacity and lipid composition, it is likely that there is cross-talk between MM cells and BMAs that leads to tumor support.

Aims: We aim to identify the direct effect of MM cells on mature BMAs and on BMA progenitors in the early stages of adipogenesis. These data could be revolutionary for our understanding of MM.

Methods: Herein, we explored the direct effects of MM cells on adipocytes before and after adipogenic differentiation using microarrays, cytokine arrays, qRT-PCR, Oil Red O lipid staining, and seahorse metabolic analyses. 3T3-L1 pre-adipocytes or primary mouse or human BM-MSCs were differentiated into adipocytes and co-cultured with MM cell lines (MM.1S, MM.1R, 5TGM1, and OPM2). To investigate the effect of MM cells on MSC adipogenic differentiation capacity, pre-adipocytes were grown to confluency and then myeloma cells were added for a 3-day exposure period prior to adipogenic differentiation. Direct and indirect (MM conditioned media (CM) and transwell cultures) effects of MM cells on MSC adipogenesis were assessed. We also developed a novel, tissue-engineered (TE) 3-D *in vitro* MAT model, as published[1], and used this for co-cultures with MM cells. BMA changes were also analyzed with histology in the MM1S Scid-Beige and the 5TGM1 C57BL/*KaLwRij* mouse models.

Results: The influence of MM cells on BMAs before or after differentiation was readily observed in multiple assays. Indirect co-culture of 3T3-L1 adipocytes with MM cells significantly reduced both adipogenic transcription factors (*Pparγ* and *Cebpα*) as well as mature adipocyte markers (*Adipoq*). This effect was also observed in BM-MSCs differentiated into adipocytes from mouse (*Pparγ and Adipoq*) and human donors (*PPARγ* and *FABP4*), suggesting a common mechanism of MM-induced inhibition between peripheral adipocytes and BMAs. Lipids were also reduced in 3T3-L1 adipocytes in direct co-culture with MM.1S cells for 7-14 days. Co-culture with MM cells reduced the expression of genes involved in lipogenesis and fatty acid oxidation, including *Srebf1*, *Cpt1*, and *Fasn,* in many adipocyte cell types, suggesting a suppression of adipogenic function as well as phenotype. Importantly, similar delipidation was also observed in TE-MAT co-cultures with myeloma cells. *In vivo,* MM decreased BM adiposity in the MM1S/SCID-Beige and 5TGM1/KaLwRij models (Figure 1).

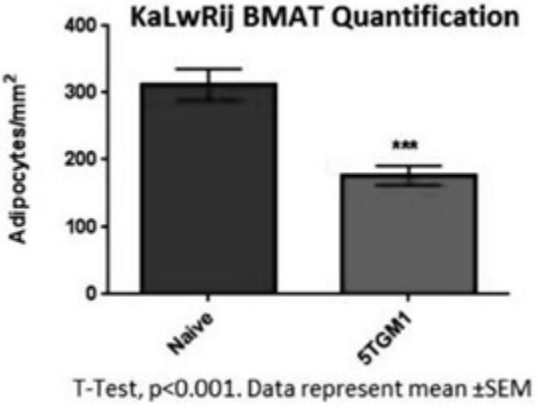

Figure 1.

Summary/Conclusion: These results indicate an adverse effect of MM cells on adipocytes and illuminate the BMA as a new member of the complex BM milieu in which the myeloma cells thrive. MM-induced reductions in BM adiposity may contribute to the skeletal changes induced by MM, i.e. suppressed bone formation and increased osteoclast activation. Our data support the need for further research into the roles of BMAT in MM pathogenesis, osteolysis and chemoresistance.

Reference

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PS1286

PROTEIN KINASE CK1 ALPHA MODULATES PROSURVIVAL AUTOPHAGY IN MULTIPLE MYELOMA

M. Carrino[1,2,*], S. Manni[1,2], L. Quotti Tubi[1,2], S. Canovas Nunes[1,2], K. Gianesin[1,2], G. Barilà[1,2], L. Trentin[1,2], R. Zambello[1,2], G. Semenzato[1,2], F. Piazza[1,2]

[1]Department of Medicine, Hematology and Clinical Immunology Branch, University of Padova, Padova, Italy, University of Padova, Padova, Italy, [2]Venetian Institute of Molecular Mediicine, Padova, Italy

Background: Multiple Myeloma (MM) is a tumor of antibody-secreting plasma cells (PCs) that accumulate in the bone marrow. As MM cells massively produce and secrete immunoglobulins, they are prone to endoplasmic reticulum stress, therefore the ubiquitin-proteasome system, the unfolded protein response and the autophagic pathway cooperate in order to avoid proteotoxicity. Autophagy is a fundamental process for both normal and malignant PCs and its impairment results in loss of PC differentiation and MM cell death. Recently, CK1α, a kinase critical for MM cell survival, was

described as a novel regulator of the autophagic pathway in RAS-driven cancers. CK1α, by phosphorylating S318/321 of the proautophagic transcription factor FOXO3a, promotes its nuclear exclusion, leading to a reduction in autophagic genes transcription.
Aims: In this study, we aimed at investigating the role of CK1α in the modulation of prosurvival autophagy in MM cells.
Methods: We inhibited CK1α with the chemical D4476 and we silenced CK1α through RNA interference (electroporation of ds CK1α siRNA or expression of an IPTG inducible CK1α shRNA). We generated MM cell clones stably expressing the mCherry-eGFP-LC3B fusion protein, which allows monitoring the autophagic flux, since double fluorescent (yellow) autophagosome become mCherry-only fluorescent (red) after their fusion with the lysosome due to an acidic pH dependent quenching of eGFP fluorescence. The effects of CK1α inactivation on autophagy related proteins were investigated by WB and qRT-PCR. The localization of FOXO3a was evaluated by nuclear proteins extraction and WB. Subcellular localization of LC3B and LAMP-2 was evaluated by immunofluorescence.
Results: In mCherry-eGFP-LC3B MM clones, upon treatment with D4476, yellow vesicles accumulated suggesting an impairment in the autophagic flux. In order to understand if autophagosomes were able to fuse with lysosomes, we stained autophagic vesicles and lysosomes respectively with an anti-LC3B and an anti-LAMP-2 antibody finding that autolysosomes were formed in D4476-treated cells, indicating that an alteration of the lysosomal pH was probably responsible of the impairment of the autophagic flux. D4476 treatment reduced FOXO3a phosphorylations, which accumulated in the nucleus, allowing the transcription of autophagic genes. Differently, upon CK1α silencing, the autophagic flux was induced as judged by the accumulation of red vesicles in mCherry-eGFP-LC3B MM clones. However, pFOXO3a S318/321 levels did not change and the transcription factor did not accumulate in the nucleus. As a consequence, the increase in transcription of autophagy-related genes was not observed.
Summary/Conclusion: We demonstrated that in MM cells the chemical inhibition or the silencing of CK1α induced apoptosis, however the two modalities of CK1α inactivation affected differently the autophagic pathway. A possible explanation is that D4476 causes accumulation of ineffective autophagic vesicles provoking an engulfment of the autophagic/lysosomal machinery that culminates in cell death. Differently, upon CK1α silencing, the autophagic flux is correctly triggered, however a genetic program backing up the autophagic flux is not activated. In this latter case, it is possible that proteins important in the autophagic machinery are progressively depleted and autophagy breaks down with the occurrence of cell death. Taken together our results suggest that CK1α supports MM cells survival also by modulating the prosurvival autophagic pathway.

PS1287

SERUM LIPID METABOLOMICS AS AN USEFUL BIOMARKER PREDICTING FOR THE EFFICACY OF BORTEZOMIB TREATMENT AND PERIPHERAL NEUROPATHY IN PATIENTS WITH MULTIPLE MYELOMA

M. Ri[1,*], K. Maekawa[2], M. Nakajima[3], A. Sekine[4], R. Ueda[5], M. Tohkin[6], N. Miyata[7], Y. Saito[8], S. Iida[1]

[1]Department of Hematology and Oncology, Nagoya City University Graduate School of Medical Sciences, Nagoya, [2]Department of Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Doshisha Women's College of Liberal Arts, Kyoto, [3]Drug Metabolism and Toxicology, Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa, [4]Center for Preventive Medical Science, Chiba University, Chiba, [5]Department of Tumor Immunology, Aichi Medical University School of Medicine, Nagakute, [6]Department of Regulatory Science, Graduate School of Pharmaceutical Sciences, [7]Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, [8]Division of Medicinal Safety Science, National Institute of Health Sciences, Kawasaki, Japan

Background: A comprehensive analysis of metabolites (metabolomics) in biofluids is a promising new strategy for liquid biopsy, as well as for biomarker analyses to predict the response to or adverse events of specific treatments. With respect to the metabolic pathways in multiple myeloma (MM) cells, only a few studies have applied metabolomics for the analysis of MM cells or serum/plasma samples from the patients to elucidate MM pathogenesis or the mechanisms underlying the transformation of MM cells. In addition, no metabolomic profile has been characterized for the response to or adverse events of MM treatments.
Aims: In this study, we performed a lipid metabolomics analysis of sera from patients with MM who received bortezomib (BTZ) plus low-dose dexamethasone (Bd) therapy to identify predictive markers for response to Bd therapy and the severity of BTZ-induced peripheral neuropathy (BiPN).
Methods: Sixty serum samples were collected prior to Bd therapy and subjected to lipid metabolomics analysis. Levels of phospholipids, sphingolipids, and neutral lipids and polyunsaturated fatty acids (FAs) and their oxidation products were measured using UPLC-TOFMS and UPLC/MS/MS, respectively. We then evaluated whether the levels of specific lipid metabolites were associated with the response to Bd therapy and the grade of peripheral neuropathy.
Results: Approximately 2000 ion peaks of phospholipids or triglycerides (PT) and 50 FAs were detected from the metabolomics analysis of 60 serum samples. In the 54 Bd samples evaluable of the response to Bd therapy, the levels of 46 PTs and 2 FAs were identified to be associated with the response. Among these, the levels of 2 FAs (TXB_2 and 12-HHT) were highly correlated with the response to Bd therapy: low levels of these 2 FAs were observed in poor responders, i.e. SD or PD patients. These 2 FAs were regulated by the same enzyme, thromboxane A synthase, which is anchored at the endoplasmic reticulum membrane, and are involved in platelet aggregation, vasoconstriction, and the progression and metastasis of several tumors.
Next, we evaluated the association of the lipid metabolites with the grade of peripheral neuropathy by lipid metabolomics analysis of all 60 serum samples; 5 PTs and 11 FAs were identified as markers of grade 2 or a higher degree of BiPN. Of the 5 PTs, a higher level of one lysophosphatidylcholine (LPC) species, which is considered as a factor promoting neuropathic pain, was observed in patients with grade 2 or higher peripheral neuropathy, suggesting an association between serum LPC and the severity of BiPN. In addition, two FAs, 14,15-DiHETE and 19,20-DiHDoPE, were present at higher levels in patients with grade 2 or higher peripheral neuropathy. These two FAs belong to the -3 FA group and have been reported to inhibit the activity of natural killer (NK) cells. The association between reduced NK cell activity and the severity of BiPN is currently under investigation (Figure 1).

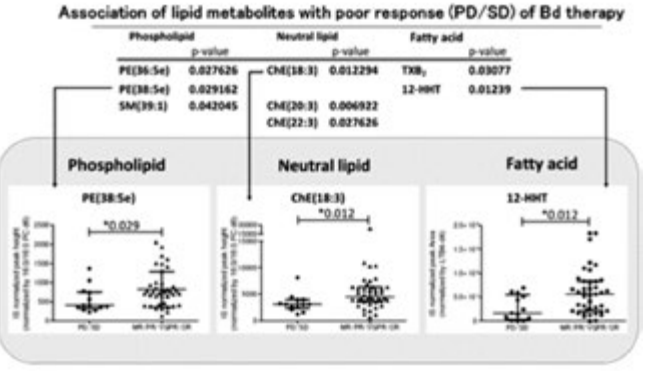


Figure 1.

Summary/Conclusion: Using serum lipid metabolomics, we have demonstrated for the first time that the specific lipid metabolite levels in the serum are associated with the response to Bd therapy and the severity of BiPN in patients with MM. These metabolites may serve as candidate biomarkers for predicting the efficacy of BTZ treatment against MM.

PS1288

CIRCULATING SOLUBLE UROKINASE-TYPE PLASMINOGEN ACTIVATOR RECEPTOR LEVELS REFLECTS RENAL FUNCTION IN NEWLY DIAGNOSED PATIENTS WITH MULTIPLE MYELOMA WHO ARE TREATED WITH BORTEZOMIB-BASED THERAPY

E. Terpos[1,*], G.-P. Papassotiriou[1], E. Kastritis[1], A. Margeli[2], N. Kanellias[1], E. Eleutherakis-Papaiakovou[1], M. Migkou[1], I. Ntanasis-Stathopoulos[1], M. Gavriatopoulou[1], E. Psimenou[1], I. Papassotiriou[2], M.A. Dimopoulos[1]

[1]Department of Clinical Therapeutics, National and Kapodistrian University of Athens, School of Medicine, [2]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece

Background: Renal impairment (RI) is a common complication of multiple myeloma (MM). Soluble urokinase-type plasminogen activator receptor (suPAR) is the circulating form of a glycosyl-phosphatidylinositol-anchored three domain membrane protein that has been implicated in the pathogenesis of kidney disease of different etiology (Hayek *et al.* N Engl J Med 2015;373:1916).
Aims: The aim of this study was to investigate a possible link between suPAR circulating levels and RI in newly diagnosed patients with symptomatic MM (NDMM) before and after frontline therapy with bortezomib-based regimens.

Methods: We studied 47 NDMM patients (26M/20F, median age 69.5 years) before the administration of any kind of therapy and after best response to bortezomib-based therapy. Thirty (64%) patients had IgG MM, 7 (15%) IgA and 10 (21%) had light-chain only MM; 13 (28%) patients had ISS-1, 19 (40%) ISS-2 and 15 (31%) had ISS-3 MM. Twenty-seven (57%) patients had eGFR <60 ml/min/1.73m^2, 23 (49%) had eGFR <50 ml/min/1.73m^2 and 10 (21%) had eGFR <30 ml/min/1.73m^2; no patient was on dialysis. suPAR was measured in the serum of all patients and of 24 healthy individuals of similar age, gender and body index (controls), using an immunoenzymatic assay (ViroGates, Denmark) along with a series of other markers of renal function (Cystatin-C), renal injury (Neutrophil Gelatinase-Associated Lipocalin, NGAL), inflammation (hs-CRP and IL-6) and cardiac function (hs-Troponin-T and NT-proBNP). eGFR values were calculated based on CKD-EPI/Cystatin-C equation (Inker *et al.* N Engl J Med 2012;367:20).
Results: suPAR levels were elevated in MM patients at diagnosis compared to controls (mean±SD, range: 4.1±2.2 pg/mL (1.4-13.0 pg/mL) vs. 1.8±0.3 pg/mL (1.1-2.6 pg/mL), p<0.001). Similarly, all other markers of cardio-renal dysfunction and inflammation were elevated in MM patients compared to controls (p<0.01 for all comparisons). suPAR levels strongly correlated with disease stage (ISS-1: 2.4±1.2 pg/mL; ISS-2: 3.6±1.8 pg/mL and ISS-3: 5.1±2.2 pg/mL; p-ANOVA <0.001). After bortezomib-based frontline therapy (VCD=32, VTD=7, VMp=7, VD=1), 9 (19%) patients achieved a CR, 11 (23%) vgPR and 19 (40%) PR. Of 23 patients with eGFR <50 ml/min/1.73m^2, 18 (78%) showed at least minor renal response to bortezomib-based frontline treatment, according to IMWG criteria. However, at patients' best response no significant changes of suPAR (4.4±2.7 pg/mL) levels were observed (p=0.31). On the other hand, suPAR levels both at diagnosis and at best response strongly correlated with eGFR values (r=-0.700, p<0.001 and r=-0.890, p<0.001, respectively) and NGAL levels (r=0.657, p<0.001 and r=0.586, p<0.001, respectively). suPAR levels at diagnosis and at best response also correlated positively with log(IL-6) and log(hs-CRP) values (p<0.001) and markers of cardiac function hs-Troponin-T and NT-proBNP (p<0.001).
Summary/Conclusion: We conclude that suPAR levels are associated with renal function in MM both at diagnosis and at best response to bortezomib-based frontline therapy. Although suPAR correlates with disease stage, confirming previous observations (Rigolin *et al.* Br J Haematol 2003;120:953), responders to anti-myeloma therapy continued to have elevated circulating suPAR, possibly reflecting persistent kidney damage, despite their renal response. Furthermore, suPAR correlated with the degree of inflammation and heart dysfunction in these patients. Further studies are needed to explore whether changes in suPAR may reflect increased risk for renal failure and/or progression in MM patients.

PS1289

PAN-ANTI-FGF-BASED THERAPY AS A NOVEL THERAPEUTIC INTERVENTION IN WALDENSTROM'S MACROGLOBULINEMIA

A. Sacco[1,*], G. Ghedini[2], G. Lanzi[3], A. Giacomini[2], M. Motta[4], A. Anastasia[4], C. Ghidini[5], R. Ronca[2], S. Giliani[3], L. Imberti[5], A. Montanelli[5], N. Bozza[6], R. Castelli[6], G. Rossi[4], M. Mor[6], M. Presta[2], A.M. Roccaro[1]

[1]Clinical Research Development and Phase I Unit; CREA Laboratory, ASST Spedali Civili di Brescia, [2]Dept. of Molecular and Translational Medicine, University of Brescia Medical School, [3]Institute od Molecular Medicine "A. Nocivelli", [4]Hematology, [5]Clinical Chemistry Laboratory, Diagnostic Department; CREA Laboratory, ASST Spedali Civili di Brescia, Brescia, [6]Dept of Food and Drug, University of Parma Medical School, Parma, Italy

Background: Bone marrow (BM) angiogenesis plays a crucial role in several hematologic malignancies, including both myeloid neoplasms and lymphoproliferative disorders. Nevertheless, the contribution of angiogenesis in patients with lymphoplasmacytic lymphoma/Waldenstrom's Macroglobulinemia (WM) and how it might be targeted for potential therapeutical intervention has not been described.
Aims: 1) To dissect the role of angiogenesis in supporting WM pathogenesis; 2) To investigate the anti-WM activity of a novel anti-pan-FGF molecule.
Methods: Publically available mRNA profiling datasets of primary bone marrow (BM)-derived CD19 WM cells were used (i.e. GSE6691; GSE9656). The Gene Set Enrichment Analysis (GSEA) computational method was adopted (FDR<0.25; p<0.05). Co-culture proliferation and adhesion assays were performed using available WM (BCWM.1; MWCL1; WMWSU), IgM secreting cell lines (MEC1), and primary WM bone marrow (BM)-derived mesenchymal stromal cells (MSCs). Cell viability of primary CD19 positive-selected BM WM cells and peripheral blood-derived mononuclear cells (PBMC) was assessed by MTS assay. Modulation of signaling pathways was studied at protein level using western blot analysis. A pan-FGF trap NSC12 molecule was identified by a virtual screening of a NCI small molecule library: it acts a trap able to block the FGF2/FGFR interaction. NSC12 was tested in order to define its potential anti-WM activity *in vitro*.
Results: GSE6691 gene expression dataset was used to investigate the most differentially expressed mRNA in WM cells as compared to their normal cellular counterpart: the top two pathways (ranked based on the normalized enrichment score and p-value) were related to "TNF-alpha signaling via NF-kB" and "angiogenesis". It has been already reported on the constitutive activation of NF-kB in WM; and therefore selected the "angiogenesis cascade" for further investigation (FDR 0.10; P 0.03). FGFR-1 was one of the most up-regulated genes in WM cells. We therefore looked at FGF- and FGFR-related mRNAs, using an independent GEP data set (GSE9656); and found FGFR-1, FGF-2, -12, -17 and -18 to be significantly enriched in WM cells as compared to their normal cellular counterpart (p<0.05). We next sought to determine the functional sequelae induced by the NSC12-mediated FGF2/FGFR blockade on WM cells. NSC12 exerted a direct anti-WM activity against all the cell lines tested (IC50: 3mM; p<0.05). Importantly, NCS12-induced cytotoxicity was demonstrated using WM patients' BM-derived CD19+ cells. NSC12 selectively targeted the FGF/FGFR axis, as demonstrated by inhibition of phospho(p)-FGFR1, as well as of downstream targets such as p-FSR2, p-JAK2, p-STAT3, and p-ERK. Of note, NSC12 inhibited WM cell proliferation even in a co-culture system with primary WM BM-MSCs. BM-MSC-induced up-regulation of p-FGFR1, p-STAT3, p-AKT was inhibited in WM cells exposed to increasing NCS12 doses, thus suggesting the activity of the compound even in the presence of the supportive BM milieu. We further confirmed NSC12-dependent induction of pro-apoptotic pathways, as shown at protein level by enhanced caspase-3- and PARP-cleavage. Notably, NSC12 did not induce cytotoxicity against healthy donor-PBMC.
Summary/Conclusion: These findings provide novel insights into the pathogenesis of WM, describing the up-regulation of the FGF/FGFR axis in WM cells, thus providing the preclinical rational for targeting FGF in this disease. Consistently, the novel pan-FGF trap NSC12 demonstrated an anti-WM activity, through both a direct and an indirect effect against WM cells and the WM cell/BM interaction, respectively.

PS1290

EVALUATION OF THE ACTIVITY OF IMIDS IN MM PROLIFERATING CELLS

J. Ballesteros[1,*], B. Puła[2,3], R. Pawel[3,4], A. Walter-Croneck[3,5], A. Druzd-Sitek[3,6], D. Dytfeld[3,7], J. Martínez López[8], P. Hernández[1], J. Gorrochategui[1], J.L. Rojas[1], D. Primo[1], K. Jamroziak[2,3]

[1]Vivia Biotech, Tres Cantos, Madrid, Spain, [2]Department of Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, [3]Polish Myeloma Consortium, Poznan, [4]Department of Hematology, Medical University of Lodz, Copernicus Memorial Hospital, Lodz, [5]Department of Haematooncology and Bone Marrow Transplantation, Medical University of Lublin, Lublin, [6]Department of Lymphoid Malignancies, Maria Sklodowska-Curie Memorial Institute and Oncology Centre, Warsaw, [7]Department of Hematology and Bone Marrow Transplantation, University of Medical Sciences in Poznan, Poznan, Poland, [8]Hospital Universitario 12 de Octubre, Madrid, Spain

Background: Treatment of multiple myeloma (MM) has evolved from non-specific chemotherapeutic agents to novel targeted therapy. Among these new treatment approaches, the immunomodulatory drugs (IMiDs) represent a good therapeutic option for many MM patients. Several mechanism of action has been proposed to explain the direct and indirect effect of IMiDs, such as antiangiogenic, proapoptotic, antiproliferative and immunomodulatory. Although there are 3 IMID drugs approved (Thalidomide, Lenalidomide, Pomalidomide), *in vitro* assays to capture the IMiDs activities are still in development. Precision medicine tests evaluating these drugs could represent a valuable tool to assess the subgroup of patients who could benefit from these treatments.
Aims: The goal of this study is to design an ex vivo model with the PharmaFlow technology, that allows to simultaneously measure cytotoxic activity and cell cycle arrest, to identify the antiproliferative effect of IMiDs agents, such as lenalidomide (LEN), in plasma cells (PC) from whole bone marrow MM samples, as a putative secondary reflect of IMiDs activity, that could predict the clinical response ex vivo.
Methods: Bone marrow samples from 16 MM patients were sent to Vivia from Polish Myeloma Consortium (PMC) and Spanish hospitals within 24h after extraction. Whole sample were incubated for 96h in well plates containing 8 concentrations of LEN. To induce plasma cell proliferation, PC were resuspended in IMDM medium supplemented with 20% autologous plasma. The number of proliferating and non proliferating live leukemic cells was determined using the CFDA dye, and the PC population and viability was determined labeling with monoclonal antibodies and Annexin V. Dose response curves of LEN for proliferating and non proliferating live

pathological cells were measured, and pharmacological responses were calculated using pharmacokinetic population models.
Results: A clear difference in the sensitivity of proliferating vs. non-proliferating MM cell subsets was observed. LEN was more active towards proliferative live tumor cells, with a median maximum effect (Emax) of 74% vs 49% and a 2 fold more sensitive potency (EC50) of 0.05 mM vs. 0.1 mM, concentrations achievable clinically. This effect was probably due to a cell cycle arrest (Figure 1, left panel). Interestingly, there is a great heterogeneity in the potency and effect (panel right, Figure 1) in all samples, pointing the possibility of patient selection for LEN treatment. In addition, the efficacy was incomplete in many patients (median Emax of proliferating live tumor cells equal to 49) leaving a significant number of proliferating cells, still even at the higher drug concentrations, that suggest the use of additional targets.

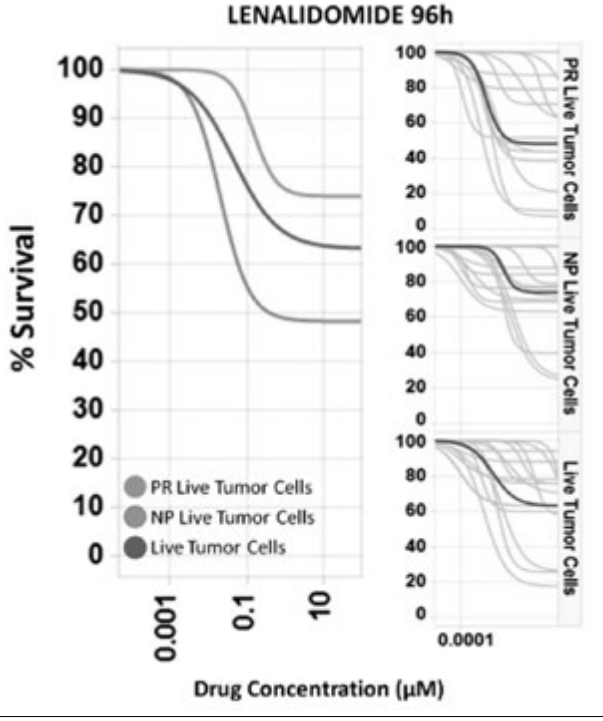


Figure 1.

Summary/Conclusion: We report a novel *ex vivo* assay incorporating autologous serum that enables the measurement of the effects of IMiDs on MM patients and thereby, a more accurately simulation of the *in vivo* conditions. This innovative assay offers better opportunities for *ex vivo* pharmacology in particular for the IMiDs, drugs with antiproliferative properties. The interpatient variability in the pharmacological profile for the LEN, if clinically validated, could help guiding a personalized treatment selection for IMiDs.

Myeloma and other monoclonal gammopathies – Clinical

PS1291

ADDITION OF VORINOSTAT TO LENALIDOMIDE MAINTENANCE IN NEWLY DIAGNOSED MYELOMA DOES NOT IMPROVE OUTCOMES: RESULTS OF THE MYELOMA XI TRIAL

M. Jenner[1,*], A. Striha[2], F. Davies[3], D. Cairns[2], C. Pawlyn[4,5], J. Jones[4], B. Kishore[6], M. Garg[7], C. Williams[8], K. Karunanithi[9], J. Lindsay[10], N. Russell[8], G. Cook[11], M. Drayson[12], M. Kaiser[4,5], R. Owen[13], W. Gregory[2], G. Morgan[3], G. Jackson[1,4]

[1]Haematology, University Hospital Southampton NHS Foundation Trust, Southampton, [2]Clinical Trials Research Unit, University of Leeds, Leeds, United Kingdom, [3]Myeloma Institute, University of Arkansas for Medical Sciences, Little Rock, United States, [4]The Institute of Cancer Research, [5]The Royal Marsden Hospital, London, [6]Heart of England Foundation Trust, Birmingham, [7]Leicester Royal Infirmary, Leicester, [8]Centre for Clinical Haematology, Nottingham University Hospital, Nottingham, [9]University Hospital of North Midlands, Stoke-on-Trent, [10]Kent and Canterbury NHS Trust, Canterbury, [11]Leeds Institute of Cancer and Pathology, University of Leeds, Leeds, [12]Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, [13]St James' University Hospital, Leeds, [14]Department of Haematology, Newcastle University, Newcastle upon Tyne, United Kingdom

Background: We previously reported in the Myeloma XI trial the improvement in PFS with lenalidomide maintenance versus observation of 38.9 versus 20.0 months. Vorinostat is an HDAC inhibitor that has shown improved PFS combined with bortezomib compared with bortezomib alone in relapsed myeloma.
Aims: We will present results of the Myeloma XI study in which patients were randomised between lenalidomide alone or lenalidomide and vorinostat maintenance.
Methods: The phase III NCRI Myeloma XI study recruited newly diagnosed myeloma patients from over 100 centers across the UK. Following protocol amendment between September 2011 and June 2013, patients were randomised in a 1:1:1 ratio comparing maintenance lenalidomide (len: 10 mg, days 1-21/28), lenalidomide plus vorinostat (len-vori: 10 mg, days 1-21/28 and 300 mg, days 1-7 and 15-21) and observation (obs) until disease progression, stratifying for induction treatments and response prior to therapy. There was an intensive pathway for transplant eligible (TE) and non-intensive pathway for transplant non eligible (TNE) patients. Primary analysis combined these pathways. The study was powered for both PFS and OS, with these being co-primary endpoints. 614 patients (395 TE, 219 TNE) were randomised, median age 66 years, between len (307) and len-vori (307). Groups were well balanced between arms.
Results: In the overall intention to treat (ITT) population, participants randomised to len received a median of 28 cycles (range 1, 60) compared with a median of 17 cycles (range 1, 63) in the len-vori group. In the TE population participants received a median of 30 cycles (range 1, 60) of len and 21 cycles (range 1, 63) of len-vori. In the TNE population participants received a median of 23 cycles (range 1, 56) of len and 13 cycles (range 1, 54) of len-vori. Overall 8.8% of participants discontinued within the first 3 cycles of maintenance in the len group compared with 22.1% in the len-vori group. 171 participants (55.7%) in the len group had dose modifications and 241 (78.5%) in the len-vori group. In the len-vori group, 197 (64.2%) had dose modification of lenalidomide and 230 (74.9%) had dose modification of vorinostat. Of those that stopped therapy, 114 (37.1%) in the len group stopped due to disease progression and 29 (9.4%) because of toxicity whereas 103 (33.6%) stopped for disease progression and 68 (22.1%) because of toxicity in the len-vori group. At a median follow up of 41 months, there were 151 PFS events (49.2%) in the len group and 168 PFS events (54.7%) in the len-vori group. PFS was not significantly different between the len group, PFS 40.2 months [95% CI 35.2, 52.6] and the len-vori group, PFS 33.1 months (95% CI 28.7, 40.6), p-value 0.1457. The hazard ratio for len-vori was 1.18 (95% CI 0.94, 1.47) indicating no PFS benefit of len-vori maintenance over len alone. There were 76 deaths (24.8%) in the len group and 75 deaths (24.4%) in the len-vori group. OS was not significantly prolonged in the len-vori group, 60.7 months, compared with not reached in the len group, p-value 0.7499 and hazard ratio 0.95 [95% CI 0.69, 1.31]. Full subgroup analysis will be presented during the meeting including of the factors influencing prolonged duration of maintenance with len-vori.
Summary/Conclusion: Addition of vorinostat to lenalidomide maintenance does not improve outcomes compared with lenalidomide alone in newly diagnosed myeloma yet despite earlier discontinuation and greater dose reductions outcomes of the combination remain favourable compared to observation alone.

PS1292

POMALIDOMIDE + LOW-DOSE DEXAMETHASONE + DARATUMUMAB IN PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA AFTER LENALIDOMIDE-BASED TREATMENT FAILURE

D. S. Siegel[1,*], G. J. Schiller[2], C. Samaras[3], M. Sebag[4], J. Berdeja[5], S. Ganguly[6], J. Matous[7], K. Song[8], C. S. Seet[9], G. Talamo[10], S. Srinivas[11], M. Acosta-Rivera[12], M. Bar[13], D. Quick[14], B. Anz[15], G. Fonseca[16], D. Reece[17], F. Zafar[18], W. Chung[18], N.J. Bahlis[19]

[1]John Theurer Cancer Center, Hackensack University Medical Center, Hackensack, [2]David Geffen School of Medicine at University of California, Los Angeles, [3]Cleveland Clinic, Cleveland , United States, [4]McGill University Health Centre, Montreal, Canada, [5]Sarah Cannon Research Institute, Nashville, [6]The University of Kansas Cancer Center, Fairway, [7]Colorado Blood Cancer Institute, Denver, United States, [8]Vancouver General Hospital, Vancouver , Canada, [9]University of California, Los Angeles Medical Center, Los Angeles, [10]Penn State Hershey Cancer Institute, Hershey, [11]Veterans Affairs New Jersey Health Care System, East Orange, United States, [12]Fundación de Investigación, San Juan, Puerto Rico, [13]Stamford Hospital, Stamford, [14]Joe Arrington Cancer Research and Treatment Center, Lubbock, [15]Tennessee Oncology, Chattanooga, [16]Florida Cancer Specialists, St. Petersburg, United States, [17]Princess Margaret Cancer Centre, Toronto, Canada, [18]Celgene Corporation, Summit, United States, [19]University of Calgary, Calgary, Canada

Background: MM-014 (NCT01946477) was designed to assess outcomes with pomalidomide (POM)-based treatment in patients with lenalidomide (LEN) treatment failure immediately before study entry. POM + low-dose dexamethasone (LoDEX) + daratumumab (DARA) was approved in the United States for use in patients with multiple myeloma (MM) who have received ≥ 2 prior therapies, including LEN and a proteasome inhibitor. However, data on the use of this triplet regimen in earlier lines of therapy and immediately after LEN-based treatment are limited. Cohort B of the MM-014 trial is investigating POM + LoDEX + DARA in this setting.
Aims: To present efficacy and safety analyses of cohort B of the MM-014 trial in which POM + LoDEX + DARA was given as second-line or greater treatment in patients with relapsed and/or refractory MM (RRMM).
Methods: Patients with RRMM who had received 1 or 2 prior lines of treatment, had a LEN-based treatment immediately before the study, and had progressive disease (PD) were eligible. In 28-day cycles, patients received POM 4 mg/day orally on days 1 to 21 + LoDEX 40 mg/day (20 mg/day if aged > 75 years) on days 1, 8, 15, and 22 + DARA 16 mg/kg intravenously on the LoDEX dosing days of cycles 1 and 2, then days 1 and 15 of cycles 3 to 6, then day 1 of cycles 7 onward. Thromboprophylaxis was mandatory. The primary objective was overall response rate (ORR) by modified International Myeloma Working Group criteria. All patients provided informed consent.

Table 1. Efficacy and safety.

Outcomes, %	N = 46
Efficacy	
ORR	71.7
CR	4.3
VGPR	21.7
PR	45.7
Minimal response	6.5
Clinical benefit (CR + VGPR + PR + MR)	78.3
SD	8.7
PD	6.5
1-year PFS	76.9
Safety	
Grade 3/4 TEAEs (occurring in ≥ 10% of patients)	
Neutropenia	71.7
Thrombocytopenia	23.9
Anemia	17.4
Infection	28.3

CR, complete response; MR, minimal response; PFS, progression-free survival; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease; TEAE, treatment-emergent adverse event; VGPR, very good partial response.

Results: The intention-to-treat population (ITT) included 46 patients. The median follow-up was 7.8 months; 13 patients discontinued treatment because of PD (n = 7), adverse events (n = 2), or other reasons (n = 4). Patients were refractory to (n = 36 [78%]) or had relapsed after (n = 10 [22%]) LEN-based treatment. The median duration of prior LEN-based treatment was 23.6 months, and 20 patients (43%) received LEN 25 mg/day as their last LEN-based treatment. Efficacy outcomes in the ITT population and grade 3/4 treatment-emergent adverse events in the safety population (n = 46; defined as all patients who received ≥ 1 dose of study drug) are shown in Table 1. The ORR was 76.7% in the efficacy-evaluable population (n = 43; defined as all patients who received ≥ 1 dose of study drug and had ≥1 post-baseline assessment for response), 72.2% in LEN-refractory patients, and 75.0% in patients who received LEN 25 mg/day as their last LEN-based treatment. Clinical benefit (defined as complete response, very good partial response, partial response, or minimal response) was achieved in 78.3% of patients, and the 1-year progression-free survival rate was 76.9%. At baseline, 10 patients (21.7%) had grade ≥ 2 neutropenia. Grade 3/4 pulmonary embolism and peripheral neuropathy occurred in 1 patient each. Any-grade infusion-related reactions occurred in 13 patients. The primary reasons for dose interruptions were neutropenia (POM and DARA) and infusion-related reactions (DARA only).
Summary/Conclusion: These results indicate that POM + LoDEX + DARA is an effective and tolerable regimen when sequenced in earlier lines of therapy in patients with RRMM and in whom first- or second-line therapy with a LEN-based treatment failed.

PS1293

DOUBLET VS TRIPLET LENALIDOMIDE-CONTAINING REGIMENS FOLLOWED BY MAINTENANCE: SUBGROUP ANALYSIS BY FRAILTY STATUS AFTER A MEDIAN FOLLOW-UP OF 5 YEARS (EMN01 PHASE III STUDY)

S. Bringhen[1,*], N. Giuliani[2], A. Rambaldi[2], F. Bonello[1], S. Ballanti[2], P. Tosi[2], A. Belotti[2], F. Ciambelli[2], S. Caltagirone[1], S. Ronconi[2], A.M. Cafro[2], C. Cangialosi[2], D. Vincelli[2], C. Cellini[2], F. Morabito[2], M. Girotto[2], M. Grasso[2], A. Palumbo[3], R. Hajek[4], M. Boccadoro[1], A. Larocca[1]

[1]Myeloma Unit, Division of Hematology, University of Torino, A.O.U. Città della Salute e della Scienza di Torino, Torino, [2]Italian Multiple Myeloma Network, GIMEMA, [3]University of Torino, A.O.U. Città della Salute e della Scienza di Torino - Currently Takeda Pharmaceuticals Co., Torino, Italy, [4]Department of Hematooncology, University hospital Ostrava, Ostrava, Czech Republic

Background: The frailty status was associated with an increased risk of death, progression, non-hematologic adverse events (AEs) and treatment discontinuation. The "one size fits all" is no longer a suitable approach, and many recommendations suggested that fit patients (pts) may benefit from triplet regimens, while intermediate and frail pts may benefit from doublet regimens. There are no data from prospective trials supporting these recommendations.
Aims: To evaluate the impact of patient frailty status on outcome of different lenalidomide-containing regimes.
Methods: 662 pts with newly diagnosed MM were randomized to receive 9 28-day cycles of Rd (lenalidomide 25 mg/day for 21 days; dexamethasone 40 mg on days 1,8,15,22 in pts 65-75 years old, 20 mg in those >75 years), MPR (melphalan orally 0.18 mg/Kg for 4 days in pts 65-75 years old and 0.13 mg/Kg in those >75 years; prednisone 1.5 mg/Kg for 4 days; lenalidomide 10 mg/day for 21 days) or CPR (cyclophosphamide orally 50 mg/day for 21 days in pts 65-75 years old and 50 mg every other day in those >75 years; prednisone 25 mg every other day; lenalidomide 25 mg/day for 21 days). After induction, pts were randomized to receive maintenance with R alone (lenalidomide 10 mg/day for 21 days) or RP (lenalidomide 10 mg/day for 21 days; prednisone 25 mg every other day), until disease progression. At diagnosis, a geriatric assessment had been performed to assess cognitive and physical status, and comorbidities with Katz's Activity of Daily Living (ADL), Lawton's Instrumental Activity of Daily Living (IADL) and Charlson comorbidity index (CCI).
Results: 217 pts in Rd, 217 in MPR and 220 in CPR arms were evaluable. According to the IMWG frailty score, 284 (43%) pts were fit, 205 (31%) intermediate and 165 (25%) frail. After a median follow-up of 5.5 years, MPR significantly prolonged PFS from 18 months to 22 months compared to CPR (HR 0.78, p=0.02) and Rd (HR 0.83, p=0.09). This difference was evident only in fit pts (5-year PFS: 37% vs 17%, HR 0.70, p=0.03), no difference was observed in intermediate and frail pts. Median OS was 66 months in MPR pts, 67 in CPR pts and 62 in Rd pts. In fit pts a survival benefit was evident: MPR and CPR prolonged OS compared to Rd (83 months vs 50 months; HR 0.69, p=0.09). Hematologic toxicity was the most frequent toxicity and was mainly related to MPR, regardless of frailty status. As expected, the occurrence of AEs and the risk of discontinuation due to AEs increased with the worsening of fitness status. At the end of induction, 402 pts were eligible for maintenance, 204 with R and 198 with RP. 192 (48%) pts were fit, 121 (30%) intermediate and 89 (22%) frail. After a median duration of maintenance of 22.0 months, PFS from start of maintenance was 22.2 months with RP vs 17.6 months with R (HR 0.80, p=0.07). This difference was significant only in fit pts (median PFS 24 vs 18 months, HR 0.70, p=0.03), no difference was seen in intermediate and frail pts. No difference in OS was observed. The occurrence of AEs and the risk of discontinuation due to AEs increased with the worsening of fitness status. (Table 1).

Table 1.

	FIT			INTERMEDIATE			FRAIL		
Arm	PFS Median	OS 5 yrs	Disc. for AEs	PFS Median	OS 5 yrs	Disc. for AEs	PFS Median	OS 5 yrs	Disc. for AEs
MPR	25.6	62%	23 (26)	20	53%	24 (30)	21.5	33%	19 (39)
CPR	21.7	58%	18 (18)	20.9	63%	19 (28)	13.8	39%	20 (38)
Rd	20.5	46%	17 (18)	16.6	67%	14 (25)	15.8	44%	15 (24)

Summary/Conclusion: This subgroup analysis showed for the first time that fit pts benefit from triplet full-dose regimens, while intermediate and frail pts benefit from a gentler doublet regimen. The higher incidence of hematologic AEs with MPR should be considered. In the future, the triplet regimen containing proteasome inhibitors and lenalidomide could be the best option in fit pts. All pts, even frail ones, benefit from continuous treatment with lenalidomide.

PS1294

POMALIDOMIDE, CYCLOPHOSPHAMIDE AND DEXAMETHASONE IN CASE OF SUBOPTIMAL RESPONSE TO POMALIDOMIDE AND DEXAMETHASONE IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA: RESULTS OF THE GMMG-PERSPECTIVE TRIAL

K. Weisel[1,*], H. Salwender[2], C. Scheid[3], M. Zago[1], B. Besemer[1], M. Haenel[4], J. Duerig[5], M. Munder[6], H.-W. Lindemann[7], A. Seckinger[8], C. Kunz[9], A. Benner[9], D. Hose[8], A. Jauch[8], L. Kanz[1], H. Goldschmidt[8]

[1]University Hospital of Tuebingen, Tuebingen, [2]Asklepios Clinic, Hamburg, [3]University of Cologne, Cologne, [4]Clinic of Chemnitz, Chemnitz, [5]University of Essen, Essen, [6]University of Mainz, Mainz, [7]Catholic Hospital Hagen, Hagen, [8]University of Heidelberg, [9]German Cancer Research Center, Heidelberg, Germany

Background: Pomalidomide (POM) + dexamethasone (Dex) is a standard treatment in relapsed and refractory multiple myeloma (RRMM) resulting in an overall response rate (ORR) of approximately 30% and a median progression-free survival of 4.0 months (San Miguel *et al.*, Lancet Oncol 2013). Patients responding to POM + Dex show extended progression-free (PFS) and overall survival (OS) (Moreau *et al.*, Leuk Lymphoma 2016). Cyclophosphamide (CY) is an alkylating agent showing anti-myeloma activity with potential synergistic effects to immunomodulating agents (IMiDs) as POM and lenalidomide.
Aims: The GMMG-PERSPECTIVE trial (Eudra-CT No. 2013 003678 29) is a phase II multicenter investigator initiated trial in RRMM patients investigating efficacy when CY is added to POM + Dex standard treatment in case of primary progression or less than partial remission (PR) after 3 treatment cycles of POM + Dex. The primary objective of the trial is to demonstrate that the objective response rate (ORR) exceeds 30%, key secondary endpoints are PFS, time to next treatment (TTNT), OS and safety.
Methods: 60 patients with RRMM after at least 2 prior treatment lines including bortezomib and lenalidomide and not responding to the last prior regimen were included. All patients were included in safety analyses. 59 patients were eligible for the intention-to-treat (ITT) analysis of primary and secondary endpoints. Patients received standard dose of POM + Dex (POM 4 mg day 1-21, Dex 40 mg day 1, 8, 15, 22 of a 28-day cycle, Dex 20 mg weekly in patients > 75 years). In case of no PR after 3 cycles and/or disease progression (PD) during cycle 1-3, CY in a dose of 500 mg/m² intravenously day 1 and 15 per cycle was added. Patients were treated until PD or unacceptable toxicity. ORR as primary endpoint was evaluated after a median follow-up of 20.1 months. After a median follow-up of 32.8 months PFS, PFS from start of CY, OS, TTNT as secondary endpoints and safety data were reported.
Results: 60 patients were included into the trial. Median age was 67.0 (range 47-81) years, median number of prior treatment lines was 3 (2-5). 45% of evaluable patients had cytogenetic high-risk disease. ORR (≥ PR) was 39% (not significant with a lower 95% confidence bound of 29.2%). PR was reached in 14 (23.7%), very good partial remission (VGPR) in 7 (11.9%) and complete remission (CR) in 2 (3.4%) patients. 36/59 patients received CY after a median time of 2.9 months (1.1-4.4 months). At the time of addition of CY 16 patients had PD, 15 stable disease (SD) and 5 showed minimal response (MR). 13/36 patients with addition of CY to POM + Dex achieved a response: 8 PR (22.2%), 3 VGPR (8.3%), 2 CR (5.6%). Median PFS of the ITT population was 6.4 months. Median PFS from start of CY was 4.8 months. Median TTNT was 11.0 months. Median OS was 18.3 months. Main hematologic toxicities ≥ grade 3 were neutropenia in 26 (43.3%), anemia in 16 (26.7%), leukopenia in 15 (25.0%) and thrombocytopenia in 9 (15.0%) patients. Main non-hematologic toxicities ≥ grade 3 were pneumonia in 10 (16.7%) patients. All other toxicities ≥ grade 3 were ≤ 5%.
Summary/Conclusion: In RRMM patients, addition of CY to standard POM + Dex treatment is able to overcome lack of response or primary resistance to POM + Dex in a marked proportion of patients. PFS, TTNT and OS of the IIT compares favorably with published data for POM + Dex alone. With a favorable toxicity profile of the triple combination, primary addition of CY to POM + Dex should be considered in future to further optimize efficacy and durability of responses in POM + Dex standard treatment.

PS1295

SKY92 RISK STRATIFICATION AT RELAPSE PROVIDES ADDITIONAL PROGNOSTIC INFORMATION FOR STANDARD-RISK MULTIPLE MYELOMA PATIENTS

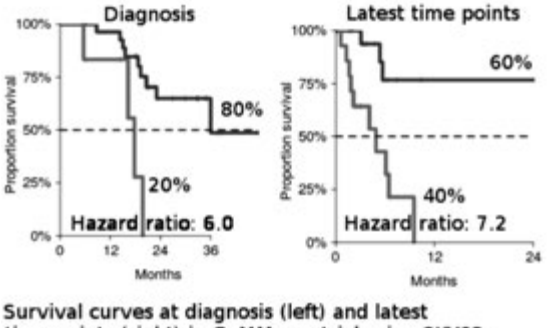
R. Kuiper[1,2,*], E. H. van Beers[3], M. H. van Vliet[2]

[1]Department of Hematology, Erasmus MC Cancer Institute, [2]Department of Bioinformatics, [3]Department of Oncogenomics, SkylineDx, Rotterdam, Netherlands

Background: Multiple myeloma (MM) is a plasma cell cancer with a disease course of recurrent relapses, resulting in a 5 yr median overall survival (OS). Gene expression (GEP) classifiers, such as SKY92, are able to differentiate high-risk (HR) and standard-risk (SR) patients at diagnosis and relapse. The SKY92 has been validated in several independent cohorts, identifying on average 18% of patients as HR. Although those cohorts differed in treatment, age of the patients and disease stage, the classifier was prognostic in all of them. However, it is unknown how the SKY92 risk classification evolves as the disease progresses.
Aims: In this study, we present the first longitudinal analysis of SKY92 by comparing classifications of samples obtained at diagnosis and relapse of the same patient.

Table 1. SKY92 risk stratification matrix in CoMMpass and TTx for newly diagnosed MM vs. relapse.

TTx					CoMMpass				
		Relapse					Latest time point		
		SR	HR	Total			SR	HR	Total
NDMM	SR	100(69%)	23(19%)	**88%**	NDMM	SR	20(57%)	8(23%)	**80%**
	HR	4 (3%)	13(9%)	**12%**		HR	1 (3%)	6(17%)	**20%**
	Total	**72%**	**28%**			Total	**60%**	**40%**	



Survival curves at diagnosis (left) and latest time points (right) in CoMMpass trial using SKY92. High-risk in red, standard-risk in blue.

Figure 1.

Methods: Two publically available MM patient data sets were used: the Multiple Myeloma Research Foundation (MMRF) CoMMpass trial (NCT145429) and the University of Arkansas for Medical Sciences (UAMS) total therapy cohorts (TTx). In the TTx cohort, microarray RNA expression data from tumor cells were determined at both diagnosis and relapse in 146

patients. OS was measured from diagnosis but not from relapse. The CoMMpass is a longitudinal study in 1000 NDMM patients, receiving treatment containing a proteasome inhibitor, IMiD or both. For 632 patients RNA-Seq was performed at diagnosis. A subset of 35 patients were sequenced at a later time point as well and had OS available as of both time points. RNA-Seq data was analyzed using the converted SKY92 classifier for that platform (SKY92r). In brief, 87 of 92 probesets of the microarray method were mapped to the RNA-Seq data; the original microarray classifier was corrected for this composition, and the classifier was then used on RNA-Seq data. For the TTx studies, microarray data is available, and SKY92 was calculated according to the algorithms routinely used in our lab for microarrays. For clarity we indicate the use of both classifiers below as SKY92.

Results: In the TTx cohorts, 12% of patients were HR at diagnosis, increasing to 28% at relapse (McNemar test $p=4.7x10^{-5}$; Table 1). In the CoMMpass cohort, 20% HR was seen at diagnosis increasing to 40% at the latest time point ($p=0.046$); all patients classified as HR at the late time point died within 1 yr from that time point (Figure 1). For 22% and 26% of patients in the TTx and CoMMpass, respectively, risk status changed, of which 19% and 23% transformed to higher risk. This suggests that in MM risk evolution, HR patients predominantly remain HR while SR patients mostly progress toward HR.

Summary/Conclusion: We observed the SKY92 risk classification for the first time in a longitudinal analysis. Reclassification at relapse provides additional prognostic insight, mostly for patients initially classified as SR at diagnosis

PS1296

CARFILZOMIB, CYCLOPHOSPHAMIDE, AND DEXAMETHASONE IN TRANSPLANT ELIGIBLE NEWLY DIAGNOSED MULTIPLE MYELOMA: PRELIMINARY RESULT OF SGHMM1 TRIAL, HIGH-RISK COHORT (NCT02217163)

Y. Chen[1], S. Gopalakrishnan[2], M. Ooi[3], N. Grigoropoulos[1], Y.S. Lee[1], C. Phipps[1], Y.T. Goh[1], W. J. Chng[3], W. Hwang[1], C. Nagarajan[1,*]

[1]Haematology, Singapore General Hospital, Singapore, Singapore, [2]Haematology, Health Sciences North, Sudbury, Ontario, Canada, [3]Haematology, National University Hospital, Singapore, Singapore

Background: Outcomes for high-risk MM patients is generally poor and could be improved through attainment of deeper responses. Carfilzomib is a second generation proteasome inhibitor with the ability to attain deep responses. Its efficacy and safety in the upfront setting for transplant-eligible high-risk MM patients is explored in this study.

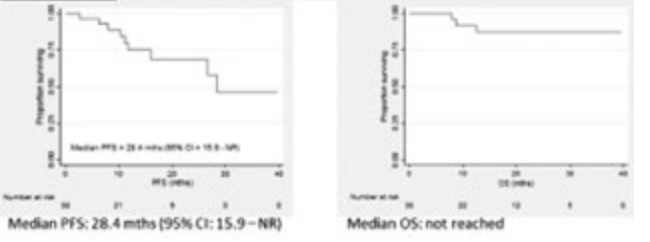
Aims: The primary objective was to assess the progression free survival (PFS) in autologous stem cell transplant (ASCT) - eligible, high-risk, newly diagnosed MM (NDMM) patients treated with the combination of Carfilzomib, Cyclophosphamide, and Dexamethasone (KCd). The secondary objective was to determine the overall survival and to assess minimal residual disease (MRD) status by multi parameter flow cytometry (MPFC) which was used to decide whether patients will receive maintenance Carfilzomib or not.

Methods: This IRB approved, open-label, Phase II study was conducted at 2 transplant centers in Singapore. Patients under 70yrs of age with high-risk NDMM (defined as any of: ISS 3, del17p, t(4;14), t(14;16)or 1q21amp) who were eligible for ASCT were enrolled. Eligible patients received six 28-day induction cycles of KCd - Carfilzomib 20/56 mg/m^2 (D1,2,8,9,15,16), Cyclophosphamide (flat dose) 500mg per week (D1,8,15,22), Dexamethasone 20 mg (D1,2,8,9,15,16). Following a safety review the dose escalation for Carfilzomib was capped at 36 mg/m^2 after 6 patients were recruited. After induction, patients had high dose Melphalan (200 mg/m^2) with stem cell rescue and consolidation with 2 cycles of KCd after three months as per above dose schedule. After consolidation, subjects who had at least a VGPR underwent disease assessment and MRD analysis by MPFC. Patients who achieved MRD negativity (MRD-) were managed expectantly whereas patients who were MRD positive (MRD+) received maintenance (Carfilzomib 36 mg/m^2 on D1,8,15 every 28 days) for 2 years or till disease progression.

Results: Median age of the patients was 63.0 years and 51.7% were Male. As of 31 December 2017, 32 patients were screened and 30 patients went on to receive KCd (2 patients were ineligible). 19 patients had completed ASCT at time of analysis, 6 patients were withdrawn from the study during induction (5 due to toxicity and 1 patient withdrew consent). With a median follow-up of 19.8 months, the median PFS was 28.4months (95% CI = 15.9 - not reached). Overall responses are shown in Table 1. Amongst the 13 patients who had completed consolidation and had MRD assessment done, 7 were MRD+ and went on to receive maintenance whilst 6 were MRD- and received no maintenance. Grade 3/4 haematologic treatment emergent adverse events (TEAEs) were seen in 9 (30%) patients: anaemia (16.7%), neutropenia (16.7%) and thrombocytopenia (13.3%). Other common TEAE were LRTI/pneumonia (23.3%), diarrhoea (10%) and acute kidney injury (13.3%). These are consistent with other reported studies with the same triplet combination. There were 6 TEAEs that lead to treatment discontinuation (3 thrombotic microangiopathies, 1 NSTEMI, 1 pulmonary oedema and 1 CMV retinitis).

Table 1.

	Post-induction (N=30) n (%)	Post-ASCT (N=25) n (%)	Post-consolidation (N=21) n (%)
MRD-	N.A.	N.A.	6 (28.6)
≥CR	10 (33.3)	11 (44.0)	12 (57.1)
≥VGPR	19 (63.3)	18 (72.0)	15 (71.4)
ORR (≥ PR)	26 (86.7)	21 (84.0)	17 (81.0)



Median PFS: 28.4 mths (95% CI: 15.9 – NR) Median OS: not reached

Summary/Conclusion: Carfilzomib, cyclophosphamide and dexamethasone (KCd) is an effective and generally well tolerated combination. Deep responses including MRD negativity are achievable with this triplet combination even in high-risk MM patients. Preliminary results from this ongoing study, support continued investigation of KCd, as a more economical alternative first-line treatment regimen (for example as compared to VRD) in high risk NDMM. Updated data based on longer follow-up and QOL data will be presented subsequently.

PS1297

WEEKLY CARFILZOMIB, LENALIDOMIDE, AND DEXAMETHASONE (KRD) IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA (RRMM): A PHASE 1B STUDY

N. Biran[1,*], D. Siegel[1], J.G. Berdeja[2], N. Raje[3], R.F. Cornell[4], M. Alsina[5], T. Kovacsovics[6], B. Fang[7], A. Kimball[7], O. Landgren[8]

[1]John Theurer Cancer Center at Hackensack University Medical Center, Hackensack, [2]Sarah Cannon Research Institute, Nashville, [3]Massachusetts General Hospital Cancer Center, Boston, [4]Vanderbilt University Medical Center, Nashville, [5]Moffit Cancer Center, Tampa, [6]Huntsman Cancer Institute, Salt Lake City, [7]Amgen Inc., Thousand Oaks, [8]Memorial Sloan Kettering Cancer Center, New York, United States

Background: KRd is approved for treatment of RRMM patients (pts). Under the approved KRd regimen, carfilzomib is given twice weekly (20/27 mg/m^2; 10-min IV infusion).

Aims: Here we present updated results from a dose-finding study assessing weekly KRd.

Methods: This study consisted of a dose-evaluation component and a dose-expansion component in both RRMM and newly diagnosed MM. Results for RRMM pts are presented here. Two dose levels were evaluated: carfilzomib 56 mg/m^2 and carfilzomib 70 mg/m^2. All pts received carfilzomib (30-min IV infusion on days [D] 1, 8, and 15; 20 mg/m^2 on C1D1), lenalidomide 25 mg (D1–21), and dexamethasone 40 mg (D1, 8, 15, and 22) on a 28-day cycle (dexamethasone was not given on D22 for cycles 9+). Pts in the expansion arm received the selected KRd regimen on the same schedule used for dose evaluation. Response was assessed by investigators.

Results: Twenty-two RRMM pts were enrolled in the dose-evaluation part and received study drug (56 mg/m^2, n=10; 70 mg/m^2, n=12). The maximum tolerated dose of carfilzomib was not reached; the 70 mg/m^2 dose was selected for dose expansion and 34 additional RRMM pts received this dose. Results are presented for pts who received carfilzomib 56 mg/m^2 during dose evaluation (n=10; median 2 prior regimens [range 1-3]) and for pts who received carfilzomib 70 mg/m^2 during dose evaluation or expansion (n=46; median 1 prior regimen [range 1-5]). Median (mean) average carfilzomib dose was 53.2 (52.8) mg/m^2 in the 56 mg/m^2 group and 62.4 (61.3) mg/m^2 in the 70 mg/m^2 group. Pt incidence of grade ≥3 adverse events was 70.0% (56 mg/m^2) and 71.7% (70 mg/m^2). Pt incidence of carfilzomib discontinuation due to adverse events was 20.0% (56 mg/m^2) and 17.4% (70 mg/m^2). There were three total deaths in the 70 mg/m^2 group (one each due

to cardiac arrest, cardiac disorder, and progressive disease). Overall response rates were 90.0% (56 mg/m^2) and 89.1% (70 mg/m^2); 20.0% (56 mg/m^2) and 30.4% (70 mg/m^2) of pts achieved a complete response (CR) or stringent CR.

Summary/Conclusion: Once weekly KRd was effective and had manageable toxicity in pts with RRMM. As a weekly carfilzomib dosing regimen could offer pts greater convenience, these results support further clinical evaluation.

PS1298

EXTENDED 5-Y FOLLOW-UP OF PHASE 3 ELOQUENT-2 STUDY: ELOTUZUMAB PLUS LENALIDOMIDE/DEXAMETHASONE VS LENALIDOMIDE/DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

K. Weisel[1,*], M.A. Dimopoulos[2], S. Lonial[3], D. White[4], P. Moreau[5], M.-V. Mateos[6], J. San Miguel[7], K. Anderson[8], O. Shpilberg[9], S. Grosicki[10], I. Spicka[11], A. Walter-Croneck[12], H. Magen[13], A. Belch[14], D. Reece[15], M. Beksac[16], S.G. Shelat[17], S. Mekan[17], O. Sy[17], A.K. Singhal[18], P.G. Richardson[8]

[1]University of Tübingen, Tübingen, Germany, [2]National and Kapodistrian University of Athens School of Medicine, Athens, Greece, [3]Winship Cancer Institute, Emory University, Atlanta, United States, [4]Queen Elizabeth II Health Sciences Centre and Dalhousie University, Halifax, Canada, [5]University Hospital, Nantes, France, [6]University Hospital of Salamanca–Instituto de Investigación Biomédica de Salamanca (IBSAL), Salamanca, [7]Clínica Universidad de Navarra, Centro de Investigación Médica Aplicada, IDISNA, CIBERONC, Pamplona, Spain, [8]Dana-Farber Cancer Institute, Boston, United States, [9]Institute of Haematology, Assuta Medical Centers, Tel Aviv, Israel, [10]Medical University of Silesia, Katowice, Poland, [11]Charles University in Prague and General Teaching Hospital, Prague, Czech Republic, [12]Medical University of Lublin, Lublin, Poland, [13]Sheba Medical Center Hospital at Tel HaShomer, Ramat Gan, Israel, [14]Cross Cancer Institute and University of Alberta, Edmonton, [15]Princess Margaret Hospital, Toronto, Canada, [16]Ankara University, Ankara , Turkey, [17]Bristol-Myers Squibb, Princeton, [18]AbbVie Inc., Redwood City, United States

Background: Elotuzumab, an immunostimulatory monoclonal antibody targeting SLAMF7, has a dual mechanism of action, directly activating natural killer cells and tagging myeloma cells for death via antibody-dependent cell-mediated cytotoxicity. In the phase 3 ELOQUENT-2 study (NCT01239797) of elotuzumab plus lenalidomide/dexamethasone (ELd) in patients (pts) with relapsed/refractory multiple myeloma (RRMM), ELd showed a 30% reduction in the risk of disease progression or death vs lenalidomide/dexamethasone (Ld) alone at 2-y follow-up (FU); this was sustained at 3-y (27%) and 4-y (29%) FU. ELd also demonstrated a favorable trend in overall survival ahead of the final analysis planned at 427 deaths.
Aims: To evaluate the progression-free survival (PFS) and safety of ELd at 5 y, a milestone timepoint in cancer survival analyses.
Methods: Pts with RRMM and 1–3 prior lines of therapy were randomized 1:1 to receive ELd or Ld in 28-d cycles until disease progression, unacceptable toxicity, or withdrawal of consent. Coprimary endpoints were PFS and overall response rate (ORR) as assessed by independent review committee. Safety was an exploratory endpoint. All pts provided written informed consent.
Results: Of 646 pts, 321 were randomized to ELd and 325 to Ld. At database lock (Nov 29, 2017), 13% (ELd) vs 7% (Ld) of pts remained on treatment; disease progression (ELd 55% vs Ld 56%) was the most common reason for treatment discontinuation. At 5-y FU (minimum 60 mo), ELd demonstrated a relative improvement of 50% in PFS compared with Ld (18% vs 12%) and a 27% reduction in risk of progression or death vs Ld (hazard ratio [HR] 0.73; 95% CI 0.60, 0.87; Figure 1). Pts with very good partial response or better (ELd 36% vs Ld 30%) had the greatest reduction in risk of progression/death (HR 0.63; 95% CI 0.44, 0.89). Median (95% CI) PFS was 19 (17, 22) mo with ELd and 15 (12, 17) mo with Ld. ORR was higher with ELd vs Ld (79% vs 66%), and median duration of response was also longer (22 vs 17 mo) with ELd. All-cause grade 3–4 adverse events (AEs; ELd vs Ld) included blood and lymphatic system disorders (46% vs 46%), infections (35% vs 27%), vascular diseases (11% vs 8%), second primary malignancies (SPMs; 10% vs 6%), and cardiac disorders (5% vs 8%). The most common treatment-related grade 3–4 AEs were blood and lymphatic system disorders (35% vs 38%) and infections (16% vs 11%). Rates of all-cause, any-grade infections (84% vs 75%) and SPMs (17% vs 11%) were higher with ELd than Ld, which may reflect longer median duration of treatment (17 vs 12 mo for each individual agent in the ELd vs Ld regimens). Fewer deaths occurred in the ELd than the Ld arm (193 vs 208). Of all treated patients, most deaths (ELd vs Ld) were due to disease progression (39% vs 42%) or infection (8% vs 5%).

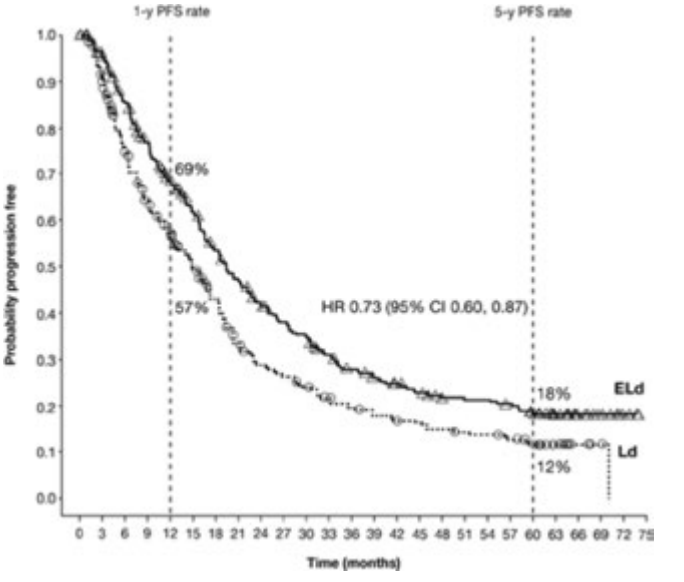


Figure 1.

Summary/Conclusion: At the milestone timepoint of 5 y, elotuzumab in combination with Ld showed durable, clinically relevant improvement in PFS and a 27% sustained reduction in the risk of progression/death vs Ld. Safety was consistent with previous findings, with minimal incremental AEs with ELd vs Ld despite longer exposure. ELd continues to show long-term treatment benefits, with the longest median FU of an immuno-oncology agent in MM.
Writing support: L Yee, Caudex, funded by BMS.

PS1299

LENALIDOMIDE-PREDNISONE VS LENALIDOMIDE ALONE MAINTENANCE IN NEWLY DIAGNOSED MULTIPLE MYELOMA: INDIVIDUAL PATIENT DATA META-ANALYSIS OF 2 RANDOMIZED PHASE III TRIALS

F. Gay[1,*], L. De Paoli[2], A. Larocca[1], C. Musolino[2], M. Genuardi[1], V. Montefusco[2], S. Spada[1], R. Centurioni[2], E. Aitini[2], A. Malfitano[1], G. Latte[2], G. De Sabbata[2], F. De Santis[1], N. Pescosta[2], P. Musto[2], G. Benevolo[2], T. Guglielmelli[2], N. Cascavilla[2], F. Patriarca[2], C. Conticello[2], R. Hajek[3], A. Spencer[4], M. Boccadoro[1]

[1]Myeloma Unit, Division of Hematology, University of Torino, A.O.U. Città della Salute e della Scienza di Torino, Torino, [2]Italian Multiple Myeloma Network, GIMEMA, Italy, [3]Department of Hematooncology, University hospital Ostrava, Ostrava, Czech Republic, [4]Alfred Health - Monash University, Melbourne, Australia

Background: Lenalidomide maintenance is approved in newly diagnosed multiple myeloma (NDMM) patients (pts) who received high-dose therapy (HDT) and autologous stem cell transplant (ASCT).
Aims: The primary aim was to compare progression-free survival (PFS), time to next therapy (TTNT), PFS2 and overall survival (OS) of NDMM pts randomized to lenalidomide-prednisone (RP) vs lenalidomide alone (R) maintenance. The secondary aim was to identify the main predictors of long term endpoints (PFS2 and OS) in pts receiving lenalidomide-based maintenance.
Methods: Data of 2 phase III multicenter randomized trials (EMN441 and EMN01) enrolling NDMM pts were pooled together. Pts received lenalidomide-based upfront treatment (EMN441 - transplant eligible pts: lenalidomide-dexamethasone [Rd] induction followed by 2 courses of HDT-ASCT or 6 cycles of cyclophosphamide-dexamethasone-lenalidomide; EMN01 - transplant ineligible pts: 9 cycles of Rd or melphalan-prednisone-lenalidomide or cyclophosphamide-prednisone-lenalidomide); after lenalidomide-based upfront treatment pts were randomized to maintenance with R alone (10 mg day 1-21 every 28) or plus prednisone (RP) (EMN441: 50 mg every other day [eod]; EMN01: 25 mg eod) until progression (PD)/tolerated. Data cut-off was October, 2017.
Results: 1051 pts were enrolled; 625 were eligible for maintenance: 315 received RP and 310 R. Median follow-up was 5 years. Median age was 69 vs 70 years in RP and R, respectively; 24% vs 21% of pts in RP and R had Revised-International Staging System (R-ISS) stage I, 69% vs 73% stage II

and 7% vs 6% stage III. Median duration of maintenance was 27 months (m) in RP and 22 m in R. At data cut-off, 78% vs 77% of pts discontinued RP and R; main reasons were PD (68% vs 75% in RP and R) and adverse events (15% in each groups). A trend towards improved PFS was seen with RP vs R (median 25 vs 19 m; HR 0.85, p=0.08). Median TTNT was 35 m with RP vs 30 m with R (HR 0.96, p=0.66), resulting in about a 10 m improvement from median PFS in both groups. There were no significant differences in PFS2 (median 57 vs 49 m; HR 0.99, p=0.947) and OS (5-year 60% vs 65%, HR 1.26, p=0.10) between the 2 arms. In multivariate cox regression analysis, the main baseline independent predictors of PFS in this lenalidomide-treated population were R-ISS Stage (I vs II: HR 0.52, p<0.001; I vs III: HR 0.40, p<0.001) and achievement of sCR/CR before maintenance (sCR/CR vs VGPR/PR: HR 0.67, p=0.04); R-ISS Stage was the main predictor of TTNT (I vs II: HR 0.48; p<0.001; I vs III: 0.34, p<0.001), PFS2 (I vs II: HR 0.38, p<0.001; I vs III: HR 0.26, p<0.001) and OS (I vs II: HR 0.39, p<0.001; I vs III: HR 0.23, p<0.001). In multivariate cox-regression analysis, the main independent factor predicting a longer time from first PD to start of second-line therapy was duration of maintenance (13-24 vs ≤12 m: HR 0.66 p<0.001; >24 vs ≤12: HR 0.30, p<0.001). Duration of maintenance had a similar impact on second PFS (13-24 vs ≤12 m: HR 0.73 p=0.002; >24 vs ≤12 m: HR 0.30, p<0.001) and on OS from relapse (13-24 vs ≤12 m: HR 0.64 p=0.008; >24 vs ≤12 m: HR 0.20, p<0.001).

Summary/Conclusion: Despite a moderate PFS benefit with RP vs R, the lack of PFS2 and OS advantage confirmed R alone as the standard maintenance. The 10-m median improvement from PFS to TTNT, plus the positive impact of prolonged duration of maintenance on time from first PD to start of second-line therapy, as well as its impact on second-PFS and OS from relapse, suggest no significant chemo-resistance with prolonged therapy and support lenalidomide use until PD.

PS1300

A GLOBAL TREATMENT STANDARD IN MULTIPLE MYELOMA (MM) REMAINS ELUSIVE DESPITE ADVANCES IN CARE OVER 15 YEARS: FIRST RESULTS FROM INSIGHT MM, THE LARGEST GLOBAL PROSPECTIVE, OBSERVATIONAL MM STUDY

M. Boccadoro[1,*], S.Z. Usmani[2], A. Chari[3], R. Rifkin[4], R. Abonour[5], J. Berdeja[6], G. Cook[7], C. Costello[8], H. Goldschmidt[9], R. Hajek[10], V. Hungria[11], H. C. Lee[12], X. Leleu[13], J. Omel[14], A. Spencer[15], M. A. Thompson[16], J. Vela-Ojeda[17], J. A. Zonder[18], N. Puig[19], A. Aguarón[20], F. E. Davies[21], B. Demers[22], K. Ren[22], S. Yu[23], T. Skacel[22], D. M. Stull[22], E. Terpos[24]

[1]Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino, Turin, Italy, [2]Levine Cancer Institute, Charlotte, [3]Mount Sinai School of Medicine, New York, [4]US Oncology Research/Rocky Mountain Cancer Centers, Denver, [5]Indiana University School of Medicine, Indiana Cancer Pavilion, Indianapolis, [6]Sarah Cannon Research Institute, Nashville, United States, [7]University of Leeds, Leeds, United Kingdom, [8]Moores Cancer Center, University of California San Diego, La Jolla, United States, [9]University Clinic of Heidelberg, Heidelberg, Germany, [10]University Hospital Ostrava, Ostrava, Czech Republic, [11]Clinica São Germano, São Paulo, Brazil, [12]The University of Texas MD Anderson Cancer Center, Houston, United States, [13]CHU Poitiers and Inserm CIC 1402, Poitiers, France, [14]The Central Nebraska Myeloma Support Group, Grand Island, United States, [15]The Alfred Hospital/MONASH University/Australian Centre for Blood Diseases, Melbourne, Australia, [16]Aurora Cancer Center, Aurora Health Care, Milwaukee, United States, [17]La Raza Medical Center, IMSS, México City, Mexico, [18]Karmanos Cancer Institute Wayne State University School of Medicine, Detroit, United States, [19]IBSAL, Hospital Universitario de Salamanca, Salamanca, Spain, [20]Myeloma Patients Europe (MPE), Brussels, Belgium, [21]University of Arkansas for Medical Sciences (UAMS), Little Rock, [22]Millennium Pharmaceuticals, Inc., Cambridge, MA, USA, a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, [23]Takeda Development Center Americas, Inc., Deerfield, IL, USA, a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Deerfield, United States, [24]National and Kapodistrian University of Athens, School of Medicine, Athens, Greece

Background: Therapeutic advances in the past 15 years have resulted in significantly improved outcomes for MM patients (pts). However, in worldwide routine clinical practice, there is no standard of therapy in the management of newly diagnosed or relapsed/refractory MM (NDMM/RRMM). Outcomes reported in real-world clinical practice versus controlled clinical trials show markedly shorter treatment durations, progression-free and overall survival. Understanding the global availability and effectiveness of treatment regimens is critical.

Aims: INSIGHT MM (NCT02761187), the largest global, prospective, observational MM study to date, aims to understand disease and pt characteristics at presentation and relapse, treatment and clinical outcomes, and the association of treatment with tolerability, effectiveness, quality of life, and healthcare resource utilization. This pre-specified first interim analysis focused on global and regional treatment patterns.

Methods: More than 4000 adult pts with NDMM or RRMM (1–3 prior therapies) will be enrolled from 15 countries in Europe (EU), the United States (US), Asia and Latin America (LA). Pts will be followed prospectively for ≥5 yrs.

Results: At data cut-off, 1000 (535 NDMM, 465 RRMM) pts had been enrolled in 13 countries. RRMM pts had received a median of 2 prior therapies. Overall, 476 (48%) pts were from EU (8 countries), 337 (34%) from the US, 94 (9%) from Taiwan, 93 (9%) from LA (3 countries); 77% White/Caucasian, 11% Asian, 8% Black/African American. Median age was 63 (range 32–89) and 67 (36–94) yrs for NDMM and RRMM pts, respectively; 68 (13%) and 91 (20%) were aged >75 yrs (19%/15%/11%/10% in EU/US/Taiwan/LA). 35% and 38% of NDMM and RRMM pts, respectively, had Charlson Comorbidity Index scores ≥1. 36% and 45% of RRMM pts had received prior SCT in 1st line and any line of therapy, respectively. Overall, 60% were treated at academic centers (96%/83%/29%/24% in Taiwan/EU/US/LA); 85% were treated outside of clinical trials. 29% were diagnosed unexpectedly during blood tests/radiological exams; other common reasons for pts seeking care were bone pain (34%), weakness/fatigue/anemia (13%), and kidney problems (6%); this breakdown was similar in academic and community centers. In RRMM pts, time to relapse decreased with each subsequent line of therapy (median times to relapse during 1st/2nd/3rd line therapy were 23.4/16.6/10.3 months). At start of treatment, 31% (NDMM) and 63% (RRMM) of physicians selected a treat-to-progression regimen. The most commonly administered regimens are shown in the Table 1. For NDMM pts triplet regimens dominated over doublets/monotherapy (41% vs 15%); bortezomib was most commonly used (65%). For RRMM doublets/monotherapy were more common than triplets (32% vs 14%); lenalidomide-based therapy was preferred at relapse (40%). 50% of pts who received a proteasome inhibitor (PI) at 1st line, received a PI-based regimen again at 2nd line; 32% of pts who received an immunomodulatory drug at 1st line received an immunomodulatory drug at 2nd line. Efficacy data are awaited.

Table 1.

Most common regimens for NDMM patients overall and by region/countries					
Rank	Overall (13 countries) N=535	Europe (8 countries) n=243	United States n=175	Taiwan n=71	Latin America (3 countries) n=46
1	VCD 89 (17%)	VCD 60 (25%)	VRD 68 (39%)	Td 28 (39%)	VCD 18 (39%)
2	VRD 79 (15%)	VTD 36 (15%)	Vd 21 (12%)	VTD 12 (17%)	CTD 6 (13%)
3	VTD 49 (9%)	Rd 20 (8%)	Rd 18 (10%)	VCD-T 10 (14%)	VMP 3 (7%)
Most common regimens for RRMM patients, overall and by region/countries					
Rank	Overall N=452	Europe n=227	United States n=157	Taiwan n=23	Latin America n=45
1	Rd 90 (20%)	Rd 49 (22%)	Rd 23 (15%)	Rd 13 (57%)	VTd 7 (16%)
2	Kd 35 (8%)	IRd 21 (9%)	Kd 14 (9%)		VCd, Vd-other, Rd Each n=5 (11%)
3	IRd 26 (6%)	Kd 18 (8%)	Vd 12 (8%)		Kd 3 (7%)

C, cyclophosphamide; d, dexamethasone; D, daratumumab; I, ixazomib; K, carfilzomib; M, melphalan; P, prednisone; R, lenalidomide; T, thalidomide; V, bortezomib.

Summary/Conclusion: There is no global treatment standard in NDMM/RRMM; marked regional differences exist, potentially due to differences in drug/trial availability and treatment patterns at academic vs community centers. Our data confirm PIs and immunomodulatory drugs as backbones of MM therapy, with bortezomib- and lenalidomide-based treatment dominating the NDMM and RRMM settings, respectively. New-generation agents, plus drugs with new mechanisms of action, are used less frequently and mostly in later lines of therapy.

PS1301

TREATMENT AND SURVIVAL OF PATIENTS WITH PRIMARY PLASMA CELL LEUKEMIA: A NATIONWIDE POPULATION-BASED STUDY AMONG 179 PATIENTS DIAGNOSED IN THE NETHERLANDS FROM 1989 TO 2015

M. Brink[1,*], O. Visser[2], S. Zweegman[3], P. Sonneveld[4], A. Broijl[4], N. van de Donk[3], A. Dinmohamed[1,4,5]

[1]Research, [2]Registration, Netherlands Comprehensive Cancer Organisation (IKNL), Utrecht, [3]Hematology, VU University Medical Center, Amsterdam, [4]Hematology, Erasmus MC Cancer Institute, [5]Public Health, Erasmus University Medical Center, Rot-

terdam, Netherlands

Background: Primary plasma cell leukemia (pPCL) is a very rare plasma cell dyscrasia with a very poor outcome. At present, there is a paucity of data on the outcome of pPCL, as prospective intervention studies in pPCL are very scare. Population-based studies can provide valuable information complementary to prospective intervention studies to assess treatment strategies and outcome in patients at the population level.
Aims: The aim of this nationwide, population-based study was to assess trends in primary therapy and survival among pPCL patients diagnosed during a 27-year period in the Netherlands.
Methods: We selected all pPCL patients diagnosed between 1989-2015 from the nationwide Netherlands Cancer Registry. Patients were categorized into three periods (1989-2000, 2001-2007, and 2008-2015) and two age groups (≤65 *v* ≥66 years). The periods were chosen based on the implementation of autologous stem cell transplantation (ASCT) and novel agents for multiple myeloma (MM) in the Netherlands. Data on primary therapy – i.e. no therapy and anti-pPCL therapy with or without a SCT – were available for individual patients. The primary end point was overall survival (OS), defined as the date from pPCL diagnosis to all-cause death. Patients alive were censored at February 1, 2017. Multivariable evaluation of OS was performed using Cox regression. p<0.05 indicates statistical significance.
Results: Our analytical cohort included 179 pPCL patients (median age, 65 years; age range, 34-91 years; 53% males; 79% diagnosed in non-academic centers; 8% with a prior malignancy). Patient characteristics were comparable across the three study periods. Overall, the application of anti-PCL therapy (including SCT) increased modestly from 88 to 92% among patients aged ≤65 (p=0.640) and from 67 to 71% among patients aged ≥66 (p=0.047) between 1989-2000 and 2008-2015, respectively. As for no therapy, the corresponding proportions were 12 and 8% among patients aged ≤65 and 33 and 29% among patients aged ≥66, respectively. What was noteworthy was that the application of SCT increased from 23 to 59% among patients aged ≤65 (p=0.004) and from 0 to 12% among patients aged ≥66 (p=0.084) between 1989-2000 and 2008-2015, respectively. During follow-up, 155 (87%) patients died. The median OS increased from 12.2 to 28.4 months for patients aged ≤65 (p=0.012; Figure 1A) and from 2.0 to 5.3 months for patients aged ≥66 (p=0.044; Figure 1B) between 1989-2000 and 2008-2015, respectively. Overall, when adjusted for the covariates listed in Table 1, the multivariable Cox model demonstrated an improvement of survival over time and an adverse effect of older age. However, when information on treatment was added to that model, the wider use of therapy explained the improved survival over time.

Table 1. Results of the multivariable Cox regression.

Covariate	Model without primary therapy HR[a]	95% CI	p[b]	Model adjusted for primary therapy HR[a]	95% CI	p[b]
Period of diagnosis						
1989-2000	1.26	0.83-1.91	0.279	1.41	0.92-2.17	0.113
2001-2007	1			1		
2008-2015	0.61	0.41-0.90	0.013	1.04	0.68-1.58	0.856
Female sex	1.05	0.76-1.45	0.753	0.92	0.67-1.28	0.628
Age ≥66 years at diagnosis	2.02	1.45-2.81	<0.001	1.24	0.87-1.78	0.235
Diagnosis in academic center	0.82	0.55-1.22	0.328	0.97	0.65-1.45	0.868
Previous malignancy	1.38	0.79-2.43	0.256	1.22	0.70-2.14	0.483
Primary therapy						
No therapy				2.64	1.78-3.93	<0.001
Anti-pPCL therapy without SCT				1		
Anti-pPCL therapy with SCT				0.34	0.20-0.57	<0.001

Abbreviations: HR, hazard ratio; CI, confidence interval; pPCL, primary plasma cell leukemia; SCT, stem cell transplantation.
[a]All covariates are simultaneously adjusted.
[b]P-values are compared with the reference category.

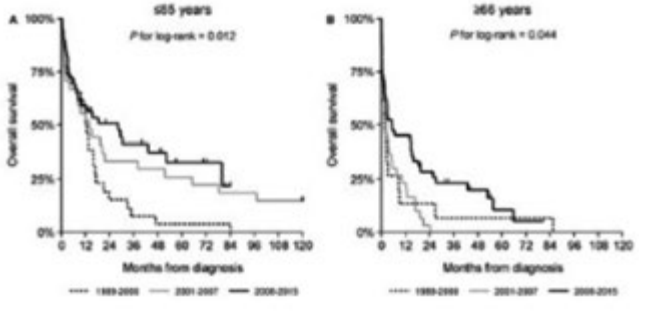


Figure 1.

Summary/Conclusion: In this nationwide, population-based study, we demonstrated that survival in pPCL improved over time. The improvement is largely explained by the wider application of SCT over time, especially among patients aged ≤65, and the introduction of novel agents. Collectively, the therapeutic advances achieved in MM seem to gradually translate into benefits for pPCL patients. Nevertheless, outcome remains disappointingly poor among patients aged ≥66. Therefore, prospective intervention studies in pPCL are imperative to further improve outcome.

PS1302

NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN AS A BIOMARKER IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA AND RENAL IMPAIRMENT: DATA FROM A PHASE II TRIAL OF POMALIDOMIDE PLUS DEXAMETHASONE

K. Weisel[1,*], M. A. Dimopoulos[2], F. Bridoux[3], N.W. Van de Donk[4], K. Ramasamy[5], E. Kueenburg[6], A. Di Micco[6], B. Rosettani[6], P. Bacon[6], P. Sonneveld[7]

[1]University Hospital of Tubingen, Tubingen, Germany, [2]National and Kapodistrian University of Athens, Athens, Greece, [3]Department of Nephrology and National Referral Center for AL Amyloidosis, CHU, Poitiers, France, [4]Department of Hematology, VU University Medical Center, Amsterdam, Netherlands, [5]Department of Hematology, Oxford University Hospitals NHS Foundation Trust, Oxford, [6] NIHR BRC Blood Theme, United Kingdom, [6]Celgene International, Boudry, Switzerland, [7]Erasmus MC Institute, Rotterdam, Netherlands

Background: Renal impairment (RI) is a common comorbidity in patients (pts) with multiple myeloma (MM) and is present in 20 to 30% of pts with newly diagnosed MM; approximately 10% require hemodialysis. Urine neutrophil gelatinase-associated lipocalin (NGAL), a protein that is released from injured nephron epithelia, has been studied as a biomarker of RI and MM disease burden. Pomalidomide (POM) is approved by the US Food and Drug Administration and European Medicines Agency for use in pts with RI, including those with severe RI on hemodialysis. A phase 2 trial investigated the safety and efficacy of POM plus low-dose dexamethasone (LoDEX) in pts with relapsed or refractory MM (RRMM) and moderate or severe RI, including those on hemodialysis. Additional markers, such as NGAL, were assessed for further understanding of their role in a population of pts with RRMM and RI.
Aims: To demonstrate the potential of NGAL as a prognostic biomarker in pts with RRMM and RI treated with POM + LoDEX.
Methods: Pts with RRMM and RI were enrolled in 3 cohorts: moderate RI (estimated glomerular filtration rate [eGFR] 30 to < 45 mL/min/1.73 m^2), severe RI (eGFR < 30 mL/min/1.73 m^2), and severe RI requiring hemodialysis. Pts provided informed consent. Urine samples were collected at baseline and sent to a central facility for NGAL analysis. Median NGAL across cohorts was 58.5 µg/L (range, 1.0-4096.0 µg/L). This median was used to delineate between low (baseline NGAL ≤ 58.5 µg/L) and high (baseline NGAL > 58.5 µg/L) NGAL levels.

Table 1.

Response	Low NGAL (n = 37)	High NGAL (n = 36)
Overall renal response rate	12 (32.4)	5 (13.9)
Renal CR	5 (13.5)	0
Renal PR	1 (2.7)	1 (2.8)
Renal MR	6 (16.2)	4 (11.1)
Stable	23 (62.2)	26 (72.2)
Worsening	2 (5.4)	3 (8.3)
Overall clinical response rate[a]	20 (54.1)	15 (41.7)
Overall response rate[b]	15 (40.5)	11 (30.6)
CR	0	0
VGPR	6 (16.2)	4 (11.1)
PR	9 (24.3)	7 (19.4)
MR	5 (13.5)	4 (11.1)
SD	15 (40.5)	15 (41.7)
PD	1 (2.7)	4 (11.1)
NE	1 (2.7)	2 (5.6)

[a] ≥ MR. [b] ≥ PR.
CR, complete response; MR, minimal response; NE, not evaluable; NGAL, neutrophil gelatinase-associated lipocalin; PD, progressive disease; PR, partial response; SD, stable disease; VGPR, very good partial response.

Key words: Pomalidomide, relapsed/refractory multiple myeloma, neutrophil gelatinase-associated lipocalin, renal impairment

Results: This analysis included 73 pts with urine NGAL measurements at baseline: 37 had low NGAL and 36 had high NGAL. Median age was similar between pts with low vs high NGAL (72.0 vs 71.0 yrs). In the low-NGAL group, 64.9% of pts were male vs 55.6% in the high-NGAL group. The median duration of MM was 48.2 mos in the low-NGAL group vs

43.2 mos in the high-NGAL group, and the median duration of RI was 35.7 vs 26.9 mos, respectively. Fewer pts in the low-NGAL group than in the high-NGAL group had an ECOG performance status of 2 (13.5% vs 27.8%) or had ISS stage III disease (78.4% vs 97.2%). Median percentage of plasma cells in the bone marrow aspirate at baseline was similar between the groups (20.0% vs 22.0%, respectively). Among 55 pts with cytogenetic data (low NGAL, 25; high NGAL, 30), fewer pts with low NGAL had del17p (8.0% vs 16.7%). At baseline, median involved serum κ free light chain (FLC) levels were 1446.50 mg/L and 3580.0 mg/L in the low-NGAL and high-NGAL groups, respectively, and λ FLC levels were 2365.0 mg/L and 2630.0 mg/L, respectively. Baseline median eGFR was 35.0 mL/min/1.73 m2 in pts with low NGAL and 17.5 mL/min/1.73 m^2 in pts with high NGAL. Treatment duration with POM + LoDEX was longer in pts with low NGAL than in pts with high NGAL (median, 31.0 vs 18.8 wks); these pts also had more cycles of treatment (median, 7.0 vs 4.5, respectively). The overall renal response rate was higher in the low-NGAL group (32.4% vs 13.9%, respectively; Table 1), as was ORR (40.5% vs 30.6%, respectively).

Summary/Conclusion: Low urine NGAL levels at baseline were associated with better response to POM + LoDEX treatment, as reflected in the higher ORR and overall renal response rate vs pts with high urine NGAL levels. Further, pts with low NGAL had longer times from diagnoses of MM and RI to study inclusion, while pts with high NGAL had worse renal function and more advanced disease at baseline. These findings suggest that higher NGAL may be related to more aggressive disease. NGAL may be a promising prognostic biomarker in pts with RRMM and RI.

PS1303

REAL WORLD CLINICAL IMPLICATIONS OF THE LIMITATIONS ASSOCIATED WITH THE REVISED INTERNATIONAL STAGING SYSTEM: UNSELECTED PATIENTS WITH MULTIPLE MYELOMA TREATED WITH NOVEL AGENTS IN AGEING SOCIETY

Y. Abe[1,*], K. Narita[1], H. Kobayashi[1], A. Kitadate[1], M. Takeuchi[1], K. Matsue[1]

[1]Division of Hematology/Oncology, Department of Internal Medicine, Kameda Medical Center, Kamogawa, Japan

Background: The Revised International Staging System (R-ISS) was developed in 2015 for more accurate prognostication by combining widely used International Staging System (ISS) with high-risk cytogenetic abnormalities (CA) [t(4;14), t(14;16) and del(17p)] as well as elevated serum lactate dehydrogenase. However, both the original and validation studies of R-ISS included relatively young patients with a median age of approximately 65 years, and more than half of the patients included in these studies were treated without bortezomib.

Aims: We aimed to investigate the superiority of R-ISS to ISS in a real world setting of an ageing society, by determining the usefulness of both R-ISS and ISS in patients with multiple myeloma (MM) treated with novel agents.

Methods: Data for 245 consecutive patients with newly diagnosed MM who were treated with chemotherapy between January 2006 and October 2017 at our institution were retrospectively analyzed. Diagnosis and treatment response were evaluated per IMWG criteria. We included patients who only received novel agents (bortezomib and/or lenalidomide).

Results: In contrast to previous studies, the median age of all 245 patients included in our study was 73.3 years, and bortezomib was used in most of the patients (96.7%). The number of patients categorized as ISS stage I, II, and III were 50 (20.4%), 66 (26.9%) and 129 (52.7%) with a median age of 69.9, 74.7 and 74.4 years, respectively. The number of patients categorized as R-ISS stage I, II, and III were 33 (14.3%), 140 (60.9%) and 57 (24.8%) with a median age of 70.3, 74.5 and 70.3 years, respectively. There was a significant difference in age between R-ISS stage II and III patients; The R-ISS stage II patients were significantly older than the R-ISS stage III patients (p=0.014). There was no significant difference in overall survival (OS) between R-ISS stage II and III patients (median OS, 73.2 and 52.1 months, respectively; p=0.192); however, a significant difference in OS was detected between ISS stage II and III patients (p=0.048) (Figure 1). Patients who were recategorized from ISS stage III to R-ISS stage II were significantly older than those who were recategorized from ISS stage III to R-ISS stage III (median age, 76.5 and 70.3 years, respectively); however, no significant difference in OS was detected between these patients. Patients with t(4;14) or t(14;16) were significantly younger than those without (median age, 74.0 and 69.3 years, respectively; p=0.012). Accordingly, there was no significant difference in OS regardless of harboring any of the high-risk CA (median OS, 60.3 and 86.4 months, respectively; p=0.572). Furthermore, patients with t(4;14) had rather favorable OS than those without, although the difference was not statistically significant (median OS, not reached and 58.3 months, respectively; p=0.096).

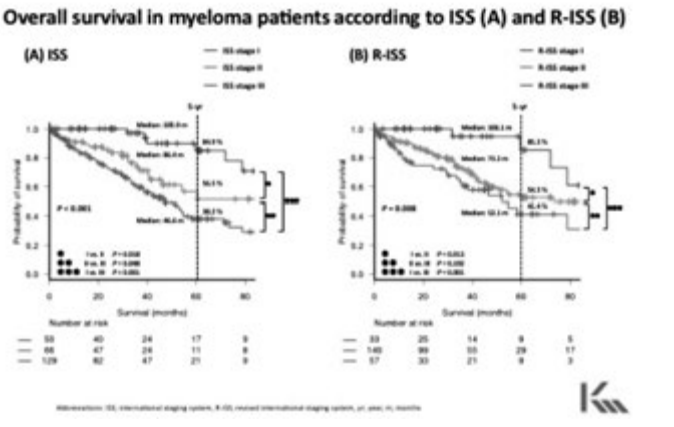


Figure 1.

Summary/Conclusion: To our knowledge, this is the first report on the limitations associated with R-ISS over ISS when used in patients with MM with a wide age-range who are treated with bortezomib-containing regimens in an ageing society. This limitation may be attributed to adopting t(4;14) as a high-risk CA for R-ISS classification. This potential limitation should be widely considered in risk stratification for MM patients because many developed countries including Western countries will successively enter the period of super-ageing society, like Japan.

PS1304

EVALUATION OF MINIMAL RESIDUAL DISEASE USING NEXT GENERATION FLOW CYTOMETRY IN PATIENTS WITH LIGHT CHAIN (AL) AMYLOIDOSIS

E. Kastritis[1], I. Kostopoulos[2,*], E. Terpos[1], D. Fotiou[1], M. Gavriatopoulou[1], N. Kanellias[1], D. C. Ziogas[1], M. Roussou[1], M. Migkou[1], E. Eleutherakis-Papaiakovou[1], O. Tsitsilonis[2], M.A. Dimopoulos[1]

[1]Department of Clinical Therapeutics, [2]Department of Biology, National and Kapodistrian University of Athens, Athens, Greece

Background: Complete hematologic response (hemCR) is associated with better survival and organ function improvement in patients with AL amyloidosis. Hematologic relapses occur even in pateinst with a hemCR and organ function may continue to deteriorate due to small residual clones that may lead to disease recurrence or may produce very small amounts of toxic light chains, which go undetectable by conventional techniques. Next generation flow cytometry (NGF) is a very sensitive method to evaluate the presence of minimal residual disease (MRD) and is a standard method for the assessment of MRD in patients with myeloma.

Aims: The aim of the current study was to evaluate feasibility and applicability of MRD by NGF in patients with AL at complete hematologic response.

Methods: We evaluated MRD in 24 patients with AL amyloidosis in sustained hemCR. MRD was assessed in BM samples according to the Euroflow guidelines, using two independent 8-color panels, both containing CD19-PC7, CD27-BV510, CD38-FITC, CD45-PerCPCy5.5, CD56-PE, CD138-BV421, and additionally CD117-APC and CD81-APCC750 only in the surface tube or CyIgκ-APC and CyIgλ-APCC750 only in the intracytoplasmic tube. A median number of 5 million events (range $3.9x10^6$-$6.1x10^6$) were acquired for each tube in a BD FACSCantoII cytometer and data analysis was conducted with Infinicyt software, that allowed merging of the two panels based on the 6 backbone markers, offering a median sensitivity level of $2.3x10^{-6}$ (range $2x10^{-6}$-$3.1x10^{-6}$).

Results: The median age at diagnosis was 59 years (range 42-75), 75% had λ-light chain, 90% had renal, 15% liver and 35% had cardiac involvement; 40% were Mayo stage-1, 50% stage-2 and 10% stage-3. At diagnosis median dFLC was 94 mg/L (range 17-879) and 15% had negative serum and urine immunofixation. Median clonal plasma cell infiltration was 8%. Primary treatment was bortezomib-based in 88% and MDex in 11%, while 33% had received ASCT. At the time of MRD testing, 50% of the patients had achieved a renal response, 4/7 (57%) patients with cardiac involvement had a cardiac response, and 1/3 (33%) a liver response. Ten (42%) patients were MRD negative (MRD^{neg}) and 14 (58%) were positive (MRD^{pos}). Notably, 6/14 (43%) MRD^{pos} cases had very low residual tumor load at levels $<3x10^{-5}$. Median time from CR to MRD testing was 36 months (39 vs 35 months for MRD^{pos} vs MRD^{neg} patients). MRD was positive in 3/4 patients with negative baseline serum and urine immunofixation and in 2/3

patients with baseline dFLC<40 mgL. Responses of at least one involved organ were documented in 70% of patients and in particular in 70% of MRD^{pos} patients vs 67% of MRD^{neg}. Among cardiac responders (n=4), 3 were MRD^{neg}. Renal responses occurred in 67% of MRD^{pos} and 75% of MRD^{neg} patients. MRD^{neg} patients had more often response in more than one organ (in all a cardiac and a renal response) while among MRD^{pos} patients all had responses to a single organ. We found no significant differences in baseline characteristics (age, dFLC, BM infiltration, NTproBNP, Mayo stage) among MRD^{neg} and MRD^{pos} patients. Among patients who received ASCT, 2/8 (25%) were MRD^{neg} vs. 6/12 (50%) that were MRD^{neg} among patients who did not have ASCT.

Summary/Conclusion: Among patients with AL amyloidosis in sustained hemCR, assessed with high sensitivity NGF 42% were MRD^{neg} and 58% were MRD^{pos}. This finding may have implications in the management of patients with AL who achieve a hemCR, especially among those who fail to achieve an organ response and may also have implication for their management, in an era of expanding treatment options.

PS1305

EARLY LIGHT CHAIN KINETICS AND DEPTH/DURATION OF HEMATOLOGIC RESPONSES TO DARATUMUMAB IN PREVIOUSLY TREATED LIGHT CHAIN AMYLOIDOSIS

G. Kaufman[1,*], S. Schrier[1], R. Witteles[2], P. Ulloa[1], M. Lugtu[1], S.A. Arai[3], M. O'Shaughnessy[4], R. Lafayette[4], M. Liedtke[1]

[1]Hematology, [2]Cardiology, [3]Blood and Marrow Transplantation, [4]Nephrology, Stanford University, Palo Alto, United States

Background: Daratumumab (dara) can produce rapid and deep ≥VGPR in pts with previously treated light chain amyloidosis (AL). However, around 1/3 of pts experience non-response (NR) or plateau at partial response (PR). Furthermore, little is known about hematologic response duration. Biomarkers correlating with depth and duration of response would be clinically useful, as have been identified for myeloma (Moritz B. ASH 2017 abs 4355).
Aims: To evaluate the relationship between early changes in disease specific light chains and long term response and clinical outcomes of AL pts treated with dara.
Methods: We retrospectively studied 44 pts with biopsy proven AL, previously treated, who subsequently received dara (with dexamethasone) at our institution between 1/2016 and 2/2018. Dara was given intravenously at a dose of 16 mg/kg weekly for 8 weeks, then biweekly for 16 weeks, and monthly thereafter. We evaluated differential free light chain (dFLC = involved–uninvolved serum light chain) changes at one month into therapy (d28), in relation to heme depth of response per consensus criteria and time to next therapy (TNT) defined as time to addition of, or change to alternative plasma cell directed agents, or death due to any cause. The Wilcoxon each pair method was used to compare change in dFLC at d28 with depth of response achieved to dara. Proportional hazards models were constructed to analyze the impact of early dFLC changes with TNT.
Results: Forty-four pts received a median 2 prior lines of therapy (1-6), including bortezomib or carfilzomib in 43 (97.8%), and lenalidomide or pomalidomide in 24 (55%). Median follow up from dara initiation was 9 months (1-24). Prior to dara, median dFLC was 4.9 mg/dL (0.4-46.7). For all pts, median d28 reduction in dFLC was 71% and maximal median reduction in dFLC during dara was 87%. Of 8 NR pts, the median d28 reduction in dFLC was 13%, of 7 pts achieving a maximum PR the median d28 dFLC reduction was 63% (p<0.01 vs NR), whereas for the 29 pts with VGPR or CR the median d28 reduction in dFLC was 81% (p<0.0001 vs NR, p=0.04 vs PR). Of 12 pts achieving less than 50% reduction in dFLC at d28, only 4 (33%) went on to achieve ≥PR. Failure to achieve at least 50% reduction in dFLC at d28 was associated with a significantly increased risk of being a dara non-responder (RR 3.67, CI 1.7-7.1, p<0.001). Median TNT was not reached for the cohort and at 9 months 70% of pts had not met criteria for TNT failure (Figure 1). Failure to achieve ≥50% reduction in dFLC at d28 was associated with shorter TNT (HR 4.28, CI 1.2-13.7, p=0.02). Of importance, median number of dara infusions after d28 was 6 in the group achieving less than 50% reduction in d28 dFLC, and 12 in the group achieving >50% reduction in d28 dFLC. Among the deep responding subgroup of 29 pts with ≥VGPR, there was no relationship between early TNT failure and d28 dFLC reduction (HR 1.005, CI 0.95-1.08, p=0.86) though the number of events was low (n=4) and 25/29 pts had not met TNT failure at last follow up.
Summary/Conclusion: In pts with pretreated AL receiving dara, responses stratify after the first treatment cycle, and change in dFLC by d28 was significantly associated with longer term treatment response. Pts achieving <50% dFLC reduction at d28 were unlikely to experience a deep heme response with further dara monotherapy. For the subgroup of deep dara responders (≥VGPR), d28 dFLC reduction did not correlate with TNT though number of events was low and longer follow up is needed.

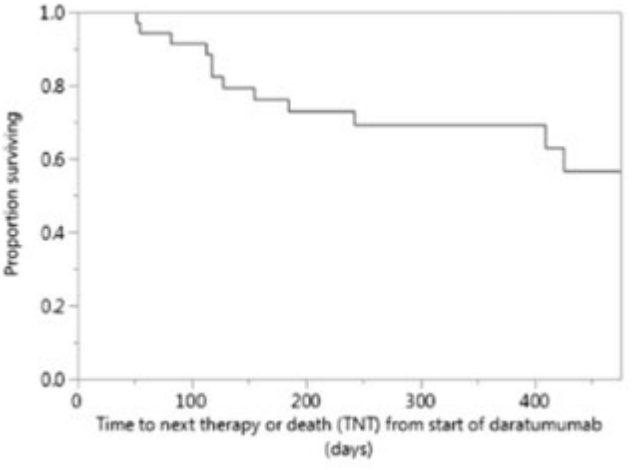


Figure 1.

PS1306

EFFICACY AND TOLERABILITY OF DARATUMUMAB AFTER ALLOGENEIC TRANSPLANTATION FOR HEAVILY TREATED MULTIPLE MYELOMA

A. P. Gonzalez-Rodriguez[1,*], L. Lopez Corral[2], A. Bermudez[3], T. Zudaire Ripa[4], P. Santos Diaz[5], A.J. Gonzalez-Huerta[1], P. Palomo[1], M.V. Mateos[2], S. Gonzalez-Muñiz[1]

[1]Hematology- Stem cell transplantation Unit, Hospital Universitario Central de Asturias, Oviedo, [2]Hematology, Hospital Universitario de Salamanca, Salamanca, [3]Hematology, Hospital Universitario Marques de Valdecilla, Santander, [4]Hematology, Complejo Hospitalario de Navarra, Pamplona, [5]Hematology, Complejo Hospitalario Universitario de Pontevedra, Pontevedra, Spain

Background: Allogenic stem cell transplantation (allo-STC) in multiple myeloma (MM) in Europe has increased in the last decades and although it is not a curative approach, the allo-SCT contributes to prolong the overall survival (OS) mainly because of the responses so far reported with novel agents after allo, through the enhancement of the graft versus myeloma. Daratumumab targets CD38 on the plasma cells resulting into a direct antitumor activity but also reduces local immune suppression within the bone marrow microenvironment, facilitating the expansion of positive immune effector cells, contributing to the antimyeloma response.
Aims: In spite of its efficacy, there are not many data about its efficacy and safety of Daratumumab in patients that had been previously received allo-SCT especially because this population was not allowed to be included in the trials and most All-SCT relapsed and we must decide the next treatment.
Methods: In this multi-center retrospective analysis, we collected and analyzed efficacy and safety data of patients receiving Daratumumab for relapsed MM following allo-SCT.
Results: Eleven patients were included (males, n=9) and the median age was 57 years (range 34-66). Eight out of 11presented with high risk features (2 plasma cell leukemia, and 6 genetic features of high risk) and 3 with early relapsed (≤ 12 months after allo-SCT). Patients were heavily pretreated and received a median of 6 prior lines (4-10). Fifty four percent were bortezomib-refractory and 81% lenalidomide-refractory. The median time from allo-STC to first dose of Daratumumab was 25 moths (7-96). Median number of Daratumumab infusions was 13 (range 4-26). Ten patients were evaluable for response and 5 (50%) achieved at least partial response (1 complete response, 1 very good partial response, 3 partial response). Three additional patients achieved stable disease so daratumumab single agent did control the disease in 80% of the patients.
Safety profile was acceptable, only two patient had infusion related reaction during the first infusion (grade 1). GVHD reactivation was only reported in a patient with prior history of acute GVHD but daratumumab did no trigger a flare for cGVHD. Four patients developped infections during treatment (pseudomona, enterobacter, CMV, influenza B). Four deaths have been

so far reported, 3 due to progression and one due to CMV infection. There are 4 patients that are ongoing receiving daratumumab. The median of PFS was 11months and median overall survival not reached at a median of follow-up of 9 moths.

Summary/Conclusion: In conclusion, we confirmed the efficacy of Daratumumab in the setting of MM relapsing after Allo-SCT in heavily treated patients with overall response of 50% and disease control rate of 80%. This encouraging efficacy is associated with a safety profile consistent with other daratumumab studies and no new safety signal. All these findings would need to be confirmed in large trials but Daratumumab can be used after allo-SCT without risk of GVHD reactivation.

PS1307

CHARACTERISTICS, TREATMENT PATTERNS, AND SURVIVAL OUTCOMES OF PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) IN NORTH AMERICA AND EUROPE: FINDINGS FROM THE PREAMBLE STUDY

R. Vij[1,*], C. Chen[2], C. Davis[2], H. Kim[3], B. Durie[4], G. Cook[5], H. Goldschmidt[6]

[1]Washington University School of Medicine, St Louis, [2]Bristol-Myers Squibb, Princeton, [3]PAREXEL, Billerica, [4]Cedars-Sinai Medical Center, Los Angeles, United States, [5]University of Leeds, Leeds, United Kingdom, [6]Heidelberg University Hospital, Heidelberg, Germany

Background: With improved treatment strategies and access to therapies that have novel mechanisms of action (MoAs), prolonging survival for patients (pts) with MM has become a realistic goal. Proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs), and PI+IMiD combinations have become the standard of care in MM. In addition, the monoclonal antibodies (mAbs) elotuzumab and daratumumab are a relatively new class of approved agents. Despite increasing treatment options, access to therapies may vary between North America (NA) and Europe, thereby affecting outcomes.

Aims: To evaluate the impact of regional treatment differences on outcomes in RRMM.

Methods: Pts aged ≥18 y with RRMM from NA (US and Canada) and Europe (France, Germany, Italy, and the UK) were identified from PREAMBLE (NCT01838512), an ongoing, prospective, observational study. Pts had ≥1 prior therapy, and began treatment with a PI, IMiD, PI+IMiD combination, or new agent (those with newer MoAs, such as mAbs, histone deacetylase inhibitors, and novel combinations) ≤90 d before or 30 d after informed consent. Data were collected at every healthcare visit for 3 y or until the end of follow-up (FU). Baseline characteristics and length of FU were assessed using descriptive analysis. Statistical comparisons were made using *t* tests, Mann–Whitney *U* tests (continuous variables), and chi-square tests (categorical variables); 2-sided p-values were obtained. Overall survival (OS) was analyzed using Kaplan–Meier (K-M) and multivariate Cox regression (adjusted from covariates selected using stepwise regression) techniques.

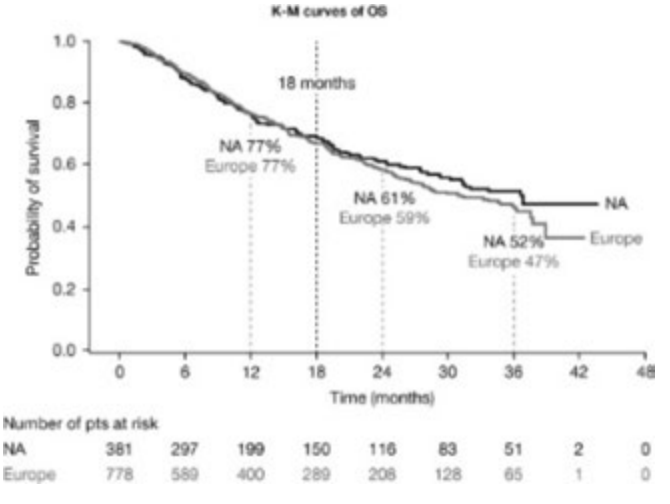


Figure 1.

Results: At database lock (Nov 30, 2017), 381 pts in NA and 778 pts in Europe were identified. In total, 58% of pts in NA and 48% of pts in Europe withdrew from the study due to death or other reasons; most deaths were due to disease progression (NA, 68%; Europe, 67%). Median (range) FU was 13 (7–27) mo and 12 (6–25) mo for pts in NA and Europe, respectively. Pts in NA vs Europe were younger at enrollment (median 66 y vs 70 y; p<0.0001), but more likely to have comorbidities (94% vs 80%; p<0.0001), including vascular (63% vs 42%), metabolic and nutritional (46% vs 27%), and musculoskeletal/connective tissue (42% vs 22%) disorders. Prior to enrollment, a greater proportion of pts in NA than Europe received ≥2 therapies (62% vs 52%; p=0.004), PI+IMiDs (29% vs 16%; p<0.0001), and new agents (3% vs 1%; p=0.0390). After enrollment, a greater proportion of pts in NA vs Europe received PIs (56% vs 46%), PI+IMIDs (29% vs 9%), and new agents (21% vs 5%; p<0.001 for all). Median (95% CI) OS was 37 (29, not estimable) mo in NA and 31 (27, 38) mo in Europe. In a multivariate analysis, risk of death was 17% lower in pts in NA vs Europe (hazard ratio [95% CI]: 0.83 [0.66, 1.04]; p=0.110). A significant separation of OS K-M curves in favor of NA was seen from 18 mo onward (p=0.0402), but not across the entire FU period (Figure 1).

Summary/Conclusion: Real-world data suggest that pts with RRMM in NA had greater access to newer agents and PI+IMiD combination regimens than pts in Europe. Survival analysis identified a potential but non-significant reduction in the risk of death for pts in NA vs Europe. OS was significantly higher from 18 mo onward for pts in NA vs Europe, which may be partly due to possible benefits from newer agents often reserved for later lines of therapy. Further analysis is warranted.

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PS1308

COMPLETE RESPONSE AND INDUCTION TREATMENT DOES NOT AFFECT SURVIVAL IN MYELOMA PATIENTS RELAPSING WITHIN 18 MONTHS FOLLOWING UP-FRONT HDM-ASCT. A POPULATION-BASED STUDY FROM 1994 TO 2015

A. Vangsted[1,*], S.N. Clemmensen[1], K. Dimoupolous[1], U.C. Frølund[2], C. Helleberg[3], N.F. Andersen[4], N. Abildgaard[5], P. Gimsing[1], H. Gregersen[6], T.W. Klausen[3]

[1]Department of Hematology,, Rigshospitalet, Copenhagen University, Denmark, Copenhagen, [2]Department of Hematology,, Department of Hematology, Roskilde Hospital, Roskilde, [3]Department of Hematology,, Herlev University Hospital, Copenhagen, [4]Department of Hematology, Aarhus University Hospital, Aarhus, [5]Department of Hematology,, Odense University Hospital, Odense, [6]Department of Hematology,, Aalborg University Hospital, Aalborg, Denmark

Background: Despite the introduction of proteasome inhibitors (PI) as induction treatment preceding high-dose melphalan an autologous hematopoietic stem cell transplantation (HDM-ASCT) for patients with multiple myeloma (MM), a subgroup of these patients experiences early relapsed myeloma (ER) leading to poor overall survival (OS).

Aims: To analyze if there is a difference in OS for MM patients achieving greater-than or equal to complete remission (CR) compared to patients achieving less than or equal to partial remission (PR) among newly diagnosed MM patients with ER within 18 months after up-front HDM-ASCT.

Methods: We analyzed the importance of achieving at least CR in a population-based cohort of 929 patients uniformly treated with HDM-ASCT. Patients treated in the high-dose trials conducted by the Nordic Myeloma Study Group (NMSG no. 5/94, 7/98 and 11/00) between 1994 and 2004 and patients registered prospectively in the Danish National Multiple Myeloma Registry between 2005 and 2015 were included. The median follow-up for OS was 63.0 months (43.6-91.8 months). Induction treatment was vincristine, adriamycin, and dexamethasone; cyclophosphamide and dexamethasone; bortezomib and dexamethasone with and without cyclophosphamide.

Results: Among patients experiencing ER, 32.3% achieved ≥ CR and 67.7% achieved PR, compared to patients with a later relapse where ≥ CR was seen in 45.3% and PR in 54.7% (p=0.0002). In patients with an ER, the fraction of patients with ≥ CR was higher (38.5%) for patients treated with a bortezomib-based induction as compared to other regimens (28.8%). There was no difference in the fraction of patients with an ER achieving ≥CR or PR in relation to bortezomib-based treatment and other treatments (OR: 1.8; CI 1.1-2.8) versus (OR: 1.7; CI: 1.1-2.4) (p=0.84), respectively. Furthermore, the fraction of patients achieving ≥CR did not differ in relation to the calendar period 1994-2004 (OR: 1.6; CI: 0.8-3.1) and 2005-2013 (OR: 1.8; CI: 1.3-2.5)(p=0.73). Interestingly, for patients with an ER, achieving ≥ CR did not result in better OS after HDT-ASCT or OS after their first relapse post-HDT-ASCT (Figure 1).

Summary/Conclusion: This population-based study shows, that among patients with an ER within 18 months, achievement of CR does not translate into improved OS irrespectively of the inclusion of PI in induction

treatment. Whether these patients have a unique resistant disease or still are MRD (minimal residual disease) positive after HDM-ASCT remains to be analyzed.

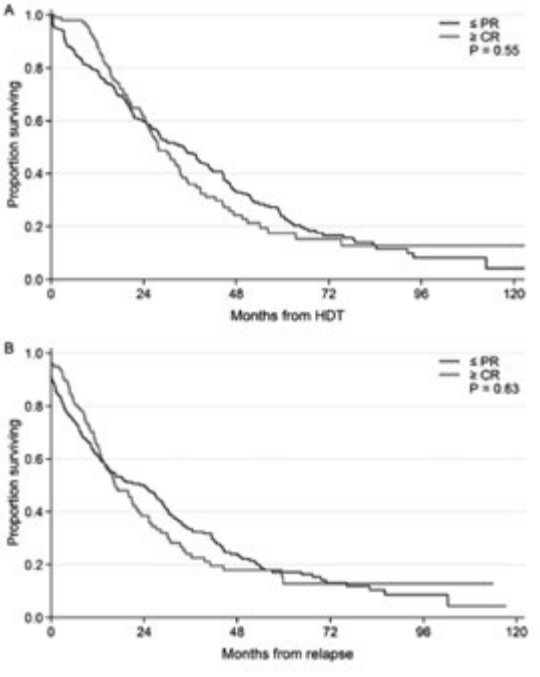

Figure 1. Overall survival for patients with an ER within 18 months depending on response after HDM-ASCT (≥ CR or ≤ PR).The upper panel shows OS after HDT-ASCT and the lower panel shows OS after first relapse after HDM-ASCT. The red line is patients achieving ≥ CR; the blue line is patients achieving ≤ partial remission (PR).

PS1309

OVERALL SURVIVAL OF PATIENTS POST-TRANSPLANT: STUDY RESULTS FROM TWO PHASE 3 TRIALS ASPIRE AND ENDEAVOR

H. Goldschmidt[1,*], M.-V. Mateos[2], D. Siegel[3], R. Abonour[4], H. Ludwig[5], M. Obreja[6], K.S. Iskander[6], P. Hari[7]

[1]University Hospital Heidelberg, Heidelberg, Germany, [2]Hematology Service, University Hospital Salamanca, Salamanca, Spain, [3]John Theurer Cancer Center, Hackensack University Medical Center, Hackensack, [4]Indiana Cancer Pavilion, Indianapolis, United States, [5]Department of Medicine, Wilhelminen Cancer Research Institute, Wilhelminenspital, Vienna, Austria, [6]Amgen Inc., Thousand Oaks, [7]Medical College of Wisconsin, Milwaukee, United States

Background: In the phase 3 ASPIRE (carfilzomib-lenalidomide-dexamethasone [KRd] vs lenalidomide-dexamethasone [Rd]) and ENDEAVOR trials (carfilzomib-dexamethasone [Kd56] vs bortezomib-dexamethasone [Vd]), carfilzomib-based regimens demonstrated superior overall survival (OS) vs standard therapies in relapsed/refractory multiple myeloma (RRMM).
Aims: In this subgroup ad-hoc analysis of ASPIRE and ENDEAVOR, OS was evaluated based on prior autologous stem cell transplant (ASCT) status.
Methods: OS was summarized using the Kaplan-Meier method.
Results: Of the patients with prior ASCT in ASPIRE (n=446) and ENDEAVOR (n=538), baseline patient and disease characteristics were generally balanced between treatment groups. In ASPIRE, patients with prior ASCT treated with KRd had an 11.4 month improvement in median OS and, for those with first relapse after ASCT, an 18.6 month improvement in median OS (57 months vs 39 months; HR=0.71 [0.48, 1.0]). In ENDEAVOR, a consistent OS benefit was observed for Kd56 vs Vd across ASCT subgroups; the benefit was more evident in the no prior transplant groups. In the updated safety analysis, adverse events were comparable between patients with and without prior ASCT and consistent with previous reports (Hari *et al.*, *Leukemia* 2017) Table 1.
Summary/Conclusion: These results suggest carfilzomib-based regimens led to clinically meaningful improvements in survival for patients who had received prior ASCT. For patients who were treated at first relapse, more differences were observed for KRd vs Rd after ASCT and Kd56 vs Vd for transplant-ineligible patients.

Table 1.

	ASPIRE[1]				ENDEAVOR[2]			
	Prior ASCT		No Prior ASCT		Prior ASCT		No Prior ASCT	
	KRd	Rd	KRd	Rd	Kd56	Vd	Kd56	Vd
ITT population, n	217	229	179	167	264	272	198	193
Median OS (95% CI), months	52 (46, 66)	41 (31, 48)	40 (32, 49)	40 (31, 46)	48 (44, NE)	41 (33, 42)	51 (40, NE)	37 (25, NE)
Median PFS (95% CI), months	26 (23, 32)[3]	18 (14, 22)[3]	26 (21, 31)	17 (14, 22)	NE (16, NE)[3]	10 (9, 12)[3]	18 (14, NE)	9 (8, 10)
First relapse, n	88	78	96	79	123	141[4]	109	90
3-year OS (95% CI), %	68 (57, 76)	52 (40, 63)	51 (41, 61)	48 (36, 58)	66 (56, 74)	64 (55, 71)	64 (54, 73)	57 (45, 68)
Median PFS (95% CI), months	30 (21, 39)[3]	18 (13, 26)[3]	28 (20, NE)	17 (13, 26)	NE (17, NE)[3]	11 (9, 15)[3]	19 (15, NE)	10 (7, 13)
Patients with at ≥1 treatment-related adverse event, n	215	224	177	165	266	268	197	188
Grade ≥3	73%	60%	66%	64%	58%	51%	62%	57%
Patients discontinuing due to AEs	8%	10%	9%	12%	19%	21%	18%	20%

[1]Data cutoff 28 Apr 2017.
[2]Data cutoff 3 Jan 2017.
[3]Hari et al, *Leukemia* 2017.
[4]For PFS, n=142.
ASCT, autologous stem cell transplant; ITT, intent-to-treat; Kd56, carfilzomib-dexamethasone; KRd, carfilzomib-lenalidomide-dexamethasone; NE, not estimable; OS, overall survival; Rd, lenalidomide-dexamethasone; Vd, bortezomib-dexamethasone.

PS1310

DURABLE PROGRESSION-FREE SURVIVAL BENEFIT IN EARLY RESPONDERS TO ELOTUZUMAB PLUS LENALIDOMIDE AND DEXAMETHASONE FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA: ANALYSIS OF 4-YEAR DATA FROM ELOQUENT-2

C. Scheid[1,*], C. Röllig[2], K. Weisel[3], H.-J. Salwender[4], M. Gramatzki[5], K. Edmaier-Schröger[6], O. Sy[7], H. Goldschmidt[8]

[1]University Clinic Cologne, Cologne, [2]University Clinic Dresden, Dresden, [3]University Clinic Tübingen, Tübingen, [4]Asklepios Klinik Hamburg, Hamburg, [5]University Clinic Schleswig-Holstein, Kiel, [6]Bristol-Myers Squibb, Munich, Germany, [7]Bristol-Myers Squibb, Princeton, United States, [8]University Clinic Heidelberg, Heidelberg, Germany

Background: Despite therapeutic advances, most patients with multiple myeloma (MM) ultimately experience relapse. In the phase 3 randomized ELOQUENT-2 study (NCT01239797) of elotuzumab combined with lenalidomide and dexamethasone (ELd) vs Ld for relapsed/refractory (RR) MM, overall response rate (ORR) was 79% vs 66% and median duration of response (DOR) was 21 vs 17 months at 4-year follow-up. Durable benefits in progression-free survival (PFS) vs Ld were reported, with a 29% reduction in the risk of progression/death and an overall survival (OS) benefit with ELd in the 4-year analysis (Dimopoulos MA *et al.* EHA 2017 [Oral S456]; final OS analysis planned at 427 events). MM is typically more aggressive with each relapse, and survival shorter with each failed therapy, presenting a challenge for clinicians treating RRMM (Dimopoulos MA *et al. Nat Rev Clin Oncol* 2015;12:42–54). Clinical decisions are often made in a timeframe of ~3 months after initiation of treatment, and responses to ELd typically begin early; in ELOQUENT-2, median time to first response (TTR) was 2 months and median time to best response was 3 months.
Aims: To analyze PFS in patients achieving early responses (within 3 months of treatment initiation) to either ELd or Ld, with an extended 4-year follow-up of ELOQUENT-2.
Methods: In ELOQUENT-2, patients ≥18 years of age with RRMM and 1–3 prior lines of therapy were randomized 1:1 to either ELd or Ld, after informed consent. Coprimary endpoints were PFS and ORR; DOR and TTR were exploratory endpoints. In this landmark analysis, PFS was analyzed by TTR for patients who achieved ≥partial response (PR) or ≥very good PR (VGPR).
Results: At database lock (Oct 18, 2016), 321 patients had been randomized to ELd and 325 to Ld; minimum follow-up was 48 months. Baseline characteristics were similar between arms; median age (ELd vs Ld) was 67 vs 66 years, 21% (both arms) had stage III disease, 52% vs 57% had prior stem cell transplantation, 35% (both arms) had refractory disease, and median time from diagnosis was 3.5 years (both arms). Patients with TTR (≥PR) of <3 months (ELd vs Ld: 210 [65%] vs 172 [53%]) showed a durable PFS benefit, with a 27% reduction in the risk of progression/death sustained throughout treatment (hazard ratio [HR] 0.73 [95% CI 0.58, 0.91]; p=0.0062) and median (95% CI) PFS for ELd vs Ld of 23.1 (20.3, 28.1) vs

18.5 (15.7, 20.4) months. Similarly, patients who achieved ≥PR and who achieved ≥VGPR within 3 months (ELd vs Ld: 102 [32%] vs 82 [25%]) showed a 39% reduction in the risk of progression/death (HR 0.61 [95% CI 0.43, 0.87]; p=0.0065) and median (95% CI) PFS for ELd vs Ld of 33.4 (30.3, 51.2) vs 23.4 (20.4, 29.5) months (Figure 1).

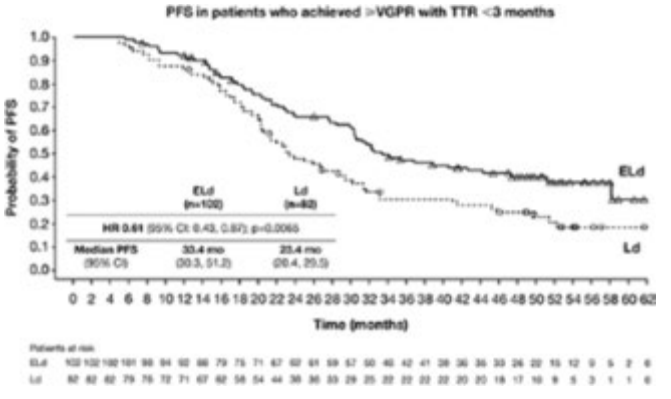

Figure 1.

Summary/Conclusion: 65% of patients achieved ≥PR to ELd within 3 months, compared with 53% of patients who received Ld. Treatment with ELd was associated with a 27% and 39% reduction in the risk of progression/death in patients with ≥PR and ≥VGPR, respectively, within 3 months, throughout an extended 4-year follow-up. These data suggest that patients with an early response to ELd may derive particular benefit from continued ELd treatment. Results of this analysis using response within 3 months may aid timely clinical decision-making in the increasingly complex treatment landscape of RRMM.

Writing support: A. Gill, Caudex, funded by BMS.

PS1311

SERIAL SERUM FREE LIGHT CHAIN ASSAY CAN REDUCE THE NEED FOR 24-HOUR URINE PROTEIN ASSESSMENT FOR RESPONSE ASSESSMENT IN MULTIPLE MYELOMA

M. Tschautscher[1,*], V. Rajkumar[2], A. Dispenzieri[2], M. Lacy[2], M. Gertz[2], F. Buadi[2], D. Dingli[2], L. Hwa[2], A. Fonder[2], M. Hobbs[2], S. Hayman[2], S. Zeldenrust[2], J. Lust[2], S. Russell[3], N. Leung[4], P. Kapoor[2], R. Go[2], Y. Lin[2], W. Gonsalves[2], T. Kourelis[2], R. Warsame[2], R. Kyle[2], S. Kumar[2]

[1]Internal Medicine, [2]Hematology, [3]Molecular Medicine, [4]Nephrology and Hypertension, Mayo Clinic, Rochester, United States

Background: According to current IMWG guidelines, detection of myeloma progression (PD) through disease surveillance relies on serial 24-hr urinary M protein levels in patients without measurable serum, but with detectable urine M protein. Only in those patients without measurable serum *and* urine M protein can the criteria involving difference in free light chain (dFLC) levels be used to monitor for and confirm PD.
Aims: Since serial 24-hr urine collections are bothersome and time consuming for our patients, we examined whether serial dFLC levels could be used as a surrogate for serial 24-hr urine protein measurements in monitoring for PD in patients with baseline measurable urine M protein.
Methods: A retrospective cohort study was conducted on all patients diagnosed with MM between 2003 and 2015. Only patients who had evidence of both confirmed (at least two consecutive measurements) and unconfirmed (<2 measurements) PD (defined by ≥25% increase in urinary protein and a minimum of 200mg protein over a 24-hr period) and sFLC measured at PD were included for the analysis. First, dFLC levels were recorded as absolute and percentage increases. Then, the quantity of patients who met dFLC thresholds of >10 mg/dL and ≥25% increase from the nadir was determined.
Results: A total of 811 24-hr urine protein collections were available for analysis of which 122 patients were identified for our study who developed PD (confirmed or unconfirmed) using 24-hr urine M protein, and had serial dFLC levels available for analysis at PD. At PD, the median percentage increase in dFLC was 110% with an interquartile range (IQR): 55-312. The median absolute increase in dFLC at PD was 74 mg/dL with an IQR: 34-168. Among our first population, 89% of patients had dFLC>25%, 94% had dFLC>10mg/dL from the nadir, and 98% met at least one of these two criteria. In our second population consisting of patients with baseline sFLC defined as a dFLC>10 mg/dL at diagnosis (n=118), 89% had dFLC>25%, 97% had dFLC>10 mg/dL increase from the nadir, and 98% met either of these two criteria. Finally, in our third population consisting of patients who met criteria for confirmed PD (≥2 urine protein assessments), 35/36 patients had >10mg/dL increase in dFLC at PD.
Summary/Conclusion: Taken together, we conclude that serial dFLC assessments can be performed in place of serial 24-hour urine protein assessments during myeloma surveillance to monitor for PD. Specifically, once these patients have an increase in dFLC of >10 mg/dL from the nadir, a 24-hour urine collection can then be assessed for M protein to evaluate for and confirm PD as described in the most current IMWG criteria, likely leading to better patient compliance, ease of testing, and reduced financial burden.

PS1312

NOVEL THERAPIES DIFFERENTIALLY IMPACT POLYCLONAL IMMUNOGLOBULIN RECOVERY IN MYELOMA PATIENTS: A LONGITUDINAL STUDY

M. Michallet[1,*], C. Chapuis-Cellier[2], C. Lombard[3], M. Sobh[1], T. Dejoie[4], H. Caillon[4], M. Attal[5], P. Moreau[6], H. Avet-Loiseau[7]

[1]Department of Haematology, Centre de Lutte contre le Cancer Leon Berard de Lyon, [2]Immunological Laboratory, Centre Hospitalier Universitaire Lyon Sud, [3]Immunological Laboratory, Centre Hospitalier Lyon Sud, Lyon, [4]Biochemistry Laboratory, Centre Hospitalier Universitaire de Nantes, Nantes, [5]Institut Universitaire du Cancer de Toulouse-Oncopole, Toulouse, [6]Department of Haematology, Centre Hospitalier Universitaire de Nantes, Nantes, [7]Unite de Genomique du Myelome, Institut Universitaire du Cancer de Toulouse-Oncopole, Toulouse, France

Background: The majority of myeloma patients present with suppressed levels of polyclonal immunoglobulins. Reconstitution to normal concentrations provides insight into the patient's immune status; however, studies in the era of novel agents are scarce and have mostly focused on single timepoints, limiting our understanding of the kinetics and significance of immunoglobulin recovery after treatment.
Aims: We studied the pattern and clinical impact of polyclonal immunoglobulin suppression in consecutive samples from patients treated with novel agents±ASCT.
Methods: We included 509 newly diagnosed patients (393 IgG, 116 IgA) from the IFM2009 trial treated with VRD±ASCT. Median follow-up was 36(2-57) months. Sera were analysed for heavy+light chain (HLC) levels using Hevylite® (The Binding Site Group Ltd, UK) at 16 consecutive timepoints spanning nearly 3 years from diagnosis. Median number of patients at each time-point was 364(224-509). HLC-pair suppression was defined as an abnormal HLC ratio and uninvolved HLC (uHLC) levels below the lower limit of normal (IgGκ<3.84, IgGλ<1.91, IgAκ<0.57 and IgAλ<0.44 g/L); and severe HLC-pair suppression as uHLC levels >50% below normal. Responses were determined by IMWG criteria. Minimal residual disease (MRD) was determined by 7-colour flow cytometry.
Results: 475/509(93%) patients presented with HLC-pair suppression. Treatment positively impacted immunoglobulin recovery; however prevalence of uHLC suppression remained significantly higher in non-transplant patients at all time-points. By the end of treatment, 12% transplant and 17% non-transplant patients remained suppressed; this percentage was sustained in the transplant arm for the following (out-of-treatment) year, but increased to 28% in the non-transplant group (p=0.003) (Figure 1).

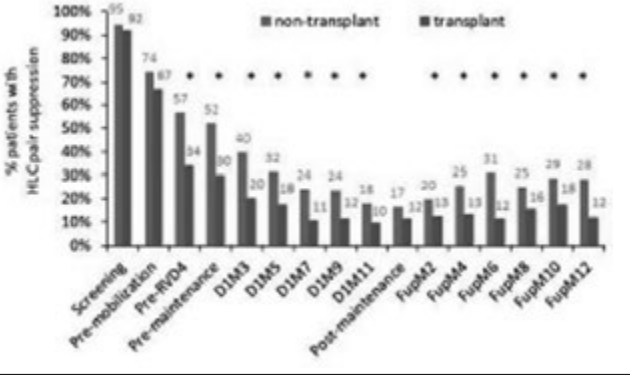

Figure 1.

Treatment also positively influenced levels of polyclonal immunoglobulin recovery. At diagnosis, uHLC levels were suppressed by a median 86% below normal. Severity of suppression gradually attenuated after transplant and throughout consolidation, reaching a plateau during maintenance after

which uHLC levels remained suppressed by about 20-30%. By contrast, consolidation in non-transplant patients did not prompt polyclonal immunoglobulin recovery; and post-maintenance uHLC levels remained moderately more suppressed (30-40% below normal) compared to the transplant arm. Similar results were obtained separately in IgG and IgA patients, although prevalence of suppression was significantly higher in the former throughout monitoring. HLC-pair suppression negatively impacted PFS during follow-up; and in multivariate analysis, severe suppression post-consolidation associated with shorter PFS in IgA patients (hazard ratio: 3.4; p=0.010). Conversely, normalisation of uHLC prior to consolidation therapy in all patients associated with higher odds of achieving ≥VGPR (odds ratio (OR): 11.6), ≥CR (OR: 3.7), and MRD negativity (OR: 2.5) post-consolidation (p≤0.001).

Summary/Conclusion: Long-term HLC-pair suppression is a relatively common occurrence in the era of novel agents; and associates with poorer outcomes. Stem cell transplantation and lenalidomide maintenance can mitigate partly the negative impact of immunoglobulin suppression through better reconstitution of humoral responses, which may result in more efficient disease control and improve clinical outcomes. Early recovery of uHLC levels alongside standard monitoring of the M-protein might provide relevant information about treatment efficacy and response outcomes.

PS1313

REAL-WORLD OUTCOMES IN TRANSPLANT INELIGIBLE NDMM PATIENTS: RESULTS FROM A LARGE UK COHORT

F. Djebbari[1,*], F. Sharpley[1], S. Fourali[1], G. Vallance[1], S. McLain-Smith[2], J. Kothari[1], K. Ramasamy[1]

[1]Haematology, Oxford Cancer and Haematology Centre, Oxford, [2]Data Analysis, Ph Associates, Marlow, United Kingdom

Background: Fixed duration therapy (FDT) is the UK standard of care for transplant-ineligible (TNE) newly diagnosed multiple myeloma (NDMM). Treatment objective is disease control whilst maintaining QoL, which translates into improved survival. QoL is often reported to be better during treatment free intervals (TFI).

Aims: We performed a retrospective analysis in TNE NDMM patients to evaluate the influence of age and choice of upfront therapy on clinical outcomes. We quantified treatment free interval (TFI).

Methods: 292 TNE NDMM patients treated between 2009-2017 within Thames Valley Cancer Network (UK) were eligible for inclusion. Data was collected from the chemotherapy database and patient records. Primary outcome was OS and the secondary outcomes were PFS, TTNT, TFI and response rates for the total cohort. Subgroup analyses were performed based on first line treatment: thalidomide-based (THAL) vs. proteasome inhibitor-based (PI), and age at presentation: ≤75 years vs. >75 years. Differences between subgroups were tested using an unstratified log-rank test. Medians and Cox hazard ratios (HR), with 95% confidence intervals (CIs) were calculated and survival estimates are presented.

Results: At start of first line therapy, median age was 75.1 years (IQR: 69 to 81), ≤75: n=144, >75: n=148, male 57%, female 43%, and ISS stage (I: 18%, II: 16%, III: 48%, unknown: 18%), serum creatinine ≥ 140micromol/L was recorded in 28% of patients. Upfront therapy was THAL: 61%, PI: 22%, alkylator-based: 10%, lenalidomide-based: 7%. Treatment was a triplet regimen (70%) and doublet (30%). Patient numbers within treatment subgroups were: THAL: n=178, PI: n=64. Median follow-up for the total cohort was 22.5 months (IQR 11.2 to 41.1). Median length of upfront FDT was 4.6 months (IQR: 2.5-5.7). Overall response rate (ORR) for first-line therapy in all patients was 66%, with responses categorised as CR: 21%, VGPR: 16%, PR: 30%, SD: 18%, PD: 8% and unknown 7%. Both gender distribution and therapy proportions (doublet, triplet) were comparable across the 2 age groups: ≤75 (56% M, 44% F, doublet 31%, triplet 69%), >75 (57% M, 43% F, doublet 29%, triplet 71%), and treatment groups: THAL group (54% M, 46% F), PI group (53% M, 47% F). ORR between subgroups was as follows: (THAL: 73% vs. PI: 72%) and (≤75: 70% vs. >75: 63%). Patients aged ≤75 showed a statistically significant improvement in OS and PFS compared to those >75: OS (49.0 vs. 22.4 months, p<0.0001, HR: 2.08, 95% CI: 1.53-2.83), PFS (9.7 vs. 8.0 months, p<0.01, HR: 1.47, 95% CI: 1.13-1.89). Survival rates at 1 year and 2 years between ≤75 and >75 were (80.4% vs. 75.9%) and (64.8% vs. 46.2%) respectively. Differences in OS and PFS were not statistically significant between the THAL and PI subgroups: OS (32.6 vs. 30.2 months, p=0.644, HR 1.09, 95% CI: 0.75-1.58), PFS (9.2 vs. 8.4 months, p=0.155, HR 1.25, 95% CI: 0.92-1.70). Difference in TTNT was not statistically significant between subgroups. The longest TFI during FDT was following first-line therapy with a median of 6.9 months (IQR: 1.4-16.9, n=190), reducing to 1.8 and 0.6 after 2nd and 3rd lines (Figure 1).

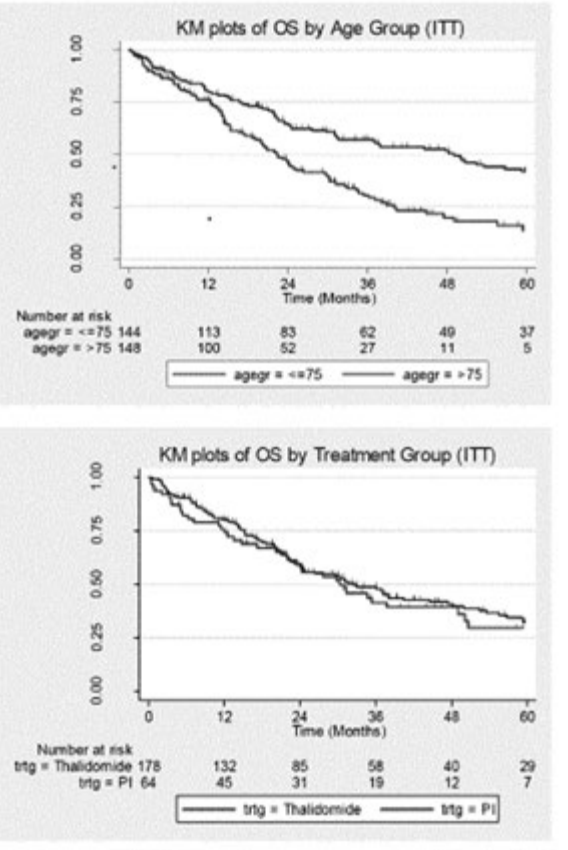

Figure 1.

Summary/Conclusion: This large real-world data study showed significantly improved OS and PFS for patients aged ≤75 compared to those >75 years, whilst choice of therapy (THAL or PI) had limited impact, when evaluated as individual factors. Clinically meaningful TFI was only recorded after first line FDT. This data suggests that a continuous therapeutic approach with manageable toxicities may be more appropriate in TNE myeloma patients.

PS1314

BONE MARROW PLASMA CELL INFILTRATION IN LIGHT-CHAIN AMYLOIDOSIS: IMPACT ON ORGAN INVOLVEMENT AND OUTCOME

L. G. Rodríguez-Lobato[1,*], N. Tovar[1], M. T. Cibeira[1], L. Magnano[2], I. Isola[1], L. Rosiñol[1], J. Bladé[1], C. Fernández de Larrea[1]

[1]Hematology, [2]Hematopathology Unit, Hospital Clínic, Barcelona, Spain

Background: Prognosis of immunoglobulin light-chain (AL) amyloidosis depends mainly on the presence of cardiac involvement and the disease burden. A higher bone marrow plasma cell (BMPC) burden has been suggested as an adverse prognostic factor.

Aims: The aim of our study was to analyze the correlation between the BMPC infiltration, clinical features and outcomes in patients with AL amyloidosis at a single institution.

Methods: We reviewed the clinical records of patients with AL amyloidosis diagnosed from January 2006 to April 2016. Seventy-nine patients (42F/37M; median age at diagnosis 65) were the final study population. Initial baseline demographics, clinical and laboratory data, treatment and follow-up were collected. Median follow-up for alive patients was 45 months. BMPC were identified by optic microscopic revision of May-Grünwald–Giemsa–stained BM smears processed according to standard procedures. All 79 patients had BM samples with adequate cellularity and BMPC infiltration was evaluated by senior cytologists. A second independent revision was performed. The concordance between the results obtained by both group of cytologists was excellent (R=0.949; p<0.001).

Results: Median BMPC infiltration at diagnosis was 11% (interquartile range 7-18). Eighteen patients (23%) presented more than 20% BMPC infiltration. BMPC infiltration significantly correlated with the serum free light-chain difference (R=0.551; p<0.001). Patients with more than 10%

BMPCs had more frequent cardiac involvement (86% *vs.* 63%; p=0.015), more stage III or IV of the revised Mayo risk stratification system (61% *vs.* 29%; p=0.01), a trend towards a higher early mortality (27% *vs.* 11%; p=0.08) and a significantly shorter progression-free survival (PFS) (median of 18 *vs.* 48 months, p=0.02) and overall survival (OS) (median of 33 months *vs.* not reached; p=0.046) (Figure 1A and 1B). Interestingly, those patients with lower BMPC infiltration were associated with kidney involvement (86% *vs.* 48%; p=0.001). Two multivariate Cox proportional hazards models were built. The first one included the 2004 Mayo Clinic staging system, BMPC infiltration and the dFLC; and the second included the revised Mayo risk stratification system and BMPC infiltration. In both analysis BMPC infiltration > 10% retained its independent prognostic value for worse PFS (HR=1.86; 95% CI, 1.013-3.73; p=0.049 and HR=2.26; 95% CI, 1.048-4.866; p=0.038, respectively) but not in OS. The use of new drugs (bortezomib or lenalidomide) seemed to overcome the negative prognostic impact of a higher BMPC infiltration (3-year PFS of 47% in ≤ 10% BMPC *vs.* 35% in > 10% BMPC; p=0.29 and 3-year OS of 62% in ≤10% BMPC *vs.* 57% in > 10% BMPC; p=0.58) (Figure 1C and 1D).

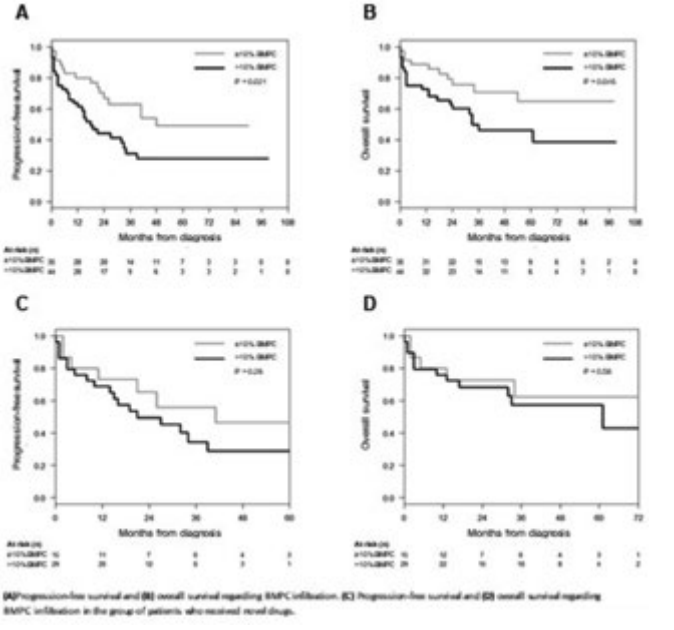

Figure 1.

Summary/Conclusion: Higher BMPC infiltration in patients with AL amyloidosis was associated with increased systemic organ damage, particularly cardiac involvement, but this fact was not related to the development of myeloma features.

PS1315

TREATMENT OF IGM-ASSOCIATED SYSTEMIC AL AMYLOIDOSIS WITH RITUXIMAB-BENDAMUSTINE

R. Manwani[1,*], D. Foard[1], S. Mahmood[1], S. Sachchithanantham[1], T. Lane[1], F. Sharpley[1], C. Quarta[1], T. Rezk[1], A. Martinez De Azcona Naharro[1], H. Lachmann[1], J. Gillmore[1], M. Fontana[1], C. Whelan[1], P. Hawkins[1], A. Wechalekar[1]

[1]National Amyloidosis Centre, University College London, London, United Kingdom

Background: Systemic AL amyloidosis is characterised by fibrillary deposition of misfolded immunoglobulin light chains within viscera. IgM-associated AL amyloidosis (IgM AL) is rare, accounting for 5-7% of patients with AL. There is a paucity of data on optimal therapy in IgM AL and outcomes are poor, with very few complete responses. Bendamustine, which has features of both an alkylating agent and purine analog, is increasingly used alongside rituximab in the treatment of non-Hodgkin's lymphoma. There have been no studies to date focusing on the use of bendamustine-rituximab (BR) in upfront or relapsed/refractory IgM AL.
Aims: To assess efficacy of BR in treatment-naïve and relapsed/refractory IgM AL. Primary outcome variables: haematological and organ response; overall survival (OS); progression free survival (PFS, progression defined as time to next treatment or death).
Methods: 27 patients with AL treated with BR from October 2011-June 2017 were included; 22 received BR as first-line treatment and 5 as second-line. 25 had a serum IgM M-protein. Of the remaining two, one had a serum IgG M-protein and the other had both a lambda and IgG M-protein; both were treated with BR first-line and both had bone marrow infiltration with lymphoplasmacytic lymphoma (LPL).
Results: Median age was 70 years (range 56-86). The number of patients with Mayo Stage I, II and III was: 9 (33%), 12 (45%) and 6 (22%). The number of patients with cardiac, renal, liver, peripheral nerve, autonomic, soft tissue and lymph node involvement was: 9 (33%), 17 (63%), 6 (22%), 6 (22%), 4 (15%), 2 (7%) and 13 (48%). Median NT-proBNP was 978ng/L (42-5708ng/L). Median M-protein was 11.5g/L (1-30g/L); 19 had a serum free light chain excess (12 lambda, 7 kappa) and median dFLC was 59.8mg/L (2.2-856mg/L). 21 patients had available bone marrow data: 3 normal, 1 plasma cell infiltrate, 14 LPL and 3 NHL not specifically classified. Five were treated second-line with BR for refractory disease, previous therapy included bortezomib-cyclophosphamide-dexamethasone (2 patients); rituximab-bortezomib-dexamethasone (1 patient); rituximab-cyclophosphamide-vincristine-prednisolone (1 patient); rituximab-cyclophosphamide-dexamethasone (1 patient); median number of previous cycles was 6 (4-8). Patients received a median of 5 cycles of BR (1-8).
Haematological responses on an ITT basis (by AL criteria) were: complete response (CR) 3 (11%), very good partial response (VGPR) 10 (37%), partial response (PR) 3 (11%), non-response (NR) 11 (41%, including 6 deaths). In the first-line group, haematological responses were: CR 3 (14%), VGPR 7 (32%), PR 3 (14%) and NR 9 (40%, including 4 deaths). In the second-line group, 3 (60%) achieved VGPR and 2 were non-responders (including 1 death). 11% of patients with cardiac involvement achieved a cardiac response and 18% patients achieved a renal response by amyloidosis consensus criteria. Median follow up was 18 months (range 3-55). In patients treated with BR first-line, median OS was not reached and median PFS was 34 months. In the second-line group, median OS was not reached and median PFS was 17 months.
Summary/Conclusion: The treatment approach in IgM AL has been heterogenous due to scant data and poor haematological responses historically. Data from this small retrospective study demonstrate an excellent overall response rate (ITT) of 59% in patients treated with BR, much higher than previously reported in this cohort, with a higher proportion achieving VGPR or better. This study suggests that first-line BR may be the treatment of choice for IgM AL if these findings are confirmed in a larger series.

PS1316

TANDEM TRANSPLANTATION OVERCOMES POOR PROGNOSIS IN NEWLY DIAGNOSED EXTRAMEDULLARY MYELOMA WITH HIGH-RISK CYTOGENETICS: A RETROSPECTIVE STUDY BY THE CMWP-EBMT

N. Gagelmann[1,*], D.-J. Eikema[2], L. Koster[2], D. Caillot[3], P. Pioltelli[4], J. Bargay Lleonart[5], P. Reményi[6], D. Blaise[7], N. Schaap[8], M. Trneny[9], J. Passweg[10], R. Parody Porras[11], J.Y. Cahn[12], M. Musso[13], X. Poiré[14], G. Kobbe[15], M. Itälä-Remes[16], V. Pavone[17], L. Fouillard[18], H. Schoemans[19], D. Bron[20], A. Pouli[21], W. Schroyens[22], L. Garderet[23], N. Kröger[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [2]EBMT data office, Leiden, Netherlands, [3]CHU Dijon, Dijon, France, [4]Ospedale San Gerardo, Monza, Italy, [5]Hospital son Llatzer, Mallorca, Spain, [6]St. István & St. Laszlo Hospital, Budapest, Hungary, [7]Institut Paoli Calmettes, Marseille, France, [8]Radboud University - Nijmegen Medical Centre, Nijmegen, Netherlands, [9]Charles University Hospital, Prague, Czech Republic, [10]University Hospital, Basel, Switzerland, [11]ICO – Hospital Duran i Reynals, Barcelona, Spain, [12]CHU Grenoble, Grenoble, France, [13]Ospedale La Maddalena, Palermo, Italy, [14]Cliniques Universitaires St. Luc, Brussels, Belgium, [15]University Hospital Düsseldorf, Düsseldorf, Germany, [16]Turku University Hospital, Turku, Finland, [17]Hospital C. Panico, Tricase, Italy, [18]Grand Hôpital de l`Est Francilien, Meaux, France, [19]University Hospital Gasthuisberg, Leuven, [20]Institut Jules Bordet, Brussels, Belgium, [21]St.Savvas Oncology Hospital, Athens, Greece, [22]Antwerp University Hospital, Antwerp, Belgium, [23]Hopital St. Antoine, Paris, France

Background: Evidence on the impact of high-risk cytogenetics, such as del(17p), in newly diagnosed multiple myeloma (NDMM) patients with extramedullary disease (EMD) after transplantation is limited.
Aims: We aimed to analyze the impact of cytogenetics on outcome after single-, tandem-autologous (tandem-auto) and autologous/reduced-intensity allogeneic (auto-allo) transplantation in NDMM with EMD.
Methods: Within the EBMT registry, we identified 488 patients (59% male, 41% female) with available data on extramedullary involvement and cytogenetics at diagnosis who received first-line single-auto (n = 373), tandem-auto (n = 84) or auto-allo (n = 31) between 2003 and 2015. Extramedullary involvement was defined as manifestations resulting from bone lesions (paraskeletal, n = 376) or hematogenous spread into different organs (n = 85), or both (n = 27). High-risk cytogenetics were defined as presence of at

least one of the following abnormalities by using FISH: del(17p) (n = 70), t(4;14) (n = 83), t(14;16) (n = 10), t(14;20) (n = 6), and abn(1) (n = 25); and was thus detected in 40% of the patients. The remaining patients had normal cytogenetics (n = 250) or other (n = 44), including other translocations or deletions, hyper- or hypodiploidy.
Patients receiving auto-allo were younger (median, 49 years) vs. single-auto (60 years) and tandem-auto (60 years; p<0.001). Before transplant, complete remission was achieved by 24% (single-auto), 13% (tandem-auto) and 19% (auto-allo; p=0.08). Median follow-up was 49.3 months.
Results: In univariate analysis, high-risk cytogenetics showed significant lower PFS and OS of 28.4% (19.6-37.2) and 48.2% (40.0-56.4) vs. 48.5% (41.4-54.8) and 78.0% (72.5-83.5; p<0.001, respectively). Evaluating outcome according to del(17p) and t(4;14) vs. normal cytogenetics showed worse PFS and OS of each abnormality (p<0.001, respectively). Progression-free survival appeared to be better after tandem-auto and auto-allo with 51.5% (39.3-63.7; p=0.06) and 60.7% (32.7-88.7; p=0.14) versus 38.3% (32.0-44.6) for single-auto while OS was significantly better for tandem-auto with 77.8% (68.4-87.2) vs. single-auto showing 62.1% (56.2-68.0; p=0.04), and not significant for auto-allo (81.1%, 66.0-96.2) vs. single-auto (p=0.17). In patients with EMD and high-risk cytogenetics overall, tandem-auto resulted in a significantly improved PFS and OS (p=0.02 and p=0.001) in comparison to single-auto while auto-allo showed significantly improved OS vs. single-auto (p=0.05). In a subgroup analysis, tandem-auto overcame poor prognosis of high-risk vs. normal or other cytogenetics in the univariate (PFS at four years: 50.4% vs. 53.6%, p=0.49; OS: 80.8% vs. 80.4% p=0.92) as well as in the multivariate analysis in terms of PFS (hazard ratio, 1.17; p=0.70) and OS (hazard ratio, 0.92; p=0.90). Regarding del(17p) and t(4;14), single-auto did not influence onset poor prognosis when compared with normal cytogenetics (p<0.001, respectively) while tandem-auto improved outcome of each of the present abnormalities resulting in similar 4-year survival vs. normal cytogenetics (p=0.83 and p=0.89).
Summary/Conclusion: High-risk cytogenetics are seen in nearly 40% of NDMM patients with EMD significantly influences PFS and OS. In comparison to single autografting, tandem-auto improves survival and overcomes poor prognosis of high-risk cytogenetics, especially in patients presenting with del(17p) and t(4;14).

PS1317

VENETOCLAX MONOTHERAPY AND COMBINED WITH DEXAMETHASONE AS TARGETED THERAPY FOR RELAPSED/REFRACTORY T(11;14) MULTIPLE MYELOMA

J. Kaufman[1,*], C. Gasparetto[2], J. Mikhael[3,4], P. Moreau[5], C. Touzeau[5], R. Vij[6], T. Facon[7], B. Pegourie[8], L. Benboubker[9], L. Boise[1], W. J. Hong[10], Y. Jiang[10], S. Alzate[11], M. Dunbar[11], J. Pesko[11], K. Friese[11], J. Ross[11], P. Maciag[11], S. Kumar[12]

[1]Winship Cancer Institute of Emory University, Atlanta, [2]Hematologic Malignancies & Cellular Therapy, Duke University, Durham, [3]Mayo Clinic, Scottsdale, [4]International Myeloma Foundation, Los Angeles, United States, [5]Centre Hospitalier Universitaire (CHU) de Nantes, INSERM, Universite de Nantes, Nantes, France, [6]Washington University School of Medicine, St. Louis, United States, [7]Centre Hospitalier Regional Universitaire (CHRU) Lille, Hopital Huriez, Lille, [8]Department of Hematology, Centre Hospitalier Universitaire (CHU) , Grenoble, [9]Centre Hospitalier Regional Universitaire (CHRU) Tours, Tours, France, [10]Genentech Inc., South San Francisco, [11]AbbVie, Inc, North Chicago, [12]Mayo Clinic, Rochester, United States

Background: The BCL-2 inhibitor venetoclax (Ven) induces cell death in multiple myeloma (MM) cell lines and primary samples, particularly those with t(11;14). Based on data indicating that dexamethasone (Dex) enhances BCL-2 expression and priming to sensitize the MM cell to Ven, Ven + Dex may provide a novel targeted combination in patients (pts) with t(11;14) MM.
Aims: To report safety, efficacy, exploratory biomarkers, and pharmacokinetics evaluated for Ven monotherapy and Ven + Dex for pts with relapsed/refractory (R/R) t(11;14) MM.
Methods: In the ongoing phase 1, open label study, pts received Ven once daily as monotherapy (n=30; 300, 600, 900, 1200 mg) or Ven 800mg + Dex 40mg (n=20). Responses were assessed by investigators using IMWG criteria. Minimal residual disease (MRD) was assessed at time of suspected complete response (CR) by next-generation sequencing in a subset of pts with available bone marrow specimens. *BCL2:BCL2L1* gene expression in bone marrow plasma cells in baseline samples were determined by quantitative real-time PCR. Results are based on a 1Dec2017 data cut.
Results: For 50 pts enrolled with t(11;14), pts had a median of 4 prior therapies (range: 1–10), with 88% prior bortezomib (68% refractory), 90% prior lenalidomide (80% refractory), and 76% prior autologous stem cell transplant. In addition to t(11;14), 6 (12%) pts had 17p deletion. Exposure to Ven was highly variable, with similar mean trough values with 800mg Ven in combination therapy and 1200mg Ven monotherapy.
Median time on study for pts with t(11;14) was similar for Ven monotherapy vs Ven + Dex (8 [range: .4–36] vs 9 [range: .1–15] months, respectively). 35 pts discontinued, with 32 related to disease progression. The most common any-grade AEs in pts with t(11;14) were diarrhea (36%), nausea (36%), neutropenia (30%), decreased white blood cell count (26%), thrombocytopenia (24%), hypophosphatemia (24%), hypokalemia (24%), upper respiratory tract infection (24%), fatigue (22%), and cough (22%). Common grade 3/4 AEs in these pts were neutropenia (22%), and thrombocytopenia (16%), and decreased lymphocyte count (16%). The most common serious AEs included pneumonia and sepsis (6% each). 2 pts had grade 3 laboratory tumor lysis syndrome that resolved with temporary interruption of Ven. No pts with t(11;14) had dose-limiting toxicities. The overall response rate (ORR) for pts with t(11;14) MM was 50% (25/50; 30% had ≥very good partial response [VGPR]). For Ven monotherapy, ORR was 40% (12/30; 27% had ≥VGPR). On Ven + Dex, ORR was 65% (13/20; 35% had ≥VGPR). ORR were consistent across subgroups within each treatment approach (Ven monotherapy vs Ven + Dex; see Table 1). Of 5 pts evaluated (2 CR, 3 VGPR), MRD negativity (10^{-4}) was observed in 2 pts (1 had CR with 1200mg Ven monotherapy and 1 had VGPR with Ven + Dex). As previously reported, response to Ven monotherapy was associated with a higher *BCL2:BCL2L1* gene expression ratio and improved ORR (high vs low ratio: 89% vs 27%). For 17 pts with evaluable bone marrow who received Ven + Dex, there was no observed difference in *BCL2:BCL2L1* expression in this small group of pts with ≥partial response (median 2^{-DDCt}: 0.067; n=12) vs those who did not achieve a response (median 2^{-DDCt}: 0.017; n=5).

Table 1.

	Ven n=30	Ven + Dex n=20
Months on study, median (range)	7.7 (.4 – 36.4)	9.3 (.07 – 14.5)
Overall response rate, n (%)	12 (40)	13 (65)
Stringent complete response	1 (3)	0
Complete response	3 (10)	0
Very good partial response	4 (13)	7 (35)
Partial response	4 (13)	6 (30)
Median (range) time to progression, months	8.3 (3.9, 11.5)	12.5 (3.6, -)
Overall response rates by subgroups, number of pts with response/total number of pts per subgroup by treatment group (%)		
No. prior therapies		
1 – 3	3/9 (33)	7/11 (64)
≥4	9/21 (43)	6/9 (67)
Refractory to last therapy	8/19 (42)	10/17 (59)
Bortezomib refractory	8/22 (36)	9/12 (75)
Lenalidomide refractory	8/23 (35)	11/17 (65)

Summary/Conclusion: Ven monotherapy and Ven + Dex had tolerable and comparable safety profiles in these heavily pre-treated pts. With ORR of 40% with Ven monotherapy and 65% with Ven + Dex, these results confirm the efficacy of Ven in pts with t(11;14) MM and support ongoing study of Ven + Dex.

PS1318

SUBCUTANEOUS DARATUMUMAB (DARA SC) + CYCLOPHOSPHAMIDE, BORTEZOMIB, AND DEXAMETHASONE (CYBORD) IN PATIENTS WITH NEWLY DIAGNOSED AMYLOID LIGHT CHAIN (AL) AMYLOIDOSIS: SAFETY RUN-IN RESULTS OF ANDROMEDA

G. Merlini[1,*], E. Kastritis[2], M. Maurer[3], J. Zonder[4], M.C. Minnema[5], S. Schönland[6], A. Wechalekar[7], G. Palladini[1], X. Qin[8], S. Y. Vasey[8], I. Khan[9], J. M. Schecter[9], R.L. Comenzo[10]

[1]Amyloidosis Research and Treatment Center, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo, and Department of Molecular Medicine, University of Pavia, Pavia, Italy, [2]Department of Clinical Therapeutics, National and Kapodistrian University of Athens School of Medicine, Athens, Greece, [3]Presbyterian Hospital and Vanderbilt Clinic, New York, NY, [4]Barbara Ann Karmanos Cancer Institute, Detroit, MI, United States, [5]UMC Utrecht Cancer Center, Utrecht, Netherlands, [6]Heidelberg Univ. Hospital, Heidelberg, Germany, [7]Univ. College London and the Royal Free London NHS Foundation Trust, London, United Kingdom, [8]Janssen Research & Development, LLC, Spring House, PA, [9]Janssen Research &

Development, LLC, Raritan, NJ, [10]Division of Hematology/Oncology, John C. Davis Myeloma and Amyloid Program, Tufts Medical Center, Boston, MA, United States

Background: Systemic AL amyloidosis is characterized by deposition of insoluble amyloid fibrils, which are derived from immunoglobulin light chains produced by clonal CD38+ plasma cells, into tissues and organs.
Aims: We present the safety run-in findings of DARA SC + CyBorD in patients with newly diagnosed AL amyloidosis in ANDROMEDA (NCT03201965).
Methods: Eligible patients had ≥1 involved organ, Eastern Cooperative Oncology Group (ECOG) score ≤2, absolute neutrophil count ≥1.0×10^9/L; hemoglobin ≥8.0 g/dL; platelet count ≥50×10^9/L; estimated glomerular filtration rate ≥20 mL/min/1.73m^2, and NT-ProBNP ≤8,500 ng/L. In the safety run-in, patients received a concentrated co-formulation of DARA (1,800 mg in 15 mL) and recombinant human hyaluronidase enzyme (rHuPH20; 30,000 U) in a single, pre-mixed vial, given by manual SC injection qw in Cycles 1-2, q2w in Cycles 3-6, and q4w thereafter ≤2 y. Cyclophosphamide 300 mg/m^2 PO or IV and bortezomib 1.3 mg/m^2 SC were given on Days 1, 8, 15, 22 of each 28-day cycle for ≤6 cycles and dexamethasone 40 mg was given qw. Dosing was staggered ≥48 hours between patients to assess infusion related reactions (IRRs). Safety was evaluated after ≥10 patients received ≥1 treatment cycle.
Results: Patients (n = 15) had a median age of 63 (range 35-77) years and a median of 58 (range 15-157) days from diagnosis. Patients had a median of 1 (range 1-3) involved organ, with kidney involvement affecting 67% of patients, cardiac involvement in 33% of patients, and ≥2 organ involvement in 40% of patients. At baseline, 33%, 60%, and 7% of patients were grouped into Mayo Clinic cardiac stage I, II, and IIIa, respectively, and 93% of patients had an ECOG score of ≤1. Among the 14 patients with available data, baseline creatinine clearance was ≥60 mL/minute in 78.6% of patients and <60 mL/minute in 21.4% of patients. Patients received a median of 2 (range 1-4) treatment cycles and a median of 5 (range 1-10) DARA injections. At the cutoff, the most common (>2 patients) treatment emergent adverse events (TEAEs) were nausea (53%), diarrhea (40%), fatigue (33%), injection site erythema (27%), and constipation, headache, anemia, rash, and cough (20% each). Dyspnea and peripheral edema were reported in 1 patient (7%) and 2 patients (13%), respectively. One grade 3/4 TEAE (hypertension; unrelated to treatment) and no serious TEAEs occurred. IRRs occurred in 1 (6.7%) patients (all grade 1). Additional data will be presented at the meeting.
Summary/Conclusion: DARA-CyBorD is tolerable in patients with AL amyloidosis, with a low IRR rate and no new safety signals. The limited incidence of dyspnea and peripheral edema indicate a low risk for volume overload.

PS1319

DISEASE ASSESSMENT BY PET-CT PLUS FLOW CYTOMETRY IN MULTIPLE MYELOMA IDENTIFIES PATIENTS WITH DIFFERENT SURVIVAL OUTCOMES

R. Alonso Fernández[1,*], M.T. Cedena[1], R. Ríos[2], J.M. Moraleda[3], V. Cabañas[3], M.J. Moreno[3], J. López-Jiménez[4], A. Sanz[4], F. Martín[4], A. Valeri[1], A. Jiménez[1], A. Gómez-Grande[1], J.J. Lahuerta[1], J. Martínez-López[1]

[1]Hematology Department, Hospital Universitario 12 de Octubre, Madrid, [2]Hematology Department, Hospital Universitario Virgen de las Nieves, Granada, [3]Hematology Department, Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, [4]Hematology Department, Hospital Universitario Ramón y Cajal, Madrid, Spain

Background: The prognostic value of Minimal Residual Disease (MRD) assessed by NGS or flow cytometry (FCM) in Multiple Myeloma (MM) has been confirmed in different works. In this way, currently, achieving MRD- is considered a surrogate marker for survival and could be a primary endpoint with clinical relevance. Recently, it has been identified the effect on survival related to persistence of active tumor burden in PET-CT after treatment. However, the available information about complementarity of both techniques and prognosis in discordant cases (MRD-/PET+ or MRD+/PET) is limited.
Aims: To improve knowledge in the prognostic value of combined PET-CT and FCM in disease assessment
Methods: We have performed an observational study collecting retrospective information from 148 MM patients treated between 2008 and 2018 in 4 Spanish hospitals. The main selection criterion was to perform FCM in bone marrow and PET-CT jointly in order to assess response at different points in the same line of treatment, regardless of treatment regimen used. We obtain data about clinical and biological parameters at baseline and response assessment by FCM and PET-CT at the beginning and on-treatment period or during follow-up. Statistical analysis was realized with SPSS 21.0 version (SPSS, Inc., Armonk, NY).
Results: Our study was performed in 148 patients with a median age of 61 years-old (68,2% ≤65 years-old) and 1-5 lines of treatment (74,3% of patients were at first line). By using diferent treatment regimes, 72,3% of patients achieved Complete Response (CR), with median Progresion-Free Survival (PFS) of 54 months and median Overall Survival (OS) not reached (NR). 73% of the patients assessed by PET-CT were PET-; meanwhile 56,1% became MRD- by FCM. By combining both techniques in response assessment to treatment (available data in 122 patients), 55,4% of patients were MRD-/PET-, 26,4% were MRD+/PET-, 10,7% were MRD-/PET+ and 7,4% were MRD+/PET+ (67, 32, 13 and 9 patients, respectively). Given the absence of significant differences in survival results and the reduced sample size of patients with PET+, we consider MRD-/PET+ and MRD+/PET+ together for the analysis. In terms of PFS we detected a significant difference (p<0,001) between patients who became MRD-/PET-, with a median PFS NR, and MRD+/PET- patients (median PFS 45 months); both have significant prolonged PFS than PET+ patients (median PFS 28 months). These differences in PFS were similar when the analysis was performed separately un patients who received a transplant or those who achieved CR at first line. In the same way, we observed significant difference in terms of OS between MRD-/PET- and MRD+/PET- patients (both median OS NR) versus PET+ patients (median PFS 64 months) (Figure 1). When the analysis employed techniques to remove guarantee-time bias (conditional landmark analysis) the effect of MRD-/PET- on PFS and OS continued to be observed. In multivariant analysis, only MRD by FCM (HR 2,14, CI95% 1,12-4,10;p=0,021) and PET+ (HR 4,15, CI95% 2,02-8,55; p<0,001) were independently associated with higher risk of progression or death.

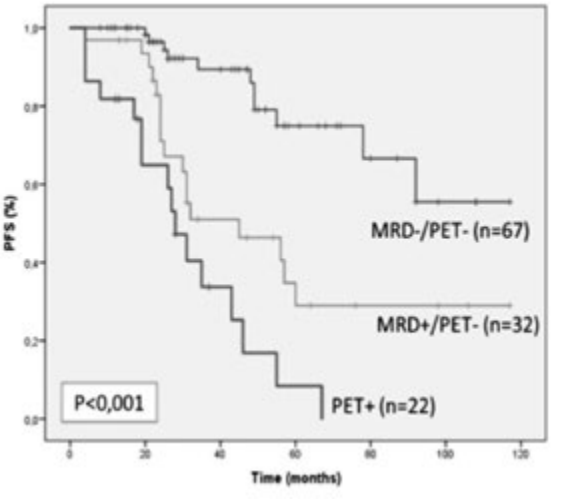

Figure 1.

Summary/Conclusion: Patients with persistent positive PET-CT, regardless of MRD status, have significant worse prognosis; meanwhile those who become both MRD and PET-CT negative, show longer PFS and OS. Both techniques are complementary and jointly provide information with prognostic relevance.

PS1320

PREDICTORS OF HEMATOLOGIC AND ORGAN RESPONSE IN AL AMYLOIDOSIS

G. Palladini[1,*], P. Milani[1], M. Basset[1], A. Foli[1], R. Mussinelli[1], P. Stefano[2], G. Merlini[1]

[1]Department of Molecular Medicine, University of Pavia, Pavia, Italy, [2]Department of Internal Medicine, University of Pavia, Pavia, Italy, Amyloidosis Research and Treatment Center, Foundation IRCCS Policlinico San Matteo, Pavia, Italy, Pavia, Italy

Background: Response to therapy favorably impacts prognosis of patients with AL amyloidosis, but cannot be predicted at the time of diagnosis.
Aims: We explored the baseline variables that predict response to upfront therapy in newly diagnosed AL amyloidosis patients.
Methods: The prospectively maintained database of the Pavia Amyloidosis Center was systematically searched for patients surviving until the first assessment of response (3 months after diagnosis). Baseline variables were

tested by logistic regression for their ability to predict hematologic, cardiac or renal response and best cutoffs were identified by ROC analysis.
Results: A total of 782 consecutive patients diagnosed between 2004 and 2015 were included. Overt multiple myeloma was excluded. Median age was 64 years (IQR 56-70 years) and 447 patients (57%) were males. Involved organs were heart (79%), kidney (69%), soft tissues (18%), liver (12%), and peripheral nervous system (11%). Cardiac stage was: I, 20%; II, 49%; IIIa, 18%; IIIb, 13%. Renal stage was: I, 48%; II, 39%; III, 13%. Upfront therapy was melphalan/dexamethasone (MDex) in 37% of patients, cyclophosphamide/ bortezomib/dexamethasone (CyBorD) in 27%, BMDex in 13%, immune modulatory drugs-based in 9%, BDex in 4%, treatment for IgM-AL amyloidosis in 3%, ASCT in 2%, other in 5%. Overall hematologic response rate was 68% [complete response (CR) 15%, very good partial response (VGPR) 39%], and cardiac (CardResp) and renal (RenResp) responses were achieved in 26% and 32% of evaluable patients, respectively. At multivariate analysis, baseline variables predicting achievement of CR/VGPR were difference between involved and uninvolved free light chain (dFLC) <130 mg/L (OR 3.70, p<0.001), BMPC<13%, (OR 1.54, p=0.043) and exposure to B (OR 2.00, p=0.001). Rate of CR/VGPR was 70%, 36%, and 23% with MDex, and 81%, 60%, and 42% with BMDex or CyBorD in patients with 0, 1, and 2 predictors, respectively. The only baseline variable associated with achievement of CardResp was female sex (OR 1.92, p=0.001). Interestingly, cardiac stage did not predict CardResp that was 21% in males and 35% in females (p=0.001). Females were more likely to obtain CardResp (OR 1.75, p=0.009) also in a multivariable analysis including achievement of CR/VGPR (OR 4.55, p<0.001). RenResp was significantly more likely in patients with baseline proteinuria <5 g/24h (40% vs 24%, p<0.001) and in those with dFLC >130 mg/L (39% vs 25%, p<0.001), and was reached in 47%, 30%, and 20% of subjects with 0, 1, and 2 risk factors, respectively. At multivariate analysis proteinuria <5 g/24h (OR 2.22, p<0.001) and dFLC >130 mg/L (OR 2.24, p<0.001) remained independent predictors of RenResp also when achievement of CR/VGPR (OR 2.20, p<0.001) was included in the model.
Summary/Conclusion: In patients surviving until the first response assessment, CR/VGPR is predicted by low baseline clonal burden and use of bortezomib. Reversibility of cardiac dysfunction is more likely in females, independently of cardiac stage and hematologic response. Investigation of sex-related factors in recovering of amyloid cardiomyopathy is warranted. The cutoff of 5 g/24h used in staging also identifies reversibility of renal damage. Renal damage associated with higher baseline dFLC (but not BMPC) was more amenable of rapid recovery after FLC reducing chemotherapy. This warrants further investigation of the role of FLC in the pathogenesis of kidney involvement.

PS1321

IXAZOMIB IN COMBINATION WITH THALIDOMIDE AND DEXAMETHASONE FOR INDUCTION AND IXAZOMIB MAINTENANCE THERAPY IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

H. Ludwig[1,*], W. Poenisch[2], A. Egle[3], S. Knop[4], M. Schreder[4], D. Lechner[5], R. Hajek[6], E. Gunsililus[7], M. Fridrik[8], A. Petzer[9], K. Weisel[10], D. Niederwieser[2], R. Greil[3], N. Zojer[11]

[1]Wilhelminen Cancer Research Institute, Vienna, Austria, [2]Department of Hematology, University of Leipzig, Leipzig, Germany, [3]Third Medical Department, Paracelsus Medical University Salzburg, Salzburg, Austria, [4]Department of Internal Medicine II, Wuerzburg University Medical Center, Wuerzburg, Germany, [5]Internal Department I - Hematology with Stem Cell Transplantation, Hemostaseology and Medical Oncology, Hospital Elisabethinen Linz, Linz, Austria, [6]Fakultní nemocnice Ostrava, Ostrava, Czech Republic, [7]Department of Internal Medicine V, Hematology and Oncology, Medical University Innsbruck, Innsbruck, [8]Department of Internal Medicine 3, Kepler Universitaetsklinikum GmbH, Med Campus III, [9]Department of Internal Medicine I, Ordensklinikum Linz - Barmherzige Schwestern, Linz, Austria, [10]Department of Interal Medicine II, University Hospital Tuebingen, Tuebingen, Germany, [11]Department of Medicine I, Center for Oncology and Hematology, Wilhelminenspital, Vienna, Austria

Background: Ixazomib is an effective oral proteasome inhibitor with a favorable toxicity profile. Recent studies showed significant activity in combination with lenalidomide-dexamethasone in RRMM pts. and in combination with thalidomide-dexamethasone in NDMM.
Aims: To evaluate the activity and tolerability of ixazomib-thalidomide-dexamethasone (IxaThalDex) in pts. with RRMM.
Methods: Patients with RRMM after ≥1 prior line of therapy (TX) meeting the following criteria were eligible: Measurable disease, ECOG PS ≤2, ANC ≥1000/µL, platelet count ≥50.000µL, GFR ≥15mL/min. TX regimen: Ixazomib (4mg, d 1, 8 and 15), thalidomide (100mg/d), and dexamethasone (40mg once/week); in pts aged ≥75 years: thalidomide (50mg/d) and dexamethasone (20mg); 8 cycles induction TX, followed by 12 cycle of ixazomib maintenance therapy (1 year). Primary objective: PFS, secondary objectives: ORR, OS, impact of cytogenetic risk and of renal impairment, safety and myeloma frailty status and QoL.
Results: 94 planned pts have been enrolled, 4 pts. did not meet the inclusion criteria and were excluded. 90 pts comprised the ITT group: age (median): 67, (44-84) years, ISS stage I: 37, II 30, III: 22, not known: 1, median number of prior TX lines: 1 (range: 1-8). 13 pts discontinued TX before completion of 2 cycles (7 progressions, 2 deaths due to myeloma). 38 pts. have already started ixazomib maintenance TX after completion of 8 cycles of induction TX; 7 pts. have not yet completed induction TX. Median FU is 11 mos., and median number of cycles is 6 (range 0-8). PR or better was achieved in 47 pts of 77 pts (61%), nCR: 7 pts (9%), VGPR: 11 (14%), PR: 29 (38%), MR: 5 (7%); clinical benefit rate (CBR) of 68%. FISH data are available in 63 of the 77 pts. ≥PR was seen in 16/26 (62%) pts with *t (4; 14)* and/or *t (14; 16)* and/or *del17p* and in 22/37 (60%) with standard risk cytogenetics. Response rates were similar in the 8 pts. classified as unfit or frail (7/8 vs 40/69, p=0.1399). Similarly, response rates did not differ between the 27 (35%) pts. with GFR <60 ml/min and those with higher levels (62.9% vs 60%, p=1.00). Median PFS at the time of reporting is 9.3 mos. (95% CI 6.9 – 11.5) in the ITT group (Figure 1). PFS was similar in pts. with and without high-risk cytogenetics (9.3 and 9.0 mos., p=0.8209, Figure 2). PFS tended to be higher, though not significantly (18.0 mos. *vs.* not reached, p=0.2942) in the non-fit/frail pts.; likewise PFS did not differ between pts. with GFR ≤60 ml/min or higher (8.3 vs 9.3 mos., p=0.2785). Median OS has not been reached (Figure 1) and was similar between cytogenetic high- and standard-risk pts. No significant difference in OS was observed between those with GFR ≤ 60 ml/min or higher (18.0 mos. *vs.* not reached, p=0.0709). Neutropenia was noted in 41%, (22% grade 2, and 3% grade 3). Leucopenia was seen in 56%, (29% grade 2, and 5% grade 3). 91% had anemia (26% grade 2, and 17% grade 3). The most frequent non-hematological toxicity was fatigue observed in 32% of pts. Infections were noted in 53% (17% grade 3). Polyneuropathy was seen in 18% (15 grade 1 or 2% grade 3).

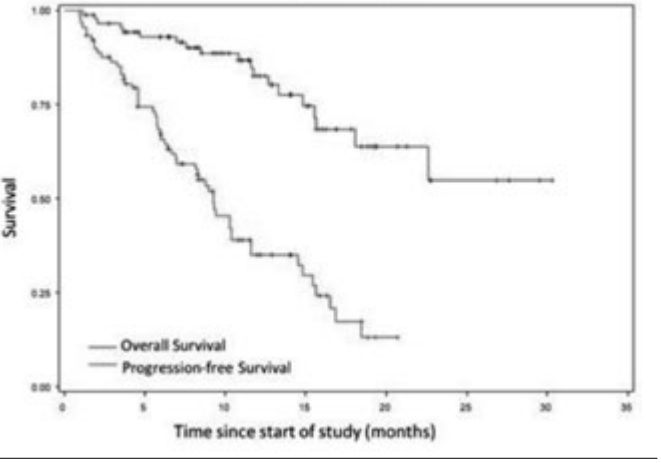

Figure 1. PFS ans OS IxaThalDex in RRMM.

Summary/Conclusion: The all oral IxaThalDex regimen showed an ORR of 61% with no difference in pts with/without high-risk cytogenetics, a CBR of 68%, and a PFS of 9.3 mos. in pts with RRMM. The regimen was well tolerated and was associated with a low incidence of mainly grade ≤2 PNP, which required dose reduction in one patient only. Response rates improved with continuation of therapy and treatment was associated with an increase in health related QoL.

PS1322

SELINEXOR COMBINED WITH LOW DOSE BORTEZOMIB AND DEXAMETHASONE (SVD) INDUCES A HIGH RESPONSE RATE IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA (MM)

N. Bahlis[1,*], H. Sutherland[2], D. White[3], M. Sebag[4], S. Lentzsch[5], R. Kotb[6], C. Venner[7], C. Gasparetto[8], G. Schiller[9], R. LeBlanc[10], W. Bensinger[11], B. Lipe[12], A. Del Col[13], M. Kauffman[14], S. Shacham[14], J. Jeha[14], J.-R. Saint-Martin[14], J. Shah[14], C. Chen[15]

[1]Southern Alberta Cancer Research Institute, Calgary, [2]Vancouver General Hospital,

Vancouver, [3]Dalhousie University and QEII Health Sciences Center, Halifax, [4]Royal Victoria Hospital, Montreal, Canada, [5]Columbia University, New York, United States, [6]Cancer Care Manitoba, Manitoba, [7]Cross Cancer Institute, Edmonton, Canada, [8]Duke University Cancer Center, Durham, [9]UCLA Ronald Reagan Medical Center, Los Angeles, United States, [10]Hôpital Maisonneuve-Rosemont, Montreal, Canada, [11]Swedish Cancer Center, Seattle, [12]University of Rochester Medical College, New York, United States, [13]Myeloma Canada, Laval, Canada, [14]Karyopharm Therapeutics, Newton, United States, [15]Princess Margaret Cancer Center, Toronto, Canada

Background: Selinexor is a first-in-class Selective Inhibitor of Nuclear Export (SINE) compound that binds and inactivates Exportin 1 (XPO1). Twice weekly (BIW) bortezomib (bort) in combination with dexamethasone (Vd) is an established therapy in relapsed and refractory multiple myeloma (RRMM). While the activity of bort BIW in combination with other agents is efficacious, prolonged use is limited due to peripheral neuropathy (PN, 50-60%) as well as acquired resistance to bort. New dosing regimes with improved tolerability and the ability to overcome resistance are needed. Preclinical studies have shown that selinexor, in combination with bort, can restore sensitivity of bort-resistant MM, in murine MM xenografts.
Aims: This Ph 1b/2 (NCT02343042), dose escalation study was designed to determine the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) for the safety, tolerability and efficacy of the combination of selinexor, bort and low dose dex (SVd) in patients (pts) with RRMM.
Methods: The study enrolled pts with RRMM after ≥ 1 prior therapy. Pts with prior exposure or refractory to proteasome inhibitors (PI) were included, provided they were not refractory to bort as a last therapy. Selinexor was dosed escalated in once-weekly (QW, 80; 100 mg) or twice-weekly (BIW, 60; 80 mg) regimens. Bort (1.3 mg/m^2 sc) was administered QW or BIW. Dex was given orally 40 mg per week.
Results: As of Feb 27th, 2018, 42 pts were enrolled; 22 pts in the dose escalation and 20 pts in the expansion (RP2D) cohort. Median age was 64 years; 23 M 19 F, median of 3 (range, 1 – 11) prior trement regimens. The MTD was not reached. Three pts in the BIW bort cohort were dose reduced to QW bort after Cycle 1. Common treatment related grade 1/2 adverse events (AEs) included: anorexia (57%), nausea (57%), fatigue (45%), and diarrhea (36%). Grade 3/4 AEs included: thrombocytopenia (45%), neutropenia (24%) and anemia (12%). Importantly, PN across all pts was limited to 6 patients (14%) (G1 4pts, G2 2 pts) of which 5 had prior bort exposure. Based on tolerability and efficacy, the RP2D of SVd is selinexor 100 mg, bort 1.3 mg/m^2 and dex 40 mg, all QW (40% less bort and 25% less dex compared to the standard, approved BIW schedule of Vd). Median PFS in PI relapsed or naïve pts is >13 months. Median PFS in PI refractory pts is 6.4 months. The median duration of response is ~12 months. Response rates can be seen in Table 1.

Table 1. SVd Best Response in PI non-Refractory & PI-Refractory Patients.

Category	N	ORR (%)	CBR (%)	CR (%)	VGPR (%)	PR* (%)	MR (%)	SD (%)	PD (%)
PI Relapsed or Naïve	19	16 (84%)	16 (95%)	2 (11%)	5 (26%)	9 (47%)	2 (11%)	1 (5%)	–
PI Refractory	21	9 (43%)	14 (67%)	1 (5%)	4 (19%)	4 (19%)	5 (24%)	6 (29%)	1 (5%)
PI Relapsed or Naïve, ≤ 3 Prior Treatments	18	15 (83%)	16 (89%)	2 (11%)	6 (33%)	7 (39%)	1 (6%)	2 (11%)	–

*1 PR unconfirmed

Summary/Conclusion: Selinexor in combination with weekly bort and dex is well tolerated and highly active in RRMM. The high ORR with SVd is achieved with 40% less bort and 25% less dex and no overt major organ toxicities. Furthermore, in pts with PI refractory MM, the ORR of 43% and CBR of 67% which support preclinical findings that selinexor re-sensitizes and overcomes resistance to PIs. This data supports the ongoing Ph 3 BOSTON study examining SVd vs Vd.

PS1323

THE MAJORITY OF NEWLY DIAGNOSED MYELOMA PATIENTS DO NOT FULFIL THE INCLUSION CRITERIA IN CLINICAL PHASE III TRIALS

T. W. Klausen[1,*], H. Gregersen[2], N. Abildgaard[3], N.F. Andersen[4], U.C. Frølund[5], P. Gimsing[6], C. Helleberg[1], K. F. Iversen[7], P.T. Pedersen[8], R.S. Pedersen[9], A.J. Vangsted[6]

[1]Department of Hematology, Herlev Hospital, Herlev, [2]Department of Hematology, Aalborg University Hospital, Aalborg, [3]Department of Hematology, Odense University Hospital, Odense, [4]Department of Hematology, Aarhus University Hospital, Aarhus, [5]Department of Hematology, Roskilde Hospital, Roskilde, [6]Department of Hematology, Rigshospitalet, University of Copenhagen, Copenhagen, [7]Department of Hematology, Vejle Hospital, Vejle, [8]Department of Hematology, Esbjerg Hospital, Esbjerg, [9]Department of Hematology, Regional Hospital West Jutland, Holstebro, Denmark

Background: Randomized phase III trials on multiple myeloma (MM) have strict patient inclusion and exclusion criteria and currently it is unknown how many patients with MM that are represented in these clinical trials.
Aims: To analyze the fraction of a population based cohort of newly diagnosed younger and older myeloma patients that fulfil the in- and exclusion criteria in clinical trials and to analyze the overall survival (OS) according to these criteria.
Methods: Four clinical trials for elderly (Hovon 87 trial; VISTA trial; FIRST trial and the SWOG S0777 trial) and younger patients (STaMINA trial; IFM2013-04 trial; IFM DFCI 2009, and the EMN02 trial) were selected. Twelve predefined in - and exclusion criteria, found in 3 out of 4 trials in each age group, were selected. The three inclusion criteria were: CRAB, measurable disease, plasma cells in bone marrow (PLC) ≥ 10% and the nine exclusion criteria were: Kidney failure, WHO performance status (PS) > 2, amyloidosis, acute myocardial infarction (AMI) within last 6 months, human immunodeficiency virus (HIV), hepatitis B or C, cancer within 5 years (excluding stage 0-1 cervical cancer, some skin cancers), severe comorbidity (congestive heart failure, dementia, severe diabetes, moderate or severe lever disease, hemiplegia). The patients were separated into two cohorts, those that were eligible for protocol inclusion (IC) and those that were not (non-IC). The IC cohort and non-IC cohort were compared using the Kaplan-Meier statistics and Cox proportional hazard models with OS as outcome. The Danish Multiple Myeloma Registry (DMMR) was used to include patients with treatment demanding MM. The Danish National Patient Registry (DNPR) was used for collection of co-morbidity data.
Results: 2189 patients, 1425 patients 65 years and 764 patients ≤ 65 years were registered in the DMMR. In the elderly cohort 522 (36.6%) fulfilled IC whereas 903 (63.4%) were non-IC. The most common reason for non-IC was kidney failure (26.1%) followed by severe comorbidity (18.5%) and PS > 2 (16.4%). Patients in the non-IC cohort had significantly worse OS compared to the IC cohort, median OS 21.3 mo. versus 44.0 mo., HR =1.7 (95% CI: 1.5-1.9), p<0.0001 (Figure 1A). In the younger cohort 345 (45.2%) fulfilled IC whereas 419 (54.8%) were non-IC. The most common reason for non-IC was kidney failure (22.2%) followed by PS > 2 (14.8%) and PLC < 10% (13.1%). Patients in the non-IC cohort had shorter OS compared to the IC cohort (median OS was 65.5 mo. versus 78.4 mo., HR 1.3 (95% CI: 1.0-1.5), p=0.021) (Figure 1B).

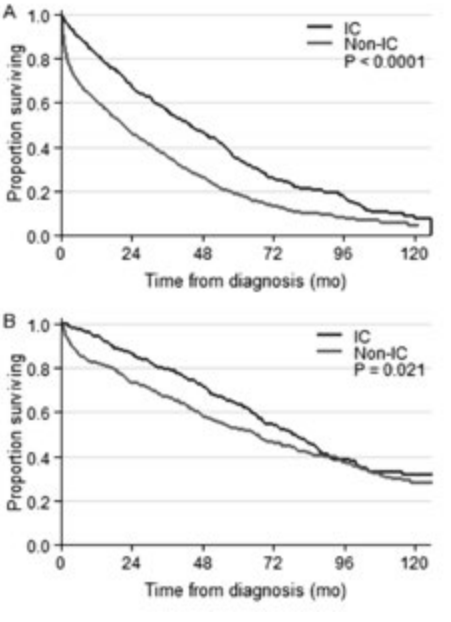

Figure 1. Figure shows OS IC vs non-IC for A) the >65 years cohort and B) the ≤65 years cohort. The blue line shows patients that fulfil the inclusion criteria the red line shows patients that do not.

Summary/Conclusion: The majority (63.4%) of elderly MM patients in a population cohort is not eligible for inclusion in most clinical trials. Elderly patients not eligible for inclusion in clinical trials have worse OS than those who are. For patients ≤ 65 years, 54.8% did not fulfill the inclusion criteria and these patients also have significantly shorter OS. These observations

affect the application of data from clinical trials to the real life MM population.

PS1324

COMPARISON OF LYMPHOTRACK® MISEQ® ASSAYS AND FLOW CYTOMETRY FOR CLONALITY AND MINIMUM RESIDUAL DISEASE ASSESSMENT IN MULTIPLE MYELOMA

Y. Huang[1,*], J. Panganiban[1], A. Jacobsen[1], M. Kaminsky[1], K. Hutt[1], J. Heilman[1], F. W. Martinez[1], M. Blankfard[1], T. Stenzel [1], A. Medina[2], C. Jiménez[2], R. García-Sanz [2], J. E. Miller[1]

[1]Invivoscribe, San Diego, United States, [2]Hospital Universitario de Salamanca-IBSAL, Salamanca, Spain

Background: Multiple myeloma (MM), characterized by presence of excess plasma cells (PCs) in bone morrow (BM), accounts for about 10% of all hematological malignancies. Multiparameter flow cytometry (MFC) is a standard tool used to detect and monitor MM in patients. Since PCR-based NGS methods can identify clonal rearrangements within the immunoglobulin (*Ig*) loci, international organizations such as NCCN, IMWG and ESMO have recently included NGS as a recommended tool for MRD assessment in MM. Recently we developed NGS-based LymphoTrack® Assays and bioinformatics software to detect and track clonal rearrangements within the *Ig* genes in B-cells. The *IGH* FR1, *IGH* FR2, *IGH* FR3 and *IGK* assays detect and track clonal rearrangements in B-cell malignancies. Here we compare the performance of LymphoTrack® MiSeq® Assays and MFC by testing 101 anonymized, paired diagnostic and MRD MM specimens.
Aims: To assess performance of LymphoTrack® MiSeq® assays in detecting and tracking clonotypes in MM samples.
Methods: 101 paired BM samples from MM patients were tested in this study. The MFC methods utilize 8-color direct immunofluorescence technique. Genomic DNA from the same collected specimens were extracted, anonymized and blinded prior to testing with the LymphoTrack® *IGH* and *IGK* MiSeq assays. Libraries were pooled and sequenced on a single MiSeq run. LymphoTrack Software was used to sort data by target and index. Diagnostic specimens were tested using all LymphoTrack® MiSeq B-cell assays to identify a MM-specific clonotype, which was tracked using a single assay in subsequent samples.
Results: Out of 101 diagnostic specimens, 84 (83%), 80 (79%), 63 (62%) and 87 (86%) samples were detected as clonal positive by *IGH* FR1, FR2, FR3 and *IGK*, respectively. When combining all 4 targets, 100% (101/101) clonal positivity was achieved.
Using LymphoTrack® MRD Software to identify clonotype sequence identified in diagnostic samples, we performed MRD assessment on 84 samples that tested positive using *IGH* FR1. 41 (49%) were positive, 42 (50%) were negative, and 1 (1%) was not evaluable, due to insufficient reads. MRD results by MFC on the same 84 patients showed 40 (48%) positive, 36 (43%) negative, and 8 (9%) not evaluable samples. Excluding samples with DNA less than 700 ng (~100,000 cell equivalents), concordance between these two methods was 85.4%. Three samples were detected by MCF but not by NGS while four samples were detected by NGS but not by MCF.
Summary/Conclusion: LymphoTrack assays detected clonotype sequences in 100% of MM diagnostic samples and achieved 85.4% agreement with MFC in detecting MRD. This suggests LymphoTrack assays are a useful tool in identifying and tracking disease status in MM samples. Unlike MFC, the LymphoTrack assays and accompanying bioinformatics software can be submitted for approval to regulatory authorities worldwide.

PS1325

QUALITY OF HEMATOLOGIC RESPONSE BUT NOT DEPTH OF NT-PROBNP RESPONSE IMPROVES SURVIVAL OF PATIENTS WITH AL AMYLOIDOSIS WHO ACHIEVE CARDIAC RESPONSE

P. Milani[1,*], M. Basset[1], A. Foli[1], R. Mussinelli[1], P. Stefano[2], G. Merlini[1], G. Palladini[1]

[1]Department of Molecular Medicine, University of Pavia, Pavia, Italy, [2]Department of Internal Medicine, University of Pavia, Pavia, Italy, Amyloidosis Research and Treatment Center, Foundation IRCCS Policlinico San Matteo, Pavia, Italy, Pavia, Italy

Background: Markers of clonal and organ disease allow accurate assessment of response to therapy in AL amyloidosis. However, while hematologic response is graded, cardiac response is defined as present or absent, and it is unclear whether the depth of NT-proBNP reduction further improves outcomes.
Aims: We evaluated the impact of depth of NT-proBNP reduction at the time of hematologic response in AL amyloidosis.
Methods: The prospectively maintained database of the Pavia Amyloidosis Research and Treatment Center was systematically searched for patients who obtained cardiac response after frontline therapy, defined as a >30% and >300 ng/L of NT-proBNP decrease compared to baseline. Cardiac response was assessed at the time of best hematologic response. Survival was calculated from the time of achievement of best hematologic response to the time of death or last contact. Cutoffs of NT-proBNP absolute values and percent reduction best predicting survival were searched by ROC analysis based on death at 1 year.
Results: A total of 130 patients evaluable for cardiac response (baseline NT-proBNP >650 ng/L) who achieved NT-proBNP response were included. Other organs involved were kidney (68%), soft tissue (24%), and liver (16%), peripheral nervous system (15%), and more than 2 organs were involved in 87% of patients. The median follow-up of living patients was 49 months [interquartile range (IQR): 36-78 months] and 105 patients (42%) died. The median time to achievement of cardiac response was 7.8 (IQR: 5.5-10.3 months). The median maximal reduction in NT-proBNP from baseline was 55.8% (IQR: 46-70%). The ROC analyses failed to identify cutoffs of NT-proBNP reduction able to significantly discriminate the outcome. Thus, patients were grouped according to tertiles and quartiles of NT-proBNP percent reductions. However, there was no significant difference in patient survival between subgroups. The best cutoff predicting survival from best response was an absolute concentration of NT-proBNP after therapy below 2100 ng/L (median survival 86 vs. 57 months, p=0.047). However, when the analysis was limited to patients who had a baseline value of NT-proBNP >3000 ng/L (required to be classified as responders with a post treatment value of 2100 ng/L), no difference in survival was observed, indicating that the impact on survival of the absolute NT-proBNP value actually reflects pre-treatment severity of cardiac dysfunction. A normalization of NT-proBNP was observed only in 9 (7%) of patients and it was not associated with a significant improvement in survival compared to other responders. Obtaining a complete response and/or very good partial response was associated with a significantly better survival from response (median survival 45 vs. 86 months, p=0.016).
Summary/Conclusion: Among cardiac responders, the depth of reduction of NT-proBNP at the time of best hematologic response does not add additional prognostic information. Even in this group of patients, severity of cardiac involvement at diagnosis retains its prognostic impact also in cardiac responders. Moreover, the achievement of a profound hematologic response identifies cardiac responders with a significantly longer survival. Thus, once cardiac response is reached, the decision on whether to discontinue treatment should be based on the depth of hematologic response aiming at VGPR or better.

PS1326

SUSTAINED MRD NEGATIVITY AT 12 MONTHS POST-ASCT PREDICTS OUTCOMES FOR MYELOMA PATIENTS: A REAL WORLD STUDY

M. Austin[1,*], C. Pawlyn[1,2], H. Woods[1], R. Morilla[1], M. Potter[1], M. Ethell[1], R. Saso[1], M. Kaiser[1,2], K. Boyd[1]

[1]Haematology, The Royal Marsden NHS Trust, [2]Myeloma group, The Institute of Cancer Research, London, United Kingdom

Background: Minimal residual disease (MRD) negativity by multiparameter flow cytometry (MFC) at 3 months post autologous stem cell transplantation (ASCT) in newly diagnosed multiple myeloma (MM) is recognised as an independent predictor of progression free survival (PFS) and overall survival (OS) in clinical trials. Additional analysis of MRD at 1-year post-ASCT, is less well established. There is also little available data on the use of MRD in the relapse setting, especially in the context of second ASCT, or from real-world clinical practice.
Aims: This study was performed to characterise the prognostic benefit of multiple time point MRD monitoring by MFC and the impact of sustained MRD negativity on outcomes after first and second ASCT in a real-world dataset.
Methods: We retrospectively analysed data for 232 consecutive MM patients who underwent ASCT between January 2012 and December 2014 at the Royal Marsden Hospital. Flow cytometry was performed at 3 and 12 months post-ASCT in the regional Haematological Malignancy Diagnostic Services Laboratory. Limit of detection was 0.01%. Baseline variable and outcome data were analysed using Kaplan-Meier survival curves and the Log-rank test using R. PFS and OS were measured from the date of ASCT using IMWG criteria.
Results: Of the 232 transplants, 188 were after first induction (ASCT1) and 44 as a second transplant after second-line or later therapy (ASCT2). Medi-

an age was 61.4 years (range 23-72). Pre-ASCT induction therapy was with IMiD (57%), proteasome inhibitor (PI, 16%) or combined IMiD+PI therapy (25%) in most cases. 81% of patients underwent a bone marrow evaluation at 3 months post ASCT and 55% at both 3 and 12 months.
56.9% of patients (n=86) were MRD- at 3 months post ASCT. Logarithmic reduction in MRD at 3 months correlated with PFS but not OS across 3 logs from <0.01% to <1% (p<0.001).
50.5% of patients (n=53) were MRD- at 12 months post ASCT. MRD- at 12 months post ASCT1 was associated with a significantly improved median PFS (55.2 vs 26.8 months p=0.007) (Figure 1a) and OS at 3 years (96% vs 92%; p=0.022). Logarithmic reduction in MRD at 1 year correlated with PFS across 5 logs from <0.01% to >10% (Figure 1b).
Longest median PFS was seen in those patients who had sustained MRD- or who deepened response from MRD+ to MRD- from 3 to 12 months. Those patients with MRD+ at both time points and those who went from MRD- at 3 months to MRD+ at 12 months had the shortest median PFS.
Post-ASCT2 at 12 months MRD- was associated with a trend to longer median PFS (32.6 vs 13.0 months, p=0.266). The longest median PFS was found in the group who remained MRD- from 3 to 12 months and the shortest in those who went from MRD- to MRD+ across these time points.

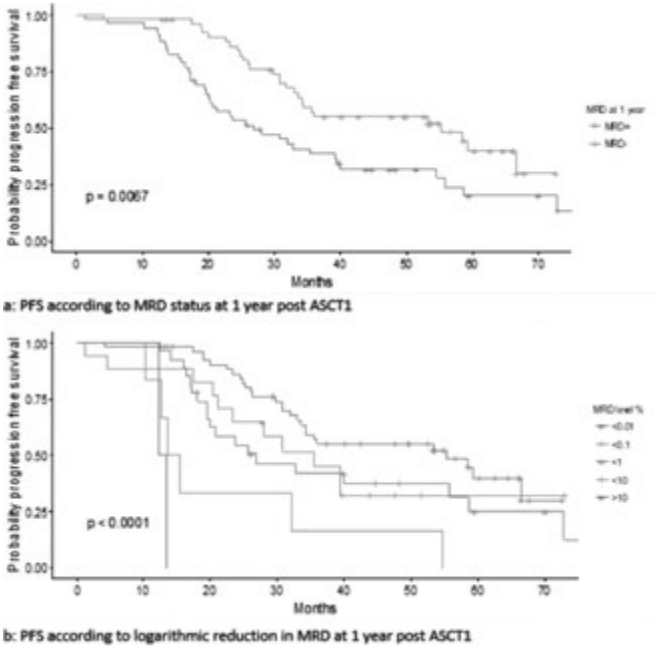

Figure 1.

Summary/Conclusion: In our real-world study, sustained MRD negativity was associated with improved survival outcomes following ASCT in both newly diagnosed MM patients and those undergoing a second transplant. Our findings support the utility of multiple time point MRD assessment in routine clinical practice and treatment strategies aiming to achieve sustained MRD- at these time points.

PS1327

MULTIPLE MYELOMA IN PATIENTS UNDER 40 YEARS OLD: A RETROSPECTIVE ANALYSIS FROM THE INTERGOUPE FRANCOPHONE DU MYELOME (IFM)

A. Caulier[1,*], B. Branco[2], J. Galtier[3], A. Perrot[4], A.-V. Michaud[5], C. Doyen[6], B. Pegourie[7], D. Caillot[8], P. Lenain[9], S. Ivanoff[10], J. Fontan[11], A.-M. Stoppa[12], S. Manier[13], L. Garderet[14], V. Richez[15], X. Leleu[15], J.-P. Marolleau[1], H. Avet-Loiseau[2], B. Royer[16]

[1]Hematology, CHU Amiens, Amiens, [2]Hematology, CHU Toulouse, Toulouse, [3]Hematology, CHU Bordeaux, Bordeaux, [4]Hematology, CHU Nançy, Nançy, [5]Hematology, CHU Nantes, Nantes, France, [6]Hematology, CHU Dinant-Godinne, Namur, Belgium, [7]Hematology, CHU Grenoble, Grenoble, [8]Hematology, CHU Dijon, Dijon, [9]Hematology, CHU Rouen, Rouen, [10]Hematology, Hôpital Avicenne, Bobigny, [11]Hematology, CHU Besançon, Besançon, [12]Hematology, Institut Paoli-Calmettes, Marseille, [13]Hematology, CHRU Lille, Lille, [14]Hematology, Hôpital Saint-Antoine, Paris, [15]Hematology, CHU Poitiers, Poitiers, [16]Hematology, Hôpital Saint-Louis, Paris, France

Background: Median age at diagnosis for multiple myeloma patients is approximately 68 years, and only 2% of them are less than 40 years old. It has been suggested that younger patients may present with different disease features and worse outcomes, but few data are available in the era of novel therapies.
Aims: Our primary objective was to describe this specific population. Secondary objectives comprised outcomes and predictive factors for survival.
Methods: We report here a multicenter retrospective study of 185 patients aged up to 40 years old, diagnosed in 37 IFM centers between 01/01/2000 and 31/12/2015. The demographics, baseline biological, cytogenetic and clinical features plus frontline therapies were analyzed using descriptive statistics. Survival outcomes (progression-free (PFS) and overall (OS) survivals) were estimated using Kaplan-Meier method.
Results: Data completion rate was 90% in patient charts and baseline characteristics are summarized in Table 1. Sex ratio M/F was 1.8. Median age was 37.6 (range 18.6-40.9) and 9% (n=16) were <31 years-old. 9 patients (5%) had primary plasma cell leukemia (pPCL) and 1 had Randall disease. Solitary Plasmacytoma was present in 3 patients (2%) at diagnosis and 9 had smoldering myeloma (SMM). Half of patients display abnormal cytogenetics. Symptomatic MM patients received induction therapy with proteasome inhibitors (PI, 73%), IMIDs (58%), or both in 53%. Overall, 88,5% of patients underwent transplant with high dose melphalan + autograft (ASCT), frontline or at time of relapse. Furthermore, 34 patients received a double intensification, and 42 underwent allogeneic transplant. Frontline response rates were good with 71% of patient in VGPR or better. Median follow-up was 5.9 years (range 0.0-16.8). None of SMM patients progressed to overt MM. Considering symptomatic MM patients, median PFS was 3.2 years (IC95:2.2-4.2). Within the whole cohort, 5- and 10-y OS were 80.5% and 61.9% respectively. Considering only symptomatic MM (excluding pPCL and SMM), 5- and 10-y OS were 81.5% and 61.3% respectively. Allogeneic transplant significantly decreased OS (5-y OS = 66.8% vs 86.2%, p<0.001), as 50% of patients died after allograft. Post-allograft median OS was 3.9 years (IC95:1.5-6.2). Age, cytopenia, renal failure, hypercalcemia and ISS score did not impact response to induction therapy. Date of diagnosis and treatment with PI or IMID did not influence OS, but higher ISS score significantly decreases OS (5-y OS =87.8%, 79.1%, 60.1% for ISS1, 2, 3 respectively, p=0.018). Strikingly, high-risk (HR) cytogenetics was associated with better response to induction therapy (VGPR/RC =87.8% in HR vs 66.7%, p=0.01) contrasting to worse OS (5-y OS =55.7% in HR vs 89.3%, p<0.001).

Table 1. Demographic and biological characteristics of the population.

Total population, n (%)	185 (100)
- Diagnosis < year 2006	44 (23.8)
- Diagnosis 2006-2010	51 (27.6)
- Diagnosis > 2010	90 (48.6)
Gender, n (%)	
- Male / Female	119 (64.3) / 66 (35.7)
Age, years median (±SD ;min;max)	37.6 (±4.1 ;18.6 ;40.9)
Type of disease, n (%)	
- symptomatic MM	162 (87.6)
- pPCL	9 (4.9)
- SMM	10 (5.4)
- Solitary plasmacytoma	3 (1.6)
- Randall disease	1 (0.5)
Calcemia , n (%)	
- ≥ 3mmol/l	13 (7.7)
- < 3 mmol/l	156 (92.3)
- ND/NA	16/0
Serum Creatinine, µmol/l median (±SD ;min;max)	81.6 (±173.7 ;43 ;1293)
Creatinine clearance. ml/mn (MDRD)	
- ≥ 60	143 (88.1)
- <60	29 (16.9)
- ND	13
ISS score 1 / 2 / 3, n (%)	89 (52) / 47 (27.5) / 35 (20.5)
- ND	14
Cytopenia, n (%)	
- Anemia / ND	59 (34.1) / 12
- Thrombopenia / ND	7 (4.1) / 13
- Neutropenia / ND	9 (5.6) / 25
Bone lesions / ND, n (%)	127 (74.7) / 15
Cytogenetics / ND, n (%)	168 (90.8) / 17
- Abnormal cytogenetics	91 (54.2)
- High-Risk (HR), including*:	48 (28.6)
del17p / t(4 ;14) / t(14 ;16) / +1q / Hypodiploidy	16 / 16 / 0 / 16 /16
*some patients display multiple abnormabilities	

Summary/Conclusion: This retrospective analysis showed that MM patients under 40 years are a heterogeneous population, in which some known prognostic factors such as ISS and HR cytogenetics remain discriminatory. Despite long survivors, survival outcome remains poor, particularly in case of HR cytogenetics. Importantly, we did not find any OS improvement for patients recently diagnosed and treated with novel agents. More aggressive

treatment strategies are required to improve survival in this young patient population.

PS1328

AMPLICON NEXT-GENERATION SEQUENCING (NGS) OF REARRANGED IMMUNOGLOBULIN (IG) LOCI IS ADVANTAGEOUS FOR CLONAL MARKER IDENTIFICATION AND MINIMAL RESIDUAL DISEASE (MRD) DETECTION IN MULTIPLE MYELOMA (MM)

M. Kotrova[1,*], F. Appelt[1], J. Wangemann[2], M. Ritgen[2], N. Darzentas[2], F. Davi[3], P. Groenen[4], M. Brüggemann[2], S. Knop[5], T. Stübig[6]

[1]Shared First Authorship, Medical Department II, Unit for Hematological Diagnostics, [2]Medical Department II, Unit for Hematological Diagnostics, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany, [3]Hopital Pitie-Salpetriere and University Pierre et Marie Curie, Paris, France, [4]Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [5]Shared Senior Authorship, Innere Medizin II, Klinikum der Universität Würzburg, Würzburg, [6]Shared Senior Authorship, Medical Department II, Unit for Hematological Diagnostics, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany

Background: Measurement of MRD has become an important tool to assess deep response to treatment in many clinical myeloma trials. Molecular techniques have been shown to have a higher sensitivity for MRD assessment compared to standard flow cytometry (FCM). However, molecular MRD quantification in MM using allele specific IG-PCR has a number of disadvantages, including failure of marker identification due to highly hypermutated IG loci and complex design of patient-specific PCR assays. Amplicon-based IG NGS has the potential to overcome such limitations and serve as a meaningful alternative to FCM-based MRD assessment.
Aims: Our aim was to evaluate the applicability of comprehensive IG amplicon NGS to detect molecular MRD markers and to compare the results of IG NGS during follow-up with those of multicolour FCM.
Methods: NGS-based marker screening was performed in pre-treatment bone marrow (BM) samples of 103 patients of the DSMM XIV phase 3 trial. Sequencing libraries were prepared using a 2-step PCR with multiplexed primer sets for IGH V-D-J (FR1, FR2 and FR3), IGH D-J, and IGK V-J and KDE loci. For MRD detection, 1-step library preparation using the same primer sets was employed. MRD was measured by NGS in 500ng BM DNA of 53 follow-up samples. The final MRD values were normalized using the IG sequences of cell-lines, which were added as spike-ins to the patient DNA in known copy numbers. The analysis of NGS MRD-negative samples was extended by NGS analysis of additional 500ng DNA. The libraries were sequenced on the Illumina MiSeq with a median coverage of 396,074 reads/sample. Raw NGS data were analysed with ARResT/Interrogate. The NGS MRD results were compared to the MRD values obtained by FCM. The flow approach consists of an optimized 2-tube 8-colour antibody panel and a bulk-lysis procedure for acquisition of 10^7 cells/sample according to the NGF EuroFlow MM MRD protocol. In the cases with discordances between NGS MRD and FCM MRD, a digital PCR (ddPCR) approach is in progress to confirm NGS MRD positivity.

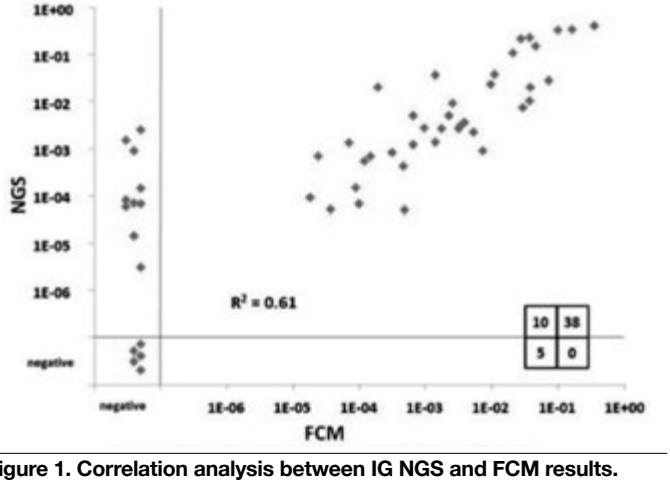

Figure 1. Correlation analysis between IG NGS and FCM results.

Results: Employing NGS-based methodology at least one clonal marker was detected in 98 of 103 patients (95%), 79/103 (77%) patients had two or more. Complete IGH rearrangements were present in 82 (80%), incomplete IGH rearrangements in 55 (53%), and IGK rearrangements in 61 (59%) patients. We detected on average 2.6 clonal markers per patient (range 0-7). From the 53 follow-up samples analysed, 48 (91%) were MRD positive using NGS. A correlation analysis between NGS and FCM is shown in Figure 1: NGS MRD data were concordant to FCM-based MRD results in 43 samples (81%) with 38 (72%) samples being MRD positive by both methods and 5 (9%) being double negative. The remaining 10 samples (19%) were only positive by NGS, of which only 2 had NGS MRD values above 0.1% (1E-03) - both positivities were confirmed by ddPCR.
Summary/Conclusion: NGS is a valuable tool for IG marker identification and MRD detection in MM, reaching at least similar sensitivities compared to standardized next generation flow (NGF). Clonal IG markers were successfully identified in >95% of patients. Still, prospective comparative analysis of unselected cases has to be performed to verify the clinical impact of NGS compared to NGF for sensitive and robust MRD assessment.

PS1329

A PHASE 1B STUDY USING THE COMBINATION OF SELINEXOR, DARATUMUMAB, AND DEXAMETHASOME IN MULTIPLE MYELOMA PATIENTS PREVIOUSLY EXPOSED TO PROTEASOME INHIBITORS AND IMMUNOMODULATORY DRUGS

C. Gasparetto[1,*], S. Lentzsch[2], G. Schiller[3], W. Bensinger[4], N. Bahlis[5], H. Sutherland[6], D. White[7], M. Sebag[8], R. Kotb[9], C. Venner[10], R. LeBlanc[11], C. Chen[12], A. Del Col[13], M. Kauffman[14], S. Shacham[14], J. Jeha[14], J.-R. Saint-Martin[14], J. Shah[14], J. Turner[15], D. Sullivan[15], B. Lipe[16]

[1]Duke University Cancer Center, Durham, [2]Columbia University, New York, [3]UCLA Ronald Reagan Medical Center, Los Angeles, [4]Swedish Cancer Center, Seattle, United States, [5]Southern Alberta Cancer Research Institute, Calgary, [6]Vancouver General Hospital, Vancouver, [7]Dalhousie University and QEII Health Sciences Center, Halifax, [8]Royal Victoria Hospital, Montreal, [9]Cancer Care Manitoba, Manitoba, [10]Cross Cancer Institute, Edmonton, [11]Hôpital Maisonneuve-Rosemont, Montreal, [12]Princess Margaret Cancer Center, Toronto, [13]Myeloma Canada, Laval, Canada, [14]Karyopharm Therapeutics, Newton, [15]Moffitt Cancer Center, Tampa, [16]University of Rochester Medical College, New York, United States

Background: Selinexor is a first-in-class Selective Inhibitor of Nuclear Export (SINE) compound that binds and inactivates Exportin 1 (XPO1). Selinexor, as a single agent, or in combination with PIs or IMiDs, has shown anti-MM activity in patients (pts) with relapsed or refractory multiple myeloma (RRMM). Daratumumab, an anti-CD38 monoclonal antibody, is approved for the treatment of RRMM. In CD138(+) myeloma cells, from newly diagnosed MM patients, were sensitized to the combination of selinexor and daratumumab as compared to single agent selinexor (p=0.005) or Dara (p=0.004).
Aims: This Ph 1b (NCT02343042), dose escalation study was designed to determine the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) for the safety, tolerability and efficacy of the combination of selinexor, daratumumab, and low dose dex (SDd) in pts with RRMM.
Methods: Pts were eligible if they had RRMM and had received ≥ 3 prior therapies, including a PI and IMiD. Selinexor was independently dosed escalated in 2 concurrent cohorts: once-weekly (QW, at 100 mg) or twice-weekly (BIW, at 60 mg) regimens. Daratumumab (16 mg/kg IV) was administered QW and dexamethasone (dex) was given orally 40 mg QW or 20 mg BIW.
Results: As of Feb 27th 2018, 13 pts (7 males / 6 females) have been enrolled. Three pts have been enrolled into the 60 mg BIW and 10 pts in the 100 mg QW cohorts. Pts have a median age of 67 years and a median of 4 (range, 2 – 10) prior treatment regimens. Adverse events include: fatigue (9 pts), thrombocytopenia (8 pts), neutropenia (7 pts), and nausea (5 pts). Two DLT's were reported in the 60 mg BIW cohort: G3 thrombocytopenia and G2 fatigue requiring dose reduction in selinexor to 100 mg QW. In the 100 mg QW cohort, 6 pts enrolled, 5 evaluable, with no DLTs; enrollment in this cohort is ongoing. A total of 10 pts were evaluable for response. In 8 dara-naïve pts, the ORR was 88% (4 VGPR, 3 PR, 1 PD), two PRs unconfirmed, and responses usually occurred within 1 cycle of treament. In the 2 pts with daratumumab refractory MM, there was one PD and one SD. Based on tolerability and efficacy, the RP2D of SDd is selinexor 100 mg, daratumumab 16 mg/kg and dex 40 mg, administered QW.
Summary/Conclusion: Selinexor 100 mg QW, can be combined safely with standard dose/schedule daratumumab and dex. The preliminary activiy with an ORR of 88% in RRMM patients who are dara naïve is promising. Enrollment is ongoing and updated data from the full phase 1 will be presented.

PS1330

A REAL WORLD RETROSPECTIVE ANALYSIS OF EXTRAMEDULLARY DISEASE FROM BALKAN MYELOMA STUDY GROUP AND BARCELONA UNIVERSITY: CLINICAL FEATURES AND OUTCOME

M. Beksac[1,*], N. Kanellias[2], L. Rosinol[3], G. S. Cengiz[1], G. Ozet[4], V. Goranova[5], D. Coriu[6], J. Bila[7], H. Ozsan[8], L.I. Balic[9], A. Ivanaj[10], E. Kastritis[2], J. Blade[11], M. Dimopoulos[2]

[1]Hematology, Ankara University, Ankara, Turkey, [2]Hematology, National and Kapodistrian University of Athens Alexandra Hospital, Athens, Greece, [3]University of Barcelona, Department of Hematology, Barcelona, Spain, [4]Hematology, Numune Hospital, Ankara, Turkey, [5]Hematology, University of Pulovdiv, Pulovdiv, Bulgaria, [6]Hematology, Fundeni Clinical Institute, Bucharest, Romania, [7]Hematology, University of Belgrade, Belgrade, Serbia, [8]Hematology, Dokuz Eylül University, Izmir, Turkey, [9]Hematology, University of Sarajevo , Sarajevo , Bosnia and Herzegovina, [10]Hematology, University of Tirana, Tirana, Albania, [11]Hematology, University of Barcelona, Barcelona, Spain

Background: Extramedullary disease (EMD), defined as a clonal plasmacytic infiltration at anatomic sites distant from the bone marrow or adjacent soft tissue, appear to account for 6-7.5% of the total myeloma population at diagnosis and tend to increase at relapse with an unfavorable prognosis (Blade, Usmani).
Aims: Here, following an international collaborative effort, we are reporting the clinical features and outcome of myeloma patients with EMD or paraosseous (PO) involvement.
Methods: This multicenter retrospective study conducted in 16 centers from 11 countries included 206 adult patients diagnosed with EMD between January 2010 and November 2017. The diagnosis of EMD was rendered in accordance with the International Myeloma Working Group Guidelines. Eligibility criteria included EMD at any time following the initial diagnosis of Multiple Myeloma (MM) excluding plasma cell leukemia or solitary plasmacytoma. Those patients with pathological or radiological evidence of neoplastic plasma cells in the soft tissues adjacent to axial skeleton were deemed to have PO involvement of locally-advanced myeloma, but not EMD. Outcome was determined as response to treatment, progression free survival (PFS) and overall survival (OS) in months by Kaplan-Meier analysis using SPSS (IBM SPSS Statistics 21; IBM Corp., Chicago, IL) statistical tool kit. We also compared the PFS and OS between the paraosseous and EMD cohorts.

Table 1. Baseline characteristics of the patients.

Caracteristic (n=195)		Results	
Age, years, Median (range)		62 (34-87)	
ISS stage (at myeloma diagnosis)			
Stage I, n (%)		66 (33.8%)	
Stage II, n (%)		58 (29.7%)	
Stage III, n (%)		65 (33.3%)	
Number of FISH abnormalities			
No abnormalities, n (%)		50 (49.2%)	
1 abnormality, n (%)		28 (27.4%)	
2 abnormalities, n (%)		12 (11.7%)	
≥3 abnormalities, n (%)		12 (11.7%)	
Del17p, n (%)		10 (9.8%)	
Del13q, n (%)		20 (19.6%)	
t(4;14), n (%)		8 (7.8%)	
t(14;16), n (%)		2 (2%)	
t(11;14), n (%)		4 (4%)	
Anatomical locations of EMD			
Soft tissue(muscle/skin) , n (%)		51 (26.2%)	
Lymph nodes, n (%)		20 (10.3%)	
Chest, n (%)		19 (9.3%)	
Liver, n (%)		17 (8.7%)	
Central nervous system, n (%)		14 (7.2%)	
Oropharynx, n (%)		8 (4.1%)	
Lung, n (%)		6 (3.1%)	
Abdominal, n (%)		6 (3.1%)	
Testis, n (%)		4 (2.1%)	
Others, n (%)		5 (2.6%)	
Initial therapy for EMD			
Systemic chemo with radiotherapy, n (%)		55 (28.2%)	
PI, n (%)		104 (55%)	
IMIDs, n (%)		53 (27.3%)	
Only radiotherapy, n (%)		9 (4.8%)	
Monoclonal antibodies (CD38,CS1), n (%)		8 (4.2%)	
Lines of therapy (Myeloma+EMD)			
1-2 lines, n (%)		75 (56.4%)	
>2 lines, n (%)		58 (43.7%)	
Autologous stem cell transplantation, n (%)		76 (39%)	
CR diagnosis	EMD, % (n)	18.3%(15/82)	p=n.s
	PO, % (n)	28.1%(9/32)	
CR relapse	EMD, % (n)	2.9 % (20/69)	p=0.001
	PO, % (n)	41.7% (5/12)	
PFS diagnosis	EMD, Mean±SD	52.2±5.3	p=n.s
	PO, Mean±SD	75.9±11.4	
PFS relapse	EMD, Mean±SD	21.3±5.0	p=n.s
	PO, Mean±SD	20.8±4.2	
OS diagnosis	EMD, Mean±SD	55.7±6.2	p=0.02
	PO, Mean±SD	108.7±13.0	
OS relapse	EMD, Mean±SD	37.9±7.9	p=0.03
	PO, Mean±SD	30.1±4.7	

Results: A total of 195 patients met the predetermined criteria for inclusion. Baseline characteristics of the patients are summarized in Table 1. The median age at diagnosis of EMD was 62 years (range 34-87 years). Out of 114 patients at diagnosis EMD/PO were 82/32 and of the 81 patients at relapse 69/12 respectively. The median time from MM diagnosis to the development of EMD in the relapse/progression group was 28.4 months (range 1-157 months). Imaging approach for EMD was CT (n:109), PET-CT (n:48) or MRI (n:30). The most common locations for EMD at the time of diagnosis of MM were the soft tissues ie muscle and/or skin (26.2%) and lymph nodes (10.3%). FISH analyses prior to the diagnosis of EMD were available for 102 patients (52.3%). The entire group received a median number of two lines of treatment following the diagnosis of EMD/MM. Although response was higher for PO vs EMD at relapse, PFS was similar (Table 1). At the time of this report, 101 patients (52.3%) have died. The estimated median OS from time of diagnosis was 2.2 years (EMD) and 6.3 (PO) years (p=0.001). For both PO and EMD, outcomes DFS and OS were better when detected at diagnosis vs relapse (p=0.000). In addition, PO disease had a better outcome(OS) compared to EMD at diagnosis (p=0.02) and relapse (p=0.032). We could not find an association between ISS.
Summary/Conclusion: EMD is an uncommon, but by no means rare, manifestation of MM. This cohort of 195 patients represents a large group of patients with EMD demonstrating CR as a reachable but not sustainable target for both PO and EMD. EMD at relapse, but not at diagnosis, is the worst group with the poorest response and survival.
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PS1331

AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN AL AMYLOIDOSIS: SURVIVAL IMPROVEMENT IN DIFFERENT RISK CATEGORIES IN A SINGLE CENTER EXPERIENCE

A. Belotti[1,*], R. Ribolla[2], C. Crippa[2], V. Orlando[2], M. D'Adda[3], A. Re[2], C. Cattaneo[2], G. Rossi[2]

[1]Hematology Division, Spedali Civili di Brescia, [2]Hematology Division, Brescia, Italy, [3]Hematology Division, Hematology Division, Brescia, Italy

Background: Treatment of AL amyloidosis (ALA) is based on anti-myeloma therapy but there is no standard and the outcome of high risk patients still remains poor: autologous stem cell transplantation (ASCT) is recommended for patients with low organ damage, whereas in intermediate and high risk patients concerns about treatment-related toxicity limit this approach. Moreover, ALA may occur in association with symptomatic (active) multiple myeloma and the concomitant high risk ALA features that can be observed require caution when transplant stategy is considered.
Aims: Aims: we analysed the outcome of patients with ALA treated at our center according to their disease risk category. Furthermore, we analysed the outcome according to the use of ASCT as part of first line treatment strategy.
Methods: Of 66 patients newly diagnosed between Jan 1999 to december 2017 (median age 63 years), 24 (36%) underwent first line ASCT, upfront (6) or following induction chemotherapy (18). The 42 patients (63%) who didn't receive ASCT (control group) were treated with CyBorD regimen (24), melphalan-dexamethasone (9), VMDex (6), VD (1) or lenalidomide-dexamethasone (2). Risk groups were identified as follows: cTnI >0.1ng/ml and/or ECOG PS ≥3: high risk; age ≤65 years with normal cTnI levels, ECOG PS <3 and eGFR > 50ml/min: low risk. Intermediate risk patients were defined if not meeting criteria for high or low risk. Haematological and organ response were analyzed; Log-rank (Mantel-Cox) test applied to the Kaplan-Meyer method was used to estimate overall survival (OS) and event free survival (EFS: time to 2nd line therapy or death). Multivariate analysis was performed using Cox proportional hazard models to compare the influence of risk categories and treatment strategy on outcome.
Results: median follow up was 2.7 years. Patient characteristics are shown in Table 1. Overall response rate (ORR) and complete remission (CR) were not significantly different between the two groups: 82% and 45% in the ASCT group and 74% and 26% in the control group, respectively. Organ response (OR) rate was significantly higher in the ASCT group (82% vs 38%, p 0.0009). Toxicity was manageable in both groups, with day 100 post-ASCT mortality rate of 4.5%. Superior EFS was observed in low risk patients compared to high risk patients (median 8.9 vs 2.58 years, p 0.01; HR 0.36, 95% CI 0.17-0.79), whereas no difference was observed between low and intermediate risk patients. Superior OS was observed as well in low risk patients compared to high risk patients (median not reached vs 2.72 years, p 0.04; HR 0.37, 95% CI 0.13-0.99), with no difference if com-

pared with intermediate risk patients. Patients receiving ASCT as part of first line treatment strategy significantly improved both EFS (median 8.0 vs 2.2 years, p 0.001; HR 0.49, 95% CI 0.26-0.89) and OS (median not reached vs 2.73 years, p 0.0007; HR 0.23, 95% CI 0.10-0.52), compared to patients in the control group. Considering intermediate/high risk categories and transplant strategy in a multivariate analysis, ASCT independently affected EFS (p 0.04; HR 1.97, 95% IC 1.03-3.77) and OS (p 0.004, HR 6.19, 95% CI 1.81-21.73).

Table 1. Patients characteristics.

	Overall (N: 66) N, %	ASCT group (N: 24, 36%)	Control Group (N: 42, 63%)	P value	Concomitant Multiple Myeloma (N, %)
Median age (range)	63 (43-86)	58 (43-72)	66 (47-86)	0.001	
Risk category					
Low	14 (23%)	9 (36%)	7 (17%)	NS	11 (16%)
Intermediate	22 (33%)	6 (28%)	14 (33%)	NS	13 (20%)
high	29 (44%)	9 (36%)	21 (50%)	NS	19 (29%)
Concomitant Multiple Myeloma	43 (65%)	18 (75%)	25 (60%)	NS	

Summary/Conclusion: Conclusion: with the limit of a small case series we provide evidence of a significant OR and survival benefit with the inclusion of ASCT in first line treatment strategy, even in the intermediate and high risk group, thus reflecting the importance of an accurate re-assessment of the patient for ASCT eligibility after induction therapy and achievement of haematological response

PS1332

COMPARABLE OUTCOMES USING PROPYLENE GLYCOL-FREE MELPHALAN FOR AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA

K. Miller[1,*], H. Sidiqi[1], M. Gertz[1], F. Buadi[1], S. Hayman[1], M. Lacy[1], A. Dispenzieri[1], D. Dingli[1], P. Kapoor[1], W. Gonsalves[1], T. Kourelis[1], W. Hogan[1], S. Kumar[1]

[1]Division of Hematology, Mayo Clinic, Rochester, United States

Background: A propylene glycol-free version of melphalan (PG-free MEL; Evomela™) was recently approved by the United States Food and Drug Administration as a conditioning regimen for autologous stem cell transplantation (ASCT) in patients with multiple myeloma. PG-free MEL incorporates a modified cyclodextrin, making it more stable and soluble than propylene glycol-solubilized melphalan (PG-solubilized MEL; Alkeran™) (Koltun *et al.*, 2010). As such, there is speculation that PG-free MEL may be less toxic than its PG-solubilized counterpart.
For example, it was noted that patients treated with PG-free MEL had a relatively low incidence of severe oral mucositis in the study which lead to its approval (Hari *et al.*, 2015). However, this was a single-arm, Phase IIb trial; hence, it is unclear if ASCT outcomes differ significantly between patients treated with either PG-free or PG-solubilized MEL in clinical practice.
Aims: In pursuit of this, we compared the clinical course of multiple myeloma patients who underwent ASCT before and after an institutional switch to PG-free MEL.
Methods: After institutional review board approval, we conducted a retrospective chart review of 416 patients who underwent ASCT for the treatment of multiple myeloma at Mayo Clinic Rochester from October 15, 2015 to October 27, 2017. The switch from PG-solubilized MEL (Alkeran™; GlaxoSmithKline, Research Triangle Park, NC, USA) to PG-free MEL (Evomela™; Spectrum Pharmaceuticals, Inc., Irvine, CA, USA) occurred on October 10, 2016. Thus, 200 patients in our study received PG-solubilized MEL; 216 received PG-free MEL. The Mann-Whitney *U* test and Fisher's Exact test were used to compared continuous and categorical variables, respectively. Oral mucositis was graded according to the World Health Organization (WHO) criteria. The presence of oral ulcers, or severe pain resulting in a significant limitation in oral intake was graded as ≥2. Moreover, patients with severe esophagitis that significantly limited oral intake were graded as ≥2. Statistical analysis was completed using JMP 13 (SAS Institute Inc., Cary, NC, USA).
Results: Patients conditioned with PG-free and PG-solubilized MEL were balanced in terms of age, sex, time to ASCT, serum creatinine, disease status, and melphalan dose (Table 1a).
After ASCT, there were no significant differences between patients treated with either PG-free or PG-solubilized MEL in terms of hospitalization, neutropenic fever, WHO Grade ≥2 oral mucositis/esophagitis, narcotic usage, IV fluid utilization, IV granisetron requirement, transfusions, and time to engraftment (Table 1b). Moreover, there were no significant differences in Day +100 hematologic responses between the two groups.

Table 1.

Baseline characteristics and ASCT outcomes for patients conditioned with PG-free MEL (Evomela™) and PG-solubilized MEL (Alkeran™).

1a. Baseline Characteristics	PG-free MEL (Evomela™) n=216	PG-solubilized MEL (Alkeran™) n=200	*P*-value
Age at ASCT (years), median (range)	62 (31-75)	61 (29-77)	0.76
Male, n (%)	132 (61%)	116 (58%)	0.55
Months to ASCT from diagnosis, median (IQR)	6 (5-10)	6 (5-9)	0.92
Serum Creatinine prior to ASCT (mg/dL), median (IQR)	1 (0.8-1.2)	1 (0.8-1.2)	0.51
Disease status prior to ASCT, n (%)			0.31
Stable or Progressive Disease	21 (10%)	26 (13%)	
PR	72 (33%)	77 (39%)	
VGPR	83 (38%)	61 (31%)	
CR	17 (8%)	11 (6%)	
sCR	23 (11%)	25 (13%)	
Melphalan dose, n (%)			0.10
200 mg/m^2	161 (75%)	164 (82%)	
140 mg/m^2	27 (13%)	22 (11%)	
Other (e.g. w/ carfilzomib or bortezomib, BEAM)	28 (13%)	14 (7%)	
1b. ASCT Outcomes	**PG-free MEL (Evomela™)**	**PG-solubilized MEL (Alkeran™)**	***P*-value**
Days to ASCT dismissal visit (from Day 0), median (IQR)	20 (18-21)	20 (18-22)	0.79
Hospitalization during ASCT, n (%)	92 (43%)	78 (39%)	0.49
Number of nights in hospital, median (IQR)	6 (3-10)	6 (3-9)	0.67
Neutropenic Fever, n (%)	133 (62%)	126 (63%)	0.84
w/ Bacteremia	38 (29%)	37 (29%)	0.80
Oral/Esophageal Mucositis (WHO Grade ≥2), n (%)	52 (24%)	40 (20%)	0.35
Narcotic requirement during ASCT, n (%)	153 (71%)	144 (72%)	0.30
Oral narcotics (tramadol, oxycodone, etc.)	108 (50%)	90 (45%)	
Fentanyl (patch)	22 (10%)	33 (17%)	
Fentanyl, Dilaudid, or Morphine IV	23 (11%)	21 (11%)	
IV Fluid requirement (liters), median (IQR)	7 (3-12)	8 (3-11)	0.45
Granisetron IV requirement (# of days on therapy after Day 0), median (IQR)	10 (3-13)	9 (1-13)	0.48
Granisetron IV cumulative dose (mg), median (IQR)	12.5 (3-20)	11 (1-18)	0.36
RBC transfusion requirement (# of units), median (IQR)	0 (0-1)	0 (0-1)	0.79
Time to ANC engraftment (>0.5 x 10^9/L) (days), median (IQR)	16 (14-17)	15 (14-17)	0.74
Platelet transfusion requirement (# of units), median (IQR)	1 (1-2)	1 (1-2)	0.45
Time to platelet engraftment (>20 x 10^9/L) (days), median (IQR)	16 (16-17)	16 (15-18)	0.77
Hematologic response at Day +100, n (%)			0.73
Stable or Progressive Disease	4 (2)	2 (1)	
PR	58 (27)	65 (33)	
VGPR	72 (33)	62 (31)	
CR	36 (17)	33 (17)	
sCR	46 (21)	38 (19)	
Day +100 All-Cause Mortality, n (%)	1 (1)	1 (1)	1.0

Summary/Conclusion: ASCT outcomes for patients conditioned with either PG-free or PG-solubilized MEL were comparable, including for the incidence of WHO Grade ≥2 oral mucositis/esophagitis (24% *vs.* 20%, respectively, p=0.35). Further studies should investigate if using PG-free MEL alters other ASCT outcomes or impacts survival in multiple myeloma.

PS1333

ACTIVITY AND SAFETY OF DARATUMUMAB MONOTHERAY IN PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA REQUIRING DIALYSIS: PRELIMINARY RESULTS OF A SPANISH, RETROSPECTIVE, MULTICENTER TRIAL

M.-J. Cejalvo[1,*], M. Legarda[1], E. Abella[2], E. Cabezudo[3], C. Encinas[4], A. García-Feria [5], B. Íñigo[6], J. Martín [7], P. Ribas[1], M.-Á. Ruíz[8], Y. González[9], J. Cornago[10], Á. Ramírez[11], P. Fernández[12], L. Rubio[1], J. de la Rubia [1]

[1]Hospital Dr. Peset, Valencia, [2]Hospital Del Mar, [3]Hospital De Manresa, Barcelona, [4]Hospital Gregorio Marañón, Madrid, [5]Hospital De Manises, Valencia, [6]Hospital Clínico San Carlos, Madrid, [7]Hospital Virgen Del Rocío, Sevilla, [8]Hospital Francesc De Borja, Gandía, [9]Hospital Dr. Josep Trueta, Gerona, [10]Hospital La Princesa, Madrid, [11]Hospital Central De Oviedo, Asturias, [12]Hospital General De Alicante, Alicante, Spain

Background: Daratumumab (DARA) has been approved for patients with relapsed and refractory myeloma multiple (RRMM) that have received ≥3 prior lines of therapy including proteasome inhibitor (PI) and an immunomodulatory (IMID) agent. It is not necessary to adjust the dosage of DARA in patients with creatinine clearance 30-60 mL/min, there are no published data available in patients requiring dialysis.
Aims: The objective of this retrospective, multicenter, open-label study is to report the safety and efficacy of DARA in patients with RRMM with end-

stage renal failure requiring dialysis.
Methods: This study included patients ≥18 years, documented progression of MM following the most recent therapy including a PI and an IMiD, ECOG 0-2, absence of chronic obstructive pulmonary disease, and no prior exposure to anti-CD38. Patients received DARA at the standard dose (16 mg/kg/IV/weekly/8 weeks, then every 2 weeks/16 weeks, and then every 4 weeks) until disease progression or unacceptable toxicity. Premedications with antihistamines, montelukast, antipyretics and corticosteroids and post-infusion medications with corticosteroids were administered. Data on efficacy and adverse events (AEs) including grade 3-4 AEs and infusion related reactions (IRRs) were collected.
Results: The study, including 12 Spanish centers is ongoing and we report here the preliminary results of the first 12 patients included from January 2017 to February, 2018. There were 8 male and 4 female with a median age of 62 years (51-71) and with a median time from diagnosis to initiation of DARA of 2.24 years (0.47 – 13.78). MM was BJ in 6 patients, IgG type in 3, IgA type in 2, and IgD in 1 patient. According to the ISS, 2 patients were in stage I, 1 patient was in stage II, and the remaining 9 patients were in stage III. The median number of prior lines was 3 (2 -6); 58% of patients received ≥3 prior lines. All patients were double refractory to both a PI and IMiD, 3 patients were also refractory to carfilzomib. Four patients had received previous stem-cell transplant (3 autologous and 1 allogeneic). Five out of the 12 patients had high-risk cytogenetics (Table 1). In 11 patients, end stage renal failure was myeloma-related (4 patients with acute renal injury at time of diagnosis), while the remaining patient was already on dialysis at time of diagnosis MM. The median time on dialysis at study entry was 2 months (range, 0.36 - 91). The median (range) number of cycles of DARA administered was 6 (1-15). The ORR and the clinical benefit rate (SD or better) was 50% (2 VGPR, 2 PR, and 2 SD). With a median follow-up of 12 months (range 7 - 15), no patient has become dialysis independent, 6 patients have died, 4 patients due to disease progression, 1 patient due to toxicity, and 1 patient due to non-myeloma related causes (bowel perforation). The remaining 6 patients are still alive with ongoing DARA treatment. No patient discontinued treatment due to AEs. Grade 1-2 IRRs were only during the first cycle (5 patients). Anemia (n=4, 50% grade ≤2), thrombocytopenia (n=4, 25% grade ≤2), and neutropenia (n=3, 33% grade ≤2).

Table 1. Baseline characteristics.

Age, years	
Median (range)	62 (51-71)
Sex	
Male/female	8/4
ECOG	
0/1/2	1/4/4
Type of Myeloma	
Bence Jones/IgG/IgA/IgD	6/3/2/1
ISS	
I/II/III	2/1/9
ISS-R	
I/II/III	2/3/4
No. prior lines, median (range)	3 (2-6)
≥3 prior lines, N (%)	7 (58)
Refractory to, N	
Bortezomib	12
Carfilzomib	3
Lenalidomide	12
Pomalidomide	2
Thalidomide	6
Alkylating agent only	6
ASCT	3
Allogenic-SCT	1
Cytogenetic profile, N	
t(11;14)	3
del17p	1
t(14;16)	1
add(1q)	3
Median time from diagnosis to initiation of DARA, years (range)	2.24 (0.47-13.78)
Median time on dialysis at beginning DARA, months (range)	2 (0.36-91)
No. of cycles of DARA, median (range)	6 (1-15)
Response	
ORR (≥PR)	6
VGPR	2
PR	2
SD	2
PD	4
NA	2
AEs, N, (alls, grade ≤2)	
Anemia	4 (2)
Thrombocytopenia	4 (1)
Neutropenia	3 (1)
IRRs, N, (alls, grade ≤2)	5 (5)

ECOG: Eastern Cooperative Oncology Group; ASCT: autologous stem cell transplantation; DARA: daratumumab; AEs: Adverse Events; ORR: overall response rate; CR: complete response; VGPR: very good partial response; PR: partial response; SD: stable disease; PD: progressive disease; NA: not evaluable; IRRs: infusion-related reactions; DARA: daratumumab.

Summary/Conclusion: Although still preliminary, our data suggest that DARA monotherapy is an efficacious and safe therapeutic option for patients with RRMM and end-stage renal failure including dialysis. AE profile seems to be similar to that previously observed in patients with normal or moderately impaired renal function.

PS1334

OPTIMISING TREATMENT FOR HIGH-RISK MYELOMA IN A STRATIFIED MULTI-CENTRE TRIAL: EXPERIENCE FROM MUKNINE OPTIMUM STUDY

V. Shah[1,2], S. Hinsley[3], A. Sherborne[1], S. Ellis[1], A. Price[1], J. Kendall[1], D. Johnson[1], R. Owen[4], M. Drayson[5], L. Flanagan[3], D. Sherratt[3], W. Gregory[3], G. Jackson[6], G. Pratt[7], G. Cook[8], S. Brown[3], M. Jenner[9], M. Kaiser[1,2,*]

[1]The Institute of Cancer Research, [2]The Royal Marsden Hospital, London, [3]Leeds Institute of Clinical Trials Research, Leeds University, [4]St James University Hospital, Leeds, [5]University of Birmingham, Birmingham, [6]Newcastle University, Newcastle, [7]University Hospitals Birmingham NHS Foundation Trust, Birmingham, [8]Leeds Institute of Cancer and Pathology, University of Leeds, Leeds, Leeds, [9]University Hospital Southampton, Southampton, United Kingdom

Background: Outcomes for newly diagnosed myeloma (NDMM) patients with high risk molecular tumor features remain unsatisfactory.
Aims: Insights from trials in relapsed myeloma and tumor evolutionary studies suggest that multi-targeted combination therapies could improve outcomes through eradication of rapidly evolving tumor sub-clones. We report here an update on one of the first multi-centre clinical trials for high risk NDMM, Myeloma UK (MUK) nine: OPTIMUM (NCT03188172).
Methods: MUKnine OPTIMUM investigates the five-drug combination Daratumumab, Cyclophosphamide, Bortezomib, Lenalidomide, Dexamethasone (DCVRd) followed by high dose melphalan + ASCT augmented by Bortezomib, intensified consolidation (DVR) and maintenance (DR) in high risk NDMM. Challenges inherent to high risk sub-group trials were addressed in line with recommendations for trials in rare cancers (Bogaerts, Eur J Canc 2015). Outcomes for DCVRd are compared using a Bayesian strategy against molecularly matched near-concurrent high risk NDMM treated on the Myeloma XI trial. Precise molecular definition of high risk is key and based on evidence from independent Myeloma IX and Myeloma XI trials demonstrating the specific association of presence of 2 or more ('double-hit') high-risk genetic lesions (Adverse translocations; del(1p32); gain(1q21); del(17p)) AND/OR a SKY92 High Risk GEP Profile with adverse PFS and OS. Molecular risk screening is performed in a central laboratory using a combination of molecular genetic (MRC Holland) and gene expression (SkylineDx) assays. Primary endpoint of MUKnine is a 25% improvement in PFS over to the molecularly matched comparator high risk group from Myeloma XI. Central longitudinal flow-based MRD (10^{-5}) is assessed for early surrogate PFS endpoint assessment and monitoring of disease dynamics. Up to 620 newly diagnosed patients will be molecularly screened and up to 105 patients with high risk status will receive OPTIMUM DCVRd therapy. Patient reported outcomes and clinical outcomes for non-high risk patients form part of the analysis.
Results: OPTIMUM opened to recruitment in October 2017 and at the time of abstract submission 41 patients have been registered for central molecular risk profiling. For 37 patients screening results were successfully generated, 4 samples failed due insufficient bone marrow myeloma cell content. Turnaround time for central screening results was on average 17 days (range 6-33 days), shorter than the protocol-defined maximum of 56 days. If clinically indicated, patients received up to two cycles of local standard induction therapy, e.g. VTD, whilst central screening was performed. Tumour cells from 18 patients were found to carry molecular high risk features as defined above, 7 were high risk by 'double-hit' genetics and SKY92, 3 by 'double-hit' only and 8 by SKY92 only. This overlapping but non-identical identification of high risk by genetic and GEP profiling prospectively confirms findings from MRC Myeloma IX and NCRI Myeloma XI and supports combined molecular testing, in particular for molecularly stratified trials. At the time of abstract submission, ten high risk patients had been registered for DCVRd therapy. Updated screening and recruitment results will be presented at the meeting.
Summary/Conclusion: We provide a first update on central molecular screening feasibility for stratification in the multi-centre UKMRA MUKnine OPTIMUM trial investigating five-drug combination therapy in high risk NDMM.

PS1335

ASYMPTOMATIC HEART INVOLVEMENT IN AL AMYLOIDOSIS

M. Basset[1,*], P. Milani[1], A. Foli[1], R. Mussinelli[1], P. Stefano[2], G. Palladini[1], G. Merlini[1]

[1]Department of Molecular Medicine, University of Pavia, Pavia, Italy, [2]Department of Internal Medicine, University of Pavia, Pavia, Italy, Amyloidosis Research and Treatment Center, Foundation IRCCS Policlinico San Matteo, Pavia, Italy, Pavia, Italy

Background: Late recognition of advanced cardiac dysfunction is still responsible for a high proportion early deaths in AL amyloidosis. However, NT-proBNP allows early recognition of cardiac amyloidosis before it manifests clinically.
Aims: In the present study we describe the clinical presentation and outcome of patients with asymptomatic cardiac AL amyloidosis.
Methods: The prospectively maintained database of the Amyloidosis Research and Treatment Center was searched for patients with cardiac amyloidosis, defined as mean left ventricular wall (mLVW) thickness >12 mm or NT-proBNP >332 ng/L in the absence of other causes, who did not present symptoms or signs of heart involvement. Baseline variables were tested by Cox univariate and multivariate analysis and best cutoffs were identified by ROC analysis.
Results: A total of 155 consecutive patients diagnosed between 2004 and 2015 were included in the study. Fifteen subjects had isolated cardiac involvement and were diagnosed because elevated NT-proBNP was detected during screening for MGUS. In 140 patients (90%) AL amyloidosis was suspected because of clinical manifestation of involvement of organs other than the heart, namely kidney in 78% of subjects, liver in 15%, soft tissues in 14%, and peripheral and autonomic nervous system in 11% and 7% of patients, respectively. Median age was 65 years and 57% of patients were males. Cardiac stage was II in all patients, representing 33% of the stage II patients diagnosed at our center in the study timeframe. Median mLVW thickness was 12.6 mm (range 9-17 mm) and median ejection fraction was 60 (range 52-71%). Median NT-proBNP was 1102 ng/L (range 349-6770 ng/L). Renal stage was I in 34% of subjects, II in 38%, and III in 28%. Upfront therapy was cyclophosphamide/bortezomib(B)/dexamethasone (CyBorD) in 33%, melphalan/dexamethasone (MDex) in 29% of patients, BMDex in 11%, B/dexamethasone in 6%, treatment for IgM-AL amyloidosis in 6%, immune modulatory drugs-based in 5%, autologous stem cell transplant in 2%, other in 9%. Overall hematologic response rate was 65% [complete response (CR) 15%, very good partial response (VGPR) 39%], and cardiac and renal responses were achieved in 28% and 29% of patients with measurable organ involvement, respectively. After a median follow-up of living patients of 36 months, median survival was 57 months, which was significantly longer than that (15 months) of 639 patients with symptomatic heart involvement diagnosed in the same timeframe (p<0.001). The baseline variables associated with survival were age, NT-proBNP, bone marrow plasma cell infiltrate (BMPC), and difference between involved and uninvolved free light chain (dFLC). The multivariate model best predicting survival included age >75 years [hazard ratio (HR) 3.38, p=0.001], NT-proBNP >850 ng/L (HR 2.29, p=0.011), and BMPC >10% (HR 2.15, p=0.026). Only 7 patients (4%) presented with all the 3 risk factors, and they all died a cardiac death after a median of 8 months. The median survival of patients with 0, 1, and 2 risk factors was not reached, 56, and 45 months, respectively.
Summary/Conclusion: Patients with AL amyloidosis diagnosed at an asymptomatic stage have a significantly better outcome than those who present with heart failure. However, advanced age, relatively high NT-proBNP concentration and plasma cell burden identify a minority of subjects who are still at high risk of early death.

PS1336

RANDOMIZED CLINICAL TRIAL (RCT) REPRESENTATIVENESS & OUTCOMES IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) REAL WORLD (RW) PATIENTS: COMPARISON OF ASPIRE, TOURMALINE-MM1, POLLUX, & ELOQUENT RCTS

A. Chari[1,*], D. Romanus[2], E. Farrelly[3], H. Huang[2], M. Blazer[3], A. Raju[3], S. Noga[2], P. Richardson[4]

[1]Icahn School of Medicine at Mount Sinai, New York, [2]Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, [3]Xcenda, LLC, Palm Harbor, [4]Dana Farber Cancer Institute, Boston, United States

Background: Only 3% of patients (pts) participate in oncology trials in the United States (US).[1] Strict eligibility criteria and high enrolment ex-US limit the generalizability of trial results and applicability of the benefit/risk treatment profile to RW pts.[2]
Aims: To estimate the proportion of US RW pts who are representative of eligibility criteria in recently completed trials of triplet lenalidomide/dexamethasone (Rd)-based regimens in RRMM and to compare the baseline characteristics and overall survival (OS) between RCT-eligible and RCT-ineligible pts within each trial group.
Methods: RRMM pts were followed retrospectively in a large US electronic health record (EHR) database.[3] Included were pts initiating treatments consistent with either the experimental or control regimen in 2nd-4th line of therapy (LOT) based on 4 RCTs: 2 proteasome inhibitor-based (PI) (ASPIRE; TOURMALINE-MM1) and 2 monoclonal antibody-based (mAb) triplet regimens with an Rd backbone (POLLUX; ELOQUENT). RW pts were categorized into "RCT-**elig**" vs "RCT-**inelig**" based on whether eligibility criteria for each of the 4 trials in RRMM were met vs not. Unadjusted Cox PH models were performed to estimate OS from start of the index regimen to death between RCT-**elig** vs RCT-**inelig** pts in each trial group. Observations were censored at time of loss to follow up/end of study period (6/2017).
Results: Applying the individual trial criteria, 25% (ASPIRE), 35% (TOURMALINE), 41% (ELOQUENT), and 47% (POLLUX) US RW pts met the eligibility criteria. In addition to exclusion criteria related to prior treatment history of/refractory status to an immunomodulatory drug (21% [ELOQUENT], 8% [other trials] of RCT-**inelig** pts), the most common reasons for ineligibility across trial groups were: low creatinine clearance (27% [ASPIRE]; 9% [other trials]) and other cancer diagnoses (12% [all trials]). Refractory status to a PI as an exclusion criterion in the PI-based trials applied to 54% of ASPIRE & TOURMALINE RCT-**inelig** pts. Less than 1% of pts were RCT-**inelig** using the POLLUX & ELOQUENT mAb-based trial criterion of prior exposure to a mAb. Clinically relevant recent cardiovascular comorbidities accounted for 12% (ASPIRE), 9.2% (TOURMALINE), 9.0% (ELOQUENT), and 5.9% (POLLUX) RCT-**inelig** pts. RW pts who were RCT-**elig** were generally significantly younger, treated in earlier LOT, more likely to have a history of stem-cell transplant, had a lower comorbidity burden. A lower proportion also had known ISS III disease vs RCT-**inelig** pts (Table). Except in the ELOQUENT group, the 3-year OS was significantly higher among RCT-**elig** v RCT-**inelig** pts but the difference in OS varied across trial groups: 77% v 57% (ASPIRE, p<0.001), 75% v 60% (TOURMALINE, p<0.001), 70% v 63% (POLLUX, p<0.01) and 67% v 65% (ELOQUENT, p=0.16) (Table 1).

Table 1.

Variable	ASPIRE N=822			TOURMALINE MM1 N=656			POLLUX N=813			ELOQUENT N=823		
	RCT Eligible N=232 (29%)	RCT Ineligible N= 590 (71%)	P-value	RCT Eligible N=298 (45%)	RCT Ineligible N=354 (55%)	P-value	RCT Eligible N=382 (47%)	RCT Ineligible N=431 (53%)	P-value	RCT Eligible N=334 (41%)	RCT Ineligible N=489 (59%)	P-value
Age, median (IQR)	65 (57, 73)	70 (61, 79)	<0.001	68 (60, 75)	71 (62, 79)	0.004	68 (61, 76)	71 (62, 79)	0.019	68 (61, 77)	70 (62, 78)	0.100
Age Group, %												
18-64 yrs	47	32	<0.001	38	31	0.003	38	30	0.008	38	31	0.060
65-74 yrs	36	30		34	31		33	31		32	31	
≥75 yrs	17	38		27	39		30	39		30	37	
CCI Score, %												
0	54	28	<0.001	49	27	<0.001	45	27	<0.001	46	27	<0.001
1	15	10		15	9		13	9		12	10	
≥2	31	63		35	64		41	64		41	63	
ISS Stage II, %	1	5	0.007*	2	5	0.034*	2	4	0.052*	2	4	0.137*
High risk (cytogenetics), %	15	15	0.912*	13	14	0.618*	13	13	0.855*	14	13	0.712*
CrCl (ml/min), median	83.6	63.1	<0.001	73.8	67.5	0.031	74.0	63.8	0.003	73.0	68.2	0.148
Hgb (g/dl), median	12.4	11.3	<0.001	12.1	11.4	<0.001	12.0	11.4	<0.001	11.9	11.6	0.050
Plts (K/L), median	187	175	0.026	184	176	0.150	187	170	0.003	184	172	0.039
LOT prior to index LOT, %	38	21	<0.001	31	21	<0.001	28	23	0.085	28	24	0.208
Time from dx to index LOT (mon), median	20.2	19.4	0.531	22.2	20.2	0.250	20.0	20.6	0.549	19.5	21.4	0.212
TPI (mon), median	7.3	1.6	<0.001	8.0	2.1	<0.001	6.1	4.1	0.023	5.6	4.6	0.307
Index LOT, %												
2LT	91	70	<0.001	89	69	<0.001	82	71	<0.001	81	73	0.007
3LT	9	22		10	23		15	21		15	20	
4LT	1	7		1	8		3	8		3	7	
PI refractory *, %	0	54	<0.001	0	54	<0.001	31	40	0.01	33	38	0.29
OVERALL SURVIVAL												
3-yr OS rate, %	77	57	<0.001	75	60	<0.001	70	63	0.004	67	65	0.158
HR, overall mortality risk, RCT-eligible vs RCT-ineligible (95% CI)	0.45 (0.32, 0.63)		<0.001	0.50 (0.36, 0.69)		<0.001	0.66 (0.49, 0.87)		0.004	0.82 (0.61, 1.08)		0.158

*Stage I&II vs III; #High Risk vs Standard Risk/Unknown; §High-risk cytogenetics was defined as presence of del(17p), t(4;14), t(14;16), and/or 1q21 gain. ¥ Baseline levels are relative to 6 months prior to initiation of index LOT, with the level closest to index LOT initiation, used; *Patients were defined as refractory if any of two definitions were met: 1. Duration of therapy of current regimen <60 days AND none of the current drugs in next regimen 2. Time to next regimen <60 days AND none of the current drugs in next regimen[3]

Key: 2LT – second-line therapy; 3LT – third-line therapy; 4LT – fourth-line therapy; CCI – Charlson Comorbidity Index; CI – confidence interval; CrCl – creatinine clearance; dx – diagnosis; Hgb – hemoglobin; IQR – interquartile range; ISS – International Staging System; LOT – line of therapy; mon – months; TPI – treatment-free interval (defined as time from end of previous LOT to index LOT); yrs – years

REFERENCES: 1) Institute of Medicine (US). https://www.ncbi.nlm.nih.gov/books/NBK83089/; 2) Kim ES et al. JCO Oct 2, 2017 (online). 3. Optum's de-identified EHR dataset (2007-2017) 4. Usmani S et al. Oncologist 2016; 21: 1-7

Summary/Conclusion: 53-75% of RW US pts do not meet eligibility criteria of recently completed trials of novel-triplet regimens in RRMM in large part due to refractoriness to lenalidomide and also PIs, for the PI containing studies. Importantly, OS between RCT-**elig** vs RCT-**inelig** pts varied based on trial eligibility characteristics. A limitation of the data is that treatment refractory disease was defined using a proxy[4] and may overestimate the true proportion of ineligible pts. Future work will evaluate the impact of individual eligibility criteria on OS.

PS1337

PHARMACOKINETIC EVALUATION OF TEST DOSE PREDICTIONS OF HIGH DOSE MELPHALAN EXPOSURE IN PATIENTS WITH MULTIPLE MYELOMA

J. Trotman[1,2,*], P. Shaw[34], C. Tiley[5,6], S. Ramanathan[7], A. Grigg[8,9], P. Presgrave[10,11], E. Newman[1], J. Estell[1,2], S.-J. Ho[7], S. Larsen[12], C. Nath[3,4]

[1]Concord Repatriation General Hospital, Concord, [2]University of Sydney, Sydney, [3]The Children's Hospital at Westmead, Westmead, [4]University of Sydney, Camperdown, [5]Gosford Hospital, Gosford, [6]University of Newcastle, Newcastle, [7]St George Hospital, Kogarah, [8]Austin Hospital, Heidelberg, [9]Univeristy of Melbourne, Melbourne, [10]Wollongong Hospital, Woollongong, [11]University of Wollongong, Wollongong, [12]Royal Prince Alfred Hospital, Camperdown, Australia

Background: High-dose melphalan (HDM) and autologous transplantation (ASCT) is used to treat multiple myeloma (MM). We have previously shown that the standard dose of 200 mg/m^2 results in a 5 fold variation in melphalan exposure, with area under the concentration versus time curve (AUC) ranging from 5 to 25 mg/L.h in 114 patients (pts). AUC above the median (12.8 mg/L.h) was associated with improved OS (8.5y vs. 5.4 y, HR 0.4, p<0.001).[1]

Aims: The aim of this pilot study was to evaluate the feasibility and accuracy of test dose pharmacokinetic (PK) predictions of subsequent high dose melphalan exposure in patients with MM.

Methods: Thirty-five pts (age range: 44 to 71y, median 60y) were recruited from 6 hospitals located up to 860km from the PK laboratory. A test dose of melphalan (20mg/m^2) was administered 1-3 days prior to the remaining 180 mg/m^2. Melphalan was infused over 30-36 min for the test dose and from 15-45 min for the subsequent dose. Blood was collected at: 5,15,30,40,75 and 150 min after completion of the infusions. Plasma melphalan concentrations were measured by HPLC-UV detection. Test dose AUC was calculated using the trapezoidal rule and used to predict the AUC for the subsequent dose, assuming linear PK. Percentage deviation of actual-from-predicted AUC was calculated as% deviation = (actual AUC – predicted AUC) / predicted AUC*100. Predicted vs actual AUC were compared using the paired Wilcoxin Rank Sign Test. Linear regression was used to test whether age, gender, renal function or body weight were associated with% deviation of actual versus predicted AUC.

Results: All sites promptly delivered all samples to the PK lab. Figure 1 shows predicted and actual melphalan AUC values listed consecutively with the first pt at each institution marked with an asterisk. Median (range) for test dose AUC was 1.32(0.98-2.03) mg/L.h. For the subsequent dose, the median predicted AUC was significantly (p<0.001) higher than the actual AUC; 11.9 (8.8–18.6) mg/L.h versus 11.0(6.3-16.0) mg/L.h. The% deviation of actual from predicted values was median (range) -10.7%, (-44% to 11%), with predictions being within±15% for 24 pts (69%). The median% deviation for the first pt in each centre (n=6) was -22; for subsequent pts (n=29) it was -9.5%, (p=0.2) and of these, 22 (76%) had full dose AUC values within±15%. Age, height ,weight and renal function did not significantly impact on the predictions. Total melphalan exposure was median 12.2 (7.6 – 17.8) mg/L.h; in 18(of 35) pts it was below 12.8 mg/L.h, the value previously associated with inferior OS.[1]

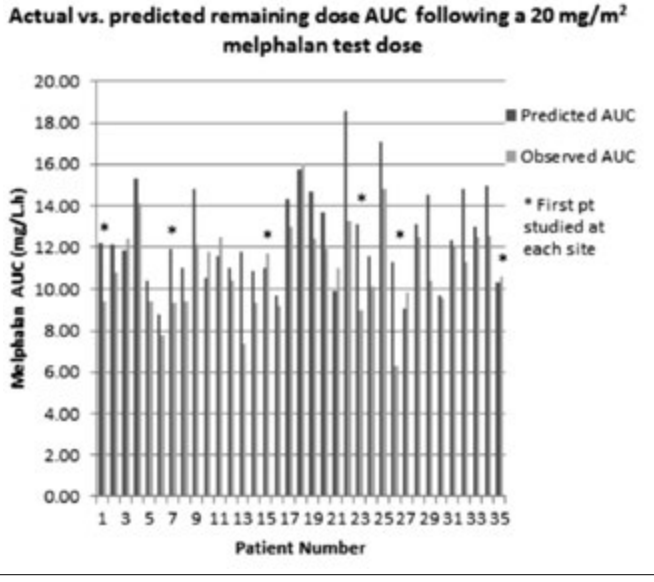

Figure 1.

Summary/Conclusion: Test-dose PK predictions of melphalan exposure were feasible and accurate to within ±15% for 69% of pts. The predictive value increased with experience possibly reflecting improved consistency in blood sampling. Further improvements might be obtained by standardizing HDM preparation and administration times, since melphalan solution is unstable, and there may have been different rates of degradation of the test and subsequent doses. The common over-prediction of the full dose AUC could lead to potential below-target dosing; conversely no patient had an actual AUC more than 11% of predicted. Investigation of infusion stability at different melphalan concentrations is underway. PK directed dosing of HDM to achieve a desirable AUC has the potential to provide more consistent melphalan exposure than the current BSA-based approach but further work to define the desirable AUC and the optimal adjustment strategy based on initial PK is needed.

Reference

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PS1338

A PHASE II STUDY OF SELINEXOR(KPT-330) COMBINED WITH BORTEZOMIB AND DEXAMETHASONE (SVD) FOR INDUCTION AND CONSOLIDATION FOR PATIENTS WITH PROGRESSIVE OR REFRACTORY MULTIPLE MYELOMA: THE SELVEDEX TRIAL

A. Broijl[1,*], E. Asselbergs[1], M. Minnema[2], S. Lonergan[1], B. van der Holt[3], N. van de Donk[4], S. Zweegman[4], P. Sonneveld[1]

[1]Department of Hematology, Erasmus MC Cancer Institute, Rotterdam, [2]Department of Hematology, UMC Utrecht Cancer Center , Utrecht, [3]HOVON Data Center, Erasmus MC Cancer Institute, Rotterdam, [4]Department of Hematology, VU Medical Center, Amsterdam, Netherlands

Background: Selinexor is an oral selective exportin-1 (XPO-1) inhibitor that forces nuclear retention and activation of many tumor suppressor proteins (TSPs) and IkB and is active as monotherapy and combined with dexamethasone in multiple myeloma (MM). Addition of proteasome inhibitors to selinexor increases levels and activity of TSPs/IkB leading to synergistic MM cell death.

Aims: A dose escalating phase I/II study was performed in patients with progressive and/or refractory MM, evaluating feasibility (phase I) and efficacy (phase II) of selinexor at 3 dose levels combined with twice weekly (BIW) bortezomib and dexamethasone (SVd).

Methods: The maximum tolerated dose (MTD) and recommended dose level (RDL) were determined using a 3+3 dose escalation scheme. Patients could be included if they had received at least one prior anti-myeloma regimen, and were not refractory to bortezomib. Selinexor on days 1,8,15,22, bortezomib 1.3 mg/m^2 subcutaneously on days 1,4,8,11, dexamethasone 40 mg on days 1,8,15,22 in a 28-days schedule.

Results: Seven patients were treated at the first selinexor dose level of 45 mg/m^2. The median number of prior treatments was 3 (range 2-7). Three patients were lenalidomide refractory during their last treatment. Two patients were deemed ineligible after inclusion, one patient had a transformation to plasma cell leukemia, one was bortezomib refractory. Two out of 5 eligible patients completed treatment as planned. Only one patient completed selinexor at the preplanned dose of 45 mg/m^2. Reasons for discontinuation were excessive toxicity(n=1), progressive disease(1), other reasons not specified(1). Two patients experienced a DLT, one grade 4 thrombocytopenia with epistaxis, one patient had an ischemic cerebrovascular event. Adverse events (AE) CTC grade 3-4 were observed in all treated patients (Table 1). The study continued with a lower dose level of selinexor of 30 mg/m^2 with adjustment of bortezomib frequency to once weekly on days 4 and 11. The median number of prior treatments was 3 (range 1-7). Three patients were lenalidomide refractory. Two out of 6 patients who started treatment completed treatment as planned. Reasons for discontinuation were neuropathy grade 4(1), pneumonia(1), progressive disease(3). Six patients were treated of whom 4 patients experienced a DLT: hyponatremia grade 3(2), polyneuropathy grade 3(1), syncope grade 3(1).The study was stopped prematurely due to unacceptable toxicity observed in the 2 different dosing schedules of SVd. In the 45mg/m^2 group 4 out of 5 (80%) patients achieved ≥PR, including 2 VGPR, and 1 sCR. Median follow-up (FU) was 27 months (range 0.6-31.5). Median PFS was 17 months, OS at 1 and 2 years was 100% and 75%, respectively. Four out of 6 (67%) patients in the 30 mg/m^2 group achieved ≥PR, including 1 VGPR; no CRs were seen. Median FU was 10.1 months (range 0.6-17.9). Median PFS was 10 months, 1 year OS was 75%.

Table 1. Adverse events CTC grade 3-4.

Adverse events CTC grade 3,4	Selinexor 45 mg/m2 (n=5)		Selinexor 30 mg/m2 (n=6)	
	Grade 3 (n)	Grade 4 (n)	Grade 3 (n)	Grade 4 (n)
Any	2	3	2	4
Infections and infestations				
Pneumonia	1		1	1
Cellulitis			1	
Urosepsis			1	
Nervous system disorders				
Ischemic cerebrovascular event		1		
Polyneuropathy	1			1
Syncope	1		1	
Blood and lymphatic system disorders:				
Leucopenia/neutropenia			2	
Investigations:				
Thrombopenia	2	2	1	2
Hyponatremia	1		3	
Metabolism and nutrition disorders:				
Dehydration	1			
Anorexia	1			
Gastrointestinal disorders:				
Diarrhea	1			
General disorders and administration site conditions:				
Fatigue	1			

Summary/Conclusion: We found that both 30 and 45mg/m^2 of selinexor in combination with either twice or once weekly bortezomib resulted in unacceptable toxicity. However, in those patients in whom the regimen was feasible we observed remarkable ORR, PFS and OS in this pretreated, predominantly lenalidomide-refractory MM population. This is consistent with results recently presented for once weekly SVd regimens. This regimen, which also showed high ORR and PFS, is currently being evaluated in the international Phase 3 randomized BOSTON study of SVd vs Vd (NCT 03110562).

PS1339

LONG-TERM OUTCOMES OF ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

Q. Bashir[1,*], K. Chamoun[1], F. H. Cheema[2], B. Oran[1], S. Ciurea[1], Y. Nieto[1], C. Hosing[1], U. R. Popat[1], P. Kebriaei[1], S. Ahmed[1], A. Olson[1], G. Al-Atrash[1], R. Mehta[1], R.Z. Orlowski[3], E.E. Manasanch[3], H.C. Lee[3], K. Patel[3], E. Shpall[1], R.E. Champlin[1], M.H. Qazilbash[1]

[1]Stem Cell Transplantation, UTMD Anderson Cancer Center, [2]Cardiovascular Research, Baylor College of Medicine, [3]Lymphoma/Myeloma, UTMD Anderson Cancer Center, Houston, United States

Background: Multiple myeloma (MM) is considered an incurable malignancy, however allogeneic hematopoietic cell transplantation (allo-HCT) can result in long-term survival in select group of patients. Long-term follow-up is needed to establish the realistic benefits of allo-HCT in myeloma patients.
Aims: To characterize the long-term outcomes of allo-HCT in patients with newly diagnosed MM>
Methods: We retrospectively analyzed all consecutive patients with newly diagnosed MM who received allo-HCT at our center between January 1994 and December 2016.
Results: Thirty-seven patients were identified. The median age was 54.6 (range= 32.4 – 68.1) years and 54.1% were male. The international staging system (ISS) stage was I, II, III and unknown in 35%, 24%, 22%, and 19% of patients, respectively. High-risk cytogenetics (IMWG definition) was identified in 19.4% of patients. Seventy-three percent had received a prior autologous HCT. A total of 24.3% received myeloablative conditioning (MAC) while 75.7% received reduced intensity (RIC) or non-myeloablative (NMA) conditioning. The graft source was match unrelated (MUD) in 16% and matched sibling donor (MRD) in 84% of patients. Prior to allo-HCT, 1 patient was in complete remission (CR), 49% were in near CR or in very good partial remission (VGPR), and 49% were in partial remission (PR). Median time from diagnosis to allo-HCT was 8.8 months. Twenty-seven percent of patients received maintenance therapy after allo-HCT. The median time to neutrophil and platelet engraftment was 12 (ANC ≥500/mL) and 13 (platelet count ≥20K/mL) days, respectively. The cumulative incidence (CI) of non-relapse mortality (NRM) was 3% at day-100 and 17% at 1-year. There was no difference in NRM between MAC or NMA conditioning. The stringent CR+CR rate was 56% (20/36) after allo-HCT. Overall response rate (PR or better) was 97%. The incidence of grade I-IV acute graft-versus host disease (GVHD) was 35%, while chronic GVHD was seen in 62%. The median follow-up in surviving patients was 123.8 months (range: 15.6-213.5). The 3, 5, and 10-year actuarial progression-free survival (PFS) were 51%, 39.1%, and 29.3%, respectively (Figure 1A). The 3, 5, and 10-year actuarial overall (OS) were 72.7%, 57.5%, and 47.9%, respectively (Figure 1B). In multivariate Cox proportional-hazards model, high-risk cytogenetics (p=0.009, Hazards Ratio (HR): 22.5, 95% CI: 2.2-236.2) independently predicted PFS whereas graft source from matched sibling donor (p=0.03, HR: 0.14, 95% CI: 0.02 – 0.85) was identified as an independent predictor for OS. At the last follow up, 51% (n=19) of patients were alive in the entire cohort, 35% (n=13) of which survived for longer than 10-years from transplant. Twenty-two percent (n=8) of patients remained alive and in remission for more than 10 years from transplant, the longest ongoing remission being 17.7 years.

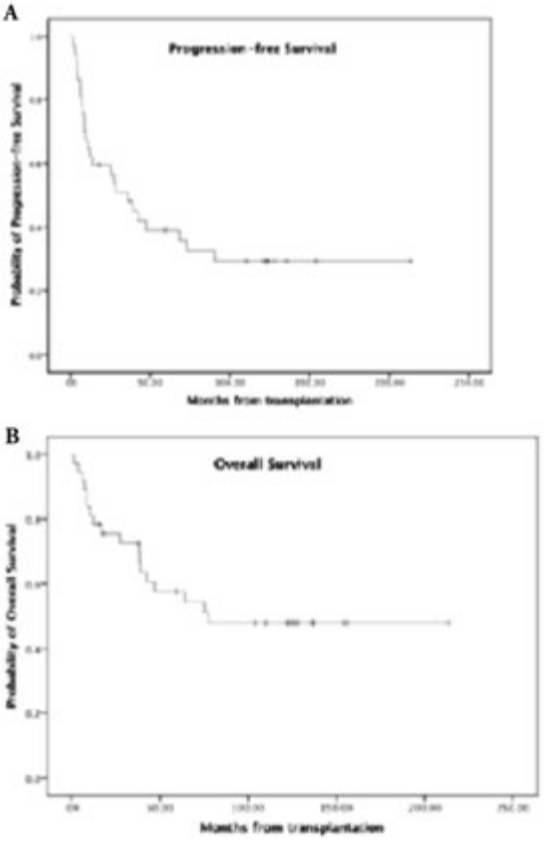


Figure 1.

Summary/Conclusion: Allo-HCT is an effective therapy for patients with MM, which can produce durable long-term remission in a significant number of patients when performed early in disease course.

Myeloproliferative neoplasms – Biology & Translational Research

PS1340

THE SCREENING OF PROTEINS SIGNATURES WITH SIGNALING PATHWAY IN POLYCYTHEMIA VERA AND INHIBITION OF PIM ENHANCES JAK2 INHIBITOR SENSITIVITY

W. Hui[1,*], F. Ye[2], W. Zhang[1], S. Wan[1], L. Su[1]

[1]Xuan Wu Hospital, Capital Medical University, beijing, China, [2]Mount Sinai School of Medicine, new york, United States

Background: The *JAK2-V617F* mutation is observed in nearly all cases of polycythemia vera (PV) and aberrant JAK2 signaling plays an etiological role. However, JAK2 inhibitors are unable to induce molecular complete remission in patients with PV, effective therapies remain needed. Targeting JAK2 downstream pathways is potential as a therapeutic approach.
Aims: This study aim to screen signaling pathway proteins in PV and explore potential therapeutic targeting.
Methods: Protein pathway array was performed to analyze the expression levels of signaling pathway proteins in peripheral blood neutrophils from 35 PV patients with *JAK2V617F* mutation and furthermore to identify the significant pathways affected by these differential expression of the proteins using Ingenuity Pathways Analysis(IPA). We hypothesized that apoptosis signaling may offer a therapeutic target for PV, apoptosis-related proteins were quantified by western blotting in an additional 38 patients with PV. Further studies using the HEL cell line, a MPN model cells which harboring the *JAKV617F* mutation, transfected with *PIM1* siRNA and treated with the JAK2 inhibitor ruxolitinib, signaling pathways and downstream effectors were characterized by Western blotting, quantitative PCR. We utilized PIM inhibitor, SGI-1776, and determined its effect on HEL cells as well as primary PV patient cells alone and in combination with ruxolitinib. The cell survival rates and the apoptosis were detected by CCK-8 and flow cytometry.
Results: There were 20 proteins were found to be dysregulated in PV patients compared with those in healthy controls ($p<0.05$). Among them, 9 proteins were up-regulated, while 11 proteins were down-regulated. These dysregulated proteins were involved in 10 major signaling pathways including apoptosis pathway. Among apoptosis-related proteins, expression of BCL-xL was correlated with the expression of PIM1 and BAD in PV patients. Furthermore, *PIM1* knockout inhibited expression of BCL-xL and BAD. Additionally, inhibition of PIM leads to an increased apoptosis and decreased cell proliferation via arrest at G1 phase in HEL cells. These data suggested PIM/BCL-xL/BAD plays an important role in promoting cell survival of PV. Furthermore, inhibition of JAK2 resulted in decreased *PIM1* mRNA expression. We found that JAK2 inhibition induces apoptosis by regulation of the PIM/ BCL-xL/BAD. PIM inhibitor synergistically enhanced JAK2 inhibitor-induced apoptosis in both HEL cells and primary cells from PV patients. In addition, SGI-1776/ruxolitinib combination therapy synergistically suppressed erythropoietin independent erythroid colony formation of primary PV cells.
Summary/Conclusion: In summary, our study demonstrated a broad dysregulation of signaling proteins and pathways including PIM/ BCL-xL/BAD in PV, and targeting PIMs may increase the efficacy of JAK2 inhibitors therapy.

PS1341

IMPACT OF MOLECULAR ANALYSIS ON PROGNOSTIC SCORES IN ESSENTIAL THROMBOCYTHEMIA: A SINGLE CENTER PROSPECTIVE COHORT EXPERIENCE

D. Luque Paz[1,2,3,*], O. Mansier[4,5,6], J. Riou[7], C. Conejero[8], L. Roy[9,10], C. Belkhodja[9], V. Ugo[11], S. Giraudier[12]

[1]UFR Santé, Université d'Angers, [2]Laboratoire d'hématologie, CHU Angers, [3]CRCINA, INSERM, Angers, [4]Laboratoire d'hématologie, CHU Bordeaux, [5]UFR Sciences de la Vie et de la Santé, [6]INSERM U1034, Université de Bordeaux, Bordeaux, [7]INSERM 1066 MINT, Université d'Angers, Angers, [8]Laboratoire d'Hématologie, [9]Service Clinique d'Hématologie, APHP, Hôpital Henri Mondor, [10]Université Paris 12, Paris, [11]Laboratoire d'Hématologie, CHU d'Angers, Angers, [12]Laboratoire de Biologie Cellulaire, APHP, Hôpital Saint Louis, Paris, France

Background: The prognosis of Essential Thrombocythemia (ET) is related to an increased risk of thrombosis and to a long-term risk of hematological evolution (myelofibrotic and leukemic transformation). Different prognosis scoring systems based on clinical and biological parameters including driver mutations have been proposed in the last few years. Additional mutations have been demonstrated as important prognostic factor in myelofibrosis but little is known in ET.
Aims: We aim to evaluate the prognostic input of additional mutations in ET in terms of survival, thrombosis and hematological transformation.
Methods: The cohort consisted in 190 ET patients diagnosed between January 2000 and December 2016. Diagnosis was based on WHO2008 criteria and the median follow-up was 6.4 years. In addition to driver mutations testing (*JAK2*V617F, *CALR* exon 9 and *MPL*W515) all coding exons of 16 genes (*ASXL1*, *EZH2*, *TP53*, *SF3B1*, *SRSF2*, *TET2*, *IDH1*, *IDH2*, *DNMT3A*, *CBL*, *NRAS*, *JAK2*, *MPL*, *SH2B3*, *IKZF1*, *KRAS*) were sequenced by NGS (TSCA and MiSeq 2x250pb, Illumina). Mutations were called with an in-house pipeline. Only exonic nonsynonymous mutations were retained and putative somatic mutations were obtained by removal of known polymorphisms. Comparisons were performed using Fisher exact test, or Student t-test or Mann-Whitney test, as appropriate. Survival curves were obtained with the Kaplan-Meier method and Cox proportional hazard regression models were performed.
Results: In our cohort, driver mutations were distributed as 116 (61%) *JAK2*V617F, 27 (14%) *CALR,* 4 (2%) *MPL*W515 and 43 (23%) triple negative. At least one additional mutation was found in 44% of the patients in the entire cohort and in 35% of the triple negative ET. No additional mutation was found in 60 *JAK2* mutated cases, 17 *CALR* cases and 28 triple negative cases. The most frequently mutated genes were *TET2* (14%), *DNMT3A* (12%) and *ASXL1* (6%). *DNMT3A, TET2, ASXL1* and *SRSF2* mutations were associated to an older age (respectively p=0.022, p=0.0066, p=0.04 and p=0.0087), while *JAK2* mutations (other than V617F) and *TET2* mutations were associated to lower leukocytes counts (respectively p=0.0014 and p=0.042). During the follow-up 16 deaths, 18 thrombosis and 11 hematological evolutions were observed. In our cohort, among classical prognostic scores (ELN, IPSET-survival and IPSET-thrombosis) the ELN scoring system was the most effective to predict survival. In univariate analysis, the presence of at least one additional mutation (p=0.012) and the number of additional mutation (p=0.019) were associated with an adverse prognosis in term of survival. In multivariate analysis including sex, ELN classification, driver mutations and additional mutation, both ELN (HR:9.6 (High risk/Low risk), p=0.029) and the presence of at least one additional mutation (HR:3.5 (Yes/No), p=0.022) were associated to a risk of death. Hematological transformation was associated with male sex (p=0.0047) and splice mutations in univariate analysis but only sex (HR:5.9 (Male/Female), p=0.029) in multivariate analysis. Only cardiovascular risk was associated with thrombosis events in univariate (p=0.013) and multivariate (HR:3.2 (Yes/No), p=0.016) analysis.
Summary/Conclusion: In our study, screening for additional mutations by NGS in ET improved the prognostic evaluation of ELN classification in term of survival. Larger studies are needed for recommending a systematic molecular screening in ET.

PS1342

THE ROLE OF THE THROMBOPOIETIN RECEPTOR (MPL) IN MYELOPROLIFERATIVE NEOPLASMS

K. Behrens[1,2,*], E.M. Viney[2], T.A. Willson[2], J.J. Babon[1,2], N.A. Nicola[1,2], W. S. Alexander[2,3]

[1]Department of Medical Biology, University of Melbourne, [2]Walter and Eliza Hall Institute, [3]University of Melbourne, Parkville, Australia

Background: Constitutive activation of the Jak/Stat signalling pathway is a hallmark of Myeloproliferative Neoplasms (MPN), with 60-90% of patients carrying the activating JAK2 mutation, $JAK2^{V617F}$. The second most frequently mutated gene is *Calreticulin* (CALR). Mutant CALR binds and activates the thrombopoietin (Tpo) receptor (Mpl), likewise inducing constitutive JAK2 activation. Several studies suggest expression of a type I cytokine receptor is essential for $JAK2^{V617F}$- and $CALR^{mut}$-mediated transformation, but the precise contribution of such receptors is unresolved.
Aims: Here we investigate the functional role of Mpl in $JAK2^{V617F}$ and $CALR^{del52}$ activity.
Methods: Mpl mutants lacking various functional domains were tested for their capacity to support either $JAK2^{V617F}$- or $CALR^{del52}$-mediated factor independence in BaF/3 cells.
Results: Similar to the wildtype (wt) receptor, a Mpl truncation mutant retaining the JAK2-interaction domain, but lacking the C-terminus, typically associated with Stat5 binding, supported $JAK2^{V617F}$-induced transformation. Interestingly, Stat5-phosphorylation was maintained. In contrast, deletion of the entire cytoplasmic receptor domain abrogated Stat5 phosphorylation and factor-independent proliferation. In contrast, in $CALR^{del52}$-expressing

BaF/3 cells, loss of a single Mpl residue, Tyrosine 599, required for Shc binding and MAKP activation, prevented factor-independent proliferation. Furthermore, pharmacological inhibition of MAPK abolished factor-independent growth of Mpl-wt CALR^{del52} cells. In factor-independent Mpl-wt CALR^{del52} cells, Tpo stimulation augmented proliferation. Stat5 phosphorylation was not observed unless Tpo was added.
Summary/Conclusion: While JAK2^{V617F} and CALR^{del52} both require Mpl to induce transformation, the cellular pathways activated downstream of the receptor differ. JAK2^{V617F} transformation is associated with Stat5 activation, although the C-terminus of the receptor, usually linked with Stat5 phosphorylation, appears dispensable. Thus, other mechanisms of Stat5 activation may exist. In contrast, CALR^{del52}-mediated transformation is dependent on Mpl-induced MAPK-signalling and can be augmented by Tpo-mediated Stat5 signalling.

PS1343

BOARDING ON THE SECRETARY PATHWAY IS REQUIRED FOR THE ONCOGENIC PROPERTY OF MUTANT CALRETICULIN

M. Araki[1,*], N. Masubuchi[2], E. Hayashi[2], Y. Yang[2], M. Imai[2], Y. Kihara[2], Y. Mizukami[2], Y. Hironaka[2], Y. Edahiro[2], A. Ohsaka[1], N. Komatsu[2]

[1]Blood Transfusion and Stem Cell Regulation, [2]Hematology, Juntendo University Graduate School of Medicine, Tokyo, Japan

Background: Recurrent somatic mutations on *calreticulin (CALR)* gene that encodes a molecular chaperone have been identified in a subset of patients with myeloproliferative neoplasms (MPNs). Studies have shown that mutant CALR interacts and constitutively activates thrombopoietin receptor MPL, which promotes the development of MPN. However, the underlying molecular mechanism of the interaction between mutant CALR and MPL was poorly understood.
Aims: We aimed to elucidate how mutant CALR interacts with MPL for the oncogenic activation in the cells.
Methods: To understand the regulatory mechanism for the interaction between mutant CALR and MPL, subcellular localization of those proteins were determined by confocal microscopic imaging in a human megakaryocytic cell line UT-7/TPO where mutant CALR exhibited the oncogenic property by activating endogenously expressed MPL. To examine the significance of subcellular localization on its oncogenic capacity, mutant CALR harboring alterations on the signal peptide sequence were expressed and examined for subcellular localization, capacity to interact with MPL, and capacity to induce factor-independent growth. To investigate the glycan-binding capacity of mutant CALR plays a role for the interaction with MPL, mutant CALR harboring mutations on glycan-binding site was expressed and examined for subcellular localization, capacity to interact with MPL, and capacity to induce factor-independent growth.
Results: Although wild-type CALR predominantly localized in endoplasmic reticulum, mutant CALR was accumulated in Golgi apparatus with MPL in UT-7/TPO cells. Deletion of signal peptide sequence from mutant CALR resulted in a loss of localization to Golgi apparatus. The mislocalized mutant CALR exhibited significantly reduced capacity to interact with MPL and no capacity to induce factor-independent growth in UT-7/TPO cells. Replacing the signal peptide sequence of mutant CALR with ones from unrelated proteins sustained the localization in Golgi apparatus, and the MPL-binding and oncogenic capacity. Mutant CALR lacking glycan-binding capacity exhibited the Golgi localization but failed to interact with MPL and to induce factor-independent growth in UT-7/TPO cells.
Summary/Conclusion: We have demonstrated that the signal peptide sequence was required for mutant CALR to localize in Golgi apparatus and to induce factor-independent growth. Consistent to previous studies, we have shown that the glycan-binding capacity of mutant CALR was required for the MPL-binding and oncogenic activation of MPL. Taken together, we proposed a model in which mutant CALR engages with MPL in the secretary pathway by recognizing N-glycosylation of MPL, which eventually induces constitutive activation of MPL.

PS1344

PHARMACOLOGICAL INHIBITION OF IGF1R/IRS TARGETING STAT3/STAT5 SIGNALING HAS ANTI-NEOPLASTIC EFFECTS IN JAK2 V617F MYELOPROLIFERATIVE NEOPLASMS

J.C. Fernandes[1,*], J.A. Machado-Neto[1], B.A. Fenerich[1], J.L. Coelho-Silva[1], A.P.N.R. Alves[1], P.S. Scheucher[1], L.L. Figueiredo-Pontes[1], E.M. Rego[1], F. Traina[1]

[1]Internal Medicine, University of São Paulo at Ribeirão Preto Medical School, Ribeirão Preto, Brazil

Background: The lack of efficiency in inducing complete remission in most patients with myeloproliferative neoplasms (MPN) treated with the JAK1/2 selective inhibitor, ruxolitinib, indicates the need to identify new therapies. IGF1 upregulation induced myeloid transformation and the multi-targeted tyrosine kinase inhibitor NVP-AEW541 notable reverted the malignant phenotype in a Jak2V617F conditional knock-in mouse model. IRS2 silencing inhibited STAT5 phosphorylation, increased apoptosis and potentiated the effects of ruxolitinib in JAK2V617F cell lines. The IGF1R/IR selective inhibitor Linsitinib (OSI-906) has anti-tumors effects *in vitro*, and clinical trials are ongoing. However, the best pharmacological strategy to target IGF1R/IRS and the molecular consequences of specific inhibitors remains undetermined.
Aims: We aimed to investigate and to compare the effects of three IGF1R/IRS pharmacological inhibitors, NT157, OSI-906 and NVP-AEW541, in Ba/F3 cells expressing wild-type (WT) and V617F mutant JAK2 and mononuclear cells from Jak2V617F and WT knockin-induced MPN mice.
Methods: Cells were cultured in the presence or not of IL-3-rich Wehi-3B-conditioned medium and the effects of inhibitors on cell viability (MTT), apoptosis (annexin V/PI and caspase 3 cleavage), proliferation (Ki-67) and protein expression (Western blot) were evaluated. Ba/F3 cells were treated with NT157, OSI-906 and NVP-AEW541 for 24, 48 and 72 hours. Bone marrow mononuclear cells from mice were treated with the inhibitors for 72 hours and cell viability was evaluated. Statistical analysis were performed by ANOVA.
Results: In Ba/F3 JAK2V617F, NT157 (≥0.4 μM), for 48 and 72 hours, significantly reduced cell viability, cell proliferation and induced apoptosis (all $p<0.05$). NT157 reduced protein expression/activation of IRS1/2, STAT3, STAT5 and P70S6K, increased ERK1/2 phosphorylation and cleaved caspase 3. OSI-906 reduced cell viability, cell proliferation, and induced apoptosis only at higher dose (40 μM) (all $p<0.05$). OSI-906 reduced protein expression/activation of IRS1/2, IGF1R, AKT1/2/3 and P70S6K, increased ERK1/2 phosphorylation and cleaved caspase 3, but did not modulated STAT5. NVP-AEW541 treatment reduced cell viability and increased apoptosis at doses ≥2 μM upon all treatment duration (all $p<0.05$) and reduced protein expression/activation of IRS1/2, IGF1R, AKT1/2/3 and STAT5, but did not modulate ERK1/2 and STAT3. In Ba/F3 JAK2WT, all inhibitors were able to prevent IL-3 dependent growth factor-induced survival, indicating that these pharmacological inhibitors potentially act in key proteins involved IL-3 signaling. In mononuclear cells from WT and Jak2V617F knockin-induced MPN mice, the three inhibitors had similar effects to those observed in cell lines. NT157 reduced cell viability at 6.4μM and OSI-906 only at higher dose (≥20 μM); while NVP-AEW541 had the best effect, reducing cell viability upon doses ≥6 μM.
Summary/Conclusion: NT157 presented anti-neoplastic effects in low doses and was able to inhibit STAT3 and STAT5. OSI-906 exerts its anti-leukemic effects only at higher doses, and did not inhibited STAT5. NVP-AEW541 demonstrated anti-leukemic effects upon all doses and time tested, probably due to its effect not only on IGF1R, but also on KIT and FLT3 signaling, as previously demonstrated (Chapuis *et al.* Haematologica, 2010). The results highlight NT157 as a potent IGF1R/IRS and STAT3/STAT5 inhibitor with anti-tumor effects in JAK2V617F MPN models.

PS1345

IN VIVO INHIBITION OF JAKS IN MYELOFIBROSIS IMPROVES MONOCYTES DIFFERENTIATION AND REACTIVATES PRO-INFLAMMATORY RESPONSE IN INFECTION-STIMULATED MONOCYTES

M. Barone[1], M. Romano[2], D. Sollazzo[1], D. Forte[3], E. Ottaviani[1], G. Auteri[1], N. Vianelli[1], M. Cavo[1], F. Palandri[1], L. Catani[1,*]

[1]Experimental, Diagnostic and Specialty Medicine, University of Bologna, Institute of Hematology "L. e A. Seragnoli", Bologna, Italy, [2]School of Immunology & Microbial Sciences, King's College London, Guy's Hospital, London, [3]Wellcome Trust-Medical Research Council Cambridge Stem Cell Institute and Department of Haematology, University of Cambridge, Bologna, United Kingdom

Background: Myelofibrosis (MF) is a clonal disorder of the hemopoietic stem cells characterized by hyperactivation of the JAK-STAT pathway and chronic inflammation. Ruxolitinib (RUX), a JAK1/2 inhibitor, exerts also immunosuppressive/anti-inflammatory activity, resulting in increased infectious risk in RUX-treated MF patients (pts). Among the cells targeted by RUX, monocytes (Mo) might be highly affected due to the importance of the JAK-STAT signalling pathway in their differentiation/function. However, the effects of RUX toward this cell remain to be clarified.
Aims: To study whether and to what extent RUX affects the ex vivo/*in vitro*

functional behaviour of circulating Mo in MF pts.
Methods: Peripheral blood (PB) was collected from 12 *JAK2*^{V617F} mutated MF pts before and after 6 months of RUX therapy and from 10 age/sex-matched healthy donors (HD). Pts were int-2 and high IPSS risk. Total Mo and subsets (Classical- CD14++/CD16-, Intermediate- CD14+/CD16+, Non-Classical-Mo CD14-/CD16++) were characterized by flow cytometry (chemokine receptors (R): CCR2, CX3CR1, CCR5; functional markers: CD86, HLA-DR, Interleukin (IL)-10R) in mononuclear cells (PBMCs). After *in vitro* stimulation of PBMCs with lipopolysaccarides (LPS), the Interleukin (IL)-1β/-6, and Tumor Necrosis Factor (TNF)-α producing Mo were measured by intracellular flow cytometry analysis. IL-10 plasma levels were measured by ELISA.
Results: We firstly observed a dysregulation of MF-Mo differentiation. During physiological maturation from Classical- to Intermediate- and Non-Classical-Mo, CCR2 is downregulated and CX3CR1 is upregulated. By contrast, in MF pts, the Intermediate- and Non-Classical-Mo maintain a high expression of CCR2 (Figure 1A) and do not upregulate CX3CR1 in Non-Classical-Mo (Figure 1B). After 6 months of therapy, RUX significantly reduced the mean percentage of CCR2+ cells (Figure 1A) and increases the intensity of CX3CR1 expression in Non-Classic-Mo (Figure 1B). In addition, RUX normalized the intensity of the motility-related CCR5 expression in all Mo-subsets (data not shown). Given the importance of IL-10 pathway in the negative feedback control of inflammatory cytokines production, we then analysed IL-10R expression in MF Mo. At baseline, the mean percentage of IL-10R+ cells was significantly increased in MF-Mo, especially Classical and Non-Classical-Mo; RUX significantly decreased the mean percentages of IL-10R+ cells in Total and Non-Classical-Mo (Figure 1C). Additionally, the increased IL-10 plasma level at baseline was not significantly modified by RUX (data not shown). Following *in vitro* LPS stimulation, total-MF-Mo at baseline showed dramatically decreased mean percentage of IL-1β, IL-6 and TNF-α producing cells. Surprisingly, RUX counteracted the LPS-mediated effects increasing and normalizing the proportion of inflammatory cytokines-producing cells in all Mo-subsets (Figure 1D, E, F). Of note, analysing baseline and RUX-treated cells, we detected an inverse correlation between the percentages of IL10R+ cells and LPS-stimulated inflammatory cytokines-producing cells ($r \leq -0.7$; $p \leq 0.04$; expressed as% IL10R/% cytokines LPS-stimulated producing cells ratio; Figure 1 G,H,I).

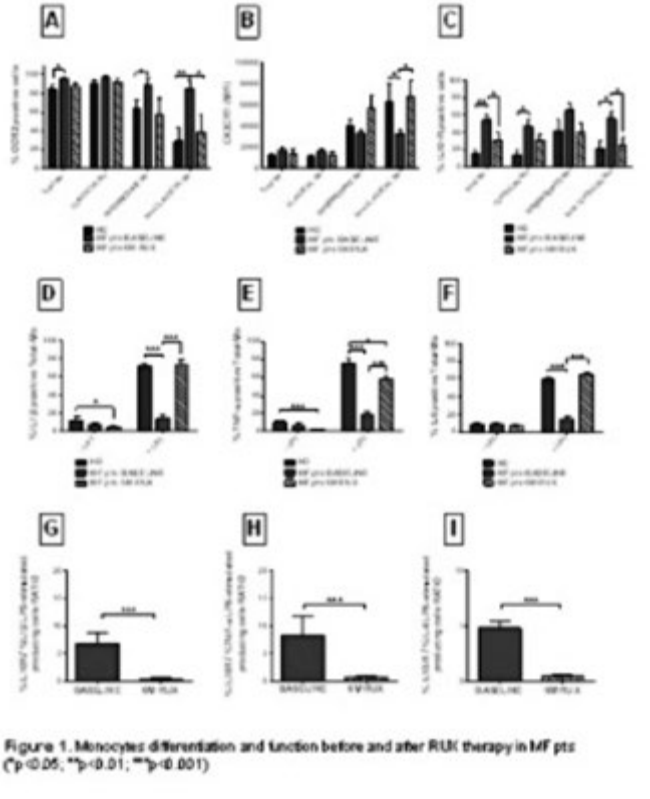


Figure 1. Monocytes differentiation and function before and after RUX therapy in MF pts (*p<0.05; **p<0.01; ***p<0.001)

Figure 1.

Summary/Conclusion: Here we demonstrated that, at variance with natural killer and T cells, which were described to be reduced in number/function by RUX in previous studies, RUX ameliorates *ex vivo* Mo differentiation/function in MF. This finding further refines the effects of RUX on MF immune system and suggests that RUX activity is cell type-dependent. A larger cohort of pts is now under evaluation to confirm these data.

PS1346

POLO-LIKE KINASE-1, AURORA KINASE A AND WEE1: NEW THERAPEUTIC TARGETS IN SYSTEMIC MASTOCYTOSIS

M. Mancini[1,*], C. Monaldi[2], S. De Santis[2], C. Papayannidis[2], M. Rondoni[3], M.C. Abbenante[2], L. Bavaro[2], M. Martelli[2], R. Zanotti[4], L. Pagano[5], M. Cavo[2], P. Valent[6], G. Martinelli[7], S. Soverini[2]

[1]Department of Experimental Diagnostic and Specialty Medicine - DIMES, Department of Experimental Diagnostic and Specialty Medicine - DIMES, University of BolognaInstitute of Hematology "L. e A. Seràgnoli", [2]Department of Experimental Diagnostic and Specialty Medicine - DIMES, Istituto di Ematologia Seràgnoli-DIMES, Bologna, [3]Azienda USL della Romagna, Ravenna, [4]Azienda Ospedaliera di Verona, Verona, [5]Fondazione Policlinico Universitario A. Gemelli , Roma, Italy, [6]Department of Hematology, Internal Medicine I, Medical University of Vienna, Vienna, Austria, [7]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (I.R.S.T.) , Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (I.R.S.T.), Meldola, Italy

Background: Systemic mastocytosis (SM) is due to the pathologic accumulation of neoplastic mast cells in one or more extracutaneous organ(s). Advanced forms of SM are associated with poor prognosis. Although midostaurin, a multikinase inhibitor active against both wild type and D816V-mutated KIT, improves organ damage and symptoms, there is still a need for new targeted agents for patients (pts) who relapse or have resistant disease. It is well known that Aurora-A overexpression promotes tumorigenesis, but the role of Aurora-A in the development of cancer has not been fully investigated. Recent studies indicate that Aurora-A may confer cancer cell chemo-resistance through dysregulation of cell cycle progression and DNA damage response. Direct evidences from literatures suggest that Aurora-A inhibits p53, but enhances Plk1, CDC25, CDK1, and cyclin B1 to promote cell cycle progression. Other studies indicate that Aurora-A suppresses RAD51, poly(ADP ribose) polymerase (PARP), and gamma-H2AX to dysregulate DNA damage response.
Aims: In this study we aimed to investigate the role of Aurora-A and Plk 1 in genomic instability and aggressiveness of advanced SM and if they may represent promising therapeutic targets.
Methods: Experiments were conducted in the HMC-1 MC leukemia cell line and in primary neoplastic MCs obtained from 7 SM patients (indolent SM, n=3; aggressive SM, n=3 and MC leukemia, n=1). Protein expression and activation was assessed by Western Blotting. Apoptotic cell death and cell cycle distribution were evaluated by flow cytometry after annexin V and propidium iodide staining, respectively. Drug cytotoxicity in *in vitro* experiments was evaluated by clonogenic assays.
Results: HMC-1 cells as well as primary neoplastic MCs displayed hyperphosphorylated AKA and Plk1. Danusertib (AKA inhibitor) and Volasertib (Plk1 inhibitor) inhibited growth and induced apoptotic cell death in HMC-1.1 (IC50=649nM and 443nM, respectively) and -1.2 cells (IC50=892nM and 808nM, respectively). The growth-inhibitory effects of Danusertib and Volasertib were found to be associated with mitotic arrest and activation of apoptosis. Cell cycle arrest was associated with increased levels of phospho (p)-Chk1 and p-Chk2, p-cyclin B1, p-cdc2 and p-Wee1. Apoptosis was demonstrated by an increase of annexin-V-positive cells and by the detection of the cleaved forms of caspase-3, -8, -9, and PARP. Wee1 inhibition by AZD1775 (500 nM) after 24h treatment with Danusertib or Volasertib (100nM), when cells were arrested in G2 phase and Wee1 was overexpressed and hyper-activated, resulted in a percentage of apoptotic cells significantly higher than that obtained from concomitant treatment with Danusertib or Volasertib (100nM) + AZD1775 (500 nM) for 48h. Moreover, both drug combinations induced a significant increase of the DNA double-strand break marker γH2AX and RAD51, suggesting that Wee1 inhibition promotes mitosis and propagates genomic instability. Finally, Danusertib and Volasertib were found to synergize with AZD1775 in inhibiting HMC-1 clonogenic potential and in inducing apoptotic cell death. Volasertib or Danusertib±MK1775 did not induce apoptosis in normal cultured cells.
Summary/Conclusion: Plk1 and AKA, alone or together with Wee1, are attractive therapeutic targets in neoplastic MCs. Repurposing Plk1 or AKA±Wee1 inhibitors in advanced clinical development for other indications is a therapeutic strategy worth to be explored in an attempt to improve the outcome of patients with advanced SM.

PS1347

NLR AS SURROGATE OF MYELOID IMMUNE SUPPRESSION CAN PREDICT EARLY SPLENIC RESPONSE TO RUXOLITINIB IN MYELOFIBROSIS

A. Bonanno[1,*], A. Romano[1], S. Stella[1], P. La Cava[1], N.L. Parrinello[1], C. Giallongo[1], D. Tibullo[1], E. Lambusta[1], M.S. Pennisi[1], F. Di Raimondo[1], G. Palumbo[1]

[1]Università degli Studi di Catania, Catania, Italy

Background: Myelofibrosis (MF), include primary myelofibrosis (PMF), post essential thrombocythemia (PET-MF) and post polycythemia vera (PPV-MF). Recently, clinical benefits have been reported in patients receiving therapy with IMIDs, that are presumably derived from immune-modulating effects of these agents; however their exact mechanism remains unclear. Ruxolitinib is the-first-in-class Jak1/2 inhibitor recently approved for treatment for MF.
Aims: We proposed to probe further into myeloid-driven immune dysfunction in MF to identify surrogate markers to apply into clinical practice.
Methods: We studied 161 patients (85 males and 76 females, median age 61 years old, range 51-90) with a diagnosis of MF, including PMF (n = 137, 85%), post-ET MF (n = 12, 7.5%), and post-PV MF (n = 12, 7.5%). Fibrosis grade 0 was found in 38%, grade 1 in 33%, grade 2 in 26% and grade 3 in 3% of patients. According to mutational status 129 (80%) patients were Jak-2 positive for the mutation V617F, 9 (5.5%) for CALR, 2 (1.5%) for MPL and 9 (5.5%) triple negative; for 9 (5.5%) patients the driver mutation status was not studied. Starting from March 2012, ruxolitinib was administered to 52/161 patients (40 of them with PMF, 8 with post-PV MF and 4 with post-ET MF) with dosage according to drug label. Spleen diameters were evaluated by physical examination before treatment and after 12 and 24 weeks of ruxolitinib continuous treatment. Neutrophils/Lymphocytes Ratio (NLR), derived Neutrophils/Lymphocytes Ratio (dNLR) Lymphocytes/Monocytes Ratio (LMR), Platelet/Lymphocytes Ratio (PLR) and Neutrophils/Platelet Ratio were calculated respectively on ANC/ALC, ANC/(WBC-ANC), ALC/AMC, PLT/AMC and ANC/PLT, as derived by hemocromocitometric exam at diagnosis and before ruxolitinib start. 40 normal volunteer ratios were used as controls.
Results: In the whole cohort of patients, NLR mean±DS was 4.7±3.5; dNLR was 2.9±1.7; LMR was 4.6±6.3; PLR was 349±429.3 and ANC/PLT ratio was 0.03±0.05. Patients with lower bone marrow fibrosis had lower mean±DS NLR (grade 0: 4.1±3.5, G1: 4.8±3.7, G2: 5.2±2.9, G3: 8.1±6.3, p<0.001). Similarly, ANC/PLT ratio had a progressive increase (from 0.015±0.020 in patients with grade 0, to 0.054±0.075, 0.089±0.303 and 0.521±0.01 respectively for grade 1, 2 and 3); conversely PLR was progressively decreased according to fibrosis grade (p<0.001). Restricting the analysis only to the 52 ruxolitinib treated patients, those who obtained a spleen reduction >50% in the first 12 weeks showed a lower NLR and ANC/PLT ratio before starting treatment (p=0.001). At 24 weeks, patients with lower NLR and ANC/PLT ratio had more chances to reduce spleen size of 100% (p<0.001). Since in clinical trials the absolute number of monocytes and lymphocytes is not often captured, in other hematological settings the derived NLR (dNLR) has been tested as surrogate of NLR. Despite dNLR was positively associated to bone marrow fibrosis grading, it was not able to predict spleen reduction from baseline in patients treated with ruxolitinib.
Summary/Conclusion: NLR and ANC/PLT ratio are increased in MF compared to normal controls and positively associated to bone marrow fibrosis. In patients treated upfront with ruxolitinib, NLR (but not dNLR) and ANC/PLT could help to predict spleen reduction both at 12 and 24 weeks. Our results need to be validated in larger cohorts, taking care to use NLR instead of dNLR.

PS1348

POSSIBLE SOMATIC MOSAICISM OF THE JAK2 GENE IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS

N. Mori[1,2,*], M. Ohwashi-Miyazaki[3], K. Yoshinaga[1], M. Shiseki[4], H. Sakura[5], J. Tanaka[4]

[1]Hematology, Tokyo Women's Medical University, [2]Internal Medicine, Tokyo Women's Medical University Medical Center East , Tokyo, [3]Hematology, Tokyo Women's Medical University, Toyko, [4]Hematology, Tokyo Women's Medical University, [5]Internal Medicine, Tokyo Women's Medical University Medical Center East, Tokyo, Japan

Background: *JAK2* V617F mutation has been identified in 95% of patients with polycythemia vera (PV) and in half of patients with essential thrombocythemia (ET) and primary myelofibrosis. The majority of patients with these myeloproliferative neoplasms (MPN) are sporadic, while a familial predisposition to MPN has been reported. Previous studies failed to identify germline *JAK2* V617F mutations in familial PV. *JAK2* SNP 46/1 is associated with a weak predisposition to MPN, but the *JAK2* 46/1 haplotype does not explain familial MPN. Germline mosaicism was reported in the *NF1* gene in hereditary neurofibromatosis and *RB1* gene in hereditary retinoblastoma, causing tumors in their children. Moreover, somatic *APC* mosaicism was estimated at a rate of about 20% in *de novo* cases of familial adenomatous polyposis.
Aims: To elucidate the presence of *JAK2* V617F mutation mosaicism, we analyzed mutations in blood and other tissues from MPN patients including siblings.
Methods: Twenty-three patients with V617F-positive PV (n=12) and ET (n=11) were analyzed. Two of the 12 PV patients were siblings (PV1 and PV2). Both of their parents had a medical history of cerebrovascular events but lacked polycythemia. Peripheral blood (n=22), bone marrow (n=1), hair (n=4), and nail (n=23) samples from the 23 patients were collected after obtaining written informed consent. Neutrophils from peripheral blood were purified by dextran sedimentation followed by hypotonic lysis and centrifugation with Ficoll-Conray. For screening of *JAK2* V617F, allele specific-polymerase chain reaction (AS-PCR) was performed. Electrophoresis was repeated at least twice using independent PCR products. Direct sequencing was performed in both directions using a 3730xL DNA Analyzer (Life technologies, Carlsbad, CA, USA). Measurement of DNA levels was based on TaqMan Mutation Detection Assays using StepOnePlus (Life technologies). Absolute quantification was conducted using the standard curve method with serially diluted DNA prepared from HEL (homozygous mutation) and HL60 (wild-type) cell lines, and commercial control (Horizon Discovery, Cambridge, UK) for *JAK2* V617F. *JAK2* SNP 46/1 was analyzed by direct sequencing. The current study was conducted with the approval of ethical committee.
Results: We first performed AS-PCR in PV1 and PV2. Sensitivity to the V617F mutation was 0.5%. Aberrant bands were observed in nail and hair DNA, while the intensity was low. We next performed AS-PCR in the other 21 patients. Aberrant bands of variable intensity were detected in 8 of the 21 nail DNA samples and one of the three hair DNA samples but not in hair DNA from the mother of PV1 and PV2. Direct sequencing showed G to T transversion at nucleotide 1849 (V617F mutation) in 22 blood DNA and 1 bone marrow DNA sample. Sensitivity of direct sequencing was 10%. The mutation was also confirmed by direct sequencing in 6 of the 8 nail DNA samples showing aberrant bands by AS-PCR, while it was unclear in nail DNA samples from PV1 and PV2. Quantitative real-time PCR of the *JAK2* gene was performed in the 23 patients. The allele burden of V617F mutation was more than 10% (12.7-38.4%) in 5 of the 6 nail DNA samples showing mutations by direct sequencing. All the 5 samples were from patients with PV. *JAK2* SNP 46/1 was analyzed and the G allele associated with V617F mutation was identified in 21 of the 23 patients.
Summary/Conclusion: Although germline V617F transmission was not observed in the siblings, detection of *JAK2* V617F mutation in nail and blood samples from the same individuals suggests somatic mosaicism of *JAK2* V617F mutation in a certain proportion of PV patients.

Myeloproliferative neoplasms – Clinical

PS1349

MULTICENTER SURVEY ON MANAGEMENT AND OUTCOME OF A RARE HEMATOLOGIC DISEASE: ADULT ONSET HISTIOCYTOSES

D. Lazzarotto[1,*], A. Candoni[1], L. Melillo[2], M. Criscuolo[3], D. Michela[4], F. Forghieri[5], P. Minetto[6], F. Vianello[7], D. Capelli[8], M. E. Zannier[1], C. Filì[1], L. Pagano[3], R. Fanin[1]

[1]Division of Hematology and Stem Cell Transplantation, Azienda Sanitaria Universitaria Integrata di Udine, Udine, [2]Division of Hematology, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, [3]Division of Hematology, Policlinico Gemelli, Rome, [4]Section of Hematology, Vito Fazzi Hospital, Lecce, [5]Section of Hematology, Department of Surgical and Medical Sciences, University of Modena and Reggio Emilia, Modena, [6]Division of Hematology, Policlinico San Martino, University of Genova, Genova, [7]Hematology and Immunology Unit, Department of Medicine, University of Padova School of Medicine, Padova, [8]Division of Hematology, Azienda Ospedaliero-Universitaria Ospedali Riuniti di Ancona, Ancona, Italy

Background: Histiocytoses are a group of diseases that mainly affect children. Adult onset Histiocytoses are very rare and their management is mainly derived from small retrospective studies, expert opinions and the pediatric experience. The history of the disease is widely variable and often unpredictable in the single patient.
Aims: For these reasons we started a multicenter survey on presentation, management and outcome of Adult Histiocytoses in the last 15 years (2002-2017). This survey is still open to recruitment.
Methods: We report the preliminary data regarding 22 adult patients (pts) diagnosed with Histiocytosis. Eighteen/22 (82%) were affected by Langerhans cells Histiocytosis (LCH), 2 (9%) by Rosai-Dorfman Disease (RDD) and 2 (9%) by Histiocytic Sarcoma (HS).
Results: Median age at diagnosis was 42 (range 18-67) and there is an important male prevalence (M/F ratio 2.7/1). Fifteen/22 (68%) pts presented with tumor mass symptoms and 10/22 (45%) with systemic symptoms. BRAF V600E mutation was tested in 8/22 pts (only in most recent cases) but none of them was positive. Eight/22 (36%) presented with a single site involvement (3 bone involvement, 3 lymph node involvement, 1 CNS involvement, 1 pulmonary involvement) [GROUP 1-Single Site]. The three pts with bone involvement were treated with surgery alone, while the others with a combination of surgery and systemic therapy (median of 1 therapy line, range 1-3). The 5-year OS of these pts is 80%, but 63% had an active disease at last follow up. Fourteen/22 (64%) pts presented with multiple site involvement (bone, lymph nodes, lung the main involved organs) [GROUP 2-Multiple Sites]. All these pts received systemic therapy (a median of 1,5 therapy lines, range 1-7) combined with surgery in 3 pts and radiotherapy in 2 pts. The best response achieved was a complete response in 4/14 pts (28%) and a partial response in 6/14 (44%) pts, 4/14 (28%) were refractory to treatments. The median duration of the best response was 24 months (range 1-83). The 5-year OS of these pts is 77% but 79% had an active disease at last follow up. OS and the percentage of pts with active disease at last follow up was not different in the two groups. Finally, 4/18 surviving pts developed irreversible organ damage (pulmonary emphysema, chronic pancreatitis, persistent diabetes insipidus, severe bone lesions) (Figure 1).

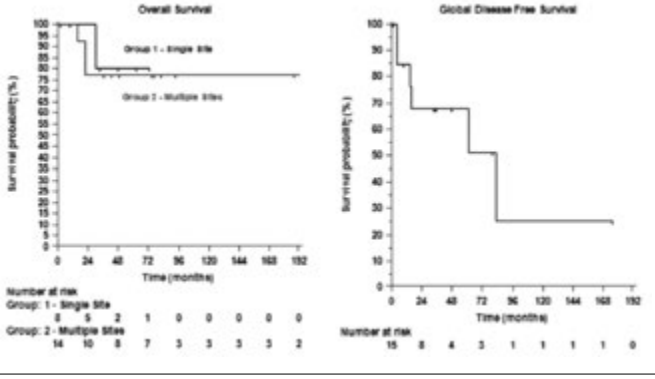


Figure 1.

Summary/Conclusion: Adult onset Histiocytoses are mainly chronic diseases in which the achievement of a complete and durable response with the current treatment options is unusual. Pts live together with their disease, its complications and the side effects of multiple therapies. Further studies are needed to discover other molecular alterations besides BRAF V600E (that is present in about 50% of cases) that can be targeted by specific therapies with the aim of improve response rates and allow pts to live without the disease and related complications.

PS1350

IS ANEMIA THE MAIN SURVIVAL MARKER IN PATIENTS WITH MYELOFIBROSIS IN THE RUXOLITINIB ERA?

M.I. Montero[1,*], E. Rodríguez-Arbolí[1], N. Alkadi[1], M. Reinoso[1], F.J. Márquez[1], E. Carrillo[1], F. de la Cruz[1], J. González[1], J. Falantes[1], I. Espigado[1], J.A. Pérez-Simón[1]

[1]Department of Hematology, Hospital Universitario Virgen del Rocío, Seville, Spain

Background: Approximately 50% of patients with myelofibrosis present with a haemoglobin concentration less than 100 g/L at diagnosis and one third of them will develop transfusion dependence during the course of their disease, which currently represents a clinical challenge without effective therapeutic options. The underlying mechanisms of anemia in this patient population are not well established, although it has been associated with ineffective hematopoiesis, elevated levels of proinflammatory cytokines, splenomegaly, mutations in several genes such as *UAF1* or downregulation of certain proteins such as in the case of GATA1.
Aims: Single centre analysis of the impact of anemia on survival and the association of response to erythropoietin treatment with JAK2 mutational status in a cohort of patients with myelofibrosis.
Methods: One-hundred nineteen patients diagnosed with myelofibrosis in our centre between June 1998 and July 2007 were included in the study. Average age at diagnosis was 67 years (range 19-88). Sixty-one percent of the patients were male and 39% female. Thirty-six percent had a previous diagnosis of another myeloproliferative neoplasm (polycythemia vera or essential thrombocythemia). Diagnosis of myelofibrosis was made based on bone marrow examination and according to the WHO criteria. V617F JAK2 status was assessed through allele-specific PCR in patients diagnosed after 2005. All patients with clinically significant anemia received treatment with erythropoietin. Statistical analyses were performed in SPSS 15.0.
Results: Average leucocyte, haemoglobin and platelet counts were $12{,}5x10^9$/L, 114 g/L and $344x10^9$/L, respectively. V617F JAK2 mutation was present in 61% of the patients. Thirty-one percent of the patients had a haemoglobin concentration less than 100 g/l at diagnosis and 47% developed transfusion dependence during the course of their disease.
Four-year overall survival (OS) was 69%. Four-year OS was 56% in patients with a haemoglobin concentration less than 100 g/l and 77% in those with a haemoglobin concentration of at least 100 g/l, statistical significance not being reached. Four-year OS in those patients who developed transfusion dependence was 60%, compared with 78%% in those without transfusion dependence (p=0.03). Only 20% of the patients with a V617F JAK2 mutation died compared with 56% of the patients with wild-type mutational status. However, four-year OS rates among patients developing transfusion dependence were similar irrespective of their mutational status (65% if V617F JAK2-positive vs. 60% if wild-type).
Summary/Conclusion: 1. Patients who develop transfusion dependence during the course of their disease show lower overall survival, with statistically significant differences, which is consistent with published data. 2. Patients with anemia and V617F JAK2 mutations respond better to erythropoietin treatment than those with JAK2 wild-type status, which indicates that the stimulation of the main pathogenic pathway in the disease may paradoxically contribute to overcome this problem.

PS1351

DEFERASIROX IN THE MANAGEMENT OF IRON OVERLOAD IN PATIENTS WITH MYELOFIBROSIS: A RETROSPECTIVE MULTICENTER EXPERIENCE OF THE RETE EMATOLOGICA LOMBARDA (IRON-M STUDY)

E. M. Elli[1,*], A. Iurlo[2], M. Caramella[3], S. Malato[4], E. Casartelli[5], M. Maffioli[6], A. Gardellini[7], M. C. Carraro[8], M. D'Adda[9], N. Polverelli[10], M. Rossi[11], N. Orofino[2], A. Carrer[12], A. Aroldi[12], B. Mora[6], L. Antolini[13], C. Gambacorti-Passerini[14], F. Passamonti[6]

[1]Hematology Division and Bone Marrow Transplantation Unit, Ospedale San Gerardo, University of Milano-Bicocca, MONZA, [2]Hematology Division, IRCCS Cà Granda - Maggiore Policlinico Hospital Foundation, [3]Department of Hematology, Ospedale Niguarda Cà granda, [4]Hematology and Bone Marrow transplantation Unit, San Raffaele Scientific Institute, Milano, [5]Transfusion Medicine , OspedaleAlessandro Man-

zoni di Lecco, Lecco, [6]Hematology, Department of Medicine and Surgery, University of Insubria & Ospedale di Circolo, ASST Sette Laghi, Varese, [7]Hematology Division, Ospedale Valduce , Como , [8]Hematology and Transfusion Medicine, ASST Fatebenefratelli Sacco , Milano, [9]Department of Hematology, ASST Spedali Civili, [10]Hematology and Bone Marrow transplantation Unit, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, [11]Cancer Center, Humanitas Research Hospital and Humanitas University, Rozzano, Milano, [12]Hematology and Bone Marrow transplantation Unit, Ospedale San Gerardo, University of Milano-Bicocca, [13]Centro di Biostatistica per l'epidemiologia clinica, Department od Health Sciences, University of Milano-Bicocca, [14]Hematology and Bone Marrow transplantation Unit, Ospedale san Gerardo, Universitàiy of Milano-Bicocca, Monza, Italy

Background: Deferasirox (DFX) is the currently available iron-chelation therapy (ICT) for the management of iron overload (IOL) in patients (pts) with myelodysplastic syndromes, while in myelofibrosis (MF) information are limited.
Aims: The primary endpoint of the study was the efficacy of DFX in terms of reduction of ferritin levels and improvement of erythroid response (according to IWG-MRT and Cheson IWG criteria). Additional endpoints were safety of DFX and survival.
Methods: We retrospectively collected 45 consecutive pts (M: 27; F: 18) with primary MF (n=30) or post-Polycythemia Vera MF (n=9) and post-Essential Thrombocythemia MF (n=6), according to WHO and IWG-MRT 2008 criteria, with transfusion-dependent anemia, treated with DFX since 2010. Data have been collected in 11 centers of Hematology belonging to the regional network REL (Rete Ematologica Lombarda). The protocol (IRON-M study) has been approved by each local ethic committee.
Results: The main features at diagnosis and at baseline of DFX treatment were reported in Table 1.

Table 1.

Clinical Features of patients at diagnosis and at baseline of Deferasirox treatment

Gender M/F, n (%)	27/18 (60/40)
Type of MF, n (%)	
Primary myelofibrosis	30 (66.6)
POST-PV myelofibrosis	9 (20)
POST-ET myelofibrosis	6 (13.3)
IPSS Classification, n (%)	
Low risk /Intermediate-1 risk	18 (40)
Intermediate-2 risk - High risk	27 (60)
Driver mutations, n (%)	
JAK2V617F positive	39 (86.6)
MPL positive	0
Calreticulin positive	3 (6.7)
Triple-negative	3 (6.7)
Median age at diagnosis, years (IR)	69.2 (40-80.4)
Median ferritin levels at diagnosis, ng/ml (IR)	446 (25-1994)
Median Hb at diagnosis, g/dl (IR)	10 (4.2 – 15.7)
Median WBC at diagnosis, /mm3 (IR)	6.68 (2.49-26.12)
Median PLT at diagnosis, /mm3 (IR)	253 (50-1360)
Median age at baseline, years (IR)	71.6 (47.6-81.5)
Median ferritin levels at baseline, ng/ml (IR)	1609 (900-4000)
Median Hb at baseline, g/dl (IR)	8.6 (5.7-10.9)
Median WBC at baseline, /mm3 (IR)	8.49 (0.78-38.63)
Median PLT at baseline, /mm3 (IR)	150 (12-1050)
Median time between initiation of transfusion dependence and start DFX, months (IR)	12.5 (1-146)
Median of packed red cells units/patient received, at baseline, n (IR)	28 (5-150)

ET: Essential Thrombocythemia; PV: Polycythemia Vera; IPSS: International Prognostic Scoring System; HB. Hemoglobin; WBC: White Blood cells; PLT: platelet count; IR: interquartile range.

According to the IPSS classification, 18 pts (40%) resulted low/intermediate-1 risk and 27 (60%) intermediate-2 risk/high-risk. Pts started DFX after a median time from MF diagnosis of 26.5 months (IR 2.6-208.7) and from transfusion dependency of 12.5 months (IR 1.2-146). Median value of packed red cells units/patient received was 28 (IR 5-150). The median ferritin levels at baseline was 1609 ng/mL (IR 900-4000). The median starting dose of DFX was 750 mg/day (10 mg/kg/day).
41/45 pts (91.1%) were evaluable for efficacy (> 3 months of DFX) and the median time of DFX exposure was 15.6 months (IR 3-59.5); among these pts, 12 (29.3%) pts obtained a stable reduction of ferritin levels < 1000 ng/mL, defined as iron chelation response (IRC). An erythroid response (ER) with DFX were observed in 18/41 pts (43.9%), 7 of them (17%) obtained transfusion independency (TI). The median time to best ER was 2.4 months. After a median follow-up of 52.8 months (IR 14.4-267.2) from diagnosis, 18/41 pts (43.9%) died, 8 of them for leukemic evolution or progression of disease. Overall, pts with ER receiving DFX showed a progressive reduction of ferritin levels at 6, 12 and 18 months respect to baseline, in contrast to non-responder (NR) pts. We did not observe a significant difference in OS from DFX initiation in ER respect to NR pts (31.7 vs 42.2 months, p=0.57). Conversely, 2-year survival from DFX initiation was 100% in pts who obtained a IRC compared to 68% in pts without IRC (p=0.001). All 45 patients were evaluable for toxicity: extra-hematological drug-related adverse events (AEs) were reported in 16 (35.5%) pts, principally consisting in renal (17.7%) or hepatic impairment (8.9%) and skin reaction (6.7%). Eleven (24.4%) pts discontinued definitively treatment due to toxicity of grade ≥2.
Summary/Conclusion: The present multicenter study reported a large retrospective series of MF pts treated with DSX. We showed that ICT with DFX is effective and safe in MF. Hematological improvement with erythroid responses can occur in a significant proportion of pts. Obtainment of ICR seems to be predictive for better survival. Further larger and prospective investigations are required.

PS1352

MYELOID AND LYMPHOID NEOPLASMS ASSOCIATED WITH PDGFRA AND PDGFRB REARRANGEMENTS: CLINICAL CORRELATES AND SURVIVAL OUTCOMES

A. Mangaonkar[1,*], N. Gangat[1], A. Al-Kali[1], K. Begna[1], M. Elliott[1], C. Hook[1], W. Hogan[1], M. Litzow[1], R. Ketterling[1], A. Tefferi[1], A. Pardanani[1], M. Patnaik[1]

[1]Hematology, Mayo Clinic, Rochester, United States

Background: Myeloid/lymphoid neoplasms with *PDGFRA* & *PDGFRB* rearrangements are incorporated under a separate category as per the 2016 World Health Organization (WHO) classification, due to their unique disease biology & sensitivity to tyrosine kinase inhibitors.
Aims: We undertook this study to describe clinical correlates and survival outcomes in patients with WHO-defined myeloid/lymphoid neoplasms associated with *PDGFRA & PDGFRB* rearrangements.
Methods: Consecutive cases of WHO-defined myeloid/lymphoid neoplasms with FISH detectable *PDGFRA* & *PDGFRB* rearrangements were identified from our institutional database, from years 1997-2017 & bone marrow biopsy/cytogenetic reports were re-reviewed.
Results: Twenty five patients were included in this study (Table 1); 23 (92%) males with a median age of 43 years (range: 19-84). Twenty one had FISH detectable *PDGFRA* [20 *CHIC2* deletions &/or t(4;12)(q12;p13) and 1 t(4;22)(q12;11.2)], while 4 had *PDGFRB* [2 *ETV6-PDGFRB* or t(5;12)(q32;p13.2), 1 t(5;19)(q32;p13.3) & 1 t(5:16)(q32;p22)] rearrangements. Of these, only 2 (9.5%) *PDGFRA* and 3 (75%) *PDGFRB* rearrangements were detected by metaphase cytogenetics. Clinical phenotypes associated with *PDGFRA* rearrangements at diagnosis included, chronic eosinophilic leukemia (CEL) 9 (43%), systemic mastocytosis with an associated hematological neoplasm 6 (29%), acute myeloid leukemia (AML) 3 (14%), myeloproliferative neoplasms-unclassifiable 2 (10%) & T-cell lymphoblastic leukemia (ALL) 1 (5%); while those for *PDGFRB* rearrangements included, chronic myelomonocytic leukemia 2 (50%), CEL 1 (25%) & B-cell ALL 1 (25%). Concurrent cytogenetic abnormalities at diagnosis were seen in 4 (18%) of 22 assessable patients and included trisomy 12, del(7q), t(2;22)(q11.2;p11.2), trisomy 5, del(9)(p21.1) & t(18;21)(q21;q22); while two patients acquired del(3q) and a FISH detectable duplication of 3q26 (3 copies of MECOM) correlating with transformation to AML. Of the 22 (88%) patients with available treatment data, 20 (91%) received imatinib, with median doses of 100 & 400 mg daily for *PDGFRA* & *PDGFRB* rearrangements respectively. Overall response rate was 100% for both *PDGFRA* & *PDGFRB* rearrangements [Complete response (CR)-15, partial response (PR)-2, hematologic response (HR)-2, & stable disease-1] with median response durations of 25 (range: 5-170) months. All 4 (20%) patients that did not achieve a CR with imatinib had *CHIC2* deletions; 3 (75%) with a CEL clinical phenotype, while one had an MPN-U. Three (12%) patients (*PDGFRA-100%)* had evidence of eosinophilic cardiomyopathy at diagnosis, 1 (4%) of whom developed worsening cardiomyopathy after starting therapy with imatinib. At last follow-up (median 40 months), 2 (9%) transformations into AML (both AML with MDS-related changes) & 3 (12%) deaths (1-AML, 1-unknown, 1-nonhematologic medical complications) were documented. Within significant limitations of a small sample size, patients with *PDGFRA* rearragments have a better median OS (median not reached) in comparison to those with *PDGFRB* rearrangements (median 66 months, Figure 1).

Table 1.

Variable; Median value (range or %)	Cohort (n=25)	PDGFRA (n=21)	PDGFRB (n=4)	P value
Age (Range in years)	43 (19-84)	43 (19-71)	47 (30-84)	0.5
No. of Males (%)	23 (92)	20 (95)	3 (75)	0.24
Hemoglobin (Range; g/dL)	12.9 (7.2-15.4)	13.3 (7.2-15.4)	8.9 (7.9-10.9)	0.05*
WBC count X 10^9 per L	10 (0.4-116.2)	10.9 (3.5-75.4)	7.2 (0.4-116.2)	0.24
Platelet count X 10^9 per L	152 (24-355)	156.5 (31-355)	103 (24-166)	0.06
Absolute eosinophil count X 10^9 per L	2 (0-23)	2 (0-23)	1.4 (0.6-3.5)	0.44
Absolute monocyte count X 10^9 per L	0.4 (0-35)	0.4 (0-1.5)	1.1 (0.4-35)	0.02
Lactate dehydrogenase (U/L)	165 (107-1517)	164 (107-1117)	823 (166-1517)	0.006*
BM blasts % (Range)	2 (0-97)	2 (0-53)	2 (0-97)	0.25
Concurrent diagnostic cytogenetic abnormalities not involving PDGFRA/PDGFRB rearrangements (%)	4 (18)	2 (10) [Trisomy 12, del(7q)]	2 (50) [t(2;22)(q11.2;p11.2), trisomy 5, del(9)(p21.1) & t(18;21)(q21;q22)]	0.1
Imatinib treatment (%)	20 (91)	17 (89)	3 (75)	0.4
Median imatinib induction dose (Range in mg)	100 (100-400)	100 (100-400)	400 (100-400)	0.02*
Transfoemation into AML after diagnosis (%)	2 (9)	2 (9.5)	0 (0)	0.4
Number of deaths (%)	3 (12)	1 (5)	2 (50)	0.03*
Allogeneic SCT (%)	2 (8)	1 (5)	1 (25)	0.24
Overall survival (OS, range in months)	Median not reached	Median not reached	66 (4-102)	-

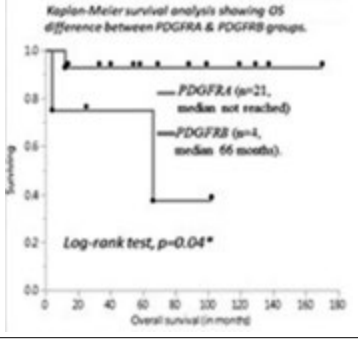


Figure 1.

Summary/Conclusion: Myeloid and lymphoid neoplasms associated with *PDGFRA* and *PDGFRB* rearrangements present with a spectrum of clinical phenotypes often characterized by eosinophilia (*PDGFRA*) and monocytosis (*PDGFRB*), can be karyotypically occult (*CHIC2* deletions), and in general have a good response to tyrosine kinase inhibitor therapy (ORR-100%). The impact of additional cytogenetic abnormalities at diagnosis, cytogenetic clonal evolution on therapy and suboptimal responses to imatinib, on outcomes, needs to be explored in a larger cohort.

PS1353

ASSESSING SERUM ALBUMIN CONCENTRATION, LYMPHOCYTE COUNT AND PROGNOSTIC NUTRITIONAL INDEX MIGHT IMPROVE PROGNOSTICATION IN PATIENTS WITH MYELOFIBROSIS

M. Lucijanic[1,*], T. Stoos-Veic[2], A. Livun[3], V. Pejsa[1], D. Cicic[4], A. Maglicic[5], R. Kusec[1]

[1]Hematology Department, [2]Cytology and Cytometry Department, [3]Clinical Department of Laboratory Diagnostics, University Hospital Dubrava, [4]Hematology department, Clinical Hospital Dubrava, Zagreb, [5]Health Care Center Vojnic, Vojnic, Croatia

Background: Primary and secondary myelofibrosis (PMF; SMF) are malignant diseases of the hematopoietic stem-cell characterized by the neoplastic myeloproliferation and strong inflammatory milieu. Prognostic-Nutritional-Index (PNI) integrates information on albumin and absolute-lymphocyte-count (ALC) and reflects inflammatory, nutritional and immune status of a patient. Clinical and prognostic significance of albumin, ALC and PNI in patients with myelofibrosis have not been previously investigated.

Aims: To assess clinical and prognostic significance of albumin, ALC and PNI in a population of myelofibrosis patients.

Methods: We retrospectively analyzed a cohort of 83 myelofibrosis patients treated in our institution from 2006 to 2017, 63 (75%) had PMF and 20 (25%) had SMF. A total of 69 (83%) patients were evaluated at the time of establishing diagnosis. Albumin, ALC and PNI were assessed in addition to other disease-specific parameters. The Kruskal Wallis test, the Mann Whitney U test, the Chi squared test, the Spearman rank correlation, the log-rank test and the Cox regression analysis were used. Optimal cut-off values were determined using the ROC curve analysis.

Results: Mean patient age was 65.6 ±10.4 years, 61% were males. Median follow up was 54 months. PMF and SMF patients had significantly lower ALC and PNI ($p<0.05$) and similar albumin (n.s.) compared to controls, there was no difference between PMF and SMF patients (n.s.). Lower albumin was significantly associated ($p<0.05$) with older age and parameters reflecting more aggressive disease biology (anemia, lower platelets, higher LDH, circulatory-blasts, transfusion-dependency, blast-phase-disease), inflammation (higher CRP, constitutional-symptoms) and higher degree of bone-marrow-fibrosis. Lower ALC was significantly associated ($p<0.05$) with lower white-blood-cells (WBC) and lower circulatory-blasts. Low PNI was significantly associated ($p<0.05$) with lower albumin, lower ALC, anemia, lower WBCs, lower serum-iron and lower transferrin-saturation. There was no difference in albumin, ALC and PNI regarding the driver-mutations. Low-albumin, low-ALC and low-PNI were all significantly associated with inferior survival ($p<0.05$) as shown in a Figure 1. In multivariate analysis adjusted for age and gender, low-albumin (HR=4.61, p=0.001), low-ALC (HR=3.54, p=0.004) and DIPSS (HR=2.45, p=0.001) were able to predict inferior survival independently of each other. Accordingly, low-PNI (HR=4.32, $p<0.001$) predicted poor survival independently of DIPSS (HR=3.31, $p<0.001$).

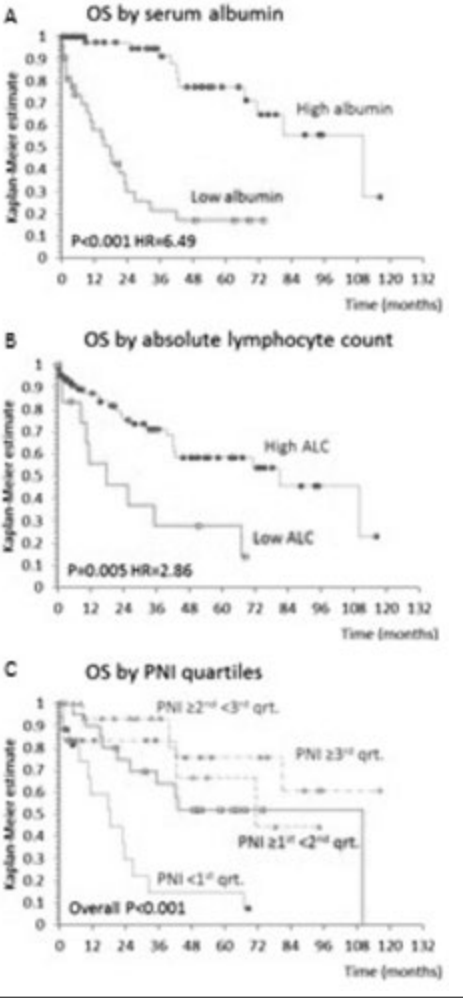


Figure 1.

Summary/Conclusion: Assessing albumin, ALC and PNI might improve prognostication in patients with myelofibrosis and could assist in recognition of patients under increased risk of death.

PS1354

LONG-TERM OUTCOME OF PATIENTS WITH SPLANCHNIC VEIN THROMBOSIS AND MYELOPROLIFERATIVE NEOPLASMS: A SINGLE CENTER EXPERIENCE

D. Lekovic[1,2,*], P. Miljic[1,2], J. Bodrozic[1], D. Culafic[2,3], D. Sefer[1], S. Bizic[1], V. Djordjevic[1], R. Jesic[2,3], M. Gotic[1,2]

[1]Clinic for Hematology, Clinical Center of Serbia, [2]Medical Faculty, University of Belgrade, [3]Clinic of Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade, Serbia

Background: According to literature data splanchnic vein thrombosis (SVT) involving portal, splenic, mesenteric or hepatic (Budd–Chiari sy,BCS) veins occur in about 1-23% of patients with MPNs. However, there is little data about influence of SVT on long-term outcome in these patients (pts).
Aims: Aim of our study was to analyze clinical course and to identify parameters influencing long-term outcomes in MPNs pts with SVT.
Methods: We retrospectively analyzed 42 consecutive MPNs pts with SVT (15 PV, 17 PMF, 3 ET, 7 latent MPN) who were diagnosed and followed in our clinic from July 1994 to November 2017. Diagnosis of MPN was established according to the WHO criteria. Latent MPN was characterized with positive *JAK2V617F* mutation or/and presence of spontaneous erythroid colony formation but without clear hematological criteria for MPNs. In all pts, SVT was confirmed by Doppler ultrasound examination of portal system or/and CT. In this study we analyzed following outcomes: occurrence of gastrointestinal (GI) bleeding episodes, SVT rethrombosis and overall survival (OS). Following parameters potentially influencing clinical outcomes were investigated: location of thrombosis, *JAK2V617* mutation, CBC, cytogenetics, treatment modalities, presence of portal hypertension, cardiovascular traditional risk factors and additional risk factors for thrombosis.
Results: Median age at MPN diagnosis was 43 years (range 19-67). Patients with BCS were significantly younger with median age of 30.5 years (p=0.007). Female gender was prevalent (59.5%). In 50% of pts, MPN was diagnosed within 3 months after onset of SVT. Additional risk factors for thrombosis had 14pts (33.3%). SVT type was BCS in 14.3% (1 latent, 4 PV, 1 ET), portal thrombosis in 83.3% (6 latent, 10 PV, 17 PMF, 2 ET) and isolated splenic thrombosis in 2.4% of pts (1 PV). According to the initial grade (Gr) of esophageal varices (EV) in 34 analyzed cases, pts were distributed as follows: Gr1 had 7pts (20.6%), Gr2 had 9 (26.5%), Gr3 had 9 (26.5%), Gr4 had 3 (8.8%), while 6pts (17.6%) didn't have EV at all. Treatment included hydroxyurea (HU) plus OACs in 16pts (38.1%), HU plus LMWH in 4 (9.5%), HU in 12 (28.6%), OACs only in 6 (14.3%), HU plus ASA in 1(2.4%), ASA only in 1(2.4%). Two pts (4.8%) were untreated. During median follow-up of 90.5 months (range 4-276) at least one GI bleeding experienced 15pts (35.7%), recurrence of SVT had 2pts (4.8%) while 10pts (23.8%) had died at median age of 51 years (range 37-72). The OS at 5, 10 and 15 years was 87.8%, 73,3% and 59,6% respectively. Median follow up until death was 76 months (range 25-192). Death was directly related to liver failure in 4 pts, GI bleeding in 3, AML in 1, while in 2 pts cause of death was unknown. Out of analyzed factors potentially influencing survival significant association with shorter OS was found for avoidance of cytoreductive treatment (p=0.001), age>60 years (p=0.005), Le>15x10^9/L (p=0.003) and initial Gr of EV>1 (p=0.019). However in multivariable Cox-regression model, cytoreductive treatment retained a statistical significance for OS only (HR 0.91, 95% CI 0.837-0.989 p=0.026).
Summary/Conclusion: Our results confirmed that SVT is often first clinical manifestation of an underlying disease, highlighting the need for thorough screening for MPNs. Introduction of cytoreductive treatment favorably influenced long-term outcomes in pts with SVT and MPN. Mortality was mainly caused by liver failure and esophageal varices bleeds, so successful management of these pts requires long-term team work of haematology and hepatology specialists.

PS1355

GENDER EFFECT ON PHENOTYPE AND GENOTYPE IN PATIENTS WITH POST-POLYCYTHEMIA VERA AND POST-ESSENTIAL THROMBOCYTHEMIA MYELOFIBROSIS: RESULTS FROM THE MYSEC PROJECT

D. Barraco[1,*], B. Mora[1], T. Giorgino[2], P. Guglielmelli[3], E. Rumi[4], M. Maffioli[1], A. Rambaldi[5], M. Caramella[6], R. Komrokj[7], J. Gotlib[8], J. J. Kiladjian[9], F. Cervantes[10], T. Devos[11], F. Palandri[12], V. De Stefano[13], M. Ruggeri[14], R. T. Silver[15], G. Benevolo[16], F. Albano[17], M. Merli[1], D. Pietra[4], T. Barbui[18], G. Rotunno[3], M. Cazzola[4], A. M. Vannucchi[3], P. Francesco[1]

[1]Hematology, Ospedale di Circolo, ASST Sette Laghi, Varese, Italy, Varese, [2]Biophysics Institute, National Research Council of Italy , Milan, [3]CRIMM-Centro Ricerca e Innovazione delle Malattie Mieloproliferative, Department of Experimental and Clinical Medicine, Azienda ospedaliera-Universitaria Careggi, University of Florence, Florence, [4]Department of Hematology Oncology, Fondazione IRCCS Policlinico San Matteo Università di Pavia, Pavia, [5]Department of Oncology-Hematology, University of Milan and BMT Unit, ASST Papa Giovanni XXIII, Bergamo, [6]Hematology, Ospedale Niguarda Cà Granda, Milan, Italy, [7]Hematology, Moffit Cancer Center, Tampa, [8]Hematology, Stanford University, Palo Alto, United States, [9]Hematology, Hôpital Saint-Louis et Université Paris Diderot, Paris, France, [10]Hematology, Hospital Clínic, IDIBAPS, Barcelona, Spain, [11]Hematology, University Hospitals Leuven and Laboratory of Experimental Transplantation, Department of Microbiology and Immunology, KU Leuven, Belgium, [12]Hematology, Policlinico S. Orsola-Malpighi, Bologna, [13]Hematology, Università Cattolica del Sacro Cuore, Rome, [14]Hematology, Ospedale S. Bortolo, Vicenza, Italy, [15]Hematology, Weill Cornell Medical College, New York, United States, [16]Hematology, SC Hematology, A.O. Città della Salute e della Scienza, Turin, [17]Hematology, Università di Bari, Bari, [18]Hematology, Research Foundation, ASST Papa Giovanni XXIII, Bergamo, Italy

Background: Survival difference between men and women in the general population is well recognized and consistent sex-discrepancies are common within hematologic malignancies. Additionally, there is developing interest in understanding how gender influences the pathogenesis, phenotype and outcome in myeloproliferative neoplasms (MPN).
Aims: The aim of the current study is to evaluate the prognostic impact of gender in the study-population of the multicentric MYSEC (Myelofibrosis Secondary to PV and ET Collaboration) project including 781 patients with post-polycythemia vera (PPV-MF) and post-essential thrombocythemia (PET-MF) myelofibrosis.
Methods: The study was approved by the Ethical Committee of each Institution and conducted in accordance with the Declaration of Helsinki. Diagnoses of secondary myelofibrosis (SMF) were performed between 1981 and 2015 and were locally reviewed according to the International Working Group on Myeloproliferative Neoplasm Research and Treatment criteria (IWG-MRT 2008). Wilcoxon rank sum and Pearson's chi-squared tests were performed to report differences between groups whereas Kaplan-Meier estimators, log-rank tests and Cox regression models were used for time-to-event analysis.

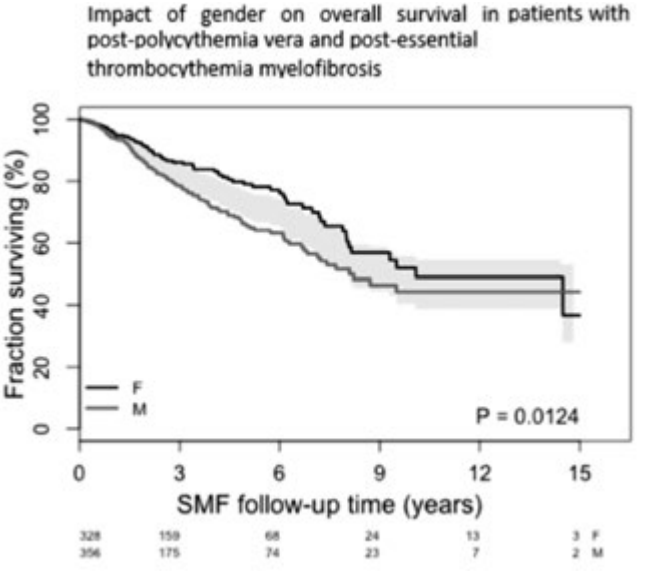


Figure 1.

Results: Within the whole cohort of 781 SMF patients, 684 had the driver mutational status available. Overall, 328 patients were female (48%): the female/male ratio was 0.92. The cohort consisted of 332 PET-MF and 352 PPV-MF, of which 167 (50%) and 161 (46%) females, respectively (p=0.23). The driver mutational status of females was *JAK2* in 257 (78%), *CALR* in 47 (14%), *MPL* in 17 (5%), triple negative in 7 (TN, 2%). Within PET-MF cohort, the distribution of driver mutations in females was as follow: 96 *JAK2*-pos (57%), 47 *CALR*-pos (28%), 17 *MPL*-pos (10%), 7 TN (4%). No significant differences have been found respect to males (p=0.57). Female gender distribution per genotypes was as follows: 46% in *JAK2*-pos PPV MF, 53% in *JAK2*-pos PET MF, 46% in *CALR*-pos, 57% in *MPL*-pos, 37% in TN cases (P =0.9). In the whole cohort, female sex was associated

with lower hemoglobin values (p=0.036), higher platelet count (p=0.041), smaller spleen size (p=0.016). Within the PET-MF cohort, female sex was significantly associated with smaller spleen size (p=0.024) and higher LDH values (p=0.021) and lower frequency of peripheral blood blasts ≥ 1% (p=0.027). No significant gender-related differences were found in terms of thrombotic events and leukemic transformation during follow-up. It is intriguing to observe that diagnosis of ET and PV occurred at younger age in females when compared to males (median 50 *vs.* 53 years, p=0.027), but the time to SMF progression resulted longer in females (median 11.3 *vs.* 10.1 years, p=0.015), eventually resulting in a superimposable age at the time of SMF transformation between gender (median 63 *vs.* 65 years, p=0.23). Overall survival from diagnosis of SMF was significantly inferior in male patients (p=0.012, log rank test, Figure 1), even after adjusting for age (HR 1.30, 95% CI 0.87-1.94; p=0.03, Cox regression model). When adding gender to the MYSEC-PM risk categories, we obtained a minor improvement of survival prediction (HR 1.36, 95% CI: 1.0-1.9; p=0.078).
Summary/Conclusion: Observations from the current study suggest that women are characterized by a specific phenotype and superior survival. Although the underlying causes remain unknown, host factors such as hormonal influences, occupational exposures, and lifestyle factors could influence the pathogenesis of MPN.

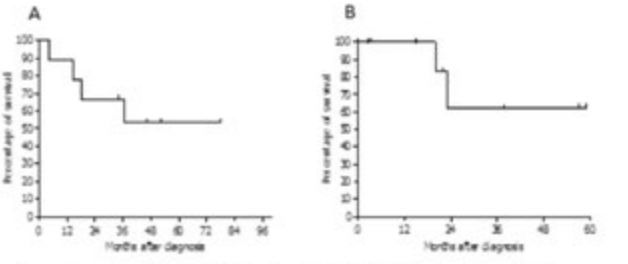
PS1356

TARGETED TREATMENT WITH DIVERSE TYROSINE KINASE INHIBITORS IN PATIENTS WITH PCM1-JAK2, BCR-JAK2 AND ETV6-ABL1 POSITIVE EOSINOPHILIA-ASSOCIATED MYELOID/LYMPHOID NEOPLASMS

G. Metzgeroth[1,*], J. Schwaab[1], N. Naumann[1], J. Luebke[1], M. Jawhar[1], A. Virchis[2], T. Somervaille[3], M. Williams[3], B. Schönberger[4], R. Frewin[5], P. J. Jost[6], A. Maschke[7], P. Keller[8], F. S. Lichtenegger[9], J. Dirk[10], K. Kratz-Albers[11], D. Kraemer[12], S. Mayer[13], N. Storch[14], A. Burchert[15], A.S. Renteria[16], P. La Rosée[17], T. Haferlach[18], H.-P. Horny[19], A. Fabarius[1], C. Haferlach[18], N.C.P. Cross[20,21], W.-K. Hofmann[1], A. Reiter[1]

[1]Department of Hematology and Oncology, University Medical Centre Mannheim, Heidelberg University, Mannheim, Germany, [2]Department of Haematology, The Royal Free & University College School of Medicine, Royal Free Campus, Universitiy College, London, [3]Department of Haematology, The Christie NHS Foundation Trust, Manchester, United Kingdom, [4]Center for Hematology and Oncology, Neuburg a.d. Donau, Germany, [5]Pathology Department, Gloucester Royal Hospital, Gloucester, United Kingdom, [6]III. Medical Department of Hematology and Oncology, Klinikum rechts der Isar, Technical University Munich, Munich, [7]Clinic for Hematology and Oncology, Helios Hospital Berlin-Buch, Berlin, Germany, [8]University Clinic of Hematology and Central Hematology Laboratory, University Hospital Bern, Bern, Switzerland, [9]Department of Hematology and Oncology, University Medical Centre Munich, Munich, [10]Clinic for Hematology and Oncology, DKD, Helios Hospital Wiesbaden, Wiesbaden, [11]Center for Hematology and Oncology, Münster, [12]Department of Hematology and Oncology, Medical Centre Oldenburg, Oldenburg, [13]Department of Hematology and Oncology, University of Regensburg, Regensburg, [14]Department of Hematology and Oncology, St. Vincenz Medical Centre, Limburg, [15]Department of Hematology, Oncology and Immunology, University of Giessen and Marburg, Marburg, Germany, [16]Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, United States, [17]Schwarzwald-Baar Klinikum, Villingen-Schwenningen, [18]Munich Leukemia Laboratory, [19]Pathology Department, University of Munich, Munich, Germany, [20]Wessex Regional Genetics Laboratory, Salisbury, [21]Faculty of Medicine, University of Southampton, Southampton, United Kingdom

Background: More than 70 different tyrosine kinase (TK) fusion genes have been identified in clinically and morphologically distinct eosinophilia-associated myeloid/lymphoid neoplasms (MLN-eo) in chronic or blast phase (BP). Targeted treatment with TK inhibitors (TKI) may be highly effective, e.g. rapid and durable complete remissions on imatinib in patients with *PDGFRA* or *PDGFRB* fusion genes. Patients with fusions involving other TK tend to have more aggressive phenotypes, but clinically significant responses have been reported with ruxolitinib (ruxo) for cases with *JAK2*, e.g. *PCM1-JAK2* in t(8;9)(p22;p24), and imatinib/nilotinib/dasatinib (nilo/ima/dasa) for cases with *ETV6-ABL1* fusions in t(9;12)(q34;p13).
Aims: We here report on phenotype and clinical course of 18 patients who have been treated with ruxo (*PCM1-JAK2*, n=8; *BCR-JAK2*, n=1) or ima/nilo/dasa (*ETV6-ABL1*, n=9), respectively.
Methods: -
Results: Figure 1 A. *PCM1*- and *BCR-JAK2* fusion genes. The median age was 63 years (range 29-73), 8/9 patients were male. Significant clinical findings included leukocytosis (9/9), eosinophilia ≥1.5x10^9/l (5/8) and splenomegaly (5/9), and in the bone marrow abnormal erythropoiesis (6/8) and marked fibrosis (7/8). At diagnosis, 2/9 patients were in BP. On ruxo (median 24 months, range 2-36), 5/9 patients achieved a complete hematologic remission (CHR) after a median of 4 months (range 2-18), and 2 of those patients even a complete cytogenetic remission (CCR). Five patients underwent allogeneic stem cell transplantation (allo SCT) because of loss of CHR (n=1), loss of CCR (n=1), clonal evolution (n=1) or as planned prior to ruxo treatment (n=2). Following allo SCT, 2 patients died due to a high-grade B-cell lymphoma with complex karyotype at month +5 or due to ruxo-resistant relapse at month +6 in a patient with initial diagnosis of BP. One patient developed a Hodgkin's lymphoma at month +43 after allo SCT while in CCR. After a median of 37 months (range 4-78) from diagnosis, 4/9 patients are alive after allo SCT (n=3) or on ruxo only (n=1), respectively. Figure 1B. *ETV6-ABL1* fusion gene. The median age was 46 years (range 20-68), 7/9 patients were male. Significant clinical findings included leukocytosis (6/7), eosinophilia ≥1.5x10^9/l (9/9) and splenomegaly (4/6). At diagnosis, 3/9 patients were in BP. All patients received a TKI up-front (ima, n=5; nilo, n=3; dasa, n=1). On ima, 4/5 patients had no response or had progressive disease while 3/4 patients on nilo (n=2) or dasa (n=1) achieved at least a CHR after a median of 5 months (range 2-18). In due course, 6/9 patients (ima, n=5; nilo, n=1) were switched to nilo or dasa because of inadequate response or progressive disease. Two patients underwent allo SCT while in CCR or because of progressive disease. After a median of 23 months (range 3-60) from diagnosis, 2 patients died because of resistant disease, 1 patient is alive with progressive disease and 6 patients are in CCR or complete molecular remission on dasa (n=3), nilo (n=2) or following allo SCT (n=1).



Overall survival (A) of 9 patients with a *PCM1-JAK2* or *BCR- JAK2* fusion gene (median follow-up 37 months, range 4-78) and (B) 9 patients with an *ETV6-ABL1* fusion gene (median follow-up 23 months, range 3-60), respectively.

Figure 1.

Summary/Conclusion: a) Patients with MLN-eo and *PCM1-JAK2*, *BCR-JAK2* or *ETV6-ABL* fusion genes are predominantly male, do not always present with eosinophilia and have an aggressive clinical phenotype, b) treatment with TKI (ruxo for *JAK2* and ima/nilo/dasa for *ABL1* fusion genes) can induce responses, however, lack of response or resistance may be observed in a significant proportion of patients, d) nilo and dasa should be preferred in *ETV6-ABL1* positive MPN-eo and e) allo SCT should be considered in all eligible patients but optimal timing remains to be defined.

PS1357

MANAGEMENT AND OUTCOME OF 25 PREGNANCIES IN WOMEN WITH POLYCYTHAEMIA VERA

I. Bertozzi[1,*], E. Rumi[2,3], C. Cavalloni[3], M. Cazzola[2,3], F. Fabris[1], M.L. Randi[1]

[1]Department of Internal Medicine - DIMED, University of Padova, Padova, [2]Department of Molecular Medicine, University of Pavia, [3]Department of Hematology Oncology, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo, Pavia, Italy

Background: Polycythaemia Vera (PV) may occur in women of childbearing age, although more rarely than Essential Thrombocythemia (ET). Pregnancy may therefore be an issue in the clinical management of rare young women with PV. In previous papers, we observed that about 1/3 of pregnancies in ET patients ends with an abortion and that there is an association between JAK2V617F mutation and late foetal loss. Data about pregnancy outcomes in PV are lacking. The largest series so far published reports 18 pregnancies in 8 PV patients with an overall rate of healthy neonates of 61% and mother complications of 22.2% (Robinson *et al.* Haematologica 2005). Then we found interesting to describe our experience in 25 pregnancies in PV.
Aims: We retrospectively analysed pregnancy outcomes and complications in our cohort of 15 young females with PV.
Methods: Our study includes 25 pregnancies in 15 females affected by PV (mean age 29.5±7.8 y at diagnosis and 34.3±3.9 y at conception; median follow-up 17 y range 2.9-33.3) who were diagnosed and followed between

1984 and 2017 at the First Medical Clinic of Department of Medicine, University of Padova and the Department of Hematology Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia. PV diagnosis has been performed or revised in agreement with WHO 2016 criteria. All but one of our 15 patients had available JAK2 mutational status: 12 (85.7%) carried V617F (mean allele burden 36±19%) and 2 carried Exon 12 mutation. Therapeutic approach has been assessed. The Fisher's exact test was adopted for the comparison of categorical variables.
Results: Within our 15 PV patients, 7 (28%) had one, 6 had two and 2 had three pregnancies. One pregnancy, ended by voluntary abortion, was excluded from the analysis of pregnancy outcome. Three out of our 15 patients developed multiple abortion during follow-up. Among 24 pregnancies, 10 (41.7%) ended with a full term and 5 (20.8%) with preterm delivery, while 9 (37.5%) were complicated by foetal loss (5 in the first-trimester, 2 in the second and 2 in the third), 4 (16.7%) by maternal complications, and 1 (5%) by intrauterine growth retardation. Each of the 2 patients with Exon 12 mutation experienced one pregnancy without complications. The total live birth rate was 62.5%. All patients received phlebotomies as required. One patient has been administered with IFN-a during both pregnancies. Eleven patients (73.3%) received antithrombotic drugs: 9 received low-dose aspirin during 16 pregnancies, 2 received low-dose aspirin and LMWH during 3 pregnancies.
Summary/Conclusion: On the best of our knowledge, this is the largest series of pregnancies described in PV patients. Our data shows that PV patients develop pregnancy complication in about 1/3 of cases, similarly to that reported by us for ET, and the live birth rate is in agreement with the paper of Robinson *et al.*; however maternal complications seem less frequent in our cohort. The high rate of late foetal loss that we have observed (16.7%) could be related to the presence of JAK2V617F mutation as described in ET. Interestingly the two patients carrying Exon12 mutation had both uneventful pregnancies. While most of our patients received antiplatelet drugs during pregnancy and post-partum LMWH, all but one was managed before the CYTO-PV study and therefore haematocrit was not aggressively controlled. Considering that at present the therapeutic approach in PV is more aggressive, we can surmise a future improvement in the outcome of pregnancy in PV patients.

PS1358

PEGYLATED INTERFERON ALPHA-2B VERSUS ALPHA-2A FOR THE TREATMENT OF ESSENTIAL THROMBOCYTHEMIA: EFFICACY AND SAFETY PROFILE

N. Pugliese[1,*], I. Cappuccio[1], A. Casoria[1], M. Raimondo[1], L. Simeone[1], G. Campagna[1], D. De Novellis[1], F. Pane[1], V. Martinelli[1]

[1]University of Naples Federico II, Naples, Italy

Background: Interferon-alfa (IFN-α) is a non-leukemogenic and effective therapy in controlling platelet count in patients with Essential Thrombocythemia (ET). However, this therapy is associated with significant adverse events and a discontinuation rate greater than 20%. Some studies demonstrated that PEGylated interferon α-2b and α-2a in ET (PEG-IFN-α-2b and PEG-IFN-α-2a) has a superior pharmacokinetic and toxicity profile compared to IFN- α. PEG-IFN has effectively been used to induce hematologic and molecular responses in ET patients. To the best of our knowledge, no study has been performed to compare the efficacy and safety of PEG-IFN-α-2a and PEG-IFN-α-2b.
Aims: The aim of our study was to compare the efficacy and toxicity profile of two formulations of PEG-IFN for the treatment of ET patients.
Methods: Patients with ET treated at our Department, Hematology of Federico II University of Naples, with PEG-IFNα-2b subcutaneously at a starting dose of 50 or 80 μg weekly, or with PEG-IFNα-2a, subcutaneously at a starting dose of 135μg weekly were examined. Therapeutic response was calculated by the revised ELN/IWG-MRT criteria including complete remission (CR), partial remission (PR) and no response (NR). Toxicity to therapy was assessed using the CTCAE 4.0 criteria.
Results: From January 2003 to January 2018, fifty-five patients received PEG-IFNα-2b, whereas forty patients received PEG-IFNα-2a. The median follow-up time was 52.1 months for the PEG-IFNα-2b group vs 38 months for the PEG-IFNα-2a group. A total of 41 (74.5%) patients in the PEG-IFNα-2b group achieved CR, as compared with 36 (90%) in the PEG-IFNα-2a group (p=0.06), the median time to achieve CR was 1 month for both groups. PR rate was higher in the PEG-IFNα-2b group compared with the PEG-IFNα-2a group (20% vs 2.5%, p=0.01), NR rate was similar (PEG-IFNα-2b 5.5% vs PEG-IFNα-2a 7.5%, p=0.79). Eleven patients who received PEG-IFNα-2b (20%) and one patient who received PEGIFN-α-2a (4.3%) discontinued the treatment because of flu like symptoms, p=0.06.
Non-hematologic adverse events of any grade occurred more frequently in the PEG-IFNα-2b group compared with PEG-IFNα-2a group: flu like symptoms (70.9% vs 57.5%, p<0.001), fatigue (58.2% vs 35%, p<0.026), injection site reaction (36.4% vs 20%, p=0.081) and autoimmune disorders (25.5% vs 20%, p=0.12), (predominantly grade 1 or 2). Lymphopenia, neutropenia and anemia were the most frequent hematologic adverse events (predominantly grade 1 or 2), and in no case have they represented a reason for treatment discontinuation.
Summary/Conclusion: Our data confirmed the efficacy and safety of both PEG-IFN for ET treatment. No difference was found regarding the CR rate between the two formulations, even if PEG-IFNα-2a showed a slightly higher rate of CR. Furthermore, PEG-IFN α-2a seems to have a better tolerability profile compared with PEG-IFNα-2b, leading to a low discontinuation rate due to adverse events.

PS1359

LIMITATIONS OF PREVIOUS PROGNOSTIC MODELS FOR THROMBOSIS AND EXPLORATION OF MODIFIED MODELS IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

Y. Hashimoto[1,*], H. Nakamae[2], T. Tanaka[1], H. Omura[1], T. Fukuta[1], M. Horiuchi[3], T. Yoshimura[3], T. Takakuwa[4], A. Mugitani[5], A. Hirose[2], M. Nakamae[2], H. Koh[2], M. Hino[2]

[1]Department of Hematology, Tottori Prefectural Central Hospital, Tottori, [2]Hematology, Graduate School of Medicine, Osaka City University, [3]Department of Hematology, Osaka City General Hospital, [4]Department of Hematology, Saiseikai Nakatsu Hospital, [5]Department of Hematology, Seichokai Fuchu Hospital, Osaka, Japan

Background: Although life expectancy of essential thrombocythemia (ET) patients is similar to that of the general population, thrombotic complications have reported to be the main causes of death in those. Therefore, the focus of treatment is prevention of complication by thrombosis. Regarding thrombosis risk stratification, conventional system is widely used in general, but recently the International Prognostic Score of thrombosis for ET (IPSET-T) and the revised IPSET-T were developed. These prognostic models are useful risk stratifications, however, the revised IPSET-T classifies young patients positive for the *JAK2* mutation, the high incidence of thrombotic events, into the low-risk category. Hence, this model may be underestimated.
Aims: We examined the prognostic factors to validate previous prognostic models for survival and thrombosis with large-scale data on Japanese patients with ET.
Methods: This study included 352 patients diagnosed with ET between 1999 and 2017. Using medical records, we investigated patient background, history of thrombohemorrhagic events (THE), causes of death, THE development after diagnosis, transformation to leukemia or myelofibrosis, and development of secondary cancer.

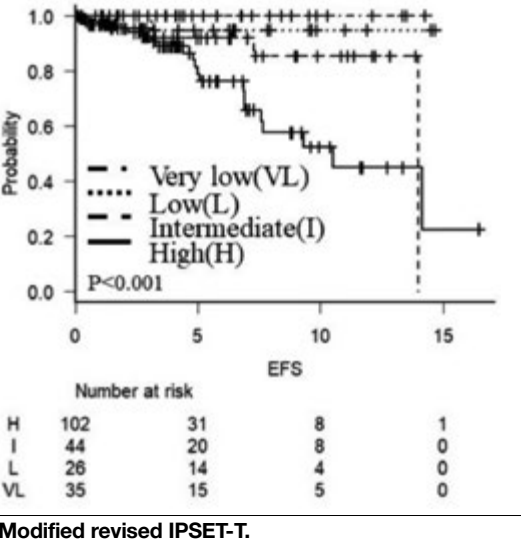


Figure 1. Modified revised IPSET-T.

Results: The median age at diagnosis was 67.0 years (range, 23-92 years). The median leukocyte count was 9.9×10^9/L; hemoglobin level, 13.5 g/dL; and platelet count, 873×10^9/L. Antiplatelet drugs were used in 69% of patients. Of the 207 patients screened for *JAK2* mutation, 139 (67%) showed positive results. Approximately 24.4% of patients had history of THE, and 13.1% and 5.1% developed THE and transformation after diag-

nosis, respectively. The reproducibility of the IPSET-survival, conventional system, IPSET-T, and the revised IPSET-T was also confirmed in Japanese patients. No significant differences were observed between patients with low scores and those with intermediate scores on the revised IPSET-T, and a comparison of patients receiving cytoreductive therapy showed that the incidence of thrombotic events was higher in those with low scores. Thus, the modified revised IPSET-T assigns 1 point for age >60 years, 2 points for history of thrombosis, and 2 points for *JAK2* mutation positivity; patients were stratified into four risk categories by total score: very low, 0 point; low, 1 point; intermediate, 2 points; and high, ≥3 points. The results demonstrated that the modified revised IPSET-T might be more useful to predict the need for treatment interventions in patients with ET than the original revised IPSET-T (Figure 1).

Summary/Conclusion: Although the revised IPSET-T is a superior risk stratification model, incidence rates of thrombotic events tended to reverse between low- and intermediate-risk categories due to the differences in treatment details. Thus, it cannot be used to compare the risk categories. The revised IPSET-T classifies 10-20% of patients with a high score on the IPSET-T into the low-risk category. They are young patients positive for the *JAK2* mutation. Attention should be paid to the high incidence of thrombotic events in these patients. Previous studies reported that advanced age, history of thrombosis, and *JAK2* mutation are strong risk factors for thrombosis. The modified revised IPSET-T allows subdivision of the risk categories on IPSET-T. Further studies with a large sample size are needed to assess the usefulness of the modified revised IPSET-T and identify patients who require antiplatelet or cytoreductive therapy.

PS1360

SERVICE EVALUATION OF PATIENTS WITH MYELOPROLIFERATIVE NEOPLASM AND SPLANCHNIC VEIN THROMBOSIS (MPN-SVT)

S. Wharin[1,*], J. Zheng[2], H. Network[3], R. Shaw[4], A. Zaidi[5], M. Desborough[6], N. M. Butt[7], M. Sekhar[2]

[1]Haematology, University Hospitals of Leicester, Leicester, [2]Haematology, Royal Free Hospital, London, [3]Haematology, National Institute Health Research Clinical Research Network, Oxford, [4]Haematology, Royal Liverpool Hospital, Liverpool, [5]Haematology, Royal London Hospital, London, [6]Haematology, Milton Keynes Hospital, Milton Keynes, [7]Royal Liverpool Hospital, Liverpool, United Kingdom

Background: SVT comprises thrombosis of hepatic vein (Budd Chiari syndrome, BCS), portal vein (PV), superior mesenteric vein (SMV) and splenic vein (SpV). The clinical and service needs of patients with MPN-SVT are complex. There is no systematic process in U.K. to assess clinical pathways and outcomes of these patients. A survey of MPN-SVT patients was undertaken at 6 sites using a validated database.

Aims: Assess feasibility of using a registry to assess outcome, clinical and service needs of MPN-SVT patients in U.K.

Methods: Between Nov 2017 and Feb 2018 patients with MPN-SVT at 6 hospitals over the past 8 years were identified. 5 hospitals were centres with specialist Haematology and interventional radiology, 1 was a regional liver transplant centre. Patient data were anonymised and analysed centrally. All data were collected by Haematology trainees via the NIHR HaemSTAR network. Service outputs were recorded including radiology and endoscopy procedures and time to referral to expert centres. User feedback was sought.

Table 1. MPN diagnosis and cytoreductive treatment.

Diagnosis	Number	HC	Rux	PEGIFN	None	Unknown
PV	9	6		1	2	
ET	3	2		1		
PMF	3	1	2			
PPV-MF	4	1	2	1		
PPV-ET	1		1			
MPN-U	11	3				8

Results: Data was obtained for 31 patients from 5 pilot centres; 16 males, 15 females. Age range 25-72 years, median 45. *MPN:* MPN diagnoses cytoreduction is shown in Table 1. 'MPN-U' includes 10 patients with *JAK2*V617f without haematological evidence of MPN. 19 patients presented with SVT. Mean time from SVT to diagnosis of MPN was 223 days (range 2- 912, median 75). *SVT:* 27 patients had PVT, 9 SpVT, 11 SMVT, 10 BCS. 15 developed varices, 6 had variceal bleeding, 9 required banding. 20 patients received warfarin, 3 warfarin+aspirin, 2 heparin, 2 DOACS, 1 antiplatelets only, 2 did not receive anticoagulation. 6 patients had a TIPPS procedure, 4 required dilation. 2 patients had a liver transplant. 26% achieved thrombus resolution or recanalization, 13% extension or recurrence, 61% had stable disease. *Patient pathways and health resources*: 24 patients were managed at an expert centre. Mean time to referral was 40 weeks (range 0- 416, median 1). Mean number of imaging episodes per patient was 5 (range 1-24, median 4). *Feasibility of database:* Case ascertainment at 4/6 sites was incomplete. Chief obstacles were lack of systematic registering of MPN-SVT patients and inaccessibility to IT infrastructure. Data was incomplete in all cases. User feedback indicated that the database was lengthy and complex. *Limitations:* 70% of participants were from one transplant centre, therefore not representative of UK practice overall.

Summary/Conclusion: Our study was initiated to test the feasibility of a national MPN-SVT registry to inform practice. The results demonstrate variation in patient pathway with respect to specialist referral anticoagulation and cytoreductive practices, reflecting lack of consensus in management of patients. MPN-SVT is a rare but serious, resource intensive complication of MPNs. Retrospective collection of data poses problems of quality and completeness. A prospective national registry with a concise dataset is likely to yield important data to address these needs.

PS1361

FAMILIAL MYELOPROLIFERATIVE NEOPLASMS: A SINGLE-INSTITUTION ANALYSIS OF 22 FAMILIES

M. Maffioli[1,*], B. Mora[1], D. Barraco[1], D. Caramazza[1], L. Elli[2], R. Accetta[2], A. Bussini[2], F. Pallotti[2,3], S. Uccella[4], R. Casalone[2], T. Giorgino[5], F. Passamonti[13]

[1]Hematology, Department of Specialist Medicine, Ospedale di Circolo, ASST Sette Laghi, Varese, Italy, [2]Cytogenetics and Medical Genetics Laboratory, ASST Sette Laghi, Varese, Italy, Varese, [3]Department of Medicine and Surgery, University of Insubria, Varese, Italy, [4]Department of Medicine and Surgery, Unit of Pathology, University of Insubria, Varese, Italy, [5]Biophysics Institute, National Research Council of Italy, Milan, Italy

Background: The majority of myeloproliferative neoplasms (MPNs), including essential thrombocythemia (ET), polycythemia vera (PV), primary myelofibrosis (PMF), and chronic myeloid leukemia (CML), have a sporadic occurrence. However, around 10% of MPNs are characterized as familial.

Aims: Familial MPNs constitute a rare opportunity to gain insight into MPN genetics. We therefore set out to characterize a cohort of familial MPN patients followed at our Institution.

Methods: We reviewed our institutional MPN database to identify familial cases (i.e., pedigrees with ≥2 subjects with an MPN). The nonparametric Wilcoxon signed rank test was used to compare age at diagnosis. The study was approved by the Institutional Ethics Committee; procedures are in accordance with the Helsinki Declaration.

Results: We identified 22 pedigrees with familial MPN, for a total of 45 MPN patients. Four pedigrees also included an individual with a non-MPN hematologic neoplasm (multiple myeloma, 1; Hodgkin lymphoma, 1; leukemia not otherwise specified, 1; acute myeloid leukemia, 1). Median follow-up of the whole cohort was 9.2 years (range, 1.2-22.0 years). The clinical and molecular features of analyzed patients are summarized in Table 1. Overall, MPN occurrence was vertical in 17 families (all but one with two consecutive generations involved), whereas in 5 families MPN cases were documented in a single generation. In families with vertical occurrence, patients of the second generation almost invariably had a younger age at diagnosis than those of the first generation (median 35 vs 65 years; paired Wilcoxon signed rank test p=0.002). Six out of seven patients with a thrombotic event were *JAK2*V617F-positive (vs 17/26 without a thrombosis). The Figure 1 reports a pedigree with phenotypically and genotypically homogeneous vertical MPN occurrence with a skipped generation (proband and grandfather both affected with *JAK2*V617F-positive PV with a splanchnic vein thrombosis – SVT – at diagnosis; mother unmutated for *JAK2* and with normal blood counts; 36-year-old sister without an MPN-phenotype but harboring *JAK2*V617F with an allele burden of 3%). Lastly, NGS analyses are ongoing in 24 subjects with available DNA (of which 4 familial pairs).

Summary/Conclusion: Within our cohort of 22 familial MPN pedigrees disease phenotype and genotype are heterogeneously distributed and occurrence is more frequently vertical. Disease anticipation is confirmed in our cases. Of great interest for perspective considerations is the family with two *JAK2*V617F-positive PV patients both experiencing SVT. It is intriguing to speculate as to whether an MPN-independent inherited genetic factor could have played a role in the pathogenesis of the thrombotic events and how this genetic event might interplay with MPN genotypes. A second point of interest is the *JAK2*V617F-positive subject without an MPN phenotype, especially considering the role of *JAK2* mutations in the context of clonal hematopoiesis of indeterminate potential.

Table 1. Clinical and molecular features of familial MPN patients (n = 45, 22 pedigrees).

Diagnosis WHO 2017: ET/PV/Pre-PMF/Overt PMF/CML/MPN-U		25 / 11 / 1 / 4 / 3 / 1
Driver mutations (n/genotyped)	- *JAK2*V617F+	27/32
	- *CALR* mut	2/12 (both Type 1)
	- *MPL* mut	2/7 (both W515L)
	- Triple negative	1/7
Homogeneous vs heterogeneous MPN phenotype within pedigree		10 vs 12
Vertical vs single generation occurrence		17 vs 5
Thrombotic events		12/38
Transformation to MF		3/38 (1 subseq. AML)
Transformation to accelerated/blast phase		5/38 (2 CML-BP)

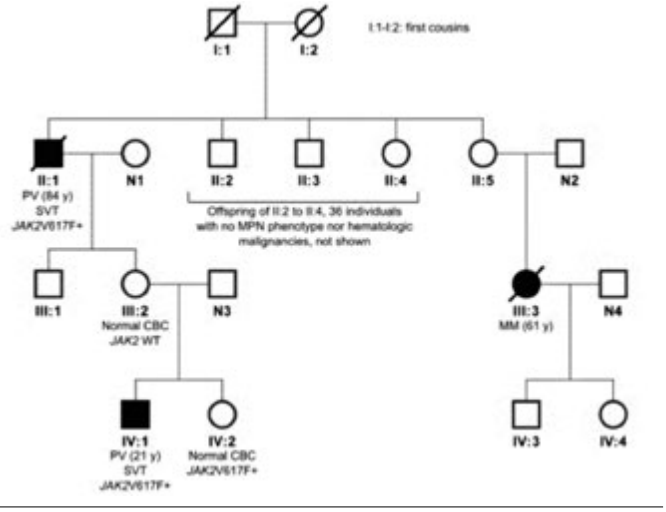


Figure 1.

PS1362

THROMBOEMBOLIC EVENTS AFTER DIAGNOSIS OF POLYCYTHAEMIA VERA IN A SINGLE CENTER POPULATION

N. Curto-Garcia[1,*], J.C. Ianotto[2], J. O'Sullivan[3], P. Harrington[1], Y. Francis[1], C. Woodley[1], D. Mclornan[1], D. Radia[1], C. Harrison[1]

[1]Haematology , Guy's and St Thomas NHS Foundation Trust, [2]Haematology , Guy's and St Thomas Foundation Trust, London , [3]Haematology , Guy's and St Thomas NHS Foundation Trust , Londonf, United Kingdom

Background: Polycythaemia vera (PV), a myeloproliferative neoplasm associated with *JAK2*V617F and Exon 12 mutations, usually affects patients >60 years old, and is associated with thromboembolic (TE) or bleeding events; and symptoms including hyperviscosity, erythromelalgia or pruritus. Progression to myelofibrosis and acute leukaemia can also occur. Studies suggest the incidence of TE events post diagnosis is up to 22% thus representing a significant problem for patients in terms of morbidity and mortality.
Aims: We report a large contemporaneous cohort of PV patients from a single centre describing TE occurring after diagnosis.
Methods: 220 patients were analysed (116 male), mean age of patients at time of diagnosis was 52 years (range 24-83).
Results: Cardiovascular (CV) risk factors were present in 106/220 (48%) of PV population, 28% patients had hypertension, 9% diabetes mellitus and 10% hypercholesterolaemia. Mean follow up was 91 months (range 2-324). Considering TE overall 94 patients had TE at any time, 42/220 of the total population (20%) experienced 50 TE events after PV diagnosis including 14 recurrent events (2 events in 5 patients and 4 in 1). Mean age at time of TE was 59 years (range 28-86). Concerning timing of TE the median time was 58 months (CI 95% (38-77) and 74% of TE had occurred by 120 months. The annual incidence of post diagnosis TE was 1.9% patient/year. CV factors were present in 15/42(35.7%) and the patient with 4 recurrent events had complicated diabetes with severe neuropathy and nephropathy, below knee amputation due to vascular disease and a renal transplant. The most frequent post diagnosis TE was in fact abdominal thrombosis (12/50), followed by cerebrovascular accident (7/50), myocardial infarct (7/50) and deep venous thrombosis (6/50). Immediately prior to TE for 26/50 events, patients were on cytoreductive agents (CRT) and antithrombotic drugs, mainly hydroxycarbamide (HC=15), ruxolitinib (Rux=4), interferon (IFN=3); 18/50 had only antithrombotic drugs (aspirin=16). For 15/50 TE events the patients had received a venesection (VS) during the year beforehand (7 had 1; 3 had 2; 4 had 3 and 1 had 4), aspirin was the most common antithrombotic therapy (n=34/50). Concerning haematological response (per European Leukemianet), at time of TE most patients were in complete or partial response (CR=14/42 and PR=19/42). For those patients on CRT (n=16) at time of TE, 10 were in CR, 5 PR, and 1 unknown. Those not on CRT, 2 were in CR, 5 in PR, 2 in NR and 2 unknown. After the occurrence of TE for 35/50 occasions, CRT was unchanged (Figure 1). Regarding recurrent events (n=14), 6 cases happened on CRT, VS and antithrombotic drugs immediately prior to event (HC=3,Rux=2, Busulfan=1), whereas 7 TE occurred without CRT; but 3/7 were treated with VS. Only 5/220 patients died during follow up and no deaths were related to TE.

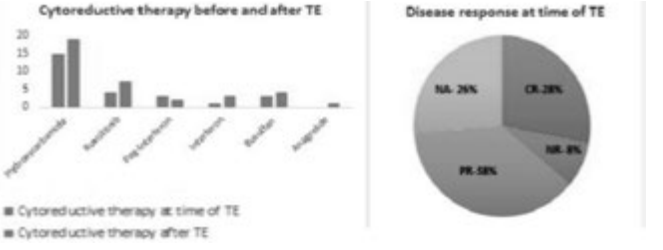


Figure 1.

Summary/Conclusion: Despite current treatments with high rates of overall responses (CR & PR per ELN), TE are a frequent complication for PV after the time of diagnosis as we have shown in our patient population; only 50% of these events occurred within the first 5 years of diagnosis, interestingly abdominal and arterial thrombosis were equally prevalent. Most patients were either in CR or PR, at the time of TE suggesting that other factors are likely to be relevant in this population in triggering thrombosis. Finally, new therapeutic approaches are required to address thrombosis free survival which remains a significant issue of concern for PV patients.

PS1363

MIPSS70 AND TRANSFUSION DEPENDENCE PREDICT SURVIVAL IN MYELOFIBROSIS PATIENTS TREATED WITH JAK1/2 INHIBITOR THERAPY

C. Mcnamara[1,*], J. Spiegel[2], E. Atenafu[3], D. Maze[1], H. Sibai[1], V. Gupta[1]

[1]Department of Medical Oncology and Hematology , Princess Margaret Cancer Centre, Toronto , Canada, [2]Department of Hematology, Stanford University, Stanford, United States, [3]Department of Biostatistics, Princess Margaret Cancer Centre, Toronto , Canada

Background: Mutation enhanced international prognostic score system for transplantation age patients with primary myelofibrosis (MIPSS70) heralds a new era in the prognostication of patients with myelofibrosis (MF). It is the first classification system to integrate clinical variables, bone marrow histology and the recent advances in molecular knowledge. Namely, the favorable risk of calreticulin (CALR) type 1-like driver mutations and the adverse impact of mutations in high molecular risk (HMR) genes both individually and cumulatively. However, transfusion status was not investigated in the construct of MIPSS70. Transfusion dependence has been independently associated with inferior survival in MF and is incorporated into the dynamic international prognostic scoring system (DIPSS) plus classification. Our group and others have shown that a high-risk DIPSS score, pre-JAK1/2 inhibitor transfusion dependence and mutations in *ASXL1/ EZH2* predicted shorter time to treatment failure (TTF) and overall survival (OS) in patients undergoing JAK1/2 inhibitor therapy.
Aims: To evaluate the impact of transfusion status and MIPSS70 on TTF and OS in MF patients treated with JAK1/2 inhibitor therapy.
Methods: Patients with a diagnosis of MPN in chronic phase and treated with JAK 1/2 inhibitor therapy were identified from the MPN database. MIPSS70 was calculated at the time of starting JAK1/2 inhibitor therapy. Molecular analysis was performed by targeted sequencing of a 54 myeloid gene panel. Univariate and multivariable analysis for TTF and OS was conducted using for the Cox proportional hazards model.
Results: One hundred patients with a diagnosis of MPN in chronic phase (2 pre-fibrotic MF; 48 overt MF; 27 PPV-MF; 23 PET-MF) and treated with JAK 1/2 inhibitor therapy (77 ruxolitinib; 23 momelotinib) were included

in the study. Forty-three patients had at least one mutation consistent with HMR profile and nine patients had ≥2 HMR mutations. CALR type 1-like mutation was present in 10 patients. As expected, this patient cohort had more advanced disease as stratified by MIPSS70 with two patients classified with low-risk, 35 with intermediate-risk and 63 with high-risk. With a median follow-up of 3.0 years, the median survival of the low/ intermediate- and high-risk groups were 6.7 and 3.0 years respectively. High-risk MIPSS70 was associated with inferior OS (HR, 2.28; 95% CI, 1.24-4.19; p=0.01; Figure 1) but not TTF. Pre-treatment transfusion dependence predicted shorter TTF (HR, 2.00; 95% CI, 1.24-3.23; p=0.01) and OS (HR, 2.10; 95% CI, 1.23-3.59; p=0.01) and remained significant on the multivariable analysis. High-risk MIPSS70 patients requiring transfusions had a shorter median survival of 2.5 years compared to 4.6 years in patients who were not requiring transfusions (p=0.002). Older age and abnormal cytogenetics were not predictive of shorter TTF or OS on multivariable analysis.

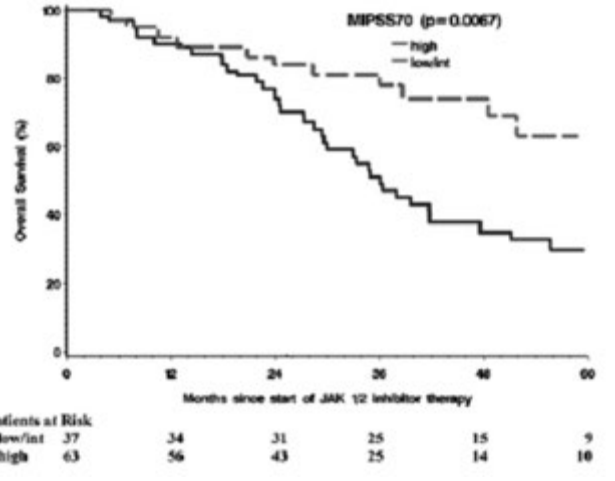


Figure 1.

Summary/Conclusion: Our data validates that MIPSS70 is useful in predicting OS in MF patients treated with JAK1/2 inhibitor therapy. Pre-treatment transfusion dependence is an independent factor for shorter TTF as well as OS. These data suggest that further enhancement of MIPSS70 may be possible by integrating transfusion status into the scoring system.

PS1364

JAK2 ALLELIC RATIO PREDICT HIGHER TROMBOTIC RISK IN MYELOFIBROSIS PATIENTS. A SINGLE CENTER EXPERIENCE ON 143 PATIENTS

M. Farina[1,*], N. Polverelli[2], E. Cerqui[1], F. Schieppati[1], M. D'Adda[1], G. Rossi[1]

[1]Hematology Unit, [2]Unit of Blood Diseases and Stem Cells Transplantation, University of Brescia, ASST Spedali Civili, Brescia, Italy

Background: Thrombosis is a recognized as a major complication in Myelofibrosis (MF) patients, representing the ultimate cause of death in about 10% of patients. So far, few studies have focused on characteristics, outcome and possible risk factors for thrombotic complications in this group of patients.
Aims: To evaluate the characteristic, incidence and prognostic factors for thrombotic events in a cohort of MF patients.
Methods: Clinical and laboratory data of 143 patients, diagnosed with MF from 2000 to 2017 at the Unit of Hematology of ASST Spedali Civili in Brescia were retrospectively collected. A competing-risk analysis was carried out to identify possible risk factors associated to thrombosis.
Results: Overall, 18 out of 143 (13%) patients developed one or more thrombosis after a median follow-up of 44 months (3-484) from diagnosis, for and cumulative incidence of 10.7%, 16.2% and 20.5% at 5, 10 and 20 years, respectively. Thrombosis frequency increased over time: 2.1% of patients developed thrombotic events during the first year, 3.15% between 1 and 2 years, 2% between 2 and 3 years, 11.25% thereafter (p=0.003). Thrombosis were venous in 11 (61%) of cases (6 splancnic, 2 deep venous thrombosis, 2 pulmonary embolism), arterial in 7 cases (3 AMI, 1 stroke, 1 TIA, 2 peripheral arterial occlusioe disease). A MF treatment was ongoing in 15 out of 18 patients (83%) at the time of thrombosis; specifically, ruxolitinib (5) and hydroxyurea (10). Clinical characteristics of the cohort are detailed in Table 1, according the occurrence of thrombotic events. Overall 58 (40.5%) patients received antiplatelet agents after MF diagnosis, in 13 cases (22.4%) after a previous thrombosis. Patients developing thrombosis had more frequently a JAK2 allele burden >75% (p=0.03), and presented a positive history for thrombotic events (p=0.05). A lower antiplatelet use was recorded among patients with thrombosis (p=0.033). At last follow-up 47 (33%) of patients had died. Causes of death were related to: AL evolution in 10 cases (21%), MF progression without evolution in 10 (21%), hemorrhage in 3 (6%), infectious complication in 7 (15%), allogeneic transplant complication in 1 (2%), unrelated 29%. Thrombosis represented the final event in 2 patients (4% of cases). Among clinical parameters, antiplatelet agents (p=0.06), JAK2 allele burden >75% (p=0.015) and previous thrombosis (p=0.084) did correlate with higher thrombotic risk. By multivariable analysis, only JAK2 allele burden maintained the statistical significance in predicting thrombotic complications (HR 4.77, CI95% 1.57-14.5, p=0.0059).

Table 1.

Patients' characteristics according to the occurrence of thrombotic event.

Characteristics	No thrombotic complications (n.125)	Thrombotic complications (n.18)	P
Male sex, n. (%)	78 (62.4%)	13 (72.2%)	0.601
Median age, years (range)	66 (28-85)	66 (26-76)	0.42
Median hemoglobin, g/dl (range)	10.8 (6.7-17.1)	10.9 (8.3-20.2)	0.55
Median leukocyte, x10³/l, (range)	9 (1.1-72.5)	9.9 (2.9-35.5)	0.86
Median platelet, x10³/l (range)	304 (11-1440)	280 (109-721)	0.76
Constitutional symptoms, n. (%)	18 (14.4%)	1 (5.5%)	0.46
Circulating blast cells, n. (%)	28 (22.4%)	3 (16.7%)	1
Palpable splenomegaly, n. (%)	80 (64%)	14 (77.8%)	0.42
PMF, n. (%)	95 (76%)	12 (66.7%)	0.39
Grade 2-3 fibrosis, n. on evaluable pts (%)	91 (72.8%)	16 (88.9%)	0.30
Intermediate2/high IPSS risk	45 (36%)	7 (38.9%)	0.59
Mutational status, n. (%)			
Jak2 mutation, n. (%)	79 (63.2%)	16 (88.9%)	0.095
Jak2 allele ratio >75%, n. (%)	5 (4%)	4 (22.2%)	**0.047**
Antiplatelet therapy, n. (%)	52 (41.6%)	6 (33.3%)	**0.033**
Previous thrombotic complication, n. (%)	22 (17.6%)	7 (38.9%)	**0.05**

Summary/Conclusion: In our experience a higher JAK2 allelic burden is the only predictive factor for thrombotic events in MF patients, confirming previous experiences in Essential Thrombocythemia and Polycythemia Vera. A more strict anti-thrombotic surveillance should be advised in this subset of MF patients.

PS1365

RUXOLITINIB AND HYDROXYUREA COMBINATION FOR THE TREATMENT OF MYELOFIBROSIS

N. Pugliese[1,*], C. Giordano[1], D. Nappi[1], L. Luciano[1], C. Cerchione[1], M. Annunziata[1], B. Casale[2], E. Crisà[3], M. R. Villa[4], L. Pezzullo[5], M. Iovine[6], M. Picardi[1], L. Simeone[7], F. Pane[1], V. Martinelli[1]

[1]University of Naples Federico II, [2]AO Ospedali dei Colli Napoli, PO Monaldi, Naples, Italy, Naples, [3]Università degli Studi del Piemonte Orientale "Amedeo Avogadro", Novara, [4]Ospedale Ascalesi, Naples, Italy, Naples, [5]Hematology and Hematopoietic Stem Cell Transplant Center. A.U.O. "San Giovanni di Dio e Ruggi D'Aragona" Salerno., Salerno, [6]AO Caserta Sant'Anna e San Sebastiano, Caserta, Italy, Caserta, [7]University of Naples Federico II, Nples, Italy

Background: Ruxolitinib, an orally bioavailable and selective inhibitor of JAK1 and JAK2, has been shown to significantly reduce splenomegaly and disease-related symptoms in patients with myelofibrosis (MF). However, no clear survival benefit has been demonstrated, which may in part reflect suboptimal drug exposure related to lower dosages needed to minimize hematological toxicity, specifically cytopenias. Furthermore, the optimal management of specific conditions such as leukocytosis or thrombocytosis is still undefined. Therefore, combining ruxolitinib with a cytoreductive agent like hydroxyurea (HU) may improve hematological response.
Aims: We evaluate the efficacy and safety of ruxolitinib in combination with HU.
Methods: This observational multi-center study analyzed 20 adult patients with a diagnosis of intermediate or high-risk primary MF, post-polycythemia

vera MF, or post-essential thrombocythemia MF requiring the addition of HU to ruxolitinib treatment to control WBC or platelet count. Ruxolitinib was initially given at a dose of 5, 15, or 20 mg twice daily, depending on baseline platelet count, adjusted in response to platelet/neutrophil count and/or lack of efficacy. HU was added when WBC or platelet counts were unsatisfactory. Patients response was assessed considering spleen size, symptoms, WBC and platelets count, according to ELN response criteria.
Results: Initially, all patients received ruxolitinib as monotherapy after a median time from MF diagnosis of 15.9 months (range 1.6–181 months). The starting dose of ruxolitinib as monotherapy was 20 mg BID in the 40% of cases, 15 mg BID in the 35%, 10 mg BID in the 5%, 5 mg BID for the 20%. Three patients temporarily discontinued ruxolitinib administration due to severe thrombocytopenia. 17 patients started HU treatment due to insufficient WBC control and 3 patients for unsatisfactory platelet control. HU was added after a median time of ruxolitinib monotherapy of 6.5 months (range 1–49.6 months). The median time of combination therapy was 14.5 months (range 2–195).
During the co-administration period, two patients temporarily reduced ruxolitinib treatment and only one discontinued ruxolitinib, due to severe thrombocytopenia. On the other hand, 8 patients reduced HU due to hematological toxicity that mostly occurred in the first 8 to 12 weeks of treatment. Toxicity was generally manageable with dose reductions and/or supportive treatment. A symptomatic response was obtained in 6 patients during ruxolitinib monotherapy and in 12 patients during combination ruxolitinib-hydroxyurea. A spleen response was obtained in 5 patients during ruxolitinib monotherapy and 8 patients with combination therapy. After a median duration of 14.5 months of combination therapy, 16/20 patients had a hematological response; 14/17 patients who had started combination therapy to control WBC count and 2/3 who started in order to reduce platelets count. The number of patients requiring ruxolitinib dosage reduction or discontinuations was lower during combination therapy, and ruxolitinib dosage was increased in 50% of patients.
Summary/Conclusion: From our experience, ruxolitinib-hydroxyurea in combination yields a clinical and hematological benefit that did not elicit cumulative or overlapping toxicities. When ruxolitinib resistance, with regard to WBC and platelet counts, is documented in the hyperproliferative phase of MF, the addition of hydroxyurea could improve hematological control and disease-related symptoms and splenomegaly.

PS1366

THE "MYMPN" PATIENT REGISTRY: VALIDATION OF A PROSPECTIVE MYELOPROLIFERATIVE NEOPLASM PATIENT REGISTRY

R. Scherber[1,*], L. Whyte[2], M. Woehrle[2], A. Dueck[3], S. Verstovsek[4], C. Harrison[5], R. Mesa[6]

[1]Hematology and Oncology, Mayo Clinic/Oregon Health and Sciences University, Portland, [2]MPN Research Foundation, Chicago, [3]Biostatistics, Mayo Clinic, Scottsdale, [4]MD Anderson Cancer Center, Houston, United States, [5]Guys St. Thomas, London, United Kingdom, [6]Mays MD Anderson Cancer Center, San Antonio, United States

Background: The myeloproliferative neoplasms (MPNs) are an uncommon hematologic malignancy that affects approximately 350,000 individuals in the United States (Blood. 2012;120(21)A2834). The disease course can be complicated for patients, including severe disease related symptom burden, thrombosis, bleeding, multimodal treatment plans, and risk of disease progression (Blood. 2017 Feb 9; 129(6): 680–92). Disease-related patient registries can be a useful and important tool for rare disease research and surveillance.
Aims: The advisors and board of the MPN Research Foundation aimed to create a prospective patient-reported MPN registry to capture demographics, disease related events, and symptom burden.
Methods: Using the platform of the Genetic Alliance an online registry was created which assessed MPN patients' baseline demographics and disease history with prospective patient-reported disease events and symptom burden. Symptom burden was assessed via the Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN SAF TSS; Blood 2011; 118(2):402-8). Approximately 50 MPN patients were utilized for beta testing of the registry. The registry was approved by the Genetic Alliance IRB prior to launch. The link to join the registry was disseminated among multiple MPN-related websites.
Results: *Accrual:* A total of 454 US MPN patients initiated the online registry process. Of these, 355 individuals completed the introductory demographic and disease information surveys. Respondents were predominantly female (63.7%). Registry enrollment by date since registry initiation is demonstrated in Figure 1a.
Disease-related information: The disease distribution of participants was similar to previously published data in the US, including 45.1% with essential thrombocythemia (ET), 39.9% with polycythemia vera (PV), and 12.6% with myelofibrosis (MF). 318/344 participants endorsed having disease-specific genetic testing. 57.4% of respondents reported having low blood counts, hemorrhage or thrombosis. Most frequent therapies included aspirin (75.5%), hydroxyurea (42.1%), JAK inhibitor (17.6%), interferon (6.0%) or no therapies (9.9%). Thus far, 303 participants prospectively reported 902 disease-related events such as blood draws (37.3%), phlebotomy (14.2%), change in MPN medicines (9.6%), bone marrow biopsies (6.9%), transfusion (4.8%), unplanned hospital stays (4.5%), thrombotic or bleeding events (2.4%), or pregnancy (0.2%).
Disease Symptom Burden: At the time of data analysis, 250 participants completed a 418 MPN-10 symptom surveys. Mean MPN10 scores were similar to previously published results for ET and PV (Figure 1b-c). Analysis to compare symptom scores for MF are ongoing.

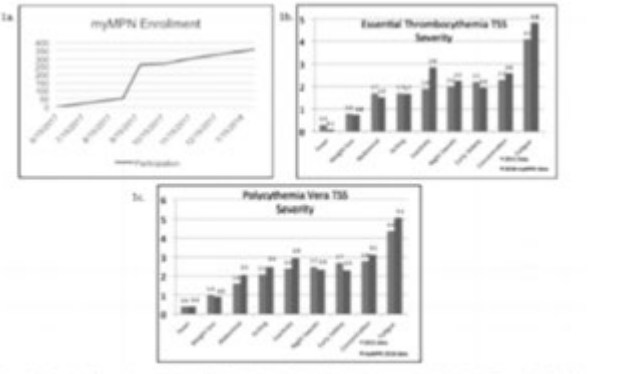


Figure 1a: Number of registrants in the MPN patient registry by date since registry initiation. Figures 1b-1c: Average MPN SAF TSS symptom score item severity as compared to previous MPN cohorts by disease subtype for essential thrombocythemia and polycythemia vera (Blood 2011; 118(2):402-8).

Figure 1.

Summary/Conclusion: An MPN patient registry has the capabilities of addressing key critical questions regarding MPN patient symptom and treatment needs. Its dynamic nature allows researchers and patient advocates to prospectively connect with individual patients regarding additional information and unmet needs in this rare population. At this time, registration is only available to individuals in the United States, although ongoing efforts are planned to recruit English-speaking MPN patients from the United Kingdom, Australia, New Zealand, and Canada. Further efforts in regards to grow patient registry enrollment and disease questionnaires are ongoing.

PS1367

THE RELATIONSHIP OF BODY MASS INDEX TO SYMPTOM BURDEN IN THE MYELOPROLIFERATIVE NEOPLASMS

R. Scherber[1,*], B. Langlais[2], H. Geyer[3], A. Dueck[2], J. Huberty[4], L. Padrnos[5], J. Palmer[5], A. Fleischman[6], R. Mesa[7]

[1]Hematology and Oncology, Mayo Clinic/Oregon Health and Sciences University, Portland, [2]Biostatistics, [3]Internal Medicine, Mayo Clinic, Scottsdale, [4]Arizona State University, [5]Hematology and Oncology, Mayo Clinic, Phoenix, [6]Hematology and Oncology, University of Califonia Irvine, Irvine, [7]Mays MD Anderson Cancer Center, San Antonio, United States

Background: The myeloproliferative neoplasms (MPNs) are a chronic hematologic malignancy with elevated symptom burden (i.e., fatigue, pain) and significantly reduced lifespan compared to general populations (JCO. 2012; 30(24):2995-3001). Elevated body mass index (BMI) is a risk factor for MPN disease development (Int. J. Cancer. 2014;134:1741–50), but little information is available on the relevance of BMI in MPN symptomatology. Understanding the relevance of BMI to symptoms may help to guide future efforts regarding disease development, progression, symptom burden or QOL.
Aims: To investigate the role of BMI in MPN related symptom burden.
Methods: This was a cross-sectional study. We used data from NUTRIENT, a large international survey conducted in MPN patients (Blood, 2017) to investigate nutrition, supplement use, and symptoms. This secondary analysis investigates the relationship of BMI to disease characteristics and symptom burden. Symptom burden was assessed using the validated MPN10 symptom assessment (JCO. 2012;30(33):4098-103).
Results: *Study group*: A total of 1,025 surveys had complete data on MPN type, weight, height, and symptom burden. MPN types included essential

thrombocythemia (ET; n=392), polycythemia vera (PV; n=390), and myelofibrosis (MF; n=243). Patients were of typical median age (60.0 years) and female predominance (74.9%) of prior MPN patient symptom investigations. *MPN Characteristics and BMI:* Overall self-reported mean BMI of respondents was overweight (25.9, SD=5.13). The distribution of respondent weight categories included 2.8% underweight, 45.7% normal weight, 32.4% overweight, and 19.1% obese. Mean BMI was not significantly different among MPN subtypes (25.8 ET, 26.3 PV, and 25.6 MF; p=0.30). *Characteristics of Patients with Elevated BMI:* Male respondents were significantly more likely to have a BMI ≥25 than female (59.9% vs 48.7%, p=0.0018). Among all MPN types, those with BMI score ≥25 demonstrated significantly higher symptom burden than those with BMI <25 (Table 1). This association persisted among patients with PV and MF subtypes, but not ET. Weak but significant positive correlations were observed between burden and BMI among all MPN subtypes (r = 0.14; p<0.001). When looking within disease subtypes, this correlation was higher for PV patients (r = 0.18; p<0.001) than for ET (r = 0.11; p<0.001) or MF patients (r = 0.11; p<0.001).

Table 1.

Differences between Overweight/Obese (BMI ≥25) vs Normal weight/underweight (<25) MPN patients (N=1025).

	BMI < 25	BMI ≥25	p
Age, mean (SD)	58.9 (12)	57.9 (12)	0.20
Female	79.3%	70.8%	0.0018
MPN10, mean (SD)	3.1 (1.6)	3.6 (1.7)	<0.001
ET subtype (n=392)	3.3 (1.6)	3.5 (1.7)	0.22
PV subtype (n=390)	2.9 (1.6)	3.7 (1.8)	<0.001
MF subtype (n=243)	3.1 (2.1)	3.5 (1.6)	0.026

Summary/Conclusion: Overweight or obese BMI is associated with significantly worsened disease-related symptom burden for most MPN subtypes. This relationship was less clear for ET, where weak but significant correlations were observed between BMI as a continuous measure and symptom burden but not when BMI was evaluated as dichotomous variable. A limitation to the data reported here is that weight and height was self-reported. A caveat to our data acquisition is the self-reported weight and height, which is unable to be externally validated in this analysis. Investigations regarding the relationship between weight loss and symptom burden during the disease course, which can be experienced by up to 67% of MF patients (Blood. 2008;112(11):A5224), should be explored further. However, these data suggest that interventions to reduce weight in MPN patients may be a strategy to combine with traditional therapy to improve symptomatology.

PS1368

MANAGEMENT AND OUTCOME OF PATIENTS DIAGNOSED WITH POLYCYTHEMIA VERA IN YOUNG AGE

G. Bogoni[1,*], G. Biagetti[1], I. Bertozzi[1], E. Cosi[1], F. Fabris[1], M. L. Randi[1]

[1]Medical Clinic 1, University of Padua, Padova, Italy

Background: Polycythemia Vera (PV) is a myeloproliferative neoplasm mainly diagnosed at a median age of 60 years. PV is rarely found in patients younger than 45 years of age and their clinical features and management is not clearly defined. Moreover, younger patients are followed for a long period of time and it is not known if their disease evolves more often in myelofibrosis or acute leukaemia compared to older patients.
Aims: In this work we evaluate the management and outcome of young patients with PV followed in a single center.
Methods: We report data of 199 patients diagnosed with PV in agreement with WHO criteria 2008 between 1986 and 2018. We divided the patients into two groups: 44 patients (23 males and 21 females, median age 37.5, range 18.6-44.9) were younger and 155 patients (81 males and 74 females median age 65 y, range 50-82) were older than 45 years at the time of PV diagnosis. We recorded the length of follow-up, the occurrence of arterial and venous thromboses both at diagnosis or during follow up, the presence of cardiovascular risk factors (diabetes, smoke, arterial hypertension, dyslipidaemia), the evolution in Myelofibrosis (MF) or Acute Leukaemia (AL). All the patients underwent phlebotomies to maintain haematocrit lower than 45% and received low-dose aspirin associated or shifted to anticoagulant in case of venous thrombosis. 135 of them received cytoreductive drugs (68%). Statistical analysis was performed using the Mann-Whitney test, the χ^2 test or Fisher's exact test and the Kaplan-Meier method.
Results: There was no difference in sex distribution, while the follow up duration was significantly longer in younger patients (median 12.8 y, range 1-31 vs median 6.7 y, range 1-22, p 0.003). 17 young (38.6%) and 45 old (29%) patients developed thrombotic complications (p= NS). Venous thromboses were more frequent in younger than in older patients (14 in young-PV and 26 in old-PV, p 0.0342), mainly due to thrombosis in unusual sites (12 in younger and 7 in older PV, p= 0.0001). Arterial thromboses (3 in young-PV and 19 in old-PV, p=NS) had a similar frequency in the two age groups, while the occurrence of cardiovascular risk factor was significantly different: 23 patients in younger PV (52.2%) and 111 in older PV (71.6%) had at least a cardiovascular risk factor (p=0.0098).
9 young and 12 old patients evolved in MF (p= 0.024). However, considering the median follow up in the two groups, the difference is not significant, as no difference was observed in AL transformation between the two groups (respectively 1 = 2.3% and 3 = 1.9%). In agreement, MF and AL free survival are the same.
Summary/Conclusion: Our study shows that there are no differences between younger than 45 years at diagnosis of PV and older patients in sex, total and arterial thrombotic events, and acute leukaemia evolution. Patients diagnosed under 45 years have an increased risk of venous thrombosis in unusual sites i.e. splanchnic venous thrombosis and cerebral veins thrombosis. Considering that cardiovascular risk factors are more common in older than in younger patients, the lack of significant difference in the occurrence of arterial thrombosis in the two groups is surprising. Indeed, in young patients with PV, the disease seems to be an independent risk factor for arterial thrombosis. Progression of myelofibrosis is more incident in young, but the matching of follow up duration and age at diagnosis suggests that the length of follow up allows more frequently the evolution.

PS1369

RUXOLITINIB IN MYELOFIBROSIS: A MULTICENTRE EXPERIENCE IN ENGLAND, NORTHERN IRELAND AND WALES

J. Russell[1,*], A. Daly[2], R. Fisken[3], A. Dicu[4], J. Cunningham[5], D. Sparksman[4], S. Docherty[4], A. Santarsieri[6], C. Saha[6], I. Lentell[6], Y.J. Lim[7], K. Maw[5], A. Fowler[8], S. Knapper[9], A. Collins[4], N. Butt[7], F. Wadelin[3], I. Whalley[10], E. Gudgin[6], A. Godfrey[6], M.F. McMullin[2], S. Sadullah[5]

[1]Haematology, Cambridge University Hospitals NHS Foundation Trust, Cambridge, [2]Belfast City Hospital, Belfast, [3]Nottingham University Hospital, Nottingham, [4]Norfolk and Norwich University Hospitals NHS Foundation Trust, Norwich, [5]James Paget University Hospitals NHS Foundation Trust, Great Yarmouth, [6]Cambridge University Hospitals NHS Foundation Trust, Cambridge, [7]Royal Liverpool University Hospital, Liverpool, [8]Peterborough City Hospital, Peterborough, [9]University Hospital of Wales, Cardiff, [10]Ipswich Hospital, Ipswich, United Kingdom

Background: Ruxolitinib (RUX), the oral Janus kinase (JAK)-1/2 inhibitor, is licensed to treat disease-related splenomegaly or symptoms in adults with primary myelofibrosis (PMF), post-essential thrombocythaemia (PET)-MF or post-polycythaemia vera (PPV)-MF. From August 2012, MF patients with intermediate (int)-1/2 or high-risk disease, based on the International Prognostic Scoring System (IPSS), could receive RUX via the Cancer Drugs Fund (CDF) in England. At that time, access to RUX in Northern Ireland (NI) and Wales was limited mainly to clinical trials. In 2016, RUX was commissioned by the NHS for int-2 and high-risk patients, but there is increasing evidence that lower-risk patients also benefit significantly from treatment.
Aims: To assess the efficacy and safety of RUX in MF in the 'real-world' setting and compare outcomes of lower- (int-1) vs higher-risk patients.
Methods: A multicentre retrospective analysis of MF patients treated with RUX at 13 centres in England, Wales and NI from Jan 2011-Dec 2017. Data were collected using electronic and paper records. Survival analysis was estimated using the Kaplan-Meier method and standard log-rank test.
Results: We identified 158 patients and excluded 8 with insufficient data and 2 who had received another JAK inhibitor. Of 148 evaluable patients, the median age at the start of therapy was 69 years (29-91) and 58% were male. There were 67 (45%) PMF, 40 (27%) PPV-MF, 30 (20%) PET-MF and 11 (7%) post-myeloproliferative neoplasm-unclassified MF patients, consisting of 1 (1%) low-risk, 29 (20%) int-1, 56 (38%) int-2 and 62 (42%) high-risk patients. A driver mutation was found in 111 patients (103 JAK2+, 6 CALR+, 2 MPL+). RUX was used first-line in 39 (32%) patients, and 111 (75%) were treated via the CDF. Median OS was 3.5 years (0–7) for all patients, 1.5 years for high-risk patients and not reached for low- or int-1/2 risk patients. IPSS/DIPSS remained prognostic with 3-year OS of 77%, 67%, and 29% for int-1, int-2 and high-risk patients (HR 2.75; 95% CI 1.8–4.1, p<0.0005). Median time on RUX was 2.2 years with estimated 1, 3 and 5-year discontinuation rates of 31%, 59% and 72%. Data collection of objective response measures, including spleen size, weight and symptom-burden, is underway. As previously observed, spleen reduction (56 of 86 patients; p=0.051) and weight gain (40 of 57 patients; p=0.07) were associated with improved survival. The most common grade 3/4 AEs were thrombocytopaenia (27%) and anaemia (42%) and 37 patients required dose modification.

Infections were common, with 53 patients receiving antimicrobial therapy. At the time of analysis, 82 (55%) patients had stopped RUX; 31 had progressive disease, 25 had died, 17 had AEs, 7 had stem cell transplantation and 2 were patient choice. Median survival after stopping was 2.3 months, but only 10 days for the 13 patients who transformed to acute leukaemia. In total, 62 (42%) patients have died; including 16 from disease progression, 14 from infection, 5 from bleeding, 2 from second cancers, and 1 from myocardial infarction. Sixty-six (45%) patients remain on therapy.

Summary/Conclusion: Our data reflect contemporary clinical trials that show the efficacy of RUX in improving MF-related splenomegaly and symptoms in both int-1/2 and high-risk patients. As expected, haematological AEs were common but readily managed. Longer follow-up is needed to assess the impact on survival and further studies into the use of RUX in lower-risk patients are required.

PS1370

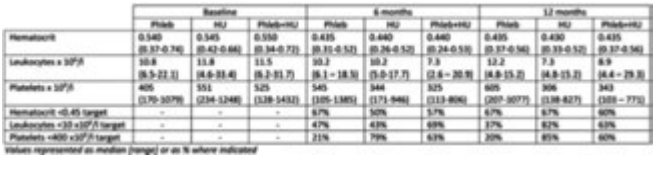
TREATMENT STRATEGIES FOR POLYCYTHEMIA VERA: OBSERVATIONS IN A DUTCH 'REAL-WORLD' COHORT STUDY

C. van de Ree- Pellikaan[1], A. de Kreuk[2], C. Schaar[3], A. Beeker[4], E. Dompeling[5], C. Gerrits[6], A. van Houten[7], M. Schipperus[8], L. Strobbe[9], W. Posthuma[10], K. Klauke[11], P. Westerweel[1,*]

[1]Internal Medicine, Albert Schweitzer Hospital, Dordrecht, [2]Internal Medicine, OLVG, Amsterdam, [3]Internal Medicine, Gelre Hospital, Apeldoorn, [4]Internal Medicine, Spaarne Gasthuis, Hoofddorp, [5]Internal Medicine, Isala Klinieken, Zwolle, [6]Internal Medicine, Ziekenhuisgroep Twente, Hengelo, [7]Internal Medicine, Maasstad Hospital, Rotterdam, [8]Hematology, Haga Teaching Hospital, The Hague, [9]Internal Medicine, Gelre Hospital, Zutphen, [10]Internal Medicine, Reinier de Graaf Hospital, Delft, [11]Novartis Pharma B.V., Arnhem, Netherlands

Background: Major thrombotic events are the main cause of mortality in Polycythemia Vera (PV) and therefore the primary focus of therapy. Treatment of PV requires control of circulating erythrocyte volume in addition to antiplatelet and/or anticoagulant treatment. Reduction of erythrocyte volume may be achieved by phlebotomy/erythrocytaferesis (Phleb), hydroxyurea treatment (HU) or a combination of both (Phleb+HU). Other treatments such as JAK2 inhibition and interferon are used in selected cases. In the absence of data from clinical trials comparing treatment strategies, there is a large variation in clinical practice.
Aims: Assessment of 'real-world' clinical practice of choice of treatment strategies for cytoreduction in PV patients and effects on the course of haematological control and patient outcome.
Methods: A retrospective chart review was performed in 150 PV patients from 10 major non-academic hospitals in the Netherlands to reflect a 'real-world' population of non-referred patients. Patients were required to meet the WHO 2008 diagnostic criteria. All patients provided written informed consent. Data were retrospectively collected from the time of diagnosis to inclusion based on the hospital records by trained data managers.

Table 1. Laboratory parameters at baseline and after 6 and 12 months of tretment.



Results: Patients had a median age of 64 (range 30-91) at diagnosis and 49% was male. 96.4% was JAK2 mutation positive. There was a high prevalence of a history of prior vascular events (37%) and concomitant cardiovascular risk factors (hypertension 59%, hypercholesterolemia 20%, diabetes 12%). A majority of patients received a combination of Phleb+HU (n=60, 40%), while others received HU only (n=32, 21%), only Phleb (n=37, 35%) or other treatment combinations (n=21, 14%). Table 1 shows the haematological parameters at baseline and at 6 and 12 months for patients treated with Phleb, HU or Phleb+HU. There were no statistically significant differences. The average (±SD) time to achieve a hematocrit target of 0.45 was the shortest in Phleb only patients (148±225 days) compared to HU (490±620 days) and Phleb+HU (346±426 days) treated patients. Leukocyte and platelet levels were numerically lower in HU-treated patients and ELN response targets were more often reached (p<0.05 in ANOVA). During the median follow-up period of 4.1 years after PV diagnosis, 22 patients (15%) suffered from a thrombotic vascular event despite treatment. In patients experiencing an event, the distribution between treatment strategies was similar and 59% used HU, while this was 61% in patients that remained event-free.
Summary/Conclusion: We observed major clinical variation in treatment strategies for PV in Dutch clinical practice. Although phlebotomizing patients shortens the time to achieving hematocrit control, median hematocrit levels were very similar after 6-12 months of treatment to that with HU alone or in combination with phlebotomies. In contrast, achievement of platelet and leukocyte ELN responses was higher in HU treated patients. Interestingly, the median hematocrit values achieved in Dutch clinical practice (43.0-44.0%) are comparable to the low-hematocrit arm of the CYTO-PV trial (44.4%). Nonetheless, the thrombotic vascular event rate in our cohort of Dutch PV patients remained clinically significant.

PS1371

PREGNANCY OUTCOMES IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS – PALG (POLISH ADULT LEUKEMIA GROUP) A RETROSPECTIVE ANALYSIS

B. Sokołowska[1,*], M. Sobas[2], M. Robak[3], K. Chojnowski[3], J. Treliński[3], J. Hałka[4], J. Niesiobędzka - Krężel[5], B. Stella - Hołowiecka[6], D. Jawniak[1], M. Kozioł[1], M. Hus[1], M. Bieniaszewska[7]

[1]Department of Haematooncology and Bone Marrow Transplantation, Medical University of Lublin, Lublin, [2]Department of Haematology, Blood Neoplasms and Bone Marrow Transplantation, Medical University of Wrocław, Wrocław, [3]Department of Hematology , Medical University of Łódź, Łódź, [4]Department of Hematology, Military Institute of Medicine, [5]Department of Hematology, Oncology and Internal Medicine, Medical University of Warsaw, Warsaw, [6]Outpatient Care Warsaw Katowice Branch, Medicover Poland, Katowice, [7]Department of Hematology and Transplantology, Medical University of Gdańsk, Gdańsk, Poland

Background: Philadelphia negative myeloproliferative neoplasms (MPNs) include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis(PMF). Pregnant women with MPNs are not only at a higher risk of thrombosis but also of various gestational complications such as recurrent abortions, premature deliveries and fetal growth restriction which result in a low live birth rate estimated at 60%.
Aims: The aim of the study was a retrospective analysis of pregnancy outcomes in patients with Philadelphia negative MPNs in PALG centers.
Methods: The study group comprised forty nine women treated in seven centers in Poland. The patients' mean age at diagnosis was 27 years of age (range 18-35) and at the time of pregnancy 37 years of age (range 19-49). ET was diagnosed in 38 patients (77.6%), PV in three patients (6.1%) whereas PMF in eight patients (16.3%). The JAK2V617F mutation was detected in 30 patients (61.2%), the CARL mutation (type 1 or 2) was discovered in 8 patients (16.3%) and seven patients were JAK2 negative. The mutational status was not measured in a group of 4 patients (8.25%). The retrospective analysis covered the period of 21 years when 82 pregnancies were reported. Before the pregnancies 47 women (57.3%) received cytoreductive therapy hydroksycarbamide (HU), Anagrelide(ANA) or interferon alfa (INF).
Results: Mean platelet count at the beginning of pregnancies was 451 G/l(215-1500) and at the time of delivery it reached 455G/l(153-1008). Anticoagulation therapy using LMWH was given during 19 pregnancies (23.2%), LMWH with aspirin(ASA) during 9 pregnancies (11%) and ASA alone during 24 pregnancies (29.3%). Cytoreductive therapy using interferon alfa(INF) was given in the course of 44 pregnancies (53.7%) and in one pregnancy apheresis was performed. A total of 13 abortions during the first trimester, two abortions and three cases of stillbirth during the second trimester and three cases of stillbirth during the third trimester were observed. Other complications such as threatened abortion and threatened premature delivery, hypertension, gestational diabetes and teratoma were reported in the second trimester. Until the end of gestation the number of 61 fetuses (74.4%) were alive. Cesarean sections were performed in 32 patients (39%) and 24 women delivered fetuses vaginally (29.3%) (no information about the type of 5 deliveries was provided). Delivery was usually succeeded at 37 week of gestation. Mean weight of fetus was 3230g. More than 50% of fetuses had APGAR scale ratio 10.
Summary/Conclusion: According to our results, a live birth rate was approximately 70%, more than it was previously expected. We observed a statistically significant correlation between the presence of vascular complications before pregnancy and the presence of JAK2 mutation and complications during the second trimester. Vascular complications before pregnancy caused 3.5 fold higher risk of occurrence of complications during the second trimester. A total of 92.3% of complications during the second trimester were observed in JAK2 positive women.

PS1372

SECOND MALIGNANCIES IN HIDROXYUREA AND INTERFERON TREATED PATIENTS

T. Nascimento[1,*], D. Luis[1], A. T. Simões [1], M. Coucelo [1], J.C. Almeida[1], M. L. Riberio[1]

[1]Hematology, Centro Hospitar e Universitário de Coimbra, Coimbra, Portugal

Background: Philadelphia –negative myeloproliferative neoplasm (MPNs) include Essential Trombocythaemia (ET), Polycythaemia Vera (PV) and Primary Myelofibrosis (PMF). Hydroxyureia (HU) treatment involves the concern of potential mutagenicity. Treatment with alfa 2 –Interferon (IFN) is being increasingly used.
Aims: to describe the frequencies of second malignancies in a cohort of MPNs patients treated with Hydroxyurea (HU), IFN Pegylated alfa 2 or the combination of these drugs (started with HU and subsequently Peg IFN Pegylated alfa 2) and compare the prevalence of cardiovascular risk factors and associated thrombotic events in this groups .
Methods: Observational, descriptive and retrospective study of patients with the diagnosis of myeloproliferative neoplasms (MPNs) followed in our centre between January 2011 and February 2018. Clinical records were reviewed and patients were divided in three groups, according to their therapy: Group 1 was treated with HU, group 2 with IFN, and group 3 with both agents. Data analysis with SPSS®.
Results: We Identified 100 patents diagnosed with MPNs with criteria for cytoreductive treatment, according to ELN criteria for haematologic response . 48 patients were treated with HU, 11 with IFN and 41 patients with both HU and IFN subsequently. Key characteristics of the patients and treatments are shown in Table 1.

Table 1.

	HU treatment N= 48	IFN treatment N=11	HU-IFN treatment N=41
Gender, n (%)			
Female	26 (54.2%)	8 (72, 7%)	20(48, 8%)
Male	22 (45, 8%)	3 (27, 3%)	21 (51, 2%)
Age at time diagnosis (median)	69 [36-89]	51 [30-58]	64 [44-85]
MPN diagnosis, n (%)			
TE	24(50%)	9(81, 8%)	14(34, 1%)
PV	14 (29,2)	1 (9,1)	14 (34,1)
PMF	8 (16,7)	1(9,1)	11(26.8%)
MPN unclassified	2(4.2%)		2(4.9%)
Overall haematologic response, n (%)			
Complete (CR)	37 (77%)	10 (90%)	27(65.8%)
Partial (PR)	9 (18.7%)	1 (10%)	12 (29.2%)
None (NR)	2(4.2%)		2(4,9%)
Comorbidity, n (%)			
Hypertension	29(60, 4%)	3(27, 3%)	19(46.3%)
dyslipidemia	23(47, 9%)	0(0%)	17(41, 5. %)
Diabetes	6(125%)	0(0%)	9 (22.0%)
thrombosis	9 (18, 8 %)	0 (0%)	11 (26, 8%)
Antiplatelet therapy n, %	42 (87,5%)	11 (100%)	35 (85,5)
AAS or clopidogrel			
Anticoagulant therapy n, % warfarin	7 (14,6)	0 (0%)	5 (12,2%)
Time of follow up months (median)	25 (1-192)	50 (3-144)	73 (3-216)

	HU treatment N= 48	IFN treatment N=11	HU-IFN treatment N=41
All others non haematological neoplasms n (%) (Pvalue 0,195)	11 (22, 9%)	0	7(39,9%)
basal cell carcinoma	1 (2, 1%)		1 (2, 4%)
Baselioma	1 (2, 1%)		
Spinocelular Carcinoma	8 (16,6%)		3 (7,3%)
Prostate carcinoma	1 (2, 1%)		1 (2, 4%)
Renal cells Carcinoma			1 (2, 4%)
Thyroid Papilary carcinoma			1 (2, 4%)

Summary/Conclusion: We registered an occurrence of second malignancies in a total of 11 in patients under HU (22.9%), none in patients under IFN and 7 in the HU-IFN patients group (39.9%).Median age at diagnosis of MPNs was higher in HU patients group (69 years, vs 51 for IFN and 64 years for HU-INF).As also reported by others, in our cohort there appears to be a tendency for an increased risk of developing a second neoplasm in the patients treated with HU , especially when compared to patients receiving interferon therapy (OR of 1.283 (95% CI, pvalue 0.77 (chi-square test). Patients treated with HU-IFN combination (mean 36 months treatment with HU in grupe 3) had fewer cutaneous neoplasms when compared to the group 1 (HU therapy mean 25 months) , (Mann Witney test (p value <05 com 95%).There was a better control hematological in patients of group 2 and lower cardiovascular risk, however, the median age of these patients was lower, requiring more follow-up time for evaluation.

PS1373

CORRELATIONS BETWEEN GENETIC AND CLINICAL CHARACTERISTICS IN PATIENTS WITH PRIMARY MYELOFIBROSIS

L. Polushkina[1,*], I. Martynkevich[1], V. Shuvaev[1], E. Motyko[1], M. Fominykh[1], L. Martynenko[1], M. Bakai[1], N. Tsybakova[1], Y. Ruzhenkova[1], N. Pavlenko[1], S. Voloshin[1], S. Bessmeltsev[1], A. Chechetkin[1]

[1]Russian Research Institute of Hematology and Transfusiology, Saint-Petersburg, Russian Federation

Background: Mutations in *JAK2*, *CALR*, *MPL* genes which trigger the process of autonomous cell proliferation are specific genetic lesions of classic Ph-negative myeloproliferative neoplasms including primary myelofibrosis (PMF). Also, the patients with PMF frequently have somatic defects of epigenetic regulators, primarily in *ASXL1* gene. Combined molecular abnormalities cause a diversity of tumor cells properties and determine the PMF clinical manifestations heterogeneity. The identification of "phenotypic signature" of mutations can become an important tool for clonal myelopoiesis disorders workup at the diagnosis.
Aims: The study aims to identify correlation between PMF clinical manifestations and the mutational status of *JAK2, CALR, MPL, ASXL1* genes.
Methods: We examined 110 patients with PMF (35% males). Median age was 59 years (16-82), median follow up – 2.6 years (0.1-23.0). For all patients the detection of *JAK2*V617F was done. *JAK2* negative samples were subsequently tested for *MPL* and *CALR* mutations. All patients except 4 underwent the analysis of mutations in exon 12 of *ASXL1* gene by direct sequencing. When comparing categorical variables Fisher exact test and chi-square test were used. The Kruskal-Wallis test and the Mann-Whitney U test were used for continuous data. P values less than 0.05 were considered statistically significant.
Results: Driver mutations (DM) were detected in 82% patients: $JAK2^+$ - 50%, $CALR^+$ - 26%, MPL^+ - 6% cases. No DM were found in 18% patients considered triple-negative (TN). Mutations in *ASXL1* gene were detected in 20% patients: $CALR^+$ - 29%, TN- 30%, $JAK2^+$ - 12%, MPL^+ - 29% cases. When compared TN and DM+ patients the former had lower hemoglobin level (here and after median values are shown) (10.1g/dL vs 12.1g/dL, p=0.006), lower platelet counts (266×10^9/L vs 506×10^9/L, p=0.041) and higher white blood cell counts (27×10^9/L vs 12×10^9/L, p=0.001). Blast counts ≥1% in peripheral blood was more often detected in $CALR^+$ (58%, p=0.001) and TN patients (60%, p=0.003) compared to $JAK2^+$ (20%). Hemoglobin level was higher in $JAK2^+$ patients compared with $JAK2^-$ (12.9g/dL vs 10.9 g/dL, p=0.021). *ASXL1* mutations were associated with a lower platelet counts (184×10^9/L vs 530×10^9/L, p=0.016), leukocytosis > 25×10^9/L (40% vs 16%, p=0.046), palpable splenomegaly (93% vs 68%, p=0.050) and blasts ≥1% (87% vs 27%, p<0.001). The median platelet counts differed in the $CALR^+$ and $CALR^-$ patients only when the *ASXL1* status was taken into account: 799×10^9/L vs 510×10^9/L (p=0.046) in $ASXL1^-$ and 313×10^9/L vs 164×10^9/L (p=0.175) in $ASXL1^+$ subgroup. Hemoglobin levels in TN patients were significantly lower than in the DM^+ASXL1^- group regardless of the *ASXL1* status (10.1g/dLin DM^-ASXL1^- and 100g/L in DM^-ASXL1^+ vs 124g/L in DM^+ASXL1^- group, p=0.050 and p=0.001, respectively). The maximum value of leukocyte counts was revealed in DM^-ASXL1^+ group (51×10^9/L) whereas in the DM^-ASXL^- group it was lower (22×10^9/L, p=0.155). White blood cell levels differed significantly between DM^+ and TN groups when *ASXL1* status was identical: 8×10^9/L vs 52×10^9/L in $ASXL1^+$ (p=0.050) and 12×10^9/L vs 22×10^9/L in$ASXL1^-$ (p=0.022) subgroup.
Summary/Conclusion: The presence of *JAK2*, *CALR*, *ASXL1* mutations as well as triple-negative status correlates with clinical manifestations of PMF. These genetic factors in different combinations can cause the variety in hematological features of the disease. Received data confirms the importance of both driver and *ASXL1* mutations screening at the diagnosis of PMF.

PS1374

MUTATIONAL PROFILES OF CLASSIC MYELOPROLIFERATIVE NEOPLASMS IN TAIWANESE PATIENTS: FREQUENCY AND CLINICAL CORRELATION

H.-C. Lin[1,*], Y.-C. Chang[1,2], Y.-H. Chiang[1], L. Huang[13], W.-T. Wang[1], C.-C. Cheng[1], N.-W. Su[1], C. G.-S. Chen[1,2], J. Lin[1], R.-K. Hsieh[1], M.-C. Chang[1], Y.-F. Chang[1,2], D.-Y. Hwang[4], K.-H. Lim[1,2]

[1]Department of Internal Medicine, Division of Hematology and Oncology, MacKay Memorial Hospital, Taipei City, [2]Department of Medicine, MacKay Medical College, [3]Department of Medical Research, Good Clinical Research Center Laboratory, MacKay Memorial Hospital, New Taipei City, [4]Department of Internal Medicine, Division of Nephrology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan, Republic of China

Background: The *JAK2*V617F, *CALR* exon 9 and *MPL* exon 10 mutations are three major driver mutations in classic *BCR-ABL1*-negative myeloproliferative neoplasms (MPNs). In addition, MPN patients can harbor other non-driver mutations such as *TET2*, *DNMT3A*, *AXSL1* and *TP53*.
Aims: The aims of this study were to determine the mutational profiles of MPN in a cohort of Taiwanese patients, and to correlate the mutations with clinical characteristics.
Methods: MPN patients seen at MacKay Memorial Hospital from Oct 2009 to Oct 2015 were enrolled into this study. The clinical and laboratory characteristics at the time of diagnosis or referral were determined retrospectively by chart review. Patient genomic DNA was derived from bone marrow or peripheral blood. Targeted next generation sequencing (NGS) was carried out using a customized myeloid-related panel including 33 genes. The Illumina *MiSeq* system was used to run NGS experiment. *MiSeq Reporter* Software and the Burrows-Wheeler Aligner were used for bioinformatics analysis.

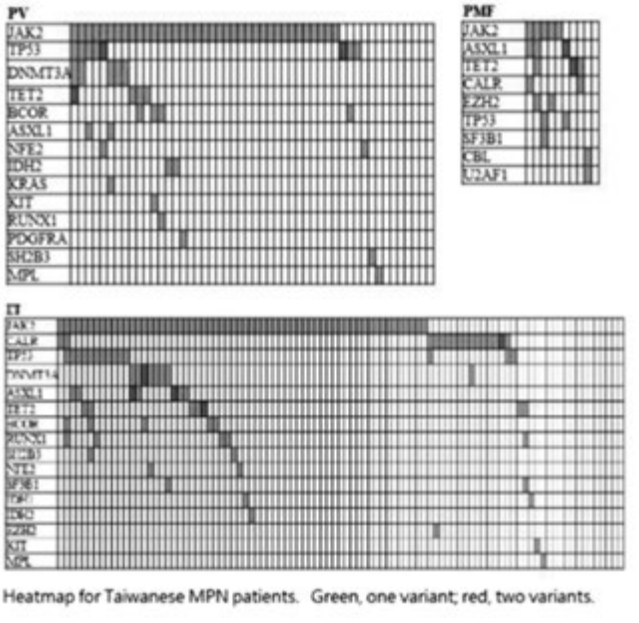


Figure 1.

Results: A total of 155 MPN patients (median age at diagnosis 57 years; 51.6% females). Our cohort was comprised of 95 essential thrombocythemia (ET, 61.3%), 50 polycythemia vera (PV, 32.3%) and 10 primary myelofibrosis (PMF, 6.5%). Frequencies of the 3 driver mutations were 67.1% for *JAK2*V617F, 11.6% for *CALR* and 1.3% for *MPL*. 22.6% of patients were classified as triple-negative. The frequencies of non-driver mutations were 15.5% for TP53, 9% TET2, 8.4% DNMT3A, 7.7% ASXL1, 5.8% BCOR, and 3.9% RUNX1 (Figure 1). The frequencies of mutations in other 11 genes occurred in less than 3% of patients. Median number of mutation was 1 (range 0-5): 12.9% no mutation, 40% 1 mutation, 27.1% 2 mutations, 14.2% 3 mutations, 3.9% 4 mutations, and 1.9% 5 mutations. Mutations in an epigenetic regulation gene (*TET2*, *DNMT3A*, *IDH1/2*) were most frequently detected (20%, one patient harbored both *TET2* and *DNMT3A* mutations) in this cohort. 9% mutations in a gene involved in histone modification/chromatin regulation (*ASXL1*, *EZH2*, one patient harbored both mutations). *JAK2*V617F mutation was significantly associated with older age at diagnosis (median 62 vs 54, p=0.02), higher hemoglobin level (median 14.7 vs 13g/dL, p=0.02) and higher leukocyte count (median 13.3 vs 8.5/uL, p<0.001). Whereas, *CALR* mutations were significantly associated with higher platelet count (median 1119 vs 731/uL, p<0.01) and lower hemoglobin level (median 11 vs 14.7g/dL, p<0.01). Patients with PMF had inferior overall survival (OS) (median 3.4 years vs. not reach vs. not reach; p<0.001). *ASXL1* mutations were associated with inferior OS (median 8.7 years vs. not reach; p<0.001). Mutations in an epigenetic regulation gene or an increased number of somatic mutations were not associated with worse OS.
Summary/Conclusion: In this cohort of Taiwanese MPNs, driver mutations correlate with clinical features. Consistence with the results from Western populations, *ASXL1* mutations had prognostic significance in MPNs.

Non–Hodgkin lymphoma – Biology & Translational Research

PS1375

Abstract withdrawn.

PS1376

CYCLIN D1-MEDIATED GENE EXPRESSION DYSREGULATION IMPACTS MANTLE CELL LYMPHOMA PATHOGENESIS

S. Demajo[1,*], R. Albero[1], G. Castellano[2], C. Capdevila[1], A. Navarro[1,3], G. Clot[1,3], F. Nadeu[1], H. Suárez-Cisneros[4], A. Enjuanes[4], M. Pinyol[4], S. Beà[1,3], E. Campo[1,3,5], P. Jares[1,3,5]

[1]Lymphoid Neoplasm Program, Institut d'investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), [2]Molecular Biology Core, Hospital Clínic de Barcelona, [3]Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), [4]Genomics Unit, Institut d'investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), [5]Laboratory of Anatomic Pathology, Hospital Clínic de Barcelona, Barcelona, Spain

Background: Mantle cell lymphoma (MCL) is a B-cell neoplasm that usually follows an aggressive clinical course. It is characterized by the t(11;14) translocation that leads to cyclin D1 overexpression. Although the classical MCL tumorigenesis model considers that cyclin D1 mediates its oncogenic effect through cell cycle activation, emerging roles of cyclin D1 in other cellular contexts suggest a more complex scenario.
Aims: To study the potential function of cyclin D1 as a transcriptional regulator in MCL and its impact in the pathogenesis of this neoplasm.
Methods: In order to identify the genes potentially dysregulated by cyclin D1 overexpression in MCL, we generated cyclin D1-silenced MCL cell lines and a lymphoid cell model of exogenous cyclin D1 overexpression and performed genome-wide expression analysis. We next combined these results with cyclin D1 ChIP-sequencing in MCL cell lines to robustly identify cyclin D1 directly regulated genes. Bioinformatic analyses of the promoters of these genes together with co-immunoprecipitation experiments were used to investigate the mechanism of cyclin D1-mediated gene regulation. We then studied the expression of the genes directly activated by cyclin D1 in MCL patients in order to define the biological and clinical relevance of this cyclin D1-regulated gene program. For that, we used different microarray data sets, including MCL primary blood (n=53) and tissue (n=106) samples, non-neoplastic lymphoid tissues (n=21), and several other B-cell lymphoid neoplasms (n=101).
Results: We identified the gene expression program transcriptionally activated by cyclin D1 in MCL, consisting of 295 genes that were downregulated in the cyclin D1 silencing cell models, upregulated in the cyclin D1 overexpression cell model and had a cyclin D1 peak in their promoters. Gene ontology analysis of these cyclin D1-activated genes showed that most of them belong to cell cycle and DNA damage response pathways. Motif enrichment analysis of cyclin D1 peaks present in their promoters together with several analyses using the ENCODE ChIP-sequencing database identified E2F4 as potential recruiting factor of cyclin D1 to its target genes. The interaction of cyclin D1 with E2F4 was confirmed by co-immunoprecipitation, as well as its interaction with the coactivator CBP, which may explain its transcriptional activation mechanism. To analyze the clinical impact of the cyclin D1-activated gene program in MCL patients, we defined a cyclin D1 signature score based on the expression of the 295 genes. We found that cyclin D1 signature was overexpressed in MCL compared with non-neoplastic samples ($p<0.001$) and with other -cyclin D1 negative- lymphoid neoplasms ($p=0.006$). Additionally, cyclin D1 signature expression levels were directly correlated to the cyclin D1 expression levels in MCL cases, both considering blood ($r=0.617$, $p<0.001$) and tissue ($r=0.493$, $p<0.001$) samples. Finally, we found that increasing cyclin D1 signature expression levels were associated with shorter overall survival in MCL patients in a continuous manner, both in blood ($p=0.001$) and tissue ($p<0.001$) samples.
Summary/Conclusion: Our results expand the oncogenic function of cyclin D1 in MCL, showing the importance of cyclin D1 as a transcriptional regulator in this disease. We have found that cyclin D1 activates a gene expression program that could have a critical impact in cell cycle and DNA damage response dysregulation in MCL and therefore in the pathogenesis of this neoplasm. Accordingly, high expression levels of cyclin D1-activated genes correlate with poor survival in MCL patients.

PS1377

SPLENIC MARGINAL ZONE B-CELL LYMPHOMAS DEPENDS ON PROGRAMMED DEATH-LIGAND 1 FOR CLONAL EMERGENCE AND ARE CONTROLLED BY T-CELLS FOR PROLIFERATION RATE

C. Vincent-Fabert[1], I. Soubeyran[2], A. Saintamand[3], F. Boyer[1], U. Zimber-Strobl[4], J. Feuillard[1], N. Faumont[1,*]

[1]UMR CNRS 7276 / INSERM U1262 - CRIBL, LIMOGES, [2]Laboratory of Pathology, Institut Bergonié, Bordeaux, France, BORDEAUX, [3]UMR INSERM U1236, University of Rennes [1], Rennes, France, LIMOGES, France, [4]Research Unit Gene Vectors, Helmholtz Center Munich, German Research Center for Environmental Health GmbH, Munich, Germany, MUNICH, Germany

Background: Marginal zone B-cell lymphomas (MZLs) are incurable indolent cancers. Presence of PD1 positive T-cells in these lymphomas has been reported. This raises the question of the escape from immune control.
Aims: To search for PDL1 expression in splenic MZLs (SMZLs) and to explore the reciprocal control between tumor cells and the immune system in a mouse model of SMZL.
Methods: Immunochemistry for PD-L1 was done on 74 ZML tumor samples. Functional studies were performed on the L.CD40 transgenic mouse model, one of the very few preclinical models for splenic MZL. These mice are characterized by B-cell specific continuous CD40 signaling responsible for a spleen indolent monoclonal B-cell tumor after one year in 60% of cases. L.CD40 mice were injected with anti-PD-L1 antibody. T-cell depletion was done by injection of a mix of anti-CD4, anti-CD8, and anti-Thy-1 antibodies. L.CD40 mice were also treated with cyclosporin A.
Results: In humans, about 60% of SZML tumor samples significantly expressed the PD-L1 molecule. Like SMZL in humans, L.CD40 mouse B-cells tumors expressed high levels of PD-L1. Treatment of L.CD40 mice with an anti-PD-L1 monoclonal antibody induced tumor regression with decreased activation, proliferation rate and spleen content of B-cells as well as a marked increase in T cell activation, that T-cells strongly expressing PD1. L.CD40 mice were next immunosuppressed (IS) either by T and NK cell depletion or with the immune suppressive drug, cyclosporin A. Spleen enlargement was increased due to increased B-cell content. Tumor B-cells from IS L.CD40 mice had an immunoblastic high-grade aspect. *In vivo* proliferation rate and expression of activation markers were also increased after IS treatment. Long term IS was associated with acceleration of B-cell clonal emergence in the spleen and blood passage of large tumor B-cells.
Summary/Conclusion: Collectively, these features suggest that emergence of SMZL is under the dependence of the PD1/PD-L1 axis and that proliferation rate of SMZL remains under the strict T-cell control. In other words, SMZL tumor B cells partially escape immune surveillance due to PD-L1 expression but immune control was still exerted against the more aggressive B-cells. Additionally, these results highlight the interest of therapies targeting the PD-1/PD-L1 axis in indolent lymphomas with PD-L1 expression.

PS1378

TPL2 KINASE HAS A TUMOR SUPPRESSIVE ROLE IN MYC-INDUCED LYMPHOMAGENESIS

E. Stagakis[1,2,*], D. Vyrla[1], H. Papadaki[2], E. Drakos[3], A.G. Eliopoulos[1]

[1]Department of Biology, National & Kapodistrian University of Athens, Athens, [2]Department of Hematology, University Hospital of Heraklion, [3]Department of Pathology, University of Crete, Heraklion, Greece

Background: MYC-induced lymphomagenesis is a complex biological process and many of its aspects remain poorly understood. Burkitt lymphoma (BL) is considered the prototypical example of a disease caused by a t(8;14)(q24;q32) translocation bringing *myc* under the control of the IgH locus. Nevertheless, other B-cell lymphomas with aggressive clinical behavior and poor treatment response can also overexpress MYC. Therefore, in order to gain a better insight into MYC-induced lymphomagenesis, it is necessary to identify new signaling pathways that cooperate with MYC. TPL2 (MAP3K8) is a MAP3 kinase with a crucial role in innate immunity and inflammation but its involvement in lymphomagenesis remains unknown. By using text mining algorithms and by inspecting transcriptome profiles registered in the Oncomine database (http://www.oncomine.org) we identified putative links between MYC and TPL2.
Aims: The aim of this study is to define the impact of and the mechanism by which TPL2 kinase affects MYC-induced lymphomagenesis.
Methods: The TPL2 mRNA and protein expression levels were assessed by qPCR and Western blot analysis, respectively. Mouse CD19+ B cells were isolated by magnetic separation from spleens of WT (C57BL/6; non-Tg) and *Eμ-myc* transgenic mice engineered to overexpress c-*myc* in B cell pro-

genitor cells under the control of the IgH chain enhancer. Mouse pre-B lymphocytes (B220+. IgM-) were isolated from bone marrow by cell sorting. The extent of apoptosis and proliferation was estimated by immunohistochemical evaluation of activated caspase-3 and phospho-histone H3 in paraffin embedded mouse lymphoma tissues. Malignant cells were cultured *ex vivo* following cytokine deprivation or treatment with chemotherapeutic reagents. Apoptosis was further assessed with flow cytometry using Annexin and PI staining. Cell proliferation was assessed using MTS assay.
Results: TPL2 mRNA levels were dramatically reduced in human Burkitt cell lines and primary BL biopsies compared to B-lymphocytes of healthy individuals. Accordingly, analysis of TPL2 expression in pre-B and B lymphomas of Eμ-myc transgenic mice revealed very low levels of TPL2 both at RNA and protein level, compared to pre-B and splenic B lymphocytes isolated from WT mice. Moreover, TPL2 expression levels correlated with the age of disease onset in Eμ-myc mice with the lowest TPL2 expression levels to be associated with a younger age of disease onset. In this regard, genetic ablation of TPL2 in *Eμ-myc* mice (*Eμ-myc/tpl2*$^{-/-}$) significantly shortened their survival to 92 days from 131 days of *Eμ-myc/tpl2*$^{+/+}$ mice ($p<0.05$). The acceleration of lymphomagenesis is not attributable to the absence of TPL2 in the tumor microenvironment as adoptive transfer of malignant *Eμ-myc* lymphocytes to mice with *tpl2*$^{+/+}$ versus *tpl2*$^{-/-}$ genetic background resulted in a similar median survival. Compared to *Eμ-myc/tpl2*$^{+/+}$, *Eμ-myc/tpl2*$^{-/-}$ lymphomas display reduced levels of activated caspase-3 and increased levels of phospho-histone H3. *Eμ-myc/tpl2*$^{-/-}$ lymphoma cells cultured *in vitro* were resistant to apoptosis following cytokine deprivation or treatment with chemotherapeutic agents, such as doxorubicin, cyclophosphamide and etoposide.
Summary/Conclusion: This work reveals a novel role for TPL2 as suppressor of myc-driven lymphomagenesis which is supported by: (a) the dramatic reduction in TPL2 expression in human and mouse lymphomas bearing oncogenic MYC activation, (b) the acceleration of disease onset in mice lacking TPL2 and, (c) the higher proliferative index and lower levels of apoptosis in MYC lymphomas that arise on a TPL2-null background.

PS1379

MECHANISMS CONFERRING RESISTANCE TO VENETOCLAX IN CHRONIC LYMPHOCYTIC LEUKEMIA AND MANTLE CELL LYMPHOMA

D. Steinbrecher[1,*], F. Seyfried[2], J. Bloehdorn[1], B.M.C. Jebaraj[1], L.-H. Meyer[2], C. Schneider[1], S. Stilgenbauer[1]

[1]Department of Internal Medicine III, [2]Department of Pediatrics and Adolescent Medicine, Ulm University, Ulm, Germany

Background: The BCL-2 family consists of important regulators of apoptosis which have been found to be deregulated in cancer. BCL-2 dependent cancers including chronic lymphocytic leukemia and mantle cell lymphoma (MCL) are highly sensitive to venetoclax (VEN). Despite presenting a highly efficient therapeutic agent, acquired resistance can develop. Understanding the underlying mechanisms of resistance to VEN is essential to identify the best possible treatment options in refractory patients.
Aims: To gain a better understanding of the molecular mechanism triggering VEN resistance, we developed *in vitro* resistance models and investigated BCL-2 family member expression changes.
Methods: In this study, we generated VEN-resistant MCL cell lines (MINO and MAVER-1) by chronic exposure to increasing doses of VEN. Apoptosis was assessed by Annexin V/7AAD staining. Protein expression of BCL-2 family members was assessed by western blot. BH3 profiling was used to assess the interplay of pro- and anti-apoptotic proteins.
Results: In VEN-resistant MINO cells, MCL-1 was not deregulated as compared to parental cells, while MCL-1 was significantly downregulated in VEN-resistant MAVER-1 cells. In variance to previous reports in diffuse large B cell lymphoma (DLBCL), linking resistance to upregulation of MCL-1 and BCL-XL (Choudhary *et al.*, Cell Death Dis 2015), our results indicate that resistance in MCL cell lines is not mediated by MCL-1. Also, dynamic BH3 profiling confirmed that VEN resistance is not mediated by and dependent on MCL-1. Actually, MCL-1 upregulation occurred in both parental and resistant cells upon treatment with VEN and normalized when treatment was stopped, suggesting that MCL-1 is stabilized by binding to proteins released from BCL-2. In line with results obtained in DLBCL (Choudhary *et al.*, Cell Death Dis 2015), we could identify a permanent BCL-XL upregulation as important mechanism conferring resistance to VEN. Additionally, BH3 profiling confirmed a dependency on BCL-XL in resistant cells. The significance of BCL-XL in mediating resistance to VEN was underlined by exposing the cells to navitoclax. In contrast to VEN, navitoclax inhibits BCL-2, BCL-W and BCL-XL and was sufficient to induce apoptosis in both parental and resistant cells. In addition to deregulation of anti-apoptotic proteins, the acquisition of resistance to VEN in MINO and MAVER-1 cells led to reduced protein expression of pro-apoptotic BCL-2 family members. In VEN-resistant MAVER-1 cells downregulation of multiple pro-apoptotic proteins including BAK, BAX, PUMA, BIM and NOXA was found, while in VEN-resistant MINO cells only pro-apoptotic BID and BAD were downregulated. Additionally, BH3 profiling confirmed this result by showing that in both resistant cell lines induction of apoptosis was hampered as compared to parental cells. In contrast to the BH3 domain inhibitor VEN, the BCL-2 inhibitor BDA-366 acts by inhibiting the BH4 domain and thereby inducing a conversion of BCL-2 into a BAX-like death molecule. BDA-366 was effectively inducing cell death in both parental and VEN-resistant cell lines (MINO and MAVER-1). Therefore, targeting BCL-2 by inhibiting its BH4 domain could be a potential alternative treatment strategy for BCL-2 dependent cancers resistant to VEN.
Summary/Conclusion: Overall, these results suggest that VEN resistance is mostly mediated by permanent upregulation of BCL-XL while MCL-1 is upregulated temporarily upon treatment in parental and resistant cells. In addition to BCL-XL upregulation, a complex deregulation of pro-apoptotic BCL-2 family members plays a role in inducing resistance to VEN.

PS1380

CLINICAL, PATHOLOGICAL AND MOLECULAR STUDY OF 46 PATIENTS WITH A DIAGNOSIS OF CD5-POSITIVE DIFFUSE LARGE B-CELL LYMPHOMA

V. Allain[1,2,*], J. Dupuis[1], L. Roulin[1], C. Robe[2], M. Baia[2], A. Nicolae[2], H. Fouché[3], M. Fusaro[3], M.-H. Delfau-Larue[3], P. Gaulard[2], C. Haioun[1], C. Copie-Bergman[2]

[1]Unité Hémopathies Lymphoïdes, [2]Département de Pathologie, [3]Département d'Hématologie et Immunologie Biologiques , Hôpital Henri Mondor - APHP, Créteil, France

Background: CD5-positive diffuse large B-cell lymphomas (CD5+ DLBCL) are aggressive lymphomas of poor prognosis, with specific clinical characteristics (adverse prognostic factors, extranodal tropism, high rate of central nervous system (CNS) relapses), and a predominant "activated B-cell like" (ABC) expression profile. However, physiopathological mechanisms explaining these peculiarities remain mostly unknown, and cure is still a therapeutic challenge.
Aims: Our goal was to describe clinical, pathological and molecular features of a CD5+ DLBCL patients series, in order to give new insights possibly leading to a better understanding of these lymphomas.
Methods: Forty six CD5+ DLBCL patients were included in this monocentric retrospective study. Association with a chronic lymphocytic leukemia or a mantle-cell lymphoma were exclusion criteria. All tumor samples were reviewed by an expert hematopathologist and classified according to the 2016 WHO classification, with an extensive immunochemistry panel and fluorescent *in situ* hybridization analysis (FISH) for *MYC*, *BCL2*, *BCL6* and *IRF4* gene rearrangements. Gene expression analysis by Reverse Transcriptase-Multiplex Ligation-dependent Probe Amplification (RT-MLPA) and mutational analysis by next-generation sequencing (NGS) on a dedicated gene panel were performed on a subset of patients.
Results: Median age at diagnosis was 68 years (range 22-83). Twenty percent of patients had a past history of solid cancer, and 17% history of autoimmune disease. DLBCL "not otherwise specified" (NOS) was the main diagnosis (89%). We found an association with a prior or concomitant low-grade B-cell lymphoma in 26% of cases. International prognostic index classified 63% of patients in the high-risk group. Extranodal tropism and a high CNS relapse rate (9%) were observed as previously described. Cell-of-origin classification revealed "non germinal-center B-cell like (non-GCB)"/ABC profile in most cases (72% by Hans algorithm and 80% by RT-MLPA). Of note, only 2 of 10 GCB CD5+ DLBCL (according to Hans algorithm) harbored a GCB profile by RT-MLPA showing the poor reliability of the Hans classifier in this specific DLBCL population. Nearly all cases (98%) showed BCL2 protein overexpression whereas *BCL2* rearrangement was rare (n=1). MYC overexpression was observed in 53% of cases. *BCL6* and *MYC* breaks were detected in 17% of cases for both genes. Preliminary results showed that none of 4 tested lymphomas harbored an *IRF4* translocation. PD-L1 was overexpressed on tumor cells in 20% of cases and a clear although heterogeneous PD1 expression by tumor cells was noted in 2/15 biopsies. Over the 36 diagnostic biopsies assessed for the presence of EBV (either by *in situ* hybridization or by RT-MLPA), 5 cases were positive (14%). The preliminary molecular analysis by NGS (performed on 32 samples) showed mutations of *MYD88* and *CD79B* in 31% and 19% of lymphomas at diagnosis, respectively. 4/6 cases with a *CD79B* mutation also harbored a *MYD88* mutation. Interestingly, all 9 *MYD88* L265P mutations were also detected by the RT-MLPA specific assay.

Summary/Conclusion: We report the clinical, pathological and molecular features of 46 CD5+ DLBCL patients. We confirm the already reported non-GCB/ABC profile and recurrent *MYD88* and *CD79B* mutations. NGS molecular analysis is still ongoing. This series highlights new findings such as a frequent association with solid cancer and PD-L1/PD1 expression in a significant number of cases which warrants further investigations.

PS1381

THE NOVEL TUMOR SUPPRESSOR SAMHD1 IS FREQUENTLY DOWNREGULATED AND CORRELATES WITH GERMINAL CENTER PHENOTYPE IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

I. Xagoraris[1,*], N. Herold[2], K. Ekstrom Smedby[3], J. Khoury[1], G.Z. Rassidakis[3]

[1]Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, United States, [2]Department of Women's and Children's Health, [3]Department of Oncology-Pathology, Karolinska Institute, Stockholm, Sweden

Background: The SAM domain and HD domain 1 (SAMHD1) protein is a deoxynucleoside triphosphate (dNTP) triphosphohydrolase, which has been initially described to restrict human immunodeficiency virus type 1 (HIV-1) in certain cell types through depletion of intracellular dNTP substrates for HIV-1 reverse transcription. Mutations of SAMHD1 gene have been linked to Aicardi-Goutières syndrome (AGS) and have been identified as putative drivers of chronic lymphocytic leukemia resulting in decreased SAMHD1 mRNA and protein levels. Therefore, SAMHD1 may play a role in oncogenesis as a tumor suppressor. In addition, SAMHD1 may confer resistance to nucleoside-based chemotherapies such as cytarabine in acute myeloid leukemia (Herold *et al.*, Nat Med 2017; 23(2):256-263). The expression levels and the potential role of SAMHD1 in the pathogenesis of diffuse large B-cell lymphoma (DLBCL) are not yet known.
Aims: To investigate the expression patterns and regulation of SAMHD1 tumor suppressor in diffuse large B-cell lymphoma (DLBCL).
Methods: SAMHD1 protein levels were assessed by Western blot analysis in 3 diffuse large B-cell lymphoma (DLBCL) cell lines including SU-DHL-4, MS, and OCI-Ly3. As our findings from in silico analysis revealed potential binding sites for MYC on the SAMHD1 gene promoter, DLBCL cell lines were treated with JQ-1, a BET and MYC inhibitor to assess possible MYC- associated regulation of SAMHD1. The patient cohort included 72 cases of *de novo* or transformed, previously untreated DLBCL diagnosed and treated at Karolinska University Hospital (Sweden) as well as 4 reactive lymph nodes for comparison. SAMHD1 expression was assessed by immunohistochemistry performed on tissue microarrays with duplicate tumor cores from each case or full tissue sections using a monoclonal antibody. The percentage of SAMHD1-positive cells was provided by counting at least 500 tumor cells in each case. Overall survival (OS) and event-free survival (EFS) were the clinical outcome endpoints. Survival analyses were performed using the Kaplan-Meier method (log-rank test) and Cox regression models.
Results: SAMHD1 was expressed in all 3 DLBCL at a variable level. Inhibition of MYC activity by JQ-1 resulted in a substantial increase in the SAMHD1 protein level suggesting a possible MYC-associated gene regulation. The highest change in the SAMHD1 levels was observed in the OCI-Ly3 cells that carries a wild-type p53 gene. In reactive lymph nodes, strong SAMHD1 expression was detected in the reactive small T-cells, whereas the large germinal center cells showed moderate expression levels. The median percentage of SAMHD1 in the neoplastic cells of DLBCL cases was 16.6% (SD=30%, range 0-95%). High SAMHD1 levels (>10% positive cells) were seen in 25% of DLBCL suggesting downregulation of SAMHD1 gene in the majority of DLBCL. The level of SAMHD1 expression (% positive tumor cells) was significantly higher in DLBCL with germinal center (GC) phenotype, in transformed versus *de novo* DLBCL, and in advanced Ann Arbor stage ($p<0.05$ in all associations). The SAMHD1 levels were also statistically associated with p53 expression as a continuous variable (% of positive cells). High SAMHD1 levels (>10%) showed a statistical trend towards worse EFS and OS, however, statistical significance was not reached ($p>0.05$) likely due to the small number of cases in the patient group with high SAMHD1.
Summary/Conclusion: SAMHD1 expression is shown for the first time in DLBCL and correlates with GC phenotype and tumor aggressiveness.

PS1382

DUAL TARGETING OF BRD4 BY AZD5153 AND BCL2 BY AZD4320 FOR HIGH-RISK B-CELL LYMPHOMAS CONCOMITANTLY OVEREXPRESSING C-MYC AND BCL2

T. Shimomura Takimoto[1,*], S. Maegawa[1], T. Tsukamoto[1], Y. Chinen[1], Y. Shimura[1], S. Mizutani[1], T. Kobayashi[1], T. Taki[2], S. Horiike[1], J. Kuroda[1]

[1]Hematology and Oncology, [2]Molecular Diagnostics and Therapeutics, Kyoto Prefectural University of Medicine, Kyoto, Japan

Background: The treatment outcomes of most B-cell lymphomas (BCLs) have been greatly improved by the advent of rituximab-containing immunochemotherapy; however, the prognoses of high-risk BCLs such as double hit lymphoma (DHL) and double expressing lymphoma (DEL) that concomitantly overexpress c-Myc and BCL2 have remained poor due to treatment resistance. Therefore, development of a new therapeutic strategy for DHL and DEL is urgently needed.
Aims: The effects of a novel BRD4 inhibitor, AZD5153, and a novel BH3-mimicking BCL2 inhibitor, AZD4320, were investigated in BCL-derived cell lines concomitantly overexpressing c-MYC and BCL2.
Methods: Conventional BRD4 inhibitors, JQ1 and I-BET151, and a BH3-mimetic, ABT-263, were obtained commercially. AZD5153 and AZD4320 were provided by AstraZeneca. Two DEL-derived cell lines, KPUM-UH1 and KPUM-MS3, established in our laboratory (Sasaki N, Exp Hematol 2011), and a DHL-derived cell line, STR-428 (JCRB Cell Bank) were used in the study. The growth inhibitory effects of the three BRD4 inhibitors and two BH3-mimetics were examined in the three cell lines using a modified MTT assay. Comparative gene expression profile (GEP) analyses and an ingenuity canonical signal pathway analysis based on the GEP results for untreated cells and cells treated with AZD5153 were performed in the three cell lines. Protein and mRNA expression levels were examined by Western blot and RT-PCR analysis, respectively.
Results: The growth inhibitory effect of AZD5153 was approximately 10 to 20 times greater than those of JQ-1 and I-BET151 in all three cell lines (IC_{50} values for AZD5151, JQ-1 and I-BET151 were 0.202-0.263mM, 2.039-4.355mM and 2.821-5.196mM, respectively). AZD4320 was approximately 2 to 100 times more effective than ABT-263 in inhibiting the growth of the three cell lines (IC_{50} values for AZD4320 and ABT-263 were 0.017-0.17mM and 0.304-1.908mM, respectively). The growth inhibitory effect of AZD5153 was mainly mediated by G1/S cell cycle blockade, while that of AZD4320 occurred through induction of apoptosis. GEP analyses identified 272 genes that were commonly upregulated by more than 1.5-fold, and 494 genes, including *c-MYC*, that were downregulated by 0.67-fold or more in the three cell lines. The ingenuity canonical signal pathway analysis of these genes revealed that BRD4 inhibition by AZD5153 inactivates the "B-cell receptor (BCR) signaling", "PI3K signaling in B-lymphocytes" and "CD27 signaling in lymphocytes" pathways. This suggests that BRD4 targeting potently inhibits pathways for B cell activation, survival and proliferation. Western blot and RT-PCR validated the finding that AZD5153 downregulated expression of genes associated with the B-cell receptor signaling pathway and c-MYC, without significant changes in expression of pro-apoptotic BH3-only proteins and anti-apoptotic BCL2 proteins such as BCL2 and $BCLX_L$. The combination of AZD5153 and AZD4320 showed synergistic and additive growth inhibitory effects in the three cell lines examined.
Summary/Conclusion: This study shows that blockade of BRD4, which potentially inhibits c-Myc and the BCR and PI3K pathways, is a rationale therapeutic strategy for DHL and DEL, and that AZD5153 may be a new BRD4 inhibitor of therapeutic interest. Furthermore, addition of AZD4320 to AZD5153 augmented the cell eradication effect in DHL- or DEL-derived cell lines. These results provide the rationale for dual targeting of BRD4 and antiapoptotic BCL2 proteins as a possible therapeutic strategy against BCLs with concomitant overexpression of c-MYC and BCL2.

PS1383

INTEGRATIVE GENOME-WIDE ANALYSIS REVEALS REGULATION OF CELL PROLIFERATION INVOLVING MULTIPLE SIGNALING PATHWAYS BY BRD4-BINDING TARGET GENES IN MANTLE CELL LYMPHOMA CELL LINES

T. Tsukamoto[1,*], S. Nakahata[2], R. Sato[3], A. Kanai[4], M. Nakano[3], T. Inaba[4], M. Taniwaki[1], K. Tashiro[3], K. Morishita[2], J. Kuroda[1]

[1]Division of Hematology and Oncology, Department of Medicine, Kyoto Prefectural University of Medicine, Kyoto, [2]Division of Tumor and Cellular Biochemistry, Department of Medical Science, Faculty of Medicine, University of Miyazaki, Miyazaki, [3]Department of Genomic Medical Sciences, Kyoto Prefectural University of Medicine,

Kyoto, [4]Department of Molecular Oncology, Research Institute of Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan

Background: Mantle cell lymphoma (MCL) remains mostly incurable, despite introduction of new therapeutic agents, and there is an urgent need to identify targetable molecules for rationale development of a more effective treatment strategy. BRD4 associates with acetylated histones and facilitates transcription of target genes. In addition, super-enhancers have been recognized as regulatory regions with a high level of acetylated histones, mediator complexes and BRD4, and super-enhancers in cancer cells are enriched at oncogenes. Importantly, several studies have highlighted the crucial functional involvement of BRD4 in oncogenes in various cancers. Therefore, broad screening of BRD4-regulated molecules might be translated into selection of therapeutic targets in MCL.
Aims: To identify therapeutic targets for MCL through broad screening of BRD4-regulated molecules using genome-wide approaches.
Methods: The human MCL-derived cell lines, Jeko-1, JVM-2, MINO and Z138, were used in the study. Growth-inhibitory effects of I-BET 151, a BRD4 inhibitor, were analyzed by MTT assay. For evaluation of apoptosis, cells were counterstained with Annexin V-FITC and propidium iodide (PI) and subjected to flow cytometric analysis. Gene expression profile (GEP) analysis was performed for JVM2 and Z138 cells with or without I-BET151 treatment. BRD4 ChIP-Seq was performed in JVM2 cells treated with 10 nM I-BET151 or DMSO. The interaction of candidate genes was examined using the Reactome Pathway Database.
Results: Treatment with I-BET151 for 72 h showed a dose-dependent inhibitory effect on cell proliferation in all of the four MCL cell lines. I-BET151 treatment induced G1/S cell cycle arrest and apoptosis in these MCL cell lines. GEP analyses revealed that 609 and 665 genes were commonly upregulated by more than 1.5-fold and downregulated by less than 0.67-fold, respectively. ChIP-Seq showed that 7988 BRD4-binding regions were dysregulated by I-BET151, with most of these sites in enhancer regions, and 547 regions were characterized as super-enhancers. Integrated analysis using the Reactome Pathway Database and the results of GEP and ChIP-Seq showed that a series of genes involved in the B cell receptor (BCR) signaling pathway and IKZF-MYC axis are regulated by BRD4, including *BLNK*, *IKZF2*, *IKZF3* and *MYB*. To confirm whether each BRD4 target contributes to survival and proliferation of MCL cells, we focused on several candidate targets: the BCR pathway, *IKZF* and *MYB*. However, ibrutinib, a Bruton kinase inhibitor, suppressed cell growth in only two of the four cell lines (MINO and JVM2), while lenalidomide, an inhibitor of the IKZF family, did not affect cell survival, despite its potency in decreasing IKZF1 and IKZF3 protein levels. *MYB* silencing using shMYB did not decrease cell proliferation in any of the four MCL cell lines.
Summary/Conclusion: Our results showed that BRD4 regulates transcription of multiple genes by binding to enhancer regions, partly involving super-enhancers and multiple known pathways such as BCR signaling and the IKZF-MYC axis. Indeed, MCL is already treated by several agents targeting these pathways in clinical practice or trial settings. The efficacies of these agents are limited because they have a single target, whereas I-BET151 concomitantly inactivated the BCR pathway and IKZF and had a high growth inhibitory efficacy in MCL cells. These results suggest that simultaneous targeting of multiple molecules involved in the BCR pathway and IKZF-MYC axis may overcome drug resistance in MCL, and that BRD4 inhibitors are promising candidates for MCL treatment.

PS1384

TRANSCRIPTOME SEQUENCING IN DIFFUSE LARGE B-CELL LYMPHOMA - RIBOSOME BIOGENESIS AND DYSREGULATION OF THE ACTIN CYTOSKELETON ARE ASSOCIATED WITH IMMUNOCHEMOTHERAPY RESISTANCE

M. Stern[1,2,*], M. Stenson[2,3], S. Bram Ednersson[2,4], A. Skaftason[5], S. Hasselblom[6], H. Herman Nilsson-Ehle[7], L. Mansouri[5], P.-O. Andersson[1,2]

[1]Section of Hematology, Department of Medicine, South Älvsborg Hospital, Borås, [2]Department of Internal Medicine and Clinical Nutrition, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, [3]Section of Hematology, Department of Medicine, Kungälv Hospital, Kungälv, [4]Department of Pathology, Sahlgrenska University Hospital, Gothenburg, [5]Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, [6]Department of Research, Development and Education, Region Halland, Halmstad, [7]Section of Hematology and Coagulation, Sahlgrenska University Hospital, Gothenburg, Sweden

Background: Diffuse large B-cell lymphoma (DLBCL) patients with early relapse or refractory disease have a very poor outcome. Immunochemotherapy resistance will, also in the era of targeted drugs, remain the major cause of treatment failure. Thus, there is a strong need to further characterize underlying molecular mechanisms. Given the heterogeneous nature of DLBCL, we hypothesized that a comparison of the transcriptome in patients with a distinctly diverse clinical response to immunochemotherapy, would give a higher probability to find relevant biological correlates.
Aims: To investigate if global RNA expression (RNA-seq) could reveal new mechanisms involved in immunochemotherapy resistance in DLBCL.
Methods: We derived total RNA from formalin-fixed paraffin-embedded (FFPE) tumour tissue from 2 groups of DLBCL patients, treated with immunochemotherapy, i) primary refractory or relapse within one year from diagnosis (n=26, REF/REL) and ii) progression-free more than 5 years after diagnosis (n=30, CURED). RNA-sequencing was performed on an Illumina HiSeq 2500 instrument (2x125bp paired end). Sequences were aligned to human reference genome version hg19 using STAR aligner and multiple quality control steps validated data integrity. Differentially expressed genes (DEGs) were identified and subjected to functional enrichment, protein-protein interaction networks and canonical pathway analyses, using NetworkAnalyst and Ingenuity Pathway Analysis (IPA).
Results: In total, we identified 327 DEGs (Benjamin-Hochberg adjusted *p*-value <0.05) of which 221 and 106 were upregulated in the REF/REL and CURED group, respectively. For genes upregulated in the REF/REL group, the most enriched pathways were RNA-transport (p=0.02) and ribosome biogenesis (p=0.03). Increased ribosome biogenesis and protein synthesis play essential roles in sustaining tumour cell growth and upregulation of ribosomal proteins has been associated chemotherapy resistance in different cancer forms. The top canonical pathway identified by IPA was actin cytoskeleton signaling (p<0.0001) and several of the enriched biological processes associated with upregulated genes in the CURED group were related to the actin cytoskeleton, e.g. focal adhesion (p<0.001), adherens junction (p=0.006), FcγR-mediated phagocytosis (p=0.01) and regulation of actin cytoskeleton (p=0.01). Emerging data indicate that remodeling of the actin cytoskeleton might play a role in chemoresistance, also for drugs included in the R-CHOP-regimen.
Among individual genes overexpressed in the REF/REL group we found several genes that previously have been described as negative prognostic factors, both in DLBCL in other malignancies, e.g. RAN, PDE4B, CD79B and PAK2. Conversely, in the CURED group, some genes have been associated with good prognosis (GDPD5, ankyrin 3), sustained remission (neuraminidase 3) or tumor suppression (USP20, HOPX) in other cancers.
Summary/Conclusion: We found an upregulation of genes related to actin cytoskeleton regulation in long-term progression-free DLBCL patients. In addition, genes upregulated in patients with early relapse or refractory disease were enriched in RNA-transport and ribosome biogenesis. In a recent study using a proteomic approach, we found a similar pattern (Stenson *et al.*, ASH 2016). Consequently, these RNA-seq data support that dysregulation of actin organization as well as ribosome biogenesis contributes to immunochemotherapy resistance in DLBCL, indicating that genes or proteins in these networks could become novel therapeutic targets.

PS1385

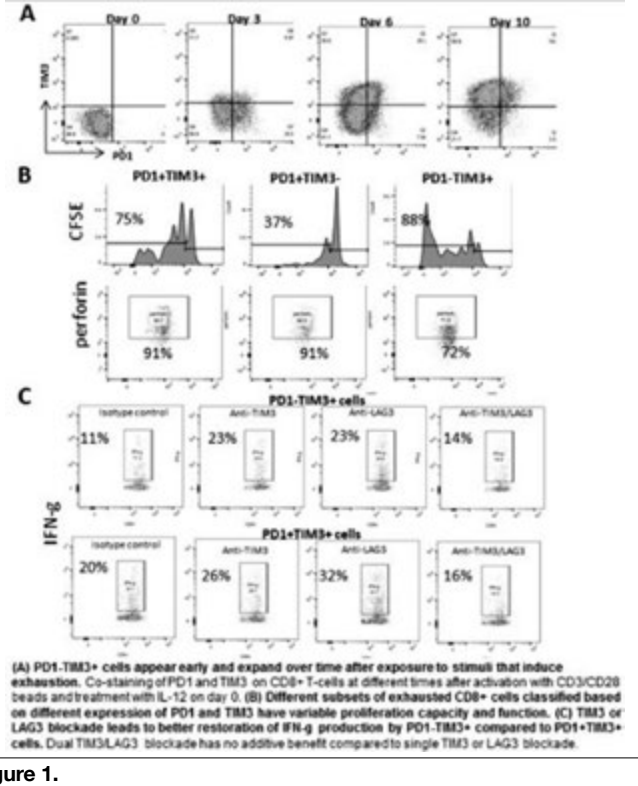
PD1-TIM3+ CELLS ARE THE PREDOMINANT POPULATION OF EXHAUSTED CD8+ CELLS IN FOLLICULAR LYMPHOMA WITH HIGH REACTIVATION POTENTIAL

T. Anagnostou[1,*], Z. Z. Yang[1], H.J. Kim[1], S. Jalali [1], J. Villasboas Bisneto[1], H. Wu[1], T.L. Price-Troska[1], A.J. Novak[1], S. M. Ansell[1]

[1]Hematology, Mayo Clinic, Rochester, United States

Background: Objective response rates to nivolumab are approximately 70% in classical Hodgkin lymphoma, but much lower in follicular lymphoma (FL) at around 11%, due to inability of these agents to activate exhausted T-cells in the tumor microenvironment (TME). The TME consists of different subsets of exhausted T-cells with variable expression of inhibitory receptors (including PD1, TIM3, LAG3) and function, which creates a spectrum of exhausted states.
Aims: In this study we compared the reactivation potential of different exhausted T-cell subsets in FL classified based on expression of PD1, TIM3 and LAG3.
Methods: We analyzed 13 healthy donor peripheral blood samples and 4 samples from FL lymph nodes. We activated healthy donor T-cells with CD3/CD28 and treated them with IL-12 to induce exhaustion as we have previously shown (Yang *et al.* J Clin Invest 2012). Flow cytometry was performed to assess expression of inhibitory receptors (PD1, TIM3, LAG3), T-cell function (perforin, granzyme B, IL-2 and IFN-g production) and proliferation (by CFSE labeling). Data was analyzed with FlowJo and we gated on different subpopulations of $CD8^+$ cells based on PD1, TIM3 and LAG3 expression. We then blocked PD1, TIM3 and LAG3 signaling, either alone and in combination at different times, and compared the reactivation poten-

tial of different CD8+ subsets.
Results: We first compared the proliferation and cytokine production between CD8+ cells activated through their T-cell receptor (TCR) with or without IL-12 treatment. We found that cells treated with IL-12 had significantly lower proliferation (60% with IL-12 vs. 84% without IL-12, p=0.003) and perforin production (16% with IL-12 vs. 24% without IL-12, p=0.002), suggesting that they were functionally exhausted. We then compared the frequencies of different T-cell subsets over time. PD1-TIM3+ cells appeared early and expanded over time following treatment with IL-12, comprising over 50% of all CD8+ cells by day 10 (Figure 1A). Similarly, in our FL samples, PD1-TIM3+ cells comprised 25-50% of all CD8+ cells. Subsequently, we compared the proliferation and function of the different exhausted T-cell subsets. T-cell subsets with variable PD1 and TIM3 expression differed in proliferation (75% in PD1+TIM3+ vs 37% in PD1+TIM3- vs 88% in PD1-TIM3+, p=0.03) and perforin production (91% in PD1-TIM3- vs 91% in PD1+TIM3+ vs 72% in PD1+TIM3- vs 78% in PD1-TIM3+, p=0.01) (Figure 1B). Of note, PD1-TIM3+ cells expressing LAG3 had lower proliferation compared to cells not expressing LAG3. Finally, we assessed the reactivation potential of different cell populations by blocking PD1, TIM3 and LAG3 alone and in combination. Although inhibitory receptor blockade was unable to prevent exhaustion, blockade of PD1, TIM3 or LAG3 on day 3 restored perforin and IFN-g production by total CD8+ cells. Since PD1-TIM3+ cells were the predominant CD8+ cell subset we tested the effect of blocking antibodies on them. TIM3 blockade was able to restore both proliferation of PD1-TIM3+ cells and IFN-g production, and LAG3 blockade had similar effects. However, TIM3/LAG3 dual blockade did not lead to greater improvement in IFN-g production compared to TIM3 or LAG3 blockade alone. Also, the effect of either TIM3 or LAG3 blockade on PD1-TIM3+ cells was higher compared to the effect on PD1+TIM3+ cells, suggesting that PD1-TIM3+ cells have better reactivation potential (Figure 1C).



(A) PD1-TIM3+ cells appear early and expand over time after exposure to stimuli that induce exhaustion. Co-staining of PD1 and TIM3 on CD8+ T-cells at different times after activation with CD3/CD28 beads and treatment with IL-12 on day 0. **(B) Different subsets of exhausted CD8+ cells classified based on different expression of PD1 and TIM3 have variable proliferation capacity and function. (C) TIM3 or LAG3 blockade leads to better restoration of IFN-g production by PD1-TIM3+ compared to PD1+TIM3+ cells.** Dual TIM3/LAG3 blockade has no additive benefit compared to single TIM3 or LAG3 blockade.

Figure 1.

Summary/Conclusion: PD1-TIM3+ cells are the predominant population of exhausted CD8+ cells in FL and have high reactivation potential, suggesting that they may serve as a target for future immunotherapies.

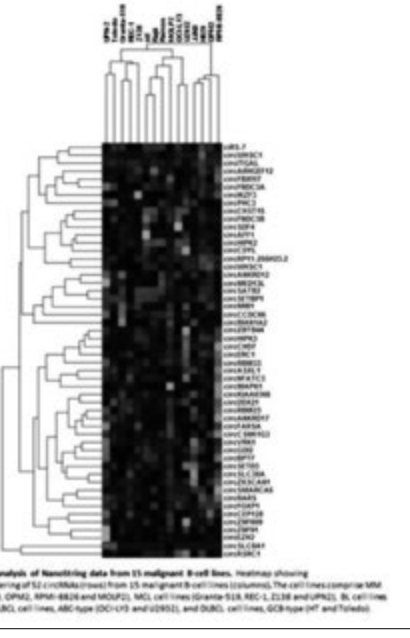
PS1386

ENZYME-FREE DIGITAL COUNTING OF ENDOGENOUS CIRCULAR RNA MOLECULES IN FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUES FROM PATIENTS WITH B-CELL MALIGNANCIES

M. Dahl[1,2,*], I. Daugaard[3,4], M. S. Andersen[3,4], T.B. Hansen[3,4], K. Grønbæk[1,2], J. Kjems[3,4], L.S. Kristensen[3,4]

[1]Department of Hematology, Rigshospitalet, [2]Biotech Research and Innovation Centre, BRIC, Copenhagen University, Copenhagen, [3]Department of Molecular Biology and Genetics (MBG), [4]Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Aarhus, Denmark

Background: Circular RNAs (circRNAs) are covalently closed endogenous RNA molecules with tissue- and disease specific expression patterns, which have potential as diagnostic and prognostic biomarkers in cancer. These molecules exert diverse regulatory functions including direct and indirect regulation of host gene expression (Ashwal-Fluss *et al.* 2014; Li *et al.* 2015), sponging of miRNAs (Hansen *et al.* 2013; Memczak *et al.* 2013), protein scaffolding (Du *et al.* 2016) and regulation of protein translation (Abdelmohsen *et al.* 2017). Several studies have reported prognostic relevance and functional significance of circRNAs in cancer (Kristensen *et al.* 2017), but no studies have yet examined the expression in B-cell malignancies. However, current methods for circRNA quantification have several limitations that prevent the development of clinically applicable assays. In particular, template switching and rolling circle transcription during reverse transcription (RT) and amplification bias during PCR may lead to misinterpretation of results (Szabo and Salzman 2016; Kristensen *et al.* 2017). Therefore, Northern Blotting, which does not rely on RT and PCR amplification, is currently the gold standard for circRNA detection. However, this method requires large quantities of RNA, is labor-intensive and not quantitatively accurate.
Aims: In this study, we aimed at developing an assay for accurate quantification of circRNAs that is clinically applicable. The NanoString platform, a technology for quantifying gene expression, is enzyme-free and suitable for low quality samples such as RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tissues. First, we examined whether the NanoString technology could be adapted for circRNA detection and quantification. Second, we examined the expression of circRNAs in different B-cell malignancies to assess whether circRNAs might represent novel oncogenic drivers in these diseases.
Methods: We profiled the genome-wide landscape of circRNA expression in cell lines of mantle cell lymphoma (MCL) and multiple myeloma (MM) using high-throughput RNA-sequencing (RNA-seq). Based on these data, we designed assays for simultaneous analyses of 52 unique circRNAs using the NanoString technology, in 15 different cell lines and paired fresh frozen and formalin-fixed, paraffin-embedded (FFPE) cell lines and patient samples including MCL, MM, follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma (BL) and chronic lymphocytic leukemia (CLL). To evaluate the specificity of the assays for circRNA detection, we treated RNA samples from two cell lines with RNase R, an exonuclease specifically degrading linear RNA.
Results: RNA-seq of the MCL and MM cell lines detected circRNAs known to be deregulated in other cancers, and identified a novel circRNA from the *IKZF3* oncogene. Interestingly, circRNA expression profiles were able to distinguish different B-cell malignancies, as shown in Figure 1. Enrichment of circRNAs upon treatment with RNase R proved that assays were specific for circRNA detection. The NanoString data were more reproducible and quantitatively more accurate than RNA-seq data and the technology works well for low quality RNA samples.



Hierarchical cluster analysis of NanoString data from 15 malignant B-cell lines. Heatmap showing expression and clustering of 52 circRNAs (rows) from 15 malignant B-cell lines (columns). The cell lines comprise MM cell lines (UM3, H929, OPM2, RPMI-8826 and MOLP2), MCL cell lines (Granta-519, REC-1, Z138 and UPN2), BL cell lines (Rajiand Ramos), DLBCL cell lines, ABC-type (OCI-Ly3 and U2932), and DLBCL cell lines, GCB-type (HT and Toledo).

Figure 1.

Summary/Conclusion: Together, we demonstrate that the NanoString technology enables specific, sensitive and accurate quantification of circRNAs in FFPE samples and provide a map of circRNA expression in B-cell malignancies. Our study provides a basis for future research focusing on circRNAs as functional drivers in lymphomagenesis and as potential novel biomarkers of prognostic relevance in these diseases.

PS1387

PROTEIN KINASE CK1 ALPHA SUSTAINS MANTLE CELL LYMPHOMA SURVIVAL BY IMPINGING ON BCR-LINKED SIGNALING PATHWAYS

S. Manni[1,2,*], E. Daniele[2], M. Carrino[1,2], K. Gianesin[2], L. Quotti Tubi[1,2], A. Visentin[1], L. Trentin[1,2], G. Semenzato[1,2], F. Piazza[1,2]

[1]Department of Medicine, Hematology and Clinical Immunology Branch, University of Padova, [2]Venetian Institute of Molecular Medicine, Padova, Italy

Background: Mantle Cell lymphoma (MCL) is a B-cell neoplasm that accounts for around 5-10% of all lymphomas, which urgently needs new therapeutic strategies. The Ser/Thr protein kinase CK1α has been found pivotal for multiple myeloma and Diffuse Large B cell Lymphoma survival. We recently demonstrated that CK1α plays a key role in myeloma cell growth and its inactivation determines myeloma cell death. CK1α is essential in the regulation of several growth signaling cascades (such as Wnt/βcatenin, NF- B, AKT, the p53-driven response), which are important for MCL clone expansion. Nevertheless, the role of CK1α in MCL pathogenesis and in associated survival signaling events has never been studied.
Aims: In this study, we have investigated CK1α expression, cellular localization and contribution to MCL survival. We evaluated the potential therapeutic role of CK1α inhibition with chemical compounds or gene silencing through RNA interference on MCL cell growth and proliferation. Moreover, the consequences on survival signaling pathways associated to resistance to chemotherapeutics have been investigated.
Methods: CK1α expression and localization were analyzed in MCL cells and controls by western blot (WB) analysis and confocal immunofluorescence microscopy. CK1α silencing was performed through the generation of CK1α IPTG shRNA inducible MCL clones or through electroporation of double strand CK1α-directed siRNA in MCL cell lines. The consequences of CK1α inhibition with D4476, a small ATP-competitive compound, or by RNA interference on MCL cell survival and proliferation were investigated with MTT test, Annexin V/Propidium Iodide labeling and cytofluorimetric analysis, analysis of PARP cleavage, analysis of pro-apoptotic/pro-survival proteins expression in WB. CK1α-dependent signaling events were analyzed by WB.
Results: We found that CK1α was highly expressed in purified primary MCL B cells and cell lines, compared with healthy controls. Differently from normal B cells, in which it was only cytosolic, in MCL cells CK1α localized both in the cytoplasm and in the nucleus. Inhibiting CK1α in MCL cells with D4476 or RNAi reduced cell viability, determined apoptosis and proliferation arrest, which were confirmed by an increase in the subG1/apoptotic peak and by the reduction of the S phase of the cell cycle. The apoptotic effects of CK1α inhibition was confirmed by an increase of the PARP cleavage and caspase activity and by a reduction of the prosurvival Mcl1 protein. From a molecular standpoint, CK1α inhibition caused a reduction of IKKα/β and RelA/p65 phosphorylation on Ser176/180 and Ser536 respectively, and of AKT phosphorylation on Ser473, indicating an important role upstream of critical B-cell receptor-dependent signaling pathways.
Summary/Conclusion: Our findings suggest that CK1α is a growth-propelling kinase in MCL, representing a new potential molecular therapeutic target. The identification of the exact mechanism(s) of action on the pathways targeted by CK1α in MCL will be the subject of future research.

PS1388

WEE1 INHIBITION ENHANCES ANTI-APOPTOTIC DEPENDENCY OF DIFFUSE LARGE B-CELL LYMPHOMA CAUSED BY CELL CYCLE ARREST AND DNA DAMAGE INDUCTION

M. De Jong[1,*], M. Langendonk[1], B. Reitsma[1], P. Herbers[1], A. Mulder[2], G. Huls[1], A. Van den Berg[3], E. Ammatuna[1], L. Visser[3], T. van Meerten[1]

[1]Hematology, [2]Laboratory medicine, [3]Pathology, University Medical Center Groningen, Groningen, Netherlands

Background: Patients with refractory or relapsed diffuse large B cell lymphoma (DLBCL) after first-line immuno-chemotherapy have a poor prognosis. Drug resistance has been attributed to intrinsic features of DLBCL such as high levels of DNA damage, impaired cell cycle checkpoints, and upregulation of intrinsic anti-apoptotic proteins. Targeting DNA repair and cell cycle checkpoint proteins and subsequently overcoming apoptotic resistance would therefore be a promising strategy. We recently demonstrated that inhibition of WEE1, a replication checkpoint kinase that prevents the onset of mitosis in cells that have incompletely replicated or have damaged genomes, resulted in apoptosis in DLBCL (MRW de Jong *et al.*, 2018). However, many tumors, including DLBCL, show apoptotic defects, which can be characterized using BH3 profiling, a rapid and functional method for determining the cellular dependency on individual pro-apoptotic and anti-apoptotic molecules (BCL-2 family proteins) and might be able to predict which treatment.
Aims: To determine the effects of WEE1 inhibition on cell cycle arrest, DNA damage and apoptosis and to determine the cellular dependency of DLBCL on individual pro-apoptotic and anti-apoptotic proteins after WEE1 inhibition, by employing BH3 profiling.
Methods: We established static BH3 profiles of eight DLBCL cell lines and DLBCL patient samples. A dynamic BH3 profile (DBP) was acquired for DLBCL cell lines SC-1, SUDHL-10 and a DLBCL patient after 18 hours of WEE1 inhibition by AZD1775 treatment. As controls, cell cycle arresting agents and/or DNA damage inducing agents Palbociclib, RO3306, Nocodazole and UV radiation were tested after 18 hours before establishing a DBP.
Results: We demonstrated that WEE1 inhibition by the anti-WEE1 inhibitor AZD1775 results in both a G2/M arrest and DNA damage. The static BH3 profiles showed that DLBCL cell lines have either a BAD-dependent profile or a BAD-independent profile. SC-1 cell line (BAD-dependent) showed increased dependency for BCL-2/BCL-XL/BCL-W, whereas SUDHL-10 (BAD-independent) showed increased dependency for MCL-1. Further validation of these anti-apoptotic dependencies showed that SC-1 had 10-fold increased sensitivity to BCL-2 inhibitor venetoclax (p=0.0012) after pretreatment with sub-optimal dose of AZD1775 (IC-50), but no increased sensitivity to MCL-1 inhibition (p=0.2370). SUDHL-10 showed 2-fold increased sensitivity to MCL-1 inhibitor A-1210477 (p=0.002), but no increased sensitivity to BCL-2 inhibition (p=0.8302) after WEE1 pretreatment. These results agree with the anti-apoptotic dependencies obtained with DBP. DBP of a chemotherapy-refractory DLBCL patient showed increased dependency BCL-2/BCL-W, which could be validated with the BCL-2/BCL-XL/BCL-W inhibitor navitoclax, but not with venetoclax, suggesting increased dependency on BCL-W after AZD1775 treatment. These DBP could be replicated by both induction of G2/M arrest and induction of DNA damage.
Summary/Conclusion: These data show that WEE1 inhibition causes both G2/M arrest and DNA damage, which leads to apoptosis in DLBCL. In addition, we demonstrate that WEE1 inhibition alters the dependence on anti-apoptotic proteins in DLBCL. Where DLBCL cell lines and patient samples exhibit unique anti-apoptotic dependencies upon WEE1 treatment, DBP was able to determine these profiles, and could therefore be used to personalize treatment of DLBCL with BH3 mimetics.

PS1389

INFLUENCE OF PLASMA EBV LOAD ON PROGNOSIS OF HIV-RELATED LYMPHOMAS

J. Muncunill[1,*], M.J. Baptista[1], A. Hernandez-Rodriguez[2], J. Dalmau[3], O. Garcia[1], G. Tapia[4], J.-M. Sancho[1], J. Martinez-Picado[3], E. Feliu[1], J.-L. Mate[4], J.-M. Ribera[1], J.-T. Navarro[1]

[1]Department of Hematology, ICO-Hospital Universitari Germans Trias i Pujol, Josep Carreras Leukaemia Research Institute, Universitat Autònoma de Barcelona, [2]Department of Microbiology, Hospital Universitari Germans Trias i Pujol, [3]AIDS Research Institute-IrsiCaixa, Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol, Universitat Autònoma de Barcelona, Institució Catalana de Recerca i Estudis Avançats (ICREA), [4]Department of Pathology, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, Spain

Background: The detection of Epstein Barr virus (EBV) in tumoral cells (EBER+) has been associated with more aggressiveness of lymphoma and poorer prognosis. The addition of rituximab to chemotherapy, seems to have improved the prognosis of EBV-related lymphomas. Some studies have shown that plasma EBV load has impact on lymphoma prognosis. However, the influence of plasma EBV load on prognosis of HIV-related lymphomas has been scarcely studied.
Aims: We aimed to evaluate the usefulness of plasma EBV load as prognostic factor in HIV-related lymphomas. Additionally, we analyzed the impact of rituximab on prognosis among NHL-HIV patients taking into account the

plasma EBV load.
Methods: Eighty HIV-related lymphoma cases (61 B-cell aggressive NHL and 19 HL) were studied. Plasma EBV load was determined at lymphoma diagnosis by a commercial real-time PCR technique (Abbott Real Time EBV, Abbott Molecular, Illinois, USA) and was considered as dichotomous variable according to two different cutoffs: detectable load provided by technique limitation (cutoff > 110 copies/ml), and high load (cutoff > 5000 copies/ml), determined by maxstat technique. We studied the association between the main clinical-biological variables and both, detectable and high EBV load in plasma. Wilcoxon test were used to compare qualitative variables. We studied the differences in overall survival (OS) and progression-free survival (PFS) of the patients regarding the EBV load, taking into account the two cutoffs, as well as the addition of rituximab to chemotherapy. Survival analyses were performed using the Kaplan-Meier method. Cox proportional risks regression models were performed for both univariate and multivariate analyses. P-values of less than 0.05 were considered statistically significant.
Results: Patients with detectable plasma EBV load (n=42) had more frequently EBV in the tumor (assessed by EBER) than patients with negative load (n=38) (68% vs 19%, p=0.005). More patients with high EBV load (n=19) had elevated LDH and EBER positivity than those with low load (n=61) (72% vs 36%, p=0.016 and 88% vs 31%, p=0.006, respectively). Patients with high EBV load had worse OS and PFS than those with low load [2-year OS (95% CI): 65% (54-78%) vs. 42% (25-71%), p=0.025, and 2-year PFS (95% CI): 55% (44-70%) vs. 42% (25-71%), p=0.05, respectively]. In the multivariate analysis, high EBV load was an independent factor for worse prognosis for both OS and PFS [HR (95% CI): 2.4 (1.1-5.3), p=0.03; 2.2 (1.01-4.6), p=0.045, respectively]. In HL patients, those with high EBV load (n= 6) had significantly worse prognosis than those with low load (n=13) for OS [2-year OS (95% CI): 33%, (11-100%) vs. 92%, (79-100%), p=0.003], and for PFS [2-year PFS (95% CI): 33% (11-100%) vs. 73% (45-100%), p=0.007]. In NHL patients, detectable EBV load had impact on OS and PFS only in those patients treated without the addition of rituximab to chemotherapy (n=32) (Figure 1A and B). The impact of EBV load remained significant in the multivariate analysis for OS and PFS [HR (95% CI): 2.7 (1.2-5.9), p=0.012; 2.4 (1.6-5.09), p=0.01, respectively]. Furthermore, high EBV load also had negative influence on prognosis of NHL lymphomas treated without rituximab.

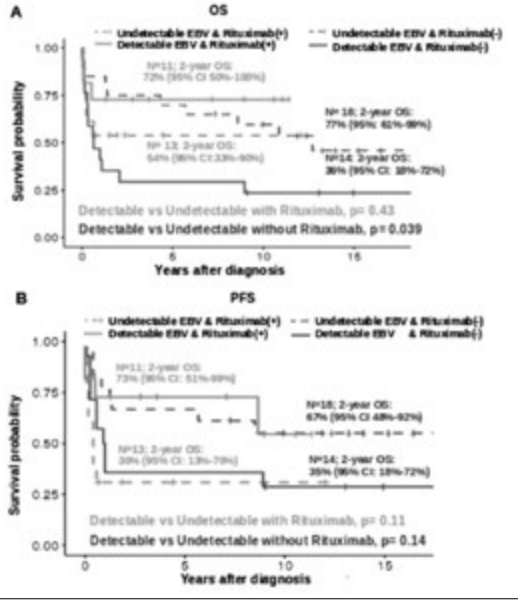


Figure 1.

Summary/Conclusion: Plasma EBV load is a prognostic factor with negative impact on HIV-related lymphoma. Among HIV-NHL cases, rituximab eliminates the negative influence of EBV load on prognosis.

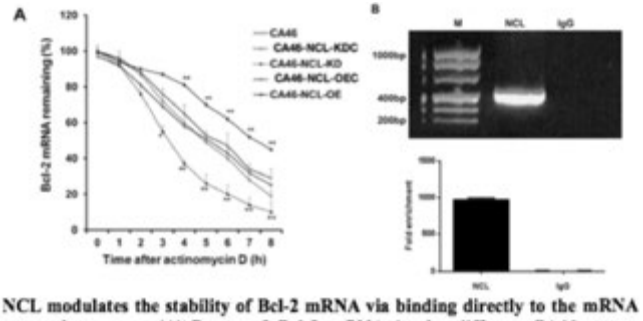
PS1390

NUCLEOLIN ENHANCED ADRIAMYCIN RESISTANCE VIA THE REGULATION OF BCL-2 EXPRESSION IN CA46 BURKITT'S LYMPHOMA CELLS

J. Hu[1,*], X. Mei[1], Y. Chen[1], Y. Chen[1], L. Wang[1], Y. Cao[1], M. Lin[1]

[1]*Department of Hematology, Fujian Institute of Hematology, Fujian Medical University Union Hospital, Fuzhou, China*

Background: The nucleo-cytoplasmic protein nucleolin (NCL, C23) is an important nucleo-cytoplasmic multifunctional protein, which is abundantly expressed in the nucleolus, the nucleoplasm, the cytoplasm and the cell membrane, playing various roles in cell proliferation, apoptosis and angiogenesis. Due to its multifaceted profile and high expression in cancer, nucleolin is considered a marker of drug resistance to chemotherapy. However, the biochemical mechanisms by which the increased expression of nucleolin suppressed the senstivity of chemotherapeutic drugs in several cancers have not been fully elucidated.
Aims: To explore the effect of NCL on drug senstivity and potential menchanism in Burkitt's lymphoma cells.
Methods: CA46 Burkitt's lymphoma cells were transfected with lentiviruses carrying the nucleolin gene (CA46-NCL-Overexpression, CA46-NCL-OE) or shRNA sequences that target the endogenous nucleolin gene (CA46-NCL-Knockdown, CA46-NCL-KD). Effect of NCL on cellular drug senstivity and apoptosis were examined by MTT assay and flow cytometry analysis respectively. Bcl-2 level was determinded using q-PCR and western blot test. Stability of Bcl-2 mRNA was measured by Actinomycin D treatment assay. Finally, RNA-immunoprecipitation assays(RIP) was used to identify the binding and interaction of Bcl-2 mRNA and NCL complexes.
Results: The results showed that CA46-NCL-OE cells exhibited significantly higher IC50 for Adriamycin compared to CA46-NCL-Overexpression negative control cells(CA46-NCL-OEC) ($p<0.01$). The IC50 of the CA46-NCL-OEC was increased from 0.39±0.08 μg/ml to 0.68±0.06 μg/ml in the CA46-NCL-OE cells. The IC50s of the CA46-NCL-KDC and CA46-NCL-KD cells were 0.30±0.04 μg/ml and 0.15±0.02 μg/ml respectively. In CA46-NCL-KD cells, the rate of apoptosis was significantly increased compared to CA46-NCL-Knockdown negative control cells(CA46-NCL-KDC) ($p<0.05$), whereas the opposite effect was noted in CA46-NCL-OE cells. A significant reduction in the mRNA and protein levels of Bcl-2 expression was observed in the CA46-NCL-KD cells compared with CA46-NCL-KDC cells ($p<0.01$), while in the CA46-NCL-OE cells the Bcl-2 expression levels were significantly increased compared with CA46-NCL-OEC cells ($p<0.01$). In addition, the decay rate of Bcl-2 mRNA in CA46-NCL-KD cells after Actinomycin D treatment was much faster than that in CA46-NCL-KDC cells. The half-life of Bcl-2 mRNA was extended from 5.2h in CA46-NCL-OEC cells to 7.4h in CA46-NCL-OE cells, whereas it was reduced from 4.9h in CA46-NCL-KDC cells to 3.2h in CA46-NCL-KD cells, suggesting that the expression of NCL could influence the stability of Bcl-2 mRNA. Finally, RNA-immunoprecipitation assays(RIP) demonstrated a significant enrichment of Bcl-2 mRNA in NCL immunoprecipitated complexes compared with control, indicating NCL could bind and interact with Bcl-2 mRNA in CA46 cells. (Figure 1).



NCL modulates the stability of Bcl-2 mRNA via binding directly to the mRNA transcript. (A) Decay of Bcl-2 mRNA in the different CA46 groups following treatment with actinomycin D (2μg/ml) for the indicated time periods. The results are expressed as percentage of the Bcl-2/β-actin ratio at the indicated time points compared with the Bcl-2/β-actin mRNA ratio at time 0 h. (CA46-NCL-KD vs CA46-NCL-KDC, CA46-NCL-OE vs CA46-NCL-OEC, *P<0.05, ** P<0.01). (B) Bcl-2 mRNA enrichment by RIP assay with an anti-NCL antibody. M:molecular marker.NCL: Bcl-2 mRNA was immunoprecipated with an anti-NCL antibody. IgG: negative control , Bcl-2 mRNA was immunoprecipated with a normal mouse IgG antibody.

Figure 1.

Summary/Conclusion: All above results indicated that nucleolin could mediate BCL-2 expression and stability, subsquently enhance adriamycin resistance in CA46 Burkitt's lymphoma cells.

PS1391

DOMINANT CYTOTOXIC NK CELL SUBSET WITHIN CLPD-NK PATIENTS IDENTIFIES A MORE AGGRESSIVE NK CELL PROLIFERATION

G. Barila'[1,2,*], A. Teramo[1,2], G. Calabretto[1,2], V. Trimarco[1], S. Carraro[1], M. Leoncin[1,2], C. Vicenzetto[1,2], V. R. Gasparini[1,2], M. Facco[1,2], F. Piazza[1,2], G. Semenzato[1,2], R. Zambello[1,2]

[1]Dept of Medicine, Hematology and Clinical Immunology section, Padua University School of Medicine, [2]Venetian Institute of Molecular Medicine (VIMM), Padua, Italy., Padua, Italy

Background: Natural Killer (NK) cells represent a class of innate cytotoxic lymphocytes characterized by CD3-/CD16+/CD56+ phenotype. Two major NK cell subsets can be recognized, $CD56^{high}/CD16^{dim/neg}$ NK cells with cytokines producing function and $CD56^{dim}/CD16^{high}$ NK cells displaying cytotoxic ability. Moreover, a subtype of NK cells with $CD56^{dim}/CD16^{high}$/CD57+ phenotype and memory properties was recently discovered. Chronic Lymphoproliferative disorder of NK cells (CLPD-NK) is a provisional entity, characterized by the chronic expansion of NK cells with restricted Killer Immunoglobuline-like Receptor (KIR) pattern. CLPD-NK still remains a heterogeneous disease with most patients being asymptomatic and the main feature of the disease being represented by neutropenia. The discovery of somatic *STAT3* and *STAT5b* mutations in CLPD-NK and T-Large Granular Lymphocyte Leukemia (LGLL) focused the attention on a constitutive activation of JAK-STAT pathway in the development of these disorders.
Aims: Using flow cytometry and regardless of KIR expression, the aim of this study was to identify different biological and clinical NK-CLPD patient subsets.
Methods: NK cells of 25 CLPD-NK patients were analysed with FACS for CD3, CD16, CD56, CD57, CD158a, CD158b, CD158e, CD94, NKG2A and NKG2C antibodies. Clinical characteristics of these patients were studied. *STAT3* exon 21 mutation analysis and *STAT5b* exon 16-18 mutation analysis was performed by Sanger sequencing and ARMS PCR
Results: By flow analysis, NK cells of 25 CLPD-NK patients were analysed for CD16 and CD56 expression, recognizing two major NK cell subsets, patients with $CD56^{dim}/CD16^{dim}$ NK cells (4/25, 16%) and patients with $CD56^{neg/dim}/CD16^{high}$ NK cells (21/25, 84%) (Panel A to C). Neutropenia (ANC<1,500/mm^3) was the most relevant feature of our cohort, detected in 10/25 patients (40%), with 5 patients (20%) presenting severe neutropenia (ANC<500/mm^3). Almost all symptomatic patients were included in $CD56^{neg/dim}/CD16^{high}$ subgroup with 8/21 (38%) patients presenting neutropenia, 5/21 (24%) severe neutropenia and 3/21 patients (14%) requiring treatment. NK cells of these patients were studied for CD57 expression and all 5 patients who experienced severe neutropenia and all treated patients presented a significantly lower CD57 mean expression toward other $CD56^{neg/dim}/CD16^{high}$ patients (12.48%±2.87 *vs* 60.43%±4.38, p<0.0001). In conclusion, three major NK cell subgroups of patients can be identified, $CD56^{dim}/CD16^{dim}$ subgroup, $CD56^{neg/dim}/CD16^{high}$/CD57- "Cytotoxic" subgroup and $CD56^{neg/dim}/CD16^{high}$/CD57+ "Memory" subgroup (Panel D-E). Among the three subgroups, no significant differences in CD94-NKG2A/C and KIR expression were found, although CD94/NKG2C phenotype and KIR restriction for CD158b and CD158e was almost exclusively a distinct feature of NK "memory" subgroup. Whereas, $CD56^{dim}/CD16^{dim}$ and NK "cytotoxic" subgroups displayed CD94-NKG2A phenotype and presented a skewed KIR pattern characterized by lack of KIR expression. Finally, *STAT3* exon 21 mutations analysis was performed with only two (8%) mutated patients found, these latters characterized by "Cytotoxic" NK immunophenotype. On the contrary, none *STAT5b* mutated patient was found (Figure 1).

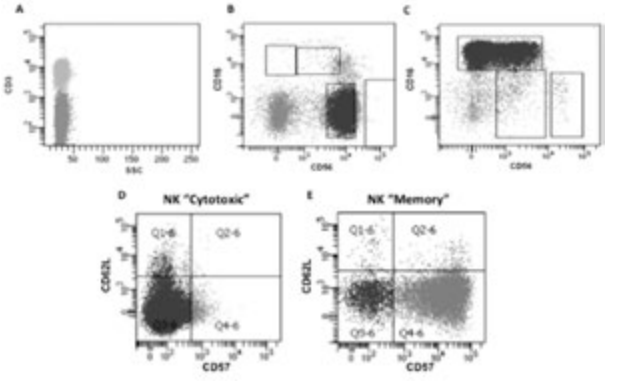


Figure 1.

Summary/Conclusion: Through NK cells flow analysis, discrete subtypes of CLPD-NK can be identified. Independently from KIR expression, patients characterized by $CD56^{neg/dim}/CD16^{high}$/CD57- cytotoxic NK cells expansion represent a unique phenotypic subgroup characterized by more symptomatic disease and the presence of *STAT3* mutation, suggesting a more aggressive proliferation of NK cells.

PS1392

IDENTIFICATION OF A MIR-146B-FASL AXIS IN THE DEVELOPMENT OF NEUTROPENIA IN T-LARGE GRANULAR LYMPHOCYTES LEUKEMIA

G. Calabretto[1,*], A. Teramo[1], B. Mariotti[2], M. Rossato[2], M. Castellucci[2], G. Barilà[1], M. Leoncin[1], C. Vicenzetto[1], V.R. Gasparini[1], M. Facco[1], G. Semenzato[1], F. Bazzoni[2], R. Zambello[1]

[1]Department of Medicine, Venetian Institute of Molecular Medicine, Padua, [2]Department of Medicine, Division of General Pathology, Verona, Italy

Background: T-Large Granular Lymphocytes (T-LGLs) leukemia (T-LGLL) is a chronic lymphoproliferation of clonal CD3+ LGLs, which can be separated in $CD3^+CD8^+CD4^-$ T-LGLs (CD8 T-LGLL) or $CD3^+CD8^{-/dim}CD4^+$ T-LGLs (CD4 T-LGLL). LGLs proliferation is maintained through an impairment of the apoptotic machinery, due to the constitutive activation of several pro-survival signaling pathways. Among these, one of the most important is the JAK/STAT axis. STAT3 over-expression represents a hallmark of leukemic LGLs and 30-40% of patients harbors STAT3 somatic mutations, that lead to STAT3 activation. These genetic lesions were recently correlated to an increased incidence of neutropenia, which represents the most frequent clinical manifestation in T-LGLL patients. The mechanism leading to neutropenia development is not completely established, although literature data provided evidence that soluble Fas Ligand (FasL) is involved in this process. Consistently, we recently demonstrated that a subset of neutropenic CD8 T-LGLL patients were characterized by high levels of FasL, whose transcription was mediated by their high STAT3 activation. These data provided a molecular explanation for the correlation between STAT3 activation and neutropenia in CD8 T-LGLL patients. However, the mechanism through which FasL production is regulated in these patients has not been clarified yet. Many microRNAs (miRNAs) are reported to act both as tumour suppressors and oncogenes in several haematological malignancies. Although STAT3 is an inducer of transcription of several oncogenes, its relationship with miRNAs has not been evaluated in T-LGLL patients yet.
Aims: The aim of this study was to investigate whether STAT3 could carry out its pathogenetic role in T-LGLL through an altered expression of miRNAs.
Methods: LGLs were purified by FACSAria cell sorter from peripheral blood mononuclear cells (PBMCs) of untreated LGLL patients and healthy donors (HD). High throughput and single miRNA analysis were carried out on purified LGLs by using the TaqMan® Human microRNA Array and Assays, respectively. Transfection with miR-146b mimic was performed in Jurkat cells or purified T-LGLs using the Amaxa Nucleofactor and the Ingenio Electroporation Solution. Transcriptional and protein expression was evaluated by real time-PCR and western blot assays.
Results: We assessed the expression of 756 mature miRNAs on purified T-LGLs, identifying two clusters: one characterized by neutropenic CD8 T-LGLL patients, with high STAT3 activation, the other characterized by HD and CD4 T-LGLL patients, with normal absolute neutrophil count (ANC) and low STAT3 activation. miRNA differentially expressed in CD8 and CD4 T-LGLs were subsequently analyzed for correlation with ANC. The expression of miR-146b, found down-regulated in patients with high STAT3 activation, was the only one correlated with ANC. Therefore, we transfected Jurkat cells and CD8 T-LGLs with a miR-146b mimic, to investigate the role of miR-146b in neutropenia development. Our data demonstrated that miR-146b downregulation increased the expression of Human Antigen R (HuR), which is a miR-146b target and a known mRNA stabilizer, reported to be required for FasL expression in T-lymphocytes. HuR protein mediated FasL mRNA stabilization, leading to increased FasL production and, consequently, to neutropenia development.
Summary/Conclusion: The current study, for the first time, demonstrated a direct role of miR-146b in the development of neutropenia reported in a subset of T-LGLL patients, representing potential targets for an individualized therapeutic approach.

PS1393

GENOMIC AND CLINICAL CHARACTERIZATION OF THE ROLE OF MYD88 MUTATION STATUS IN WALDENSTROM'S MACROGLOBULINEMIA

Z. Hunter[1,2,*], J. Castillo[3], J. Gustine[3], S. Treon[1]

[1]Harvard Medical School, [2]Dana-Farber Cancer Instutite, [3]Dana-Farber Cancer Institute, Boston, United States

Background: Waldenstrom's Macroglobulinemia (WM) is characterized by bone marrow (BM) involvement of IgM secreting lymphoplasmacytic lymphoma and somatic activating mutations in MYD88 and CXCR4 in 95% and 35-40% of patients, respectively. Deletions in in chromosome 6q were also observed in half of MYD88 mutated ($MYD88^{MUT}$) patients. Patients with wild-type MYD88 ($MYD88^{WT}$) have an increased mortality risk compared to their $MYD88^{MUT}$ counterparts (Treon *et al*., BJH 2017) and demonstrate inferior response to the BTK inhibitor, ibrutinib (Treon *et al*., NEJM 2015). These findings point to fundamental genomic differences in tumor biology between MYD88 mutated and wild-type patients.
Aims: To improve the care and clinical management of all WM patients we sought to study the clinical characteristics and genetic lesions that differ in WM patients based on MYD88 mutation status.
Methods: We identified 46 $MYD88^{WT}$ and 262 $MYD88^{MUT}$ patients diagnosed during the same time period. Clinical characteristics were compared at time of diagnosis and patients were followed for disease transformation and overall survival. Bone marrow (BM) $CD19^{+}$ WM cells were isolated from 18 $MYD88^{WT}$ patients following informed consent. DNA and RNA from these cells were submitted for next generation whole exome and RNA sequencing. Findings were compared to our existing whole genome and RNA sequencing data of 57 WM patients (Hunter *et al*., Blood 2014; 2016). A cohort of 9 $CD19^{+}CD27^{+}$ memory B-cells (MB), 9 $CD19^{+}CD27^{-}$ peripheral blood B-cells, and 16 $CD138^{+}$ plasma cells from healthy donors (HD) were also included.
Results: The median follow-up for all patients was 64.1 and 73.7 months for $MYD88^{WT}$ and $MYD88^{MUT}$ patients, respectively (p=NS). To date 11 (23.9%) and 15 (5.7%) deaths have been recorded among the $MYD88^{WT}$ and $MYD88^{MUT}$ patients, respectively (p=0.003). The estimated 10-year survival was 73% (95% CI 52-86%) for $MYD88^{WT}$ and 90% (95% CI 82-95%) for $MYD88^{MUT}$ patients (Log-rank p<0.001). Transformation to diffuse large B-cell lymphoma occurred in 7 (15.2%) of $MYD88^{WT}$ and 2 (0.76%) of $MYD88^{MUT}$ patients (p<0.0001), with an odds ratio of transformation of 23.3 (95% CI 4.2-233.8; p<0.001). Whole exome sequencing analysis of the $MYD88^{WT}$ patients revealed somatic mutations in TBL1XR1 (28%), PTPN13 (22%), KMT2D (17%), CXCR4 (17%), MALT1 (11%), NFKB2 (11%), TP53 (11%) and with BCL10, NFKB1, NFKBIB, NFKBIZ, NOTCH1, ATM, IGFR1, KDM6A, and KMT2C each observed once (6%). No deletions were observed in chromosome 6q in $MYD88^{WT}$ WM. $MYD88^{WT}$ samples displayed a distinct transcriptional profile. However, 82/100 (82%) of the top differentially expressed genes between $MYD88^{WT}$ and HD MB samples were seen in a comparison of $MYD88^{MUT}$ WM compared with HD MB. These genes included distinctive WM associated findings from our previous $MYD88^{MUT}$ analysis such as DNTT, RAG1, RAG2, CXCL12 and VCAM1. Gene set enrichment analysis indicated a down regulation of NFKB induced TNFA signaling relative to $MYD88^{L265P}$ WM and a corresponding increase in AKT/MTOR signaling compared with either HD MB cells or $MYD88^{MUT}$ WM (p<0.005) (Figure 1).

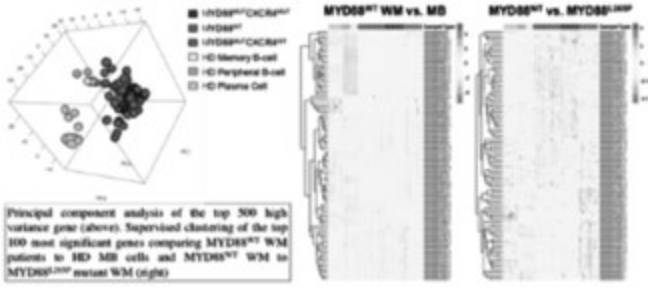


Figure 1.

Summary/Conclusion: Our findings confirm the prognostic significance for MYD88 mutation status as an important determinant of overall survival and risk of disease transformation. While genomic drivers differ between the two populations, the overall transcriptional profile seems largely comparably with the overall $MYD88^{MUT}$ gene expression signature. Additional studies and comparisons to related lymphomas are planned.

PS1394

IN SILICO DISSECTION OF DIFFUSE LARGE B CELL LYMPHOMA MICROENVIRONMENT PROVIDES A 45-GENE PANEL FOR RISK STRATIFICATION

S. Ciavarella[1,*], M.C. Vegliante[1], M. Fabbri[2], S. De Summa[3], F. Melle[2], G. Motta[2], A. Enjuanes[4], A. Gulino[5], S. Tommasi[3], A. Scattone[6], A. Zito[6], C. Agostinelli[7], U. Vitolo[8], A. Chiappella[8], E. Campo[9], C. Tripodo[5], A. Guarini[1], S.A. Pileri[2]

[1]Hematology and Cell Therapy Unit, IRCCS-Istituto Tumori "Giovanni Paolo II", Bari, [2]Unit of Diagnostic Haematopathology, European Institute of Oncology, Milan, [3]Molecular Diagnostics and Pharmacogenetics Unit, IRCCS - Istituto Tumori "Giovanni Paolo II", Bari, Italy, [4]Genomic Unit, IDIBAPS, Barcellona, Spain, [5]Tumor Immunology Unit, Dipartimento per la Promozione della Salute e Materno Infantile "G. D'Alessandro", University of Palermo, Palermo, [6]Pathology Department, IRCCS-Istituto Tumori "Giovanni Paolo II", Bari, [7]Department of Experimental, Diagnostic, and Specialty Medicine (DIMES), Bologna University School of Medicine, Bologna, [8]Hematology Unit, Azienda Ospedaliero Universitaria Città della Salute e della Scienza di Torino, Torino, Italy, [9]Hematopathology Unit, Pathology Department, University of Barcelona, Institute of Biomedical Research August Pi i Sunyer (IDIBAPS), Barcelona, Spain

Background: Diffuse large B cell lymphoma (DLBCL) comprises a large group of disease entities with high molecular heterogeneity and variable treatment responsiveness. Results from gene expression profiling (GEP) studies highlighted the role of cell of origin, namely activated B cell-like cells (ABC) and germinal center B cells (GCB), and stromal gene signatures for predicting clinical outcome and stratifying patient risk. However, GEP failed in recognizing definite target cell populations of the tumor microenvironment endowed with prognostic significance, and its clinical use was limited by the lack of standardized, commercially available assays applicable to routine - formalin-fixed, paraffin-embedded (FFPE) - tissue sample. Recently, a deconvolution method based on GEP (CIBERSORT) has been demonstrated to discriminate with high sensitivity different cell subsets within complex tissues, including tumors.
Aims: We applied CIBERSORT to dissect the DLBCL mileu and used *in silico* results to generate an easy-to-use gene panel that predicts clinical outcome of patients based on their tumor microenvironment composition.
Methods: A 1,028-gene matrix was generated to distinguish among 17 immune and stromal cell types; CIBERSORT was applied on publicly available GEP from 482 untreated DLBCL. *In silico* stratification of cases according to clinical outcome revealed significant differences among proportions of putative tumor-infiltrating cell types, whose related genes were selected and incorporated in a 45-gene panel. NanoString technology was applied to validate the prognostic power of the panel in a set of 203 FFPE samples from advanced-stage DLBCL patients treated with comparable first-line regimens.

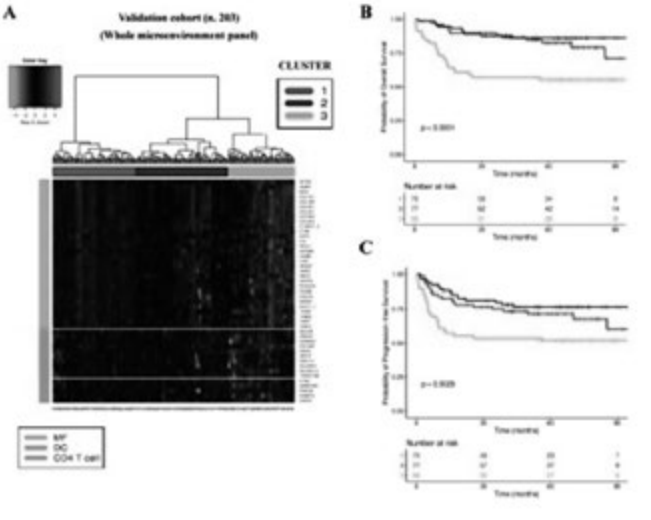


Figure 1.

Results: Higher amounts of myofibroblasts (MF), dendritic cells (DC), and CD4+ T-cells correlated with better outcomes *in silico*. Unsupervised clustering analysis stratified cases into three different subgroups with high, intermediate and low expression of genes included in the panel (Figure 1A). In particular, patients from validation cohort segregated in three separate clusters identifying two main prognostic subgroups with significantly different OS (Figure 1B) and PFS (Figure 1C). When stratified according to the expression of specific cytotype-related genes, the unsupervised clustering generated subgroups with similar prognostic trend. Interestingly, the prognostic value of microenvironment genes was independent of cell of origin categorization, and integration of the two models remarkably improved survival prediction. Furthermore, the prominent contribution of MF-related genes (30/45) in the panel, along with the results from Gene Set Enrichment Analysis (GSEA) and *in situ* immunostainings suggested a strong influence of stromal and extracellular matrix determinants of DLBCL biology.
Summary/Conclusion: Our computational dissection of DLBCL microenvironment identified new prognostic categories and provided an easy-to-apply NanoString-based gene panel that powerfully predicts patient survival. Moreover, owing to its relation with specific stromal and immune components, the panel may acquire a predictive relevance in clinical trials exploring new drugs with known impact on DLBCL microenvironment.

PS1395

GENOMIC CHARACTERISATION OF CIRCULATING TUMOUR DNA (CTDNA) PREFERENTIALLY IDENTIFIES EARLY CLINICALLY RELEVANT GENOMIC EVENTS AND PROVIDES INSIGHT INTO THE CLONAL ARCHITECTURE OF LYMPHOID MALIGNANCY

P. Blombery[1,2,*], G. Ryland[1], J. Markham[1], J. Guinto[1], M. Wall[2,3,4], M. McBean[1], K. Jones[1], E. Thompson[1,2], D. Cameron[5], T. Papenfuss[1,2,5], M. Prince[1,2], M. Dickinson[1], D. Westerman[1,2]

[1]Peter MacCallum Cancer Centre, [2]Sir Peter MacCallum Department of Oncology, University of Melbourne, [3]Victorian Cancer Cytogenetics Service, St. Vincent's Hospital, [4]St Vincent's Institute of Medical Research, [5]Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

Background: DNA shed from tumour cells (known as "circulating tumour DNA" or ctDNA) can be detected in the peripheral blood of patients with haematological malignancy offering a less invasive approach to obtaining tissue for genomic characterisation to guide management of patients.
Aims: We aimed to perform comprehensive sequence variant, copy number and translocation characterisation from ctDNA in a diverse range of lymphoid malignancies to gain insight into underlying lymphoma biology and to detect diverse genomic lesions that may impact patient care.
Methods: Libraries were made from ctDNA collected from patients at Peter MacCallum Cancer Centre (PMCC) during routine clinical sequencing. Libraries were captured using the PMCC PanHaem hybridisation-based next generation sequencing panel and sequenced on an Illumina NextSeq. The PMCC PanHaem panel targets 313 genes recurrently mutated in haematological malignancy, provides genome-wide copy number calls as well as detects translocations involving IGH/TCR loci.
Results: ctDNA from patients with a broad range of lymphoid malignancies (including diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma (BL), chronic lymphocytic leukaemia (CLL), mantle cell lymphoma (MCL), cutaneous T-cell lymphoma (CTCL), peripheral T-cell lymphoma not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL) and multiple myeloma(MM)) was assessed. Comprehensive genomic characterisation including detection of sequence variants, genome-wide copy number changes and IGH/TCR translocations was possible from ctDNA and revealed significant differences in the spectrum and relative burdens of sequence variants, copy number changes and translocations between the tumour and the ctDNA compartments across all histologies.
Genomic lesions that are classically considered "early" establishing events in lymphomagenesis (e.g. IGH-CCND1 in MCL/ MM, IGH-MYC in BL, TET2 mutations in AITL) were uniformly detectable in both ctDNA and biopsied tumour compartments. Moreover, these early lesions were present at relatively high allelic burden in the ctDNA compared to genomic lesions that are classically considered to occur later in lymphomagenesis (e.g. RAS mutations and TP53 mutations/ copy number changes). This phenomenon was most pronounced in the observation of a marked difference in the spectrum of aberrant somatic hypermutation (aSHM) that was observed between the biopsied tumour and ctDNA compartments including identification of multiple individual variants and genes exclusively mutated in ctDNA versus the biopsied tumour compartment (including BCL2, SOCS1, PIM1 and MYC). This is supportive of the hypothesis of aSHM being an ongoing and, in some cases, a highly spatial and tumour site-specific process in lymphoid malignancy.
Summary/Conclusion: We have demonstrated that comprehensive genomic characterisation of lymphoid malignancy is possible from ctDNA and assessment of this compartment is particularly suited for detecting early clonal events in lymphomagenesis. Given the diagnostic, prognostic and therapeutic relevance of these early lesions in lymphoid malignancies this approach is of significant clinical value. Moreover, the observation of the differential representation of genomic lesions between tumour and ctDNA compartments in our data contributes to furthering the understanding of clonal architecture in lymphoid malignancy.

PS1396

THE DIALOG BETWEEN MANTLE CELL LYMPHOMA AND MONOCYTES/MACROPHAGES CAN BE DISRUPTED BY BTK INHIBITION AND MONITORED THROUGH CD163 MODULATION *IN VIVO*

A. Papin[1,*], C. Bellanger[1,2], B. Tessoulin[1,2], M. Amiot[1], S. Le Gouill[1,2], C. Pellat-Deceunynck[1], D. Chiron[1]

[1]CRCINA, INSERM, CNRS, Université de Nantes, Université d'Angers, [2]CHU de Nantes, Nantes, France

Background: The aggressive clinical behavior of mantle cell lymphoma (MCL) and its short response to current treatment highlight a need for continuing exploring rational novel option. Recently it has been demonstrated that tumor microenvironment strongly influences MCL expansion and chemoresistance. However, there is little information regarding the nature of extrinsic signaling and their resulting molecular regulations.
Aims: Here we aimed to study the dynamic interactions between primary MCL cells and its myeloid microenvironment through 3 axes: 1) Determine the protumoral role of myeloid cells using primary coculture models *ex vivo*. 2) Characterize the phenotype of MCL associated monocytes (M-MCL) *in vivo*. 3) Assess the role of targeted therapies to disrupt the dialog between MCL and monocytes/macrophages.
Methods: Primary MCL cells (n>20) were cocultured 7 days with monocytes or pre-differentiated M1 (GMCSF, IFNg) or M2 (MCSF, IL10) macrophages. Macrophages derived from monocytes cocultured with MCL cells (M-MCL) were characterized using a panel of markers (CD14, CD68, CD163, CD11b, CD86, HLA-DR, CD16, CD23) as well as cytokine production (IL10, TGFb, IL6, BAFF, IGF1). To validate the specific phenotype of M-MCL *in vivo* the same markers were assessed on circulating monocytes of MCL patients at diagnosis (n= 10), or treated with a combination of ibrutinib and obinutuzumab (n= 9; OASIS trial NCT02558816).
Results: Whereas primary MCL cells alone rapidly undergo spontaneous apoptosis *ex vivo*, we observed that M-MCL and M1/M2 macrophages greatly support cell survival after 7 days of coculture in all samples tested (n=14; p<.0005). Of interest, both M-MCL and M2, but not M1, macrophages induced cell-cycle progression in clinically aggressive (n=6) but not indolent (n=4) MCL subtype. Multiple factor analysis highlights that M-MCL were closely related to M2 ($CD163^{high}$, $CD11b^{low}$, $IL10^{+}$, $IGF1^{+}$). We next confirmed that primary MCL cells overexpressed the M2-promoting factors MCSF and IL10 compared to normal B cells (p<.005), the level of MCSF being higher in aggressive forms of MCL (p< .05). Accordingly circulating monocytes from MCL patients expressed a higher level of CD163 compared to healthy donors (median 9.7 vs. 6.2; p<.05), suggesting that MCL reprograms monocytes into M-MCL *in vivo*.
We then demonstrated that targeting BTK with ibrutinib (500nM) inhibited IL10 and MCSF secretion by MCL *in vitro* and consequently IL10/MCSF-mediated CD163 induction on M-MCL, suggesting that BTK inhibition disrupts molecular communication between both cell types. Accordingly, ibrutinib counteracted monocyte-dependent survival (n=9; p< .05) and proliferation (n= 4) of primary MCL cells. These observations were confirmed *in vivo* through analysis of the peripheral blood of patients included in the OASIS trial. We observed a decrease of the CD163 level on circulating monocytes (median: -49%, range: -16% to -98%) in 8 patients and an increase in 1 patient (+ 27%) after 8 days of ibrutinib (560 mg/day) and obinutuzumab (1000 mg/8 days). Importantly, longitudinal follow-up after several cycles highlighted that CD163 increase at day 8 (n=1) was associated to clinical progression whereas CD163 decrease (n=3) was associated to complete response.
Summary/Conclusion: Through secretion of IL10 and MCSF, MCL reprograms monocytes into M2 macrophages that in turn favor tumor survival and proliferation. Ibrutinib counteracts this dialog and ibrutinib-dependent CD163 modulation on circulating monocytes *in vivo* appears as an early marker of patients' response to treatment.

PS1397

MYD88 CONSTITUTIVE ACTIVATION IS DIRECTLY ABLE TO INDUCE INDOLENT LYMPHOPLASMACYTIC LYMPHOMA WITH MONOCLONAL IGM PEAK SECRETION IN 1 YEAR OLD MICE: A MODEL FOR WALDENSTROM'S MACROGLOBULINEMIA

C. Vincent-Fabert[1,*], L. Roland[1], C. Ouk[1], M. Deveza[2], N. Gachard[2], N. Faumont[1], J. Feuillard[1]

[1]Contrôle de la Réponse Immune B et des Lymphoprolifération (CRIBL) UMR CNRS 7276 INSERM 1262, Centre de Biologie et de Recherche en Santé, [2]Hématologie Biologique, CHU , Limoges, France

Background: Waldenström's Macroglobulinemia (WM) is an incurable lymphoplasmacytic indolent B cell lymphoma with a monoclonal IgM peak. The MYD88 L265P mutation, activator of NF-kappa B (NF-kB), is found in 80% to 90% of WM. So far, there is no existing animal model for WM to evaluate the role of MYD88 activation, and the only published mouse model harboring the ortholog MYD88 L252P mutation develops aggressive B-cell lymphomas in aged mice.
Aims: The aim of this study is to developp a mouse model harboring the MYD88 L252P mutation in order to better understand the oncogenic history of the disease since the aquisition of the first event, the MYD88 mutation.
Methods: The MYD88 L252P coding sequence was attached to YFP cDNA with an IRES sequence. This construct was flanked with loxP sites and inserted in the Rosa26 locus. Here, we present the first results of the corresponding CD19_CRE/LoxP MYD88 L252P mouse model.
Results: *Ex vivo* TAT-CRE induction of MYD88 L252P expression in purified B-cells from spleen induced activation, proliferation and protection against cell death. *In vivo*, B-specific expression of MYD88 L252P in CD19_CRE/LoxP MYD88 in 3.5 month old mice, led to mild splenomegaly due to expansion of B cells with an activated phenotype. In parallel, the presence of CD138 positive cells that cytologically resembled plasma cells was observed in blood. In one-year-old mice, we observed an increase in white blood cells, with again presence of CD138 positive cells. Serological studies revealed the presence of an immunoglobulin M (IgM) peak in the mutant mice associated with a decrease in circulating IgG levels. These mice had a large splenomegaly that was correlated with infiltration of tumor B-cells with a marked lymphoplasmocytic aspect at both cytological and histopathological levels. Compared to controls, the proliferative index was moderately increased, ranging 10% - 20% and spontaneous apoptosis was decreased. In parallel, we established the direct signature of MYD88 L265P deregulated genes. In addition to MYD88 itself, expression of NF-kB signature genes such as ICAM1, EBI3, FAS or TNFAIP3 was increased. MYD88 L265P also induced over expression of CCND2, of the anti-apoptotic molecules BCL2A1 and MCL1 with decreased expression of BAX, of genes involved in the formation of the Golgi apparatus, as well as of the immunosuppressive CD274 molecule.
Summary/Conclusion: These preliminary results suggest that the constitutive activation of MYD88 is able to promote B cell clonal emergence with a moderate increase of proliferation rate, protection against apoptosis, engagement in plasma cell differentiation, and monoclonal IgM peak, leading to a murine disease strongly resembling human Waldenström's Macroglobulinemia.

PS1398

CELL OF ORIGIN AND GENOMIC PROFILE OF PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA DETERMINED USING THE NANOSTRING LST ASSAY AND ULTRA DEEP TARGETED NEXT GENERATION SEQUENCING

C. Bödör[1,*], D. Alpar[1], D. Marosvari[2], H. Rajnai[2], B. Kajtar[3], B. Deak[4], T. Schneider[4], H. Alizadeh[5], A. Matolcsy[2], S. Brandner[6], J. Storhoff[7], L. Reiniger[2]

[1]1st Department of Pathology and Experimental Cancer Research, [2]1st Department of Pathology and Cancer Research, Semmelweis University, Budapest, [3]Department of Pathology, University of Pecs, Pecs, [4]National Institute of Oncology, Budapest, [5]Department of Hematology, University of Pecs, Pecs, Hungary, [6]Division of Neuropathology and Department of Neurodegenerative Disease, Institute of Neurology, University College London, London, United Kingdom, [7]Nanostring, Seattle, United States

Background: Primary central nervous system lymphoma (PCNSL) is characterized by a consistently inferior outcome compared to systemic diffuse large B-cell lymphoma (DLBCL). The distinction of DLBCL into cell of origin (COO) categories based on patterns of gene expression reminiscent of germinal center B-cell (GC-) type and activated B-cell (ABC-) type has profound prognostic and potential therapeutic implications with many novel therapies displaying differential efficacy between the GC and ABC groups.The NanoString LST assay is a digital gene-expression based test for COO assignment, representing a more accurate means of COO determination compared to standard immunohistochemistry (IHC) based algorithms, and was recently successfully applied in nodal DLBCLs.
Aims: In the present study, we tested the LST assay on a cohort of PCNSL patients and performed a complementary targeted genomic profiling on a subset of these patients.
Methods: The COO of 77 PCNSL patients was determined with the NanoString LST assay using RNAs extracted from FFPE samples and compared with the results obtained by the Hans IHC algorithm. The genomic profiles of 62 PCNSL cases were determined using ultra-deep targeted next generation sequencing (NGS) of 13 recurrently mutated target genes, including *CARD11, CCND3, CD79B, CSMD2, CSMD3, IRF4, C-MYC, MYD88, PAX5, PIM1, PRDM1, PTPRD* and *TP53* using the TruSeq Custom Amplicon approach on a HiSeq4000 Instrument (Illumina) to an average depth of 30.000x.
Results: The LST assay resulted in the following subtype calls: 80.5% ABC, 12.9% GC and 6.5% unclassified (UC). Using the Hans IHC algorithm, 94.8% (73/77) of the PCNSL cases were classified as ABC subtype with 5.2% (4/77) representing the GC subtype. Interestingly, the COO classification obtained using the LST assay showed a different outcome in 16.9% (13/77) of the cases: Out of the 12 cases classified as ABC subtype with the IHC algorithm, 7 cases presented as GC subtype and 5 showed an UC profile using the LST assay. On the other hand, one case characterized as GC subtype with the IHC assay was classified as ABC when analyzed using the LST assay. As for the mutation analysis, we detected a total of 402 somatic mutations in the 62 PCNSL cases across the 13 genes analyzed with individual cases harboring mutations in 4 genes on average (range: 1-11). The most frequently mutated genes were *MYD88* (76%), *PIM1* (55%) and *PRMD1* (50%). The mutation frequencies in the remaining genes were as follows: *CD79B* (40%), *PTPRD* (32%), *MYC* (31%), *PAX-5* (31%), *CARD11* (26%), *TP53* (26%), *IRF4* (23%), *CCND3* (19%), *CSMD2* (19%) and *CSMD3* (18%). Analyzing the mutation profiles of the ABC and GC subtypes, we observed an enrichment of *CD79B* (47% vs 20%) and *PIM1* (59% vs 20%) in cases with ABC subtype, with *IRF4* mutations being present in ABC cases only (29% vs 0%).
Summary/Conclusion: We successfully applied, for the first time, the NanoString LST assay for COO determination using FFPE derived RNA samples from a cohort of 77 PCNSL patients. Interestingly, the LST assay revealed a lower proportion of patients with ABC subtype compared our IHC findings (80.5% vs 94.8%). This may reflect the genuine biology of these cases, as the NanoString LST assay represents the most reliable COO determination approach compared to the original gold standard GEP method. The frequent mutations revealed in components of different actionable pathways including the BCR pathway, cell cycle regulation and CNS development highlights the role of these variants in the pathogenesis of PCNSL.

PS1399

IMMUNOPROFILING OF HIV-ASSOCIATED LYMPHADENOPATHY: HIGH-THROUGHPUT ANALYSIS OF THE T CELL RECEPTOR GENE REPERTOIRE SUPPORTS EPITOPE-SPECIFIC T CELL RESPONSES

E. Stalika[1,*], K. Gemenetzi[1], A. Vardi[2], F. Psomopoulos[1], E. Vlachonikola[1], C. Galigalidou[1], T. Koletsa[3], K. Stamatopoulos[1], M. Papaioannou[4], A. Hadzidimitriou[1]

[1]Institute of Applied Biosciences, CERTH, [2]Hematology Department and HCT Unit, G. Papanicolaou Hospital, [3]Pathology Department, Faculty of Medicine, Aristotle University of Thessaloniki, [4]First Department of Medicine, Medical School, Thessaloniki, Greece

Background: Non-neoplastic lymphadenopathy is a characteristic, though certainly not specific, finding in HIV/AIDS patients. It may arise with the onset of HIV viremia (acute retroviral syndrome) that can persist beyond the acute phase. Histopathological findings at this early phase mainly pertain to hyperplastic changes with large lymphoid follicles; with time, the number of lymphoid follicles diminishes, while plasma cells increase; at the extreme is a pattern characterized by sclerosis of the germinal centers in the residual follicles. Moreover, a heightened turnover of both $CD4^+$ and $CD8^+$ T cell populations in peripheral blood and lymphoid tissues of these patients has been reported. Overall, these findings allude to a dynamic, ongoing immune response that is still incompletely characterized at the molecular level.
Aims: In order to obtain a truly comprehensive view into the dynamics of the T cell responses in HIV-associated lympadenopathy, we interrogated the TR repertoire of biopsy samples from 12 patients with HIV infection.
Methods: TRBV-TRBD-TRBJ gene rearrangements were amplified on

gDNA isolated from paraffin-embedded tissues according to the BIOMED2 protocol. PCR products were subjected to NGS on the MiSeq Illumina Platform. Dedicated bioinformatics pipeline was developed for the analysis of raw NGS data which includes quality filtering, merging of pair-end read, preparation of fasta files for the IMGT/High V-QUEST tool; and, IMGT/High V-QUEST metadata analysis, interpretation and visualization.
Results: Overall, 1,474,718 productive rearrangements were included in the analysis. Rearrangements with identical TRBV gene usage and CDR3 sequence were defined as clonotypes. For repertoire analyses, clonotypes rather than single rearrangement sequences were considered. A total of 16,039 unique clonotypes were identified (median 1337; range 340-6,473/case), of which 9,882 represented singletons. Amongst the 46 functional TRBV genes identified, the most frequent were: TRBV6-5 (14.6%), TRBV19 (13.1%), TRBV29-1 (10.4%) and TRBV12-3 (9.2%), collectively accounting for 47.3% of the TRBV repertoire; the TRBV29-1 gene predominated in 7/12 cases. Analyzed HIV samples carry expanded clonotypes (median 513; range 131-2411/case). The predominant clonotype ranged in frequency from 8 to 87.5% of the total clonotypes observed in each case. Cluster analysis of the CDR3 sequences identified 564 different clonotypes shared by different patients (public clonotypes), albeit at low frequency. Of note, a particular clonotype (TRBV29-1/ SVDPSGTGGEGYT) was detected in all samples, representing the dominant clonotype in 7/12 cases. The chance that this may happen randomly is negligible. In order to confirm that this represents a true biological phenomenon rather than an artifact e.g. due to contamination, we applied PCR reaction re-set up, Sanger sequencing of monoclonal PCR products and exclusion of index hopping, all leading to findings suggestive of the former possibility. Additionally, in 4/12 cases, the above clonotype appeared at high frequency shaping further an oligoclonal profile, whereas in one case the frequency was 0,15%, arguing against contamination. Interestingly, highly similar clonotypes were also detected differing by single aminoacids within the CDR3.
Summary/Conclusion: In conclusion, the TR gene repertoire of HIV-associated lymphadenopathy appears profoundly skewed, displaying striking homology of the CDR3 regions of major clones; oligoclonal profiles; and TRBV gene usage restriction. On these grounds, we argue that this profile is likely reflecting epitope-specific T cell responses.

Platelets disorders

PS1400

INSUFFICIENT RECEPTOR EDITING LEADS TO DEFECTIVE B CELL CENTRAL TOLERANCE IN PRIMARY IMMUNE THROMBOCYTOPENIA (ITP)

Z. Sheng[1,*], Y. Gao[1], M. Hou[1], S. Li[2], J. Peng[1]

[1]Department of Hematology, [2]Department of Oncology, Qili Hospital, Shandong University, Jinan, China

Background: The antibody-mediated platelet destruction is the most important pathogenic mechanism in primary immune thrombocytopenia (ITP). However, it is not clear how these anti-platelet antibodies are generated in ITP patients. Receptor editing and VH replacement are processes to change the specificity of immature B cell receptors (BCR) by a secondary recombination of immunoglobulin genes, which are the main part of central tolerance of B cells.
Aims: The aim of this study is to investigate how the anti-platelet antibodies are generated and overcome the central B cell tolerance in patients with ITP.
Methods: We first performed next-generation sequencing on peripheral B cells of ITP patients and found a distinct immunoglobulin gene κ chain (Igκ) repertoire from the healthy control. We then performed single cell RT-PCR on naïve B cells to clone both Ig heavy chain (IgH) and Ig light chain (IgL) genes from the same cells. The paired IgH and IgL were co-transfected and the recombinant monoclonal antibodies were expressed and purified. The reactivity of these antibodies against platelet antigens was determined by ELISA, immunofluorescence assays, and flow cytometry with human platelets. Cross-reactivity of these antibodies was tested by ELISA coated with double strand DNA, insulin and lipopolysaccharides. Ig gene sequence features were analyzed by programs including IMGT, IgBLAST, and VHRFA-1.
Results: Deep sequencing revealed that the ITP-derived Igκ preferentially used the downstream Vκ (Vκ1-20) (44.65% vs. 42.20%, p=0.0210) and upstream Jκ (Jκ1-2) (27.82% vs. 23.38%, p=0.0210) segments compared with those derived from healthy controls, which indicated a defective receptor editing of B cells. Utilizing single cell RT-PCR, 228 recombinant antibodies were cloned and expressed derived from naïve B cells in 4 ITP patients and 4 healthy controls. Low numbers of somatic mutations validated that the antibodies were derived from naive B cells. ELISA assay showed that 17.0% (21/124) of the ITP-derived naïve B cells were platelet-reactive, in contrast to the 2.8% (3/104) in healthy donor-derived naïve B cells, suggesting a defective B cell tolerance in the early developing stage. The anti-platelet reactivity was also confirmed by immunofluorescence assays and flow cytometry. Cross-reactivity test revealed that the anti-platelet antibodies were polyreactive to multiple antigens. Sequence analysis revealed that positively charged amino acids were highly enriched in the complementarity determining region 3 (CDR3) of the anti-platelet antibodies, which possibly generates the potential to interact with the negatively charged glycoproteins. The Igκ genes derived from ITP patients preferentially used upstream Jκ segments (49.1% vs. 25.7%, p<0.01) compared to healthy controls, indicating an insufficient IgL receptor editing in ITP patients. In addition, the frequencies of VH replacement products were elevated in IgH genes from ITP patients (8.6% vs. 4.4%, p<0.05) compared to healthy controls.

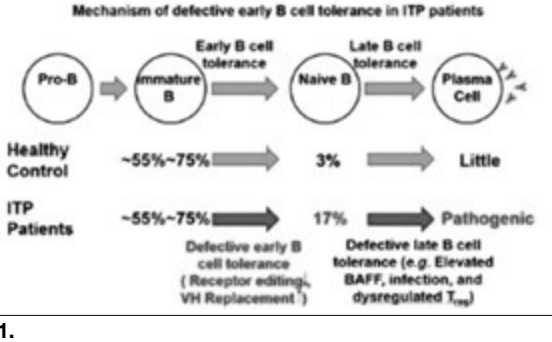


Figure 1.

Summary/Conclusion: In summary, this study provides evidence of defective early B cell tolerance and accumulation of anti-platelet naïve B cells in patients with ITP. This defect might be a result of insufficient receptor editing in IgL as well as excessive VH replacement in IgH.

PS1401

SUPERIORITY OF AVATROMBOPAG TO PLACEBO IN I REDUCING PLATELET TRANSFUSIONS IN PATIENTS WITH THROMBOCYTOPENIA AND CHRONIC LIVER DISEASE UNDERGOING SCHEDULED PROCEDURES- POOLED ANALYSIS OF 2 STUDIES

F. Poordad[1], L. F. Allen[2], K. Aggarwal[2,*], M. Vredenburg[2], N. Alkhouri[1]

[1]Texas Liver Institute, San Antonio, [2]Dova Pharmaceuticals, Durham, United States

Background: Thrombocytopenia (TCP) is common in patients with chronic liver disease (CLD) and complicates clinical management with these patients requiring multiple invasive procedures. Prophylactic platelet transfusions are used to reduce bleeding risk, but have multiple associated safety risks. Avatrombopag (AVA), an oral thrombopoietin receptor agonist, has completed Phase 3 studies as an alternative to platelet transfusions for patients with TCP and CLD.
Aims: To report the results of 2 randomized, double-blind, placebo (PBO)-controlled Phase 3 trials (ADAPT-1 and ADAPT-2).
Methods: ADAPT-1 and ADAPT-2 enrolled adults with CLD and platelet count (PC) <50x10^9/L undergoing a procedure. Patients were divided by Baseline PC into **Cohort 1**- PC <40x10^9/L and Cohort 2- PC 40 to <50x10^9/L, and randomized 2:1 to once-daily oral AVA (Cohort 1- 60 mg; Cohort 2- 40 mg) or PBO for 5 days, with the procedure 5 to 8 days after the last dose. The primary efficacy endpoint was the proportion of patients not requiring a platelet transfusion or rescue procedure. Secondary endpoints assessed the proportion of patients achieving the target PC (>=50x10^9/L) by Procedure Day, change in PC from Baseline to Procedure Day, and safety.
Results: 435 patients were randomized in the 2 studies. AVA was superior to PBO in both cohorts in the number of patients not requiring a platelet transfusion or bleeding rescue (Cohort 1: AVA- 66.9%, PBO- 28.6%; p< 0.0001; Cohort 2: AVA- 88.0%, PBO 35.8%; p< 0.0001). A greater number of AVA-treated patients also achieved the target PC in both Cohort 1 (AVA- 68.1%, PBO- 5.5%; p<0.0001) and Cohort 2 (AVA- 90.6%, PBO- 29.9%; p<0.0001). Mean increases in PCs were also greater with AVA compared with PBO in both cohorts (**Cohort 1**: AVA- 31.7x10^9, PBO- 1.8x10^9/L; **Cohort 2**: AVA- 41.0x10^9, PBO 3.5x10^9/L). The most common TEAEs were pyrexia, abdominal pain, nausea, and headache.
Summary/Conclusion: AVA was superior to placebo in reducing the need for PLT transfusions, the proportion of patients achieving the target PC, and increasing the PC.

PS1402

Abstract withdrawn.

PS1403

PREDICTIVE FACTORS FOR DEVELOPING SYSTEMIC LUPUS ERYTHEMATOSUS AFTER CHILDHOOD AUTOIMMUNE CYTOPENIA

A. Guth[1], N. Cheikh[2], R. Vieux[1], L. Thierry[3], G. Leverger[4], Y. Bertrand[5], H. Fernandes[6], E. Jeziorski[7], C. Guitton[8], B. Bader-Meunier[9], S. Bayart[10], M. Pasquet[11], F. Millot[12], Y. Perel[6], N. Aladjidi[6,*]

[1]Pediatrie 1, University Hospital of Besançon, Besançon, [2]Pediatrie [1], University Hospital of Besançon, Bordeaux, [3]Hematology Unit, APHP-Hospital Robert Debré, [4]Pediatric Onco-Hematology Unit, APHP-Hospital Armand Trousseau, Paris, [5]Pediatric Immuno-Hematology Unit, University Hospital of Lyon, Lyon, [6]pediatric oncohematology unit / CEREVANCE / CIC1401, Inserm CICP, University Hospital of Bordeaux, Bordeaux, [7]Pediatric Hematology Unit, University Hospital of Montpellier, Montpellier, [8]Pediatrics, APHP-Bicêtre, Le Kremlin-Bicêtre, [9]Immuno-Hematology Unit, APHP-Necker, Paris, [10]Pediatric Hematology Unit, University Hospital of Rennes, Rennes, [11]Pediatric Onco-Hematology Unit, University Hospital of Toulouse, Toulouse, [12]Pediatric Onco-Hematology Unit, University Hospital of poitiers, Poitiers, France

Background: Systemic lupus erythematosus (SLE) beginning at pediatric age is reported to be more severe than that SLE beginning during adulthood, and may be associated with autoimmune cytopenia (AIC). The incidence of SLE in the long-term follow-up of AIC in children is not known, especially when significant titers of anti-nuclear antibodies (ANA) are documented.
Aims: The objectives of this study were to define predictive factors to develop SLE in the follow-up of childhood AIC.
Methods: In this national prospective observational cohort, children with chronic immunologic thrombocytopenic purpura (ITP), autoimmune hemolytic anemia (AIHA) or Evans syndrome (ES) before the age of 18 and a subsequent SLE (SLICC criteria) were analyzed. A uni and multivariate study of the factors associated with the occurrence of SLE was performed.
Results: Of the 1245 children with AIC registered in this national cohort, 192 had ANA ≥ 1/160 at least once in evolution (15%) and with a median follow-up of 5.1 years (0.6-26), 49 patients (4%) developed an SLE at a median age of 14.7 years (0.9-24.5). These 49 children had chronic ITP in 22 cases, ES in 20 cases or AIHA in 7 cases. The clinical manifestations of SLE were rheumatologic in 55% of cases, cutaneous in 48% of cases, renal in 13% of cases, seritis in 8% of cases, neuro-lupus in 4% of cases. Twelve of those patients only had biological manifestations of SLE. Among the treatments proposed for those AIC, splenectomy was used in 21% of cases, rituximab in 20% of cases and hydroxychloroquine in 79% of cases. The impact of hydroxychloroquine on the clinical manifestations of SLE is currently being analyzed. In unvaried preliminary analysis, factors associated with progression from AIC to SLE are female sex, family history of autoimmune disease, advanced age at initial diagnosis (12.1 vs 5.6 years), initial severity of AIC and a high ANA titer. In multivariate analysis the female sex (RR = 7.18 (IC95% [1.99-25.8], p =0.003)) and ANA titer ≥1 / 320 (RR = 25(IC95% [8.33-50], p<10-4)) were significantly associated with SLE occurrence.
Summary/Conclusion: In this prospective national study of rare diseases, 25% of children with AIC and high ANA titers developed SLE during adolescence and young adulthood. Long-term specialized survey should be proposed for patients with CAI discovered in childhood, especially girls with ANA ≥ 1/320, with a particular attention during the transition phase between pediatrics and adult health care. Early use of hydroxychloroquine could decrease the frequency of severe organ complications of these SLEs.

PS1404

THE POLYMORPHISM OF CASPASE RECRUITMENT DOMAIN-CONTAINING PROTEIN 9 GENE MAY CONTRIBUTE TO RISK OF IMMUNE THROMBOCYTOPENIA

Z. Sheng[1,*], J. Li[1], M. Hou[1], J. Peng[1]

[1]Department of Hematology, Qilu Hospital, Shandong University, Jinan, China

Background: Primary immune thrombocytopenia (ITP) is an acquired autoimmune disease characterized by thrombocytopenia and increased risk of bleeding. The etiology remains unclear. It has been widely accepted that the genetic factors play a significant role in the pathogenesis of ITP. Single nucleotide polymorphisms (SNPs) are the most common structure of human genetic variation. There are a few studies which found that three SNPs including rs4077515 and rs59902911 in the caspase recruitment domain-containing protein 9 (CARD9) gene and rs7216796 in the mitogen-activated protein 3 kinase 14 (MAP3K14) gene were involved in the immune regulation and development of autoimmune diseases. It is well recognized that many autoimmune diseases have similar underlying etiology and have shared susceptible genes.
Aims: The aim of our study was to investigate the possible association between the three SNPs and the incidence of primary ITP in the Chinese Han population.
Methods: In this case-control study, the total subjects included 84 Chinese primary ITP patients who were admitted to Department of Hematology, Qilu Hospital, Shandong University, and 89 ethnically matched healthy controls. To explore the association between these polymorphisms and more complete clinical data, the cases were stratified by susceptibility, severity, corticosteroid sensitivity, and refractoriness based on the standardized definitions. Written informed consent was obtained from each participant in accordance with the Declaration of Helsinki. Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) that had been isolated from whole blood samples from participants by a standard protocol. These three SNPs genotyping was performed on Sanger sequencing. Statistical analysis was performed using SPSS 21.0, and associations between the SNPs and ITP were calculated by a chi-squared (χ^2) test or Fisher's exact test. A two-tailed p<0.05 (or adjusted p value by Bonferroni multiple testing) was considered statistically significant.
Results: Demographic and clinical characteristics of healthy controls and ITP patients were summarized in Table 1. Four genetic models were used to analyze the association between the three SNPs and ITP. Our results demonstrated significantly different genotype distributions of CARD9 rs4077515 between the ITP and control groups under the codominant and dominant models (p=0.001 and p=0.006, respectively, Table 2). Univariate logistic regression analysis further revealed that for rs4077515 in CARD9, AG and AA/AG genotypes were both statistically significant compared to GG after Bonferroni multiple correction (p=0.001 and p=0.005, respectively, Table 2). And, this SNP locus was a risk factor. However, there was no obvious difference in the genotype or allele frequencies on the other two genes between ITP and control groups. With regard to severity, corticosteroid sensitivity and refractoriness, we did not find any significant differences in

genotype and allele frequencies.
Summary/Conclusion: The obtained data indicate that the AG of CARD9 rs4077515 is more frequent in patients than in controls and that this polymorphism may be a genetic risk factor associated with the development of ITP in the Chinese Han population. Further studies on larger samples are needed to investigate the role of CARD9 rs4077515 polymorphism in etiology of ITP.

Table 1. Demographic and clinical characteristics.

	Controls	ITP patients
No.	89	84
Age, mean ± SD	43.75±12.93	38.17±16.83
Gender (M/F)	45/44	40/44
ITP severity, n (%)		
Severe ITP	NA	52 (61.9)
Non-severe ITP	NA	32 (38.1)
Treatment, n (%)		
No use of corticosteroid	NA	10 (11.9)
Corticosteroid-sensitive	NA	52 (61.9)
Corticosteroid-resistant	NA	22 (26.2)
Refractory ITP	NA	4 (4.8)
Non-refractory ITP	NA	80 (95.2)

M, male; F, female; NA, not applicable; ITP, immune thrombocytopenia.

Table 2. Association between selected SNPs and ITP risk.

Gene	SNP	Mode	Genotype	Controls		ITP patients		Uncorrected p value	OR(95% CI)	Adjusted p value
				Count	%	Count	%			
			GG	39	43.8	20	23.8		1	
CARD9	rs4077515	Codominant	AA	7	7.9	1	1.2	0.001	0.260 (0.029-2.354)	0.231
			AG	43	48.3	63	75		3.019 (1.530-5.959)	0.001
		Dominant	GG	39	43.8	20	23.8		1	
			AA/AG	50	56.2	64	76.2	0.006	2.613 (1.340-5.094)	0.005

SNP, single-nucleotide polymorphism; CI, confidence interval; OR, odds ratio; ITP, immune thrombocytopenia.
Bold highlights statistical significance (p < 0.05).

PS1405

LABORATORY CHARACTERIZATION OF PATIENTS WITH (SUSPECTED) INHERITED PLATELET DISORDERS: RESULTS FROM THE 'THROMBOCYTOPATHY IN THE NETHERLANDS' STUDY

M. Blaauwgeers[1,*], I. van Asten[2], A. Huisman[2], S. Korporaal[2], M. Coppens[3], M. Kruip[4], E. Beckers[5], J. Eikenboom[6], R. Tamminga[7], R. Urbanus[2], R. Schutgens[1]

[1]Van Creveldkliniek, [2]Clinical Chemistry and Hematology, UMC Utrecht, Utrecht, [3]AMC, Amsterdam, [4]Erasmus MC, Rotterdam, [5]Maastricht UMC, Maastricht, [6]LUMC, Leiden, [7]UMCG, Groningen, Netherlands

Background: Inherited platelet disorders (IPDs) are rare disorders of the primary hemostasis, caused by defects in platelet function (inherited platelet function disorders, IPFD) and/or platelet production (inherited thrombocytopenia, IT). Most IPDs are a challenge to diagnose due to a paucity of distinctive clinical and laboratory features and poor standardization of laboratory tests. However, an accurate diagnosis is essential to optimize treatment and improve patient care. There is a large variation in the diagnostic strategy used to assess platelet function, despite recommendations on a standardized approach. This study reports the results of a diagnostic strategy used in a cohort of (suspected) IPDs in the Netherlands
Aims: To describe the results of the diagnostic work-up for patients with (suspected) IPDs
Methods: Adult patients with a clinical and/or laboratory suspicion for having an IT or IPFD were included in a nationwide cross-sectional study on IPDs ('Thrombocytopathy in the Netherlands' (TiN)) between February 2016 and December 2017. All participants gave written informed consent. The ISTH-BAT was used to evaluate bleeding symptoms. Laboratory testing consisted of a complete blood count, MPV, peripheral blood smear, von Willebrand factor activity, light transmission aggregometry (LTA) (ADP/collagen/arachidonic acid/ristocetin), flow cytometry (P-selectin expression, fibrinogen binding, glycoprotein expression and mepacrine uptake), nucleotide content analysis (platelet lysates using bioluminescence) and genetic analysis (89-gene NGS platform). An IT was diagnosed if the platelet count was below the normal range and if a candidate genetic defect was identified or, in the absence of a genetic defect, when clinical characteristics and family history pointed towards a genetic origin. An IPFD was diagnosed if one or more platelet function assays and/or platelet nucleotide content were abnormal, regardless of the identification of a genetic defect
Results: A total of 159 patients with a suspected IT (n=26) or IPFD (n=133) were included. Clinical and laboratory features are shown in Table 1. In 7 of the 26 suspected IT patients (27%) a candidate genetic defect was identified: mutations were found in the genes ACTN1 (2), TUBB1 (2), MYH9 (1) and GP9 (2). Thirteen patients had a final diagnosis of IT, of whom 5 had an additional platelet function defect. We were able to diagnose an IPFD in 63 of the 133 patients (47%) with a suspected IPFD: 51 patients had abnormal LTA results, 9 patients showed abnormalities in flow cytometry analysis. One patient had normal LTA and flow cytometry results but an abnormal platelet nucleotide content. Two patients had normal platelet function assays but a possible pathogenic genetic mutation in GFI1B. In the other 70 patients with normal platelet function assays, no candidate genetic defect was found. We identified 7 patients with δ-SPD, 2 with α-SPD, 5 with an ADP-receptor defect, 2 with a thromboxane receptor defect, 1 with GPVI deficiency and 46 with uncharacterized functional defects. 1 patient had a combination of δ-SPD and an ADP-receptor defect. A genetic defect was found In 13 of the 63 (21%) patients. Mutations were found in GFI1B (2), P2RY12 (6), PTGS1 (2), RUNX1, SLFN14 and TBXA2R.

Table 1. Clinical and laboratory features of patients included in the TiN study.

	IT (n=26)	IPFD (n=133)
Female sex (%)	12 (46)	118 (89)
Age (years), median (IQR)		
Male	30 (21-46)	40 (28-59)
Female	32 (23-37)	41 (31-53)
BS, median (IQR)		
Male	4 (2-7)	7 (5-9)
Female	7 (3-12)	10 (7-13)
Platelet count (10^9/L), median (IQR)	78 (37-93)	238 (199-288)
MPV (fL), median (IQR)	9.8 (8.9 -12.6)	7.9 (7.1-8.4)
Abnormal platelet function assay, n (%)	14 (54)	61 (46)
Genetic defect identified, n (%)	7 (27)	13 (10)
Final diagnosis of IPD, n (%)	13* (50)	63 (47)
Genetic defects	ACTN1, GP9, MYH9, TUBB1	GFI1B, P2RY12, PTGS1, RUNX1, SLFN14, TBXA2R

BS: bleeding score; IT: inherited thrombocytopenia; IPD: inherited platelet disorder; IPFD: inherited platelet function disorder; IQR: interquartile range; MPV: mean platelet
* in 5 patients an additional platelet function defect was identified

Summary/Conclusion: Clinical management of patients with an inherited platelet disorder will benefit from a conclusive diagnosis. By adding flow cytometry, platelet nucleotide content and genetic analysis to the diagnostic strategy of patients with suspected IPDs, more patients will receive a definitive diagnosis, which will improve patient care

PS1406

FEATURES OF PRN1008, A BRUTON'S TYROSINE KINASE INHIBITOR, IN CLINICAL DEVELOPMENT FOR IMMUNE THROMBOCYTOPENIC PURPURA

S. Gourlay[1,*], C. Langrish[2], M. Bradshaw[3], M. Francesco[3], P. Nunn[4], D. Kuter[5]

[1]Clinical Development, [2]Immunology Research, [3]Biology, [4]Early Development, Principia Biopharma, San Francisco, [5]Center for Hematology, Massachucetts General Hospital, Boston, United States

Background: PRN1008 is an oral, reversible covalent inhibitor of Bruton's tyrosine kinase (BTK) in clinical development for the treatment of multiple autoimmune diseases. BTK is an essential signaling element downstream of the B cell receptor and Fc receptor pathways. BTK activation is critical for B cell activation, and also regulates antibody mediated activation of other immune cells, such as macrophages, neutrophils and mast cells through Fc receptor signaling. Although BTK is expressed in platelets, alternative signaling pathways exist which bypass BTK signaling to retain normal platelet function.
Aims: Assessment of the potential clinical profile of the BTK inhibitor PRN1008 for the treatment of immune thrombocytopenia.
Methods: *In vivo* assessments of PRN1008 pre-treatment were conducted in a mouse anti-CD41 ITP model and an autoantibody-mediated rat arthus model. The ex vivo effects of PRN1008 on platelet aggregation to a panel of agonists in healthy subject and ITP patient blood (n=5 each) were studied. Platelet counts and any adverse effects pertinent to bleeding or bruising were summarized from prior PRN1008 healthy volunteer and a pemphigus Phase 2 clinical study (NCT02704429). Changes in autoantibody levels over a 12-week treatment period in that study were characterized, using doses of between 400 mg and 600 mg twice daily. An adaptive, open-label, dose-finding, phase 1/2 study investigating the safety, PK, and clinical activity of PRN1008 in patients with relapsed or refractory ITP has been initiated (NCT03395210). The 12 week study has an intrapatient dose escalation design with 12 weeks of active therapy and 12 weeks of follow-up.
Results: Preclinically, treatment with PRN1008 *in vitro* profoundly inhibited B cell activation and blocked antibody-mediated activation of immune cells via Fc receptor signaling. *In vivo*, PRN1008 demonstrated a significant dose-dependent reduction of platelet-loss in an anti-CD41 induced mouse model of immune thrombocytopenia, and robust anti-inflammatory effects in an antibody driven rat arthus model. In human blood, PRN1008 treatment *ex vivo* did not interfere with platelet aggregation in healthy subjects or in ITP patients. In humans, PRN1008 has been well tolerated to date, without safety signals of thrombocytopenia, bleeding or bruising. Reductions in anti-desmoglein 3 levels within 4 weeks were seen in 10/15 (67%) of pemphigus vulgaris patients with elevated pre-treatment levels, on a background of 0-30 mg of prednisone or equivalent corticosteroid. By 12 weeks the mean reduction in both anti-desmoglein 3 and 1 antibodies was approximately 50%. The Phase 1/2 immune thrombocytopenia study builds on these data with a design that can rapidly identify doses that can safely raise platelet counts.
Summary/Conclusion: Preclinical and clinical data suggest that PRN1008 will reduce platelet destruction in ITP via inhibition of autoantibody/Fc-gamma receptor signaling in splenic macrophages, and also reduce autoantibody generation, to diminish platelet loss in ITP. Initial clinical studies have not identified lowered platelet counts, bleeding or bruising as safety signals. A Phase 1/2 clinical study has been initiated to explore efficacy and safety in immune thrombocytopenia at several dose levels.

PS1407

ATRA CORRECTS IMPAIRED MACROPHAGE FUNCTION IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA THROUGH A20/NLRP3 SIGNALING PATHWAY

Y. He[1,2,3,*], J. Xue[1,2,3], X. Liu[1,2,3], X.-L. Zhu[1,2,3], J.-M. Zhang[1,2,3], C.-C. Wang[1,2,3], Q.-M. Wang[1,2,3], Y.-N. Wang[1,2,3], Q. Chen[1,2,3], H.-X. Fu[1,2,3], L.-P. Xu[1,2,3], K.-Y. Liu[1,2,3], X.-J. Huang[1,2,3], X.-H. Zhang[1,2,3]

[1]Peking University Institute of Hematology, Peking University People's Hospital, [2]Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, [3]Collaborative Innovation Center of Hematology, Peking University, Beijing, China

Background: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by increased platelet destruction and impaired platelet production. Mesenchymal stem cells (MSCs) in ITP patients displayed enhanced senescence and apoptosis. We previously found a pathological reduction in TNFAIP3 (Tumor necrosis factor alpha-induced protein 3, A20) levels, which induced NF-κB/SMAD7 pathway activation, causing a deficiency in MSCs in ITP patients (He Y 2018). MSCs promote the balance of macrophage polarization (M1/M2) toward the M2 phenotype. Our previous clinical study finds that all-trans retinoic acid (ATRA) is safe and effective in achieving a rapid and long-lasting response in ITP (Feng FE 2017). However, there is no information available on the role of macrophage polarization in megakaryocyte maturation and platelet production in ITP patients to date.
Aims: The aim of the present study was to explore the role of macrophage polarization in megakaryocyte maturation and platelet production and explore whether ATRA has a role in macrophage polarization.
Methods: Immunofluorescence pathology analysis of the M1 (CD68 and iNOS) and M2 (CD163 and CD206) polarization of macrophages in the bone marrow (BM) of the ITP patient, and the liver, spleen and BM of murine model of ITP. Bone marrow derived MSCs, macrophage and CD34+ cells were isolated and expanded. Cell proliferation and apoptosis of MSCs were assessed. The cytokines of MSCs were detected by ELISA. Macrophages were treated with either IFN-ɣ or IL-4, and M1 and M2 activation was analyzed by flow cytometry. Western blot and RT-PCR was employed for the expression of A20, NF-kB and NLRP3 at the mRNA and protein level in the ITP macrophage. MSCs were cocultured with macrophages, and macrophages were cocultured with CD34+ cells. We compared the influence of the ITP macrophage to megakaryocytopoiesis by estimating the relative percentages of megakaryocytes and platelets using flow cytometry. Megakaryocyte ploidy and pro-platelet forming (PPF) cells were also analyzed.
Results: Immunofluorescence pathology showed an aberrantly higher M1/M2 polarization ratio both in ITP patients and mouse model. ITP macrophages showed the enhanced phagocytic activity of platelets. There was a significantly decreased expression in A20 and activated NF-κB/NLRP3 in the macrophages of ITP patients. MSCs from ITP patients showed enhanced senescence and apoptosis, as well as a higher expression of TNF-α. MSCs of ITP facilitate higher M1 polarization through secreting TNF-α compared with HC-MSCs. To assess the influence of ITP macrophages to megakaryocytopoiesis, co-cultures systems were performed. We found that when CD34+ cells were cocultured with ITP macrophage, the relative pre-platelet and platelet count were significantly lower in the ITP-macrophage group than the HC-macrophage group. The treatment of ATRA could inhibit the activated NF-κB/NLRP3 signaling in the ITP-macrophage and redress the abnormal A20/NF-κB/NLRP3 signaling pathway. ATRA modulated macrophages towards the M2-like phenotype and improved the megakaryocytopoiesis in ITP.
Summary/Conclusion: The abnormal A20/NLRP3 signaling pathway in the macrophages of ITP contribute to a higher M1/M2 polarization, which impaired the megakaryocyte maturation, PPF and platelet production in ITP patients. ATRA modulated macrophages toward the M2-like phenotype and improved the megakaryocytopoiesis in ITP.

PS1408

RH-TPO RESTORES MEGAKARYOCYTES AND BONE MARROW NICHE COMPONENTS IN THE TREATMENT OF SEVERE THROMBOCYTOPENIA IN PATIENTS WITH CIRRHOSIS ASSOCIATED WITH HEPATITIS B

W.-Y. Zhai[1,*], X. Liu[1], Q.-S. Huang[1], Q. Jiang[1], H. Jiang[1], J. Lu[1], H. Wang[2], Y.-D. Xie[2], H. Ma[2], L.-P. Xu[1], K.-Y. Liu[1], X.-J. Huang[1], X.-H. Zhang[1]

[1]Peking University Institute of Hematology, [2]Institute of Hepatic Diseases, Peking University People's Hospital, Beijing, China

Background: Hepatitis B virus is a widespread virus that severely affects people's health. People infected with hepatitis B virus may develop liver cirrhosis. Thrombocytopenia is commonly seen in patients with liver cirrhosis. Thrombocytopenia plays a key role in thrombin generation and possibly bleeding tendencies in patients who have cirrhosis. However, its pathogenesis is considered to be complicated and still remains poorly understood. Rh-TPO can promote megakaryocyte development and platelet production, regarding an elevation in platelet counts both *in vitro* and *in vivo*. Its effect of elevating platelet counts has been proven in ITP patients, post-transplantation patients and aplastic anemia patients, while there is still no information available for its effect in cirrhotic patients with concomitant thrombocytopenia.
Aims: The aim of the study is to explore the effectiveness and mechanism of action of rh-TPO in the treatment of severe thrombocytopenia in patients with cirrhosis associated with hepatitis B.
Methods: This was multi-center research conducted in the People's Republic of China between January 2005 and December 2017. Patients with HBV-related liver cirrhosis (HBV LC) accompanied with severe thrombocytopenia (defined as a platelet count of <30 000 per cubic millimeter) were enrolled and divided into 2 groups by the occurrence of splenomegaly. Each patient received nucleoside analog (NA) alone treatment, or NA plus prednisone or NA plus rh-TPO treatment. Platelet counts, serum TPO levels, immature platelet fraction(IPF), reticulated platelets, glycocalicin, bone marrow biopsy, occurrence of bleeding events and adverse events were evaluated before and after treatment. Samples provided by healthy donors were also investigated.
Results: Patients with HBV LC associated severe thrombocytopenia had significantly lower serum TPO levels, higher IPF, higher reticulated platelets and higher glycocalicin compared to healthy donors. The platelet counts of HBV LC patients with splenomegaly were lower than those without

splenomegaly. A decrease in the number of megakaryocytes accompanied by impaired differentiation and maturation, a decrease in the number of CD34+ hematopoietic stem cells (HSCs) and a decrease in the number of mesenchymal stem cells along with abnormal senescence and apoptosis could be observed in the bone marrow of HBV LC patients. M1 polarization could also be observed in both the bone marrow and spleen of HBV LC patients. After rh-TPO treatment, a decrease in IPF and reticulated platelets could be observed in patients, while there was no significant change in glycocalicin. Compared with bone marrow samples before treatment, the number of megakaryocytes was elevated, along with a promotion of their differentiation and maturation. The number of CD34+ HSCs and MSCs was also increased. The abnormally activated M1 polarization was corrected after treatment. A significant elevation in platelet counts could be observed in patients who received NA plus prednisone and patients who received NA plus rh-TPO, along with fewer bleeding events compared to the patients who received NA alone. Patients without splenomegaly had a better reaction to treatment than those with splenomegaly.
Summary/Conclusion: Treatment with rh-TPO could restore megakaryocytes and bone marrow niche components, thus elevating the platelet counts and reducing the risk of bleeding events in HBV LC with severe thrombocytopenia, especially in patients without splenomegaly.

PS1409

A HIGH-THROUGHPUT SCREENING ASSAY TO EVALUATE CHEMOTHERAPY-INDUCED MYELOSUPPRESSION USING HEALTHY PERIPHERAL BLOOD AND BONE MARROW

K.K. Javarappa[1,*], D. Tsallos[1], C. Heckman[1]

[1]Institute for Molecular Medicine, Finland (FIMM), University of Helsinki, Helsinki, Finland

Background: Myelosuppression is a major side effect of chemotherapy and can result in infections, bleeding complications, increased risk of morbidity and mortality, and also limit the drug dose and frequency of administration contributing to poorer outcomes. It is therefore essential to understand the effects of novel chemotherapies on healthy hematopoiesis to avoid drug-induced myelosuppression. High throughput flow cytometry (HTFC) technologies and methods to detect rare hematopoietic cell populations are critical in advancing our understanding of how different blood cell types in complex biological samples respond to chemotherapeutic drugs.
Aims: The aim of this study was to develop an easily implementable HTFC assay that can monitor the cytotoxic effects of multiple chemotherapeutic drugs in a wide concentration range on megakaryocytes and myeloid progenitors in healthy human peripheral blood (PB) and bone marrow (BM).
Methods: Megakaryocytes and other myeloid progenitor cells are rare in PB and can be difficult to detect. However, culturing the SPCs in supplemented serum free medium induced the expansion of megakaryocytes and myeloid cells after 8-10 days. Using the 9-color panel, we were able to detect immature megakaryocytes (CD41a+CD42b-), mature megakaryocytes (CD41a+CD42b+), classical monocytes (CD14+CD16-), intermediate monocytes (CD14+CD16+) and non-classical monocytes (CD14-CD16+). By using antibodies for CD3, CD19 and CD56 labelled with fluorophores in the same colour channel, we were able to remove other cell types that were not of interest. With the addition of drugs, we were able to identify chemotherapies that inhibited expnsion of the different stages of megakaryocytes and myeloid cell populations, and the concentration range where the cells were most affected.
Results: Megakaryocytes and other myeloid progenitor cells are rare in PB and can be difficult to detect. However, culturing the SPCs in supplemented serum free medium induced the expansion of megakaryocytes and myeloid cells after 8-10 days. Using the 8-color panel, we were able to detect immature megakaryocytes (CD41+CD42b-), mature megakaryocytes (CD41a+CD42b+), classical monocytes (CD14+CD16-), intermediate monocytes (CD14+CD16+) and non-classical monocytes (CD14-CD16+). By using antibodies for CD3, CD19 and CD56 labelled with fluorophores in the same colour channel, we were able to remove other cell types that were not of interest. With the addition of drugs, we were able to identify chemotherapies that inhibited expansion of the different stages of megakaryocytes and myeloid cell populations, and the concentration range where the cells were most affected.
Summary/Conclusion: We developed a high throughput assay based on a multicolor flow cytometry panel that allows for simultaneous detection of different stages of megakaryocytes and myeloid progenitor cell populations in healthy peripheral blood and bone marrow. Applying the assay to a HTFC platform provides a sensitive, reproducible and rapid tool for screening hundreds of chemotherapeutic drugs and understanding the cytotoxic effects on rare cell types. The assay may be a more appealing method to evaluate the myelosuppressive potential of novel drugs and drug combinations compared to traditional lower throughput, time consuming and labor-intensive methods.

PS1410

A NOVEL ACQUIRED RUNX1 VARIANT IS RESPONSIBLE FOR ANKRD26 DYSREGULATION AND THROMBOCYTOPENIA IN SPORADIC FORM OF MYELODYSPLASTIC SYNDROME

S. Ferrari[1], D. Ragazzo[2], A. Lombardi[1], E. Omenetto[1], C. Scaroni[2], G. Semenzato[3], F. Vianello[3,*], F. Fabris[1]

[1]Medicine, Chair of Medicine, [2]Medicine, Endocrinology Unit, [3]Medicine, Haematology and Clinical Immunology, Padova, Italy

Background: RUNX1 and ANKRD26 dysregulation has been shown to play a role in familial thrombocytopenia with predisposition to acute leukaemia. Whether the same mechanism may contribute to thrombocytopenia of patients with myelodysplastic syndromes (MDS) is unknown.
Aims: We aim to evaluate the genetic status of ANKRD26 and related hematopoietic transcription factors like *RUNX1* and *ETV6* in subjects with MDS, in the presence or absence of thrombocytopenia.
Methods: 31 MDS subjects have been considered for the study. 18 of them had thrombocytopenia. 7 subjects with inherited thrombocytopenia were enrolled as a control. *ETV6*, *RUNX1*, *ANKRD26* gene were investigated. Functional studies were performed in the megakaryoblastic cell line MEG-01. Gene and protein expression were evaluated by mRNA with ddPCR system analysis and WT. The impact of the RUNX1 variant was evaluated by site-specific mutagenesis of the vector containing the wild type (wt) human *RUNX1* coding sequence (hRUNX-wt-pCMV6), inserted in the pCMV6-AC-Myc-DDK plasmid vector. To evaluate whether the variant found may modulate *RUNX1* expression itself at mRNA and protein level, MEG-01 were transfected with hRUNX-wt-pCMV6 or hRUNX-mut-pCMV6 expression vectors. To investigate the effects on transcriptional activity of *RUNX1* new variant, MEG-01 cells were transfected with ANKRD26-pGL3-basic reporter vector, which contains the luciferase sequence under control of the 5'UTR of the ANKRD26 gene. Dominant negative effect of mutant RUNX1 on wild type RUNX1 was studied by transfection of MEG-01 cells with different amounts of wild type and mutant RUNX1. Finally, the effect of RUNX1 mutant on platelet factor-4 expression has been also evaluated.

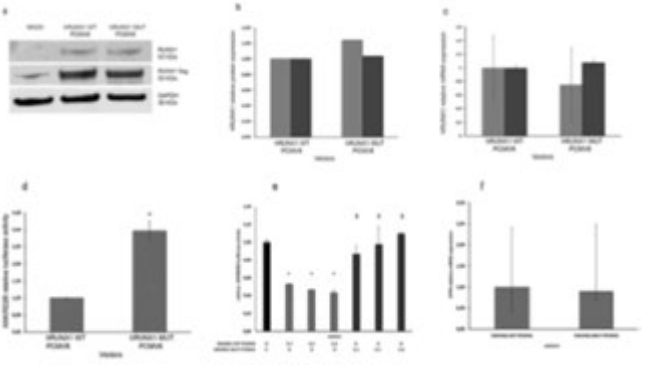


Figure 1.

Results: None of MDS and control subjects showed alterations in the *ETV6* gene or in the *ANKRD26* gene. Four patients among MDS subjects with thrombocytopenia showed previously undescribed *RUNX1* variants, namely p.Pro76Arg, p.Trp79Gly, c.934_935delA, c.1214_1215insTG and none of them was found in databases. We did not find *RUNX1* known mutations in MDS patients with normal platelet count. Not unexpectedly, all 4 patients with *RUNX1* variants progressed to AML. Expression studies showed that the variant p.Pro76Arg in *RUNX1* gene does not significantly affect the expression of *RUNX1* protein (Figure 1a,b) or the expression of MEG-01 total *RUNX1* mRNA (Figure 1c). As shown in Figure 1d, hRUNX-mut-pCMV6 vector, compared to hRUNX-wt-pCMV6, significantly increased *ANKRD26* luciferase activity, and this was achieved in a dose-dependent manner, suggesting that *RUNX1* variant p.Pro76Arg decreases the ability of *RUNX1* to bind DNA responsive elements, leading to increase transcriptional activity of *ANKRD26*.
Co-transfection of MEG-01 cells with different amounts of hRUNX-wt-

pCMV6 in presence of increasing quantities of hRUNX-mut-pCMV6 plasmid showed no dominant negative effect of mutant-RUNX1 on wt-RUNX (Figure 1e). As RUNX1 has been shown to affect PF4 expression in previous studies, we sought to evaluate if this was also the case for the mutant studied. However, we did not observe an effect of RUNX1 haplodeficiency on platelet PF4 expression (Figure 1f).
Summary/Conclusion: Our data are consistent with RUNX1 haploinsufficiency in subjects with thrombocytopenia in the context of MDS. The novel described *RUNX1* variant dysregulates *ANKRD26* by inducing its persistent expression. Whether and how *ANKRD26* dysregulation may play a role in the prognosis of these patients and in the identification of novel therapeutic targets in subjects with MDS would reserve future studies.

PS1411

FACTORS AFFECTING THROMBOPOIETIN RECEPTOR AGONIST REINITIATION AFTER DISCONTINUATION IN WELL-CONTROLLED PATIENTS WITH IMMUNE THROMBOCYTOPENIA IN THE UK: RESULTS FROM A MODIFIED DELPHI CONSENSUS PANEL

N. Cooper[1,*], Q. Hill[2], J. Grainger[3], J.-P. Westwood[4], C. Bradbury[5], D. Provan[6], J. Thachil[7], N. Ramscar[8], A. Roy[9]

[1]Hammersmith Hospital, London, [2]The Leeds Teaching Hospital, Leeds, [3]Royal Manchester Children's Hospital, Manchester, [4]University College Hospital London, London, [5]University of Bristol, Bristol, [6]Barts and the London School of Medicine, London, [7]Central Manchester University Hospital, Manchester, [8]Novartis UK, London, United Kingdom, [9]Novartis Pharmaceuticals, East Hanover, United States

Background: Immune thrombocytopenia (ITP) is an autoimmune disorder resulting in reduced platelet production and increased platelet destruction. Peer-reviewed literature indicates that in a real-world setting, prescribers are sometimes able to obtain prolonged responses in ITP using thrombopoietin receptor agonists (TPO-RAs), allowing for a 'drug holiday', with monitoring to reinitiate therapy when platelet levels fall below a threshold. However, a clear treatment framework for tapering and discontinuation is lacking. Currently it is also not possible to predict with confidence which patients with ITP will sustain their responses after going off therapy, and the subsequent length of the response. It is thus unclear which patients could be suitable candidates for TPO-RA tapering.
Aims: To develop guiding principles on determining optimal TPO-RA treatment regimens, treatment duration, criteria for discontinuation, and the approach to reintroducing treatment in relapsing patients with ITP.
Methods: Drawing on real-world experience from leading experts involved in treating patients with ITP, a consensus meeting was organized using a modified Delphi panel approach. Experts participated in a structured technology-based platform that captured individual uninfluenced opinion, followed by a panel debate and discussion in which consensus was sought. Discussion topics included criteria for tapering and discontinuation of TPO-RAs and criteria for reinitiating treatment in patients who relapse. Elsewhere we describe the outcome of the panel's discussion on the criteria for tapering and discontinuation; here we focus on the criteria for reinitiation of treatment. Prescribers with experience of using this TPO-RA discontinuation-reinitiation approach were asked to estimate the duration of discontinuation in their last 3 patients.
Results: Seven ITP experts participated in the June 2017 consensus meeting. The experts agreed that tapering and discontinuation of TPO-RAs at the point where the patient feels better and is deemed clinically improved is a rational approach. Experts reported that, in their last 3 patients, the average duration of TPO-RA discontinuation was 2–3 months, with the highest reported estimate being 4–5 years. They also reported that where therapy had to be reinstated, a decrease in platelet counts was the reason to reinstate therapy, with presence of symptoms such as clinically significant bleeding being the second most common reason. In the case of such adverse patient outcomes, the physician, in conjunction with the patient, should re-evaluate tapering and revert back to the previous TPO-RA dose. In certain patients with a high risk of life-threatening bleeding, or where effective rapid rescue therapy is unavailable, tapering and discontinuation of TPO-RAs may not be suitable.
Summary/Conclusion: A decrease in platelets is the primary driver of reinstating treatment with TPO-RAs. This suggests that physicians do not wait for symptoms before reinstating therapy and illustrates the importance of continued monitoring of lab values. In general, patients prefer treatments that they can use intermittently or even discontinue. Thus, there is a need for stronger evidence around how best to achieve sustained remission with a more real-world-aligned patient behavior.

PS1412

IMMATURE PLATELET FRACTION (IPF): AN INTERESTING TOOL TO INVESTIGATE THROMBOCYTOPENIA

Z. Van De Wyngaert[1,*], E. Fournier[2,3], V. Soenen[2], J. Gauthier[1], C. Preudhomme[2], T. Boyer[2]

[1]Maladies du Sang, CHU Lille, [2]Laboratoire d'hématologie, Centre de Biologie Pathologie, CHU Lille, Lille, [3]Laboratoire d'hématologie, CH de Dunkerque, Dunkerque, France

Background: Immature platelet fraction (IPF) is a parameter available on new generation hemocytometers. Reticulated, or immature platelets, are measured by fluorescence, and reflect bone marrow capacity to produce platelets. However, reference range on newest hemocytometers, as well as applicability of this parameter in daily practice, are not well defined so far.
Aims: We aimed at evaluating how IPF can be used in management and diagnosis of a thrombocytopenia of unknown origin.
Methods: Measurements were done on a Sysmex XN hemocytometer. First, we established reference range in normal subjects with normal complete blood counts. We then measured IPF values among patients with thrombocytopenia who underwent bone marrow aspiration.
Our objectives were to establish a cut-off value to distinguish peripheral from central causes of thrombocytopenia. We chose to exclude myelodysplastic syndromes because of the multiple mechanisms of thrombocytopenia in these patients.
Results: We analyzed 307 adult subjects, recruited inside our university hospital. Median IPF% was 3.8% [IQ 2.8 – 4.975] and min-max [0.7 – 8]. 73 patients underwent bone marrow aspiration. Median age was 59, IQR[45 – 75], 48% of patients were males. Thrombocytopenia was the only anomaly in 21 patients (29%), or associated with anemia (n=31, 42%), macrocytosis (n=3, 4%), neutropenia (n=2, 3%), or pancytopenia (n=16, 22%). Thrombocytopenia was peripheral (PT) in 49 patients (67%) and central (CT) in 24 (33%). Mean platelet count was similar in both groups (40 *vs.* 41 G/l respectively). In the PT group, mechanisms for thrombocytopenia were platelet consumption (n=23, 32%), immunological destruction (n=23, 32%) or hypersplenism (n=3, 4%). In the CT group, diagnosis were aplastic anemia (n=3, 4%), drug toxicity or malnutrition (n=10, 14%), severe sepsis in intensive care unit (n=7, 10%), absence of bone marrow recovery after stem cell transplantation (n=2, 3%).
Median IPF was 6% [3 – 8] in CT and 15% [10 – 21] in PT. Using a ROC curve, a cut-off value of IPF ≥13% predicts peripheral thrombocytopenia; specificity and predictive positive values (PPV) are 100%, sensitivity is 59% and predictive negative value is 55%.
Summary/Conclusion: An IPF% value ≥13% predicts a peripheral mechanism for thrombocytopenia (PPV = 100%). In our series of patients, bone marrow aspirations could have been avoided in 40% of cases.

PS1413

INTERLEUKIN-1 RECEPTOR ANTAGONIST GENE POLYMORPHISM IN EGYPTIAN CHILDREN AND ADOLESCENTS WITH IMMUNE THROMBOCYTOPENIA: ASSOCIATION WITH DISEASE SUSCEPTIBILITY, RESPONSE TO THERAPY AND OUTCOME

M.M. Al-Tawil[1,*], T.M. Kamal[2], S.M. Abd El-Ghany[1], O.M. Borham[1]

[1]Pediatric Hematology / Oncology, [2]Genetics, Children hospital, ain shams university, Cairo, Egypt

Background: Immune thrombocytopenia (ITP) is one of the most common hematologic disorders with poorly predictable clinical course and outcome. Scanty studies showed possible association between interleukin-1 receptor antagonist (IL-1Ra) gene polymorphism and susceptibility to ITP. No similar studies were done on Egyptian children. Moreover, no studies linked IL-1Ra gene polymorphism to disease severity or outcome
Aims: To study the distribution of IL-1Ra gene polymorphism (introns 2) among Egyptian children and adolescents with ITP and among healthy controls. Also, to correlate IL-1Ra gene polymorphism to disease severity, response to treatment modalities and outcome
Methods: This is a prospective case control study that included 60 Egyptian children and adolescents from unrelated families, diagnosed with ITP (37 females and 23 males; aged 1-17 years with mean of 9.2± 4.5 years). Patients included 22 subjects with acute ITP (disease duration less than 3 months), 7 patients with persistent ITP (disease duration 3-12 months) and 31 patients with chronic ITP (disease duration more than 1 year). The study also included 50 age and sex Egyptian matched healthy controls. subjects and controls were analyzed for IL-1Ra allele and genotype distribution using VNTR polymorphism of IL-1 receptor antagonist (IL-1Ra) intron-2. All patients were followed up for a minimum of one year to study disease outcome and

response to therapy in association with IL-1Ra genotypes and gene alleles. Bleeding everity was assessed with skin, mucosae, organs, grading (SMOG) score

Results: The frequencies of the polymorphic allele (A2) as well as genotype A1A2 were significantly higher in cases of ITP compared to controls (p value =0.0007, 0.0002; respectively). Allele A2 conferred 3.1 times increased relative risk for disease development. Also, allele A2 as well as genotype A1A2 were significantly more frequent among remitted cases (p value= 0.028, 0.029; respectively), but there was no significant difference between different genotypes and alleles regarding bleeding score(p values> 0.05). Patients with polymorphic allele A2 showed significantly better response to steroids than those with homozygous wild allele A1 (p 0.028), with insignificant difference in IVIG response (p0.44)

Summary/Conclusion: IL-1Ra polymorphism might contribute to the susceptibility to ITP in Egyptian children. The presence of A2 polymorphic allele of IL-1Ra gene was found to have better disease outcome and response to steroids than those with homozygous wild alleles

PS1414

CLINICAL CHARACTERISTICS AND TREATMENT COURSES FOR CYTOMEGALOVIRUS-ASSOCIATED THROMBOCYTOPENIA IN IMMUNOCOMPETENT CHILDREN AFTER NEONATAL PERIOD

Y.J. Shim[1,*], J.K. Suh[2], J. Y. Kim[3], J.M. Lee[4], E. J. Yang[5]

[1]Pediatrics, Keimyung University School of Medicine and Dongsan Medical Center, Daegu, [2]Pediatrics, Gachon University Gil Medical Center, Incheon, [3]Pediatrics, Kyungpook National University Hospital, [4]Pediatrics, College of Medicine, Yeungnam University, Daegu, [5]Pediatrics, Pusan National University Children's Hospital, Busan, Korea, Republic Of

Background: Cytomegalovirus (CMV) causes severe diseases in premature infants and immunocompromised hosts, and antiviral therapy is often required for disease control. However, the clinical manifestations and treatment courses for CMV-associated thrombocytopenia in immunocompetent children are unclear.

Aims: We reviewed the clinical characteristics and treatment courses for CMV-associated thrombocytopenia in immunocompetent children by conducting a multicenter retrospective study.

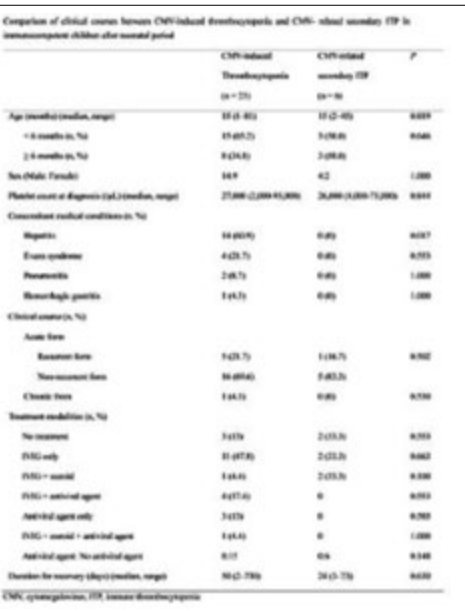
Methods: Children > 1 month and < 15 years of age with thrombocytopenia and positive CMV polymerase chain reaction (PCR) being treated at 3 tertiary medical centers in Daegu from January 2000 to March 2017 were included. Medical records of patients from all three institutes were retrospectively reviewed. This study targeted the previously healthy and immunocompetent children. Patients with primary immunodeficiency, leukemia, inherited bone marrow failure syndrome, or acquired immune deficiency syndrome were excluded from the final analysis. Additionally, patients suffering from non-CMV infections were also excluded.

CMV infection was defined as the detection of virus by quantitative or qualitative PCR in the blood or urine samples, performed on the day of detection of thrombocytopenia. CMV-induced thrombocytopenia caused by direct infection of megakaryocytes by CMV was defined as the state in which the patients showed CMV-positive PCR and CMV infection-like symptoms (fever, elevated transaminases, jaundice, hepatomegaly, splenomegaly, pneumonitis, gastritis, or myalgia) at the time of thrombocytopenia diagnosis. While, CMV-related secondary ITP caused by immunologic reactions after recent CMV infection was defined as the state in which the patients displayed CMV-like symptoms a few weeks before the diagnosis of thrombocytopenia. Altogether, in the present study, both of them referred to as CMV-associated thrombocytopenia.

Results: Among 1,065 children with thrombocytopenia, 29 (2.7%) displayed CMV-associated thrombocytopenia. The median age at diagnosis was 15 months and the median platelet count was 26,000/μL. They were classified into the CMV-induced thrombocytopenia (23/29) and CMV-related secondary immune thrombocytopenia (ITP, 6/29) groups. Fourteen subjects had hepatic dysfunction, four had Evans syndrome, two had pneumonitis, and one had gastritis. IVIG was used for 21 patients, and six patients among them showed recurrence, for whom IVIG or antiviral therapy was used. All, except one, recurrent or chronic cases belonged to the CMV-induced thrombocytopenia group. Antiviral therapy was used more frequently for the CMV-induced thrombocytopenia group (8/23, 34.8%) than for the CMV-related secondary ITP group (0/6); however, the results were not statistically significant (p=0.148) (Table 1).

Summary/Conclusion: CMV is a rare but unique etiology of thrombocytopenia, and observed even in healthy children after the neonatal period. About one-third patients need antiviral therapy for disease control. Further, CMV-induced thrombocytopenia is more complex than CMV-related secondary ITP.

Table 1.



PS1415

RAISED EXPRESSION OF APRIL IN THE PLATELET WITH IMMUNE THROMBOCYTOPENIA AND ITS CLINICAL IMPLICATIONS

Y. Hao[1,*], S. Wang[2], H. Li[2], R. Yang[2], Z. Zhou[3]

[1]Second Affiliated Hospital of Kunming Medical University, [2]Institute of Hematology, Chinese Academy of Medical Sciences, Tianjin, [3]Second Affiliated Hospital of Kunming Medical University, Kunming, China

Background: Immune thrombocytopaenia (ITP) is an antibodymediated autoimmune disease characterized by accelerated platelet destruction and suboptimal platelet production. A proliferation-inducing ligand (APRIL or TNFSF13), a member of TNF superfamily, structurally and functionally related to B cell activation factor of the TNF family (BAFF,TNFSF13b), has been shown to regulate lymphocyte survival and activation through interaction with its receptors transmembrane activator and calcium-modulating cyclophilin ligand interactor (TACI) and B cell maturation antigen (BCMA). APRIL is secreted as a soluble factor by various cells including inactive B cells, T cells, monocytes, neutrophils, macrophages, and dendritic cells, as well as by epithelial cells, osteoclasts, and megakaryocytes. APRIL has also been reported to be released by activated platelets. Recent studies have suggested an important role for APRIL, not only in normal immune responses, but also in the establishment and/or maintenance of autoimmune and inflammatory diseases. Patients with ITP has increased plasma levels of APRIL, however, the correlation between the enhanced expression of APRIL in platelets and the clinical relevance in the ITP patients is still unknown. The enhanced expression of APRIL in platelets may suggest a potential involvement of APRIL in the ITP autoimmune disorder.

Aims: To investigate the correlation between the expression of APRIL platelet levels and clinical indicators with ITP patients, and the relationship between different drug therapy and the expression of ITP platelet levels.

Methods: 1. Peripheral blood was collected from ITP patients and healthy controls with EDTA-anticoagulated vacuum tubes. Platelet-rich-plasma (PRP) was separated by centrifugation of the blood for 10 minutes at 800rpm. The PRP was then centrifuged again at 1700 g for 10 min at 20 °C to separate plasma from the platelets.

2. Flow cytometry and real time quantitative PCR were used for detecting the level of APRIL from platelets of healthy controls and ITP patients.

3. The relationship between APRIL levels and the disease severity of ITP and the platelets count and the effect of glucocorticoid treatment were analyzed.

Results: 1. The APRIL levels in the platelets from newly diagnosed ITP patients were significantly higher than those from healthy controls(p=0.00) and CITP patients (p=0.01).,while there was no significant difference between CITP patients and healthy controls. 2. Compared to healthy controls, the mRNA expression of APRIL was increased in ITP patients (p=0.04). 3. The level of APRIL in the platelets was slightly correlated with platelet count (p=0.05) and also directly correlated with the positive serum platelet-specific antibody. 4. After the treatment of conventional immuno-

suppressive drugs (glucocorticoids, GC) such as dexamethasone and prednisolone to the ITP patients, the level of APRIL was higher in NR patients than complete response (CR) patients(p=0.01),however, there was no statistics difference between CR patients and PR patients or partial response (PR) patients and no response (NR) patients Figure 1).

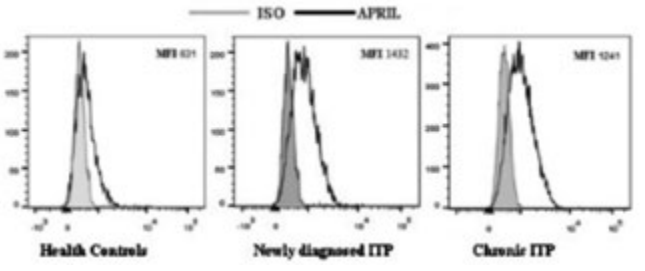


Figure 1.

Summary/Conclusion: Immune thrombocytopenia platelets showed increased levels of APRIL and the increased was associated with low platelet count, and APRIL might play a pathogenic role in the development of this disease.

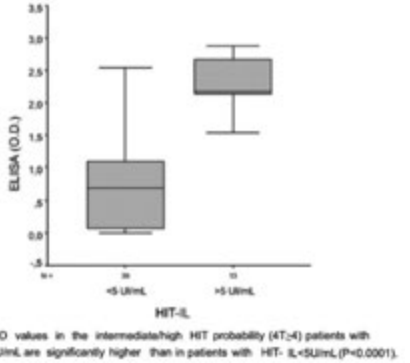
PS1416

EVALUATION OF HIT SYNDROME WITH AN AUTOMATED IMMUNOASSAY IN CORRELATION WITH THE 4T SCORE IN CARDIOVASCULAR PATIENTS

E. Iliopoulou[1], G. Soufla[1], S. Georgantis[1], T. Kanellopoulou[1,*], M. Katafygioti[1], T. Kostelidou[1]

[1]Department of Haematology, Blood Transfusion Unit and Coagulation and Haemostasis, Onassis Cardiac Surgery Center, Athens, Greece

Background: Heparin-induced thrombocytopenia (HIT), a serious complication of heparin therapy results in heparin/platelet-factor4 complexes (antiPF4/H antibodies-Abs). Upon clinical suspicion, laboratory evaluation for HIT syndrome should be performed.
Aims: To evaluate the reliability of a rapid, automated assay for HIT diagnosis along with the "4Ts" before cardiac surgery.
Methods: We analysed clinical and laboratory characteristics of 118 patients for HIT syndrome presence. Clinical suspicion was determined by "4T score" (≤3 low, ≥ 4intermediate/high probability). Patients were screened for HIT Abs with both HemosIL HIT-$Ab_{(PF4-H)}$ (HIT-IL) and ELISA-IgG (EIA). A HIT-IL value ≥1.0 U/mL was considered positive. We considered EIA (Asserachrom Stago) OD<1.0 as negative, OD 1.0-2.0 as intermediate and OD>2.0 as strongly positive for HIT-Abs presence. Functional test was performed with heparin-induced platelet aggregation test (HIPA) in selected cases to confirm a positive result.
Results: Both HIT-IL and EIA results were statistically associated (p<0.001), as well as with the "4T score" (p<0.001). In the intermediate/high probability group, 77% with a positive HIT-IL>5U/mL had an EIA OD>2.0 (p<0.0001); a positive HIT-IL between 1.8-5U/mL was correlated with 50% probability of an EIA OD>2.0, whereas HIT-IL 1.0-1.8U/mL yielded an EIA OD<1.0. Overall, in the same group, HIT-IL had a 50% positive and 84% negative predictive value of HIT-Abs presence. Interestingly in thirty patients with 4T score 3, HIT-IL had a 100% negative and 59% positive predictive value and HIT-IL>5U/mL was correlated with an EIA OD>2.0 (p=0.018) (Figure 1).



ELISA OD values in the intermediate/high HIT probability (4T≥4) patients with HIT-IL>5UI/mL are significantly higher than in patients with HIT- IL<5UI/mL (P<0.0001).

Figure 1.

Summary/Conclusion: The use of a rapid automated assay for HIT diagnosis overrides time consuming and expensive tests. Our results confirmed a strong association of HIT-IL>5U/mL with 4T≥4 and EIA OD>2.0 indicating its value in assessing strong probability of HIT. Further including patients with 4T score 3, a HIT-IL>5U/mL is still correlated with EIA OD>2.0 confirming our previous observation. HIT-IL<5U/mL should in any case be evaluated with EIA and HIPA.

PS1417

IMMATURE PLATELET FRACTION AS A MARKER OF THROMBOPOIESIS IN THROMBOCYTOPENIC PATIENTS

M. Farrugia[1], A. Gatt[1], D. J. Camilleri[1,*]

[1]Pathology Department, Mater Dei Hospital, Tal-Qroqq, Malta

Background: Thrombocytopenia manifests from a myriad of diseases most of which are secondary to peripheral platelet destruction or due to bone marrow (BM) failure. Distinguishing between the two is of utmost importance particularly for clinical management and treatment decisions. A novel parameter, the Immature Platelet Fraction (IPF), measures young reticulated platelets and is comparable to reticulocytes in erythropoiesis.
Aims: To explore the value of IPF in the diagnostic workup of immune thrombocytopenic purpura (ITP) patients, and to investigate the usefulness of IPF in the clinical management of haemato-oncology patients on chemotherapy.
Methods: Cohort A consisted of 20 patients diagnosed with refractory ITP, 6 newly diagnosed ITPs and 14 follow up cases. Cohort B comprised of 20 haemato-oncology patients undergoing chemotherapy. Twenty healthy controls were included in the study. Samples were consecutively selected with different age groups. Platelet count was measured by the fluorescent platelet channel (PLT–F) on XN-20 analyser (Sysmex, Kobe, Japan) and included an IPF–% and absolute count (IPF–A). Paired sample t-test was performed to study statistical significance at p-value<0.05 between cohorts, both for IPF–A and IPF–%.
Results: The mean platelet count (PLT-F) for cohort A and B was 30 and $25x10^9$/L respectively, and $304x10^9$/L for controls. A significant increase was observed in Cohort A when compared to Cohort B for both IPF–A and IPF–% (p=0.0009 and p = < 0.00001 respectively). ITP cohort A showed a higher IPF–% then Controls (p<0.00001) but lower IPF–A (p=0.00478). Three patients from the ITP cohort had a very low IPF–A (<$1.0×10^9$/L). BM studies were available for 2 of these patients in which megakaryocytes were normal to increased in number with atypical forms. When compared to controls, the haemato-oncology cohort B had very low IPF–A, but similar IPF–% (p=< 0.00001 and 0.8249 respectively) (Figure 1).

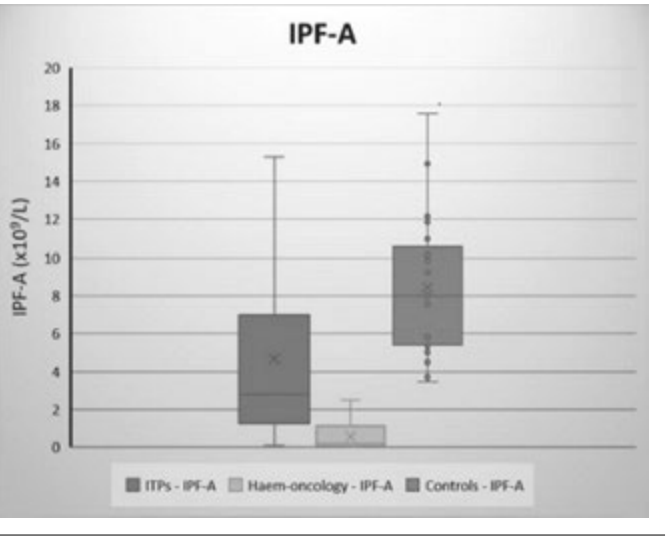


Figure 1.

Summary/Conclusion: Autoimmune idiopathic thrombocytopenic purpura (aITP) manifests in low platelet counts through increased peripheral platelet consumption, explaining the higher IPF–A/IPF–% counts when compared to patients on chemotherapy. This results from increased demand and supply of young platelets from the bone marrow. Correct distinction is of utmost importance for clinical and therapeutic decisions. However, this study possibly identified 3 ITP patients with abnormal thrombopoiesis (ITP with very

low IPF–A) possibly indicating defective platelet production due to possible dysmegakaryopoiesis. When the ITP cohort was compared to controls, IPF–A was lower for ITP, suggesting a platelet feedback loop mechanism that is different from that seen in reticulocytes. This can be related to an insignificant increase in thrombopoietin levels seen in ITP. These patients may in fact benefit mostly from thrombopoietin analogues. Since a rise in immature platelets can predict platelet recovery, the absolute count IPF–A could be useful in the clinical management of patients receiving chemotherapy. IPF–A can be useful to limit the number of platelet transfusions thereby minimizing adverse platelet reactions. Further research could establish a cut–off value for the optimum platelet recovery to administer chemotherapy and/or platelet transfusions. This study identifies a novel biomarker of BM status and activity.

PS1418

A DECREASED PLATELET-TO-LYMPHOCYTE RATIO IS ASSOCIATED WITH A LOW PLATELETCRIT IN PRE-DIABETES

B. Nkambule[1,*], Z. Mkandla[1], T. Mutize[1]

[1]Laboratory Medicine and Medical Science, University of Kwazulu-Natal, Durban, South Africa

Background: People living with type 2 diabetes are at an increased risk of cardiovascular disease. Chronic immune activation and elevated levels of circulating platelets exacerbate the inflammatory milieu in diabetic patients. Platelets play a crucial role that links inflammation with thrombosis. Different indices of platelet function indicate increased platelet activation and sequestration in diabetic individuals. The platelet-to-lymphocyte ratio (PLR) and platelet-to-neutrophil ratio (PNR) have been reported as reliable markers of systemic inflammation.
Aims: To evaluate the platelet-to-lymphocyte (PLR), platelet-to-neutrophil ratio (PNR) and plateletcrit (PCT) in prediabetes using an animal model of prediabetes.
Methods: Seventeen (n=17) male C57BL/6 mice were randomised into two separate diet groups; 10 kcal% fat control diet group (diet code: D12450J) (n=4) and a 60 kcal% fat diet group (diet code: D12492) (n=13). After 3 weeks of feeding, oral glucose tolerance testing was performed to determine the glucose metabolism status of the animals. Two hundred microliters (200µl) of blood was then collected from the lateral tail vein into 3.2% sodium citrate coated tubes. Platelet indices (mean platelet volume; platelet count); absolute lymphocyte count and neutrophil count were measured using the AcT hematology analyser. The plateletcrit, platelet-to-lymphocyte and platelet-to-neutrophil ratio were also calculated
Results: The control and pre-diabetic group showed no sign of thrombocytopenia or leukopenia as the lymphocyte and neutrophil counts were comparable between the two groups (p>0.05). However the PCT was decreased in pre-diabetic mice, mean PCT% 3.75±0.35 vs. 4.99±0.8375. The pre-diabetic had a decreased PLR; 983.4±100.02 vs.1589±393.8 control group, p=0.04. In the pre-diabetic group, decreased PLR levels correlated with; PCT% (r=0.57, p=0.03) and PNR (r=0.613,p=0.02) Furthermore, the PNR was also decreased in pre-diabetic mice, 97.20±7.41 vs. 140.1±24.45 control group, p=0.03.
Summary/Conclusion: In pre-diabetes the PCT is decreased and these decreased levels directly correlate with the decreased PLR. Moreover, pre-diabetics have a low PNR which is associated with a low PLR. These findings may suggest that in pre-diabetic individuals platelet indices are affected and these may suggest increased platelet adhesion and localised inflammation

PS1419

CHANGES IN PLATELET AGGREGATION DURING PREGNANCY AND THE IMMEDIATE POSTPARTUM PERIOD

B. Hussein[1,*], A. Maarouf[1], K. Gomez[2], J. Davies[2], D. Obeng-Tuudah[2], R. Kadir[2]

[1]Haematology Department, Nanakali Hospital for Blood Disease and Oncology, Erbil, Iraq, [2]Haemophilia Center and Thrombosis Unit, Royal Free Hospital, London, United Kingdom

Background: Platelet dysfunction is implicated in uteroplacental disorders. During the early stages of gestation platelets have important roles in the process of placentation. Platelet function contributes to enhanced haemostasis at delivery. However, there is limited data on the changes of platelet function during normal pregnancy. Understanding physiological changes of platelet aggregation during different stages of pregnancy is helpful for better understanding of pathophysiology of abnormal placentation.
Aims: To assess platelet aggregation during three trimesters of pregnancy and immediate postnatal period in normal healthy women compared to control non-pregnant group.
Methods: Cross-sectional cohort study including a total of 46 women: 10 participants for each trimester, 10 postnatal cases and 6 control non-pregnant women. Case selection was based on specific inclusion criteria. 30mL of venous blood was obtained from each participant following consent. Light transmission aggregometry was performed with Dual channel Payton 600B aggregometer using six platelet aggregating agonist (epinephrine, adenosine triphosphate, collagen, ristocetin, arachidonic acid and U46619).
Results: The findings included reduced secondary aggregation curve appearance in pregnant and postnatal women when compared to control group, which was most apparent in the third trimester. Compared to non-pregnant controls, platelet aggregation induced by ADP and collagen were reduced during third trimester while epinephrine-induced aggregation was reduced during the first trimester.
Summary/Conclusion: Reduced platelet reactivity in response to epinephrine during early pregnancy can be considered as a mechanism to reduce thrombosis and allow normal placentation while diminished ADP and collagen induced aggregation in third trimester could be a compensatory mechanism since pregnancy associated with hyper-coagulation particularly in late stages.

PS1420

CHANGING PROFILE OF PLATELET ACTIVITY AND TURNOVER INDICES DURING TREATMENT RESPONSE OF IMMUNE THROMBOCYTOPENIA: PREDICATIVE VALUE OF REPONSE BY GLYCOCALICIN AND SOLUBLE CD40 LIGAND

H. Chang[1,*], P.-L. Li[1], Y.-S. Hung[1], T.-C. Tang[1], C.-W. Ou[1], P.-N. Wang[1]

[1]Hematology, Chang Gung Memorial Hospital, Kweishan, Taoyuan, Taiwan, Republic of China

Background: Immune thrombocytopenia (ITP) is characterized by relatively active platelet function and rapid platelet turnover. Platelet-related plasma markers may be useful in distinguishing ITP from other etiologies of thrombocytopenia. Measurable plasma markers include platelet activity markers (e.g. soluble P-selectin [sP-selectin] and soluble CD40 ligand [sCD40L]) and platelet turnover markers (e.g. glycocalicin [GC]). It has been reported that some of these plasma markers change with treatment response of ITP. It is unclear whether changes of these plasma markers may be predicative of treatment response in patients with ITP.
Aims: We intended to determine changes of activity (sP-selectin, sCD40L) and turnover (GC) markers in various phases of ITP.
Methods: Patients with ITP were classified by their platelet counts in response to treatment, which included no response (NR, including new diagnosis), partial response (PR) and complete response (CR) according to previously defined criteria. Plasma markers of platelet activity (sP-selectin and sCD40L), and platelet turnover (GC) were measured by ELISA. Platelet counts and mean platelet volume (MPV) were obtained in the clinical laboratory using GenS System-2. Plasma markers in ITP subjects were compared with patients with myelodysplastic syndrome and among different phases of ITP. For patients with paired samples collected in different periods, levels of plasma markers were compared (NR *vs.* PR and PR *vs.* CR) in a paired analysis.
Results: One hundred and sixteen samples (29 CR, 32 PR, 55 NR) from 79 patients were collected for this study. Compared to myelodysplastic syndrome and acute myeloid leukemia with comparable platelet counts, ITP patients had significantly different plasma levels of sP-selectin (p=0.026). There is no difference in sCD40L, MPV, GC and GC index. Patients with increased platelet counts (PR+CR) had higher levels of sP-selectin (p=0.026) and sCD40L (p=0.001) than those with new diagnosis or NR. There was no significant difference in platelet turnover markers MPV (p=0.077) or GC (p=0.078). However, there was a marked decrease of GC index (p<0.001) in patients with PR and CR, compared to the NR group. Thirteen patients had paired samples in NR and PR. Analysis showed no difference in sP-selectin, sCD40L, MPV or GC but significant difference in GC index (p=0.017) (Figure 1A). Another 12 patients had paired samples in PR and CR. Analysis showed no difference in sP-selectin, sCD40L, MPV or GC but significant difference in GC index (p=0.029) (Figure 1B). To explore the role of biomarkers in predicting treatment response, patients with low platelet counts (NR) were divided into refractory disease and new diagnosis disease which later responded to treatment. Significant difference was found in GC (p=0.020) and sCD40L (p=0.001). Such results suggested plasma levels of sCD40L and GC may in potential predict treatment response in patients with ITP.
Summary/Conclusion: Patients with ITP have a different platelet activity profile from myelodysplastic syndrome, as characterized by plasma sP-selectin levels. Platelet activity markers (sP-selectin and sCD40L) and GC indices change in parallel with treatment response. Plasma levels of GC and

sCD40L are different between new diagnosis of and refractory ITP patients. Such findings suggest plasma GC and sCD40L not only change with treatment but also predict likelihood of treatment response.

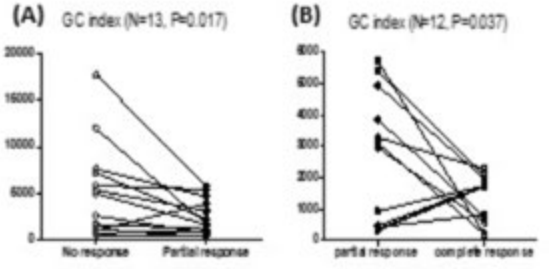


Figure 1.

PS1421

THE IMPACT OF HIGH MOBILITY GROUP BOX 1 IN THE PATHOGENESIS OF IMMUNE THROMBOCYTOPENIA

E. G. Umit[1], M. Baysal[1,*], H. Goze[1], V. Asoglu[1], A.C. Ozdover[1], O. Kirkizlar[1], M. Demir[1]

[1]Hematology, Trakya University Faculty of Medicine, Edirne, Turkey

Background: High-mobility group box 1 (HMGB1) was originally defined as a ubiquitious nuclear protein regulating chromatin structure and gene transcription. Later, new roles for a cytosolic component as inflammasome activation and autophagy were discovered. Extracellular HMGB1 was demonstrated to be related with cell signalling for certain receptors such as Toll-like receptors (TLR) which may contribute to the pathogenesis of inflammatory disorders.
Aims: The aim of our study is to evaluate the value of HMGB1 and integrate the pre-clinical knowledge regarding HMGB1 and their related receptors of TLR into the clinical knowledge of immune thrombocytopenia (ITP) to derive future therapeutic options.
Methods: Newly diagnosed and treatment naive 50 ITP patients and 30 healthy age and sex matched controls were enrolled in the study. Clinical and demographic features were recorded and whole blood counts and HMGB1 levels with ELISA method were determined.
Results: Within patients group, 34 were female (68,0%) while 16 were male (32,0%); and in control group 17 were female (56,7%) and 13 were male (43,3%). In the patient group, mean age was 53,56 years, mean values of platelet count 20,772/mm^3 MPV 10,7 fL, hemoglobin 12,1 g/dL, leucocyte 9721/mm^3 and HMGB1 was 15.093,4 ng/mL. In the control group, mean age was 53,23 years, mean values of platelet count 251.900/mm^3, MPV 8,8 fL, hemoglobin 12,9 g/dL, leucocyte 7820/mm^3 and HMGB1 was 5.606,06 ng/mL. Age was not related with HMGB1 levels in both groups. Platelet levels were significantly related with HMGB1, especially in the very low and treatment needing group of platelet <30.000/mm^3 showed the highest levels of HMGB1 (p levels <0,005). Likewise, MPV, as a surrogate for increased platelet turnover fort he pathogenesis of platelet destruction in ITP was significantly related with HMGB1 levels (p<0,005). In the control group, though not proven with logistical analysis, low normal platelet counts showed relatively higher HMGB1 levels.
Summary/Conclusion: The role of extracellular HMGB1 was demonstrated as being as a proinflammatory cytokine, secreted by activated macrophages as a late mediator of inflammation (1). The absence of HGMB1 resulted with increased vulnerability to macrophage conducted innate immunity and lack of autophagy (2). As a damage associated molecular pattern (DAMP) molecule, HMGB1 should be regarded as an inflammatory and non-inflammatory molecule and a possible therapeutic target (3). HMGB1 stimulates the release of tumor necrosis factor (TNF) alpha and other inflammatory cytokines in macrophages and has two important binding sites for TLRs and receptor for advanced glycation end products (RAGE). In our study we observed an increase in HMGB1 levels in patients with ITP, related with platelet counts which may be explained with an immune response that cannot be detected with IL-6 related acute phase proteins such as C reactive protein. Levels of HMGB1 during remission and their impact on treatment responses and relapses should be studied further with large prospective studies. Likewise, patients with ITP who reach normal platelet counts may be followed up with HMGB1 levels for relapse prediction and other thrombocytopenias which have increased destruction as their pathogenesis may be investigated for HMGB1 levels for disease and prognosis predictions.

PS1422

WHAT ARE THE UNDERLYING CAUSES AND CLINICAL CHARACTERISTICS OF CHILDREN WITH REACTIVE THROMBOCYTOSIS AT A KOREAN TERTIARY MEDICAL CENTER?

Y. J. Shim[1,*], E.J. Yang[2], N. Jung[1]

[1]Pediatrics, Keimyung University School of Medicine and Dongsan Medical Center, Daegu, [2]Pediatrics, Pusan National University Children's Hospital, Busan, Korea, Republic Of

Background: Reactive thrombocytosis (RT) is a common condition among children, although no studies have examined the etiology or clinical characteristics of RT among Korean children.
Aims: The present retrospective study aimed to determine the etiology and clinical characteristics of RT among children who were admitted to a single Korean center during a 10-year period.
Methods: This retrospective study collected data from <18-year-old children with a platelet count of >500×10^9/L who were admitted to pediatric wards in the Keimyung University Dongsan Medical Center between January 2006 to December 2016. The study protocol was approved by the institutional review board of Keimyung University Dongsan Medical Center (2017-11-038).
Cases of RT were classified as mild (grade 1, platelet count of 501–700×10^9/L), moderate (grade 2, platelet count of 701–900×10^9/L), severe (grade 3, platelet count of 901–1,000×10^9/L), and extreme (grade 4, platelet count of >1,000×10^9/L).
Analysis of variance with Bonferroni adjustment was used to compare the mean values between the groups, and intra-group comparisons of proportions were performed using the linear by linear chi-square statistic. Pearson's correlation analysis was performed to define the correlations between the variables. All statistical analyses were performed using IBM SPSS software (version 23.0; IBM Corp., Armonk, NY), and *P*-values of <0.05 were considered statistically significant.

Table 1. Comparing the variables according to the severity of reactive thrombocytosis.

	Mild (N = 3,400)	Moderate (N = 580)	Severe (N = 46)	Extreme (N = 87)	*P*
Age (years)	1.3 (1.2–1.4)	1.0 (0.8–1.2)	0.9 (0.4–1.3)	1.2 (0.7–1.7)	0.013
Sex (male : female)	1,466 : 1,934	247 : 333	25 : 21	42 : 45	0.348
WBC (×10^9/L)	12.4 (12.2–12.6)	13.6 (13.1–14.1)	14.6 (12.9–16.2)	16.2 (14.3–18.1)	< 0.001
Hb (g/dL)	11.2 (11.1–11.2)	10.9 (10.8–11.0)	10.6 (10.0–11.0)	10.7 (10.3–11.1)	< 0.001
AST (IU/L)	48 (45–51)	44 (41–47)	40 (33–47)	52 (39–66)	0.707
ALT (IU/L)	39 (36–42)	39 (35–43)	35 (25–46)	44 (29–60)	0.950
ESR (mm/h)	25 (24–26)	30 (27–33)	33 (21–44)	38 (29–46)	< 0.001
CRP (mg/dL)	1.5 (1.4–1.5)	1.6 (1.3–1.8)	1.2 (0.5–2.0)	2.2 (1.3–3.1)	0.054
LDH (IU/L)	655 (626–684)	690 (608–774)	654 (520–787)	764 (544–983)	0.540
Admission duration (days)	6.2 (6.0–6.4)	7.1 (6.6–7.5)	8.7 (6.3–11.1)	14.4 (10.6–18.2)	< 0.001

The values are presented as mean values and 95% confidence intervals.
WBC, white blood cells; Hb, hemoglobin; AST, aspartate transaminase; ALT, alanine transaminase; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; LDH, lactate dehydrogenase.

Results: During the study period, RT accounted for 13.5% of children who were admitted to the pediatric wards (4,113/30,355). The median age of children with RT was 0.3 years, and the proportion of children who were <1-year-old was 71.0%. Among the 4,133 children, 82.7% of cases involved mild RT, 14.1% of cases involved moderate RT, 1.1% of cases involved severe RT, and 2.1% of cases involved extreme RT. The underlying causes of RT were as follows: infection (78.1%), Kawasaki disease (KD) (6.0%), tissue damage (3.7%), neurologic disease (2.8%), allergy (1.9%), perinatal disease (1.8%), hemato-oncologic disease (1.5%), non-KD autoimmune inflammation (1.3%), and others (2.9%). Significant differences according to RT severity were detected for hemoglobin (Hb) levels, white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), and admission duration (Table 1). After controlling for patient age, disease category, liver function, C-reactive protein levels, and lactate dehydrogenase levels, there was a negative correlation between platelet count and hemoglobin level (r = –0.251, p=0.008). And there were positive correlations between platelet count and white blood cell count (r = 0.305, p=0.001), between platelet count and erythrocyte sedimentation rate (r = 0.252, p=0.007), and between platelet count and admission duration (r = 0.257, p=0.006).
The proportion of KD was highest in the extreme RT subgroup (p<0.001) and in the 1–7.9-year-old group (p<0.001). Non-KD autoimmune inflam-

mation (Henoch–Schönlein purpura, juvenile idiopathic arthritis, and inflammatory bowel diseases) was only observed in mild/moderate RT cases, and its proportion was highest in the 8–18-year-old group (p<0.001).
Summary/Conclusion: The most common cause of RT among Korean children was infection, and extreme RT was associated with prolonged admission. KD was prevalent in the extreme RT and 1–7.9-year-old groups. Non-KD autoimmune inflammation was prevalent in the mild/moderate RT and ≥8-year-old groups.

PS1423

EFFECT OF PERAMIVIR ON PLATELET COUNTS IN PATIENTS WITH SUSPECTED OR CONFIRMED INFLUENZA INFECTION

S.-Y. Ko[1,*]

[1]*Laboratory Medicine, Korea University Hospital, Seoul, Korea, Republic Of*

Background: Several cases and one retrospective study have been reported of an increase of platelet counts in patients with suspected influenza after oseltamivir treatment.
Aims: The purpose of this study was to assess the effect of peramivir, an intravenous neuraminidase inhibitor which was approved by FDA in 2014, on platelet counts for the first time.
Methods: We retrospectively analyzed consecutive patients who were treated with peramivir or were tested for influenza from January 2015 to December 2017 in a tertiary hospital. Patients were divided into three groups: patients with peramivir treatment and proven influenza (group 1), with peramivir treatment but without influenza (group 2), and without peramivir treatment and proven influenza which was redefined as those with oseltamivir treatment and proven influenza (group 3), since most of the influenza-proven patients without peramivir treatment were treated with oseltamivir. Platelet counts were collected from three intervals: day (-30)-(-10) (baseline), day 0, and day 5-15 (after treatment). Day 0 was the day of peramivir injection or the first day of oseltamivir treatment.
Results: A total of 1,763 patients were treated with peramivir and 3,796 were tested positive for influenza. The number of patients in each group were 1,542, 221, and 2,162, and the number of patients whose platelet counts were available from all three intervals were 347, 93, and 88 for group 1, 2, and 3, respectively. Intragroup analysis revealed significant decrease in platelet counts from baseline to day 0 in all groups. After treatment, platelet counts of group 1 and group 2 recovered to those of the baseline while of platelet count of group 3 got significantly higher (Table 1)

Table 1.

Platelet counts	Baseline (day (-30)-(-10))	Day 0	After treatment (day 5-15)	P-value between baseline and day 0	P-value between baseline and after treatment
Group 1	261.6k	204.7k	266.8k	<0.0001	0.3606
Group 2	253.4k	214.2k	252.2k	0.0034	0.8411
Group 3	249.2k	197.4k	279.5k	<0.0001	0.0106

Summary/Conclusion: The increase of platelet counts after treatment that exceeds that of the baseline was observed only in patient group treated with oseltamivir but not in those with peramivir. The exact reason for this different finding between the two neuraminidase inhibitors on platelet counts needs further investigation.

PS1424

STUDIES ON PLATELET FUNCTION IN A GROUP OF CLL PATIENTS DURING THE FIRST YEAR OF IBRUTINIB TREATMENT

B. Sokołowska[1,*], D. Jawniak[1], A. Nowaczyńska[1], A. Szymczyk[1], E. Wąsik - Szczepanek[1], E. Drab - Urbanek[1], W. Tomczak[1], J. Kozińska[1], M. Kozioł[1], M. Hus[1]

[1]*Department of Haematooncology and Bone Marrow Transplantation, Medical University of Lublin, Lublin, Poland*

Background: Ibrutinib, an inhibitor of Bruton's tyrosine kinase (BTK) has a significant activity in chronic lymphocytic leukemia (CLL). Although the treatment is well tolerated, some clinical studies reported mucocutaneous bleeding complications occurring during the first months of the treatment. Such complications may be caused by platelet or vessel abnormalities.
Aims: The aim of the study was to evaluate platelet function before and during the first year of ibrutinib therapy.
Methods: We examined 16 patients with CLL (6 females and 10 males, mean age 66 (49-82). All patients started with 420 mg Ibrutinib but then in one patient the dose was reduced to 140 because of atrial fibrillation. In all group of patients platelet aggregation studies were performed in whole blood samples using Multiplate technology, in which platelets were activated by adenosine diphosphate (ADPtest), arachidonic acid (ASPItest), collagen (COLtest), TRAP-6 (TRAPtest). Platelet function was evaluated four times: before Ibrutinib treatment, after 1month, after 2 – 4 months and after one year of the therapy. Control group (CG) consisted of 20 healthy persons: six males and 14 females, mean age 44 (26-59).
Results: Statistically significant impaired platelet function in a group of patients in all the tests and in all the points was detected compared to the control group p=0.001-0.05. Exacerbation of platelet defects in the course of the treatment measured by COLtest was observed. COLtest was diminished after 12 months compared to the value before the treatment [COLtest 1: M: 33U(6-70); COLtest 4 M: 11U(0-22). In the remaining three tests a very interesting tendency occurred. The value of the fourth point was raised indicating some improvement in ADP, arachidonic acid and TRAP induced platelet aggregation[ADPtest1: M:27U(0-58), ADPtest4: M :34U(3-84), ASPItest 1:M:45U(21-101), ASPItest4:M:56U(2-123), TRAPtest1:M: 56U(28-95), TRATtest4:M:86U(15-140).This tendency was the most pronounced in TRAPtest but not statistically significant.Bleeding complications in five patients occurring during the first three to six months of ibrutinib therapy were observed (in two patients: nose bleeding, in three patients: skin petechiae). Lower platelet count (75-85G/l) was detected in three out of five patients just before Ibrutinib therapy. Platelet count in the group of patients without bleeding complications was as follows: before ibrutinib therapy: M:141G/l (106-204) and after one year M:166.5G/l (126-209). Whereas, in the group of patients with bleeding complications was as follows: before therapy: M:85G/l(52-171) and after one-year treatment: M:132G/l(87-188).
Summary/Conclusion: We detected impaired platelet aggregation measured by Multiplate technology in all the patients. The impairment of collagen induced platelet aggregation was exacerbated during ibrutinib treatment. On the other hand, ADP, arachidonic acid and TRAP induced platelet aggregation tended to improve during the treatment. Only 31% of ibrutinib patients presented some mild bleeding complications at the beginning of the treatment.

PS1425

VALIDATION OF THE PLASMIC SCORE FOR THROMBOTIC MICROANGIOPATIES IN A SINGLE UNVERSITY HOSPITAL

M.D.C. Ballester[1,*], B. Lopez[1], J.M. Sanchez[1], G. Aspas[1], A. Perez[1], M.G. Uriol[2], S. Cabello[2], A. Sampol[1]

[1]*Hematology Deparment,* [2]*Nephrology Department, Hospital Universitario Son Espases, Palma de Mallorca, Spain*

Background: Dr.Pavan and collegues have developed a new score "the PLASMIC score" to stratify patients with thrombotic microangiopathies (TMA) according to the risk of having severe ADAMTS 13 deficiency.This will allow us to know the probability the patient has to present a Thrombotyc Thrombocytopenic Purpura (TTP) before counting on the result of ADAMTS 13 and thus facilitate therapeutic decisions.
Aims: To validate the PLASMIC score in a single center cohort.
Methods: We have collected retrospectively all consecutive 44 patients (including adults and children) presenting to one large academic center in Palma de Mallorca, SPAIN, with TMA between Jan 2010 to Feb 2018.We have collected the data proposed in the score of all patients from the date of diagnosis of the TMA. We have used contingency tables in the SPSS software v.18 to calculate the association between a high score result and the probability of having a TTP. We also calculated the sensitivity, specificity, positive and negative predictive value of the score.
Results: Our validation cohort includes previously diagnosed patients, 11 for Hemolytic uremic syndrome (SHU), 20 for atypical Hemolytic uremic syndrom (SHUa), 6 for TTP and 7 for Transplant related TMA. For the PTT group, the score identified 5 of them with high probability of presenting severe deficit of ADAMTS 13, classifying the other one of intermediate risk, with a diagnostic sensitivity of 83%. Likewise, the score identified 4 patients, 3 of them SHUA and 1 MAT-AT with a high risk of developing ADATMS 13 deficiency, these results being false positive. The specificity of the test was 89%, the positive predictive value 55% and the negative predictive value 97% (Figure 1).
Summary/Conclusion: "The plasmic" score is a very useful tool for the

early detection of patients with PTT in those centers that do not have a result of ADAMTS 13 quickly available. The score along with clinical judgment would help medical teams make quick therapeutic decisions.
Our results confirm the external validation of this score.

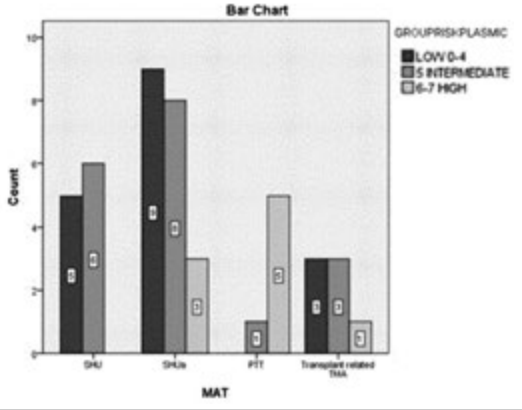


Figure 1.

PS1426

OCCURRENCE AND MANAGEMENT OF HEPATOBILIARY ADVERSE EVENTS (AES) IN ADULTS WITH IMMUNE THROMBOCYTOPENIA (ITP) DURING LONG-TERM TREATMENT WITH ELTROMBOPAG: RESULTS FROM THE EXTEND STUDY

A. Khelif[1,*], M.N. Saleh[2], R.S. Wong[3], O. Meyer[4], O. Ocak Arikan[5], J. Maier[5], J. B. Bussel[6]

[1]Hôpital Farhat Hached, Sousse, Tunisia, [2]Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, United States, [3]Sir YK Pao Centre for Cancer & Department of Medicine and Therapeutics, Chinese University of Hong Kong, Shatin, NT, Hong Kong, [4]Institute of Transfusion Medicine, Charité-Universitätsmedizin Berlin, Berlin, Germany, [5]Novartis Pharma AG, Basel, Switzerland, [6]Pediatric Hematology/Oncology, Weill Cornell Medicine, New York, United States

Background: ITP is an autoimmune disorder of reduced production and accelerated destruction of platelets. Eltrombopag (EPAG), an oral thrombopoietin receptor agonist metabolized and eliminated by the liver, is approved for previously treated chronic ITP patients (pts) aged ≥1 yr. In randomized trials of ≤6-months' duration, liver test abnormalities were recorded in 11% (EPAG) and 7% (placebo) of pts. EXTEND (Wong *et al. Blood* 2017;130:2527–2536) was an open-label, long-term extension study of ITP pts who participated in prior EPAG studies.
Aims: Describe hepatobiliary AE (HAE) occurrence and management during EXTEND.
Methods: In EXTEND, pts started EPAG at 50 mg/day, with dose titrated as appropriate to achieve platelet counts of ≥50–200x10^9/L. This analysis describes HAEs during EXTEND; HAEs with missing assessments were deemed drug-related.
Results: 302 pts received ≥1 EPAG dose: 67% female; 17% ≥65 yrs old (median, 50). Median exposure duration was 2.4 yrs (range, 2 days–8.8 yrs) and mean daily dose was 50.2 mg/day (SD, 21.6).
Forty-five pts (15%; Table 1 i) experienced 177 HAEs, and 34/45 had >1 HAE; 111/177 (63%) HAEs were considered drug-related. Most HAEs resolved (n=171, 97%) and required no change in dose (n=143, 81%); 17 (10%) led to treatment withdrawal in 7/302 pts (2%), 10 (6%) required dose interruption, and two (1%) dose reduction. Most HAEs were of mild severity (Grade 1, n=115 [65%]; Grade 2, n=44 [25%]; Grade 3, n=18 [10%]), with 17 (10%) considered serious AEs (none fatal). Most HAEs occurred within the first yr; median (range) time to first HAE in the 45 pts was 155 (7–1576) days. Increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were the most common HAEs: 25 pts had 41 AEs of increased ALT, and 23 pts had 41 AEs of increased AST (Table ii). Of 25 pts with AEs of increased ALT, 21 also had increased AST. Almost all AEs of increased ALT and AST resolved; most required no change in EPAG dose and few led to EPAG withdrawal (Table 1 ii). Seventeen pts had 22 AEs of increased blood bilirubin (Grade 1, n=15 [68%]; Grade 2, n=5 [23%]; Grade 3, n=2 [9%]), of which 95% (n=21) resolved. Most required no change in EPAG dose (n=14, 64%); four pts (18%) discontinued EPAG because of increased blood bilirubin, with increased ALT and AST also listed as concomitant causes for 3/4 pts. Twelve pts had 39 AEs of hyperbilirubinemia (Grade 1, n=28 [72%]; Grade 2, n=9 [23%]; Grade 3, n=2 [5%]); all resolved and only one (3%) led to EPAG withdrawal. Nine pts experienced ALT and/or AST >3x upper limit of normal (ULN) and total bilirubin >1.5xULN. HAEs of elevated alkaline phosphatase (n=18) were recorded in 10 pts; most resolved without a change in dose (n=16, 89%), and only one (6%) led to EPAG withdrawal (also attributed to increased ALT, AST, and bilirubin).
Summary/Conclusion: HAEs during long-term (up to 8.8 yrs) treatment of ITP pts with EPAG in EXTEND were observed in <1/6 pts, and were usually mild and transient, resolving without change in EPAG dose in ~4/5 cases. Only 2% (7/302) of pts discontinued EPAG because of HAEs. Assessment of drug-induced HAEs in this analysis is complicated by pts' various confounding factors (eg comorbidities). In line with the product label, our findings support measurement of serum ALT, AST, and bilirubin prior to initiating and during treatment with EPAG to effectively manage potential HAEs; if abnormalities arise, further liver tests should be performed and levels monitored weekly until resolved or stabilized, with dose modifications (if required) performed as appropriate.

Table 1.

Proportion of patients with a hepatobiliary adverse event during EXTEND (i) occurrence and outcomes from adverse events of increased ALT and AST (ii)

i

	Eltrombopag (N=302)	
Preferred term*	All	Drug related
Any hepatobiliary event, n (%)	45 (15)	33 (11)
ALT increased	25 (8)	16 (5)
AST increased	23 (8)	15 (5)
Blood bilirubin increased	17 (6)	12 (4)
Hyperbilirubinemia	12 (4)	9 (3)
Blood alkaline phosphatase increased	10 (3)	5 (2)
Transaminases increased	4 (1)	3 (<1)
Hepatic enzyme increased	3 (<1)	1 (<1)
Hepatic function abnormal	3 (<1)	3 (<1)
Bilirubin conjugated increased	2 (<1)	2 (<1)
Urine bilirubin increased	1 (<1)	1 (<1)
Gallbladder pain	1 (<1)	0
Abdominal discomfort	1 (<1)	1 (<1)
Abdominal pain	1 (<1)	0

*Hepatobiliary events were coded using MedDRA v18.0
Hepatobiliary events leading to withdrawal: increased ALT, n=5; increased AST, n=3; blood bilirubin increased, n=4; hyperbilirubinemia, alkaline phosphatase increased, transaminase increased, hepatic enzyme increased, bilirubin conjugated increased (all n=1)

ii

	Eltrombopag N=177 HAEs (45 patients)	
	Increased ALT N=41 HAEs (25 patients)	Increased AST N=41 HAEs (23 patients)
Median time to first HAE, days	221 (7–1814)	221 (7–1277)
Grade, n (%)		
1	24 (59)	25 (61)
2	12 (29)	11 (27)
3	5 (12)	5 (12)
Serious HAEs, n (%)	5 (12)	4 (10)
Outcome, n (%)		
Resolved	41 (100)	40 (98)
Treatment action, n (%)		
Dose unchanged	32 (78)	33 (80)
Dose interrupted	3 (7)	3 (7)
Dose withdrawn	5 (12)	3 (7)
Dose reduced	0	1 (2)
Not applicable	1 (2)	1 (2)
Related to treatment (includes records with relation to treatment 'missing'), n (%)		
Yes	22 (54)	19 (46)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HAE, hepatobiliary adverse event

Quality of life, palliative care, ethics and health economics

PS1427

BENEFIT OF EARLY TREATMENT WITH BLINATUMOMAB: LONG-TERM SURVIVAL OUTCOMES FOR ADULT PATIENTS WITH RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA RECEIVING FIRST VS SUBSEQUENT SALVAGE THERAPY

F. Severin[1,*], T. Delea[2], J. Amdahl[2], M. Hagiwara[2], D. Boyko[2], L. Sabatelli[1], S. Gonzalez-McQuire[1], Z. Cong[3]

[1]Amgen (Europe) GmbH, Zug, Switzerland, [2]Policy Analysis Inc. (PAI), Brookline, MA, [3]Amgen Inc, Thousand Oaks, CA, United States

Background: In the randomized, phase 3 TOWER trial, blinatumomab significantly improved overall survival (OS) and delayed time to clinically meaningful health-related quality of life (HRQoL) deterioration compared with standard of care (SOC) chemotherapy in adult patients with Philadelphia negative (Ph–) relapsed/refractory (R/R) B-precursor acute lymphoblastic leukemia (ALL).
Aims: To estimate long-term survival outcomes in adult patients with Ph– R/R B-precursor ALL receiving blinatumomab, and to quantify the value of treating patients with blinatumomab as first salvage (early treatment) versus subsequent salvage therapy (late treatment).
Methods: A partitioned survival model with a lifetime (50-year) time horizon was used to estimate expected life-years (LYs) and quality-adjusted LYs (QALYs) gained for blinatumomab versus SOC in subgroups of patients receiving early versus late treatment, a pre-specified 2:1 stratification factor in TOWER (blinatumomab: N = 107 early and N = 164 late; SOC: N = 54 early and N = 80 late). Model health states were defined based on response to treatment, relapse and survival. OS and event-free survival (EFS) among responders were estimated by fitting parametric survival distributions to failure-time data from TOWER (23-month maximum follow-up), with distributions selected based on visual and statistical fit (Bayesian information criterion), and plausibility of long-term projections against historical data. Based on these methods a restricted Gompertz model was selected to model OS and a restricted generalized gamma model was selected to model EFS among responders. Utility values for model health states were estimated by mapping from EORTC QLQ-C30 questionnaire data collected in TOWER to the EQ-5D, using a published response mapping algorithm and United Kingdom (UK) tariffs, then analyzed using generalized linear model regression. HRQoL for patients surviving for 4 years was assumed to be similar to that of the general population of the UK.
Results: Utility values for the same health states were higher for blinatumomab than for SOC, and for early versus late treatment. Projected OS probabilities at 5 years were 22.4% versus 10.3% for blinatumomab versus SOC in patients receiving early treatment, and 13.2% versus 7.5% in those receiving late treatment. In patients receiving early treatment, blinatumomab was projected to more than double expected LYs and QALYs over the model time horizon compared with SOC (4.21 LYs and 3.36 QALYs gained, Table 1); the projected gains in LYs and QALYs with blinatumomab in patients receiving late treatment were smaller (2.35 LYs and 1.88 QALYs gained, Table 1).

Table 1.

Expected LYs and QALYs blinatumomab vs SOC in subgroups of patients receiving early vs late treatment

	Early treatment	Late treatment
LYs		
Blinatumomab	8.41	5.79
SOC	4.20	3.44
Incremental benefit	*4.21*	*2.35*
(% compared with SOC)	*(100.2%)*	*(68.3%)*
QALYs		
Blinatumomab	6.48	4.43
SOC	3.12	2.55
Incremental benefit	*3.36*	*1.88*
(% compared with SOC)	*(107.7%)*	*(73.7%)*

LY, life-year; QALY, quality-adjusted life-year; SOC, standard of care.

Summary/Conclusion: Use of blinatumomab in Ph– R/R B-precursor ALL is projected to result in substantial gains in LYs and QALYs, with a greater projected benefit in patients treated at first salvage versus subsequent salvage therapy. These results suggest that blinatumomab should be used as early as possible in the treatment pathway for patients with Ph– R/R B-precursor ALL.

PS1428

HEALTH-RELATED QUALITY OF LIFE AND RATES OF EMERGENCY ROOM VISITS AND HOSPITALIZATION IN PATIENTS WITH AL AMYLOIDOSIS: A PROSPECTIVE ANALYSIS

K. L. McCausland[1], T. Quock[2,*], S. Guthrie[3], M. K. White[1], M. Bayliss[1]

[1]Optum, Johnston, [2]Prothena Biosciences Inc, South San Francisco, [3]Biopharma Strategic Consulting, LLC, San Francisco, United States

Background: Light-chain (AL) amyloidosis is a rare, progressive and typically fatal disease in which misfolded light chains are deposited in tissues and organs, which may lead to organ failure, disability, and death. Health-related quality of life (HRQoL) has been shown to be a significant prognostic factor associated with clinical outcomes such as survival and response to treatment. A better understanding of how HRQoL may be prospectively associated with costly healthcare resource utilization outcomes is warranted.
Aims: To examine whether health-related quality of life (HRQoL) is prospectively associated with rates of emergency room (ER) visits and hospital admissions, independent of other patient characteristics, during 12 months of observation.
Methods: A diverse sample of patients (n=224) with AL amyloidosis from the United States, Europe, and other countries participated in a non-interventional, longitudinal online study at three time-points (initial, 6-month, and 12-months). Negative binomial regression models, adjusted for potential demographic and disease characteristic confounders, were used to examine the association between initial HRQoL scores (the physical and mental component [PCS, MCS] summary scores of the SF-36v2® Health Survey) and the cumulative number of each HCRU outcome (ER visits and hospital admissions). Incident rate ratios (IRR) were interpreted in terms of 5 point decrements in HRQoL scores.
Results: A five-point lower PCS score, indicating decreased physical function, was associated with a 44% higher rate of ER visits ($p<0.001$) and a 36% higher rate of hospital admissions ($p<0.001$). A five-point lower MCS score was associated with a 27% greater rate of ER visits ($p<0.001$) and a 31% greater rate of hospitalizations ($p<0.001$).
Summary/Conclusion: Both physical and mental HRQoL impairment were significantly associated with higher rates of ER visits and hospital admissions, independent of other patient characteristics. Scores from patient-reported HRQoL surveys may serve as a proxy for disease severity and could be used to predict future ER visits and hospital admissions. Such information can provide stakeholders insight into the humanistic and societal cost associated with this condition.

PS1429

COST COMPARISON OF TREATMENT STRATEGIES FOLLOWING INITIATION WITH RD VERSUS VMP PLUS DARATUMUMAB IN NEWLY DIAGNOSED PATIENTS INELIGIBLE FOR ASCT

G. Jackson[1,*], R. Dutton[2], S. Dhanasiri[3]

[1]Clinical Hematology, NCCC Freeman Road Hospital, Newcastle Upon Tyne, [2]Dolon, London, United Kingdom, [3]Celgene International, Boudry, Switzerland

Background: For newly-diagnosed multiple myeloma patients (NDMM) ineligible to receive autologous stem cell transplant (ASCT) lenalidomide/low-dose dexamethasone (Rd) and bortezomib/melphalan/prednisone (VMP) are recommended and widely used as first-line treatments. Newer agents and combinations are in development and may become alternative options for first-line therapy. These include VMP + Daratumumab (Dara), which has demonstrated a statistically significant improvement in progression-free survival (PFS) over VMP. While clinical considerations are important in treatment decisions, combination regimens involving newer agents are costly and thus have a potential impact on health system budgets. Assessment of the economic impact of new treatments is also important in decision-making.
Aims: This analysis examines the cost impact from the perspective of the EU5 health systems (i.e. France, Germany, Italy, Spain, and the United Kingdom) of initiating therapy with VMP+Dara compared with Rd for patients ineligible to receive ASCT.
Methods: A cost pathway model was developed to follow an average patient through three lines of therapy. Possible therapies given in second- and third-

line following Rd or VMP+Dara were based on clinical guidelines or expert opinion. A pragmatic assumption around subsequent treatment distribution was made i.e. treatments given in earlier lines are not repeated. Duration of treatment (DoT) and duration of response (DoR) were taken from published sources where available. Since DoR for VMP+Dara is not reported yet, it was assumed to be the same as for Rd. Only drug acquisition costs (at list price or public prices) and administration costs were included. Two scenarios are considered: A) Rd followed by VD, DaraVd or carfilzomib/d; followed by Dara monotherapy or pomalidomide/d; or B) VMP+Dara followed by Rd or Rd combinations with elotuzumab, ixazomib, or carfilzomib; followed by carfilzomib/d or pomalidomide/d.

Results: In the Rd scenario, costs were €337,143 over an average patient pathway covering three lines of treatment, compared with €452,734 for the VMP+Dara scenario. An incremental increase of €59,145 (83%) was observed in year 1 for the VMP+Dara scenario, reflecting the higher monthly drug costs for the 4-agent regimen vs Rd (€9,830 vs €5,943), and the cost of administering Dara intravenously vs oral Rd. In years 2–4, treatment costs were similar in both scenarios, while in years 5+ costs were again considerably higher in the VMP+Dara scenario, reflecting the longer duration of treatment of 3L regimens used within the VMP+Dara scenario (Figure 1).

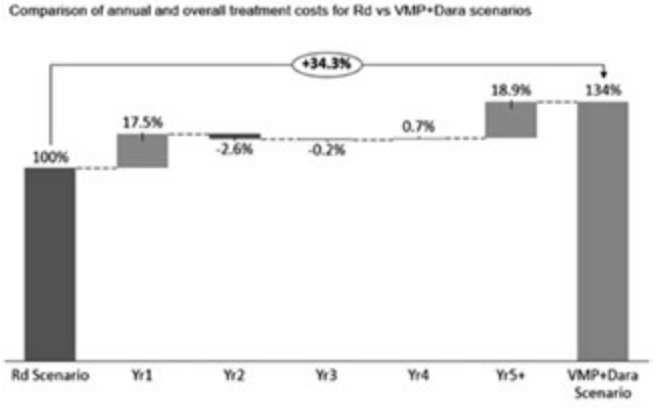


Figure 1.

Summary/Conclusion: The results of this analysis suggest that initiating therapy with VMP+Dara is likely to significantly increase treatment costs compared with Rd, both in the first year of therapy and across the total treatment pathway. Results are largely driven by the cost of VMP+Dara, administration costs of Dara and costs of combination treatments received post relapse. Costs for AE management such as infusion related or peripheral neuropathy have not been considered. Rd has a well-established acceptable safety profile, has demonstrated a 4-year OS of approximately 60%, and has been shown to delay progression to second-line therapy to a median of 69.5 months in patients achieving a ≥VGPR. Whilst VMP+Dara has demonstrated efficacy vs. VMP alone, more long-term safety and survival data are awaited. This analysis suggests that Rd is a cost-efficient use of NHS resources in EU5 for treating patients with NDMM ineligible for ASCT.

PS1430

IMPROVABLE LIFESTYLE FACTORS IN LYMPHOMA SURVIVORS

C. Minoia[1,*], S. Ciavarella[1], G. Lerario[1], A. Daniele[2], S. De Summa[3], M. Napolitano[4], A. Guarini[1]

[1]Haematology Unit, [2]Clinical Pathology Unit, [3]Molecular diagnostics and Pathogenetic Unit, National Cancer Research Centre "Giovanni Paolo II" Bari, Italy, Bari, [4]Research Coordination and Support Service, Istituto Superiore di Sanità, Rome, Italy

Background: Lifestyles (LS) represent the unique modifiable and improvable risk factors for secondary neoplasms and cardiovascular late events in cancer survivors. The impact of adherence to correct LS on quality of life (QoL) and late toxicities has been investigated mainly in breast, prostate and colorectal cancers, whereas only a minority of studies have dealt with lymphoma survivors.

Aims: As part of the "Center for Disease Control Program 2014 (CCM2014)" launched by the Italian Ministry of Health, from November 2016 to October 2017 our group evaluated LS risk factors in a population of lymphoma survivors and planned subsequent strategy for prevention.

Methods: We prospectively included in the study 70 consecutive patients, who were in continuous remission of lymphoma for at least 3 years. Patients took part in a survey including specific LS items in accordance with current international guidelines, such as smoking cessation, adherence to the Mediterranean diet (daily intake of more than 3 portions of fruit/vegetables, weekly intake of less than 2 portions of meat), physical activity (at least a moderate physical activity for 150 minutes a week) and body mass index (BMI) and to a nutritional evaluation assessing the metabolic status (anthropometry, plicometry according to Durnin-Womerslay, indirect calorimetry and plurimetabolic risk).

Results: We observed that 15.7% of subjects smoked, 89.7% did not follow the Mediterranean diet and 75.3% had insufficient physical activity. Almost two/thirds (64.7%) had at least 2 negative LS factors and 14.7% 3 negative factors. Moreover, 33% of patients presented a metabolic syndrome, 30.8% of patients were overweight (BMI 25–29.9) and 26.4% obese (BMI >30). Notably, 12 (17.1%) subjects had developed a cardiac toxicity as late event. Out of 11 subjects who had developed a therapy-related cardiomyopathy, 10 (90.9%) had at least 2 negative LS factors. Even with the limited number of subjects, some features related to the initial disease and to the administered anti-cancer treatment were found at risk for the development of metabolic syndrome, in particular a diagnosis of NHL (OR 3.9, 95%CI[1,15÷14,479], p=0.03), a anti-cancer therapy including high dose steroid (OR 3.5, 95%CI [1,06÷13,28], p=0.04) and an age at evaluation of 60 years or above (OR 8.2, 95%CI[1,69÷61,84], p=0.01), with the first two parameters directly related to an increased BMI (> 25): OR 5.7, 95%CI[1,48÷28,98] for NHL (p=0.01), and OR 3.4, 95%CI [0,98÷13,14] for high dose steroid (p=0.06).

Summary/Conclusion: The analysis provides a general evaluation of a subset of patients potentially cured from lymphoma, but who could develop late chronic morbidities which impact on quality of life and prognosis. The application of prevention strategies in this context may positively influence the clinical outcome of these patients, thus making the activation of dedicated educational programs on healthy LS desirable, with particular regard to smoking cessation, nutritional intervention and physical activity, all according to current guidelines.

PS1431

QUALITY OF LIFE IN ADVANCED-STAGE,ASYMPTOMATIC,NON-BULKY FOLLICULAR LYMPHOMA TREATED WITH RITUXIMAB SHOWS SIGNIFICANT IMPROVEMENT COMPARED WITH WATCHFUL-WAITING: PHASE 3 RANDOMISED INTERNATIONAL STUDY

M. A. V. Marzolini[1,*], W. Qian[2], L. Clifton-Hadley[3], P. Patrick[3], W. June[4], L. Stevens[3], C. Pocock[5], F. Miall[6], D. Cunningham[7], J. Davies[8], A. Jack[9], R. Stephens[10], J. Walewski[11], B. Ferhanoglu[12], K. Bradstock[13], D. Linch[14], K.M. Ardeshna[1]

[1]Haematology, University College London Hospital, London, [2]Cambridge Clinical Trials Unit - Cancer Theme, Cambridge, [3]Cancer Research UK and UCL Cancer Trials Centre, London, [4]University of Manchester, Manchester, [5]Haematology, East Kent Hospitals, Canterbury, [6]Haematology, University Hospitals of Leicester NHS Trust, Leicester, [7]Medicine, Royal Marsden Hospital, London, [8]Haematology, Western General Hospital, Edinburgh, [9]St James' Institute, Leeds, [10]MRC Clinical Trials Unit, London, United Kingdom, [11]Maria Sklodowska-Curie Memorial Institute and Cancer Centre, Warsaw, Poland, [12]Istanbul University, Istanbul, Turkey, [13]Westmead Hospital, University of Sydney, Sydney, Australia, [14]Haematology, UCL Cancer Institute, London, United Kingdom

Background: Traditionally, pts with asymptomatic, advanced stage, nonbulky follicular lymphoma (FL) have been managed with a watchful waiting approach until disease progression. The 'Watch and Wait' trial (NCT00112931) was an international Phase 3 randomised study examining whether rituximab could delay the need for chemotherapy or radiotherapy and the effect on quality of life (QoL) over time. The long term results of the QoL sub-study are presented here.

Aims: To evaluate QoL changes in pts with asymptomatic, advanced stage FL treated with rituximab monotherapy or managed using a watch-and-wait approach.

Methods: The pts were aged ≥18 years, Stage II – IV, asymptomatic FL (Grades 1, 2 & 3a) ECOG PS 0-1. Pts were randomised to either watchful waiting (**Arm A**; 187 patients) or to receive rituximab 375mg/m^2 weekly for 4 weeks followed by rituximab maintenance every 2 months for 2 years (**Arm C**; 192 patients). QoL questionnaires were completed at baseline (before & after randomisation), at Month (Mo) 7, Mo 13, Mo 25 and Mo 37. Questionnaires included the Mental Adjustment to Cancer (MAC), Illness Coping Style (ICS), the Hospital Anxiety and Depression Scales (HAD) scales and some additional concerns. All subscales (except HAD) were standardised on a 100-point score with a higher score indicating better QoL. A change of 5-10 points for a 100-point scale was regarded as a minimal clin-

ically important difference. We have previously reported QoL only at Month 7 (Ardeshna, Lancet Oncol, 2014; 15: 424-35).
Results: The pts in the maintenance rituximab arm were significantly more likely to feel in control of their situation, as demonstrated by the MAC scores, than those patients in the watchful waiting arm (Month 7: p=0.0035; Month 13, p= 0.0587; Month 25; p=0.0006; Month 37; p=0.0555).
The pts in the watchful waiting arm were significantly more likely to avoid learning or thinking about their illness and to have unpleasant connotations associated with their clinic visits, as shown by the ICS scores, when compared with those in the rituximab maintenance arm (Month 7, p= 0.0035; Month 13, p= 0.0020; Month 25, p=0.0106) though this effect was not seen at Month 37 (p= 0.2020). With respect to some specific concerns we had enquired about: the pts in the watchful waiting arm were significantly more likely to be more worried about their disease becoming more aggressive over time (Month 7, p=0.0333; Month 13, p=0.0001; Month 25, p=0.0002; Month 37, p=0.0010). They were also significantly more likely to be concerned regarding being able to support themselves or their family due to their illness over time, when compared to the patients in the maintenance rituximab arm (Month 7, p= 0.4289; Month 13, p=0.0052; Month 25, p=0.0132; Month 37, p=0.0015). The pts in the maintenance rituximab arm were less likely to worry about requiring or needing more treatment in the future when compared with the watchful waiting group (Month 7, p=0.0037; Month 13, p=0.0170; Month 25; p=0.0346; Month 37, p=0.0104).
There was no significant difference between the two study arms in the HAD-Anxiety and HAD-Depression sub-scores over time. We did not find any QoL score to be significantly worse in the rituximab maintenance group.
Summary/Conclusion: These results demonstrate improved QoL scores in the pts in the maintenance rituximab arm when compared with those in the watchful waiting arm, indicating that rituximab maintenance, and the potential side-effects associated with this, was not detrimental to the pts' quality of life and indeed resulted in an improved quality of life in some domains.

PS1432

HOW TO IDENTIFY PATIENTS WITH MALIGNANT HEMOPATHIES WHO DON'T BENEFIT FROM STANDARD DOSE CHEMOTHERAPY

S. Dubruille[1,*], C. Kenis[2], Y. Libert[3], M. Delforge[4], L. Dal Lago[5], M. Roos[5], C. Borghgraef [5], T. Pepersack[5], H. Wildiers[2], D. Bron[1]

[1]Department of Hematology, Institut Jules Bordet, Brussels, [2]Department of Oncology, University Hospitals Leuven, Leuven, [3]Clinic of Psycho-Oncology, Institut Jules Bordet, Brussels, [4]Department of Hematology, University Hospitals Leuven, Leuven, [5]Onco-geriatrics Unit, Institut Jules Bordet, Brussels, Belgium

Background: Major progresses have been achieved to identify older patients with malignant hemopathies who should be treated with standard doses of chemotherapy. However, a reliable frailty score remains urgently needed to better define the unsuspected vulnerable population that does not benefit from chemotherapy. In the literature, three clinical (functional decline, cognitive impairment (CI) and comorbidities) and two biological (low albumin level and IL-6 level) factors are frequently correlated with a poor overall survival (OS) and/or chemotherapy-related toxicities.
Aims: To investigate the reliability of a simple clinico-biological tool for the screening of frail patients with malignant hemopathies to predict unacceptable chemotherapy-related toxicity (toxic death) or poor survival (<6 months).
Methods: 270 consecutive patients (65-90yrs) with malignant hemopathies admitted to receive chemotherapy where included in a prospective multicentric study conducted in the Inst. J. Bordet (ULB, Brussels) and in the University Hospitals (KUL, Leuven). A Comprehensive Geriatric Assessment (CGA) was performed. Univariate and multivariate Cox proportional hazards model were used to evaluate the respective value of functional decline, abnormal cognitive function, comorbidities, low albumin and CRP level to predict 1-year survival.
Results: One hundred and seventy-nine patients were evaluable for the clinico-biological screening tool (NHL, n=107; CLL, n=19; MM, n=26; AML, n=14; ALL, n=3; LMMC, n=7, MDS, n=3). Eighty-five percent were considered to have a more favorable prognosis (NHL, CLL or MM). Functional decline was associated with abnormal cognitive function (p=0.026) and inflammation (p<.001). Based on our previous analyses in the Charlson Comorbidity Index we took the strongest prognostic factor: gastro-intestinal (GI) ulcer (p<.001). A "frailty" scoring system was thus developed, based on our 4 independent predictive factors for poor survival: CI (MMSE<27, n=56), presence of GI ulcer (n=23), low albumin level (alb<3.5 mmol, n=50) and surrogate of IL-6 level (CRP≥2mg/l, n=135). The population was stratified into 3 groups: "fit" (score=0-1, n=97), "vulnerable" (score= 2, n=53) and "frail" (score= 3-4, n=29). The one-year survival was 80% in "fit" and 57% in "vulnerable" patients (HR=2.59; 95% CI=1.41-4.75; p=.002). In "frail" patients 38% were alive at one-year (HR=4.83; 95% CI=2.53-9.23; p<.001) with a median survival of 5 months. Causes of death remain disease-related in a majority of the patients (73%). In our largest group of older NHL patients (n=107), the "frailty" scoring was also studied (MMSE<27, n=30; GI ulcer, n=12; alb<3.5 mmol, n=26, CRP≥2mg/l, n=80): the OS was 82% in fit (n=67) and 68% in vulnerable (n=25) (HR=2.00; 95% CI=0.82-4.90; p=.129) patients. In "frail" patients 40% were alive at one-year (n=15) (HR=5.46; 95% CI=2.29-13.03; p<.001) with a median survival of 5 months and thus, also reliable in this lymphoma population.
Summary/Conclusion: In our selected population of fit patients referred to receive chemotherapy for malignant hemopathies, our frailty score helps the clinician to predict a very poor outcome. This frailty score detects unsuspected frailty in patients who may benefit from palliative care. Further prospective analyses in a larger cohort of malignant hemopathies are ongoing to validate the reliability of this score.

PS1433

THE ROLE OF MENTAL AND BEHAVIORAL DISORDERS IN LEUKEMIA PREDISPOSITION: A NATIONWIDE CASE-CONTROL STUDY

H. Moisander-Joyce[1,*], A. Nikkilä[2], J. Raitanen[2,3], O. Lohi[4], A. Auvinen[1,2]

[1]Tampere Center for Child Health Research, Tampere University and University Hospital, [2]Tampere University, [3]UKK Institute for Health Promotion, [4]Tampere Center for Child Health Research, Tampere University Hospital and University of Tampere, Tampere, Finland

Background: Many studies have discussed the increased mental health and behavioral problems in pediatric cancer patients and survivors. Less is known, however, about the prevalence of congenital mental and behavioral disorders in the pediatric leukemia population. Their role in cancer predisposition is being actively studied: multiple cancer predisposing syndromes present with intellectual disability either as monogenic (e.g. Ataxia-telangiectasia) or chromosomal aberrations (e.g. Down syndrome).
Aims: We studied the prevalence of mental and behavioral disorders the Finnish pediatric leukemia population with the aim to assess the role of congenital mental and behavioral disorders as risk factors for leukemia.
Methods: We used a nationwide, register-based case-control study to investigate the prevalence of mental and behavioral disorders in pediatric leukemia patients. We identified all pediatric leukemia cases (0-15 year old) from 1990 to 2011 (N=1100) in Finland and randomly selected three times as many controls (N=3272) from the Population Registry, individually matched by gender and year of birth. 81% (N=885) of the cases had acute lymphoblastic leukemia and 13% (N=142) acute myeloid leukemia. We used the Care Register for Health Care by the Finnish National Institute for Health and Welfare to obtain information about ICD-10 Chapter V diagnoses (Mental and Behavioral Disorders) based on inpatient care and specialist outpatient visits for the above population. We used the Register for Congenital Malformations to exclude the patients with Down syndrome. There were no patients with other known leukemia predisposition syndromes which present with mental or behavioral symptoms. This left us with 1058 cases and 3270 controls for analysis.

Table 1. Odds ratios of mental and behavioral disorders for pediatric leukemia patients.*

	Cases	Controls	OR (95% CI)	p value
All diagnoses (0-15 year old)				
Neurotic, stress-related and somatoform disorders (F40-48)	73 (6.90 %)	42 (1.28%)	5.77 (3.87, 8.70)	<0.01
Behavioral syndromes (F50-59)	62 (5.86%)	26 (0,80%)	7.76 (4.80, 12.85)	<0.01
Mental retardation (F70-79)	16 (1.51%)	24 (0.73%)	2.07 (1.03, 4.09)	0.02
Disorders of psychological development (F80-89)	73 (6.90%)	157 (4.80%)	1.47 (1.09, 1.97)	0.01
Diagnoses > 2 years prior to leukemia diagnosis				
Neurotic, stress-related and somatoform disorders (F40-48)	1 (0.09%)	4 (0.12%)	0.77 (0.12, 7.82)	0.82
Behavioral syndromes (F50-59)	1 (0.09%)	4 (0.12%)	0.77 (0.02, 7.82)	0.82
Mental retardation (F70-79)	4 (0.38%)	3 (0.09%)	4.13 (0.70, 28.27)	0.04
Disorders of psychological development (F80-89)	10 (0.95%)	29 (0.89%)	1.07 (0.46, 2.27)	0.86

*Patients with Down Syndrome are excluded from this analysis.

Results: Overall, 708 patients (442 controls, 266 cases) had at least one ICD-10 Chapter V diagnosis. This equals 14% and 25% of the controls and cases, respectively. We focused on our study on the four areas most relevant to pediatric leukemia patients: 1) neurotic, stress-related and somatoform disorders (F40-48), 2) behavioral syndromes associated with physiological disturbances and physical factors (F50-59), 3) mental retardation

(F70-79) and 4) disorders of psychological development (F80-89). In conditional logistic regression analysis pediatric leukemia patients had significantly more mental and behavioral disorders in all the above areas (see Table 1). However, when the analysis was restricted to the period prior to the date of leukemia diagnosis (and the corresponding date for matched controls) with a two-year latency period, only mental retardation was a statistically significant risk factor for leukemia (p=0.04).
Summary/Conclusion: Our preliminary analysis suggests that mental retardation is a statistically significant risk factor for leukemia. The majority of pediatric leukemia cases were diagnosed prior to school age (mean 5.7 years, median 4.7 years) and thus, mental retardation diagnosed prior to leukemia is often of congenital origin, possibly due to yet-to-be-discovered genetic syndromes which affect both brain development and cancer predisposition.

PS1434

IMPACT OF CMV PROPHYLAXIS ON RATES OF REHOSPITALIZATION IN ADULT CMV SEROPOSITIVE ALLOGENEIC HSCT RECIPIENT: EXPERIENCE FROM THE LETERMOVIR PHASE 3 CLINICAL TRIAL

S. Mt-Isa[1], H. Wan[1], C. Badshah[1], V. Teal[1], R. Leavitt[1], J. Schelfhout[1,*]

[1]MSD, Whitehouse Station, NJ, United States

Background: In a Phase III randomized, double-blind, placebo-controlled study of cytomegalovirus (CMV)-seropositive Hematopoietic Cell Transplant (HCT) recipients, letermovir prophylaxis significantly reduced the incidence of clinically significant CMV infection through 24 weeks post-HCT. This research describes the impact of letermovir prophylaxis on the rate of rehospitalization in the clinical trial.
Aims: The aim of this research is to describe the impact of letermovir prophylaxis on the rate of rehospitalization in the clinical trial.
Methods: Rehospitalization was recorded as an exploratory endpoint in the clinical trial at end of treatment (Week 14), time of primary endpoint (Week 24) and through an extended follow-up period (Week 48). The trial further recorded whether the rehospitalization was related to CMV (referred to as CMV-related rehospitalization). Prespecified analyses describe the observed rates of rehospitalization for the letermovir and placebo groups at the specified times. Fine-Gray cumulative incidence function (CIF) regression models were used to explore the rate of all-cause, and CMV-related rehospitalization accounting for the competing risk of mortality. A multiple linear regression model was used to describe the cumulative length of stay (LOS) for all-cause rehospitalizations that occurred through Week 48 (excluding time of initial transplant stay).
Results: Observed rates of all-cause rehospitalization were lower for the letermovir group compared to placebo at end of treatment (36.6% vs. 47.6%), time of primary endpoint (48.6% vs. 55.3%), and through extended follow-up (55.7% vs. 60.6%). The CIF regression model demonstrated rates of all-cause rehospitalization were significantly lower through Week 14 (HR=0.72; p=0.021) but did not reach significance at Week 24 (HR=0.81; p=0.109) or Week 48 (HR=0.84; p=0.173); and CMV-related rehospitalizations were significantly reduced at Week 14 (0.6% vs. 7.1%; HR=0.09; p=0.001), Week 24 (2.8% vs. 7.6%; HR= 0.36; p=0.015), and Week 48 (3.1% vs. 8.8%; HR=0.34; p=0.007). The adjusted mean cumulative LOS was shorter for letermovir subjects compared to placebo subjects but did not reach statistical significance (3.1 fewer days p= 0.333).
Summary/Conclusion: Letermovir was shown to significantly reduce the rate of clinically significant CMV infection in a placebo-controlled randomized clinical trial. These analyses suggest that there is also a reduction in the rate and cumulative days of rehospitalization. Caution is warranted as the trial was not sufficiently powered to detect differences in this exploratory endpoint. Nonetheless, these data provide valuable insights into the economic burden of CMV. Real world data and findings from future clinical trials are needed to better understand the nature of the association between CMV and rehospitalizations.

PS1435

QUALITY OF LIFE IN PATIENTS WITH RARE HEREDITARY HEMOLYTIC ANEMIA, THE IMPORTANCE OF SOCIAL SUPPORT

S. Van Straaten[1,2,*], S. Hagens[1,2], J. Verhoeven[1,2], W. van Solinge[1], R. Schutgens[2], R. van Wijk[1], E. J. van Beers[2]

[1]Laboratory of Clinical Chemistry & Haematology, [2]Van Creveldkliniek, UMC Utrecht, Utrecht, Netherlands

Background: Over the past few decades, life expectancy of patients with rare hereditary hemolytic anemia has shown remarkable improvement. The availability of blood transfusions, chelation therapy and other supportive treatment options have improved life expectancy to well into adulthood. However, whether this improved life expectancy is accompanied by improved quality of life (QoL) is not well known.
Aims: The aim was to analyze QoL in patients with hereditary hemolytic anemia as compared to normative data and to define specific subgroups of patients with impaired QoL.
Methods: This is a cross sectional, observational study of 83 patients with rare hereditary hemolytic anemia. Patients were divided into 3 main categories: hemoglobin disorders (e.g. sickle cell disease(SCD), β-thalassemia), red cell enzyme disorders (e.g. pyruvate kinase deficiency) and red cell membrane disorders (e.g. hereditary spherocytosis). All patients completed FACT-An, a validated questionnaire developed within the Functional Assessment of Chronic Illness Therapy (FACIT) system. The questionnaire consists of 5 subdomains. The first 4 domains: physical, social/family, emotional and functional well-being (PWB, SWB, EWB, FWB) are combined to create a general QoL-scale (FACT-G) that can be compared to available normative data provided by FACIT, based on a sample of the general US adult population. The last domain (Fact-AnS) measures additional symptoms of anemia. To analyze clinically meaningful differences between patients and normative data we used established "Minimally Important Difference" (MID) based on anchor- and distribution-based methods. For Fact-G total score the difference was 5 points, for individual subdomains 2 points. We performed non-parametric correlation tests between QoL and documented clinical and laboratory parameters and results of the 6-minute walk test (6MWT, expressed as percentage of predicted distance). To minimize risk of confounders, patients with transfusion dependency or SCD were excluded from correlation analyses with hematological parameters.
Results: Patients had a median age of 43 years (range 18-84) and a median Hb of 11,4g/dL(range 6,4-16,4). The total patient population did not score a clinically meaningful difference on general QoL (Fact-G) compared to the normal population. However, patients with enzyme or membrane disorders and patients who had never received a transfusion had a clinically meaningful higher Fact-G score compared to normative data. Patients who were transfusion dependent had a lower Fact-G score. The subdomain SWB was the most important influencer of differences in Fact-G. There was a significant negative correlation between SWB and age(ρ=-0,428, p=0,002). There was no correlation between QoL and Hb, reticulocyte count or 6MWT. (Table 1).

Table 1.

	Haemoglobin disorders	Red cell enzyme disorders	Red cell membrane disorders	Transfusion dependent patients	Transfusion independent patients	Never transfused	All patients
PWB	**-3,5000**	0,5000	**2,0000**	-1,5000	0,5000	0,5000	0,5000
SWB	-0,6000	**3,5667**	**2,5667**	-0,6000	**2,5667**	**2,5667**	**2,4000**
EWB	**-2,0000**	0,5000	0,0000	**-2,0000**	0,0000	0,0000	-1,0000
FWB	-1,1000	**2,4000**	1,9000	0,4000	1,4000	1,4000	1,4000
Fact-G	-3,2000	**5,6000**	**6,1000**	**-5,9000**	2,1000	**5,1000**	2,1000
Fact-AnS	*52*	*58,5*	*63,5*	*51*	*60*	*60*	*59*

Differences in median scores compared to normative data of normal population. Clinically meaningful differences are marked bold.

Summary/Conclusion: Patients with enzyme and membrane disorders report higher QoL than the general population. This effect is mostly based on high scores in the social/family well-being subdomain. The same pattern is reported in patients with cancer and it might reflect a higher social support need caused by serious illness. Our study shows a decreased QoL in transfusion dependent patients, and increased QoL in patients who were never transfused. The difference is also mostly based on differences in social/family well-being. Our study clearly shows the importance of social/family well-being for QoL of patients with hereditary hemolytic anemia. Improving social support could be an interesting future focus to increase QoL, especially in older, transfusion dependent patients, or patients with hemoglobin disorders.

PS1436

HOW PHYSICIANS' AND PATIENTS' CHARACTERISTICS AFFECT THE LENGTH OF PATIENTS' VISIT IN THE HEMATO-ONCOLOGICAL CLINIC

A. Shacham-Abulafia[1,2], I. Amitai[1,*], O. Uziel[3,4], O. Pasvolsky[1,2], A. Yosefi[1], P. Raanani[1,2], U. Rozovski[1,2]

[1]Institute of Hematology, Davidoff Cancer Center, Rabin Medical Center, Petah-Tikva, [2]Sackler Faculty of Medicine, Tel Aviv University, Tel-Aviv, [3]Felsenstein Medical Research Center, Rabin Medical Center, [4]Sackler Faculty of Medicine, Tel Aviv Uni-

versity, Petah-Tikva, Israel

Background: The relationship between doctors and their patients has received philosophical, sociological and literary attention throughout history. In this study we narrowed our perspective on patient-doctor bonding to a single dimension, the length of time doctors spend with their patients. In a recently published study, doctors estimated spending on average 13 to 16 minutes on each patient's visit. However, to our knowledge, no published studies quantified the duration of patients' visit time. Numerous studies in the field of social psychology confirmed that as most of us implicitly assume, people tend to spend more time with people that resemble them.
Aims: 1. quantify length of patients' visits; 2. determine whether patients' and doctors' characteristics affect the visit time.
Methods: This study was conducted at the adult hemato-oncology department at Rabin Medical Center, an Israeli 1300 bed tertiary medical center. Since 2005 a customer-time management software (Q-Flow®) entered routine use at our institute. This software records the length of patients-doctors' meetings and provides patients' demographic data. With this software we collected data on all consecutive visits in the hemato-oncology clinics during 2017. Additional data was collected from electronic medical records.
Results: In 2017, 22,516 visits of 3314 patients that were seen by 22 doctors were recorded. The median age of the patients was 68 years (range: 17 to 99), male to female ratio was 1/1.07 and 83% (n=1722) were married. Patients were classified according to their country of origin: Israel (52%, 1734), Western countries (6%, 187), Eastern European countries (19%, 615), Arab countries (22%, 738) or others (1%, 40). The most common hematological diagnosis was lymphoma (38%, 1203) followed by plasma cell dyscrasia (16%, 498), myeloproliferative neoplasms (12%, 394) and chronic lymphocytic leukemia (10%, 316). The median length of patients'-doctors' visit was 12.3 minutes (range 3.7 to 36), and although the variance in length of visits was relatively narrow, it differed significantly according to patients' and doctors' characteristics. Fellows had the longest visits (median visit length 14.9 minutes compared to 13.2 minutes for young seniors and 10.9 minutes for older seniors, $p<0.0001$). Compared to male doctors, female doctors had longer visits ($p=0.029$). Yet, both patients' and doctors' gender predicted visits' length. The shortest being when both the doctor and the patient were males ($p<0.004$ compared to *female-patient*, *female-doctor* pair and $p<0.047$ compared to *female-patient*, *male-doctor* pair). Likewise, Country of origin also had minor, yet significant impact on length of visits, being longer for patients born in Israel of Jewish descendent compared to patients born in Israel of Arab descendent ($p= 0.002$) and shorter compared to patients of Eastern European descendent ($p=0.019$). Conversely, patients' age and family status had no impact on visits' length.
Summary/Conclusion: There is a relative narrow variance in doctors'-patients' visit time with shorter professional experience being the strongest predictor of longer visit time. Yet, doctors' and patients' characteristics impact this time significantly. As expected by the similarity-attraction theory and since most of our doctors are of Jewish-descendent and were born in Israel or in Western European countries, the length of patients' visit of similar country of origin was longest. Additionally, as expected by cultural stereotypes, when a male doctor meets a male patient the interaction time was the shortest.

PS1437

SYSTEMATIC REVIEWS OF ECONOMIC BURDEN AND HEALTH-RELATED QUALITY OF LIFE (HRQOL) IN PATIENTS WITH ACUTE MYELOID LEUKAEMIA (AML)

R. Bosshard[1,*], S. Ralston[2], K. O'Reilly[1], S. Chadda[2], D. Cork[2]

[1]Otsuka Pharmaceutical Europe Ltd, Wexford, [2]SIRIUS Market Access, London, United Kingdom

Background: AML is the most common acute leukaemia in adults, with high mortality rates, especially among elderly individuals who account for a large and increasing percentage of the AML population. Treatment of AML is ideally intensive chemotherapy followed by potentially curative haematopoietic stem cell transplant. However, elderly patients and those with significant comorbidities may be considered unfit for intensive chemotherapy (IC) because of an inability to tolerate the therapy. These patients are often treated with lower-intensity therapies such as azacitidine, decitabine, or low dose cytarabine. Due to the relapsing course of AML these patients are likely to run out of treatment options leading to death.
Aims: To conduct systematic reviews to assess firstly the impact of AML on HRQoL and secondly the economic implications at patient, carer, and health service levels for treatment of AML in elderly patients and other patients considered unfit for IC and in patients with relapsed/refractory AML (rr-AML).
Methods: Two independent systematic reviews were conducted to assess HRQoL and economic burden. Electronic databases were searched using specific search strings to identify studies between 2001-2016 that met predefined patient/intervention/comparison/outcome (PICO) criteria, including PubMed, EMBASE, and EconLit. Electronic searches were supplemented with hand searching of conference abstract databases, registries, and HTA agency websites. Conference abstracts from 2015-2016 were included.
Results: Ten studies meeting the specific criteria were identified for each systematic review. EORTC QLQ-C30 was the instrument most commonly used to measure HRQoL. FACT-Leu, FACT-An, SF-12, EQ-5D, and QOL-E were also used. No validated AML specific instruments have been identified. HRQoL was worse in patients who were considered unfit for intensive chemotherapy than in patients assigned to intensive treatments. Poor HRQoL at diagnosis was associated with shorter overall survival, especially in older patients receiving non-intensive treatments. High levels of fatigue were associated with poor HRQoL at diagnosis. Non-intensive treatments did not significantly improve HRQoL in any study, whereas HRQoL did improve in patients receiving IC. Hospitalisation was identified as having a detrimental effect on HRQoL in one study of patients receiving IC. The cost studies identified in the second systematic review reported transfusion costs as the most significant contributor for patients receiving supportive care, and drug costs as the most significant for patients receiving low intensity treatments. However, hospitalisation costs were not included in this model, and were shown to be the most significant contributor for patients receiving IC. Another study identified that hospitalisation costs were not significantly lower for older patients receiving low intensity treatment. No studies specifically assessed burden of relapsed/refractory AML, caregiver burden, or indirect costs.
Summary/Conclusion: Patients considered unfit to receive IC have poor survival and poor HRQoL, and current low intensity treatments do not appear to significantly improve HRQoL. The main cost drivers were dependent on treatment regimen, but included drugs, transfusions, and hospitalisation. Novel treatments which improve survival and HRQoL are needed in this difficult, treatment naïve population. No studies have been identified which specifically describe HRQoL or economic burden associated with rr-AML, further research to understand the burden in this population is required.

PS1438

VENETOCLAX IMPROVES QUALITY OF LIFE FOR PATIENTS WITH RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA

T. Cochrane[1,*], T. Chagorova[2], T. Robak[3], S.-P. Yeh[4], A. Masud[5], K. Sail[5], V. Komlosi[5], M. A. Anderson[6]

[1]Gold Coast Hospital, Southport, Australia, [2]Penza Regional Cancer Center, Penza, Russian Federation, [3]Medical University of Lodz and Copernicus Memorial Hospital, Lodz, Poland, [4]China Medical University, Taichung, Taiwan, Republic of China, [5]AbbVie, Inc, North Chicago, IL, United States, [6]Integrated Department of Hematology, Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Melbourne, Australia

Background: Patients with chronic lymphocytic leukemia (CLL) have significantly decreased health related quality of life (HRQoL), particularly related to severe and progressive fatigue. Side effects of chemotherapies and the emotional burden of living with an often poor prognosis disease also negatively impact patient HRQoL. Venetoclax (Ven), an oral BCL-2 inhibitor, has demonstrated high rates of deep and durable response in CLL patients, including those with 17p deletions or relapsed/refractory (R/R) disease, and has been shown to facilitate clinically relevant improvement in several key aspects of functioning and HRQoL.
Aims: To evaluate the impact of Ven monotherapy on the quality of life of patients with R/R CLL.
Methods: This is an open-label, phase 3b, multicenter study (NCT02980731) that assessed patient-reported HRQoL at baseline and every 4 weeks, using the European Organization for Research and Treatment of Cancer Quality of Life Core Questionnaire (EORTC QLQ-C30). Patients were ≥18 years old with R/R CLL, including those with 17p deletion, *TP53* mutations, and/or prior experience with B-cell receptor inhibitor-containing (BCRi) therapy, treated with Ven monotherapy. HRQoL subscales analyzed included: Global Health Status, Role Functioning, Emotional Functioning, Cognitive Functioning, Social Functioning, and Fatigue. Relevance of mean changes in HRQoL measures from baseline were analyzed based on minimum important difference (MID); a 5-10 point change was defined as MID, and >10 points was clinically meaningful. Safety and adverse events (AEs) were also monitored.
Results: Data cutoff was November 3, 2017; the current median time on study is 17 weeks. Of 92 patients, 66% were male; the median age was 64

years (range: 37–86). Among those with available data, 17p deletions and *TP53* mutations were confirmed in 35% (25/72) and 27% (8/30) of patients, respectively. Overall, 36%, 20%, and 45% of patients had one, two, and three (or more) prior lines of therapy respectively; 23% of patients had prior BCRi therapy. Clinically meaningful improvements in global health status, role functioning and fatigue were observed as early as week 12 post-treatment. Mean change in these parameters from baseline is shown in the Table 1, and mean change over time (compared to baseline) is shown in the graph. On average, patients' fatigue dropped by 37% by treatment week 12, and maintained improvement (23%) at 24 weeks. AEs occurring in ≥10% of patients were neutropenia (25%), thrombocytopenia (15%), anemia (14%), and diarrhea (14%). Serious AEs occurred in 25% of patients, of which the most common were febrile neutropenia (4%) and pyrexia (4%).

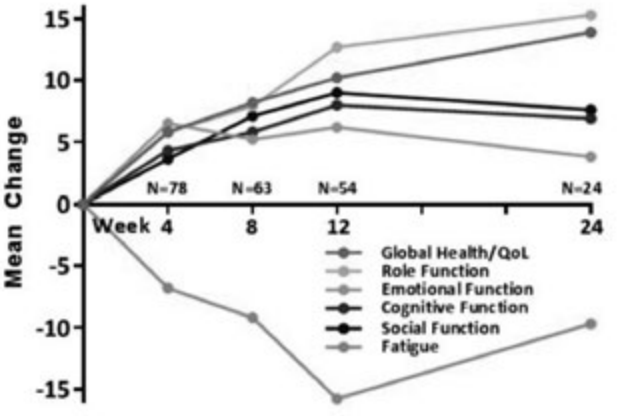


Figure 1.

Table 1.

EORTC QLQ-C30 Parameter	Baseline Mean N=91	Mean (95% CI) change, week 24 N=24
Global Health Status/QoL	57.1	+13.9 (6.1 – 21.7)
Role Functioning	65.8	+15.3 (6.7 – 23.8)
Emotional Functioning	76.6	+3.8 (-2.0 – 9.7)
Cognitive Functioning	80.8	+6.9 (0.1 – 13.8)
Social Functioning	71.1	+7.6 (1.4 – 13.9)
Fatigue*	42.7	-9.7 (-18.1 – -1.4)
* Negative mean change denotes improvement		

Summary/Conclusion: Preliminary data suggest that patients with R/R CLL, receiving venetoclax monotherapy, experience improvement in several key aspects of functioning and quality of life. These findings are consistent with previous studies of R/R CLL patients that received venetoclax monotherapy.

PS1439

NO ASSOCIATION BETWEEN INDOOR RADON AND CHILDHOOD LEUKEMIA IN FRECCLE (FINNISH REGISTER-BASED CASE-CONTROL STUDY OF CHILDHOOD LEUKEMIA)

A. Nikkilä[1,*], J. Raitanen[1], O. Lohi[2], A. Auvinen[1]

[1]University of Tamepre, [2]Tampere Center for Child Health Research, Tampere University and University Hospital, Tampere, Finland

Background: Leukemia is the most common malignancy of childhood. Its etiology remains unknown apart from few established risk factors (Down syndrome and high-dose ionizing radiation). The association between indoor radon and childhood leukemia has been studied before but the results have been conflicting[1-6].
Aims: We aim to characterize the association between indoor radon and childhood leukemia more accurately with high-quality data.
Methods: We created a log-linear model for prediction of residential radon levels based on 6008 dwellings with measured indoor radon concentrations. The predictors in the model included building type, building material, basement, radon protection, floor area, floor number, soil uranium concentration, average indoor radon by postal code area, terrain elevation and location on any landform formed by the latest ice age. Missing data of predictive variables (0 – 11%) was replaced with multiple imputation. In addition, a categorical model was created by dividing indoor radon levels into three groups: <50% (<36Bq/m^3), 50–90% (36 - 200Bq/m^3), >90% (>200Bq/m^3). The robustness of the models was evaluated with multiple sensitivity analyses. Our childhood leukemia dataset comprises of all childhood leukemia cases diagnosed in Finland between 1990 and 2011 (N=1093) and thrice as many controls matched by gender and year of birth[7]. We applied the log-linear radon prediction model to the nationwide register-based case-control dataset and estimated the cumulative indoor radon exposure throughout the study subjects' complete residential histories.
Results: The log-linear model predicted observed radon levels adequately (r^2 = 0.23). It showed robust behavior with no signs of overfitting in sensitivity analyses and k-fold cross-validation. Spearman correlation between measured and predicted values was 0.48 and Cohen's kappa for the model with categorical radon levels was 0.34. When the predicted average indoor radon concentration was divided into quartiles and the group with lowest exposure (9.0 Bq/m^3) was used as the reference group, the OR for the 2nd quartile (29 Bq/m^3) was 0.89 (95% CI 0.72, 1.10); third (45 Bq/m^3) OR=0.82 (95% CI 0.66, 1.01); and highest (68 Bq/m^3) OR=0.95 (95% CI 0.77, 1.17) relative to the lowest quartile. (Figure 1).

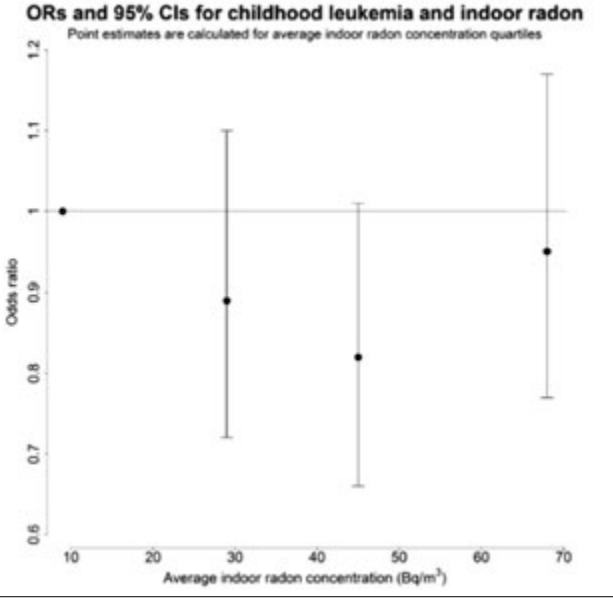


Figure 1.

Summary/Conclusion: The prediction model developed for residential radon performed reasonably well. However, it was unable to robustly identify the dwellings with the highest radon concentrations. No increase in leukemia risk with predicted indoor radon was found. The results should be interpreted cautiously as the exposure modelling has significant uncertainties.

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PS1440

CLARITHROMYCIN ADDED TO THE VCD REGIMEN CAUSES REDUCED HEALTH-RELATED QUALITY OF LIFE IN MULTIPLE MYELOMA PATIENTS

L. K. Nielsen[1,*], T.W. Klausen[2], T. Do[2], I.B. Kristensen[3], U.C. Frølund[4], N.F. Andersen[5], C.L. Andersen[4], A.J. Vangsted[6], N. Abildgaard[7], H. Gregersen[8]

[1]Quality of Life Research Center, Department of Haematology, Odense University Hospital, Odense, [2]Department of Haematology, Herlev Hospital, Copenhagen, [3]Department of Haematology, Vejle Hospital, Vejle, [4]Department of Haematology, Zealand University Hospital, Roskilde, [5]Department of Haematology, Aarhus University Hospital, [6]Department of Haematology, Copenhagen University Hospital, Copenhagen, [7]Department of Haematology, Odense University Hospital, Odense, [8]Department of Haematology, Aalborg University Hospital, Aalborg, Denmark

Background: Newly diagnosed multiple myeloma (MM) patients report improved health-related quality of life (HRQoL) during induction treatment and autologous stem cell transplantation (ASCT). The Danish Myeloma Study Group initiated the CLAIM study; a randomized, placebo-controlled, double-blinded phase II study with the aim of investigating the efficacy and safety of adding clarithromycin (CLA) or placebo (PLA) to cyclophosphamide-bortezomib-dexamethasone (VCD) induction therapy in transplant eligible, newly diagnosed MM patients. The study was prematurely stopped due to severe gastrointestinal complications in the CLA-group. The response data suggested no effect of the addition of CLA to the VCD regimen *(Gregersen et al., ASH 2017, abstract 3129).*
Aims: The objective was to compare patient-reported HRQoL between the two treatment groups.
Methods: The patients answered three validated HRQoL questionnaires at inclusion and after two and six months. The European Organisation For Research And Treatment Of Cancer Quality Of Life Questionnaire (QLQ-C30), the Multiple Myeloma module (MY20) and the Functional Assessment of Cancer Therapy/Gynecologic Oncology Group Neurotoxicity (FACT/GOG-ntx) subscale were used. Mean score for each domain in each group were calculated by mixed model repeated measures. The mean score difference between the two groups were interpreted by clinically relevant difference between groups. For the QLQ-C30 and MY20 domains the threshold of ≥ 5 points was used (*Cocks et al.*, J Clin Oncol. 2011;29:88-96) and for the FACT/GOG-ntx subscale the threshold of ≥ 11.8 was used (*Calhoun et al.*, Int J Gynecol Cancer 2003, 13,1-8). Sensitivity analyses were carried out to explore the impact of unanswered questionnaires by identifying auxiliary variables for missing response and adding this information to the analysis.
Results: Of the 58 included patients, 55 patients participated in the HRQoL reporting; 25 in the CLA-group and 30 in the PLA-group with compliance of 84% and 89% in the CLA- and PLA- group, respectively. At two months follow-up the patients in the CLA-group reported clinically relevant reduced global quality of life (QoL), physical, role, emotional and social functioning, body image and more fatigue, insomnia, disease symptoms, side effect of treatment, and peripheral neuropathy compared to the patients in the PLA-group. For physical, role and social functioning, and insomnia the reduced HRQoL were persistent at six months follow-up. In the investigation of the pattern of missing answers to questionnaires, grade 3 and 4 adverse events were identified as auxiliary variables. When these were incorporating into the analysis of the global QoL domain, the mean score difference became larger.
Summary/Conclusion: The results are underpowered because of lower sample size than planned. Still, the results demonstrate that MM patients report a clinically relevant reduced HRQoL, when clarithromycin is added to the VCD regimen. A possible explanation to these findings could be an interaction between bortezomib and clarithromycin, and the reduced HRQoL could be a result of increased exposure to bortezomib in the CLA-group. The study emphasizes the importance of including HRQoL as an endpoint in clinical trials and has a potential role as part of real-time safety monitoring of symptomatic toxicities in clinical trials. Even though the compliance rate was high, we found that unanswered questionnaires should be incorporated in the interpretation of HRQoL results.

PS1441

RELINF PROJECT: PROSPECTIVE EPIDEMIOLOGICAL REGISTRY OF LYMPHOID NEOPLASMS IN SPAIN, ON BEHALF OF GELTAMO GROUP

M. Bastos Oreiro[1,*], A. Muntañola Prat[2], C. Panizo[3], P. Balsalobre[1], M. Sanchez[4], E. Gonzalez[5], S. Gonzales de Villambrosia Pellon[6], J.L. Bello Lopez[7], R. Córdoba [8], A. Gutierrez[9], M.J. Terol[10], P. Gomez Prieto[11], E. Perez Ceballos[12], A. Salar[13], H. Luzardo Enriquez[14], R. Andreu[15], T. García Carecedo[16], J.A. Hernandez Rivas[17], R. de la Cámara[18], M.J. Ramirez[19], F. Iniesta Martínez[20], J.A. Queizán Hernandez[21], C. Grande[22], S. Novelli Canales[23], A. López[24], M. Moreno Velazquez[25], A. Domingo García[26], V. Noriega[27], E. Pardal[28], E. Donato[29], M. Polo[30], E. Del Cabo López[31], D. Caballero*[32], A. Lopez Guilllermo[33]

[1]Hematology, Hosp. Gral Gregorio Marañon Univ, Madrid, [2]Hematology, Hospital Mutua Terrasa, Terrasa, [3]Hematology, Clínica Universitaria de Navarra, Pamplona, [4]Data manager, Geltamo, Madrid, [5]Hematology, ICO Duran y Reinalds, Barcelona, [6]Hospital Marqués de Valdecillas, Santander, [7]Hematology, Hospital Clínico Universitario de Santiago, Santiago de Composterla, [8]Hematology, Fundación Jimenez Díaz, [9]Hematology, Hospital Son Espases, Madrid, [10]Hematology, Hospital Clínico de Valencia, Valencia, [11]Hematology, Hospital La Paz, Madrid, [12]Hematology, Hospital Morales Meseguer, Murcia, [13]Hematology, Hospital del Mar, Barcelona, [14]Hematology, Hospital Universitario de Gran Canaria Dr Negrin, Las Palmas de Gran Canaria, [15]Hematology, Hospital Politécnico La Fe de Valencia, Valencia, [16]Hematology, Hospital Arnau de Vilanova, Lleida, [17]Hematology, Hospital Infanta Leonor, [18]Hematology, Hospital de la Zarzuela, Madrid, [19]Hematology, Hospital Jerez de la Frontera, Jerez de la Frontera, [20]Hematology, Hospital VIrgen de la Arrixaca, Murcia, [21]Hematology, Hospital General de Segovia, Segovia, [22]Hematology, Hospital [12] de Octubre, Madrid, [23]Hematology, Hospital Santa Creu y Sant Pau, [24]Hematology, Hospital Vall d'Hebron, Barcelona, [25]Hematology, Hospital Germans Trias i Pujol, Badalona, [26]Hematology, Hospital de Granollers, Granollers, [27]Hematology, Hospital Universitario de La Coruña, La Coruña, [28]Hematology, Hospital Virgen del Puerto, Cáceres, [29]Hematology, Hospital Universitario Dr Peset, Valencia, [30]Hematology, Hospital Clínico San Carlos, Madrid, [31]Hematology, Hospital del Bierzo, Ponferrada, [32]Hematology, Hospital Universitario de Salamanca. Geltamo scientific committee president, Salamanca, [33]Hematology, Hospital Clinic de Barcelona, Barcelona, Spain*

Background: Lymphomas are a heterogeneous group of disease entities with well-defined clinical, morphological, immunophenotypic, and cytogenetic characteristics. Furthermore, regional and racial differences have been reported in their incidence and subtype distribution.
Aims: Due to the lack of epidemiological Lymphoma Registries in Spain, on 2014 GELTAMO Group start a prospective Registry with the aim of our study determine the incidence and distribution of lymphomas in Spain
Methods: We designed an online platform for the prospective registry. All GELTAMO group centers who expressed their intention to participate in the study were endowed with a password to access the platform. The start of data collection was in January 2014. Since then, all patients diagnosed with lymphoid neoplasms according to 2008 WHO classification are being consecutively registered in the platform. The data cut for this analysis was made in January 2018. The variables to collect are: alphanumeric code, reference hospital, gender, date of birth, diagnosis, date of diagnosis, status and date of last follow-up.

Table 1.

HISTOLOGICAL SUBTYPES OF LYMPHOMA	n (9163)	%
PRECURSOR (IMMATURE CELL) LYMPHOID NEOPLASMS	78	0.01
MATURE B-CELL NEOPLASMS	7387	0.81
T-CELL AND NK-CELL NEOPLASMS	551	0.06
HODGKIN LYMPHOMA	1120	0.12
POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS (PTLD)	27	0.00

PRECURSOR (IMMATURE CELL) LYMPHOID NEOPLASMS	n (78)	%
NO SPECIFIED	0	0.00
B-CELL LYMPHOBLASTIC LEU / LYM(BCLL/L) NOS	37	47.44
BCLL/L WITH GENETIC ABNORMALITIES	13	16.67
T-CELL LYMPHOBLASTIC LEU / LYM	28	35.90

MATURE B-CELL NEOPLASMS	n (7336)	%
NO SPECIFIED	78	1.06
CLL / SLL	1201	16.37
B-CELL PROLYMPHOCYTIC LEUKAEMIA	14	0.19
SPLENIC MARGINAL ZONE LYMPHOMA	253	3.45
HAIRY CELL LEUKAEMIA	49	0.67
WALDENSTRÖM'S MACROGLOBULINEMIA	221	3.01
A HEAVY CHAIN DISEASE	1	0.01
G HEAVY CHAIN DISEASE	1	0.01
M HEAVY CHAIN DISEASE	1	0.01
PLASMA CELL MYELOMA	136	1.85
SOLITARY PLASMACYTOMA OF BONE	4	0.05
EXTRAOSSEOUS PLASMACYTOMA	2	0.03
MALT LYMPHOMA	371	5.06
NODAL MARGINAL ZONE LYMPHOMA	246	3.35
FOLLICULAR LYMPHOMA	1647	22.45
PRIMARY CUTANEOUS FCCL	52	0.71
MANTLE CELL LYMPHOMA	389	5.30
DLBCL NOS	2097	28.59
DLBCL T-CELL/HISTIOCYTE-RICH	70	0.95
PRIMARY CNS LYMPHOMA	99	1.35
PRIMARY CUTANEOUS DLBCL LEG TYPE	20	0.27
DLBCL WITH CHRONIC INFLAMMATION	6	0.08
LYMPHOMATOID GRANULOMATOSIS	5	0.07
PMBCL	85	1.16
INTRAVASCULAR LARGE B-CELL LYMPHOMA	8	0.11
ALK+ LARGE B-CELL LYMPHOMA	4	0.05
PLASMABLASTIC LYMPHOMA	37	0.50
PRIMARY EFFUSION LYMPHOMA	12	0.16
LBCL ARISING IN HHV8-ASSOCIATED MCD	6	0.08
BURKITT LYMPHOMA	130	1.77
INTERMEDIATE DLBCL-BURKITT	69	0.94
INTERMEDIATE DLBCL-CHL	22	0.30

T-CELL AND NK-CELL NEOPLASMS	n (551)	%
NO SPECIFIED	11	2.00
T-CELL PROLYMPHOCYTIC LEUKAEMIA	16	2.90
T-CELL LGL LEUKAEMIA	31	5.63
AGGRESSIVE NK-CELL LEUKAEMIA	3	0.54
SYSTEMIC EBV+ TCL OF CHILDHOOD	3	0.54
HYDROA VACCINIFORME-LIKE LYMPHOMA	0	0.00
ADULT T-CELL LEUKAEMIA / LYMPHOMA	8	1.45
EXTRANODAL NK/T-CELL LYMPHOMA NASAL	33	5.99
ENTEROPATHY-ASSOCIATED TCL	12	2.18
HEPATOSPLENIC TCL	8	1.45
SUBCUTANEOUS PANNICULITIS-LIKE TCL	3	0.54
MYCOSIS FUNGOIDES	55	9.98
SÉZARY SYNDROME	19	3.45
LYMPHOMATOID PAPULOSIS	24	4.36
PCALCL	16	2.90
GD-TCL	4	0.73
PERIPHERAL T-CELL LYMPHOMA NOS	128	23.23
ANGIOIMMUNOBLASTIC TCL	98	17.79
ALCL ALK+	46	8.35
ALCL ALK-	33	5.99

HODGKIN LYMPHOMA	n (1120)	%
NO SPECIFIED	73	6.52
NODULAR SCLEROSIS CHL	634	56.61
MIXED CELLULARITY CHL	256	22.86
LYMPHOCYTE-RICH CHL	69	6.16
LYMPHOCYTE-DEPLETED CHL	18	1.61
NODULAR LYMPHOCYTE-PREDOMINANT HL	70	6.25

POST-HSCT LYMPHOPROLIFERATIVE DISORDERS (PTLD)	n (27)	%
NO SPECIFIED	0	0.00
PLASMACYTIC HYPERPLASIA	0	0.00
INFECTIOUS MONONUCLEOSIS-LIKE PTLD	0	0.00
POLYMORPHIC PTLD	4	14.81
MONOMORPHIC PTLD	22	81.48
CHL TYPE PTLD	1	3.70

Results: Since 2014 to January 2018, 9163 patients have been registered in 47 different hospital centers across Spain. The median follow up for alive patients was 18 month. The overall survival of the global series at 18 month was 85%. Median age was 54.78 years old (IQR: 38.7-67.6). Distribution by gender was 52% male and 48% female. The incidence according to histological types was: precursor B/T-cell neoplasms 0.9%, mature B-cell neoplasms (B-NHL) 80%, T and NK neoplasms (T/NK-NHL T/NK) 6%, Hodgkin (HL) 12% and post-transplant lymphoproliferative disorders (PTLDSL) 0.3%. (Table 1). As expected, within B-NHL, diffuse large B cell lymphoma NOS and follicular lymphoma were the most frequent entities reported (28.3% and 22.2% respectively) (Table 1). A total of 870 patients with marginal lymphoma (11.3% of mature B lymphomas) were registered, distributed in nodal (246, 3.3%), extranodal (370, 5%) and splenic (253, 3.4%) subtypes. Among the T/NK-NHL (Table 1), the most frequent were peripheral T lymphomas (123, 23.2%) and anaplastic lymphomas (ALK+ 46 (8.3%), ALK- 33 (5.9%). Among the HL (Table 1), the classic subtype represented 93.7% of the cases, being nodular sclerosis the most frequent

variant (60.4%). Among the PTLD groups, 88% had monomorphic subtype. In a non-detachable number of patients, the histological subtype could not be specified, 78 cases of B-NHL, 73 cases of HL and 11 cases of T-NHL. This was mainly due to the sort of biopsy (trucut), technical problems, or absence of specific diagnostic techniques.
Summary/Conclusion: This is the first prospective registry to know the incidence and distribution of lymphomas in Spain. Knowing the local epidemiology is essential to optimize the use of resources, design clinical trials and identify minority entities focus on their study our incidences are in accordance to those previously reported in occidental countries. Currently, we are adapting our platform to the new 2016 WHO classification.

PS1442

A HEALTH STATE UTILITY MODEL ESTIMATING THE IMPACT OF IVOSIDENIB ON QUALITY OF LIFE IN PATIENTS WITH RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA

E. Wehler[1], M. Storm[2,*], S. Kowal[1], C. Campbell[1], A. Boscoe[2]

[1]HEOR, IQVIA, Fairfax, [2]HEOR, Agios Pharmaceuticals Inc., Cambridge, United States

Background: Acute myeloid leukemia (AML) is the most common form of leukemia in adults in the United States (US). Prognosis is poor, especially for patients with relapsed/refractory (R/R) disease. Treatment options for R/R AML are limited and, until recently, no new drugs had been approved in over 4 decades. Ivosidenib is an oral monotherapy in clinical trials for the treatment of IDH1-mutated (mIDH1) R/R AML. The phase 1, single-arm trial showed a complete remission or complete remission with partial hematologic recovery rate of 30.4% and a favorable adverse event (AE) profile. As health-related quality of life (HRQoL) data was not collected in the trial, a modeling exercise was undertaken to understand the potential utility benefit associated with ivosidenib compared to other treatments used for R/R AML.
Aims: The objective of the study was to estimate the lifetime HRQoL impact of ivosidenib in R/R AML.
Methods: A partitioned survival model leveraging event-free survival (EFS) and overall survival (OS) curves considered the impact of clinical performance and AEs on HRQoL. No directly comparable clinical trials were found for mIDH1 patients, and available evidence for non-mIDH1 comparators had missing data metrics and poorly matched populations. As such, two modeling approaches were considered: 1. Using survival data from the phase 1 ivosidenib trial for all interventions (AE differences only) and 2. Using published survival data from a phase 3 R/R AML clinical trial of clofarabine + cytarabine (AE and EFS/OS differences) for all comparators. Grade ≥3 AEs occurring in ≥5% of patients were included. The model considered time to remission, number of treatment cycles, and two rounds of induction/consolidation therapy, where applicable. AE rates were derived from available clinical studies. Utility values for each disease phase and disutilities for each AE were from the published literature. The total disutility attributed to AEs for each intervention was calculated using an additive approach.

Table 1. Base case results.

Intervention	Using ivosidenib survival data for all treatments		Using literature-based survival for comparators		Total AE disutility
	Life-Years	QALYs	Life-Years	QALYs	
Ivosidenib	0.915	0.399	0.915	0.399	-0.129
LoDAC	0.915	0.369	0.834	0.341	-0.166
HMAs	0.915	0.301	0.834	0.278	-0.225
Daunorubicin and cytarabine fixed dose	0.915	0.264	0.834	0.245	-0.244
7+3	0.915	0.257	0.834	0.239	-0.251
HiDAC	0.915	0.135	0.834	0.123	-0.377
Other HIC	0.915	0.319	0.834	0.298	-0.187
Midostaurin+ chemotherapy	0.915	0.000	0.834	0.000	-0.570

LoDAC: low dose cytarabine; HMAs: hypomethylating agents; HiDAC: high dose cytarabine; IC: other high intensity chemotherapy

Results: Assuming ivosidenib survival is representative of other comparators (no survival benefit), ivosidenib produces slightly more quality-adjusted life years (QALYs) [range: 0.030 vs LoDAC to 0.399 vs midostaurin+ chemotherapy], largely driven by benefits of AE reductions in infections and hematologic disorders. Applying the clofarabine+cytarabine survival data to the comparators increases incremental QALYs [range: 0.058 vs. LoDAC to 0.399 vs. midostaurin+chemotherapy] due to gains in life years for ivosidenib [0.081 vs. all comparators]. Baseline estimates are conservative, as the model did not include several potential benefits of ivosidenib (e.g. impact of stable disease, hospitalization rates and transfusions). Further, given the limited published studies with both EFS and OS data, the modeled comparator data was based on a trial of patients who had received ≤2 prior regimens and thus would be expected to demonstrate better EFS and OS than patients in the ivosidenib trial who had received a median of 2 prior regimens. The additive disutility approach for AEs potentially over-estimates overall AE impact on HRQoL for more toxic therapies, however, scenario analyses comparing only the single most impactful AE event across comparators still result in incremental QALY gains for ivosidenib, reinforcing baseline findings (Table 1).
Summary/Conclusion: Given the potential for improved survival and its favorable AE profile versus other R/R AML therapies, ivosidenib is expected to improve HRQoL over patients' lifetimes in the mIDH1 R/R AML population.

PS1443

ASSESSMENT OF ADHERENCE IN HAEMOPHILIA PATIENTS - EVALUATION OF AN US INSTRUMENT AND DEVELOPMENT OF A NEW QUESTIONNAIRE FOR USE IN GERMANY

S. Von Mackensen[1,*], S. Halimeh[2]

[1]Institute of Medical Psychology, University Medical Centre Hamburg-Eppendorf, Hamburg, [2]Gerinnungszentrum Rrhein-Rruhr (GZRR), Duisburg, Germany

Background: Non-adherence is a major public health problem leading to financial burden on healthcare systems. Adherence is influenced by social, economic, healthcare system related and condition-related aspects. Due to high treatment costs, the evaluation of adherence in people with haemophilia (PWH) is important. In Europe no haemophilia-specific adherence instrument exists; only an US instrument from a different health-care system is available (VERITAS-PRO for prophylaxis PWH, VERITAS-PRN for on-demand PWH).
Aims: To verify whether this US instrument is relevant for other healthcare systems and to which extent the single items are important to PWH and Healthcare professionals (HCP). To develop an adherence questionnaire for Germany.
Methods: The study consists of three phases (evaluation of US instrument, conduct of focus groups with different stakeholders, construction of new adherence questionnaire). PWH from a haemophilia treatment centre (HTC) in Duisburg and HCP were asked to evaluate the single questions of the US instruments concerning their relevance for German PWH on a 5-point-Likert-scale (ranging from not important to very important). Suggestions for additional questions were possible. The level of agreement was based on the calculation of the content validity ratio (CVR) defined as a linear transformation of a proportional level of agreement on how many raters within a panel rate an item very important. Separate focus groups were conducted with HCP (paediatricians, internists), PWH (adults, adolescents, parents of haemophilic children) and health insurance companies.
Results: So far, the first 2 phases were performed. Fifty PWH (23 adults, 27 parents of haemophilic children) participated with a mean age of 35.78±11.0 years. Most PWH had haemophilia A (78%), were severely affected (62%) and on prophylaxis (62%). Based on the content validity ratio (CVR ≥0.300) across all respondents only 1 question ("*I use the doctor-recommended dose for infusion*") of the Veritas-PRO and 3 questions of the Veritas-PRN ("*I use the correct number of factor boxes to total my recommended dose*", "*I have enough factor and supplies at home to infuse when needed*", "*I call the treatment centre before medical interventions*") were considered as "very important" for use in Germany. Few suggestions for additional adherence questions were made by PWh, e.g. distance from home to HTC, educational level, confidence in physician, barriers of non-adherence and improvement of adherence. In general, questions concerning dosing were considered only relevant for the US. 25 out of 27 HCP sent back their evaluation forms (62.5% physicians, 25% nurses, and 2 study/haemostaseology assistants). 36% treated only adult PWH, 16% only paediatric PWH and 48% treated both. HCPs were treating PWH since 15.5±6.7 years (range 9-30). Based on the content validity ratio (CVR ≥0.440) four questions of the Veritas-PRO domains 'timing', 'planning', 'dosing' and 'communicating' (e.g., "*I call the treatment center when I have questions about hemophilia or treatment*") and seven questions of the Veritas-PRN domains 'treating', 'dosing'

(e.g. "*I infuse the prescribed dosage for bleeds*"), 'planning', and 'communicating' were considered "very important" by HCPs for use in Germany. HCP suggested adding a variety of questions concerning substitution diary, visits and commun-ication with HTC, etc. In general, questions concerning novel therapies were missing.
Summary/Conclusion: The evaluation has proven that there is a need of an appropriate adherence instrument different from the US ones applicable for the health care system in Germany.

PS1444

HEALTH-RELATED QUALITY OF LIFE (HRQOL) IN PEDIATRIC PATIENTS WITH RED CELL PYRUVATE KINASE DEFICIENCY

P. Klothaki[1,*], R. Grace[2], S. Eber[3], M. Wlodarski[4], J. Kunz[5], P. Bianchi[6], E. Fermo[7], S. Burdach[8], O. Andres[9], M. Nathrath[1], N. Kollmar[1]

[1]Pädiatrische Hämatologie und Onkologie, Klinikum Kassel, Kassel, Germany, [2]Dana-Farber Boston Children´s Cancer and Blood Disorder Center, Boston, United States, [3]Praxis für Kinder und Jugendmedizin, Munich, [4]Pädiatrische Hämatologie und Onkologie, Universitätsklinik Freiburg, Freiburg, [5]Pädiatrische Hämatologie und Onkologie, Universität Heidelberg, Heidelberg, Germany, [6]Fondazione IRCCS Ca´Granda, Ospedale Maggiore Policlinico, [7]Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico , Milan, Italy, [8]Medizinische Fakultät, Technische Universität München, Munich, [9]Erythrozytenlabor Universitäts-Kinderklinik Würzburg, Universitäts-Kinderklinik Würzburg, Würzburg, Germany

Background: Pyruvate kinase deficiency (PKD) is the most common enzymopathy of anaerobic glycolysis in erythrocytes. PKD is a rare autosomal recessive disease with a prevalence of 1 in 20.000 people causing a hereditary non- spherocytic hemolytic anemia (Zanella *et al.* 2005). There are no published studies concerning health-related quality of life (HRQoL) in patients with PKD.
Aims: This study describes HRQoL in children with PKD in Germany.
Methods: 16 pediatric patients with compound heterozygous or homozygous PKD who participated in Germany in the PKD Natural History Study were included in the analysis. The German version of the PedsQL 4.0 (Pediatric Quality of Life) questionnaire was used to assess HRQoL. The patients were divided into 3 groups according to age in years: 2-4 (n=8), 5-7 (n=2), 8-18 (n=6). 63% of the patients (n=10) had severe anemia (Hb< 8 g/dl), 4% (n=1) moderate anemia (Hb 8-10 g/dl) and 33% (n=5) mild anemia (Hb 10-12 g/dl). The questionnaire was answered by all children ages≥5 years and by parents of children of all ages. The answers were transformed into a score from 0 to 100 (with 100 representing the best HRQoL) and were compared using the Mann-Whitney Test. PedsQL≥81 is considered an indicator of a good HRQoL, 61-80 intermediate HRQoL and ≤60 poor HRQoL (Beverung *et al.* 2015).
Results: The mean parental PedsQL scores of all 16 children were 79.10 (range: 0-100) in the physical- and 79.81 (range: 21.15 -100) in the psychosocial health summary score.
The mean PedsQL scores of children≥5 years were 89.45 (range: 78.13 - 100) in the physical - and 84.10 (range: 63.33 -100) in the psychosocial health summary score. These were not significantly different in comparison to the mean scores given by their parents (83.59, range: 46.86-100 in the physical, p=0.42- and 81.02, range: 43.33-93.33 in the psychosocial health summary score, p=0.13), with the mean scores suggesting a good HRQoL. 63% (n=5) of the children in this group had mild- and 37% (n=3) severe anemia. The mean PedsQL scores reported by the parents of children <5 years were not significantly different than parental report of children ≥ 5 years (74.60, range: 59.3-100, versus 83.59, range: 46.86-100 in the physical, p=0.28 - and 78.60, range: 21.15-100, versus 81.02, range: 43.33-93.33 in the psychosocial health summary score, p= 0.33). The mean parental PedsQL scores of children <5 years are consistent with an intermediate HRQoL. 88% (n=7) of children < 5 years had severe- and 12% (n=1) moderate anemia.
Summary/Conclusion: This is the first study concerning HRQoL in pediatric patients with PKD. Using the PedsQL 4.0, mean child reported scores are consistent with a good HRQoL and parental scores are consistent with an intermediate HRQoL. Parents of children≥5 years give higher scores than parents of children <5 years. Given the severity of anemia in the children in this study, the PedsQL measure may not sufficiently detect the symptoms and impacts of children with PKD. Future studies of disease specific HRQoL measures may be helpful in detecting the impact of this rare anemia in children.

PS1445

QUALITY OF LIFE IS REDUCED IN PATIENTS WITH (SUSPECTED) PLATELET FUNCTION DISORDERS

M. Blaauwgeers[1,*], I. van Asten[2], A. Huisman[2], S. Korporaal[2], M. Coppens[3], M. Kruip[4], E. Beckers[5], J. Eikenboom[6], R. Tamminga[7], R. Urbanus[2], R. Schutgens[1]

[1]Van Creveldkliniek, [2]Clinical Chemistry and Haematology, UMC Utrecht, Utrecht, [3]AMC, Amsterdam, [4]Erasmus MC, Rotterdam, [5]Maastricht UMC, Maastricht, [6]LUMC, Leiden, [7]UMCG, Groningen, Netherlands

Background: Platelet function disorders (PFDs) are rare bleeding disorders, characterized by mucocutaneous bleeding and prolonged bleeding after surgery or childbirth. Repeated bleeds throughout life can have a significant impact on health related quality of life (HR-QoL). Studies investigating HR-QoL in patients with PFDs are limited.
Aims: To determine HR-QoL in patients with (suspected) PFDs and to assess the association between bleeding severity and HR-QoL.
Methods: Adult patients with an increased bleeding tendency, suspected for having a PFD, were included in a nationwide cross-sectional study on PFDs ('Thrombocytopathy in the Netherlands'). All participants gave written informed consent. HR-QoL was assessed using the Short Form (SF)-36 survey, including 8 domain scores ranging from 0 to 100 (optimal). Bleeding symptoms were evaluated using the ISTH Bleeding Assessment Tool (BAT), resulting in a bleeding score (BS, range 0-48 for men, 0-56 for women). Mann Whitney U test was used to compare SF36 domain scores to the general Dutch population scores. Multivariate regression analysis was used to assess the association between BS and HR-QoL.
Results: A total of 159 patients were included of whom 129 (81%) were female. The males and females had a median age of 46 (interquartile range 32-61) and 41 (interquartile range 31-55), respectively. The median BS for males and females was 9 (interquartile range 5-13) and 10 (interquartile range 8-14), respectively. Ninety-seven patients (61%) had an objective platelet function defect based on platelet function testing. Compared to the general population, patients with a (suspected) PFD showed significantly lower ($p<0.05$) scores on all domains of the SF36 except the domain role emotional. HR-QoL was not significantly different between patients with an objective platelet function defect and those with normal results. A higher bleeding score, indicating a more severe bleeding phenotype, was associated with a lower score on the domains physical functioning, role limitations due to physical functioning and general health perception ($p<0.05$).
Summary/Conclusion: Patients with PFDs have a lower HR-QoL as compared to the general Dutch population. HR-QoL is not significantly different between patients with and without an objective platelet function defect, indicating that an increased bleeding tendency has a significant impact on QoL, regardless of an objective explanatory diagnosis. A more severe bleeding phenotype was associated with a lower HR-QoL score on most of the physical domains of the SF-36.

PS1446

PALLIATIVE CARE INDICATION IN ONCOHEMATOLOGIC PATIENTS: A COMPARISON TO SOLID TUMOR PATIENTS

L. Morais[1,*], M. Diaz-Valdes[2], A. Pola[2], S. Bernal[2], S. Alonso-Alvarez[1], E. Colado[1], T. Bernal[1,2]

[1]Servicio de Hematología, Hospital Universitario Central de Asturias, [2]Departamento de Medicina, Universidad de Oviedo, Oviedo, Spain

Background: In spite of recent diagnostic and treatment progress, the prognosis of elderly patients diagnosed of acute myeloid leukemia or higher-risk myelodysplastic (AML-MDS) syndrome remains dismal; even in those patients who are fitted enough to receive chemotherapy or hypomethylating agents, median survival is near 12 months. According to actual end-of-life care (EOL) standards, this population should receive early palliative care (PC), integrated with standard oncologic care. Conversely, recent evidence shows and excess of aggressiveness in this population. The reasons of this need to be determined, but it is suggested that poor performance status and transfusion dependency of hematological patients are barriers that restrict the applicability of palliative care.
Aims: To compare EOL care in AML-MDS and stage-IV lung cancer in a tertiary care center and identify factors related to late or no palliative care (PC) referral.
Methods: Consecutive AML > 65 years and stage-IV lung cancer patients were studied. Data is shown as percentage or median (interquartilic range). Univariant comparisons of the main indicators of EOL aggressiveness between the 2 cohorts were done using Chi-square or Wilcoxon tests as

appropriate. Survival was calculated from diagnosis to death or last follow up using Kaplan-Meier curves and log-rank tests.
Results: From 1st June 2006 to 8th February 2017, 77 patients with AML-MDS and 98 stage-IV lung cancer patients were recorded. Median age at diagnosis was 75 (70-79) and 63 (59-71) years respectively (p<0.001). There were no differences in gender or ECOG. Number of comorbidities according to the Charlson comorbidity index were 1 (0-2) vs 2 (1-3), (p=0.02). Regarding first line therapy, 21% AML-MDS were treated with chemotherapy, 64% with hypomethylating agents and 15% with supportive care exclusively. Fourteen patients received second line therapy for progression or relapse after first response. Of them, 10 patients received chemotherapy and 3 hypomethylating agents. There were no differences in survival between the 2 cohorts (AML-MDS 13[10-19] vs 12[9-19] months form AML-MDS and lung cancer respectively, p=0.37). Discussion between patients and physician about prognosis was documented in 29% AML-MDS patients as opposed to 85% lung cancer patients (p<0.001). There were no statistical differences regarding the frequency of hospital admissions, days spent in hospital or the proportion of patients receiving chemotherapy within the 14 last days of life. However, more AML-MDS patients were admitted into hospital in their last month of life (86 vs 44%, p<0.001). More AML patients were transfusion dependent and received more red blood cells or platelet transfusion in their last 2 months of life. (75% vs 3%, p<0.0001.) Referral to PC Unit was documented in 19% LAM-MDS compared to 48% lung cancer patients (p<0.001).
Summary/Conclusion: Although transfusion dependency is a major need of AML patients, rate of referral to palliative care unit is disproportionately low, indicating the need of earlier PC referral.

PS1447

COST PER MEDIAN MONTH OF PROGRESSION-FREE SURVIVAL FOR DARATUMUMAB PLUS BORTEZOMIB AND DEXAMETHASONE COMPARED WITH CARFILZOMIB PLUS DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

E. Maiese[1,*], C.N. Graham[2], E. Hawe[3], J.-G. Le Moine[3]

[1]Janssen Scientific Affairs LLC, Horsham, PA, [2]RTI Health Solutions, Research Triangle Park, NC, United States, [3]RTI Health Solutions, Manchester, United Kingdom

Background: New treatments for relapsed/refractory multiple myeloma have recently been FDA approved in the United States (US). For FDA approval, these treatments are compared to historic standards of care in randomized controlled trials (RCTs), therefore, limited information regarding safety/efficacy comparisons between newer treatments is available to utilize in comparisons of costs.
Aims: We sought to compare the average cost per median month of progression-free survival (PFS) for daratumumab plus bortezomib and dexamethasone (DVd) compared with carfilzomib plus dexamethasone (Kd) in patients who have received at least one prior line of therapy.

Table 1. Results.

Cost Category	DVd	Kd
Total drug acquisition	$183,903	$161,753
Total adverse event	$4,616	$3,265
Total administration	$7,237	$5,393
Total Costs	**$195,755**	**$170,411**
Calculated median PFS (months)	**16.9**	**10.0**
Cost per median month of PFS	**$11,597**	**$16,984**

Methods: PFS for DVd was estimated via parametric survival analysis of patient-level data from the CASTOR RCT. PFS for Kd was estimated by applying the PFS hazard ratio (HR) for DVd vs. Kd (HR=0.59), calculated through a network meta-analysis (NMA), to the best-fit DVd PFS curve. Treatment duration was estimated by applying the ratio of median duration of treatment and reported median PFS from the trials to the median PFS calculated via the survival analysis and NMA. HRs, dosing amounts, dosing schedules, relative dose intensity, and adverse event (AE) incidence were extracted from the CASTOR and ENDEAVOR RCTs. Wholesale acquisition costs and administration costs were based on standard US sources. AE costs were sourced from published literature.
Results: Median PFS estimates from the analysis for DVd and Kd were 16.9 and 10.0 months, respectively. Total costs were similar between the two treatments. However, average cost per median month of PFS was lower for DVd than for Kd. Results are presented in Table 1.
Summary/Conclusion: Based on this analysis, average monthly cost per median month of PFS is lower for DVd compared to Kd in the treatment of multiple myeloma patients with at least one prior line of therapy.

PS1448

EFFICACY AND SAFETY OF FACILITATED SUBCUTANEOUS IGG ADMINISTRATION IN PATIENTS WITH SECONDARY IMMUNODEFICIENCY DUE TO HEMATOLOGICAL MALIGNANCIES. A SINGLE-CENTER RETROSPECTIVE ANALYSIS

M. Dimou[1,*], T. Iliakis[1], D. Maltezas[1], A. Bitsani[1], S. Kalyva[1], S. Kotsanti[1], A. Koudouna[1], P. Petsa[1], P. Papaioannou[1], M.-C. Kyrtsonis[1], P. Panayiotidis[1]

[1]1st Propedeutic Internal Medicine Clinic, Medical School of Athens, Laikon General Hospital, Athens, Greece

Background: Hematologic malignancies are often complicated by secondary immunodeficiency(SID), namely hypogammaglobulinemia, the cause of common and opportunistic infections. Immunoglobulin replacement with intravenous gamma globulin administration(IVIg) reduces the number of infections, antibiotics' need and hospitalization days. The subcutaneous(sc) route of IgG is equally effective, with certain constraints (reduced bioavailability, multiple injection sites, frequent infusions). The facilitated sc IgG(fSCIg) method uses the action of recombinant human hyaluronidase(rHuPH20) and has been studied in primary immune deficiency(PID) syndromes with similar efficacy to IVIg, with several advantages (self-administration, same bioavailability, long infusion intervals, few adverse drug reactions-ADRs). fSCIg has been less extensively studied in SID.
Aims: We present our retrospective single-center data from fSCIg administration in SID of hematological patients, focusing on safety and efficacy issues.
Methods: Thirty-three hematological patients with SID have been treated with fSCIg, according to ESMO 2015 guidelines, between mid-October 2015 and mid-January 2018. Baseline patients' characteristics are shown in Table 1.

Table 1. Baseline patients' characteristics.

Patients	n=33 (16 female, 17 male)
age	66,1 yr. (38-88)
type of preexisting hematologic malignancy	• CLL (n=25) • Multiple Myeloma (n=3) • B- Non-Hodgkin Lymphoma (n=3) • Hodgkin's Lymphoma (n=1)
disease treatment status before fSCIg initiation	• treatment naive (n=5 all CLL pts) • pretreated (n=28), prior treatment lines: 3(1-7)
IgG replacement before fSCIg initiation	n=13, all with IVIg median time on IVIg 26,2 months (6,2-82,2)
median IgG levels before fSCIg initiation	• pts on IVIg (n=13): 532 mg/dL (80-982) • IVIg naive pts (n=18): 403,5 mg/dl (102-632) (trough IgG levels)
Infections during the last 12 months before fSCIg initiation	7 pts (all on IVIg): no infectious episode 26 pts* (6 on IVIg, 20 without IVIg) • 15 cases with recurrent lower respiratory tract infections • 7 cases with recurrent upper respiratory tract infections • 3 cases with recurrent kidney infections • 2 cases with recurrent soft tissue infections • 1 case with multiple Herpes zoster reactivation episodes *: pts may have presented more than one type of infection

Treatment goal was IgG trough levels around 600 mg/dL. fSCIg(10% IgG) dosage was 0.4-0.8 mg/Kg/month. Treatment modifications were made according to infection occurrence. fSCIg was given with a variable rate portable pump and a sc 24G needle . First, patients were treated with rHuPH20 (1-2 ml/min). Ten min after rHuPH20, fSCIg(10%) was infused through the same sc needle, at the same injection site. The rate of the 1st fSCIg infusion was gradually increased from 10 ml/min to 300 ml/min. If well tolerated, patients were treated with the rate of 300 ml/min for all subsequent infusions. The first 4 infusions took place at our department and the patients/relatives were instructed how to use the pump. The subsequent infusions took place at patients' home.

Results: Between mid-October 2015 and mid-January 2018, 33 patients have been treated at our department with fSCIg(10%). Four hundred forty-four uncomplicated infusions were administered. Median number of infusions:11(2-31), median time of follow-up: 11,2 months(0,5-27). Available trough IgG levels at various time points are shown in Table 2. Thirty-two (97%) patients were able either to self-administer the formulation or to be treated by the aid of a relative after the 4th training session. Six patients (18,1%) presented at least 1 infection while on fSCIg. Four patients: an episode of lower tract infection, 1 patient: an episode of nail infection of a lower extremity and 1 patient: a flu-like infection and a dermal infection. All 6 patients had IgG levels <600 mg/dL at the time of infection. They started receiving fSCIg in shorter time intervals(q3W). There was no infection after this modification. Three patients(9%) presented mild ADRs(grade 1): low grade fever/headache the evening of the 1st and/or 2nd fSCIg infusion. Among the 444 fSCIg infusions, 298 took place outside hospital facilities, at patients' home.

Table 2. Madian IgG trough level at several time points of fSCIg administration.

month of treatment	IgG trough levels (mg/dL)
3rd (n=21)	787 (231-1254)
6th (n=17)	945 (664-2760)
12th (n=14)	789 (476-1290)
24th (n=5)	895 (497-1350)

Summary/Conclusion: Our retrospective single-center data from fSCIg administration in hematological patients with hypogammaglobulinemia and recurrent infections show that this method is very effective in reducing infections, with few ADRs. These findings compare favorably to those observed with IVIg method. Finally, fSCIg method manages to reduce the everyday nursery/hospital burden of a tertiary hospital.

PS1449

IMPACT OF LOW GRADE ADVERSE EVENTS OF TYROSINE KINASE INHIBITORS ON THE QUALITY OF LIFE OF PATIENTS TREATED FOR CHRONIC MYELOID LEUKEMIA

B. Sidi Mohamed El Amine[1,*], H. Asma[1], O. Fouzia[1], S.A. Nadjet[1], C. Malika[1], B. Soraya[1], Z. Zahia[1]

[1]Universitary Hospital of Sidi Bel Abbés, Sidi Bel Abbes, Algeria

Background: Patients with chronic myeloid leukemia (CML) have a better life expectancy after treatment with a tyrosine kinase inhibitor (TKIs). The possibility of adverse events (AEs) with long-term treatment may result in dose adjustments and discontinuation of therapy, or non-adherence, which may negatively affect treatment efficacy and patients' quality of life (QoL).
Aims: The aim of our work is to identify low grade AEs of TKIs and their impact on QoL of patients with CML.
Methods: Data of 79 patients with CML were analyzed. We used the FACT-Leu questionnaire, which is a validated tool for assessing QoL during leukemia treatment.
Patients were seen either at day hospital or during biological evaluations and clinical controls. The low grade of TKIs were reported at each control and then grouped according to their severity, using the NCI CTCAE terminology (version 3.0). The correlation between low grade AEs and QoL scores was assessed using the Spearman test. The ANOVA test was used to determine the change in QoL depending on the grade of TKIs side effects.
Results: Of the 79 patients, 30 (38%) experienced side effects with TKIs, with dose adjustments in 15 (19%) patients, and therapeutic discontinuation in 8 (10%). According to NCI CTCAE terminology, AEs were of grade 1 in 8 (10%) patients, and grade 2 in 8 (10%) others. The average FACT-Leu QoL score for all patients who received TKIs was 129 [84-170], lower in patients with low grade AEs (118 [87-155]) , but higher for those without AEs: 135 [84-170]. The FACT-Leu QoL score was correlated with the appearance of a TKI related low grade AEs (p=0.01). The ANOVA showed a variability in the FACT-Leu score based on severity of TKIs side effects (p=0.04).
Summary/Conclusion: This study shows that low grade AEs of TKIs can alter QoL of patients treated for CML. Their impact as well as other factors must be taken into account for the optimal treatment of newly diagnosed CML patients.

Sickle cell disease

PS1450

HEME-INDUCIBLE LIPID OXIDATION PATTERN SUPPORTS MONITORING OF CELL-FREE HEMOGLOBIN ACTIVITY IN PATIENTS WITH SICKLE CELL DISEASE

J. Deuel[1,2,*], S. Di Palma[3], A. Yalamanoglu[4], B. Paul[4], S. Dominik[1]

[1]Division of Internal Medicine, [2]Division of Haematology, University Hospital of Zurich, [3]Functional Genomics Center Zurich FGCZ, University of Zurich, Zürich, Switzerland, [4]Laboratory of Biochemistry and Vascular Biology, DBCD/ OBRR/ CBER FDA, Silver Spring MD, United States

Background: Sickle cell disease (SCD) is a genetic disease based on a hemoglobinopathy leading to sickling of red blood cells in the circulation. This phenomenon leads to a state of chronic hemolysis as well as to occasional sickle cell crises with accelerated release of cell-free hemoglobin (Hb) into plasma. Low density lipoprotein (LDL) is a lipid transport particle present at relatively high levels in human and oxidized LDL (oxLDL) is supposed to be a driver of pathology in a number of vascular diseases. The lipid core of LDL is composed of a range of different lipid classes with unsaturated and thus oxidation-prone species. Due to its specific characteristics, LDL appears to represent an interesting indicator-compartment to monitor Hb-driven lipid-oxidation in hemolytic diseases.
Aims: To characterize a specific plasma-lipid oxidative pattern as a biomarker of Hb-driven pathology in SCD-patients.
Methods: We developed a novel mass spectrometry (MS) method to *de novo* identify and quantify oxidized lipid species by detecting unique features of lipid oxidation. We used this method to characterize the degree of LDL oxidation in 10 SCD patients by comparing the oxidation-pattern to control patients without hemolytic disease. To extract and correlate a heme/Hb induced LDL oxidation pattern, guinea pigs were infused with cell-free Hb. LDL was harvested from these animals and oxidized LDL characterized and quantified with the same MS-based method.
Results: By infusing free Hb into guinea pigs, we could reproducibly induce a specific pattern of LDL oxidation. A highly similar pattern was detected very pronounced in half of the SCD patients studied. None of the control patients showed significant LDL oxidation (Figure 1).

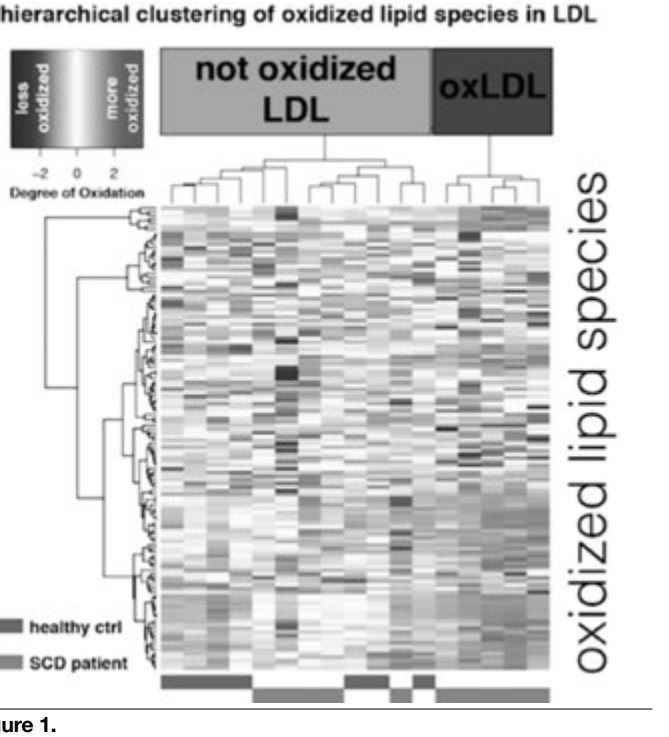


Figure 1.

Summary/Conclusion: We characterized a Hb-induced plasma-lipid oxidation pattern, which may serve as a biomarker for hemolysis-driven pathology in patients with sickle cell disease and other hemolytic conditions.

PS1451

COMPLEMENT ACTIVATION IN HOMOZYGOUS SICKLE CELL DISEASE PATIENTS IS MODULATED BY HYDROXYUREA

L. Roumenina[1,*], P. Chadebech[2,3,4], G. Bodivit[2,3,4], P. Vieira-Martins[5], L. Kiger[6], A. Jouard[2], F. Pirenne[2], F. Galactéros[7], V. Frémeaux Bacchi[5], P. Bartolucci[3,7,8]

[1]INSERM UMRS1138 Cordeliers Research Center, INSERM UMRS1138 Cordeliers Research Center, Paris, [2]Etablissement Français du Sang, Hôpital Henri-Mondor, [3] UPEC, INSERM U955 Team [2] and IMRB (Institut Modor de Recherche Biomédicale), Créteil, [4]Laboratoire d'Excellence GR-Ex, [5]Service d'Immunologie Biologique, Hôpital Européen Georges Pompidou, Assistance Publique – Hôpitaux de Paris, Paris, [6]INSERM U955, team [2], [7]Sickle Cell Referral Center, Service de Médecine Interne, Hôpital Henri-Mondor, Créteil, [8]Laboratoire d'Excellence GR-Ex, Paris, France

Background: Markers of alternative complement pathway activation have been detected in patients with sickle cell disease (SCD). The mechanism of this process is poorly understood, and could be related to complement activation at the surface of sickle red blood cells or by hemolysis-derived products. Altered membrane phospholipids exposure on sickle erythrocytes was shown to mediate complement activation. However the progression of the complement cascade to its terminal steps and its modulation by hydroxyurea have never been studied in a cohort of SCD patients.
Aims: The objective of this study was to screen for plasmatic complement biomarkers in a cohort of SCD patients, treated or not with hydroxyurea.
Methods: Blood samples were obtained from homozygous SCD patients followed in our Sickle cell referral center after receiving their written consent. Two groups were constituted according to treatment: Hydroxyurea (HU) and untreated (CT). Exclusion criteria were secondary causes of complement activation, transfusion in the 3 last months, or pregnancy. Steady state was defined as >1 month after a vaso occlusive crisis or acute event. Plasma samples were collected and stored frozen for exploration of complement. Plasmatic C3, C4 and Factor H protein levels, CH50 activity, soluble sC5b-9 and presence of anti-Factor H autoantibodies were screened. Membrane expression of CD55 and CD59 was measured on erythrocytes. All samples were analyzed at the French Sickle Cell Referral Center and at the French complement referral laboratory. The normal upper value of C5b9s was 450 ng/ml. Results are expressed as medians with [interquartile range] or percentages. Mann Whitney non parametric test, and Chi² test where used for comparison between HU and CT; Pearson's correlation coefficients were computed to assess the relationship between sC5b9 and collected biological parameters.
Results: Seventy two patients were included, 3 patients were secondary excluded due to positivity for anti-Factor H antibodies. Sixty nine patients were analyzed, with a median age of 31.4 years [23.7-40.7]; 27/69 patients were treated by HU, with similar age in HU and CT patients, (29.9 and 33 years, respectively). sC5b9 was abnormally high (>450 ng/ml) in 46 patients (66%), without significant difference between VOC (9/69) and steady state (respectively 741 and 611 ng/ml). In the group of patients treated by HU the abnormal complement activation was less frequent compared to CT patients (52% vs 76%, p=0.03) with significantly lower levels of sC5b9 (457 vs 812, p=0.04). There was no difference in CD55 or CD59 that could explain the difference between groups. Linear regression showed that sC5b9 increase was associated with lower hemoglobin concentration in the HU group (p=0.05) and higher% dense red blood cells (DRBC) (p=0.04) which present higher HbS concentration and polymerization levels. No correlation was found with LDH or HbF values.
Summary/Conclusion: Our results show that terminal complement activation is common in SCD. HU treatment, known to decrease the hemolysis level and%DRBC, decreased significantly the complement activation in association with increase of hemoglobin. Mechanisms of complement modulation by HU are not elucidated but could be related to the%DRBC decrease. Further studies are necessary to evaluate the complement activation relationship with chronic organ damages, known to be associated with hemolysis and DRBC.

PS1452

THE OXYGENSCAN: CONTINUOUS MEASUREMENT AND QUANTIFICATION OF SICKLING DURING DE- AND REOXYGENATION TO MONITOR DISEASE SEVERITY AND TREATMENT EFFECT IN SICKLE CELL DISEASE

M. Rab[1,2,*], B. van Oirschot[1], T. Merkx[1], A. van Wesel[1], S. Hendriks[3], J. de Zoeten[3], O. Abdulmalik[4], M. Safo[5], B. Versluijs[6], R. Schutgens[2], G. Pasterkamp[7], E. van Beers[8], R. van Wijk[9]

[1]Department of Clinical Chemistry and Haematology, [2]Van Creveldkliniek, University Medical Center Utrecht, Utrecht, [3]RR Mechatronics, Zwaag, Netherlands, [4]Division of Hematology, The Children's Hospital of Philadelphia, Philadelphia, [5]Medicinal Chemistry, Institute for Structural Biology, Drug Discovery and Development, School of Pharmacy, Virginia Commonwealth University, Richmond, United States, [6]Pediatric Hematology, [7]Laboratoy of Clinical Chemistry and Haematology, University Medical Center Utrecht, [8]Van Creveldkliniek, University Medical Center, [9]Laboratory of Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht, Netherlands

Background: In sickle cell disease (SCD) a single point mutation in the gene encoding for beta-globin chain underlies the production of the abnormal hemoglobin S (HbS). Upon deoxygenation HbS can polymerize, resulting in sickling of red blood cells (RBCs). These sickled RBCs have strongly reduced deformability, leading to vaso-occlusive crises and chronic hemolytic anemia. To date, there are no laboratory parameters or assays capable of predicting disease severity or directly monitoring treatment effects.
Aims: To develop and validate the Oxygenscan: a new method to investigate RBC deformability as a function of oxygen tension and thereby quantify sickling behavior in patients with SCD.
Methods: Red blood cell deformability (expressed as Elongation Index – EI) was measured as a function of oxygen tension by ektacytometry using the Laser Optical Rotational Red Cell Analyzer (Lorrca, Zwaag, The Netherlands). This 'Oxygenscan' measures EI during one round of deoxygenation (nitrogen gas) followed by reoxygenation while exposed to a fixed shear stress of 30 pascal. Main Oxygenscan read out parameters were defined as follows (Figure 1A): EImax: maximum elongation during deoxygenation, EImin: minimum elongation after deoxygenation, ΔEI: difference between EImax and EImin, Point of Sickling (POS): pO2 (mmHg) at which >5% decrease in EI during deoxygenation is observed. Blood samples of SCD patients and healthy controls were used to validate the technique.

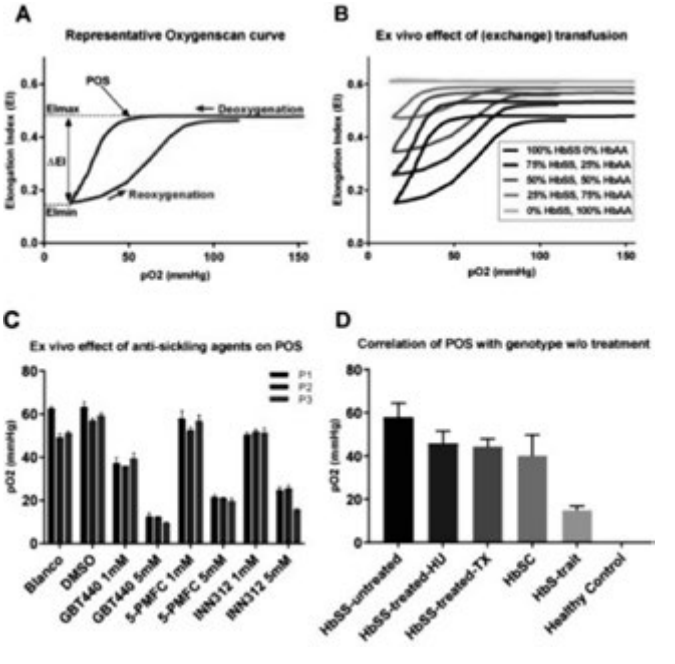


Figure 1.

Results: Oxygenscan curves were highly reproducible with a coefficient of variation (CV) below 5% for all four main parameters. See Figure 1A for a representative example. When whole blood from SCD patients (n=3) was mixed in various proportions with a ABO compatible healthy control blood sample the Oxygenscan curve accordingly shifted upwards towards normal, with a most pronounced effect on EImin and ΔEI, data of 1 patient is shown in Figure 1B. Multiple Oxygenscan variables were related with HbS% but with a linear correlation of r= 0.998 (p=0.00013) in this patient, EImin was the strongest one. This suggests that exchange transfusion primarily effects EImin. Notably, RBCs from healthy control blood samples show no change in EI during de-/reoxygenation. POS likely reflects directly the properties of an individual patient's hemoglobin dissociation curve. When factors that influence the dissociation curve were altered, e.g. temperature and pH, a shift in POS was observed. ΔEI appears to reflect the tendency of an individual patient to sickle as it increases upon higher temperatures and lower pH. Upon *ex vivo* exposure to anti-sickling agents, altering the oxygen affinity of hemoglobin, a left-shift of the POS was observed, indicating improved

deformability at lower oxygen tensions (Figure 1C). In addition, a substantial decrease in ΔEI was observed, suggesting less cells are able to sickle. When RBCs from 19 SCD patients with different genotypes and treatment regimens (homozygous SCD (HbSS), untreated, n=5; treated with hydroxyurea (HU), n=4; or with exchange transfusion (TX), n=3; compound heterozygous for HbC and HbS, n=3, heterozygous HbS, n=4; healthy controls, n=5) were analyzed the POS was highest in untreated HbSS patients (Figure 1D). Treatment with either HU or TX caused a decrease in the POS, and an increase in EImax and EImin.
Summary/Conclusion: The Oxygenscan brings the sickling assay to a new level with unparalleled repeatability, and with multiple parameters that quantify different aspects of sickling biology. We suggest that the Oxygenscan can be used to assess an individual patient's disease severity and monitor treatment effect.

PS1453

VOXELOTOR DOSE EXTRAPOLATION IN A PHASE 3, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY IN PEDIATRIC PATIENTS WITH SICKLE CELL DISEASE (GBT440-032, HOPE KIDS 2)

C. B. Washington[1,*], B. Goldstein[1], S. Dixon[1], C. Hoppe[2], R.E. Ware[3], J. Lehrer[1]

[1]Global Blood Therapeutics, South San Francisco, CA, [2]UCSF Benioff Children's Hospital Oakland, Oakland, CA, [3]Cincinnati Children's Hospital, Cincinnati, OH, United States

Background: The phase 3 HOPE Kids 2 study (GBT440-032) design includes a novel approach to determine initial voxelotor dosing in pediatric patients aged 9 months to <12 years with sickle cell disease (SCD). The dosing strategy is based on population pharmacokinetics (PPK), physiologically based PK (PBPK) modeling, and dose extrapolation from studies of voxelotor in adults and adolescents (12 to <18 years old). PBPK modeling is considered a "bottom-up" approach as it uses patient-specific parameters (eg, anatomy, physiology, and pathophysiology) and drug-specific data (eg, absorption and metabolism). In pediatrics, PBPK models provide an opportunity to integrate known developmental changes in organ function to predict drug disposition and PK exposures across age groups. Extrapolation of data from adults to children is an important emerging tool in pediatric drug development and regulatory decision making and allows for the use of fewer patients in trials.
Aims: Currently, dose selection for the phase 3 HOPE adult and adolescent study is evaluating 2 doses, 900 mg and 1500 mg, for final dose selection. One or both doses may be used. The phase 3 HOPE Kids 2 study will use the final selected dose(s) from HOPE to provide equivalent voxelotor exposures in infants and children.
Methods: Planned PK data collection will confirm exposures in infants and in children and validate the PK and PBPK models. Clinical events (eg, vaso-occlusive crises, spleen loss, dactylitis, sepsis, aplastic crisis, stroke, and silent infarcts) will also be analyzed via PK/pharmacodynamic modeling.
Results: Based on existing data, including PK data from adults, adolescents, and children (6 to <12 years of age), the PPK models for voxelotor in whole blood and plasma are 2-compartment models with first-order absorption and elimination. Simulations based on age-based and weight-based groups were compared. Although age-based groups were able to maintain the older pediatric groups (2 to <6 years and 6 to <12 years) within the target range of exposures, lower or higher weight patients within those groups were likely to fall outside the target exposure range. Baseline body weight was a statistically significant covariate on volume and clearance in the pooled analysis. Therefore, current models support a weight band-based dosing approach based on PPK and PBPK, both of which resulted in similar extrapolated dosing schemes for children (Table 1). Once a larger pediatric dataset is available, a covariate analysis will be conducted to evaluate other potential covariates (eg, age, sex, baseline hemoglobin, and concomitant hydroxyurea use) on voxelotor whole blood and plasma exposures.

Table 1.

Voxelotor Projected Doses for Children Based on PPK and PBPK Modeling

Population Weight Band	900 mg-Equivalent Adult Dose*	1500 mg-Equivalent Adult Dose*
5 to <10 kg	200 mg	400 mg
10 to <20 kg	400 mg	600 mg
20 to <40 kg	600 mg	900 mg
≥40 kg	900 mg	1500 mg

*Note that the 900 mg, 1500 mg, or both doses may be used in the HOPE Kids 2 study.

Summary/Conclusion: The HOPE KIDS 2 trial uses a novel approach to identify a weight band-based voxelotor dosing regimen for infants and children with SCD which will help simplify dosing in the pediatric population.

PS1454

EARLY RECOGNITION OF THE SICKLING PROCESS OF ERYTHROCYTES BY LABEL-FREE LIGHT MICROSCOPIC IMAGING IN PATIENTS WITH SICKLE CELL DISEASE

I. Metze[1,*], F. Borges da Silva[2], K. Metze[3]

[1]Hematology - Hemotherapy Center, [2]University of Campinas, Campinas, Brazil, [3]Pathology, University of Campinas, Campinas, Brazil

Background: Fluorescence lifetime imaging microscopy (FLIM) is able to reveal physicochemical characteristics of the cells. In former works, we have shown with this technique that does not need staining, that it is possible to identify the lineage of immature hemopoietic precursors with this technique.
Aims: Sickle cell disease is characterized by hemoglobin polymerization in erythrocytes and FLIM could be able to recognize these conformational changes.
Methods: unstained air-dried peripheral blood smears of 32 patients with sickle cell diseases (23 cases homozygous SS, 4 cases Sβ, 3 cases Sαtal and 2 SC) and of 31 patients without hematologic disease were analyzed. Images were captured with a confocal Zeiss Upright LSM780-NLO microscope and single-photon counting equipment [Becker&Hickl] to acquire the FLIM images. Pseudo-colors according to the rainbow spectrum were attributed to different fluorescence lifetimes.
Results: Normal erythrocytes (Figure 2a) from the controls had short lifetimes (205.2±51.0 ps). Normally shaped erythrocytes in smears of homozygous SS patients (Figure 2b-d) had 183.3±36.1 ps, crenated erythrocytes had 272.5±74.1 ps and drepanocytes had 345.8±85.8 ps. Concerning mixed hemoglobinopathies normally shaped erythrocytes had 241.6±19.9 ps and drepanocytes had 390.3±68.3. Normally shaped erythrocytes were significantly different among the 3 groups p=0.004 although homozygous SS patients had lower values than mixed hemoglobinopathies. However, drepanocytes had similar values for all hemoglobinopathies. We could exactly localize the regions of higher lifetime values which are equivalent to areas of hemoglobin polymerization, when compared with electron microscopy (Figure 2e).

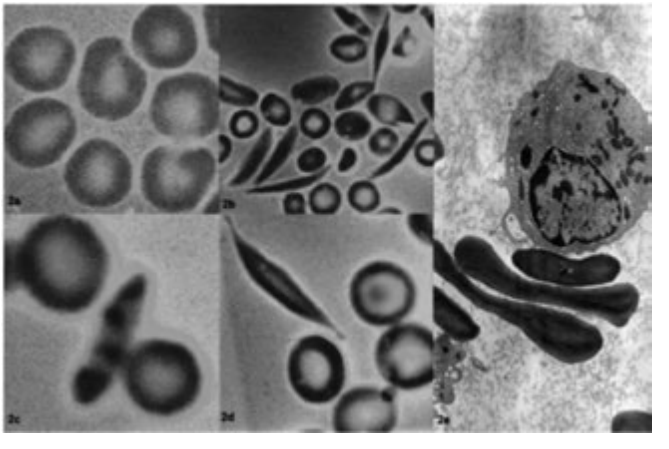


Figure 1.

Summary/Conclusion: The FLIM technique could show differences among normal cases and sickle cell diseases in normally shaped erythrocytes. It also could show increased lifetimes of hemoglobin autofluorescence in polymerized areas, thus demonstrating precisely the topographic distribution of polymerized hemoglobin.
Supported by : FAPESP (Research Foundation of São Paulo State) and CNPq (National Research Council).

PS1455

SICKLE CELL DISEASE SEVERITY MEASURE: DEVELOPMENT, TRANSLATION, AND PATIENT CULTURAL SENSITIVITY VALIDATION

L. Burke[1], K. M. Fox[2], K. Bridges[3,*], M. Kraus[3], J. Lehrer[3], J. Hobart[4]

[1]LORA Group, LLC, Royal Oak, MD, [2]HealthiVibe, LLC, Arlington, VA, [3]Global Blood Therapeutics, South San Francisco, CA, United States, [4]Plymouth University Penin-

sula Schools of Medicine and Dentistry, Plymouth, United Kingdom

Background: Sickle cell disease (SCD) is an autosomal recessive disorder where polymerization of deoxygenated sickle hemoglobin leads to red blood cell sickling, hemolysis, anemia, fatigue, tissue ischemia, severe pain crisis, and organ damage, all of which lower quality of life. The Sickle Cell Disease Severity Measure (SCDSM) is a novel 9-item electronic patient-reported outcome (PRO) instrument designed to evaluate changes in SCD symptom burden-the first created in strict accord with US Food and Drug Administration (FDA) guidance for clinical trial endpoints. Subjective language use around pain and fatigue, the core symptoms of SCD, made the proper translation and cultural adaptation of the instrument paramount for the global phase 3 Hemoglobin Oxygen affinity modulation to inhibit sickle hemoglobin PolymErization (HOPE) trial (GBT440-031) of voxelotor (GBT440).
Aims: To translate and culturally adapt the SCDSM for use in the HOPE trial.
Methods: The SCDSM was developed to assess the core symptoms of SCD according to FDA requirements (21 CFR 314.126) in patients with SCD in the US and UK. Translation and cultural adaptation adhered to the guidelines of the International Society for Pharmacoeconomics and Outcomes Research. The SCDSM was forward-translated twice by in-country native speakers of the given language, with discrepancies between translations resolved during reconciliation. The SCDSM was then back-translated into English and reviewed against the original version of the PRO, with discrepancies addressed during harmonization. The cognitive debriefing interviews were conducted face-to-face, with in-country native speakers of the target language as translators. Five qualified respondents per target country/language provided feedback on the translated material. During the interviews, each item was discussed separately to determine whether the wording made items difficult to answer or understand, and whether the respondent would have phrased the text differently. Last, the cognitive debriefing results were reviewed and finalized.
Results: The penultimate SCDSM version comprised 10 questions that were reduced to a final set of 9 when cognitive debriefing showed ambiguity around 1 question between US and UK English speakers. Longitudinal evaluation during part A of the HOPE trial involved 2821 person-screening days in the total dataset and 2515 PRO completions. Patients completed 3 anchor questions daily to evaluate sensitivity to change and estimate meaningful change score. The SCDSM quantitated daily patient symptoms reliably and precisely and detected clinically meaningful symptom changes reliably and reproducibly. Symptom exacerbations were much more frequent than vaso-occlusive crisis events. The SCDSM was translated and culturally adapted into Dutch, French, Italian, German, Turkish, Arabic, Swahili, Twi, and Luo. High fidelity of translation and cultural adaptation was found for all languages irrespective of language root or the overarching social/economic milieu of a language. The validation of the SCDSM showed that it is appropriate for use in the languages spoken in the countries enrolling adolescents and adults with SCD in the HOPE trial.
Summary/Conclusion: The SCDSM is an instrument designed to capture core signs and daily symptoms of SCD. As language and its cultural equivalence present an important hurdle to PRO instrument use across cultures, the real-world experience of developing and adapting the SCDSM is a prototype for developing PROs that meet the FDA bar for trial endpoints across therapeutic areas.

PS1456

IMPROVEMENT OF THE MULTIDISCIPLINARY MANAGEMENT OF SICKLE CELL DISEASE PATIENTS: IMPLEMENTATION OF A SICKLE CELL DISEASE PATIENT'S CLINICAL DATABASE: BELGIAN REGISTER OF SICKLE CELL DISEASE PATIENTS

S. Wambacq[1], B. Gulbis[2,*], A. Ferster[1,3], L. Phu Quoc[1,4,5]

[1]Pediatric Hemato-Oncology, Hopital des Enfants Reine Fabiola, Brussels (Laeken), [2]Department of Clinical Chemistry, LHUB-ULB - Hôpital Erasme, Brussels, [3]Faculty of Medicine, ULB, Brussels (Laeken), [4]Faculty of Medicine, ULB, Brussels, [5]Peadiatrics, HIS, Brussels (Etterbeek), Belgium

Background: Sickle Cell Disease (SCD) is the most frequent genetic disease in the Brussels region, with a total amount of cases which is keeping on growing. The disease targets merely patients of African origin. This pathology has a low recognition and disease modifying therapies have been developed only in the last 20 years. The number of cases will increase due to reduction of mortality during childhood. We do not have reliable data on the demographical profile of the Sickle Cell Anemia population in Belgium, on the survival rate, amount and type of infections, frequency of follow up, amount of patients treated with hydroxyurea, chronic transfusion or bone marrow transplantations, number of transfusions, socio-economical situations and schooling. The exploitation of the existing clinical database (2008, financed by IRIS research) allowed major improvements and gives us unique data about mortality of the Sickle Cell Anemia patients as well as the benefit of the neonatal screening.[1]
This database included patients of eight centers. From 2008 until 2012 al available data were recorded retrospectively and prospectively. Data were registered from neonatal screening or from diagnosis (first contact) until last follow-up or death. Data included diagnosis, demography, and outcome data.
Aims: 1. To keep the data of the existing register and build a new register with a larger amount of participating institutes (national). 12 centers are included, and at least 3 more centers are candidate to participate. This will result in coverage of approximatively 90% of the Belgian SCD patients (approximatively 1000 cases). 2. To maintain a biological, clinical database of Sickle Cell Disease patients and improve knowledge and collaboration in Sickle Cell Disease. The register has been adapted in a new IRIS project with following aims : a) Follow up of mortality and morbidity; b) Keep a national surveillance tool to improve the prevention and screening of complications; c) Establish new recommendations based upon the collected results; d) Most important data easy accessible and visible; e) FAIR Data – SNOMED coded; f) Management of statistics; g) Easily operated. 3. The collected data will be used to have a continued and actual vision of the number of cases and to emit public health recommendations. The results will be published in an annual report. These data will be scientifically exploited and compared with international data. This observation and collection of data will endorse communication with the actors of public health, families, patient associations and authorities. The database is secured and respects the legal requirements of privacy (anonymized).
Methods: See aims.
Results: The Belgian SCD register is operational since March 2018 at https://www.drepano.be/drepano. In 2019 a first annual report of the registered data will be generated (Figure 1).

Figure 1.

Summary/Conclusion: We developed an easily operable database to improve of the multidisciplinary management of sickle cell disease patients. Finally, this database could serve as framework for other chronical pathologies. Later on it could be picked up in the framework of the registers of 'rare diseases', shared with other EU countries and be inter-operable with other platforms respecting the legal and privacy requirements.

Reference
1. Lê P Q *et al.*, Survival among children..., Pediatr Blood Cancer 2015 Jul 14.

PS1457

VARIATIONS IN COMPLICATIONS RELATED TO VASO-OCCLUSIVE CRISES AND HYDROXYUREA USE IN PATIENTS WITH SICKLE CELL DISEASE: OBSERVATIONS FROM STUDIES IN THE USA, SPAIN AND THE GULF REGION

E. Cela[1,*], Y. Wali[2], L. Bronte[3,4], L. Debonnett[5], M. Heeney[6]

[1]Pediatric Hematology Unit, Hospital General Universitario Gregorio Marañón, Facultad de Medicina, Universidad Complutense de Madrid, Madrid, Spain, [2]Pediatric Hematology Unit, Sultan Qaboos University Hospital, Muscat, Oman, [3]Department of Epidemiology and Public Health, University of Miami, Miller School of Medicine, Miami, FL, [4]Foundation for Sickle Cell Disease Research, Hollywood, FL, [5]Novartis Pharmaceuticals, East Hanover, NJ, [6]Harvard Medical School, Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Boston, MA, United States

Background: Sickle cell disease (SCD) is a complex genetic disease and a

global health problem, with a changing geographical distribution due to migration. Vaso-occlusive crises (VOCs) can cause episodes of acute, intense pain, which are the primary cause of hospitalization in SCD and can lead to life-threatening complications such as acute chest syndrome (ACS) and stroke. Repeated vaso-occlusive episodes can result in end-organ damage. Hydroxyurea (HU) is widely used for preventing VOCs, however many pts experience recurrence while receiving HU or are unwilling to use it. Although regional variations in SCD genetics have been reported, data on regional differences in VOCs and other complications are lacking.
Aims: To evaluate variations in SCD patient characteristics from studies conducted in the USA, Spain and the Gulf region (Qatar, UAE and Oman).
Methods: We analyzed data from four observational studies. CICL670A2418 (NCT01441375) was a retrospective study of the medical records of 254 SCD pts aged ≥16 years in three US centers (US study 1). An objective of the study was to evaluate SCD complication rates and utilization of healthcare delivery, during an average of 7.7 years' observational data (Jordan *et al. Curr Med Res Opin* 2015). CICL670AUS38 (NCT01220115) was a prospective study documenting clinical outcomes in 498 SCD pts (2–<18 years, n=317; ≥18 years, n=181). Pts were enrolled from 57 US centers (US study 2), with data collected from medical histories ≤5 years before enrollment (Heeney *et al. Blood* 2014). CICL670AES15T was a retrospective/prospective study in 615 SCD pts aged <18 years enrolled in the Hemoglobinopathy Pediatric Spanish Registry. Data were collected from 50 hospitals, with a mean follow-up of 5.8 years (Cela *et al. Pediatr Blood Cancer* 2017). CICL670AAE02 was a cross-sectional study of 410 SCD pts aged 2–16 years that described SCD patient characteristics, disease and treatment profile. It was conducted in 3 centers in the Gulf region, with data collected from the previous 3 years. Assessments performed in each study included: patient characteristics; SCD genotype; the proportion of pts with VOCs, ACS, priapism and stroke; hospitalization information; and HU use.
Results: The proportion of pts with the HbSS genotype ranged from 52.9% (the Gulf study) to 87.4% (US study 1) (Table 1). The US and Gulf studies reported acute pain crisis/VOC in 74.4–84.4% of pts, compared with 21.3% in the Spanish study. ACS was reported in 15.9% to 35.1% of pts across the studies. Priapism was reported in 5.1–10.2% of pts in the US, while in the Spanish and Gulf studies these proportions were 0.3–0.7%. In US study 1, stroke was reported in 13.4% of pts compared with a range of 1.0–4.6% in the other studies. HU use ranged from 25.8% to 47.4%, whereas 66.9% to 82.9% of pts across the studies were hospitalized at least once.

Table 1.

Patient characteristics, complications, HU use and hospitalizations from four studies in the USA, Spain and the Gulf

	US study 1 (N=254)	US study 2 (N=498)*	Spain (N=615)[a]	Gulf (N=410)
Study/analysis dates	July 2011 – July 2012	Jan 2010 – Sept 2014	Jan 2014 – May 2015	Feb 2014 – Aug 2016
Patient characteristics				
Mean age ± SD, years	27 ± 11	18.5 ± 15.2	8.1	9.3
HbSS patients, n (%)	222 (87.4)	369 (74.1)	497 (80.8)	214 (52.2)
Black/African American patients, n (%)	254 (100)	469 (94.2)	NR	53 (12.9)
Complication rate				
Acute pain crises/VOCs, n (%)	189 (74.4)[b]	385 (77.3)[b]	131 (21.3)	346 (84.4)**
ACS, n (%)	44 (17.3)[b]	175 (35.1)[b]	99 (15.9)	74 (18.0)**
Stroke, n (%)	34 (13.4)[b]	23 (4.6)[b]	16 (2.6)	4 (1.0)**
Priapism[††], n (%)	13 (5.1)[b]	27 (10.2)[b]	2 (0.3)	3 (0.7)**
HU use				
Patients receiving HU, n (%)	NR	236 (47.4)[b]	159 (25.8)	184 (44.9)
Hospitalization data				
Patients hospitalized[‡‡], n (%)	170 (66.9)	413 (82.9)[b]	NR	319 (77.8)

*Data for pediatric and adult patients combined. [a]Median age shown; ethnicity not recorded in the registry. [b]Data from observation period (7.7 years); stroke data are from the history and observation period. [c]Data from the 5 years prior to enrollment. **Data from the previous 3 years. Percentages calculated based on the total number of patients, not the number of patients who experienced complications (n=222). ††Priapism calculated based on all patients. ‡‡For SCD-related complications in US study 1, for any reason in US study 2, and for VOCs or other complications in the Gulf study. ACS, acute chest syndrome; HU, hydroxyurea; NR, not reported; SD, standard deviation; VOC, vaso-occlusive crisis.

Summary/Conclusion: The results highlight the significant burden of complications related to VOCs in SCD, most notably acute pain crises and other serious manifestations. The results also indicate that many pts with SCD are hospitalized despite receiving HU. Regional differences in the frequency of complications were observed (see Table 1). These may be related, but not limited to, differences in the population characteristics (e.g. age, proportion of HbSS genotype) and the non-interventional nature of data collection. Migration patterns also contribute to the changing demographics of SCD in different countries. Individually tailored therapies are needed to help improve well-being in this under-served patient population.

PS1458

CORRELATION BETWEEN HEMOLYSIS AND HYPERCOAGULABILITY MARKERS AND EFFECTS OF HYDROXYUREA THERAPY IN SICKLE CELL PATIENTS

W. V. Tonassé[1,*], F. Chénou[1], I. F. Domingos[2], T.H.C. Batista[2], R.M. Santana[2], A. S. Araújo[3], M.A.C. Bezerra[2], F.F. Costa[4], M.F. Sonati[5], M.N.N. dos Santos[5]

[1]Department of Clinical Pathology, School of Medical Sciences, State University of Campinas-UNICAMP, CAMPINAS, [2]Department of Genetics, Federal University of Pernambuco (UFPE), [3]Hematology and Hemotherapy Foundation of Pernambuco (HEMOPE), Recife-PE, [4]Hematology and Hemotherapy Center, State University of Campinas-UNICAMP, [5]Department of Clinical Pathology, School of Medical Sciences, State University of Campinas-UNICAMP, Campinas-SP, Brazil

Background: Sickle cell anemia (SCA) is characterized by chronic hemolysis and vasculopathy complications as the process of hemolysis with the release of hemoglobin (Hb) contained in erythrocytes is an important mediator in vascular diseases. Plasma Hb (pHb) and its heme component have been found to trigger specific pathophysiologies, such as acute and chronic vascular disease, inflammation, abnormal endothelial activation, vasoocclusion, thrombosis, stroke and renal impairment, all associated with adverse clinical outcomes in patients with hemolysis. However, the role of intravascular hemolysis (IH) on hemostatic alterations remains unclear.
Aims: This cross-sectional study aimed to evaluate the relation between plasmatic levels of hemolysis and hypercoagulability markers in Brazilian patients with SCA hydroxyurea-naïve and SCA patients on hydroxyurea therapy.
Methods: A total of 62 adults (19 – 42 years) SCA patients: 32 hydroxyurea-naïve (HbSS) and 30 on hydroxyurea therapy (HbSS-HU) were included in the study, all in steady state, followed in Pernambuco s Blood Center HEMOPE. The Hb profile was investigated by high-performance liquid chromatography. Levels of pHb and Lactate Dehydrogenase (LDH), as IH markers, were measured by enzyme-linked immunoassay and Cobas C501 analyzer, respectively. Hypercoagulation markers such as D-dimer and fibrinogen levels were quantified using Dade Thrombin Reagent Multifibren® U and immunoturbidimetric assay Innovance D-DIMER in Sysmex® CA-1500. Data were analyzed with GraphPad Prism 6 and SPSS v23 and significance set at $P \leq 0.05$. Correlation coefficients (r) and P values are based on Spearman correlation test. Mann-Whitney U test was used to compare data between HbSS and HbSS-HU groups.
Results: The study findings suggest that there is a significant positive correlation between pHb and LDH (r=0.396, p=0.030) and a negative correlation between both pHb, LDH and fibrinogen in HbSS patients. [r = -0.377 (p=0.036) and r = -0.405 (p=0.032) respectively]. In addition, HbSS patients had higher hemolysis rate in comparison to HbSS-HU patients as evidenced by pHb levels (3.29 mg/dL vs 1.59 mg/dL, $P_{pHb} = 0.003$) and LDH levels (644.0 U/L vs 426.5 U/L, $P_{LDH} = 0.05$). Similarly, D-dimer levels were higher in HbSS when compared to HbSS-HU patients (2144 ng/mL vs 1496 ng/mL, p=0.045). Although, there was no statistically significant difference between fibrinogen levels in HbSS patients and HbSS-HU patients (Median: 238,8 mg/dl and 279.8 mg/dl, p=0.06).
Summary/Conclusion: Overall, higher IH rate was associated with more activation of coagulation in SCA patients and fibrinogen consumption, suggesting an important role of the pHb in the activation process of coagulation in this disease. Hydroxyurea therapy is associated with a reduction in the IH and hypercoagulation state in SCA patients, suggesting a new clinical benefit for the use of hydroxyurea in SCA patients.

PS1459

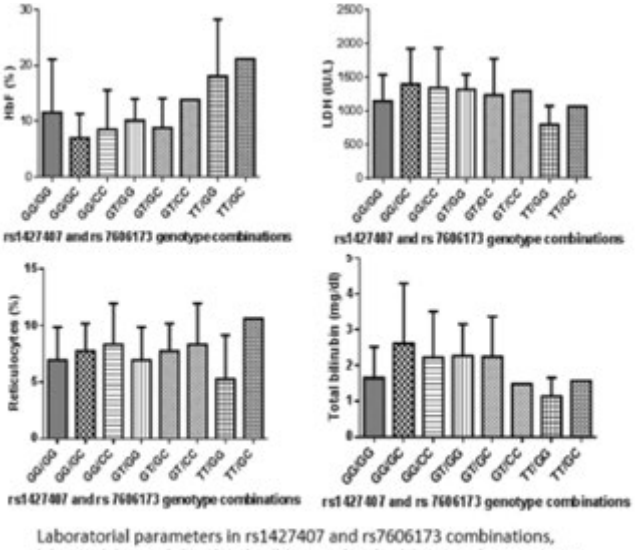
INFLUENCE OF BCL11A POLYMORPHYSMS ON FETAL HEMOGLOBIN LEVELS AND HAEMOLYSIS MARKERS IN SICKLE CELL ANEMIA PEDIATRIC PATIENTS NOT RECEIVING HYDROXYUREA

R.M.L. Rahimy[1,2], C.S.A. Adanho[1,2], R.P. Santiago[2,3], C. Vieira[2], C.C. Guarda[1], S.S. Santana[1,2], S.C.M.A. Yahouedehou[1,2], K.B.C. Caetano[1,4], T.N. Pitanga[1], J.R.D. Ferreira[4], L.D.C. Oliveira[3], V.V. Maffili[1], I.M. Lyra[2], D.L. Zanette[1,*], M.S. Goncalves[1]

[1]Laboratory of Investigation in Genetics and Translational Hematology, Gonçalo Muniz Institute / Fiocruz, [2]Faculty of Medicine, Federal University of Bahia, [3]Laboratory of Investigation in Genetics and Translational Hematology , Gonçalo Muniz Institute /Fiocruz, [4]Faculty of Pharmacy, Federal University of Bahia, Salvador, Brazil

Background: Fetal hemoglobin (HbF) levels are one of the main determinants of the clinical presentation of sickle cell anemia, since high levels of HbF are associated with reduced polymerization of hemoglobin S, thus alle-

viating vaso-occlusion and hemolysis. Certain polymorphisms of the *BCL11A* gene are capable of modulating basal levels of HbF, even in the absence of any therapeutic intervention.
Aims: The main objective of this study was to analyze the influence of two polymorphisms in the *BCL11A* gene (rs1427407 and rs7606173), on the levels of HbF, bilirubin, lactate dehydrogenase (LDH) and reticulocytes count in pediatric patients with sickle cell anemia that were not under hydroxyurea treatment.
Methods: Whole blood and serum samples were used for hematological and biochemical analysis. DNA was extracted from EDTA-whole blood samples, and the fragments containing the gene polymorphisms were amplified by PCR followed by Sanger sequencing with specific primers. Free and informed consent was obtained from the legal guardians of all patients included in this study.
Results: Seventy-three patients fulfilled the inclusion criteria, aged 2 to 17 years. The frequency of the mutant T allele of rs1427407 was 0.22 and that of the wild-type G allele of rs7606173 was 0.57. The TT mutant genotype of rs1427407 showed the highest HbF levels as compared to GT and GG genotypes (p=0.0382). On the other hand, for the wild-type GG genotype of rs7606173 no significant increase in HbF level was observed, when compared to the GC and CC genotypes. The mean reticulocyte and LDH values were lower in the mutant TT and wild-type GG genotypes of rs1427407 and rs7606173, respectively, although the differences were not significant. The lowest bilirubin levels were found in the mutant TT genotype of rs1427407 and wild type GG polymorphisms of rs7606173, when compared to the other genotypes. Total and indirect bilirubin levels were significantly different between GG and GT genotypes of rs1427407 for (p=0.0102 and p=0.0108, respectively). Regarding bilirubin levels, it is important to take into consideration the high frequency of rs8175347 polymorphism of *UGT1A1* gene in the population analyzed in previous observations of the research group. This polymorphism corresponds to the presence of additional thymine-adenine (TA) repeats in the promoter region of *UGT1A1*, which results in increased indirect bilirubin levels. Lower levels of indirect bilirubin were found in individuals that simultaneously carried the TT mutant genotypes for *BCL11A* rs1427407 and *UGT1A1* rs8175347 (7 to 8 TA repeats). By combining the genotypes of the two polymorphisms of *BCL11A*, rs1427407 and rs7606173, no individuals with the mutant TT / CC association were found. The individuals carrying the TT/GG combination had lower percentage of reticulocytes and lower levels of LDH and bilirubin; while HbF average levels were twice higher than in individuals carrying the GG/CC combination. Accordingly, the mean number of painful crises was three times lower in the GG / CC group in the year prior to data collection. Figure 1 summarizes the data described here.



Laboratorial parameters in rs1427407 and rs7606173 combinations, (a): Fetal hemoglobin levels. (b): LDH levels. (c): Reticulocytes count. (d): Total bilirubin. HbF: Fetal hemoglobin, LDH: lactate dehydrogenase

Figure 1.

Summary/Conclusion: The mutant T allele of rs1427407 and the ancestral G allele of rs7606173 of *BCL11A* gene were associated with higher HbF levels and lower values of some laboratory hemolysis markers, showing more evident effect for rs1427407 T allele. We also report that the effect of rs1427407 and rs7606173 appears to overcome the effect of rs8175347 on bilirubin levels in patients with $(TA)_{7/7}$ and $(TA)_{7/8}$ polymorphisms in the promoter region of the *UGT1A1* gene.

PS1460

TICAGRELOR VERSUS PLACEBO FOR THE REDUCTION OF VASO-OCCLUSIVE CRISES IN PEDIATRIC SICKLE CELL DISEASE: DESIGN OF A RANDOMIZED, DOUBLE-BLIND, PARALLEL-GROUP, MULTICENTER PHASE 3 STUDY (HESTIA3)

M.M. Heeney[1,*], M.R. Abboud[2], C. Amilon[3], M. Andersson[4], J. Githanga[5], B. Inusa[6], J. Kanter[7], M. Leonsson-Zachrisson[8], A. D. Michelson[9], A.R. Berggren[8]

[1]Hematology, Sickle Cell Program, Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Harvard Medical School, Boston, United States, [2]American University of Beirut Medical Center, Beirut, Lebanon, [3]Early Clinical Development, IMED Biotech Unit, [4]Global Medicines Development, AstraZeneca, Gothenburg, Sweden, [5]Haematology and Blood Transfusion Unit, Department of Human Pathology, University of Nairobi, Nairobi, Kenya, [6]Paediatric Haematology, Evelina London Children's Hospital, Guy's and St Thomas' NHS Trust, London, United Kingdom, [7]Sickle Cell Disease Research, Pediatric Hematology-Oncology, Medical University of South Carolina, Charleston, United States, [8]Global Medicines Development, AstraZeneca R&D, Gothenburg, Sweden, [9]Center for Platelet Research Studies, Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Harvard Medical School, Boston, United States

Background: There is a high unmet need for therapies to reduce the complications of sickle cell disease (SCD), including vaso-occlusive crises (VOCs), in pediatric patients. Activated platelets contribute to the formation of cell aggregates during sickling. Platelet inhibition, therefore, has the potential to reduce VOC rates. Ticagrelor is an oral, direct-acting, and reversible $P2Y_{12}$ receptor antagonist that inhibits adenosine diphosphate–mediated platelet activation and aggregation. Ticagrelor has a wealth of data in adult cardiac patients and is approved to reduce the rate of cardiovascular death, myocardial infarction (MI), and stroke in adults with acute coronary syndrome (90 mg BID) and adults with a history of MI (60 mg BID). The HESTIA program is currently ongoing to explore the potential therapeutic benefits of ticagrelor for the reduction of VOCs in SCD; the pharmacokinetics (PK), pharmacodynamics (PD), and tolerability of a broad range of ticagrelor doses were investigated in phase 2 studies in children (3-17 y; HESTIA1) and young adults (18-30 y; HESTIA2) with SCD. Though ticagrelor was well tolerated in SCD patients, larger and longer-term studies are needed to assess its safety and ability to reduce VOC rates.
Aims: HESTIA3 will evaluate the efficacy, safety, and tolerability of ticagrelor *versus* placebo over a period of 1 y and up to approximately 2 y in pediatric patients with SCD
Methods: Planned enrollment is approximately 182 patients with SCD (randomized 1:1 to receive either ticagrelor or placebo) confirmed for homozygous sickle cell (HbSS) or sickle beta zero thalassemia (HbS/β^0) and with ≥2 VOCs in the year prior to Visit 1 (Figure 1). At least 50 evaluable patients in each age group (≥2 to <12 y and ≥12 to <18 y) will be recruited from approximately 20 countries worldwide, including Ghana, Kenya, South Africa, Tanzania, Egypt, Lebanon, Canada, US, Italy, UK, and India. The study drug will be given in addition to standard, locally available treatments for SCD (*e.g.*, stable dose hydroxyurea). Body-weight adjusted ticagrelor doses (15, 30, or 45 mg BID) were identified based on PK/PD modeling and simulation of phase 2 data. The selected ticagrelor doses are projected to achieve greater platelet inhibition than that observed in earlier efficacy trials of platelet inhibition in pediatric SCD. The predicted level of platelet inhibition with the selected doses is ~35% to 80% reduction in $P2Y_{12}$ reaction units from baseline compared with placebo.

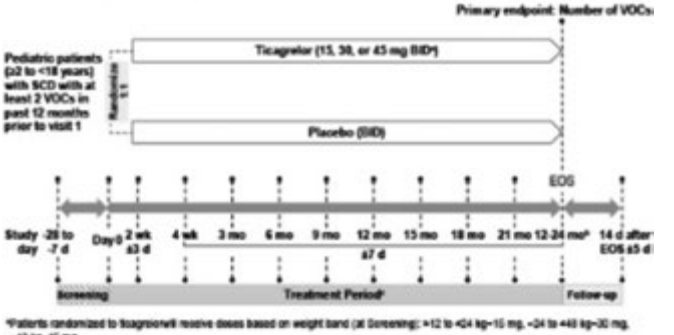


Figure 1. HESTIA3 study design.

Results: The primary endpoint is the number of VOCs (the composite of a

painful crisis of ≥2 h duration [treated in a medical setting or at home] and/or acute chest syndrome). Secondary endpoints include hospitalizations, symptomatic disease burden, pain intensity and analgesic use during VOCs, acceptability of formulation, and health-related quality of life as measured by the Pediatric Quality of Life Inventory (PedsQL) SCD Module and Fatigue total score and by dimension using the PedsQL Multidimensional Fatigue Scale. Safety will be assessed by adverse events, including bleeding events, and laboratory assessments. Platelet inhibition data, measured by the vasodilator-stimulated phosphoprotein (VASP) assay, will be collected for exploratory purposes.
Summary/Conclusion: HESTIA3 will provide long-term data on the effects of ticagrelor in the management of VOCs in pediatric patients with SCD. This trial will target therapeutic platelet inhibition, which is expected to translate into improved clinical results. The first patient is expected to be enrolled during the second quarter of 2018.

PS1461

NOVEL TRIAL DESIGN TO EVALUATE ORAL VOXELOTOR FOR THE TREATMENT OF SICKLE CELL DISEASE: THE PHASE 3 HEMOGLOBIN OXYGEN AFFINITY MODULATION TO INHIBIT SICKLE HEMOGLOBIN POLYMERIZATION (HOPE) TRIAL

K. I. Ataga[1,*], C.C. Hoppe[2], R.E. Ware[3], J. Howard[4], M. Tonda[5], J. Ganju[5], J. Lehrer[5]

[1]University of North Carolina Comprehensive Sickle Cell Program, Chapel Hill, NC, [2]UCSF Benioff Children's Hospital Oakland, Oakland, CA, [3]Cincinnati Children's Hospital, Cincinnati, OH, United States, [4]Guy's and St Thomas' NHS Foundation Trust and King's College London, London, United Kingdom, [5]Global Blood Therapeutics, South San Francisco, CA, United States

Background: Design of clinical trials for sickle cell disease (SCD) is challenging in part because of a lack of consistent end point definitions. Vaso-occlusive crisis (VOC), the most frequently used end point, does not measure full disease burden and reflects health care utilization and attitudes of patients and health care professionals. In phase 1/2 studies of patients with SCD, treatment with voxelotor (GBT440), an inhibitor of sickle hemoglobin polymerization, has resulted in increased hemoglobin (Hb) levels and reduced hemolysis and was well tolerated. Voxelotor is being evaluated in the ongoing HOPE trial using Hb as the primary study end point, along with novel secondary end points.
Aims: The innovative phase 2/3 HOPE trial design with novel primary and secondary end points to accelerate drug development will be presented.
Methods: HOPE (NCT03036813) is a randomized, placebo-controlled, multicenter study of oral voxelotor in patients with SCD (aged 12-65 years) with baseline Hb 5.5-10.5 g/dL and 1-10 episodes of VOC in the previous year. The study population consists of 3 patient cohorts: Group 1, Group 2, and Group 3. To accelerate clinical development of a potential SCD therapy, the study seamlessly combines phase 2 dose selection (Groups 1 and 2) and the phase 3 pivotal cohort (Groups 2 and 3). Patients in Group 1 (n=60) and Group 2 (n=180) will be randomly assigned 1:1:1 to voxelotor 900 or 1500 mg/day or placebo. Patients in Group 3 will be randomly assigned 1:1 to the selected voxelotor dose or placebo. Patients in Group 2 may be combined (up to 90 patients) with those in Group 1 for the dose selection analysis to provide flexibility in the sample size of the dose selection portion of the study. Analysis for voxelotor dose selection will occur when the final patient (up to 150 total patients from Groups 1 and 2) has received 12 weeks of treatment. Group 2 patients randomized to placebo and the selected dose who were not unblinded for the dose selection analysis will become part of the phase 3 pivotal cohort. Group 2 patients randomized to the nonselected dose will be offered enrollment in an open-label extension study. The primary end point, an increase in Hb >1 g/dL from baseline to 24 weeks, is an objective and clinically relevant laboratory measure of disease modification based on voxelotor mechanism of action. This trial is the first to use the 9-item Sickle Cell Disease Severity Measure, a patient-reported outcome (PRO) specifically developed for the HOPE study following FDA guidance, as a secondary end point. This novel electronic PRO will evaluate changes in severity and exacerbation of core SCD symptoms (eg, pain and fatigue-related symptoms) from baseline to 24 weeks. Additional secondary end points include measures of hemolysis, rates of VOC, transfusions, and opioid use. The study is designed to enable selection of PRO-defined symptom exacerbations or traditionally defined VOC as the key secondary end point based on the Group 1 analysis.
Results: This study is ongoing.
Summary/Conclusion: The HOPE trial, expected to complete enrollment by late 2018, will evaluate the efficacy and safety of voxelotor compared with placebo in patients with SCD. The phase 2/3 design with novel clinical end points may accelerate voxelotor development and provide objective and relevant measures of efficacy and clinical outcomes.

Stem cell transplantation – Clinical

PS1462

OUTCOME OF PHILADELPHIA CHROMOSOME-POSITIVE ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS RECEIVING TYROSINE KINASE INHIBITOR MAINTENANCE THERAPY AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

T. Tapaninen[1,*], A. Nihtinen[1], R. Niittyvuopio[1], V. Lindström[1], J. Heiskanen[1], E. Martelin[1], M. Itälä-Remes[1]

[1]Helsinki University Hospital Comprehensive Cancer Center, Helsinki, Finland

Background: Prognosis of Philadelphia chromosome-positive adult acute lymphoblastic leukemia (Ph+ALL) has improved with allogeneic hematopoietic stem cell transplantation (HSCT) and the addition of tyrosine kinase inhibitors (TKIs) to the front-line therapy. However, relapses occur. The role of post-transplant TKI maintenance therapy is unclear.
Aims: This study aimed to evaluate the efficacy of TKI maintenance after HSCT in adult patients with Ph+ALL.
Methods: A retrospective study of all adult Ph+ALL patients undergoing HSCT in Helsinki University Hospital between 2004-2015 was performed (Group 1). According to local practice, TKI maintenance for at least two years was recommended to all patients after HSCT regardless of the PCR-based minimal residual disease-status (MRD). Imatinib was the primary drug in 2004-2011, and since 2012, dasatinib was preferred. All patients in Group 1 had received TKI prior to HSCT. The outcomes were compared to a TKI-naïve historical cohort from 1996-2002 (Group 2).
Results: Group 1 consisted of 29 patients (62% male) with a median age at HSCT of 44 years (range 21-65). Median post-HSCT follow up of the living patients was 8.4 years (2.1-14). 34% had a sibling donor and 24% a bone marrow graft. All patients received a myeloablative conditioning (MAC), and 79% received TBI 12 Gy. Group 2 included 19 patients (58% male) with a median age of 47 years (19-60) and a median follow up of 18 years (15-20). 63% had a sibling donor and 53% a bone marrow graft. All had MAC, and 95% received TBI. 24 of the 29 patients (83%) in Group 1 had TKI maintenance. 15 patients received imatinib (max. 400 mg/day for all), and 9 dasatinib (median max. 70 mg/day). Median time to TKI initiation after HSCT was 7.6 weeks (2.9-68), and the median duration of maintenance 22 months (2.1-93). TKI was not initiated for 5 patients due to transplant-related complications. OS rates for Group 1 (n=29) and Group 2 (n=19) were 72.4% vs 36.8% (p=0.048), and the respective TRM rates 14.5% vs 16.1% (p=0.819). Subsequently, outcome of the 24 patients receiving TKI maintenance in Group 1 was studied according to their MRD status. MRD was detectable before HSCT in 17 patients and negative in 7. OS rates for these patients were 76.5% vs 85.7% (p=0.566), and PFS 58.8% vs 71.4% (p=0.578), respectively. For the 6 patients who were MRD-positive at 12 weeks and 18 that were MRD-negative, OS were 66.7% vs 83.3% (p=0.289), and PFS 50% vs 66.7% (p=0.307), respectively. Imatinib maintenance was started before the 12-week sample in 12 patients and dasatinib in 7. Latest relapse occurred 2.7 years after HSCT. Intolerance to TKI lead to interruption of the maintenance at some point in 11 of the 15 patients (73%) receiving imatinib (most commonly due to neutropenia) and 6 of the 9 patients (67%) receiving dasatinib (neutropenia or infection). Thus, 7 of the 29 patients (24%) in Group 1 had full-term TKI maintenance. OS for the imatinib-treated vs dasatinib-treated patients were 86.7% vs 66.7% (p=0.271), and PFS 73.3% vs 44.4% (p=0.126), respectively.
Summary/Conclusion: Outcome of Ph+ALL patients treated with TKI before and after HSCT was superior compared to TKI-naïve patients, explained by reduced leukemia-related mortality. In the TKI-era, MRD status prior to HSCT did not affect the outcome of patients receiving MAC. Due to the small sample size, no conclusions can be drawn about the potential benefits of starting TKI maintenance to all Ph+ALL patients regardless of their MRD status after HSCT.

PS1463

PD-1 OVEREXPRESSION ON BONE MARROW RESIDENT CD8+ MEMORY T-CELL SUBSETS COULD BE A PREDICTABLE MARKER FOR LEUKEMIA RELAPSE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

N. Popova[1], M. Drokov[1,*], L. Kuzmina[1], Y. Davydova[2], N. Kapranov[2], E. Mikhalcova[1], O. Koroleva[1], V. Vasilyeva[1], Z. Konova[1], D. Dubnyak[1], E. Usikova[1], A. Sidorova[1], I. Galtseva[2], E. Parovichnikova[1], V. Savchenko[1]

[1]Bone marrow transplant, [2]Immunophenotyping laboratory , National Research Center for Hematology, Moscow, Russia, Moscow, Russian Federation

Background: Programmed cell death protein 1 (PD-1) is a surface receptor expressed normally on activated T- and B-cells, NK, dendritic cells and monocytes and is known to be a negative immune regulator of these immune cells that has detrimental effects on anti-tumor immunity. Ability of leukemia cells exploiting PD-1-axis to escape the immune system through expression of PD-1-ligand is associated with developing of a relapse. Leukemia relapse after allogeneic stem cell transplantation (allo-HSCT) is still the main post-transplant problem and its solving has not been found yet.
Aims: To evaluate PD-1 expression on bone marrow (BM) resident CD8+ memory T-cell subsets in leukemia patients after allo-HSCT and to assess its impact on a relapse developing after allo-HSCT.
Methods: 0,5 ml of BM was obtained from 19 leukemia patients with a median age of 36 years old (range, 20-60) who underwent allo-HSCT. All patients engrafted before day +30 and stayed in remission within the follow-up period in 100 days after allo-HSCT. The analysis was carried out on day +90. White blood cells count (WBC) in BM samples was evaluated by Sysmex XE-2100 hematology analyzer. 1×10^6 of WBC (excluded nucleated red blood cell) from BM were stained using "lyse-wash-stain" standard protocol. Flow cytometry analysis was performed on BD FACS Canto II (Becton Dickinson, USA). 7-AAD was used to exclude dead cells from the analysis. The anti-CD8-APC-Cy7, anti-CCR7-PE-Cy7, anti-CD28-PE, anti-CD45R0-FITC, PD1-APC antibodies (Becton Dickinson, USA) were used to define BM resident CD8+ memory T-cell subsets and expression of PD-1: T naive and T stem cell memory (Tnv+Tscm) – CD8+CD45R0-CCR7+CD28+PD-1+; T central memory (Tcm) - CD8+CD45R0+ CCR7+CD28+PD-1+; T transitional memory (Ttm) - CD8+CD45R0+ CCR7-CD28+PD-1+; T effector memory (Tem) - CD8+CD45R0+CCR7-CD28-PD-1+; T terminal effector (Tte) - CD8+CD45R0-CCR7-CD28-PD-1+.
Results: According to our data PD-1 was overexpressed on all BM CD8+ memory cell subsets on day +90 in those patients who relapsed after the day +100 compared to the patients who stayed in complete remission after the day +100. (Table 1).

Table 1.

Expression of PD-1 on BM resident CD8+ memory cell subsets on day +90 in leukemia patients after allo-HSCT.

Subsets on day +90	Patients in complete remission, n=12 mean (standard error)	Patients relapsed after +100 day, n=7 mean (standard error)	p-value
CD8+ PD1+	79,2% (9,4%)	91,3% (4,9%)	> 0,05
Tnv+scm PD1+	61% (16%)	85% (9%)	> 0,05
Tcm PD1+	81% (9%)	94% (4%)	> 0,05
Ttm PD1+	85% (9%)	96% (3%)	> 0,05
Tem PD1+	79% (12%)	91% (7%)	> 0,05
Tte PD1+	71,8% (9,8%)	79,9% (9,5%)	> 0,05

Summary/Conclusion: Overexpression of PD-1 on BM resident CD8+ distinct memory cells seems to be a useful predictable marker that can help to start preventive therapeutic approaches with anti-PD-1 activities (checkpoint inhibitors) and in its turn to improve posttransplant results. But this hypothesis needs to be confirmed in the largest cohort of patients.

PS1464

OUTCOME OF SECOND SOLID CANCER AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION: A COHORT STUDY ON 4065 PATIENTS ON BEHALF OF THE COMPLICATIONS AND QUALITY OF LIFE WORKING PARTY OF THE EBMT

A. Tichelli[1,*], E. Beohou[2], M. Labopin[2], G. Socié[3], A. Rovó[4], M. Badoglio[2], A. van Biezen[5], P. Bader[6], R.F. Duarte[7], G. Basak[8], N. Salooja[9]

[1]Hematology, University Hospital, Basel, Switzerland, [2]EBMT Paris Study Office, Hôpital Staint Antoine, [3]Dept. of Hematology, Hôpital St Louis, Paris, France, [4]Hematology and Central Hematology Laboratory , University Hospital Bern, Bern, Switzerland, [5]EBMT data office Leiden, LUMC, Leiden, Netherlands, [6]Hospital for Children and Adolescents, Frankurt a. Main, Germany, [7]Hematology, Hospital Universitario Puerta de Hierro, Majadahonda, Spain, [8]Hematology, Oncology and Internal Medicine, Central Clinical Hospital of the Medical University , Warsaw, Poland, [9]Center of Haematology, Imperial College, London, United Kingdom

Background: With the improved outcome of patients treated with hematopoietic stem cell transplantation (HSCT) the number of long-term survivors is steadily increasing. However, HSCT survivors are at risk of late complications. Second solid cancers are feared late complications after HSCT. Their incidence and risk factors have been established, but little is known on their outcome.
Aims: We assessed the outcome of second solid cancers from their time of diagnosis in HSCT survivors.
Methods: We performed a retrospective cohort study of patients registered with the EBMT who developed a second solid cancer between 2000 and 2014. Median and 5-year age-standardized overall survival (OS) from time of diagnosis of the second cancer, causes of death, risk factor multivariate analysis and standardized mortality ratio (SMR) were calculated for 18 different cancer types.
Results: There were 4065 second solid cancers (1443, allogeneic; 2622, autologous). Primary diagnoses were acute leukemia (23%), chronic leukemia (9%), lymphoma (34%), plasma cell disorders (22%), solid tumors (4%), myelodysplastic syndromes and myeloproliferative neoplasms (6%) and acquired marrow failure syndromes (1%). The median age at HSCT and at diagnosis of second cancer was 53 years (range 0.7-70) and 59 years (3.2-82), respectively. The median follow-up time from HSCT and diagnosis of second cancer was 121 months (range 3.6-409) and 34 months (range 0-187), respectively. The 5-years age-standardized OS was 47% (95% CI 45-49). Survival outcome was mainly depended on the type of second cancer (Table 1). 5 cancer types had a favourable outcome with median survival >10 years (thyroid, cervix, prostate, breast, melanoma), 7 had an intermediate outcome with median survival >1-9 years (renal, oropharyngeal, bladder, ovarian, sarcoma, endometrial, gastric), and 6 had poor outcome with median survival ≤1 year (brain, esophageal, hepatobiliary, lung, pancreas). Survival risk factors after diagnosis of second cancer were older age at HSCT, type of cancer, donor type, and graft-versus-host disease. Overall, 1777 (43·9%) patients died: 74·9% as a result of the second cancer. SMR was increased for melanoma, prostate, breast, renal, bladder, colorectal, and endometrial cancer, but normal for other second cancers.

Table 1.

Outcome of the different second solid cancer types after HSCT

outcome	Second cancer	No. patients	Median survival (y)	5-year OS % (95% CI)	Cumulative incidence* (95% CI)
favourable	Thyroid	149	nr	83 (76-92)	9 (4-17)
	Cervix	57	12.4	70 (57-86)	15 (6-26)
	Prostate	410	9.8	69 (64-75)	14 (10-18)
	Breast	547	10.6	69 (64-74)	13 (10-17)
	Melanoma	343	nr	68 (62-74)	22 (17-27)
intermediate	Kidney	177	5.2	55 (47-65)	24 (17-32)
	Oropharyngeal	207	8.2	53 (46-62)	31 (24-39)
	Bladder	144	4.9	49 (39-62)	25 (17-35)
	Ovarian	77	2.2	43(32-58)	42 (29-54)
	Sarcomas	215	3.3	42(34-51)	47 (39-55)
	Colorectal	446	3.2	41 (36-48)	39 (33-44)
	Endometrial	46	3.4	40 (26-63)	45 (27-61)
poor	Gastric	158	1	29 (21-39)	58 (48-66)
	Brain	156	0.9	21 (15-30)	63 (53-71)
	Oesophageal	88	0.9	21 (13-36)	61 (48-72)
	Hepatobiliary	90	0.9	18 (11-31)	74 (61-84)
	Lung	597	0.9	14 (11-19)	74 (69-78)
	Pancreas	145	0.6	8 (3-18)	89 (79-94)

nr: not reached; * of death due to second cancer

Summary/Conclusion: The outcome of second solid cancers after HCT is mainly dependent on cancer type. SMR is increased for some but not all cancers, which develop after HCT and risk factors for poor survival include age at HSCT, GVHD and donor type. Early diagnosis is likely to be facilitated by life-long follow up. There is no reason to treat these patients differently from the general population, but close cooperation between oncologists and hematologists is mandatory.

PS1465

SIMILAR OUTCOMES OF REDUCED INTENSITY HAPLOIDENTICAL TRANSPLANTATION WHEN COMPARED TO MATCHED UNRELATED DONOR TRANSPLANT IN DIFFUSE LARGE B CELL LYMPHOMA. A JOINT ANALYSIS FROM CIBMTR-LC & EBMT-LWP

A. Sureda[1,*], P. Dreger[2], K. W. Ahn[3], H. Finel[4], J. Tischer[5], L. Castagna[6], D. Blaise[7], A. Dominietto[8], C. Schmid[9], F. Ciceri[10], A. Boumedil[4], C. Litovich[3], S.M. Smith[11], T. Fenske[3], S. Dietricht[2], S. Robinson[12], S. Montoto[13], M. Hamadani[3]

[1]Institut Català d'Oncologia, Barcelona, Spain, [2]University of Heidelberg, Heidelberg, Germany, [3]Medical College of Wisconsin, Milwaukee, United States, [4]EBMT Office, Paris, France, [5]Klinikum Grosshadern, Muenchen, Germany, [6]Istituto Clinico Humanitas, Milano, Italy, [7]Institut Paoli Calmettes, Marseille, France, [8]Ospedale San Martino, Genoa, Italy, [9]Klinikum Augsburg, Augsburg, Germany, [10]Ospedale San Raffaele, Milano, Italy, [11]University of Chicago, Chicago, United States, [12]University Hospitals, Bristol, [13]St. Bartholomew's Hospital, Barts Health NHS Trust, London, United Kingdom

Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative treatment for patients with relapsed diffuse large B cell lymphoma (DLBCL). In the absence of an HLA-identical sibling, the use of 8/8 matched unrelated donors (MUDs) has been considered the standard of care. Preliminary data suggest that haploidentical donors could be an alternative to MUDs in lymphomas.
Aims: The aim of this study was to compare the long-term outcome of MUD-HSCT with that of haplo-HSCT with cyclophosphamide post-transplant (Cy-post) for the first time specifically in patients with DLBCL.
Methods: 913 adult patients with relapsed DLBCL treated with allo-HSCT [132, haplo-HSCT; 403, MUD with anti-thymocyte (ATG) / Campath and 378, MUD without (w/o) ATG / Campath] were reported from 2008 to 2015 to CIBMTR and EBMT databases. Only reduced intensity conditioning regimens, Cy-post in the haplo group and additional prophylaxis with calcineurin inhibitors in the MUD group were included. There were significant differences amongst the 3 groups of patients (Haplo-HSCT, MUD with ATG/Campath and MUD w/o ATG/Campath) with respect to age (yrs) [58 (20-75) *vs* 55 (19-75) *vs* 56 (23-73), p=0.003], Karnofsky score at HSCT ≥ 90% [73% *vs* 62% *vs* 57%, p<0.001], distribution of HSCT-comorbidity score (p<0.001) and a prior autologous-HSCT [43% *vs* 59% *vs* 61%, p=0.001]. As expected, total body irradiation and bone marrow stem cells were more frequently used in the haplo-HSCT setting (86% and 76% *vs* 7%, and 7% *vs* 32% and 5%, p<0.001 both, respectively).
Results: 28-day cumulative incidence (CI) platelet recovery was significantly lower in haplo-HSCT [61 (50-71)% *vs* 89 (85-92)% *vs* 89 (84-92)%, p<0.001]. Conversely, haplo-HSCT was associated with a lower 180-day CI of grade 3-4 acute graft versus host disease (aGVHD) [7 (3-12)% *vs* 13 (10-16)% *vs* 19 (15-23)%, p<0.001] and 2-yr chronic GVHD (cGVHD) [18 (12-26)% *vs* 32 (27-38)% *vs* 57 (52-63)%, p<0.001]. With a median follow up (range) for surviving patients of 47 (5-73), 39 (<1-100) and 36(3-96) mo for the 3 cohorts, there were no significant differences in terms of 3-yr non relapse mortality (NRM) [22 (15-30)% *vs* 26 (21-31)% *vs* 30 (25-35)%], relapse/progression (RP/P) [41 (32-49)% *vs* 38 (33-43)% *vs* 34 (29-39%)], progression free survival (PFS) [38 (29-47)% *vs* 36 (31-41)% *vs* 37 (31-42%)] and overall survival (OS) [46 (37-55)% *vs* 43 (38-49)% *vs* 46 (41-52)%]. Multivariate analysis indicated that the use of haplo donors was associated with a significant lower incidence of grade 3-4 aGVHD [RR 0.36 (0.18-0.73), p=0.0071] and that both haplo and MUD with ATG/Campath were associated with a lower incidence of cGVHD [RR 0.25 (0.16-0.38), p<0.001 and RR 0.53 (0.41-0.67), p<0.001, respectively]. Type of donor did not significantly influence any of the four major outcomes. Time from diagnosis to HSCT > 24 mo was the only independent prognostic factor for NRM (p=0.001) while R/P was adversely affected by both time between diagnosis and HSCT > 24 mo and by not being in complete remission (p=0.002 and p<0.001, respectively). Disease status at HSCT was the only independent prognostic factor predicting for both PFS and OS.
Summary/Conclusion: Haplo-HSCT with the Cy-post platform results in similar long-term outcomes than MUD-HSCT in patients with relapsed DLBCL with a lower incidence of grade 3-4 aGVHD and cGVHD. Our results suggest that haplo donors are an adequate alternative to MUD in this setting.

PS1466

TARGETING EXPANDED GUT HOMING EFFECTOR T CELL LINEAGES IN ACUTE INTESTINAL GRAFT VERSUS HOST DISEASE: IMPLICATIONS FOR VEDOLIZUMAB

P. Pavlidis[1,*], V. Mehra[2,3], S. Kottoor[4], E. Alberts[4], E. Shabbir[5], J. DigbyBell[4], T.-L. Tsui[6], C. Scotta[6], E. N. Lamperti[6], G. Emlyn[7], H. Abu Arqoub[7], C. Rice[2], L. Barber[6], D. Mclornan[8], H. de Lavallade[9], V. Potter[9], M. Silverman[5], Y. Jethava[5], T. Pagliuca[9], G. Mufti[3], K. Raj[2], G. Lombardi[6], B. Hayee[1], N. Powell[4]

[1]Gastroenterology, [2]Haematology, Kings College Hospital, London, [3]Division of Cancer Studies, [4]Department of Experimental Immunobiology, Kings College London, London, United Kingdom, [5]University of Iowa Hospitals and Clinics,, Roy and Lucille Carver College Of Medicine, University of Iowa, Iowa City, United States, [6]Kings College London, [7]Histopathology, Kings College Hospital, London, [8]Haematology, Kings College London, [9]Kings College Hospital, London, London, United Kingdom

Background: Acute graft versus host disease may affect the gastrointestinal tract (aGI-GvHD) in up to 60% of allogeneic haematopoetic stem cell transplant (HSCT) recipients who develop GvHD resulting in significant morbidity. Half of these patients will develop steroid refractory disease which is associated with high non-relapse mortality due to the lack of safe and efficacious therapies. Targeting gut homing lymphocytes with vedolizumab, an anti-α4β7 monoclonal antibody, has proven safe and effective for managing intestinal inflammation in the context of inflammatory bowel disease (IBD).
Aims: We tested the hypothesis that the gut trafficking α4β7/MADCAM-1 pathway is important in the pathogenesis of human aGI-GvHD and report our clinical experience of vedolizumab use for steroid dependent/refractory aGI-GVHD.
Methods: Prospective study (UK REC reference: 15/LO/1998), recruiting HSCT recipients with aGI-GvHD (n=10) and controls [IBD (n=36) and non-IBD controls (n=32)]. Samples collected included peripheral blood and distal colonic biopsies. The β7+ CD4+ compartment in peripheral blood was phenotyped with a multiparametric flow cytometry panel. MADCAM-1 and S100A8 (calprotectin subunit, biomarker of intestinal damage) expression in the gut were tested with RT-PCR. Clinical data on steroid dependent/refractory severe aGI-GvHD patients treated with vedolizumab (n=12) were retrospectively collected.

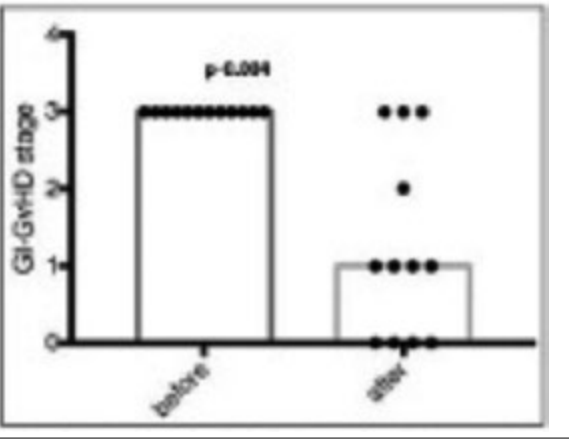


Figure 1. 75% median reduction in staging score of aGI-GvHD post Vedolizumab.

Results: Within the circulating effector memory population (CD3+CD4+CD45RA-CD45RO+CCR7-) there was significant enrichment of β7+ effector memory cells in both inflammatory conditions (IBD: 24% ±2.7, aGI-GvHD: 29%±6.5 *vs.* controls: 17%±1.1, both p<0.05). Analysis of each individual subtype of the effector T cell lineages demonstrated that β7 expression was especially enriched in Th1 (CXCR3+CCR6-), Th17(CCR6+CXCR3-) and Th1/17 (CXCR3+CCR6+) (p=0.0034). MADCAM-1 expression in aGI-GvHD is upregulated in comparison to non-IBD controls [fold change: 2(range 0, 5), p=0.006)] and at similar levels to patients with IBD, [fold change: 2 (range 0, 9)]. Levels of MADCAM-1 expression correlated to the expression of the calprotectin subunit S100A8 [r=0.90, 95%CI (0.34, 0.99), p=0.014]. Twelve patients with steroid refractory severe aGI-GvHD following T-deplete HSCTs were treated with vedolizumab, a monoclonal antibody targeting α4β7 on a compassionate basis. Nine patients had a sustained clinical improvement in gut symptoms (75% median reduction in clinical score, p=0.004, Figure 1) with a median aGI-GvHD free period of 5 months (range 0.5-28m) with 4 patients reporting rapid & complete resolution of GI symptoms. Seven of 12 patients are still alive at median follow up of 10 months (range 1-28m) with 3 patients dying of refractory GVHD, 1 from recurrent urosepsis and 1 from primary disease progression. Median number of vedolizumab doses required were 2 (range 1-15). Vedolizumab use was clinically safe and patients did not

suffer any infusion related adverse events. None of the patients had recurrence of symptoms on discontinuation of therapy.
Summary/Conclusion: We show that aGI-GvHD is associated with significant expansion of gut homing effector T cell lineages, most notably Th1 and Th17 cells. Interestingly the ligand for the α4β7 integrin (MAdCAM-1) is also highly expressed in gut tissue from aGI-GvHD patients, further supporting therapeutic targeting of this gut homing pathway. Our promising clinical data on vedolizumab, with its gut specific activity and low risk of systemic immunosuppression have the potential to change the landscape of treatment in this condition.

PS1467

ELTROMBOPAG TO IMPROVE PLATELET RECOVERY IN HAPLOIDENTICAL STEM CELL TRANSPLANTS (HAPLO-SCT): RESULTS OF A PHASE II TRIALPLUS A COMPARISON TO CONTROL PATIENTS

U. Popat[1,*], R. Bassett[1], F. Aung[1], B. Valdez[1], S. Ahmed[1], A. Alousi[1], Q. Bashir[1], C. Hosing[1], P. Kebriaei[1], I. Khouri[1], D. Marin[1], Y. Nieto[1], A. Olson[1], B. Oran[1], M. Qazilbash[1], K. Rezvani[1], R. Mehta[1], E. Shpall[1], S. Ciurea[1], B. Andersson[1], R. Champlin[1]

[1]*The University of Texas MD Anderson Cancer Center, Houston, United States*

Background: Slow platelet recovery is common after Haplo-SCT done with post-transplant cyclophosphamide (PCy) and bone marrow as a graft source. Improved platelet recovery may reduce the need for platelet transfusions and improve outcomes. Eltrombopag, a thrombopoietin receptor agonist, is indicated for use in ITP and aplastic anemia.
Aims: In this phase II study and comparison to a control group, we investigated the safety and efficacy of eltrombopag after Haplo-SCT.
Methods: Patients older than 18 receiving Haplo-SCT with PCy based GVHD prophylaxis were eligible for study. Patients received eltrombopag 300 mg per day for 60 days starting on day 5 after transplant. Median age was 57 (range 20-62). Primary endpoint of the study was to estimate the rate of platelet count > 50,000/μl on post-transplant day 60 without platelet transfusion support in the prior 7 days. We compared 37 patients who received at least one dose of eltrombopag to a control group of 83 patients transplanted during the same time period. Conditioning regimen was fludarabine, melphalan, and either thiotepa or 200cGy TBI and the graft source was bone marrow in all patients.
Results: All serious adverse events (SAEs) seen were those inherent in Haplo-SCT procedure. No eltrombopag-related ≥ grade 4 SAEs were seen. Patient characteristics were similar between eltrombopag and control groups, and study results are summarized in Table 1. Overall survival, progression free survival, GVHD rate, relapse rate, and non-relapse mortality were similar in the two groups.

Table 1.

	Control (n=83)	Eltrombopag (n=37)	P value
Primary Endpoint: number and proportion of successes	39 (47%)	24 (65%)	0.08
Median Time to platelet engraftment(>20K/μl)-days	34	30	0.05
Median Time to neutrophil engraftment	20	19	0.38
Cumulative incidence of platelet engraftment(>20K/μl)	63% (52-73)	81% (68-94)	0.05
Median platelet transfusions	19	12	0.20

Summary/Conclusion: Eltrombopag 300mg/day for 60 days is safe and appears to improve platelet recovery in patients receiving Haplo-SCT with PCy.

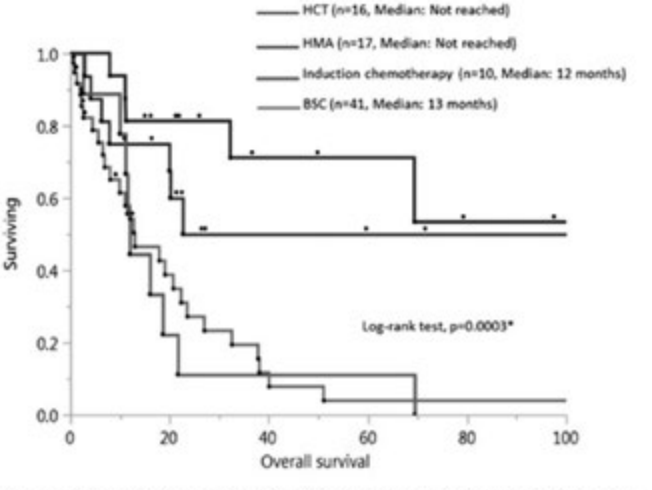
PS1468

ALLOGENEIC STEM CELL TRANSPLANTATION OVERCOMES THE NEGATIVE PROGNOSTIC IMPACT OF ASXL1 MUTATIONS IN TRANSPLANT ELIGIBLE PATIENTS WITH CHRONIC MYELOMONOCYTIC LEUKEMIA

P. Pophali[1,*], A. Mangaonkar[1], N. Gangat[1], A. Al-Kali[1], M. Elliott[1], K. Begna[1], H. Alkhateeb[1], W. Hogan[1], M. Litzow[1], M. Patnaik[1]

[1]*Hematology, Mayo Clinic, Rochester, United States*

Background: Chronic myelomonocytic leukemia (CMML) is an aggressive myeloid neoplasm with a median survival of <36 months. Mutations involving *TET2* (~60%), *SRSF2* (~50%) and *ASXL1* (~40%) are frequent, with frame shift and nonsense mutations in *ASXL1* independently and adversely impacting survival. Allogeneic stem cell transplantation (HCT) remains the only curative approach, with hypomethylating agents (HMA) not being able to modify disease biology.
Aims: We carried out this study to specifically assess the impact of HCT on survival outcomes in *ASXL1* mutant CMML patients.
Methods: Consecutive cases of CMML were identified from our CMML data base. Only patients below the age of 75 years were included (upper age limit for HCT). Targeted exome sequencing data for 36 genes was available at CMML diagnosis in all cases.
Results: 185 patients with a diagnosis of CMML with available molecular data at diagnosis were included; median age 67 years (range: 24-75); 122 (66%) males. Of these, 26 (14%) underwent allogeneic HCT (median age 57, range 26-74 years), while the remaining 159 (86%) patients (median age 68, range 24-75 years) were managed with HMA (n= 36), AML-like induction chemotherapy (n=18), and best supportive care (BSC, n=87). The most frequent genomic alterations in the cohort were *ASXL1* (49%), *SRSF2* (44%), *TET2* (35%), *NRAS* (16%), *SETBP1* (14%), and *CBL* (14%). In comparison to the non HCT group, patients in the HCT group were younger (p=0.0001), had lower HB levels (p=0.003), higher PB (p=0.0001) and BM (p=0.01) blast%, more likely to be classified as CMML-2 (p=0.01), had higher risk stratification by the Mayo model (p=0.005) and the Mayo Molecular Model (p=0.003), and were more likely to have received AML-like chemotherapy (p=0.0005). There were no differences in the cytogenetic risk stratification, with 53 (29%) patients having an abnormal karyotype.
In the HCT group, 11 (44%) patients received myeloablative conditioning (MAC) while 14 (56%) received reduced intensity conditioning (RIC). Donor sources included matched siblings (32%), matched unrelated (52%), mismatched unrelated (4%) haploidentical donors (8%), and umbilical cord blood (4%) (PB- 88%, BM-8% and cord blood- 4%). Acute GVHD grade 2-4 was reported in 58% patients while moderate to severe chronic GVHD was reported in 31% patients. Post-HCT disease relapse was seen in 6 (24%) patients. At a median follow up of 50 months, there were 102 (55%) deaths and 36 (19%) leukemic transformations. The median OS for all patients with *ASXL1* mutations was 20 months (95% CI: 12- 27 months). The median OS for *ASXL1* mutant patients that underwent HCT was not reached (95% CI: 32.2- NR months), while for those treated with non HCT options was 16 months (95% CI: 11.1-21.6 months) (p=0.0009) (Figure 1). There was no difference in survival between MAC vs RIC (p=0.34) or between the different graft sources. In the non-HCT cohort, patients treated with HMA had a median OS that was not reached (95% CI: 7.7- NR months), in comparison to 12 months (95% CI: 2.5-21.6 months) for AML type induction chemotherapy and 13 months (95% CI: 6.8-22.3 months) for BSC (p=0.02). Among the *ASXL1* mutant patients, the 3 year survival rate was 19%; 43% in HCT vs 13% in non HCT group (p=0.009).



Kaplan-Meier survival analysis for *ASXL1* mutated CMML patients treated with HCT and non-HCT therapies.

Figure 1.

Summary/Conclusion: Allogeneic HCT in eligible patients was able to overcome the adverse prognostic impact of *ASXL1* mutations. While HMA therapy does improve survival, the fact that it does not alter disease biology results in inevitable disease progression. Prospective studies are needed to validate our results.

PS1469

ASSOCIATION OF SOCIO-ECONOMIC FACTORS WITH SURVIVAL OF PATIENTS WHO EXPERIENCE SEVERE CLASSIC ACUTE GVHD AFTER ALLO-HSCT. A RETROSPECTIVE STUDY BY THE TRANSPLANT COMPLICATIONS WORKING PARTY, EBMT

A. Frankiewicz[1,*], C. Peczynski[2], S. Giebel[1], A. Harrington[3], G. Socié[4], D. Niederwieser[5], C. Scheid[6], M. Bornhäuser[7], N. Kröger[8], A. Elmaagacli[9], B. Afanasyev[10], P. Dreger[11], C. Rössig[12], D. Blaise[13], C. Kratz[14], I. Yakoub-Agha[15], B. Kremens[16], B. Strahm[17], G. Wulf[18], R. Arnold[19], H. Greinix[20], G.W. Basak[21]

[1]Bone Marrow Transplantation and Oncohaematology, Centre of Oncology - Institute, Maria Sklodowska - Curie Memorial, Gliwice Branch, Gliwice, Poland, [2]2EBMT Paris Study Office / CEREST-TC, [3]2EBMT Paris Study Office / CEREST-TC, Hôpital Saint Antoine, [4]Dept.of Hematology – BMT 1, 3Hopital St. Louis, Paris, France, [5]Div. Hematology, Oncology and Hemostasiology , University Hospital Leipzig , Leipzig, [6]1Dept. of Medicine, University of Cologne , Cologne, [7]Medizinische Klinik und Poliklinik, Universitaetsklinikum Dresden, Dresden, [8]Department of Stem cell Transplantation, 7University Hospital Eppendorf Hamburg, [9]Department of Haematology, Asklepios Klinik St. Georg , Hamburg, Germany, [10]Research Institute for Paediatric Oncology, Hematology, and Transplantation, First State Pavlov Medical University of St. Petersburg Raisa Gorbacheva Memorial , St. Petersburg, Russian Federation, [11]Medizinische Klinik u. Poliklinik V, University of Heidelberg , Heidelberg, [12]Pädiatrische Hämatologie und Onkologie, 11Universitaetsklinikum Muenster Klinik für Kinder- und Jugendmedizin, Muenster, Germany, [13]12Programme de Transplantation&Therapie Cellulaire Centre de Recherche en Cancérologie de Marseille, Institut Paoli Calmettes, Marseille, France, [14]Dept. Pediatric Haematology/Oncology, Hannover Medical University , Hannover, Germany, [15]UAM allo-CSH CHRU, Hôpital HURIEZ , Lille, France, [16]Dept. of Pediatric Hematol./Oncol, University Hospital, Essen, 17Pediatric Hematology and Oncology Department, Pediatrics and Adolescent Medicine University Medical Centre, Freiburg, [18]Abteilung Hämatologie und Onkologie , Universitaetsklinikum Goettingen, Goettingen, [19]Campus Virchow Klinikum Medizinische Klinik m. S. Hämatologie/Onkologie, Charité Universitätsmedizin Berlin, Berlin, Germany, [20]19Klinische Abteilung für Hämatologie, Universitätsklinik für Innere Medizin, Graz, Austria, [21]Department of Haematology, Oncology and Internal Medicin, Medical University of Warsaw, Warsaw, Poland

Background: Acute graft-versus-host disease (GvHD) remains one of the main causes of non-relapse mortality (NRM) after allogeneic hematopoietic cell transplantation (alloHCT). The treatment of severe acute GvHD is complex and costly. The outcome may depend on many patient-, donor-, and transplant-related factors but potentially also on center activity and socio-economic issues.
Aims: The aim of this study was to identify factors associated with prognosis of patients who experience severe acute GvHD after alloHCT, including socio-economic measures and center experience.
Methods: Patients and methods: 4152 adults with hematological malignancies who received alloHCT from either HLA-matched sibling (n=1328) or unrelated donor (n=2824) between year 2011 and 2015, and developed grade 3 or 4 acute GvHD, were included in the analysis. Non-relapse mortality (NRM) was the primary study end-point. The following independent variables were considered together with clinical factors: current Health Care Expenditure (HCE), public HCE, private HCE, HCE as% of Gross Domestic Product per capita (GDP), Human Development Index (HDI), team experience. Median values were used as cut-off points for the analysis.
Results: In a univariate analysis, the probability of NRM at 2 years was increased for countries with lower current HCE (≤median vs. >median, 60% vs. 53%, respectively, p=0.009), lower HCE as% of GDP (57% vs. 52%, p=0.002), lower public HCE (60% vs. 53%, p=0.009) and lower HDI (57% vs 52%, p=0.007). Neither private HCE nor center experience affected NRM. In a multivariate model, among socio-economic factors, the risk of NRM was most strongly predicted by current HCE (>median vs. ≤median; HR=0.72, p=0.0007). In addition, the risk of NRM was increased with increasing patient age (HR=1.23 per each 10 years, p<0.0001), advanced disease stage (HR=1.17, p=0.005), previous autologous HCT (HR=1.33, p=0.011) and the use of unrelated donors (HR=1.3, p<0.0001), while reduced for CMV neg/neg donor/patient serological status compared to other combinations (HR=0.82, p=0.0007), the diagnosis of plasma cell disorders (HR=0.66, p=0.008), and the use of reduced intensity vs. myeloablative conditioning (HR=0.82, p=0.0005). HCE >median was also associated with reduced risk of the overall mortality (HR 0.73, p=0.0002) and reduced risk of treatment failure (either relapse or NRM, inverse of progression free survival; HR 0.78, p=0.003). No association of socio-economic factors with the risk of relapse or chronic GvHD could be documented.
Summary/Conclusion: Country-specific socio-economic factors, in particular current HCE, are strongly associated with survival of patients who experience severe acute GvHD. Our findings should be considered in interpretation of clinical studies in the field of alloHCT.

PS1470

COMPARISON OF REDUCED-INTENSITY CONDITIONING REGIMENS IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA > 45 YEARS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION – A RETROSPECTIVE STUDY BY THE ALWP/EBMT

Z. Peric[1,*], M. Labopin[2], C. Peczynski[2], E. Polge[2], J. Cornelissen[3], B. Carpenter[4], M. Potter[5], R. Malladi[6], N. Russel[7], H. Schouten[8], N. Fegueux[9], G. Socié[10], M. Rovira[11], J. Kuball[12], M. Gilleese[13], S. Giebel[14], M. Mohty[15], A. Nagler[16]

[1]Dpt of internal medicine, School of Medicine, University of Zagreb, Zagreb, Croatia, [2]EBMT Paris study office / CEREST-TC, Saint Antoine Hospital , Paris, France, [3]Erasmus MC Cancer Institute, University Medical Center Rotterdam, Rotterdam, Netherlands, [4]Dpt of hematology, University College London Hospital, [5]Leukaemia Myeloma Units, Royal Marsden Hospital, London, [6]Dpt of hematology, University Hospital Birmingham NHSTrust, Queen Elizabeth Medical Centre, Birmingham, [7]Nottingham University Hospital, Nottingham, United Kingdom, [8]Dpt of internal medicine, University Hospital Maastricht, Maastricht, Netherlands, [9]Dpt of hematology, University Hospital Centre Lapeyronie, Montpellier, [10]Dpt of hematology, Hospital St. Louis, Paris, France, [11]BMT unit, Institute of Hematology & Oncology, IDIBAPS, Barcelona, Spain, [12]Dpt of hematology, University Medical Centre, Utrecht, Netherlands, [13]Haematology Department Bexley Wing, St James's Institute of Oncology, Leeds, United Kingdom, [14]M. Sklodowska-Curie Institute - Oncology Center, Gliwice Branch, Gliwice, Poland, [15]Dpt of hematology, Saint Antoine Hospital, Paris, France, [16]Dpt of hematology, Chaim Sheba Medical Centre, Tel-Hashomer, Israel

Background: The optimal reduced-intensity conditioning (RIC) for patients (pts) >45 years (y) with acute lymphoblastic leukemia (ALL) undergoing allogeneic stem cell transplantation (allo-HSCT) is not defined.
Aims: We compared the 3 most popular RIC protocols used in this pts category.
Methods: 417 pts with ALL, age ≥ 45 y, who underwent a matched-sibling or unrelated allo-HSCT in first complete remission between 2005 and 2016 and reported to the EBMT were included. Allo-HSCT outcomes were assessed comparing fludarabine/busulfan (FLUBU, Bu at 6.4 mg/kg, n=127) vs fludarabine/melphalan (FLUMEL, Mel at 130-140 mg/m2, n=190) vs fludarabine-TBI (FLUTBI, TBI at 2 Gy, n=100).
Results: All pts received peripheral blood stem cell grafts. Most pts in the FLUBU group received ATG (88%), while most of the FLUMEL pts received Campath (71%) as GVHD prophylaxis. Only 12% of the patients received *in vivo* T cell depletion in the FLUTBI group (11 ATG and 1 campath). Pts in the FLUBU group were significantly older (median 59 y, range 45-71) than pts in the FLUMEL (median 54 y, range 45-74) and the FLUTBI (median 57 y, range 45-72) groups, respectively (p=0.001). Incidence of Ph+ ALL was lower in the FLUMEL group compared to FLUBU or FLUTBI groups (52% vs 69%, p<10-3). The rest of the demographics and transplant characteristics were comparable between the three groups.
At 2y , there were no significant differences between the groups in terms of cumulative incidence (CI) of relapse (FLUBU 40%, 95%CI 30-49; FLUMEL 36%, 95%CI 28-44; FLUTBI 41%, 95%CI 30-51, p=0.21); non-relapse mortality (NRM) (FLUBU 18%, 95%CI 11-26; FLUMEL 22%, 95%CI 16-29; FLUTBI 14%, 95%CI 8-22, p=0.09); overall survival (OS) (FLUBU 55%, 95%CI 45-65; FLUMEL 50%, 95%CI 42-59; FLUTBI 60%, 95%CI 49-70, p=0.62) or leukemia-free survival (LFS) (FLUBU 43%, 95%CI 33-52; FLUMEL 42%, 95%CI 34-51; FLUTBI 45%, 95%CI 35-56, p=0.99). In addition, all groups had similar CI of grade II-IV acute GVHD (FLUBU 23%, 95%CI 16-31; FLUMEL 27%, 95%CI 20-33; FLUTBI 32%, 95%CI 23-42, p=0.33). However, the CI of chronic (c) GVHD was significantly higher in the FLUTBI group (55%, 95%CI 44-65) in comparison to FLUBU (37%, 95%CI 27-46) and FLUMEL group (37%, 95%CI 28-46)(p=0.03). This difference resulted in significantly lower GVHD-relapse-free survival (GFRS) in the FLUTBI group (18%, 95%CI 10-26) compared to the FLUBU (35%, 95%CI 25-44) and the FLUMEL groups (28%, 95%CI 20-36) (p=0.02). In the multivariate analysis, there were no more differences in cGVHD and GRFS between the 3 conditioning regimens when adjusting for the use of *in vivo* T-cell depletion. Finally, the FLUMEL regimen (vs FLUBU) was shown to be an independent risk factor for a higher NRM (HR 1.97, 95% CI 1.05-3.72, p=0.04) mostly due to infections with CI of 11%, 95% CI 7-16 at 2 years.
Summary/Conclusion: Based on our current study the three most popular RIC preparative regimens (FLUBU, FLUMEL and FLUTBI) administred pre allo-HSCT for ALL patients >45y yield similar transplantation outcomes as for relapse, OS and LFS. The higher incidence of cGVHD and lower GRFS observed by univariate analysis in the FLUTBI group is mainly related to the low use of *in vivo* T-cell depletion in this group. Collectively, our results suggest that chemo/immunotherapeutic strategies prior and/or post-transplant should be improved to reduce relapse rather than adapting or intensifying the transplant-conditioning regimen.

PS1471

A PHASE I/II TRIAL OF INTRAVENOUS AZACITIDINE FOR ACUTE GVHD PROPHYLAXIS IN PATIENTS UNDERGOING MATCHED UNRELATED STEM CELL TRANSPLANTATION: INTERIM PHASE II RESULTS

M. Schroeder[1,*], J. Choi[1], M. Cooper[1], S. Marriott[1], K. Gerbic[1], J. Liu[1], A. Ghobadi[1], K. Stockerl-Goldstein[1], R. Vij[1], A. Cashen[1], A. Camille[1], M. Jacoby[1], I. Pusic[1], R. Romee[2], G. Uy[1], P. Westervelt[1], J. DiPersio[1]

[1]Washington University School of Medicine, Saint Louis, [2]Dana Farber Cancer Institute , Boston, United States

Background: The negative impact of acute graft-versus-host disease (GvHD) on morbidity and mortality after allogeneic transplant is significant; thus, finding a means to harness the beneficial Graft versus tumor effect (GVT) while reducing or eliminating GvHD is a major goal of transplant trials. Alterations in immune subsets present after transplant can work to suppress allo-reactive T-cell responses by increasing regulatory T-cells and suppressing allo-reactive T-cell proliferation. Azacitidine (AZA) treatment in pre-clincal models resulted in an increase in regulatory T-cells, a decrease in allo-reactive T-cell proliferation and prevention of acute GvHD while preserving GVT effects (Choi *et al.* Blood 2010, Cooper *et al.* J Immunol 2017). Based on these results a phase I/II study was designed to test the safety and efficacy of AZA administered shortly after transplant for the prevention of acute GvHD and relapse in subjects receiving transplants from matched unrelated stem cell donors. We are reporting the interim results for Phase II.
Aims: To define the maximum tolerated dose of AZA and the effect on grade II-IV GvHD when given after matched unrelated donor transplant (MUD).
Methods: Patients with hematologic malignancies in remission age 18 – 70 were eligible. Myeloablative or reduced intensity conditioning without antithymocyte globulin was used. All recipients in phase II were required to receive G-CSF mobilized peripheral blood grafts with at least 4×10^6 CD34/kg and have at least 1×10^6 CD34/kg cryopreserved as back up in case of primary graft failure. AZA was administered intravenously on day +7 for five consecutive days and repeated every 28 days for a total of 4 cycles after allogeneic transplant from a 10/10 HLA matched unrelated donor (Figure 1). GvHD prophylaxis with mini-methotrexate and tacrolimus was given. Phase I, 3+3 dose escalation design of 4 cohorts (AZA dose levels 15, 30, 37.5, and 45 mg/m^2) was used to determine the recommended phase II dose (RP2D). The primary outcome for phase II is the rate of grade II – IV acute GvHD at day 180 after transplant.
Results: The RP2D from phase I was 45 mg/m^2 (Schroeder *et al.* ASH 2015) with one DLT observed secondary to primary graft failure. To date in phase II we have transplanted 35 of 46 planned subjects and all 35 have received study drug. Recipient characteristics include: median age 59 (range 24 – 70); 54% male; diagnoses - AML in CR (22), MDS (13); conditioning - reduced intensity (12), myeloablative (23). Primary graft failure has occurred in one subject in phase II related to HHV6 infection. Median ANC engraftment was 14 days (range excluding case of graft failure 10 - 18 days). Median platelet engraftment was 19 days (range 10 – NR). To date, the phase II acute GvHD incidence grade II – IV has occurred in 15 (43%) and grade III/IV in 7 (20%). The majority of cases have responded to steroids with 7 cases of steroid refractory aGvHD. With a median follow up of 248 days (range 27 - 891), 4 subjects have relapsed and 25 (71%) remain alive. The most common non-hematologic grade 3 or 4 AEs were gastrointestinal toxicity, electrolyte abnormalities, and infections.

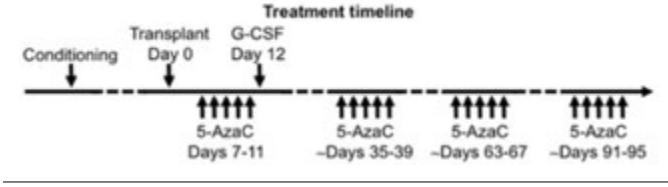


Figure 1.

Summary/Conclusion: In conclusion, AZA can be given safely starting at day +7 after MUD transplant up to a dose of 45mg/m^2. Phase II enrollment and follow up is ongoing and will be updated at the meeting. Correlative studies from banked bio-specimens evaluating T-cell subsets and methylation before and after treatment are ongoing.

PS1472

BUSULFAN FLUDARABINE (BU-FLU) COMPARED TO THIOTEPA BUSULFAN FLUDARABINE (TBF) FOR ALLOGENEIC TRANSPLANTS IN ACUTE MYELOID LEUKEMIA (AML) OR REFRACTORY ANEMIA WITH EXCESS BLASTS (RAEB) IN REMISSION

A. Bacigalupo[1,*], F. Sora'[1], S. Sica[1], C. Di Grazia[2], P. Chiusolo[1], N. Mordini[3], A. Olivieri[4], A.P. Iori[5], E. Angelucci[2]

[1]Hematology, Pliclinico Gemelli, Roma, [2]Hematology, IRCCS Azienda Ospedaliera Universitaria San Martino – IST, Genova, [3]Hematology, Ospedale S Croce, Cuneo, [4]Hematology, Ospedali Riuniti, Ancona, [5]Hematology, Policlinico Umberto 1, Roma, Italy

Background: The combination of Busulfan and fludarabine (BU-FLU) is considered a standard conditioning regimen for patients with acute myeloid leukemia undergoing an allogeneic hemopoietic stem cell transplant (HSCT), especially in patient older than 40 years of age. However, in a recent prospective study, designed for patients in first or second remission (CR1 or CR2) the incidence of leukemia relapse at 3 years was 38%, both for patients receiving BU-FLU as well as for patients randomized to BU-CY (Lancet Oncol 2015;16:1525). The addition of thiotepa to a BU-FLU regimen (referred to as TBF) has shown promising control of leukemia, and has emerged as a standard regimen in cord blood (CB) transplants (BMT 2012; 47: 1287). The question is whether this holds true also for transplants other than CB grafts.
Aims: to compare Bu-FLU with TBF in patients with AML or RAEB, grafted in remission from related or unrelated donors.
Methods: Eligible for this study were 336 patients allografted between January 2008 and April 2017 with AML or RAEB in hematologic remission : 165 patients received the BU-FLU regimen (busulfan 3.2 mg/kg/day x4; and fludarabine 40 mg/m^2/day x4), and 171 received TBF(thiotepa 5mg/kgx2, busulfan 3.2 mg/kg/dayx3, fludarabine 50 mg/m^{2}x3). Graft-versus-host disease (GVHD) prophylaxis was as follows: in HLA identical sibling transplants , cyclosporin (CyA) and Metothrexate (MTX); in unrelated donor transplants CyA+MTX+ antithymocyte globulin (ATG) and for family haploidentical donors (HAPLO) , CyA, mycophenolate mofetil (MMF) and cyclofosphamide 50mg/kg given on day +3 and +5, after transplantation (PT-CY) . Supportive care and infectious disease prophylaxis, or pre-emptive therapy, were provided as per Institutional protocols. The two groups (BUFLU and TBF) were comparable for age (p=0.3). The TBF group had more CR2 (27% vs 10%, p=0.001), more RAEB/sAML (29% vs 12%, p<0.01), more haploidentical transplants (62% vs 2%) as compared to BU FLU
Results: With a median follow up of 486 and 513 days (range, 8-3044 and 2-2322 respectively) after transplantation, the 5-year cumulative incidence of TRM was 15% and 9% for BU-FLU and TBF (p=0.1); the 5-year cumulative incidence of leukemia relapse related deaths (RRD) was 22% and 8% (P<0.001). The 5-year actuarial survival was 53% (43-62%) and 77% (69-85%) (p<0.001) respectively for the BUFLU and the TBF group. The survival advantage was seen also when looking only at HLA identical siblings and UD, excluding HAPLO grafts (52% vs 78%, p=0.01).
In a multivariate Cox analysis, after correcting for patients age, interval diagnosis transplant, first or second remission , and donor type, the use of TBF reduced the risk of death as compared to BU-FLU (RR 0.41, p=0.01) . In a Cox analysis on RRD, TBF showed a reduced risk (RR 0.35, p=0.04). The only significant predictor of TRM was age over 50 years (RR 2.5, p=0.01).
Summary/Conclusion: Superior survival of patients receiving TBF , as compared to BU-FLU appears to be due to reduced relapse, whereas TRM is comparable. The survival advantage is independent of donor type, and therefore of GvHD prophylaxis.

PS1473

EFFICACY AND SAFETY OF DEFIBROTIDE BY VENO-OCCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME (VOD/SOS) DIAGNOSTIC CRITERIA IN AN EXPANDED-ACCESS (T-IND) STUDY

S. Corbacioglu[1,*], N. Kernan[2], A. Pagliuca[3], R. Ryan[4], R. Hume[4], W. Tappe[4], P. Richardson[5]

[1]Department of Pediatric Hematology, Oncology and Stem Cell Transplantation, University of Regensburg, Regensburg, Germany, [2]Pediatric BMT Service, Memorial Sloan Kettering Cancer Center, New York, United States, [3]King's College Hospital, London, United Kingdom, [4]Jazz Pharmaceuticals, Inc., Palo Alto, [5]Jerome Lipper Multiple Myeloma Center, Division of Hematologic Malignancy, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, United States

Background: Hepatic VOD/SOS is a potentially life-threatening complication of conditioning regimens for hematopoietic stem cell transplant (HSCT). VOD/SOS with multi-organ dysfunction (MOD; renal and/or pulmonary) may be associated with >80% mortality. Traditionally, VOD/SOS has been clinically diagnosed with Baltimore criteria (bilirubin ≥2 mg/dL [34 µmol/L] plus two or more of hepatomegaly, ascites, and weight gain ≥5%), modified Seattle criteria (2 or more of bilirubin >2 mg/dL, hepatomegaly or right upper quadrant pain, and weight gain >2% [sometimes >5%]), or by biopsy. VOD/SOS is dynamic and may be progressive, and traditional criteria require overt clinical manifestation (eg, Baltimore criteria requires hyperbilirubinemia), which may occur relatively late or not at all, even in the course of severe disease. Defibrotide is approved to treat severe VOD/SOS post-HSCT in patients aged >1 month in the European Union, and VOD/SOS with renal or pulmonary dysfunction post-HSCT in the United States and Canada.
Aims: To compare Day +100 survival in subgroups of post-HSCT patients who were diagnosed with VOD/SOS by Baltimore criteria, modified Seattle criteria, or biopsy, and were treated with defibrotide in the expanded-access T-IND program (2007-2016).
Methods: The original T-IND protocol (to 2012) required VOD/SOS post-HSCT per Baltimore criteria or biopsy, and MOD; it was amended to include patients without MOD, with VOD/SOS per modified Seattle criteria. Diagnosis criterion used was selected on the case report form by the investigator. Patients received defibrotide 25 mg/kg/d (6.25 mg/kg q6h) recommended for ≥21 days.
Results: Of 1000 patients in the T-IND with VOD/SOS post-HSCT, 635 (63.5%) were reported as being diagnosed by Baltimore criteria, 331 (33.1%) by modified Seattle criteria, and 34 (3.1%) by biopsy. MOD was present in 512 (51.2%) of all patients (378 [59.5%] in the Baltimore group, 112 [33.8%] modified Seattle group, and 22 [64.7%] biopsy group). Kaplan-Meier estimated Day +100 survival among all defibrotide-treated patients with VOD/SOS post-HSCT was 58.9% (95% CI, 55.7% 61.9%) with 51.6% (95% CI, 47.6% 55.5%) for the Baltimore group, 72.3% (95% CI, 67.0% 76.8%) for the modified Seattle group, and 67.6% (95% CI, 49.2% 80.6%) for the biopsy group. Similar patterns of Day +100 survival among patients diagnosed by Baltimore vs modified Seattle criteria were shown in subgroups with and without MOD (Figure 1). Treatment-emergent and treatment-related adverse events (AEs) occurred in 66.4% and 18.8% of all patients, 71.6% and 17.9% of Baltimore patients, 61.2% and 20.5% of modified Seattle patients, and 50.0% and 8.3% of biopsy-proven patients. Hemorrhage occurred in 25.4% of all patients, 28.4% of Baltimore criteria patients, 22.4% of modified Seattle patients, and 16.7% of biopsy-proven patients.

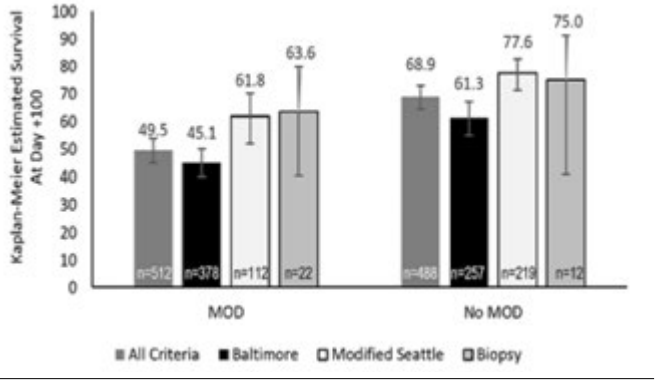


Figure 1.

Summary/Conclusion: Patients diagnosed by the more stringent Baltimore criteria, which requires hyperbilirubinemia, had lower survival rates vs patients diagnosed using modified Seattle criteria or biopsy. Treatment-emergent AEs were consistent with previous reports of defibrotide treatment. The lower overall survival in the Baltimore group suggests that requiring hyperbilirubinemia for VOD/SOS diagnosis may result in patients with more severe disease, leading to worse outcomes. These results corroborate data published by Yakushijin *et al.* (*Bone Marrow Transplant*, 2016).

PS1474

PLASMA PRESEPSIN LEVEL IS A PREDICTIVE MARKER OF SEVERE ACUTE GVHD AFTER ALLO-HSCT: USEFULNESS AS A BIOMARKER OF ALLO-HSCT RELATED COMPLICATION

T. Oyake[1,*], A. Otsu[1], M. Nishiya[1], T. Shimoyama[1], N. Sugawara[1], R. Sasaki[1], W. Izumita[1], Y. Tsukushi[1], S. Kowata[1], Y. Ishida[1], T. Ishida[1]

[1]Department of Hematology and Oncology, Internal Medicine, Iwate Medical University School of Medicine, Morioka, Japan

Background: Infectious complications including febrile neutropenia (FN) are often observed in patients with hematological disease after allogeneic hematopoietic stem cell transplantation (allo-HSCT). These Infectious complications sometimes cause of graft versus host disease (GVHD) or other allo-SCT related complications. Presepsin (Pre-SEP) is a subtype of soluble CD14, which is a receptor for lipopolysaccharide (LPS)/LPS-binding protein complexes and is expressed on the surface of monocytes and neutrophils. In cases of bacterial infections, Pre-SEP is produced from monocytes and neutrophils, and quickly elevated in plasma, due to phagocytes of bacteria by monocytes or neutrophils. Recently, it has been shown to be a useful biomarker for assessing the severity of sepsis and has come into use in the field of critical care medicine. However, little is known about the biological characteristics of Pre-SEP in patients with neutropenia including FN and other infectious complications after allo-HSCT.
Aims: we tried to clarify where it is a useful marker for diagnostic assessment of FN after allo-HSCT, and for predictive assessment of GVHD and other allo-HSCT related complications.
Methods: We prospectively measured the plasma concentration of Pre-SEP in the patients who received allo-HSCT, at Day-7, 0, 4, 7, 14, 21 and 28 after allo-SCT in our single institute: Iwate Medical University Hospital (N=30). And we evaluated the association between the Pre-SEP level and allo-HSCT related complications such as infections, GVHD and others. Furthermore, we analyzed the clinical significance of Pre-SEP as biomarker of life-threating complications after allo-HSCT. This study was approved by the institutional review board in Iwate Medical University.
Results: In most of cases after allo-SCT, Pre-SEP was elevated from the time at FN onset, and decreased to normal level along with improvement of FN due to the treatment of antibiotics and/or recovery from neutropenia. (N=30, Day-7: 182.5 pg/ml (104-356), Day0: 209 pg/ml (120-910), Day4: 258 pg/ml (145-862), Day7: 353 pg/ml (122-551), Day14: 470 pg/ml (165-835), Day21: 346 pg/ml (130-707), Day28: 348 pg/ml (114-861), median (range), cut off value: 314 pg/ml) Otherwise, Pre-SEP was continuously elevated in some cases after improvement of FN, and these continuously elevated cases tIended to be often accompanied with allo-HSCT related complications such as GVHD. In patients suffered from acute GVHD (grade2-4), the average of Pre-SEP level from the six points of following time in each case: at day0, Day4, day7, day14, day21 and day28 after allo-SCT were significantly higher compared to that in cases with no acute GVHD (p= 0.025). Similarly, in patients suffered from acute GVHD (grade3-4), the average of Pre-SEP level from the six points of following time in each case: at day0, day7, day14, day21 and day28 after allo-SCT were significantly higher compared to that in cases with acute GVHD (grade1-2) (p= 0.015). On the other hand, there was no deference in plasma Pre-SEP level at the above period after allo-SCT, between cases with chronic GVHD and without chronic GVHD.
Summary/Conclusion: Plasma Pre-SEP level is not only a reliable marker of FN after allo-SCT, but a predictive marker of severe acute GVHD. Closer monitoring of Pre-SEP could be a good help for the predictive assessment for allo-SCT related life-threating complications.

PS1475

ROLE OF IFN-GAMMA IN IMMUNE-MEDIATED GRAFT FAILURE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

P. Merli[1,*], I. Caruana[1], R. De Vito[2], F. Del Bufalo[1], L. Strocchio[1], G.A. Weber[1], A. Pitisci[1], V. Cirillo[1], F. Galaverna[1], M. Algeri[1], G. Palumbo[1], P. Montanari[3], M. Ballabio[3], F. Locatelli[1,4], C. De Min[3]

[1]Department of Pediatric Hematology and Oncology, [2]Histopathology Unit, IRCCS Bambino Gesù Children's Hospital, Rome, Italy, [3]Novimmune SA, Geneva, Switzerland, [4]University of Pavia, Pavia, Italy

Background: Data from animal models suggested that interferon gamma (IFNγ) plays a central role in graft failure (GF), an important cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT).
Aims: To characterize the pathophysiology of GF *in vivo*.
Methods: We characterized the cytokine/chemokine profile of patients experiencing GF after HSCT, through serial sampling (at day 0, +3, +7, +10, +14 and +30 after transplantation), comparing the results with matched-controls who achieved sustained donor engraftment. Cytokine levels in peripheral blood (PB) was evaluated using validated MesoScale Discovery

platform-based immunoassays (MSD, Rockville, USA). In 7 patients experiencing GF who underwent bone marrow sampling when GF was suspected, we also studied the lymphocyte infiltrate through flow-cytometry.
Results: Between December 2015 and August 2017, 15 patients developed GF, 14 primary and 1 secondary. Median age at transplant was 2.6 years (range 0.3-16). Patient's original diagnoses were: severe aplastic anemia (4 patients), familial hemophagocytic lymphohistiocytiosis (HLH, 3 patients), metabolic disorders (2 patients), acute lymphoblastic leukemia, acute myeloid leukemia, thalassemia major, congenital amegakaryocytic thrombocytopenia, malignant osteopetrosis, combined immunodeficiency (1 patient each). The donor was a partially matched family donor in 13 cases; either a matched unrelated donor or an unrelated cord blood unit was used for the remaining 2 patients. 50% of patients presented anti-HLA antibodies. Fifteen paired patients who underwent HSCT with sustained donor engraftment in the same period were used as controls.
The levels of IFNγ, CXCL9, CXCL10, sIL2Rα, IL10, sCD163 and TNFα differed significantly between GF patients and controls (Figure 1, panels A-C). In patients experiencing GF, IFNγ, CXCL9 and IL10 levels started to increase 3 days after infusion of stem cells. In details, IFNγ levels at day +3 were 8859±7502 pg/ml vs 0 pg/ml for controls (p=0.02), while CXCL9 levels were 1514.0±773 pg/ml vs 233.6 ±50.1 pg/ml for controls (p=0.0006). Regarding CXCL9 levels at day+3, ROC analysis showed an area under the curve of 0.905 (95% Confidence Interval 0.709-0.987) (p<0.0001) (Figure 1D); a cut-off value of 274.5 pg/ml had a sensibility of 88.89% and a specificity of 78.57%.
BM lymphocyte infiltrate showed an increased number of CD8+ positive cells; these cells displayed an effector memory phenotype (CD45RO+/CCR7-). Moreover, they expressed markers of both activation (e.g. over-expression of CD95 and down-regulation of CD127) and exhaustion (CD57, CD279 (PD1), CD223 and CD366). Finally, when the T-cell repertoire was analyzed by the evaluation of the Vβ chain on the CD3+ population, a polyclonal distribution of spectratypes across the TCR Vβ gene families was found.

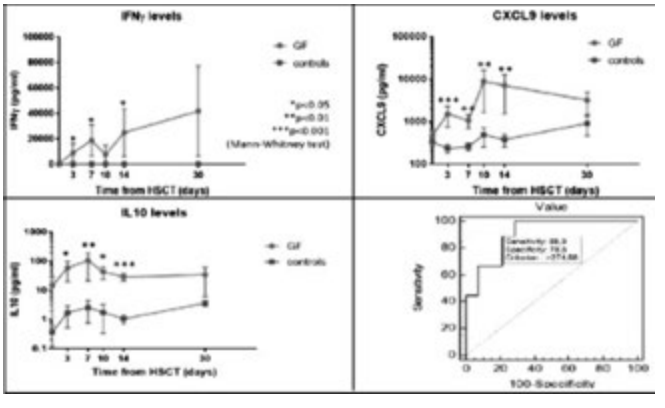


Figure 1.

Summary/Conclusion: We demonstrated a peculiar cytokine/chemokine profile in patients experiencing immune-mediated GF, characterized by high levels of IFNγ and CXCL9, as well as other "HLH-like" factors. These data suggest that the activation of IFNg-pathway, as shown by high CXCL9 levels, represents a biomarker for early diagnosis of GF. Moreover, our results offer the rationale for designing a study aimed at exploring the therapeutic/preventive role of a targeted neutralization of IFNg in patients undergoing HSCT using a monoclonal antibody such as emapalumab, already being studied for the treatment of HLH.

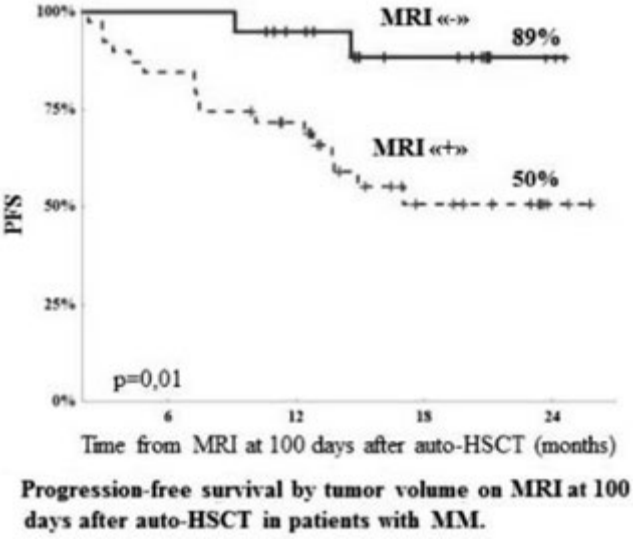
PS1476

EFFECTS OF POST-AUTO-HSCT MRI BONE MARROW LESIONS ON PROGRESSION-FREE SURVIVAL IN PATIENTS WITH MM

M. Solovev[1,*], L. Mendeleeva[1], G. Yatsyk[2], N. Lutsik[2], M. Firsova[1], V. Savchenko[1]

[1]Bone Marrow Transplantation, [2]Magnetic Resonance Tomography, National Research Center for Hematology, Moscow, Russian Federation

Background: In view of the pathogenic mechanisms of bone resorption by myeloma cells, visualization of lesions by MRI, the most sensitive method for the detection of infiltration, may become one of the criteria for subsequent differential treatment of patients with .
Aims: To determine the effect of bone marrow lesions detected by MRI in patients with following auto-HSCT on progression-free survival (PFS).
Methods: 60 patients with MM (26 male and 34 female) aged 36 to 66 years (median age 56) were enrolled in a prospective study in the period from December 2015 to June 2017. The ISS stages at the time of diagnosis were as follows: stage I in 22 patients, stage II in 22, and stage III in 16 subjects. Soft tissue components were observed in 23 patients (38%). All patients received induction therapy with bortezomib, immunomodulatory drugs were used in 10 cases. Following induction therapy, single auto-HSCT was performed in 47 patients and tandem auto-HSCT in 13. MRI of the spine and pelvic bones was carried out with a GE Signa Profile system without contrast enhancement 100 days after the first auto-HSCT. The nature of the lesions was determined and the bone marrow infiltration lesions (≥ 5 mm) counted. The total lesion volume was determined as follows: identified foci and bone marrow infiltration lesions were labeled based on signal intensity intervals using the Regions of interests tool; 3d reconstruction and the LINS MACHAON software were used for segmentation, with subsequent labeling of identified bone marrow lesions and comparison with the total labeled volume and the original image. After labeling of the infiltrative lesions, the abnormal MR signal area was calculated and recorded as the total tumour volume. Statistical analysis was done using Statistica 10.
Results: After auto-HSCT, the following antitumour response rates were observed: stringent CR in 19 patients (32%), CR in 13 (22%), VGPR in 23 (38%) and PR in 5 subjects (8%). MRI demonstrated bone marrow lesions in 47 patients: the number of lesions ranged from 1 to 56 (average 6). The distribution of subjects by number of detected lesions was as follows: 1 – 10 in 40 patients (85%), 11 - 20 in 5 (11%), and over 20 lesions in 2 cases (4%). The total tumour volume varied from 0 to 103 cm^3 (average 10 cm^3). At the time of the analysis, 58 patients were alive and 33 of them (57%) had maintained a CR for 13 to 29 months (median 17 months); 20 patients had a relapse 5 to 26 months after auto-HSCT (median 8 months). The two-year PFS for the entire sample was 55% (the median was not reached). In 20 out of 60 patients (33%), the tumour volume was found to be less than 1 cm^3, which was regarded as no minimal residual tumour or MRI negativity. Comparative PFS analysis conducted in patients with MM depending on the tumour volume at 100 days after auto-HSCT revealed a statistically significant difference (p=0.01): the two-year PFS in MRI-negative patients after auto-HSCT was 89% vs. 50% in MRI-positive subjects (Figure 1).



Progression-free survival by tumor volume on MRI at 100 days after auto-HSCT in patients with MM.

Figure 1.

Summary/Conclusion: MRI negativity after auto-HSCT is a favourable prognostic factor predicting long progression-free survival.

PS1477

COMBINED EVALUATION OF DISEASE RISK INDEX AND ABCG2 EXPRESSION IDENTIFY AML PATIENTS AT A VERY HIGH RISK OF RELAPSE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

D. Damiani[1,*], M. Tiribelli[1], A. Michelutti[1], A. Sperotto[1], A. Geromin[1], E. Toffoletti[1], R. Fanin[1]

[1]Division of Hematology and BMT, Azienda Sanitaria Universitaria Integrata di Udine, Udine, Italy

Background: Despite the increased use of allogeneic stem cell transplantation (SCT) as a curative option in high risk hematologic malignancies, disease relapse remains a major challenge for long term survival and prediction of relapse risk after SCT is therefore a pivotal issue. In the past years, the disease risk index (DRI) has been specifically developed to predict transplantation failure focusing mainly on disease recurrence rather then on transplant-related toxicities. We have previously reported a significant correlation between ABCG2 overexpression and relapse probability in patients undergoing allogeneic SCT for acute myeloid leukemia (AML).
Aims: In this work we evaluated the impact of ABCG2 expression on the outcome of SCT in AML patients classified according to DRI.
Methods: We retrospectively analyzed 184 patients (87 males and 97 females, median age 50 years, range: 17-22) receiving allogeneic SCT for high risk AML; according to conditioning regimen, 108 patients underwent myeloablative (MAC) and 76 reduced intensity (RIC) transplant. Competing risk analysis and Fine-Gray proportional hazard regression were employed to explore the impact of ABCG2 expression, DRI risk group, and the most common patient-, donor- and transplant-related variables on cumulative incidence of relapse (CIR) and non-relapse mortality (NRM).
Results: At the time of analysis 71/184 (38.5%) patients were alive in complete remission (CR), 71/184 (38.5%) relapsed after SCT and 42/184 (23%) died without relapsing. In univariate analysis, factors affecting relapse were status at transplant (62%, 95%CI 52-75 in patients with detectable AML vs 24%, 95%CI 17-33 in CR patients; p=0.00001), ABCG2 over-expression (49%, 95%CI 39-61 vs 28%, 95%CI 21-38 in ABCG2- patients; p=0.005), non-sibling donor (43%, 95%CI 24-53 vs 30%, 95%CI 21-42 in SCT from HLA-identical sibling; p=0.046), absence of chronic GVHD (44%, 95%CI 35-55 vs 20%, 95%CI 11-33 in patients with chronic GVHD; p=0.003), DRI (70%, 95%CI 52-83 in very high risk vs 57% 95%CI 47-70 in high risk vs 16%, 95%CI 10-26 in intermediate risk vs 11%, 95%CI 2-18 in low risk; p<0.0001). In multivariable analysis statistical significance was retained by ABCG2 positivity (HR=2.7, 95%CI 1.71-4.30; p=0.0002) and DRI risk group (HR=1.77, 95%CI 1.36-2.30; p=0.0002). Combination of ABCG2 and DRI resulted in four groups with significantly different relapse risk (Figure 1). Notably, ABCG2 overexpression identified among DRI intermediate patients a subgroup with significantly higher relapse probability (28%, 95%CI 16-49 vs 16%, 95CI 10-26 of the whole intermediate DRI group), and a small cohort of DRI very high patients with dismal outcome.

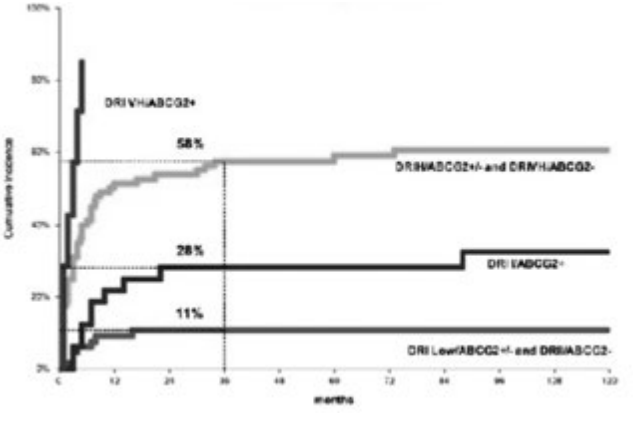


Figure 1. Relapse risk according to DRI and ABCG2.

Summary/Conclusion: ABCG2 overexpression confirmed its negative prognostic role after allogeneic SCT in AML and could be combined to DRI. A refined patient's stratification could be useful to tailor transplantation platforms to eradicate minimal residual disease or to develop preemptive therapies avoiding overt relapse.

PS1478

AFTER ALLOGENEIC HEMATOPOIETIC TRANSPLANTATION, A COMPOSITE SCORE BASED ON PLASMATIC LEVELS OF IL-2 RECEPTOR AND TIM-3, CAN PREDICT, AT DAY +18, TRM AND SEVERE A-GVHD

G. Milone[1,*], S. Leotta[1], A. Cupri[1], R. Crocchiolo[2], A.E. Marchese[3], G. Sapienza[4], A. Spadaro[4], G. Avola[4], L. Scalise[4], G. Tripepi[5]

[1]Hematology and BMT, Policlinico , Catania, [2]Hematology, Azienda Sanitaria Bergamo ovest, Bergamo, [3]Laboratory Medicine, [4]Hematology and BMT, Azienda Policlinico Vittorio Emanuele, Catania, [5]CNR, Ospedali Reggio Calabria, Reggio Calabria, Italy

Background: We have previously demonstrated that, at expected time of engraftment (day +18), patients that later showed acute GVHD, already at day +18, have a reduced colony growth in their marrow (Exp Hem 2015, 43:430) suggesting that at this time various degree of alloreactivity are already acting. On this basis we hypothesized that may be possible to identify early, at the time of engraftment (day +18), patients having distinctive levels of alloreactivity and hence distinctive prognosis.
Aims: In a prospective study, we determined, the prognostic value of plasma cytokines (IL-6, rec IL-2, ST-2, ICAM, IFN gamma, TIM-3) measured at expected time of engraftment (day+18/+19).
Methods: Plasma cytokines (IL-6, rec IL-2, ST-2, ICAM, IFN gamma, TIM-3) were measured using a ELISA assay, plasma was obtained on day+18/+19 after allogeneic hematopoietic transplantation. Eighty-five patients were studied, Mean age was 45 years (18-67), 53 were affected by myeloid malignancies, 32 by lymphoma or myeloma, donor type for allogeneic hematopoietic transplantation was in 38% an HLA-identical sibling and in 62% an alternative donor (MUD/HAPLO).
Results: In univariate analysis, negative factors important for OS were age (HR: 1.024, p=0.11), Alternative Donor (HR 1.913 p=0.07), Source of HSC from BM (HR 1.735, p=0.13), high level of Interleukin-2 receptor (HR 3.329, p=0.0004) and high level of TIM-3 in plasma. (HR: 10.087, p=0.002). In multivariable analysis high level of Interleukin-2 receptor and high level of TIM-3 were retained important for OS, (REC IL2: HR 3.140, p= 0.004; TIM-3: HR 10.940, p=0.003). ROC curve were used to identify in TIM-3 and REC IL-2 levels a cutoff having the best combination of sensitivity and specificity in respect to death for any cause and this cut-off value was used to divide continuous data in groups. A composite score from value on day +18 of TIM-3 and REC IL2 was developed and patients were classified in three groups based on number of cytokines over cut-off (none, one or two). Patients having both cytokines over the cut-off (24/85 patients) had a TRM of 40% versus a TRM of 12% in the group of remaining patient (61/85 pts), Gray test p=0.002. GVHD caused death in 25% of patients of high risk group while caused death in 6.8% of low risk patients.
Summary/Conclusion: The study of TIM.3 and REC IL-2 in plasma at day +18 allows the early identification of patients at high risk for NRM and of severe a-GVHD, such patients need increase of immunosuppressive therapy.

PS1479

A NEW ULTRASONOGRAPHY-BASED SCORING SYSTEM IS EFFECTIVE DIAGNOSTIC TOOL FOR SINUSOIDAL OBSTRUCTION SYNDROME AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

M. Nishida[1,2,*], K. Kahata[3], E. Hayase[1,3], M. Sato[2], Y. Kudo[1,2], S. Omotehara[1,2], T. Iwai[2], S. Shiratori[3], K. Okada[3], H. Goto[3], J. Sugita[3], M. Onozawa[3], D. Hashimoto[3], T. Endo[3], T. Kondo[3], H. Shibuya[2], C. Shimizu[1], T. Teshima[3]

[1]Division of Laboratory and Transfusion Medicine, [2]Diagnostic Center for Sonography, Hokkaido University Hospital, [3]Department of Hematology, Hokkaido University Faculty of Medicine, Sapporo, Japan

Background: Sinusoidal obstruction syndrome (SOS) / veno-occulusive disease (VOD) is a well-documented complication after hematopoietic stem cell transplantation (HSCT). The diagnostic criteria of SOS/VOD are based on clinical findings. Considering the pathogenesis of SOS/VOD, that is endothelial damage in sinusoid leading to impairment of blood flow and congestion, transabdominal ultrasonography (US) would be a very feasible modality thanks to its capacity to detect alteration of blood flow.
Aims: This study aimed to establish a new diagnostic scoring system based on US findings by prospective study.
Methods: The patients who underwent allogeneic HSCT in our institute were enrolled. US scanning method consisting of 17 items was applied to all the patients before HSCT and on day 14 or when the signs of SOS/VOD appeared after HSCT. Based on the findings obtained from 17-item method, new diagnostic method by US was established and assessed its effectiveness.
Results: In total 106 patients were analyzed, of which 10 patients were diagnosed as SOS/VOD by Baltimore criteria, Seattle criteria or The European Society for Blood and Marrow Transplantation criteria. As a result of univariate analysis between control and SOS/VOD group, 10 findings and parameters were selected and weighed according to its significance to establish scoring system. This new scoring system (US-10) which consists of ten findings including hepatomegaly, gall bladder wall thickening, portal hypertention appeared in portal vein, paraumbilical vein and hepatic artery resitive index, and ascites, was assessed for its effectiveness to detect SOS/VOD. When cut-off value of US-10 was set 5, the sensitivity and specificity was 100% and 95.8%, respectively. Two patients underwent US when the weight gain and slight elevation of serum bilirubin before meeting diagnostic cri-

teria, and they exhibited US-10 scores higher than 5. This suggests that our new scoring system could catch SOS/VOD earlier than clinical criteria. Furthermore, two patients marked 5 before HSCT and both of them developed SOS/VOD after HSCT, on the other hand, none of the control group exceeded 5 before HSCT. US-10 scores before HSCT might predict SOS/VOD.
Summary/Conclusion: US-10 scoring system showed good correspondence with widely used diagnostic criteria. It would be a powerful diagnostic tool for SOS/VOD. In addition, it is expected that new scoring system detects early stage of SOS/VOD prior to clinical criteria and predicts the risk of SOS/VOD before HSCT.

PS1480

THE SIGNIFICANCE OF PERI-TRANSPLANTATION MINIMAL RESIDUAL DISEASE ASSESSED BY MULTIPARAMETER FLOW CYTOMETRY ON OUTCOMES FOR ADULT AML PATIENTS RECEIVING HAPLOIDENTICAL ALLOGRAFTS

J. Liu[1,*], R. Ma[1], Y.-R. Liu[1], L.-P. Xu[1], X.-H. Zhang[1], H. Chen[1], Y.-H. Chen[1], F.-R. Wang[1], W. Han[1], K.-Y. Liu[1], Q.-Z. Fan[2], X.-J. Huang[1], Y.-J. Chang[2]

[1]Haematology Department, [2]Peking University People's Hospital, Beijing, China

Background: Allogeneic stem cell transplantation (Allo-SCT) is an effective therapy for acute myeloid leukemia (AML) patients. Several researchers have suggested that minimal residual disease (MRD) detected by multicolor flow cytometry (MFC) before and after SCT identifies a subgroup of patients at high risk of hematological relapse and poor outcomes in human leukocyte antigen (HLA)-matched transplantation. But there was no effect of pre-transplant MRD determined by MFC on relapse of patients with AML receiving haploidentical allografts.
Aims: To explore the impact of post-MRD status and peri-transplant MRD kinetics, especially in adult AML patients on transplant outcomes in haplo-SCT settings.
Methods: Adult AML patients, 18 years of age or older, in their first or second morphologic remission, who received haplo-SCT in Peking University People's Hospital from 1 Jan 2012 to 30 June 2016 were included in this retrospective study. Relationship of pre-MRD status, post-MRD status, or MRD dynamics peri-SCT and transplant outcomes were analyzed using the Kaplan-Meier method and a Cox proportional hazards model with time-dependent variables.
Results: A retrospective study (n = 460) was performed to assess the relationship between MRD and transplant outcomes in a haplo-SCT setting. Patients from the pre-MRDneg group and the pre-MRDpos group had comparable outcomes. Compared to post-MRDneg patients, post-MRDpos patients had a higher incidence of relapse (100% vs. 8.3%, p=0.000), lower incidences of OS (16.9% vs. 78.2%, p=0.000) and LFS (0% vs. 76.5%, p=0.000), and comparable probability of NRM (13.4% vs. 16.9%, p=0.560). In a second set of analyses, all adult AML patients undergoing haplo-SCT were classified into MRDneg/MRDneg group, MRD descending group, and MRD ascending group according to MRD dynamics by flow cytometry peri-SCT. Compared to other two groups, patients from the MRD ascending group had higher cumulative incidences of relapse (MRD ascending, 100%; MRDneg/MRDneg, 9.6%; MRD descending, 19.2%; p=0.000) and worse probabilities of OS (MRD ascending, 28.5%; MRDneg/MRDneg, 76.3%; MRD descending, 76.0%; p=0.000) and LFS (MRD ascending, 0.0%; MRDneg/MRDneg, 73.9%; MRD descending, 74.0%; p=0.000).
Summary/Conclusion: The results indicated that unmanipulated haplo-SCT is superior to MSDT in eradicating pre-transplantation MRD, and MRD assessment peri-SCT is useful for risk stratification from a practical perspective.

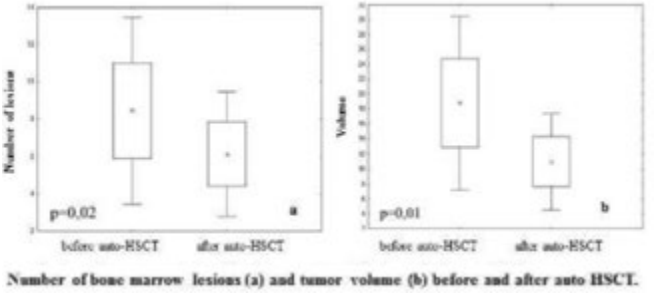
PS1481

CHANGES IN THE NUMBER OF BONE MARROW LESIONS AND TUMOUR MASS ON MRI IN PATIENTS WITH MM FOLLOWING AUTO-HSCT

M. Solovev[1,*], L. Mendeleeva[1], G. Yatsyk[2], N. Lutsik[2], M. Firsova[1], V. Savchenko[1]

[1]Bone Marrow Transplantation, [2]Magnetic Resonance Tomography, National Research Center for Hematology, Moscow, Russian Federation

Background: Despite the availability of results from several studies that evaluated the role of MR images in the diagnosis of MM, data on the value of MRI after antineoplastic treatment, in particular after auto-HSCT, are limited.
Aims: To determine the nature and scale of bone marrow involvement in patients with MM before and after auto-HSCT using MRI.
Methods: 40 patients with MM (15 male and 25 female) aged 36 to 66 years (median age 56 years) were enrolled in a prospective study in the period from December 2015 to March 2017. MRI of the spine and pelvic bones was performed before auto-HSCT and 100 days after it to track changes in bone marrow involvement after transplantation. MRI was carried out with a GE Signa Profile system without contrast enhancement. The nature of the lesions was determined and the bone marrow infiltration lesions (≥ 5 mm) counted. The total lesion volume was determined as follows: identified foci and bone marrow infiltration lesions were labeled based on signal intensity intervals using the Regions of interests tool; 3d reconstruction and the LINS MACHAON software were used for segmentation, with subsequent labeling of identified bone marrow lesions and comparison with the total labeled volume and the original image. After labeling of the infiltrative lesions, the abnormal MR signal area was calculated and recorded as the total tumour volume. Statistical analysis was done using Statistica 10.
Results: After completion of induction therapy, the following antitumour responses (according to the IMWG criteria) were observed: stringent CR in 7 patients (17.5%), CR in 9 (22.5%), VGPR in 17 (42.5%) and PR in 7 subjects (17.5%). Prior to auto-HSCT, normal bone marrow MR images were observed in 7 patients and focal lesions in 33: the number of lesions ranged from 1 to 80 (average 10). The distribution of subjects by number of detected lesions was as follows: 1 lesion in 10 out of 25 patients (75%), 11 - 20 in 5 (15%), and over 20 lesions in 3 cases (10%). The pre-auto-HSCT total lesion volume varied from 0 to 212 cm^3 (average 19 cm^3). At 100 days after auto-HSCT, a stringent CR was observed in 14 patients (35%), CR in 10 (25%), VGPR in 12 (30%) and PR in 4 subjects (10%). Post-auto-HSCT MRI revealed bone marrow lesions in 33 patients as well, their number ranged from 1 to 56 (average 7). The distribution of subjects by number of lesions detected after auto-HSCT was as follows: 1 lesion in 10 out of 27 patients (82%), 11 - 20 in 4 (12%), and over 20 lesions in 2 subjects (6%). The post-auto-HSCT total lesion volume varied from 0 to 103 cm^3 (average 10 cm^3). Analysis of changes in the number of lesions and tumour volume based on MRI findings revealed a lower number of lesions after auto-HSCT in 17 patients (52%) and a reduction of tumour volume in 30 cases (91%). High-dose chemotherapy with subsequent auto-HSCT was followed by a statistically significant (p=0.02) decrease in the number of bone marrow lesions on MRI (Figure 1a). Comparison of the tumour volumes detected by MRI before and after auto-HSCT also demonstrated a significant (p=0.01) reduction of the total lesion volume after auto-HSCT (Figure 1b).



Number of bone marrow lesions (a) and tumor volume (b) before and after auto HSCT.

Figure 1.

Summary/Conclusion: Auto-HSCT is an effective treatment stage for patients with MM that affects bone remodeling through reduction in bone marrow lesions and tumour volume.

PS1482

ACUTE INTESTINAL GRAFT-VERSUS-HOST DISEASE AMONG PATIENTS UNDERGOING ALLOGENEIC CELL TRANSPLANT FOR HEMATOLOGICAL MALIGNANCY IN THE UNITED STATES: A RETROSPECTIVE CLAIMS DATABASE ANALYSIS

A. Taylor[1,*], B. Johnson[2], G. Kim[2], J. Drahos[3], J. Yang[3], M. Akbari[3], N. Shah[4]

[1]Takeda Pharmaceuticals International, London, United Kingdom, [2]IBM Watson Health, Cambridge, [3]Takeda Pharmaceuticals International, Boston, [4]Medical College of Wisconsin, Milwaukee, United States

Background: While allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only curative option for many hematological malignancies, its effectiveness is limited by the occurrence of serious infections and graft-ver-

sus-host disease (GvHD) which presents in 30-80% of allo-HSCT recipients. There are limited data on real-world outcomes after allo-HSCT, however, of allo-HSCT complications, acute intestinal GvHD is associated with the highest risk of mortality and morbidity.
Aims: Using a large, healthcare claims database this study aims to understand infectious complications and healthcare utilization among patients who received an allo-HSCT who developed acute intestinal GvHD.
Methods: Patients receiving an allo-HSCT procedure were identified in the MarketScan® Research Databases between 2009-15. From those identified, those developing acute GvHD (aGvHD) post allo-HSCT were selected using diagnosis codes. A claims algorithm, which ranked the severity of aGvHD symptoms, was developed to identify intestinal aGvHD grade. Patients were followed for 1 year, end of continuous insurance enrollment, end of study period (9/30/16) or death, whichever occurred first. Demographic and clinical characteristics were identified at index and six months prior to allo-HSCT, respectively. Generalized linear regression was used to estimate the probability of having a subsequent inpatient (IP) admission, emergency room (ER) visit and developing infection post-allo HSCT.
Results: A total of 1,288 patients (median age 54, range 53 to 55) m*et al.*l selection criteria; 43.9% (n=565) were identified as having aGvHD, of those 59.8% (n=338) were identified as having intestinal aGvHD. Age, gender and overall comorbidity were similar between those with intestinal aGvHD and those without aGvHD. The proportion of patients with an IP admission and ER visit after discharge from allo-HSCT was significantly higher among those with intestinal aGvHD compared to those without aGvHD (82.2% vs 54.8%) and (45.6% vs. 32.5%), respectively, both p<.0001. The most common infections in allo-HSCT patients were pneumonia (52.4%), septicemia (27.4%) and cytomegalovirus (22.1%) which were also significantly higher in patients with intestinal aGvHD compared to those without aGvHD (63.9% vs 46.9%), (39.3% vs. 23.1%) and (38.2% vs. 16.2%), respectively, all p<.0001. Patients with intestinal aGvHD were 4 times more likely to have a subsequent IP admission (odds ratio [OR], 4.02; 95%CI, 2.88-5.62; p<.0001) and nearly twice as likely to have an ER visit (OR, 1.78; 95%CI, 1.34-2.36; p<.0001) after discharge from their allo-HSCT than patients who did not develop aGvHD. Patients with intestinal aGvHD were nearly 4 times more likely to develop infections than patients who did not develop aGvHD (OR, 3,77; 95%CI, 2.43-5.86; p<.0001) (Table 1).

Table 1.

SELECTED DESCRIPTIVE METRICS	Intestinal aGVHD Patients	Non-aGVHD Patients	P Value
PATIENT CHARACTERISTICS MEASURED IN THE BASELINE PERIOD	N = 338	N = 723	
Age [Mean, SD]	56.3 (14.9)	56.9 (14.5)	0.5325
Male [N, %]	190 (56.4)	418 (57.8)	0.7565
Deyo Charlson Comorbidity Index [Mean, SD]	3.6 (1.9)	3.6 (2.0)	0.3594
Hematological Malignancy [not mutually exclusive] [N, %]			
Acute lymphoblastic leukemia	116 (32.5)	196 (27.1)	0.0086
Acute myeloid leukemia	222 (65.7)	444 (61.4)	0.2063
Chronic lymphocytic leukemia	39 (11.5)	87 (12.0)	0.8265
Multiple myeloma and plasma cell neoplasms	36 (10.7)	96 (13.4)	0.2991
Myelodysplastic syndrome	103 (30.5)	224 (31.0)	0.8672
Other (including lymphosarcoma and other connective and soft tissue neoplasms	7 (2.1)	9 (1.3)	0.3836
HEALTHCARE UTILIZATION MEASURED POST-INDEX			
Patients with at least one ER visit subsequent to discharge from allo-HSCT [N, %]	154 (45.6)	235 (32.5)	<0.0001
Number of ER visits [Mean, SD]	1.6 (2.5)	1.1 (2.3)	<0.0001
Patients with at least one inpatient hospitalization subsequent to discharge from allo-HSCT [N, %]	278 (82.2)	396 (54.8)	<0.0001
0 additional hospitalization (only initial index allo-HSCT)	60 (17.8)	327 (45.2)	<0.0001
1 additional hospitalization	76 (22.5)	176 (24.4)	0.4478
2 additional hospitalizations	59 (17.5)	104 (14.4)	0.1962
3 additional hospitalizations	60 (17.8)	48 (6.6)	<0.0001
4 additional hospitalizations	33 (9.8)	33 (4.6)	0.0011
5+ additional hospitalizations	50 (14.8)	33 (4.6)	<0.0001

Summary/Conclusion: In this US claims analysis, patients who developed intestinal aGvHD after allo-HSCT had increased morbidity. Patients with intestinal aGvHD were nearly 4 times more likely to develop a major infection, 4 times more likely to have a subsequent IP admission and nearly twice as likely to have an ER visit than patients who did not develop aGvHD. Using real-world evidence to study patients undergoing allo-HSCT for hematological malignancy can augment clinical research leading the way to a better understanding of the therapeutic gaps for these patients. New strategies are indicated to optimize health care and minimize risk of morbidity among patients with intestinal aGvHD.

PS1483

ALLOGENEIC HEMATOPOIETIC STEM CELLS TRANSPLANTATION FOR NON-HODGKIN'S LYMPHOMA IN SWITZERLAND. 30 YEARS OF EXPERIENCE 1985-2017

E. Chigrinova[1,*], P. Stakia[2], M. Nabergoj[3], M. Medinger[4], H. Baldomero[5], S. Masouridi-Levrat[3], C. Dantin[3], K. Ritter[6], A. Stern [4], A. Rovó[1], U. Schanz[6], J. Passweg[4], Y. Chalandon[3]

[1]Hematology, University Hospital of Bern, Bern, [2]Hematology, Hôpital du Valais, Sion [3]Hematology, Geneva University Hospitals (HUG), Geneva, [4]Hematology, [5]Swiss National Stem Cell Transplant Registry/SBST, University Hospital of Basel, Basel, [6]Hematology, University Hospital of Zurich, Zurich, Switzerland

Background: Because of the relatively high transplant-related mortality rate, allo –HSCT in NHL often remains the ultimate line of treatment. However, despite of a quick development of new efficient targeted therapies, allo-HSCT represents so far the only potentially curable treatment for nearly all types of NHL. Introduction of reduced intensity conditioning regimens, graft's T-depletion, better supportive care and graft-versus-host disease (GvHD) prophylaxis and treatment have decreased non-relapse mortality (NRM) rates, making use of allo-HSCT an important alternative therapeutic option in the setting of relapsed/refractory NHL.
Aims: We report here the 30 years of Swiss experience in allo-HSCT for NHL.
Methods: The study included 254 allo-HSCT episodes performed in 3 University Hospitals of Switzerland (Zürich, Basel and Geneva) between September 1985 and August 2017 for NHL including: chronic lymphocytic leukemia (n=66), diffuse large B-cell lymphoma (n=60), mantle cell lymphoma (n=31), follicular lymphoma (n=22), marginal zone lymphoma (n=7), burkitt lymphoma (n= 8), Waldenstrom's macroglobulinemia (n=6), hairy cell leukemia (n=1), T-cell lymphoma, NOS (n=33), anaplastic large T-cell lymphoma (n=7) and angioimmunoblastic T-cell lymphoma (n=13). All patients relapsed after standard therapy. Impact on OS, DFS, RI and NRM was assessed in univariate and multivariate analysis for: year of allo-HSCT before and after the 2000; prior auto-HSCT or allo-HSCT; disease status including CR, PR, SD, PD; number of remission: 1st vs >1st; Karnofsky index >80% vs <80%; donor's type: identical siblings, MUD, MMUD; donor's age: >60 vs <60; stem cell source: BM vs PBSC; no vs graft's T-depletion; conditioning type: RIC vs MAC; no vs TBI use; no vs acute/chronic GvHD; T vs B-cell origin; indolent vs aggressive NHL; NHL type.
Results: Median follow up was 18.5 months (5-72) with 25 years of the maximum follow up. Median OS=168 months, DFS= 107 months. Patient's median age at the time of allo-HSCT was 50.7 (43.7-57.2). 5 and 10-years OS, DFS and NRM were 57±4%, 53±4%, 23±3% and 51±4%, 46±4%, 25±3.0% respectively. RI at 5 and 10 years was 30±3% and 38±4%. In univariate analysis, no prior auto/allo HSCT, Karnofsky > 80%, 1st remission at HSCT (Figure 1) and disease status (CR) were associated with both improved OS and DFS (p<0.05). TBI use showed also a trend toward better OS and DFS (p=0.058 and 0.06 respectively). Related donor (vs unrelated) was associated with better OS (p=0.017). In multivariate analysis Karnofsky > 80%, CR and PR, absence of prior auto/allo-HSCT were the most significant factors influencing OS and DFS (p<0.05). NRM was influenced by donor's type, with the worse survival for the MUD vs Identical-sibling (p=0.001) and >1st remission status (p=0.03). The highest RI was found in cases transplanted before the year 2000 (p=0.03) non being in 1st remission (p=0.01) and those with aGvHD (p=0.007).

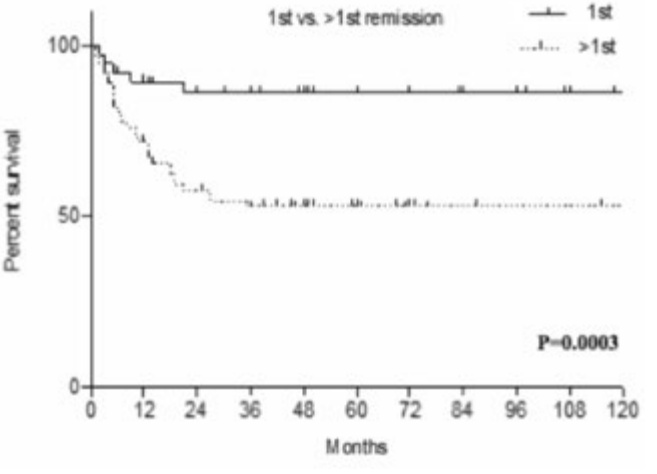


Figure 1.

Summary/Conclusion: Our analysis of a mixed-type NHL cohort at 25 years of maximum follow-up confirms the efficacy of allo-HSCT in pretreated/refractory patients showing promising 5 and 10 years OS and acceptable NRM rate. Interestingly, OS was found to be independent of NHL type and GvHD presence. Patient's status according to Karnofsky index, remission quality (CR and PR) and absence of heavy pretreatment were associated with better OS and DFS. These results should be interpreted

carefully due to the retrospective nature of the analysis; the heterogeneity of NHL and the conditioning regimen.

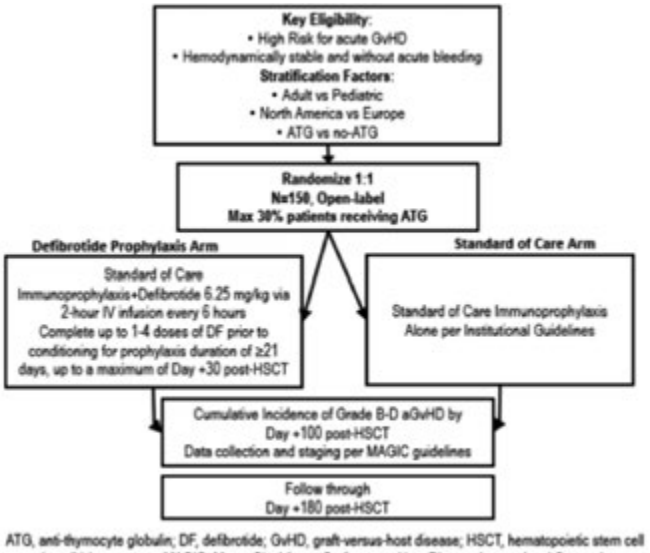
PS1484

PHASE 2, OPEN-LABEL STUDY OF DEFIBROTIDE FOR PREVENTION OF ACUTE GVHD FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT (HSCT)

S. Corbacioglu[1,*], P. Kim[2], R. Hume[2], W. Tappe[2]

[1]Department of Pediatric Hematology, Oncology and Stem Cell Transplantation, University of Regensburg, Regensburg, Germany, [2]Jazz Pharmaceuticals, Inc., Palo Alto, United States

Background: GvHD, a life-threatening complication of HSCT, causes mortality in ~20% of patients who receive allogeneic HSCT (aHSCT). Current immunoprophylactic therapies for GvHD show variable efficacy and safety, indicating a need for additional strategies. Acute GvHD (aGvHD) usually arises within the first 100 days post-aHSCT. Defibrotide (DF) is approved for the treatment of severe VOD/SOS post-HSCT in patients aged >1 month in the European Union and VOD/SOS with renal or pulmonary dysfunction post-HSCT in the United States and Canada; the use of defibrotide (DF) for prophylaxis of aGvHD or VOD/SOS is off-label. In a study of DF for VOD/SOS prophylaxis (Corbacioglu, *Lancet* 2012), a secondary endpoint analysis suggested that DF may reduce aGvHD incidence; children receiving DF had a significantly reduced incidence of aGvHD at Day +100 post-HSCT versus controls. The hypothesis of DF activity in aGvHD based on this finding warranted further study.
Aims: To compare the efficacy of DF added to standard-of-care (SOC) immunoprophylaxis vs SOC immunoprophylaxis alone for the prevention of aGvHD.
Methods: This phase 2, prospective, randomized, open-label study (Figure 1) is targeting enrollment of 150 pediatric and adult patients with acute leukemia or myelodysplastic syndrome who are at high risk of aGVHD due to planned myeloablative or reduced-intensity conditioning prior to non-manipulated bone marrow or CD3+ T-cell replete peripheral blood stem cell grafts from unrelated donors. Inclusion criteria are leukemia in morphologic complete remission, normal liver function (total bilirubin <2x the upper limit of normal [ULN]; alanine aminotransferase and aspartate aminotransferase <3x ULN), and HLA-matched or single allele mismatch graft (high-resolution DNA-based typing). Exclusion criteria are prior HSCT, acute bleeding ≤24 hours before start of treatment, use of medication that increases bleeding risk, comorbid condition, pregnancy, lactation, and psychiatric illness. Diagnosis and staging of aGvHD follow the recommendations of the Mount Sinai aGvHD International Consortium. Grading of aGvHD uses the International Bone Marrow Transplant Registry Severity Index. Randomized patients (1:1) are administered SOC immunoprophylactic therapies without (control) or with DF (25 mg/kg/day) in four 2-hour infusions of 6.25 mg/kg every 6 hours beginning before the start of conditioning regimen (up to 4 doses) for a recommended duration of ≥21 days up to Day +30 post-HSCT.



ATG, anti-thymocyte globulin; DF, defibrotide; GvHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplant; IV, intravenous; MAGIC, Mount Sinai Acute Graft-versus-Host Disease International Consortium.

Figure 1. Study design.

Results: The primary outcome measurement is the cumulative incidence of Grade B D aGvHD in the DF-treated and control groups by Day +100 post-HSCT. Secondary endpoints include Grade B–D aGvHD-free survival by Days +100 and +180 post-HSCT; cumulative incidence of Grade B–D aGvHD by Day +180 post-HSCT, Grade C–D aGvHD by Days +100 and +180 post-HSCT, and cumulative incidence of relapse by Days +100 and +180 post-HSCT; and comparison of health-related quality of life and safety profile. Enrollment is ongoing.
Summary/Conclusion: Results will help determine the safety and efficacy of DF for the prevention of aGvHD in high-risk pediatric and adult patients receiving aHSCT.

PS1485

PROGNOSTICATION OF CHRONIC GRAFT VERSUS HOST DISEASE: THE IMPACT OF IMMUNE RECONSTITUTION

F. Serpenti[1,2,*], F. Lorentino[1], R. Milani[3], R. Greco[1], M. Marcatti[1], A. Assanelli[1], C. Messina[1], D. Mannina[1], M. carrabba[1], F. Giglio[1], M. Sarah[1], F. Lunghi[1], E. Guggiari[1], C. Corti[1], M. Bernardi[1], J. Peccatori[1], C. Bonini[2,4], F. Ciceri[1,2], M.T. Lupo-Stanghellini[1]

[1]Haematology and BMT Unit, [2]Vita-Salute San Raffaele University, [3]Immuno-Haematology and Transfusion Medicine Unit, [4]Experimental Haematology Unit, Division of Immunology, Transplantation and Infectious Diseases Programm in Immunology and Bio-Immunotherapy of Cancer (PBIC), San Raffaele Scientific Institute IRCCS, Milano, Italy

Background: Allogeneic stem cell transplantation (HCT) survivors are at risk of developing long-term complications such as chronic GvHD (cGvHD); a precise prognostic stratification of these patients (pts) emerged in recent years. Since 2011, the CIBMTR risk score is a validated tool to assess the prognosis of pts developing cGvHD, based on 10 clinical and easy-to-collect variables. A revised version has been published more recently taking into account the number of lymphocytes and eosinophils. Immune reconstitution (IR) impairment affects HCT survivors at very long-term follow-up (LTFU) which justifies the occurrence of late fatal infection especially in presence of GVHD-history.
Aims: We aimed at prospectively validating the CIBMTR score in our Center, plus finding correlation with IR data at time of cGvHD diagnosis to enhance prognostication.
Methods: We analyzed 111 consecutive adult pts who underwent an HCT between July 2012 and December 2016 at our Institution and developed cGvHD at any time point and of any severity (according to NIH 2014 guidelines). A written consent was given for the use of medical records for research in accordance with the Declaration of Helsinki. Median follow-up for surviving pts was 3.2 y (range 1.1-5.5). For the univariate analysis of overall survival (OS), we considered the following covariates: patient age, female to male gender mismatch, disease risk index, type of donor (matched-related vs matched-unrelated vs haploidentical), GvHD prophylaxis (ATG- vs CTX-based), Karnofsky performance status (PS), history of prior acute GvHD, lymphocytes and eosinophils counts at cGvHD diagnosis and IR variables at time of cGvHD onset. We built a multivariate model considering all variables significantly associated with OS (log rank p-value <0.05) and, once we identified the variables independently predicting OS, we derived a formula of a risk index predicting survival by using the B-coefficients (β) found in the model. Each pts was assigned a score and, then, we defined three prognostic risk-groups (low, intermediate, high) by dividing the population into three classes using the first and third quartiles.
Results: Our data confirmed the validity of the CIBMTR risk score - both the standard and the revised versions - in particular in identifying very high risk patients – ref Figure 1. We identified 4 variables that significantly ($p<0.05$) proved to be independently correlated to OS in our multivariate model: CD3CD4>233/mcL (HR 11,4, β 2.4, p 0.003), NK cell<115/mcL (HR 8.1, β 2.1, p0.003), IgM<0,45 g/L (HR 8, β2.1, p 0.007), Karnofsky PS<80% (HR 72, β 4.3, $p<0.001$). Final score was calculated as follows: 2,4 (if CD4>233/mcL) + 2,1 (if NK<115/mcL) + 2,1 (if IgM<0,45 g/L) + 4,3 (if Karnofsky<80%). Patients were stratified into 3 risk classes: low risk if score ≤2,4; intermediate risk if 2,4≤4,5; high risk if >4.5. These 3 different risk classes significantly predicted transplant-related mortality (TRM) and OS: the 3.5y cumulative incidences of TRM were 3.6%, 16.6% and 28.6% for low, intermediate and high-risk pts, respectively (p=0.006). OS at 3.5y was 96%, 71% and 27% for low, intermediate and high-risk pts, respectively ($p<0.001$).
Summary/Conclusion: Irrespective of donor, cGvHD affects 20-50% of HCT survivors. The CIBMTR risk score is able to provide a clear prediction of outcome in these pts, allowing a personalized therapeutic intervention. Monitoring of IR after HCT is strongly informative and could be an adjunc-

tive tool to identify high-risk patients at cGvHD onset.

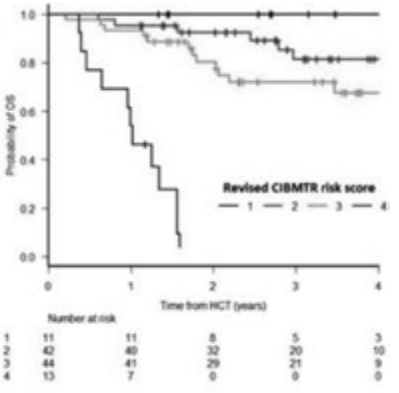


Figure 1.

PS1486

UTILIZATION AND OUTCOMES OF FERTILITY PRESERVATION TECHNIQUES IN WOMEN UNDERGOING ALLOGENIC HEMATOPOIETIC CELL TRANSPLANT

A. Higgins[1,*], Z. Khan[1], C. Coddington[1], S. Hashmi[1], M. Hefazi[1], H. Alkhateeb[1], M. R. Litzow[1], W.J. Hogan[1], E. Cathcart-Rake[1], C. Thompson[1], M.M. Patnaik[1]

[1]Mayo Clinic, Rochester, United States

Background: Iatrogenic menopause with consequent infertility is a major complication in reproductive aged women undergoing hematopoietic stem cell transplantation (HCT). Recent American Society of Clinical Oncology guidelines recommend a discussion of the possibility of infertility and the options for fertility preservation as part of informed consent before initiation of any cancer-directed therapy, including HCT.
Aims: We carried out this study in order to i) describe disease and reproductive variables in a cohort of reproductive aged women undergoing allogeneic HCT, ii) assess rates of fertility counseling provided, and iii) document utilization and outcomes of fertility preservation techniques.
Methods: After IRB approval, women aged 15-49 years at the time of allogeneic HCT, between the years 2001 and 2017, were identified from the Mayo Clinic Rochester institutional HCT database. Medical records were abstracted to obtain demographic data, treatment details, reproductive variables, and fertility counseling and outcomes. Data was analyzed primarily using descriptive statistics and chi-square test was used to compare frequencies.
Results: One hundred seventy-seven women were age eligible, of whom 49 (28%) were excluded due to documented post-menopausal state or prior hysterectomy. The median age of the cohort was 31 years (range, 15-49) with median gravidity and parity being G1P1 (range, G0-8P0-6). Fifty-four (42%) women were nulligravid at the time of HCT. Reasons for HCT included AML 49%, ALL 23%, MDS/MPN 13%, chronic myeloid neoplasms 2%, Hodgkin's lymphoma 2%, and non-malignant hematological disorders 11%. Eighty-six percent underwent a myeloablative conditioning (MAC) (65% CY+TBI 1200-1320cGY and 32% BU+CY), while 14% received a reduced intensity conditioning (RIC) (24% FLU+MEL, 20% CY+ATG, 4% FLU+BU, and 48% other). Only 34 women (27%) had documented fertility counseling within 72 hours of diagnosis and a total of 61 (48%) prior to HCT. Thirty-eight women (30%) were referred to a reproductive endocrinologist, of whom 13 (10%) underwent assisted reproductive technologies (ART; 9 oocyte cryopreservation, 4 embryo cryopreservation). Of these, 7 were successful, 4 were unsuccessful, and 2 had been previously completed at an outside institution prior to HCT. The median time to successful completion of the successful 7 ART procedures was 13 days. The remaining 25 women could not undergo ART due to disease severity (68%), low antral follicle count (12%), and financial barriers (20%). Ninety-three women (72.7%) received Lupron for ovarian suppression prior to conditioning although not all of these women had documented discussion of all fertility preservation options. Three (3%) of 105 women who underwent MAC had spontaneous menstrual recovery after HCT (median 14 months; range, 6-21 months), in comparison to 10 (43.5%) of 23 women who underwent RIC ($p < 0.0001$) (median 21.5 months; range, 5-83 months). In this cohort, there were two spontaneous pregnancies (8.7%), both occurring after RIC (CY+Campath+400 cGy TBI and CY+ATG) at 71 and 72 months after HCT, respectively.
Summary/Conclusion: Oncofertility is an emerging field due to an increasing number of young cancer survivors. Herein, we document that even at a large tertiary HCT center the rate of fertility counseling and reproductive endocrinology referrals was low and the rate of successful assisted reproductive interventions was even lower. Spontaneous menstrual recovery was rare, but more likely in the setting of RIC HCT. A concerted multidisciplinary effort is needed to improve oncofertility referral and outcomes.

PS1487

LOW INCIDENCE OF SYMPTOMATIC OSTEONECROSIS AFTER ALLOGENEIC HSCT IN CHILDREN WITH HIGH-RISK OR RELAPSED ALL – RESULTS OF THE ALL-SCT2003 TRIAL

M. Kuhlen[1,*], P. Bader[2], M. Sauer[3], M.H. Albert[4], B. Gruhn[5], T. Güngör[6], G. Kropshofer[7], P. Lang[8], A. Lawitschka[9], M. Metzler[10], F. Pentek[11], C. Rössig[12], P. Schlegel[13], M. Schrappe[14], J. Schrum[15], A. Schulz[16], W. Schwinger[17], A. von Stackelberg[18], B. Strahm[19], M. Suttorp[20], I. Teichert-von Luettichau[21], W. Wößmann[22], A. Borkhardt [1], R. Meisel[1], U. Poetschger[9], E. Glogova[9], C. Peters[9]

[1]Department of Pediatric Oncology, Hematology and Clinical Immunology, Center for Child and Adolescent Health, Medical Faculty, Heinrich Heine University Duesseldorf, Duesseldorf, [2]Department for Children and Adolescents, Division for Stem Cell Transplantation and Immunology, University Hospital Frankfurt, Frankfurt, [3]Department of Pediatric Hematology and Oncology, Hannover Medical School, Hannover, [4]Department of Pediatrics, Dr. von Hauner University Children's Hospital, Ludwig-Maximilians Universität, München, [5]Department of Pediatrics, Jena University Hospital, Jena, Germany, [6]Division of Stem Cell Transplantation, University Children's Hospital Zürich, Zürich, Switzerland, [7]Department of Pediatrics, Medical University Innsbruck, Innsbruck, Austria, [8]Department of General Paediatrics, Oncology/Haematology, Tübingen University Hospital for Children and Adolescents, Tübingen, Germany, [9]Children's Cancer Research Institute (CCRI), St. Anna Kinderkrebsforschung, Department of Paediatrics, Medical University of Vienna, Vienna, Austria, [10]Department of Paediatrics and Adolescent Medicine , University Hospital Erlangen , Erlangen, [11]Department of Pediatric Hematology and Oncology, Kinderklinik III, Universitätsklinikum-Essen and the University of Duisburg-Essen, Essen, [12]Department of Pediatric Hematology and Oncology, University Children´s Hospital Muenster, Muenster, [13]University Children's Hospital, Pediatric Oncology, Hematology and Stem Cell Transplantation, University of Würzburg, Würburg, [14]Department of Pediatrics, University Hospital Schleswig-Holstein, Campus Kiel, Kiel [24105], Kiel, [15]Department of Pediatric Hematology and Oncology, University Children's Hospital Hamburg-Eppendorf, Hamburg, [16]Department of Pediatrics, University Medical Center Ulm, Ulm, Germany, [17]Department of Paediatrics and Adolescent Medicine, Medical University of Graz, Graz, Austria, [18]Department of Paediatric Oncology/Haematology, Charité Universitätsmedizin Berlin, Charité Campus Virchow, Berlin, [19]Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology, Medical Centre, Faculty of Medicine, University of Freiburg, Freiburg, [20]Department of Pediatrics, University Clinic Carl Gustav Carus, Technische Universität Dresden, Dresden, [21]Children's Hospital Medical Center, Technische Universität München, München, [22]Department of Pediatric Hematology and Oncology, Justus-Liebig-University, Giessen, Germany

Background: High-risk acute lymphoblastic leukemia (ALL) can currently be cured in up to 70% of children using hematopoietic stem cell transplantation (HSCT). However, long-term side-effects seriously impacting quality of life remain substantial. Although osteonecrosis (ON) accounts for one of the most debilitating long-term sequelae, reliable data remain scarce.
Aims: To determine incidence of and risk factors for symptomatic ON (sON) in children and adolescents undergoing allogeneic HSCT for high-risk or relapsed ALL in the ALL-SCT 2003 trial.
Methods: Children and adolescents with ALL enrolled in the prospective ALL-SCT-BFM 2003 trial between September 2003 and September 2011 were eligible for this analysis. ON assessment was performed prospectively by means of adverse event and routine follow-up reporting. In patients in whom sON had been reported, additional data were retrieved retrospectively via an ON-specific questionnaire.
Results: 557 patients were analyzed. Median age at HSCT was 10.3 years (range 0.5-26), median time from HSCT until last follow-up was 5.8 years (range 0.06-11.7). 55 patients were diagnosed with sON resulting in a CI of 7% at 5 years (SD 1%). Median time from HSCT to diagnosis of sON was 13.4 months (range 1-80). 43% of those patients were diagnosed within the first year after HSCT, 67% within two years. 14 patients had sON prior to HSCT. Most prominent localizations of sON were knees (26; 65%) followed by hips (20; 50%), and feet (18; 46%).
In univariate analysis, the CI of ON in children younger than 10 was 2% (SD 1%) at 5 years after HSCT. The CI in children aged 10-15 years and 16 years or older was 8% (SD 2%) and 11% (SD 4%) respectively

(p<0.001). Children with a history of ON prior to HSCT (CI of ON 45% SD 17% vs. 8% SD 2% without ON prior to HSCT, p<0.001) and those with aGvHD of grade 3 or 4 (hazard ratio (HR) 1.69; 95% CI, 1.2-2.7; p=0.017) or cGvHD (hazard ratio (HR) 1.655; 95% CI, 1.2-2.7; p=0.009) were at higher risk to develop ON. Neither gender (CI 8% (male) SD 1% vs. 5% (female) SD 2%, p=0.387), first or later remission (CI 7% SD 2% vs. 7% SD 1%, p=0.741), donor type (MSD CI 9% SD 2% vs. MD CI 6% SD 1% vs. MMD CI3% SD 2%, p=0.110), stem cell source (BM CI 6% SD 1% vs. PB CI 8% SD 2% vs. other CI 0% SD 0%; p=0.7662) or type of conditioning regimen (with TBI CI 5% SD 2% vs. without TBI 7% SD 1%; p=0.390) were significant risk factors.
In multivariate analysis, age at HSCT (10-15 years vs. <10 years HR 4.49, p=0.011; >15 years vs. <10 years HR 6.92, p=0.001), diagnosis of sON prior to HSCT, and cGVHD (yes vs no HR 2.99, p=0.020) proved to be significant independent risk factors for the development of ON.
Summary/Conclusion: CI of osteonecrosis was 7% in patients enrolled in the ALL-SCT 2003 trial. Apart from preexisting sON prior to HSCT, age and cGVHD turned out to be the most important prognostic factor for the development of sON. The impact of acute GvHD of grade 3 or 4 on the development of ON requires further study.

PS1488

EARLY BLOOD STREAM INFECTION AFTER BMT IS ASSOCIATED WITH CYTOKINE DYSREGULATION AND POOR OVERALL SURVIVAL

E. Catchpoole[1], C. Thirunavukarasu[2], A. Varelias[3], S. Schlebusch[4], S. Olver[5], N. Zomerdijk[2], E. Osland[2], G. Kennedy[2], S.-K. Tey[5], G. Hill[5], K. Markey[5,*]

[1]Pathology Queensland, [2]Royal Brisbane and Womens Hospital, [3]QIMR Berghofer Medical Resarch Institute, [4]Mater Pathology, [5]QIMR Berghofer Medical Research Institute, Brisbane, Australia

Background: The key complications of allogeneic bone marrow transplant (BMT) remain graft-versus-host disease (GVHD) and opportunistic infection.
Aims: We examined the rates of confirmed blood stream infection (BSI) in allogeneic BMT patients, the organisms isolated, and the association of BSI with other post-transplant outcomes and the dysregulation of inflammatory cytokines.
Methods: 184 patients in a prospective observational cohort of allogeneic BMT recipients treated at the RBWH between 2009 and 2015 were studied. Patients underwent either myeloablative conditioning (MAC; cyclophosphamide and total body irradiation) or reduced intensity conditioning (RIC; fludarabine and melphalan). We examined all blood cultures collected between day -7 and day 100, and assessed the results, along with other infectious complications and overall transplant outcome. IL-1, IL-6, IL-8, TNF and IFNγ were analysed in plasma samples collected at day -7, 0, +3, +7, +14, +21, +30, +60, +90 and +120 using cytokine bead array.
Results: Median patient age was 52±14 years (58.7% male), with 35.3% of transplants for acute myeloid leukemia, 18.5% for acute lymphoblastic leukemia and 16.8% for myelodysplastic syndrome. 167 of the 184 patients (91%) had blood cultures collected, and 69 (38%) patients had a confirmed BSI. *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Enterococcus* spp. and viridans *Streptococcus* spp. were the most commonly isolated organisms. Gender, conditioning (RIC vs. MAC) and donor type (sibling vs. unrelated) did not differ significantly between those with and without confirmed BSI. Elevated temperature (>38°C) at the time of culture collection was associated with an almost 2-fold increased rate of returning a positive blood culture (16.8% vs 9.3%; OR 1.9, 95% CI 1.21 – 3.23; p=0.0058). On a per-patient basis, CMV reactivation at any time before day 100 was not associated with BSI (OR 1.38, 95% CI 0.71 – 2.65; p=0.39). BSI-free status was associated with a significant improvement in 2 year overall survival (HR 0.59, 95% CI 0.37-0.95; p=0.01), due to a significant reduction in non-relapse mortality predominantly unrelated to the primary BSI.
The presence of a BSI prior to engraftment was associated with the dysregulation of IL-6 and IL-8. Significantly higher levels of IL-6 were seen at day 14 in recipients of RIC allografts with pre-engraftment BSI compared to those without. IL-8 was significantly elevated in patients who received either RIC or MAC allografts in the setting of pre-engraftment BSI, at day 7 in the RIC recipients and day 30 in the MAC group.
Summary/Conclusion: Our findings suggest that BSI early after BMT defines a group of high-risk patients with enhanced cytokine dysregulation and poor transplant outcome. This is the first study reporting the BSI-specific induction of IL-6 and IL-8 in the setting of the broader cytokine dysregulation after BMT. The contribution of BSI to cytokine dysregulation and transplant-related mortality now needs to be studied in large prospective multicenter studies, ideally in conjunction with microbiome analysis.

PS1489

REGRESSION TREE BASED MODEL FOR PREDICTING NON-RELAPSE MORTALITY IN AML PATIENTS ALLO-TRANSPLANTED IN FIRST COMPLETE REMISSION

A. Nair[1,2,*], G. Hill[3], D. Ritchie[4], J. Butler[5], A. Spencer[1,2], T.-Y. Wang[6], J. Muirhead[7], A. Mclean[6], K. Mason[8], J. Reynolds[2], D. Curtis[1,2]

[1]Haematology, Alfred Health, Melbourne, [2]Monash University, Melbourne, [3]QIMR Berghofer Research Institute, Brisbane, [4]Haematology, Royal Melbourne Hospital, Melbourne, [5]Haematology, [6]Royal Brisbane Hospital, Brisbane, [7]Alfred Health, Melbourne, [8]Royal Melbourne Hospital, Melbourne, Australia

Background: Allogeneic stem cell transplantation in first remission (CR1) remains the treatment of choice for many AML patients. However, the treatment is associated with significant complications and the decision to transplant should be based on pre-transplant prediction of procedure related mortality weighed against the benefit of potential cure. Previously published prognostic scores such as HCT-CI and EBMT score can be used for risk prediction, however, none of them has comprehensively addressed all the potential elements of risk. For example, HCT-CI does not address the donor and procedure related factors whereas EBMT score does not take the patient comorbidity into account.
Aims: To identify factors significantly associated with non-relapse mortality (NRM) in AML patients undergoing allogeneic stem cell transplantation in CR1 and to construct a simplified tool for risk stratification using regression tree approach.
Methods: We did a retrospective review of 242 AML patients transplanted in CR1 from HLA-matched sibling or unrelated donors at 3 Australian centres from 2008 to 2014. The factors considered for analysis were age at transplant, disease risk (complex and monosomal karyotypes, FLT3 ITD positivity, secondary and therapy related AML were considered high risk), comorbidity index (HCT-CI), donor age, sex, donor type, CMV status and conditioning intensity. We performed univariate analysis and multivariate competing risk regression, taking NRM and relapse as competing events. We then did recursive partitioning using regression tree model to further classify patients into risk groups based on NRM.

Table 1.

Total		242
Patient Age	Median (range)	53 (17-69)
Donor Age	Median (range)	43 (20-66)
Patient sex	Male (%)	132 (55)
Sex mismatch	Female donor for male	44
Disease Risk	High	157
HCT-CI score	0	76
	1	32
	2	32
	3	31
	> 3	30
	Unknown	41
Conditioning	MAC	110
	RIC	107
	NMA	25
Donor type	Sibling	123
	Unrelated	119
CMV status	D-/R-	59
	D+/R-	29
	D+/R+	85
	D-/R+	60
	Unknown	9

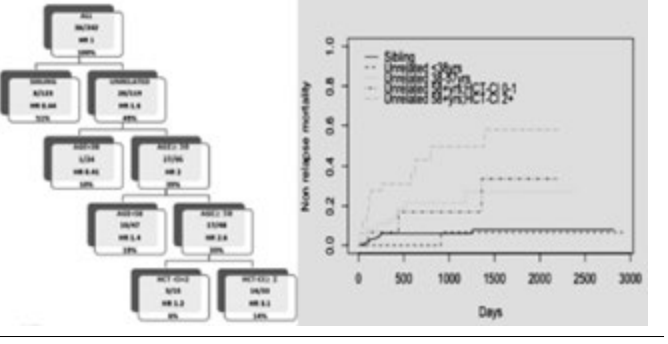


Figure 1.

Results: Patient characteristics are given in the Table 1. A significantly higher proportion of patients with lower HCT-CI (0-1) received myeloablative conditioning (66% vs 32%, p=<001). After a median follow up of 43.8 months, a total of 36 non-relapse deaths occurred (overall NRM-14.9%). Primary causes of death were GVHD (22), Infection (7), idiopathic pneumonia syndrome (3), graft failure (1), multi-organ failure (1), veno-occlusive disease

(1) and pulmonary embolism (1). On univariate analysis, cumulative incidence of NRM was significantly higher in patients older than 60 (p=0.02), HCT-CI≥2 (p=0.008), unrelated donor (p=0.0002) or less intense conditioning (p=0.01). On competing risk regression, age at transplant, as a continuous variable (HR: 1.04; CI (1.005-1.07); p=0.03) and unrelated donor (HR: 3.64; CI(1.65-8.02); p=0.001) were significant determinants of NRM. HCT-CI was no longer an independent predictor of NRM. However, using a regression tree model, we were able to formulate a step-wise risk stratification that incorporated donor type, age and HCT-CI (Figure 1). Three distinct risk groups were recognised where all patients with sibling donors and younger patients with unrelated donors were at the lowest risk and older patients with high HCT-CI having unrelated donors were at the highest risk of NRM.

Summary/Conclusion: Estimation of NRM is an important prerequisite to allogeneic stem cell transplantation. Here we show that a regression tree-based approach is an alternative and perhaps superior method of predicting NRM for patients with AML, allotransplanted in CR1.

PS1490

COMPARISON OF BUSULFAN/CYCLOPHOSPHAMIDE ALONE AND IN COMBINATION WITH THIOTEPA OR ANTI-THYMOCYTE GLOBULIN ON HEMATOPOIETIC STEM CELL TRANSPLANTATION OUTCOME AMONG BETA-THALASSEMIA PATIENTS

S. H. Ansari[1,*], T. Shamsi[1], T. Farzana[1], U. Zaidi[1], S. Siddiqui[1], A. Hanifa[1], T. Khan[1], B. Kaleem[1], J. Hassan[1], N. Anwar[1]

[1]National Institute of Blood Disease & Bone Marrow Transplantation, Karachi, Pakistan

Background: Beta thalassemia major (bTM) is one of the most common indications for hematopoietic stem cell transplantation (HSCT) in Pakistan. Prior to HSCT, the recipient is given a conditioning regimen, the goal of which is to provide sufficient immunoablation to prevent graft rejection.The most commonly used conditioning regimen combinations are (i)Busulfan and Cyclophosphamide (Bu/Cy), (ii) Busulfan, Cyclophosphamide and Thiotepa (Bu/Cy/Thiotepa) and (iii) Busulfan, Cyclophosphamide and Antithymocyte globulin (Bu/Cy/ATG).

Aims: The purpose of this study is to compare the disease free survival rates between patients receiving Bu/Cy, Bu/Cy/Thiotepa or Bu/Cy/ATG.

Methods: We used data of 78 patients who underwent HSCTs between 2000-2017. Of 78 patients 25 were on Bu/Cy, 21 were on Bu/Cy/Thiotepa while 32 were on Bu/Cy/ATG. The doses of these conditioning regimens are as follows: *Bu/Cy group:* Bu(4 mg/kg/day) for 4 Days, Cy(50 mg/kg/day) for 4 Days; *Bu/Cy/Thiotepa:* Bu(3.5mg/kg/day) for 4 Days, Cy(50 mg/kg/day) for 4 Days, Thiotepa(10 mg/kg)on day-6; B*u/Cy/ATG:* Bu(3.5mg/kg/day) for 4 Days, Cy(50 mg/kg/day) for 4 Days, ATG(10 mg/kg/day) for 4 Days.

Results: The disease free survival rate was 56% in Bu/Cy, 71.4% in Bu/CY/Thiotepa and 84% in Bu/Cy/ATG. Primary graft failure in Bu/Cy group was 12%, while 9.5% in Bu/CY/Thiotepa and 9% in Bu/Cy/ATG group. Relapse of the disease occurred in 20% of Bu/Cy group patients and 9.5% of Bu/CY/Thiotepa group patients, however, 6% patients in Bu/Cy/ATG group had relapse of beta-thalassemia major. In terms of overall survival, Bu/CY/ATG(90%) was better, however, 2 out of 21 patients had relapse of the disease compared to no relapse in Bu/Cy/Thiotepa group. The primary graft failure was similar among both groups.

Summary/Conclusion: The combination of Bu/Cy/ATG showed mos effective results in terms of disease free survival rates.

PS1491

SECOND TRANSPLANT FOR PRIMARY GRAFT FAILURE , FOLLOWING HAPLOIDENTICAL GRAFTS WITH POST-TRANSPLANT CYCLOPHOSPHAMIDE

S. Giammarco[1,*], A. M. Raiola[2], F. Sorà[3], S. Sica[3], C. Di Grazia[2], L. Laurenti[3], E. Angelucci[2], A. Bacigalupo[3]

[1]Fondazione Policlinico Universitario A Gemelli, Rome, [2]Hematology, IRCCS San Martino, Genova, [3]Hematology, Fondazione Policlinico Universitario A Gemelli, Rome, Italy

Background: Rejection, following haploidentical transplants (HAPLO) with post-transplant high dose cyclophosphamide (PT-CY) has been described and has been associated with donor specific antibodies (DSA) (BMT 2015;21:1392).

Aims: To assess the incidence of rejection in patients with hematologic malignancies , prepared with a myeloablative conditioning regimen and the outcome of a second transplant.

Methods: We studied 403 patients with hematologic malignancies, receiving an unmanipulated HAPLO marrow graft. Median age was 52 (17-74) and 38% had advanced disease. All patients received GvHD prophylaxis with Cyclosporin, mycophenolate and PTCY. The conditioning regimen used, was one of the following three: # Fludarabine (FLU) 40 mg/m^{2}x3 + Total body irradiation (TBI) 9.9-12 Gy (n=75)# Thiotepa (THIO) 5mg/kgx2, busulfan (BU) 3.2 mg/kg/dayx3, FLU 50 mg/m^{2}x3 (TBF) (n=180)# THIO 5mg/kgx2, BU 3.2 mg/kg/dayx2, FLU 50 mg/m^{2}x3 (n=148)

Results: *Rejection* was seen in 0/75 patients after TBI (0%), 5/180 after TBF with 3 days of Busulfan (2,7%) and in 10/148 in patients receiving TBF with 2 days of Busulfan (p=0.02). We could not find a correlation of rejection with donor age, patients age, diagnosis, phase of the disease, ABO mismatch, or bone marrow cell dose infused. *Busulfan.* We did find a trend for more rejections in Rome (6,8%) , where BU is given on 2 divided daily doses (q12 h), as compared to Genova (3,2%), where BU is given in a single daily dose, (q24h): rejection with full dose TBF (BU3 days) was 3/47 for patients receiving BU q12h (6.38%) , vs 2/133 (1,50%) for BU given q24h (p=0.08). *Second transplant:* 15 patients failed to recover neutrophils by day +28, and were found to have 100% recipient chimerism. Of these 15 patients, one was infused with CD34 selected cells from the same donor, without a conditioning regimen, and died without recovery, after having failed a third transplant.14 patients received a second graft (at a median interval of 43 days, range 33-66) with the Baltimore protocol and PB from the same donor (n=11) or another family member (n=3). GvHD prophylaxis was again CyA and PTCY. 11/13 had trilineage recovery (85%) and became 100% donor: a neutrophil count of 0.5x10^9/L was reached on day 18 (16-21) and a platelet count of 20x10^9/L on day 35 (18-80). Acute GvHD grade I occurred in 46% and grade II in 8%. Moderate-severe chronic GvHD developed in 23%. One year actuarial survival of the 14 patients who received a second graft is 85% (Table 1).

Table 1. Clinical characteristics of 403 patients.

conditioning	FLU-TBI 9.9-12 Gy	TBF (BU3)	TBF (BU2)
Number	75	180	148
Recipient's age	30 (15-58)	49 (17-72)	61 (18-74)
Year of Transplant	2012(11-17)	2015(11-17)	2015(11-17)
Advanced disease (>CR2)	24%	30%	44%
Donor : HLA HAPLO family	100%	100%	100%
Stem cell source BM	100%	100%	100%
Rejection : n.pts (%)	0 (0%)	5 (2,7%)	10 (6,7%)
Second Transplant (patients)			
Interval 1st- 2nd Tx :days (range)			43 (33-66)
Donor of 2nd Tx other HAPLO /id SIB/ same		2/ 1/ 11	
Engraftment : patients (%)			12 (85%)

Summary/Conclusion: (a) the risk of rejection is dependent on the intensity of the conditioning regimen: it does not occur after TBI, and is low with full dose TBF (2.7%); (b) there is a trend for more rejection with Busulfan given q12h; (c) full engraftment can be achieved with a second transplant, in over 80% of patients rejecting. Mortality due to rejection, in this series of 403 patients, was 0.75%.

PS1492

SUBPOPULATIONS OF MONOCYTES AND B REGULATORY CELLS IN PATIENTS WITH CHRONIC GRAFT-VERSUS-HOST DISEASE

A. Babic[1,*], L. Kuric[1], L. Desnica[2], M. Milosevic[3], R. Serventi Seiwerth[2], N. Druakovic[4], Z. Peric[4], M. Mravak Stipetic[5], E. Bilic[6], R. Ceovic[7], T. Klepac Pulanic[8], I. Petricek[9], T. Vukic[10], I.B. Sepec[11], L. Rajic[12], E. Bilic[12], N. Saban[3], M. Baresic[13], D. Ljubas Kelecic[14], S. Mazic[15], I. Bojanic[15], R. Zadro[16], R. Vrhovac[4], S.Z. Pavletic[17], D. Batinic[16], D. Pulanic[4]

[1]Department of Laboratory Medicine, [2]Department of Internal Medicine, Division of Hematology, University Hospital Center Zagreb, [3]Medical School University of Zagreb, [4]Department of Internal Medicine, Division of Hematology, University Hospital Center Zagreb and Medical School University of Zagreb, [5]University Hospital Center Zagreb and School of Dental Medicine University of Zagreb, [6]Department of Neurology, [7]Department of Dermatology, University Hospital Center Zagreb and Medical School University of Zagreb, [8]Community Health Center Zagreb East, [9]Department of Ophtalmology, University Hospital Center Zagreb and Medical School University of Zagreb, [10]Department of Rheumatology and Rehabilitation, [11]Department of Neurology, University Hospital Center Zagreb, [12]Department of Pedi-

atrics, University Hospital Center Zagreb and Medical School University of Zagreb, [13]Department of Internal Medicine, Division of Clinical Immunology and Rheumatology, [14]Department of Internal Medicine, Section for Clinical Nutrition, [15]Department of Transfusion Medicine and Transplantation Biology, University Hospital Center Zagreb, [16]Department of Laboratory Medicine, University Hospital Center Zagreb and Medical School University of Zagreb, Zagreb, Croatia, [17]National Cancer Institute, National Institutes of Health, Bethesda, United States

Background: Chronic Graft-versus-Host Disease (cGVHD) is a major late complication after allogeneic hematopoietic stem cell transplantation influencing immune reconstitution and affecting different organs and tissues. Since cGVHD is a complex multisystemic allo- and autoimmune disease, different clinical presentations of cGVHD might have different biology with different immunological disturbances which may lead to specific target therapies.
Aims: To address this question, we analyzed the associations of subsets of monocytes and putative (by immunophenotype) B regulatory cells (Bregs) with organ-specific and other cGVHD manifestations.
Methods: Patients with cGVHD were enrolled into the multidisciplinary prospective study at the University Hospital Center Zagreb, Croatia, with extensive clinical assessments of different specialists, and with laboratory data collection. Since 2017 laboratory analyses included immunophenotypic subpopulations of monocytes (classical (CD14++CD16-), intermediate (CD14++CD16+) and non-classical (CD14+CD16++)) and Bregs (CD27-, CD27+, total Bregs) in peripheral blood by flow cytometry what we correlated with demographic, transplant and other cGVHD-related data. Descriptive and univariate analyses were undertaken to determine associations between monocytes and Bregs subsets and outcomes of interest.
Results: Twenty two adult patients with cGVHD were analyzed. The median age was 45 years [34-57], 54.5% were female. All were transplanted for hematologic malignancies, 50% underwent myeloablative conditioning, 63.6% had unrelated donor transplant, 81.8% received peripheral blood stem cells as a graft source, and 65% had previous acute GVHD. The median time from cGVHD diagnosis to this analysis was 23 months [1-60.1]. At the moment of analysis, 59.1% were receiving systemic immunosuppression, 40.0% had severe and 45.5% moderate NIH cGVHD global score, and 36.4% had active disease according to clinical impression. The most frequent organ involvement with cGVHD were eyes (68.2%), skin (55.5%), lung (55.5%) and liver (40.9%). Patients who had active disease by clinical impression had lower total B lymphocytes (p=0.031). Lower total Bregs (p=0.026) but not total B lymphocytes (p=0.715) were associated with worse cGVHD severity according to 10 point scale. Those who were receiving higher intensity of immunosuppression had lower total B lymphocytes (p=0.042), Bregs (p=0.043) and its subsets CD27- (p=0.039) and CD27+ (p=0.021). Higher Karnofsky score was associated with higher total (p=0.007), CD27- (p=0.008) and CD27+ (p=0.019) Bregs, and with higher percentage of non-classical monocytes (p=0.020). Patients with lung cGVHD had lower total (p=0.015), CD27- (p=0.021) and CD27+ (p=0.037) Bregs, those with liver cGVHD had lower percentage of intermediate (p=0.007) and non-classical (p=0.043) monocytes, but higher percentage of classical monocytes (p=0.004), those with joint/fascia cGVHD had lower percentage of non-classical monocytes (p=0.007), and those with skin cGVHD had lower percentage of CD27- (p=0.032) and lower percentage of total Bregs (p=0.029). Patients with mouth cGVHD (p=0.043) and those with more severe global NIH score (p=0.040) had higher classical monocytes.
Summary/Conclusion: This preliminary analysis suggested that several different organs affected by cGVHD had distinct associations with different subpopulations of monocytes and Bregs. Intensity of immunosuppression influenced levels of Bregs and its subsets, but not levels of monocytes and its subsets. Further study with larger cGVHD cohort is necessary for further research of complex cGVHD biology.

PS1493

LIFESTYLE BEHAVIOR AMONG LYMPHOMA SURVIVORS AFTER HIGH DOSE THERAPY WITH AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

H.S. Bersvendsen[1,2,*], H.S. Haugnes[3,4], U.-M. Fagerli[5,6], Ø. Fluge[7], H. Holte[8], K. B. Smeland[9], T. Wilsgaard[10], C.E. Kiserud[11]

[1]Oncology, University Hospital of North Norway, [2]Clinical Medicine, UIT- The Arctic University of Norway, Tromsoe, [3]Clinical Medicine, UIT-The Arctic University of Norway, [4]Oncology, Universityhospital of North Norway, Tromsø, [5]Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, [6]Oncology, St. Olav`s Hospital, Trondheim, [7]Oncology, Haukeland University Hospital, Bergen, [8]Oncology, [9]National Advisory Unit for Late Effects After Cancer, Department of Oncology, Oslo University Hospital, Radiumhospitalet, Oslo, [10]Community Medicine, UIT- The Arctic University of Norway, Tromsø, [11]National Advisory Unit for Late Effects After Cancer, Department of Oncology, Oslo University Hospital, Radiumhospitalet, Oslo, Norway

Background: Lymphoma therapy is associated with increased risk of late effects such as secondary malignancies, cardiovascular disease, chronic fatigue (CF) and reduced quality of life. Due to the intensity and accumulated doses of chemotherapy given, the prevalence of late effects are presumably even higher after high dose therapy with autologous hematopoietic stem cell transplantation (auto-HSCT). A healthy lifestyle may positively influence the presence and outcome of late effects.
Aims: To assess prevalence of lifestyle behavior and associated risk factors among auto-HSCT treated lymphoma survivors.
Methods: We conducted a national cross-sectional study on an unselected group of lymphoma survivors treated with auto-HSCT from 1987 to 2008. Survivors under active lymphoma treatment were excluded. Among 399 eligible participants, 312 (78%) completed a questionnaire reporting on lifestyle factors, personality trait, CF, somatic and mental illness. We constructed a summary score on somatic and mental burden, (cut off ≥4 and ≥2 illnesses, respectively). The degree of unhealthy lifestyle was a sum score of physical inactivity (defined as moderate physical activity for <150 minutes/week or strenuous physical activity for <75 minutes/week), overweight (defined as body mass index >25), smoking and an alcohol consumption exceeding recommendations (defined as > 10 g and 20 g alcohol/week for women and men, respectively). Descriptive statistics, logistic and ordinal regression were used. Multivariable analyses were adjusted for age, gender and socioeconomic status (education and household income).
Results: Among auto-HSCT treated survivors; mean age 54.6 years and 60% men, 55% were physical sedate, 55% were overweight, 18% were smoking and 5% had an alcohol consumption exceeding recommendations. A sedate lifestyle was associated with older age (OR 1.23, 1.00-1.51 for every 10 years increase in age), low household income (OR 1.97, 1.18-3.29), CF (OR 2.30, 1.31-4.04) and somatic illness ≥4 (OR 2.39, 1.21-4.74) in multivariable analyses. In adjusted analyses male gender was associated with an increased risk of being overweight (OR 1.77, 1.10-2.86) while indolent non-Hodgkin lymphoma was associated with a lower risk (OR 0.39, 0.21-0.72). Smoking was more frequent among survivors <40 years (24%) than among those >65 years (7%). In multivariable analyses older age was related to lower risk of smoking (OR 0.70, 0.54-0.90 for every 10 years increase of age), while not being in a relationship (OR 2.38, 1.15-4.94) or CF (OR 2.12, 1.10-4.06) increased the risk of smoking. A less healthy lifestyle was associated with male gender (OR 1.79, 1.13-2.84), CF (OR 2.37, 1.44-3.88) and higher somatic burden (OR 2.62, 1.45-4.75), whereas ≥3 regimens prior to auto-HSCT was related to a more healthy lifestyle (OR 0.28, 0.14-0.59), on adjusted analyses.
Summary/Conclusion: The majority of auto-HSCT survivors were physical sedate and overweight. Surprisingly, one quarter of younger survivors were smoking. A higher burden of morbidity (somatic illness and CF) was associated with a less healthy lifestyle. Presumably, these survivors need more attention during follow-up in order to prevent and mitigate late effects, and to increase quality of life. Our results illustrate the necessity of incorporating recommendations and support for improving health-related behavior into cancer survivorship plans for auto-HSCT survivors.

PS1494

CTLA-4 GENE POLIMORPHISM INFLUENCE THE OUTCOME OF HEMATOPOIETIC STEM CELL TRANSPLANTATION

P. Kovy[1,*], N. Meggyesi[1], K. Balassa[2], L. Varga[2], A. Bors[1], A. Batai[3], E. Torbagyi[3], L. Gopcsa[3], L. Lengyel[3], A. Barta[3], A. Tordai[4], T. Masszi[5], P. Remenyi[3], H. Andrikovics[1]

[1]Laboratory of Molecular Genetics, Central Hospital of Southern Pest National Institute of Hematology and Infectious Diseases, [2]School of PhD Studies, Semmelweis University, [3]Department of Haematology and Stem Cell Transplantation, Central Hospital of Southern Pest National Institute of Hematology and Infectious Diseases, [4]Department of Pathophysiology, [5]3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary

Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a major curative therapeutic option for malignant hematopoietic diseases. T cell activation plays an important role in the development of graft versus leukemia and graft versus host disease (GvHD). Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is an inhibitory regulator of T cell activation, that influence the predisposition to autoimmunity and the outcome hematopoietic stem cell and solid organ transplantation. Several single nucleotide polymorphisms (SNPs) have been reported to influence the function of CTLA-4, with contradictory results in the publications.
Aims: The aim of this study was to investigate the role of CTLA-4 +49A>G

(rs231775) polymorphism in the outcome of HSCT.
Methods: We investigated the correlation between recipient and donor CTLA-4 +49A>G and allo-HSCT outcome in a cohort of 411 adult patients who underwent first allo-HSCT between January 2007 and December 2013 at our single center. Donor type was HLA-matched sibling in 210 and matched unrelated donors (MUD) in 201 patients. Myeloablative conditioning (MAC) was performed in 263, and reduced intensity conditioning (RIC) in 148 patients. For identification of CTLA-4 from genomic DNA we applied LightCycler melting curve analysis (LightCycler 480II, Roche Diagnostics).
Results: We did not find any correlation between recipients' CTLA-4 +49A>G polymorphism and HSCT outcome. Nevertheless recipients who received graft from G allele carrier donors showed increased relapse rate in line with increasing G allele dosage [AA: 22% (38/171), AG: 26% (51/193), GG: 40% (19/479), p=0.043 in the genotypic model; p=0.019 in the recessive model]. On contrary, the frequency of the acute GvHD grades III-IV and cytomegalovirus (CMV) reactivation/disease decreased according to the presence of the G allele in the donor CTLA-4 genotype [aGvHD: AA: 20% (34/171), AG: 11% (22/193), GG: 6% (3/47); p=0.018; CMV: AA:24% (41/171), AG:16% (31/192), GG:9% (4/47); p=0.027] Overall survival was not different in patient subgroups according to donor genotypes. Cause of death was altered, patients with G allele carrier donors were more frequently died of the underlying malignant disease [AA: 30% (25/82), AG: 41% (39/95), GG: 59% (16/27); p=0.026]. Donor genotype similarly influenced outcome in acute leukemia, MUD donor, MAC conditioning subgroups.
Summary/Conclusion: In our patients cohort with hematologic malignancies treated with HSCT, CTLA-4 +49A>G polymorphism of HSCT donors influenced risk of relapse, aGvHD, CMV and cause of death, but not overall survival. The genotyping of CTLA-4 +49A>G polymorphism in donors may help in the choice of GvHD prophylaxis and personalised treatment.

PS1495

ELDERLY PATIENTS WITH MULTIPLE MYELOMA: LONG-TERM OUTCOME FOLLOWING INDUCTION WITH NOVEL DRUGS AND AUTOLOGOUS STEM CELL TRANSPLANT

S. Rassool[1,*], L. Foster [1], Y. Li [1], S. Muhammad[2], A. Zhelyazkova[3], C. Patalappa[4], S. Jowitt[5], P. Carrington[2], H. Patel[6], E. Davies[2], P. Bulger[2], M. Pervaiz[3], S. Burns[2], C. Humphrey[2], N. Khaliq[2], A. Allameddine[3], S. Ravenscroft[5], H. Greenfield[3], J. Frazer[2], W. Ogden[2], E. Tholouli[2], A. Rocci[2,7]

[1]Medical School, The University of Manchester, [2]Haematology, Manchester Royal Infirmary, Manchester University NHS Foundation Trust, [3]Haematology, Royal Oldham Hospital, The Pennine Acute Hospitals NHS Trust, [4]Haematology, Royal Bolton Hospital, Bolton NHS Foundation Trust, [5]Haematology, Salford Royal Hospital, Salford Royal NHS Foundation Trust, [6]Haematology, Royal Albert Edward Infirmary, Wrightington, Wigan and Leigh NHS Foundation Trust, [7]Faculty of Biology, Medicine and Health, School of Medical Science, Division of Cancer Sciences, University of Manchester, Manchester, United Kingdom

Background: The autologous stem cell transplant (ASCT) is still the cornerstone of treatment for multiple myeloma (MM) despite the introduction of several novel agents. Initially limited to patients up to 65 years of age, the use of ASCT in patients over this age is now increasing. In this population, ASCT shows a similar safety profile to younger patients, however there are limited data available on the long-term outcome of patients above the age of 65 years receiving induction with novel-drugs and ASCT.
Aims: To evaluate the efficacy and safety of ASCT in patients over 65 years of age.
Methods: Over 300 patients underwent ASCT as first-line treatment for MM at Manchester Royal Infirmary between 01/01/2010 and 31/12/2016 and a randomly chosen sample from this was included in this study. Patients received 200 mg/m^2 of Melphalan, unless creatinine clearance was <30 ml/min or serum creatinine was >200 µmol/L, in which case they received 140 mg/m^2. No age-adjustment dose of Melphalan was performed. Kaplan–Meier curves were used to determine Time to Next Treatment (TTNT) and Overall Survival (OS), using log-rank test to compare results.
Results: One hundred and fifty-nine patients have been included in this study: 107 were ≤65 years old and 52 were >65 years old. Patients received a median of 6 cycles of induction treatment containing Thalidomide (102 patients), Bortezomib (52), Lenalidomide (39), Carfilzomib (14), Cyclophosphamide (132) and Doxorubicin (19). After induction treatment, among patients ≤65 years, 36% achieved CR, 45% VGPR, 17% PR, 1% MR and 1% had PD. Three months after the ASCT, the quality of response increased in all categories: CR 49%, VGPR 42% and PR 9%. In patients >65 years, after induction treatment 27% achieved CR, 55% achieved VGPR and 18% achieved PR. Three months after the ASCT, these increased to 42%, 42% and 12%, respectively, with 4% achieving MR. After a median follow-up of 50.6 months, the median TTNT was 87.3 months for those ≤65 years and 85.2 months for those >65 years (HR 1.025, CI 0.475-1.574, p=0.366). The 5-year OS was 87% vs 76% respectively (HR 0.509, CI 0.231-1.122, p=0.094). The length of hospitalisation was similar between the 2 groups (median of 19 days for ≤65 years vs 20 for >65 years) but the readmission rate in the 3 months following ASCT was higher in those >65 years at 22%, compared to 9% in those <65. However, the median length of readmission was the same, at 7.9 days per patient in both groups. Interestingly, the median number of days per patient with C-reactive protein >50 mg/L was similar in both groups (5 days vs 6 days respectively) but the proportion of patients transferred to intensive care was much higher in the younger population (8%) than in the older group (2%), likely reflecting the tendency to perform ASCT in younger patients even in the presence of several comorbidities. The haematopoietic recovery showed no differences between the ≤65 years group and the >65 years: median number of days before Absolute Neutrophil Count >1x10^9/L was 13 vs 12 respectively; median number of days before platelets >100x10^9/L was 19 vs 21. The Transplant Related Mortality at 3 months was 0% in both groups, whilst the rate of death at 12 months was 2.8% for patients ≤65 years old vs 1.9% for patients >65 years.
Summary/Conclusion: ASCT is feasible and safe in patients >65 years following treatment with induction regimens including novel agents. Consolidation with ASCT improves the depth of response in patients >65 years and translates into similar TTNT and OS compared to patients ≤65 years.

PS1496

SEVERE PRE-ALLO-HSCT IRON OVERLOAD (IO), MEASURED BY LIVER MRI, SIGNIFICANTLY IMPAIRED THE PROCEDURE LONG-TERM OUTCOME

L. Aguirre[1], E. Salvador[2], J.M. Alustiza[2], C. Lombardi[1], A. Ugarte[3], A. Bizjak[1], N. Argoitia[1], N. Uresandi[1], M. Araiz[4], I. Urreta[5], J.I. Emparanza[5], C. Vallejo[1,*]

[1]Hematopoietic Transplant Unit, [2]Osatek, [3]Radiology Department, [4]Hematology Department, [5]Clinical Epidemiology, Donostia University Hospital, Donostia/San Sebastián, Spain

Background: Pre-transplant IO has been suggested to have a detrimental effect on long-term survival of HSCT recipients.
Aims: In this analysis, we wanted to check if IO affected adversely to the long-term outcome of our series de patients.
Methods: We retrospectively studied pts who consecutively underwent allo-HSCT in our center during the period 2015-2017. A Signal Intensity Ratio (SIR) method was employed to estimate the liver IO at pre-HSCT.
Results: 160 pts were studied. One hundred and nineteen were male, and 41 female. Median age was 52 years (7-69). The baseline diseases were: 62 AML, 30 NHL, 23 ALL, 13 MDS, 10 MM, 10 cMPD, 6 BMF, 4 cLPD, and 2 graft failure. One hundred and nine had underwent alternative donor transplants (81 unrelated, and 28 haplo-identical), and 51 HLA-id family donor transplants. Stem cell source was PB in 148, and BM in 12 cases. Conditioning regimen was intensive in 73 pts, and RIC in 87; no non-myeloablative allo-HSCT were performed. Pt CMV serology was positive in 133 (83.13%) pts. Majority of pts had been heavily transfused pre-HCST: 102 pts (73.75%) had received more than 15 PRBCs. The median of PRBCs was 20 [0-147]. Median pre-HSCT ferritinemia was 1208 ng/mL (18-6195), and 90 pts (56.96%) had > 1000 ng/mL. Strikingly, pre-HSCT chelation had been employed only in 23 pts (22.55% of the heavily transfused pts). Pre-HSCT MRI, performed in 71 pts, showed severe IO (LIC > 4.5 mg/g) in 21 pts (29.58%), moderate IO (LIC 2.2-4.4 mg/g) in 32 (45.07%) and no significant IO in 18 (25.35%). The numbers of pts with a follow-up > 180 days and > 365 days (or death before) were 130 and 112, respectively. Overall mortalities at day +180 and day +365 were 16.15% (21 pts), and 28.57% (32 pts), respectively. Long-term mortalities (at day +180, and day +365) of pts with pre-HSCT liver MRI are reflexed in the Tables 1 and 2.

Table 1. Mortality at day +180 based on pre-allo-HSCT iron overload (liver MRI).

	Pts without severe iron overload in pre-HSCT liver MRI	Pts with severe iron overload in pre-HSCT liver MRI	p
N	36	14	
Mortality	1 (2.78%)	3 (21.43%)	< 0.05

Only pts with a follow-up > 365 days (or death before).

Table 2. Mortality at day +365 based on pre-allo-HSCT iron overload (liver MRI).

	Pts without severe iron overload in pre-HSCT liver MRI	Pts with severe iron overload in pre-HSCT liver MRI	p
N	19	11	
Mortality	4 (21.05%)	6 (54.54%)	< 0.05

Only pts with a follow-up > 365 days (or death before).

Summary/Conclusion: 1) Our data shows that pre-allo-HSCT iron-overload is a major risk factor for post-transplant mortality. 2) Our real-life study also reflects that only a minority of the heavily transfused pts had received chelation therapy previously to the allo-HSCT. Considering the relevance of pre-allo-HSCT iron overload, we strongly suggest referring physicians to employ chelation therapy for patient candidates for transplant during the treatment of the underline disease.

PS1497

INCIDENCE AND RISK FACTORS FOR THE DEVELOPMENT OF HEMORRHAGIC CYSTITIS ON HAPLOIDENTICAL TRANSPLANTATION

M. Perera Alvarez[1,*], M. Saez-Perdomo[2,3], A. Suárez Cabrera[1], M. Torres Ochando[2], L. Guerra[2], E. González Pérez[2], C. Acosta Fleitas[2], S. Jiménez Bravo de Laguna[2]

[1]Hematology, Hospital Universitario de Gran Canaria Dr. Negrín, [2]Hematology, Hospital Universitario de Gran Canaria Dr Negrín, [3]Universidad de las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain

Background: Hemorrhagic cystitis (HC) is a serious complication ocurring after allogenic hematopoietic stem cell transplantation (HSCT) more frequent on haploidentical (haplo) HSCT, with an incidence of 10% to 70% associated mainly with the effect of cytotoxic agents such as Cyclophosphamyde (Cy). The conditioning regimen, BKV infection and graft versus host disease have an implication in the incidence. Other authors related the reactivation of CMV and a previous transplantation as risk factors to HC development.
Aims: With this study we aim to describe the HC incidence and risk factors in our serie of haplo-HSCT.
Methods: We analyzed all consecutive haplo-HSCT from family donors performed at our Hospital between 2013 and January 2018. The conditioning regimen used for the transplant depend on the age, comorbidities and state of malignance disease. High dose Cy (50 mg/kg on days 3 and 4) posttransplantation (PTCy), cyclosporine and Micofenolate mofetil was used to prevent GVHS acute. We used as HC prophylaxis intense hydratation on the Cy administration day and the following 24 hours (using bladder wash only in 1 patient with cardiac dysfunction) and perfused MESNA at 100% of Cy dose beginning 15 minutes before the Cy administration on 16 pts and at 20% of the last dose at 0, 4 and 8 hours on all pts. We used SPSS V.23 to determine the cumulative incidence (CI) of HC.
Results: We performed 43 haplo-HSCT, of which 25 were males and 18 were women. The mean age was 43 (range 16-68). The pts presented the following diagnosis: AML (24), HD (6), ALL (3), LPCS (3), MDS (3), AA (2), NHL (2). 11 pts developed HC (28.7% CI) (Figure 1A) with a median time from haplo-HSCT to onset of 7 days (range 0-42). The grade of HC was: 1 (9%) grade I, 6 (55%) grade II and 4 (36%) grade IV. 7/11 (63,3%) developed HC before day +30 and 4/11 (36,3%) after that day. The group of pts who develop early HC, 2 had received another HSCT before and 4 (57%) were BKV PCR positive. All the pte developed HC after day +30 were BKV PCR positive and one was CMV positive in urine too. 1 (9%) was grade I, 6 (55%) grade II and 4 (36%) grade IV. No pts died due to HC and all cases resolved without sequelae. 26 pts received PTCy and 17 pts received Cy pre- and post-transplant. The CI at day +80 for the pts with PTCy was 26.1% and for Cy pre- and post-transplant 32.8% (Figure 1B). We found no statistically significant difference on the CI of HC between these two groups. The development of HC was related to Cy and thrombocytopenia in the first 30 days, for the rest of the pts (after day +30) the HC was related to BKV infection, as a consequence of the immunosuppression state of the patient, we observed that all these pts had positives. No pts died due to HC and all cases r esolved without sequelae.
Summary/Conclusion: The incidence of HC associated to post-HSCT high Cy dose in our series is 15% lower than other ones. Most of them on grade 1 or 2 and without mortality associated. The risk of HC is high, particularly in the setting of highly pre-treated patients (especially those undergoing a 2nd transplant). The development of HC after day +30 is evidently associated to BKV as a contributing factor for continuous inflammation and CMV reactivation (as an immunosuppression marker).

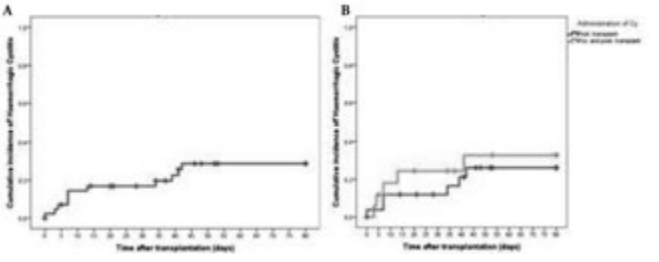


Figure 1.

PS1498

THREE MONTH FULL WHOLE BLOOD AND MIXED T CELL CHIMERISM IS ASSOCIATED WITH SUPERIOR OUTCOMES IN REDUCED-INTENSITY ALEMTUZUMAB-BASED ALLOGENEIC STEM CELL TRANSPLANTS FOR HAEMATOLOGICAL MALIGNANCIES

A. Kanellopoulos[1,*], G. Mcilroy[1], S. Paneesha[1], S. Elmoamly[1], B. Kishore[1], M. Kaparou[1], K. Holder[1], E. Nikolousis[1]

[1]Hematology, Birmingham Heartlands Hospital, Birmingham, United Kingdom

Background: Chimerism kinetics and HCT-CI are important tools for the success of reduced intensity conditioning (RIC) allogeneic stem cell transplants (SCT), although their impact on patient outcomes has been scarcely validated in Alemtuzumab based conditioning.
Aims: Exploratory Analysis of RIC Alemtuzumab based allogeneic stem cell transplants.
Methods: Retrospective analysis of 132 consecutive patients with myeloid (n=67) and lymphoid (n=65) malignancies receiving alemtuzumab-based RIC allogeneic SCT from matched sibling (32/132, alemtuzumab dose 30mg), or matched unrelated (97/132, alemtuzumab dose 50mg) donors. Median recipient age was 59 years (range 22 to 72) and 48 (36%) were female. For myeloid disease conditioning consisted of fludarabine and melphalan (34/67), fludarabine and busulphan (32/67) or fludarabine and cyclophosphamide (1/47); whilst lymphomas were treated with fludarabine and melphalan (32/65), BEAM (10/65), fludarabine and BEAM (22/65), or fludarabine, melphalan and gemcitabine (1/65).

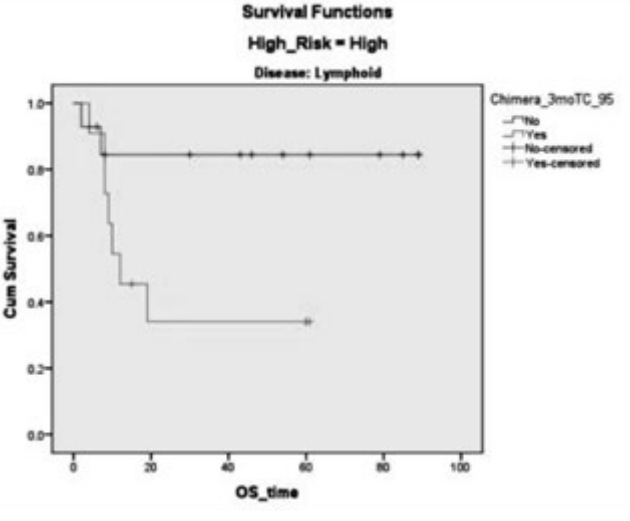


Figure 1.

Results: With a median follow-up of 13 months, the median overall survival (OS) and relapse free survival (RFS) were 24 months and 15 months, respectively. There were no statistical differences in outcomes between two disease categories (p=0.86). Time to transplant was significantly longer for lymphoid than myeloid transplants (median 25.5 vs 7 months, p<0.001) but it was not associated with the OS. OS was worse in patients with high-risk lymphoid disease (high-grade, T-cell, or lymphomas in partial remission at transplant) (HR 3.1 p=0.039). Estimated 2-year TRM was 21% (95% CI 13%

to 29%). Adjusting for age (OR 1.08, 0.01 to 1.14) and sex (p=0.46), 2-year TRM is associated with lymphoid disease, compared with myeloid (OR 3.33, 1.01 to 10.1). 2-year OS – 50% (40% to 60%); Myeloid – 51% (37% to 66%) and Lymphoid – 49% (35% to 63%). 2-year PFS – 58% (48% to 68%) whereas grade 2-4 acute GvHD was 44% (36% to 53%). Adjusting for age, sex, and disease class, full WB chimerism is associated with better OS (HR 0.24, 0.082 to 0.699, p= 0.009), whereas full T-cell chimerism is associated with worse OS (HR 2.97, 1.095 to 8.049, p=0.032). Similarly, full WB chimerism is associated with better PFS (HR 0.27, 0.11 to 0.638, p= 0.003), but full T-cell chimerism is associated with worse PFS (HR 2.48, 1.095 to 5.635, p= 0.029). 2-year TRM is higher in full T-cell chimeras (8/26, 31%), than mixed T-cell chimeras (0/34) (p=0.001). 2-year relapse-related mortality tends to be lower in full T-cell chimeras (4/26, 15%), than mixed T-cell chimeras (9/34, 26%) (p=0.358). Adjusting for age (60 years) (OR 1.065, 1.011 to 1.121, p=0.017), HCT-CI is not associated with OS (comparing 0-1 with 2+, p=0.34). Finally, age predicted OS (univariate, over vs under 60, HR 1.62, p=0.056; univariate, age as continuous variable, HR 1.034, 1.007 to 1.061, p=0.012). HCT-CI did not predict OS, comparing 0-1 with 2+ (p=0.66) (Figure 1).
Summary/Conclusion: In our patient series of RIC alemtuzumab-based allogeneic transplants, three-month full donor status in whole blood and three-month mixed T-cell chimerism are associated with favourable RFS and OS. HCT-CI was not predictive irrespectively of patient age.

PS1499

OUTCOMES OF SECOND ALLOGENEIC STEM CELL TRANSPLANTATION FOR RELAPSE OF HEMATOLOGICAL DISEASES OR GRAFT FAILURE: A SINGLE-CENTRE EXPERIENCE

M. Poiani[1,2,*], D. Salvatore[13], G. Battipaglia[1,3], R. Duléry[1], F. Giannotti[1], F. Malard[1], E. Brissot[1,4], S. Sestili[1], R. Belhocine[1], F. Isnard[1], S. Lapusan[1], R. Adaeva[1], O. Legrand[1,4], M. Labopin[1,4], A. Ruggeri[1], M. Mohty[1,4]

[1]Hopital Saint Antoine, Paris, France, [2]AOU Ospedali Riuniti, Ancona, [3]Federico II University, Naples, Italy, [4]Sorbonne Université UPMC Univ Paris 06, INSERM, Centre de recherche Saint-Antoine, Paris, France

Background: Second allogeneic hematopoietic stem cell transplantation (HSCT) might be considered an option in patients experiencing disease relapse or graft failure after first HSCT. However, its feasibility is hampered by high rates of toxicity and mortality.
Aims: We retrospectively analyzed outcomes of 44 consecutives patients (26 males and 18 females)
Methods: They underwent 2nd HSCT either for disease relapse (group 1, n=28) or graft failure (group 2, n=16) between 2005 and 2017.
Results: Median age at 2nd HSCT was 39 (range 15-69) years. Diagnosis were acute myeloid leukemia (group 1: n=20; group 2: n=5), acute lymphoblastic leukemia (group 1, n=3; group 2, n=4), myelodysplastic syndrome (group 1, n=1; group 2, n=2), myeloproliferative neoplasm (group 1, n=4; group 2, n=1), bone marrow failure (group 1, n=0; group 2, n=4). Median time from 1st HSCT was 23 (range 2.6-161) months in group 1 and 2.2 (range 1.6-17.3) months in group 2. All but 5 patients received graft from a different donor than 1st HSCT. Graft source were: matched related bone marrow (BM, group 1, n=1; group 2, n=0), matched related peripheral blood stem cells (PBSC, group 1, n=2; group 2, n=2), haploidentical BM (group 1, n=3; group 2, n=1), haploidentical PBSC, (group 1, n=8; group 2, n=2), cord blood (group 1, n=8; group 2, n=10), matched unrelated PBSC (group 1, n=6; group 2, n=1). At time of 2nd HSCT, 14 patients were in complete remission and 14 presented active disease in group 1. All but two patients underwent chemotherapy for disease relapse before 2nd HSCT. Conditioning regimen was myeloablative in 6 patients (group 1, n=5; group 2, n=1), reduced intensity (RIC) in 36 (group 1, n=23; group 2, n=13). A sequential schema consisting of a combination of thiotepa, etoposide and cyclophosphamide followed by a fludarabine and busulfan based RIC was used in 8 out of 14 patients with active disease in group 1. *in vivo* T-cell depletion was used in 22 patients (group 1, n=15; group 2, n=8). All but 2 patients engrafted (1 in each group), in a median time of 18 (range 6-51) days. Cumulative incidence (CI) of day 100 grade II-IV acute graft versus host disease (GVHD) and 5-years (5y) chronic GVHD were 32% (95% CI: 18-48) and 39% (95% CI: 24-55), respectively, for the entire cohort. With a median follow-up of 58 (range 2-107) months, 5y non-relapse-mortality (NRM) and relapse incidence (RI) were 33% (95% CI: 18-49) and 30% (95% CI: 16-45), respectively. At 5-years disease-free (DFS) and overall survival (OS) were 37% (95% CI: 21-53) and 40% (95% CI: 24-55). Globally, 25% patients died of infections (n=10), relapse (n=7), GVHD (n=3), SOS/VOD (n=2), failure/rejection (n=1) or for unknown causes (n=2). At 5 years, outcomes of patients in group 1 were: RI 33% [14-53], NRM 30%[11-51], aGVHD 36%[17-56], cGVHD 50%[27-70], DFS 37%[15-58], OS 41%[20-61], respectively. Outcomes of patients in group 2 were: RI 25% [7-48], NRM 38%[14.5-61], aGVHD 27%[8-51], cGVHD 25%[7-49], DFS 38%[14-61] and OS 38%[14-61].
Summary/Conclusion: Despite hampered by a non-negligible morbidity and mortality, 2nd HSCT might be considered as an option to rescue a certain number of patients experiencing disease relapse or graft failure, for which prognosis is very poor. Decision is to be discussed on a case-to-case basis. Further advances in supportive care and post-transplant treatment strategies, might be helpful in order to improve outcomes and to propose this strategy in a larger number of patients.

PS1500

CLOFARABINE- BASED CYTOREDUCTIVE TREATMENT FOLLOWED BY MYELOABLATIVE CONDITIONING AND ALLOGENEIC STEM CELL TRANSPLANTATION IN CHILDREN WITH HIGH-RISK, RELAPSED OR REFRACTORY HEMATOLOGIC MALIGNANCIES

D. Medina Valencia[1,*], M. Estacio[1], M. Rosales[1], L. Perafan[2], E. Manzi[2]

[1]Stem cell transplant, [2]Research department, Fundacion Valle del Lili, Cali, Colombia

Background: Patients with relapse/ refractory or MDR-high hematologic malignancies have a poor prognosis. Allogeneic stem cell transplantation (SCT) is the only curative option, however, this treatment is associated with a low survival and high risk of relapse posttransplant in this group of patients[1,2]. Previous studies in adults reported the feasibility of a sequential therapy consisting of a cytoreductive chemotherapy followed by SCT with different strategies, including clofarabine-based, with encouraging results[3–7]. Nevertheless, there are few reported of this strategy in the pediatric setting.
Aims: The aim of this retrospective analysis was to report outcomes using clofarabine-based cytoreduction followed by myeloablative intensity conditioning and allogeneic transplantation.
Methods: We retrospectively reviewed all pediatric with hematologic malignancies who underwent allogeneic SCT after treatment with clofarabine, between years 2012 and 2017. In patients with ALL the sequential regimen consisted of clofarabine (30 mg/m2/day or 40mg/m2/day) and steroid (Dexamethasone or methylprednisolone) plus cytosine arabinoside (2 g/m2/day) for 5 consecutive days. Additional patients with AML or MDS received G-CSF priming. Followed, after a 3-day rest, by myeloablative conditioning (BU-FLU-TBI 400 cgy or MEL) and allogeneic stem-cell transplantation.
Results: Eighteen patients received a clofarabine-based Cytoreduction. Were included 10 acute myeloid leukemia, 4 acute lymphoblastic leukemia, 2 juvenile myelomonocytic leukemia, 1 myelodysplastic síndrome and 1 myeloid sarcoma. Median age was 4.9 years (Range 1.6 -16.1). Donors were Haploidentical (n=12) and Identical sibling (n=6). The median pre-transplant marrow blast percentage was 1.65 (range 0.08- 89). At day +30, 14 patients were evaluable for response, 8 patients were in negative MDR and 6 patients were in positive MDR with a median marrow blast percentage of 0.45 (range 0.3-1.2). Full donor chimerism was found in all patients at day +30. The cumulative incidence of acute GVHD II-IV was 59%, only one patient has severe GVHD and for chronic GVHD was 27%. The 1-year overall (OS) was 59.5% (95% CI:30-80%) and event-free survival (EFS) was 46% (95% CI:18-70%). Transplant related mortality (TRM) at 100 days was 11.1%. Eleven patients developed liver toxicity, 2 grade III, 1 grade II and 8 grade I. We don't found increased in creatinine.
Summary/Conclusion: Clofarabine- based cytoreductive treatment can be safe and feasible option for children with high-risk, relapsed or refractory hematologic malignancies. However, our results require further support from prospective randomized studies to improve the efficacy of this strategy.

Stem cell transplantation – Experimental

PS1501

SELECTIVE INHIBITION OF OXIDATIVE PHOSPHORYLATION PREVENTS LETHAL GRAFT-VERSUS-HOST DISEASE WHILE MAINTAINING ANTITUMOR IMMUNITY

Z. Mciver[1,*], R. Blevins[1], B. Coe[1], M. Detty[2], J. Grayson[3]

[1]Hematology and Oncology, Wake Forest School of Medicine, Winston Salem, NC, [2]Chemistry, University at Buffalo, Buffalo, NY, [3]Microbiology and Immunology, Wake Forest School of Medicine, Winston Salem, NC, United States

Background: Pathogenic T cells play a central role in many immunological disorders. In hematopoietic stem cell transplant (HSCT), acute graft-versus-host-disease (GVHD) is caused by an attack on the recipient's tissues by donor allogeneic T cells. *Ex vivo* application of phototherapy to selectively deplete GVHD-causing cells prior to transplant may prevent GVHD. However, none of the photosensitive agents in use today have demonstrated selectivity without significant toxicity occurring in resting cells. We have designed the first-in-class, novel photosensitizer 2-Se-Cl with the ability to accumulate in pathogenic T cells in proportion to degree of oxidative phosphorylation (OXPHOS). Unique to 2-Se-Cl is the ability to potently stimulate P-glycoprotein (P-pg). Enhanced P-gp activity promotes the efficient removal of photosensitizer not sequestered in mitochondria, and protects resting lymphocytes essential for antipathogen and antitumor responses.
Aims: We have previous demonstrated the ex vivo photodepletion of alloreactive T cells using 2-Se-Cl successfully prevented GVHD in a lethal model of the disease with full retention of antipathogen immunity and 3rd party responses. The aim of our study is to employ 2-Se-Cl to prevent acute GVHD while retaining robust antitumor activity in well established animal models of complete MHC-mismatched and MiHA-mismatched HSCT.
Methods: To evaluate for the retention of antitumor immunity, both MHC-mismatched (C57BL/6 ($H2^b$)→Balb/c (H2d)) and MiHA-mismatched (C3H.SW → C57Bl/6) transplantations were preformed. To prepare the cells for photodepletion, donor splenocytes were cocultured for 4 days with irradiated (20 Gy) recipient splenocytes, and then photodepleted (PD) with 2-Se-Cl. On the day of HSCT, recipient mice were irradiated (8.5Gy for Balb/c and 10.5Gy for C57BL/6), and received 10^7 T cell-depleted (TCD) donor-derived bone marrow cells accompanied by 5 x10^6 PD - treated cells, or recipient specific (A20, Balb/c-derived and FLT3+ AML C57BL/6-derived) leukemia cells engineered to express firefly luciferase, or both. To evaluate for the retention of 3rd party responses, C3H/HeJ were irradiated (9.5 Gy) and received a combination of 10^7 TCD donor-derived bone marrow cells accompanied by 5 x10^6 PD - treated cells. All mice were monitored for signs of GVHD according to a well-established mouse GVHD grading system, and all mice that received leukemia cells were evaluated weekly by IVIS for evidence of disease progression. Three to 5 animals underwent HSCT in each group in 3 independent experiments.
Results: Recipient mice that received PD-treated splenocytes survived > 60 days without evidence of GVHD or leukemia, equivalent to controls (recipients of TCD bone marrow cells only). In contrast, mice that were inoculated with leukemia at time of HSCT without the addition of PD-treated splenocytes died of leukemia progression. Additionally, all third-party C3H/HeJ mice that received the same number of PD-treated cells died of lethal GVHD, consistent with the selective depletion of the alloimmune response towards first-party recipient mice by 2-Se-Cl.
Summary/Conclusion: These results demonstrate that ex vivo application of 2-Se-Cl protects against lethal GVHD while maintaining antitumor immunity in both the complete MHC-mismatch and MiHA-mismatched settings. The high selectivity of this novel photosensitizer may have broad applications, including the rapid production of immune effector cell products with potent antitumor activity and a low potential for toxicity.

PS1502

AN UNBALANCED MONOCYTE MACROPHAGE POLARIZATION IN THE BONE MARROW MICROENVIRONMENT MAY CAUSE THE OCCURRENCE OF POOR GRAFT FUNCTION AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

H.-Y. Zhao[1,*], Z.-S. Lyu[1,2], C.-W. Duan[3], Y. Song[1,2], T.-T. Han[1], X.-D. Mo[1], Y. Wang[1], L.-P. Xu[1], X.-H. Zhang[1], X.-J. Huang[1,2], Y. Kong[1]

[1]Peking University People's Hospital, Peking University Institute of Hematology, [2]Peking-Tsinghua Center for Life Sciences, Peking University, Beijing, [3]Key Laboratory of Pediatric Hematology and Oncology Ministry of Health and Pediatric Translational Medicine Institute, Shanghai Jiao Tong University School of medicine, Shanghai, China

Background: Poor graft function (PGF) remains a life-threatening complication following allogeneic hematopoietic stem cell transplantation (allo-HSCT), and the underlying mechanisms have not yet been elucidated. Considerable evidence from murine studies has demonstrated that effective hematopoiesis depends on the specific bone marrow (BM) microenvironment, where hematopoietic stem cells reside. In this regard, we previously reported that PGF patients had impaired BM endosteal cells and endothelial progenitor cells, as well as dysregulated T cell responses in the BM microenvironment, which may be involved in the occurrence of PGF. Macrophage(MΦ) commonly exist in two distinct subsets: M1 and M2, is one of the important components of BM immune microenvironment. Meanwhile, Increasing murine studies show that BM resident MΦs are indispensable for HSCs function and BM erythroid output. However, little is known about the quantity and function of BM MΦs and whether BM MΦs directly interact with HSCs in PGF patients post allo-HSCT.
Aims: To compare the number and function of BM MΦs between patients with PGF and GGF after allo-HSCT. Moreover, to investigate whether the BM MΦs directly interact with HSCs in PGF patients.
Methods: This prospective nested case-control study enrolled 30 PGF patients, 60 matched patients with good graft function (GGF), and 30 healthy donors (HD). Standard monocyte subsets, defined by cluster of differentiation CD14 and CD16, as well as M1(CD68+CCR2+) and M2 (CX3CR1+CD163+) from BM samples were analyzed by flow cytometry. The functions of BM derived MΦs were evaluated by migration assay, phagocytosis assay and cytokine profile. CD34+ cells from HDs were co-cultured respectively with BM derived macrophages from PGF and GGF patients, and the levels of reactive oxygen species (ROS) and apoptosis in CD34+ cells were analyzed by flow cytometry after 5 days co-culture. Besides, colony-forming unit (CFU) assay was performed. Finally, intracellular proteins levels of CD34+ cells were evaluated by LSRFortessa software and expressed as the mean fluorescence intensity.
Results: Alterations in standard monocyte subsets (classical, intermediate and non-classical) were found when comparing subjects among PGF, GGF and HDs. Moreover, PGF patients displayed an unbalanced M1/M2 ratio compared with the GGF and HDs, attributable to an increased number of M1 and a reduced number of M2. Dysfunctional BM derived MΦs, which were characterized by impaired proliferation, migration and phagocytosis, were revealed in PGF patients. Moreover, PGF BM derived MΦs produce more proinflammatory cytokines than GGF BM derived MΦs, including IL-12 and TNF-α. While, GGF BM derived MΦs have a high production of TGF-β. The BM CD34+ cells co-cultured with PGF BM derived MΦs, characterized by higher levels of ROS and apoptosis, had a deficit in CFU outgrowth at baseline level compared with those cells co-cultured with GGF BM derived MΦs. Subsequently, the higher p-p38 and p-JNK expression was observed in the BM CD34+ cells which were co-cultured with PGF BM derived MΦs.
Summary/Conclusion: In summary, the current study demonstrated an unbalanced M1/M2 number and dysfunction of BM MΦs in PGF patients. Moreover, BM derived MΦs from PGF patient have a negative impact on CD34+ cells *in vitro* study. Therefore, it would be of value to investigate the appropriate approach to balance the M1/M2 and enhance function of BM MΦs, which may be a promising therapeutic approach for PGF patients.

PS1503

COMPLEMENT-RELATED GENETIC VARIANTS IN ADULT ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANT RECIPIENTS WITH TRANSPLANT-ASSOCIATED THROMBOTIC MICROANGIOPATHY

E. Gavriilaki[1,*], T. Touloumenidou[1], I. Batsis[1], D. Mallouri[1], A. Vardi[1], A. Marvaki[1], M. Koutra[1], P. Zerva[1], E.-E. Koravou[1], A. Papalexandri[1], M. Stamouli[2], K. Gkirkas[2], P. Tsirigotis[2], E. Yannaki[1], I. Sakellari[1], A. Anagnostopoulos[1]

[1]Hematology Department - BMT Unit, G. Papanicolaou Hospital, Thessaloniki, [2]Second Department of Internal Medicine, Division of Hematology, ATTIKO University Hospital, National and Kapodistrian University of Athens, Athens, Greece

Background: Transplant-associated thrombotic microangiopathy (TA-TMA) is a potentially life-threatening complication of allogeneic hematopoietic cell transplantation (HCT). In light of encouraging results by complement inhibition treatment, a few reports have documented complement-related genetic variants in TA-TMA patients. However, their incidence and the clinical importance of pre-transplant genetic profiling remain unclear.
Aims: To detect complement-related variants in TA-TMA patients using next-generation sequencing and investigate their association with clinical

outcomes.
Methods: We enrolled consecutive allogeneic HCT recipients diagnosed with TA-TMA according to the IWG criteria from 2014 to 2017. Patients' DNA was obtained from pre-transplant peripheral blood samples. Probes were designed using Illumina's Design studio. The amplicons cover exonic regions of complement regulatory genes (*CFH, CFH-related, CFI, CFB, CFD, C3, CD55, C5, MCP, thombomodulin, ADAMTS13*) spanning 15 bases into the intronic regions. We used 10ng of initial DNA material. Libraries were sequenced on a MiniSeq System in a 2x150 bp run. Analysis was performed using the TruSeq Amplicon application. We performed variant calling with the Illumina-developed Somatic Variant Caller in germline mode and variant allele frequency higher than 20%. Ensembl and Refseq were used for annotation of the output files. Variants were further annotated using the Variant Interpreter (Illumina).
Results: We studied 16 patients that presented TA-TMA at median + 80 (9-540) days, after full hematopoietic constitution. Patients were transplanted from sibling (4), matched (8) or mismatched (3) unrelated or haploidentical (1) donors, mainly with a myeloablative conditioning (8). The majority presented infectious complications (10) and severe acute (14) or extensive (11) chronic graft-versus-host disease.
In genetic analysis, patients presented heterogeneous variant profiles including pathogenic, likely pathogenic, benign and variants of unknown significance (median number of variants: 75, range: 53-99). Pathogenic complement-related variants were found in 15/16 patients. The majority (13/16) harbored pathogenic variants (SNVs) in *complement-factor H (CFH)*, that have been previously reported in patients with atypical hemolytic uremic syndrome (aHUS). 4 patients presented additional pathogenic variants in *ADAMTS13* and 2 patients in *CFB*. One patient presented pathogenic variants only in *ADAMTS13* and 1 only in *CFB*.
Regarding clinical outcomes, only 3 patients responded to conventional treatment. Interestingly, these patients harbored only 1 pathogenic variant (1 patient in *ADAMTS13*, 1 in *CFB* and 1 in *CFH*). Based on physician's decisions, 4 refractory patients received complement inhibition treatment. These patients harbored more than one pathogenic variant. Although all patients showed initial hematologic response to eculizumab treatment, TA-TMA resolved only in 1 patient after 4 eculizumab doses. 12/16 patients succumbed to transplant-related mortality.
Summary/Conclusion: The identification of pathogenic complement-related variants in TA-TMA patients suggests genetic susceptibility to impaired complement regulation. Despite increased morbidity and mortality, complement inhibition seems promising in selected patients. In this complex setting, genetic variants need to be further investigated in control groups in order to confirm their clinical importance and usefulness in early recognition of patients who may benefit from complement inhibition.

PS1504

MURINE MODELS FOR ESTABLISHING STEROID REFRACTORY GVHD

T. Toubai[1,*], C. Rossi[2], I. Tawara[3], C. Liu[4], C. Zajac[5], K. Oravecz-Wilson[5], D. Peltier[5], Y. Sun[5], H. Fujiwara[5], J. Wu[5], M. Riwes[5], I. Henig[5], S. Kim[5], K. Ishizawa[1], P. Reddy[5]

[1]Yamagata University, Yamagata, Japan, [2]University of Heidelberg, Heidelberg, Germany, [3]Mie University, Tsu, Japan, [4]Rutgers-Robert Wood Johnson Medical School, New Brunswick, [5]University of Michigan, Ann Arbor, United States

Background: Steroids are the first line therapy for acute graft-versus-host disease (GVHD). However, the outcome of steroid refractory GVHD (SR-GVHD) is poor due to the lack of effective treatments. The development of therapies for SR-GVHD is limited by an incomplete understanding of its pathophysiology because of the lack of clinical animal models.
Aims: Here we address the need for a SR-GVHD animal model by developing a major histocompatibility (MHC) matched, multiple minor histocompatibility antigens (miHAs) mismatched model as well as a MHC mismatched haploidentical murine bone marrow transplantation (BMT) models.
Methods: We utilized the clinically relevant major histocompatibility (MHC) matched, multiple minor histocompatibility antigens (miHAs) mismatched model as well as a MHC mismatched haploidentical murine bone marrow transplantation (BMT) models. Dexamethasone (DEX) was administered from day +7 to day +21 at a dose of 0.1 mg/kg.
Results: We observed three phenotypes in response to DEX: 1) Steroid refractory (REF): progressive GVHD characterized by severe peak GVHD clinical scores, progressive weight loss and early mortality; 2) stable GVHD (ST) characterized by moderate GVHD clinical scores, moderate body weight loss, and minimal late mortality; 3) steroid responsive (RES) GVHD characterized by near complete normalization of GVHD clinical scores, near complete normalization of weight loss, and no mortality. We demonstrate that animals can develop SR-GVHD regardless of whether steroids are initiated early or late post allogeneic bone marrow transplantation (allo-BMT). In general, we observed increased GVHD specific histopathological damage of target organs in SR-GVHD animals relative to steroid responsive animals. Interestingly, we found no significant differences in donor T cell characteristics between steroid refractory and responsive animals
Summary/Conclusion: We propose using our models to decipher SR-GVHD pathogenesis and to develop SR-GVHD treatment. However, these models, like any model system, show some distinction from complex clinical realities and will need to be continuously optimized. Nonetheless, in this study, we established SR-GVHD models and found that donor T cell characteristics were not significantly different between groups treated with steroids. These data suggest that T cell independent mechanisms may also contribute to steroid refractoriness more so than was previously considered.

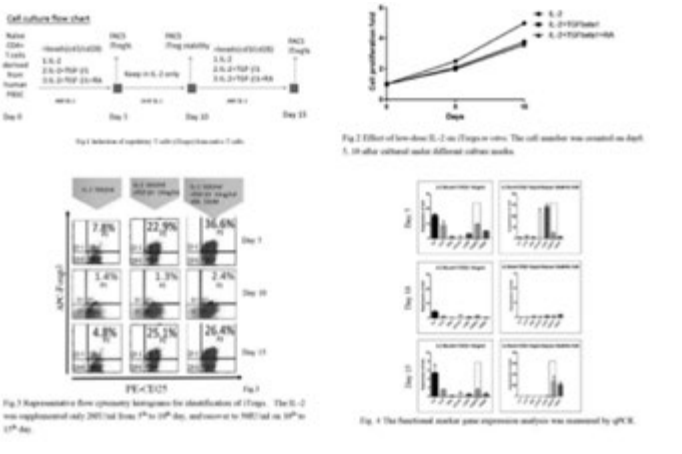
PS1505

LOW-DOSE INTERLEUKIN-2 RESTORING HUMAN REGULATORY T CELL FUNCTION *IN VITRO* AS A POTENTIAL APPLICATION FOR GVHD THERAPY AFTER TRANSPLANTATION

T.-J. Chiou[1,*], H.-H. Lin[1], W.-H. Wu[1], J.-Y. Jian[1], S.-T. Chu[2], W.-F. Tzeng[3]

[1]Division of Transfusion Medicine, Department of Medicine, Taipei Veterans General Hospital and National Yang-Ming University School of Medicine, [2]Institute of Biochemisty, National Taiwan University, Taipei, [3]Department of Life Science, Fu-Jen University, New Taipei City, Taiwan, Republic of China

Background: Currently, isolated and *in vitro*-expanded natural regulatory T cells (nTregs) was shown to be a potentially effective therapy for patients with GVHD after transplantation. However, shortage of nTregs in peripheral blood and time consumption of expansion *in vitro* would limit the clinical application. The activity of induced regulatory T cells (iTregs) to suppress the responder T cells should be maintained for longer time to improve the GVHD therapeutic efficiency. Low-dose IL-2 administration was ever tried to induce the functional Tregs *in vivo*, and improve the therapy of cGVHD. IL-2 may expand the T lymphocytes and restore the functional iTregs in a short time *in vitro*, then to ensure the successful therapy for acute GVHD.
Aims: As known, iTreg cells would decay after a period of time *in vivo* or *in vitro*. In order to provide more number of effective iTregs for clinical use, we try to develop a method for induction and expansion of iTregs via IL-2 restores Tregs *in vitro*.
Methods: Human PBSC were prepared from peripheral blood of healthy donor by Ficoll-Hypaque density gradient centrifugation. Naïve T cells were isolated by negative selection. The harvested naïve T cells were cultured and stimulated under cytokines-containing RPMI1640 medium. The flow chart was shown in Figure 1. For cell proliferation and activity maintenance, the cells were cultured under IL-2 supplemented medium. The harvested cells were analyzed by flow cytometry with fluorescence-conjugated CD antibodies, including CD4, CD25, CD127 and FoxP3. The identification of iTregs function was analyzed via gene marker qPCR analysis.



Figures 1,2,3,4.

Results: Amplification of the T cell number obtained more iTreg cells; therefore, cultivation of the iTreg cells under low-dose IL-2-containing medium would stimulate the T cell proliferation to about 4-fold on the 10th-day

(Figure 2). After the first cytokines stimulation, we harvested the iTregs, and then, keep the cells in low-dose IL-2 only medium for another 5 days to expand and maintain activity of the T cells. On the 10th-day, the expanded T cells would be performed with anti-CD3/CD28 antibodies and with cytokines supplement for the further induction. The IL-2 expanded-T cells would be further induced to iTregs (Figure 3). *FoxP3* gene and other marker genes expression measurement were as functional iTregs indicators, and showed high expression on the 5th- and 15th- day iTregs but lower on the 10th-day (Figure 4). Our data showed that the proliferation of cells would be maintained within the period under low-dose IL-2 medium, the *FoxP3* gene expression would decrease, but restored on 15th-day. Therefore, we could harvest more functional iTregs after the low-dose IL-2 incubation, because of the cell expansion during IL-2 medium incubation for 5 days. Using marker gene qPCR analysis, we have confirmed all of these functional iTregs, and the MLR assay also showed the suppression of the responder T cells.

Summary/Conclusion: Our study showed that after the activation and induction of naïve T cells, we could maintain the cells with IL-2 containing medium. After the second induction, we could restore the iTreg cells. Therefore, we may provide more functional iTregs cells for the potential use in the therapy of GVHD patients continuously in the future. [This study got grant support from LIHPAO Life Science (R12004), Ministry of Science and Technology (MOST 105-2314-B-075-062-MY2), and Taipei Veterans General Hospital (V107C-018)].

PS1506

HYPOFRACTIONATED TOTAL LYMPHOID IRRADIATION BY HELICAL TOMOTHERAPY AND HIGH DOSE CHEMOTHERAPY AS CONDITIONING FOR AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH RELAPSED/REFRACTORY LYMPHOMA

F. Guolo[1,*], S. Vagge[2], A. Pepe[1], P. Minetto[1], A. Dominietto[3], F. Ballerini[1], C. Ghiggi[3], A. Ibatici[3], E. Angelucci[3], R. Corvo'[2], R.M. Lemoli[1], M. Gobbi[1]

[1]Clinic of Hematology, Department of Internal Medicine (DiMI), University of Genoa, [2]Department of Radiation Oncology, [3]Hematology Unit and Bone Marrow Transplantation, Ospedale Policlinico San Martino, IRCCS per l'Oncologia, Genoa, Italy

Background: Salvage chemotherapy followed by autologous stem cell transplantation (ASCT) is the standard of care for patients with relapsed/refractory (R/R) Hodgkin's Lymphoma (HL) and non-Hodgkin lymphoma (NHL). However conventional conditioning regimens have low efficacy in R/R patient and only a minority of them is cured by this approach. Promising results with hyperfractionated total lymphoid irradiation (TLI) followed by high-dose chemotherapy and ASCT have been reported in advanced HL patients, despite relevant toxicity due to radiation-induced damage of healthy tissues. Helical tomotherapy (tomo-TLI) is a novel rotational technique that delivers highly conformal radiation dose with significant sparing of critical organs thanks to integrated fan beam CT scan.
Aims: Assessing feasibility of a conditioning strategy based on high dose chemotherapy and tomo-TLI as preparation for ASCT in patients affected by R/R lymphoma.
Methods: From February 2011 to December 2017, 15 patients with R/R HL (n=7), diffuse Large B Cell NHL (n=6) and T-cell NHL (n=2) were treated. Median age was 42 years (range 20-68) and median number of previous lines of therapy was 3 (range 2-4). Of note, 4 patients had already received prior ASCT. Salvage chemotherapy was decided case by case and clinical response was determined by functional imaging prior to ASCT. Conditioning chemotherapy consisted of high-dose Bendamustine (400 mg/sqm) and Melphalan 140 (mg/sqm) for patients older than 40 years (n=10) and conventional FEAM (Fotemustine, Ethoposide, Cytarabine and Melphalan) for younger patients. Hypofractionated tomo-TLI at a daily dose of 400 cGy for 3 consecutive days was scheduled prior to chemo-conditioning. The total dose of 1200 cGy, biologically equivalent to 1600 cGy in classical daily fractionation, was delivered to all nodal sites including the spleen, with a simultaneous integrated boost over the region of residual disease. High dose chemotherapy was administered 4 days after tomo-TLI.
Results: Salvage chemotherapy induced CR in 7 patients (5 HL, 2 NHL), PR in 5 (2 HL,3 NHL), less than PR IN 3. Conditioning strategy was well tolerated. Six patients (40%) experienced fever of unknown origin and 5 patients (33%) developed grade 3/4 mucositis. None experienced grade 3/4 extra-hematological toxicity. The median number of CD34+ cells infused was $5{,}5\times10^6$/kg (range 2,1 -10,0). All patients showed complete engraftment and median time to neutrophil and platelet recovery was 11 (range 9-21) and 12 days (range 9-21) respectively. Median follow-up was 38 months (CI 95%: 1.3–66.1 months). All patients in PR or less before transplant achieved CR. There were no cases of treatment-related death. The 3-year overall PFS and OS were 78% and 93% (Figure 1) respectively.

Post-ASCT relapse occurred in 3 patients (HL=2 and NHL=1) at a median time of 8 months and 1 NHL patient subsequently died of progressive disease 7 months after ASCT.

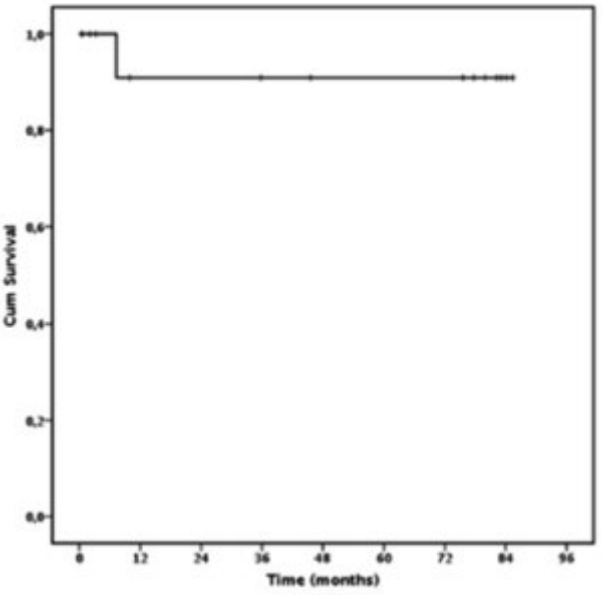


Figure 1. Overall survival.

Summary/Conclusion: Our preliminary results show that tomo-TLI can be safely used in advanced lymphomas. It greatly reduces the damage to healthy tissues and allows to deliver sequential high dose chemotherapy as combined conditioning. With the limit deriving from the small size of this series, we observe that all patients achieved CR after the procedure, even if heavily pretreated, and that relapse rate was low. Overall these results encourage the implementation of tomo-TLI in the standard conditioning for R/R lymphomas.

PS1507

THE RELATIONSHIP BETWEEN SELECTIVE PRE-TRANSPLANTATION IMMUNE PARAMETERS AND EARLY INFECTIONS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES UNDERGOING ASCT – PRELIMINARY REPORT

J. Mańko[1], A. Szymczyk[2,*], A. Szymańska[2], A. Bojarska-Junak[3], A. Walter-Croneck[1], W. Legieć[2], D. Jawniak[1], J. Roliński[3], I. Hus[2]

[1]Chair and Department of Haematooncology and Bone Marrow Transplantation, [2]Independent Clinical Transplantology Unit, [3]Chair and Department of Clinical Immunology, Medical University of Lublin, Lublin, Poland

Background: Patients with hematological malignancies have a particularly high risk of infectious complications that is a consequence of impaired immunity resulting from the nature of the underlying diseases, co-morbidities as well as the therapy. Hematopoietic stem cell transplantation is currently the standard treatment for many hematological diseases and it is associated with long-term immunity deficits, resulting in severe, life-threatening bacterial, viral and fungal infections. The risk of infections is much lower after autologous transplantation (ASCT) compared to allogeneic transplantation, however serious life-threatening infections are observed in some patients after ASCT.
Aims: The aim of the study was to find the relationship between quantitative and qualitative disturbances concerning T-cells, B-cells, NK cells and monocytes, the concentration of intracellular cytokines and the frequency and severity of early infectious complications (until hematopoietic recovery) in patients with hematological malignancies after ASCT.
Methods: The study group consisted of 47 patients aged 18-68 years (51.2±13.9 years) who underwent ASCT. Eight patients were diagnosed with Hodgkin's lymphoma (HL), 13 with non-Hodgkin lymphoma (NHL), 26 patients with multiple myeloma (MM) and 1 patient with amyloidosis. Subpopulations of peripheral blood lymphocytes (B, Th0, Th1, Th2, Tc, Treg, anergic lymphocytes T, NKT) and monocytes (Tie2+, CD163+, HLA-DR+, CD1d+, 163+) were evaluated before ASCT and at the time of hematopoietic recovery using flow cytometry. For intracellular detection of cytokines (IFN-gamma, IL-4, IL-6, IL-10, IL-12, IL-17, TNF, TGFβ, IL-1β,) in the studied cell populations the Cytofix/Cytoperm Kit (BD Biosciences)

was used according to the manufacturer's recommendations.
Results: Early infectious complications were more frequent in the group of patients with HL or NHL than in the MM patients (11 vs. 5 cases). The percentage of CD3+/CD69+ (3.6±1.9% vs. 3.0±3.1) and CD3+/CD56+ T cells (6.8±5.0 vs. 3.4±3.0) decreased and the percentage of CD14+/TNF+ (1.5±1.6 vs. 2.0±1.8) and CD14/IL1β+ (2.0±2.2 vs. 3.3±2.2) monocytes increased after ASCT. In patients with post-transplantation early infections, the percentage of monocytes expressing pro-inflammatory cytokines, such as CD14+/TNF+ (1.0±1.5% vs. 1.8±1.7%), CD14+/IL1β+ (0.8±0.9% vs. 2.5±2.5%) and CD14+/IL-6+ (1.1±1.7% vs. 2.5±2.6%) before ASCT was significantly higher than in patients without infections ($p < 0.05$). In patients without colonization with highly pathogenic bacteria the percentage of CD14+/Tie2+ cells before transplantation was higher (53.6±19.7% vs. 33.8±15.5%, $p < 0.05$) than in patients with colonization. Before ASCT, the percentage of CD14+/IL12+, CD14+/TNF+, CD14+/TGFβ+, CD14+/IL1β+ cells was statistically lower ($p < 0.05$) in patients with mucositis after the transplantation comparing to the patients without mucositis, while the differences were more significant in patients with more severe mucosal lesions (stage III or IV according to WHO classification) compared to the group in which this complication was not observed.
Summary/Conclusion: Assessment of qualitative and quantitative disturbances of lymphocytes, monocytes expressing pro-inflammatory cytokines may allow to determine the factors predisposing to greater susceptibility to early infections after ASCT. However, in case of mucositis mechanism of development seems to be different. Further studies are necessary for a larger group of patients and assessing a larger number of parameters.

PS1508

SELECTIVE HSC-ABLATION USING ANTI-CD117 ANTIBODY DRUG CONJUGATE ENABLES SAFE AND EFFECTIVE AUTOLOGOUS AND ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

A. Czechowicz[1,*], R. Palchaudhuri[2], Z. Li[3], A. Scheck[4], Y. Hu[5], J. Hoggatt[6], B. Saez[7], M.K. Mansour[8], F. Winau[9], P. Murphy[10], D.T. Scadden[7], D.J. Rossi[5]

[1]Pediatrics, SCTRM, Stanford University School of Medicine, Stanford, [2]Magenta Therapeutics, Cabridge, MA, [3]NHLBI, National Institutes of Health, Bethesda, MD, [4]Pediatrics, SCTRM, Stanford University School of Medicine, Stanford, CA, [5]PCMM, Boston Children's Hospital, Boston, [6]CRM, Massachusetts General Hospital, Boston, MA, [7]SCRB, Harvard University, Cambridge, MA, [8]Massachusetts General Hospital, Boston, MA, [9]PCMM, Boston Children's Hospital, Boston, MA, [10]NIAID, National Institutes of Health, Bethesda, MD, United States

Background: Hematopoietic stem cell transplantation (HSCT) can be curative for many blood and immune diseases. However, today HSCT is primarily restricted to a small number of patients with deadly malignancies that have few other options. If made safer, HSCT could be applied to many more patients and disease settings. A large amount of toxicity of the procedure comes from the irradiation/chemotherapy conditioning currently employed to enable donor HSC engraftment. Eliminating genotoxic conditioning would dramatically improve HSCT, which would be especially beneficial in gene therapy/gene editing settings where this is the major limitation to expanded use. We and colleagues have previously developed multiple antibody-based conditioning approaches, but they each have had unique limitations.
Aims: Our goal is to develop a single-agent, potent, safe and effective conditioning approach that functions across all-diseased settings, that could be applied to non-malignant and malignant diseases and enables improved autologous or allogeneic HSCT.
Methods: We have previously shown that competition with host HSC limits donor HSC engraftment, and that antibodies depleting host HSC can be safe alternatives (Czechowicz, Science 2007). These antagonistic anti-CD117 antibodies were shown to be effective in immunodeficient mice, but additional strategies/agents were needed to enable donor engraftment in wild-type settings which caused toxicities (Xue Blood 2010, Chhabra Sci Trans Med 2016). Anti-CD45 antibody-drug conjugates overcame this limitation, however these induced a lymphopenia which is not desirable in many settings (Palchaudhuri, Nat Biotech 2016). To overcome this, here we generated an anti-CD117 antibody-drug conjugate (CD117-ADC) by linking anti-CD117 antibodies to protein synthesis toxin saporin. We tested this conditioning agent in syngeneic HSCT and in combination with transient immunosuppression in allogeneic HSCT.
Results: The CD117-ADC led to >99.9% depletion of host HSCs and enabled >99.9±0.1% engraftment of syngeneic donor murine whole bone marrow cells and >69.0±12.8% engraftment of syngeneic donor murine purified HSCs. The CD117-ADC in combination with transient immune suppression also induced robust mixed chimerism even in HLA-mismatched HSCT settings (Balb/c into B6 mice). Importantly and uniquely, this agent did not cause any significant toxicities. Rather it grossly spared red blood cells, platelets, and all major immune cells. The treated animals did not require transfusions, and immunity remained functionally intact. No gross tissue toxicity was observed apart from mild transient transaminitis, and animals remained fertile and did not require transplantation to maintain hematopoiesis.
Summary/Conclusion: CD117-ADCs provide the possibility of safe and effective autologous and allogeneic transplantation with a single-agent, one-time injection without major toxicities, cytopenias or perturbations to immunity. These agents are likely to become important agents in all forms of clinical transplantation, from malignant to non-malignant settings, and serve as important tools to study hematopoietic biology. As multiple anti-CD117 antibodies and ADCs are in development and being tested in clinical trials, such an approach may be rapidly translatable a range of patients with blood and immune diseases including leukemia, sickle cell anemia, beta thalassemia, immunodeficiencies, HIV and many others. Such agents may be especially beneficial in patients with high sensitivity to traditional conditioning, such as bone marrow failure patients with Fanconi Anemia or other DNA-repair defects.

Thalassemias

PS1509

HEMATOLOGICAL PHENOTYPES OF DIFFERENT ALPHA-THALASSAEMIA GENOTYPES CAUSING HYPOCHROMIC MICROCYTIC ANEMIA AT NEONATAL PERIOD

T. Suksangpleng[1,*], S. Ekwattanakit[1], S. Riolueang[1], V. Viprakasit[2]

[1]Thalassemia Center, [2]Division of Hematology/Oncology, Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Background: Thalassaemia (thal) is highly prevalent throughout subtropical regions including Thailand. In particular α-thal characterized by mutations and/or deletions involved the α globin genes (αα/αα) is extremely common in Southeast Asia in which 30-35% of the population carry either α^0-thal (--/αα) or α^+-thal (-α/αα). Homozygous or compound heterozygous α°-thal lead to severe anemia *in utero* and a fatal thal syndrome; Hb Bart's hydrops (100% of Hb Bart's). This serious condition is due to the fact that the α-globin genes are switched on and expressed early and throughout fetal haematopoiesis. However, the effects of less severe α-thal syndrome; Hb H disease (--/-α) and α°- or α^+-thal carriers for fetal haematopoiesis remain to be elucidated. Moreover, Hb Bart's could be detected in other α-thal conditions with various quantities and this could be used to screen α-thal at newborn screening program.
Aims: To determine hematological parameters in Thai neonates with different types of α-thal and identify a new screening tool for α-thal at birth.
Methods: After informed consent, 5 dried blood spot (DBS) and 300-µl EDTA whole blood samples were collected from healthy newborns (aged 48 hrs) by heel prick puncture. This study was approved by our local ethical committee at Siriraj Hospital. All of DBS were used for hemoglobin (Hb) analysis by using capillary electrophoresis (CE) and isoelectric focusing (IEF) followed by genomic DNA extraction for PCR-based molecular analysis including α-thal GAP-PCR, α-thal ARMS-PCR, β-thal ARMS-PCR, and PCR-RFLP for Hb E to detect >98% of abnormal globin alleles found in Thailand. In addition, complete blood count (CBC) was determined in whole blood EDTA samples using COULTER A^c·T 5diff Autoloader Hematology Analyzer (Beckman Couter, Brea, USA). The reference ranges and cut-off levels of hematological parameters were statistically analyzed by using PASW Statistics 18 Software (SPSS Inc, 2015).

Table 1.

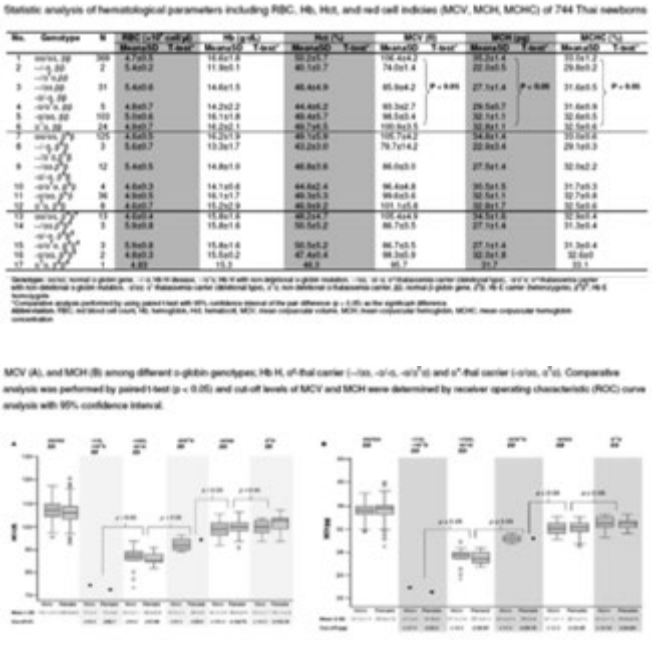


Figure 1.

Results: Average (mean) and standard deviation (SD) of hematological parameters (RBC, Hb, Hct, red cell indicies) of 744 newborns (excluding 5 individuals with HbE/β thal and β thal traits) based on different α and β globin genotypes were shown in Table 1. Out of 744 neonates, 188 (25.26%) were HbE carriers while 22 (2.95%) were homozygous Hb E (Hb EE). In neonates with normal β globin genes, we found red cell indicies; MCV, MCH, MCHC to be significant differences (t-test: $p<0.05$) among different α-globin mutation types. Interestingly, the levels of Hb and Hct seem to be positively correlated with number of intact normal α globin genes; those with 3 genes affected (Hb H disease) had the lowest Hb, Hct and all RBC indicies (Table 1). Coinheritance of Hb E, either Hb E trait or HbEE, appeared to have no additional effect on haematological parameters supporting the fact that the β globin genes switched on only at perinatal period but would be fully operational within the first two years of life. This finding was observed at both genders (Figure 2A and 2B).
Summary/Conclusion: For the first time, we showed that other forms of α-thal beyond Hb Bart's hydrops can also cause hypochromic microcytic anemia *in utero* resulting in neonatal anemia at 48 hrs. Mutations in the β globin genes showed no effects on neonatal haematology. Reductions in RBC indicies can be used as a screening tool for α thalassaemia in addition to the detection of Hb Bart's at birth by CE or IEF. A combination of both parameters (RBC indicies and Hb Bart's) could provide a better screening tool for α-thal in particular those with α^+-thal carriers.

PS1510

SERUM YKL-40 LEVELS IN PATIENTS WITH B-THALASSEMIA: RELATION TO VIRAL HEPATITIS, LIVER STIFFNESS AND HEPATIC IRON CONCENTRATION

S. Mostafa[1,2,*]

[1]Paediatrics, Cairo University, [2]Paediatrics, National Institute of Hepatology, Cairo, Egypt

Background: YKL-40 is an inflammatory glycoprotein expressed by infiltrating macrophages in various inflammatory conditions. It has been found to be elevated in patients with different pathological conditions like acute and chronic inflammations, increased remodeling of the extracellular matrix (ECM), development of fibrosis and cancer. Several studies have found elevated YKL-40 concentrations in sera of patients with liver diseases such as hepatic fibrosis by hepatitis C virus.it has been suggested that YKL-40 concentrations reflect the degree of liver fibrosis.
Aims: To evaluate serum YKL-40 Levels in patients with β-thalassemia and its relation to viral hepatitis, liver stiffness as assessed by Transient elastography (FibroScan, FS) and Hepatic Iron Concentration
Methods: A prospective study included 100 patients with β- TM (43 males and 57 females) with mean age 13.8±2.7 years (range: 5-18 years). Serum ferritin level, Liver enzymes (ALT and AST), HBs Ag, Anti HCV Ab and Serum YKL-40 using ELISA kit were evaluated. All patients were subjected to Liver MRI T2* to detect liver iron content by the sequence and Transient elastography (FibroScan, FS) to assess degree of liver stiffness.
Results: Mean fibroscan value was (10.99±11.5) kPa with a median 6.7 (range 1.3 to 47) kPa. 64 (64%) patients were categorized as F0-1 and 17 (17%) were stage F2-3, 19 (19%) patients had severe fibrosis. Their median serum ferritin was 3100 ng\ml, with 61 (61%) patients had values exceeding 2500 µg/L. Median cardiac T2* was 24.2 with 30 patients had values below 20 ms, and the median LIC was 16.21-mg/g DW with 68 patients showed readings above 7 mg/g dw. NYL-40 was evaluated as a marker of inflammation and liver fibrosis and showed mean value 1505.1 (±960.9) pg/ml, and range from 500 to 3529 pg/ml. Mean YKL-40 was significantly higher among males (p=0.03), patients on chelation therapy (p=0.002), patients on DFS ($p\leq0.001$), in those with abnormal liver enzymes, splenectomised patients, patients with HBV sero-positivity, those with moderate elevation of T2* and patients with high grades of liver fibrosis ($p<0.05$). YKL-40 showed positive correlation with the rate of transfusion, LIC, ferritin, ALT and AST but negative correlation with weight, height and T2*. ROC curve analysis revealed that the cutoff value of YKL-40 at 1500 pg/mL could differentiate β-TM patients with and without viral hepatitis with 86.7% sensitivity and specificity of 91.4%, area under the curve (AUC) 0.933, positive predictive value 81.2 and negative predictive value 94.1 ($p<0.001$). ROC curve analysis revealed that the cutoff value of YKL-40 at 1600 pg/mL could detect β-TM patients with liver cirrhosis with 93.4% sensitivity and specificity of 97.1%, area under the curve (AUC) 0.972, positive predictive value 93.7 and negative predictive value 97.1 ($p<0.001$).
Summary/Conclusion: Serum YKL-40 Levels are elevated in patients with β-thalassemia and can detect patients with active viral hepatitis and liver stiffness.

PS1511

THE STRONG LINK BETWEEN PANCREAS AND HEART IN THALASSEMIA MAJOR

A. Meloni[1,*], L. Pistoia[1], A. Spiga[2], M. Fortini[3], L. Sardella[4], D. Maddaloni[5], C. Argento[6], M. Laura[7], M.G. Sanna[8], N. Dello Iacono[9], P. Fina[10], A. Riva[11], V. Positano[1], A. Pepe[1]

[1]Fondazione G. Monasterio CNR-Regione Toscana, Pisa, [2]P.O. Pediatrico Microcitemico "A.CAO", Cagliari, [3]Azienda Ospedaliero-Universitaria "S.Anna", Cona (FE), [4]A.S.L. di Bari, Bari, [5]Ospedale "Engles Profili", Fabriano, [6]Ospedale "San Giovanni Di Dio", Agrigento, [7]Ospedale "Sant'Eugenio", Roma, [8]ASL N°1 Sassari, Sassari, [9]Ospedale Casa Sollievo della Sofferenza IRCCS, San Giovanni Rotondo (FG), [10]Ospedale "Sandro Pertini", Roma, [11]Ospedale "SS. Annunziata" ASL Taranto , Taranto, Italy

Background: Some preliminary data have postulated a correlation between pancreatic iron overload and heart iron and function in thalassemia major (TM) patients.
Aims: In the present study we explored systematically in a multicenter study the link heart-pancreas in a large cohort of TM patients .
Methods: We considered the first 232 TM patients (129 M, mean age 36.95±9.83 years) enrolled in the E-MIOT (Extension-Myocardial Iron Overload in Thalassemia) project. T2* measurements were performed over pancreatic head, body and tail and global value was the mean. Myocardial iron overload (MIO) was quantified using a T2* segmental approach. Biventricular function parameters were assessed by cine images. Late gadolinium enhancement (LGE) images were acquired to detect myocardial fibrosis.
Results: A significant correlation between pancreatic and cardiac iron was reconfirmed in this more numerous population and a normal pancreas T2* showed negative predictive value of 100% for cardiac iron. Pancreatic iron was correlated to the LV ejection fraction (EF), but not to the right ventricular (RV) EF. LGE sequences were acquired in 101 TM patients and 43 (42.57%) of them showed macroscopic myocardial fibrosis. Global pancreas T2* values were significantly lower in patients with fibrosis (6.27±4.12 ms vs 11.15±9.23 ms; p=0.021). Twenty-two patients showed cardiac complications (11 arrhythmias, 6 heart failure, 2 pulmonary hyperthension, 1 vascular disease, and 2 others) and of them 21 had pancreatic iron. Patients with cardiac complications showed a significant lower global pancreas T2* (7.55±6.11 ms vs 14.31±13.39 ms; p=0.024).
Summary/Conclusion: Pancreatic iron is a strong predictor not only for cardiac iron, but also for cardiac complications supporting a more profound link between pancreatic iron and heart disease in TM. More studies are needed to evaluate the prognostic role of pancreatic iron on cardiac complication.

PS1512

A SIMPLE NGS METHOD FOR DETECTION OF MUTATIONS CAUSING ALPHA AND BETA THALASSEMIA

M. Uzunel[1,*]

[1]Devyser, Hägersten, Stockholm, Sweden

Background: Thalassemias are inherited blood disorders characterized by abnormal hemoglobin production. Depending on the type and number of mutations, the symptoms can vary from none to severe forms where stem cell transplantation is the only curative treatment. Today, many different methods are available for mutation analysis of thalassemia patients. These include ARMS-PCR, restriction-enzyme PCR, GAP-PCR, Sanger sequencing, LIPA and MLPA. The use of different methods may be time-consuming and expensive, especially when analyzing less common deletions.
Aims: To develop a simple NGS method for detection of mutations causing alpha and beta Thalassemia.
Methods: We have developed an amplicon based NGS method using only ONE oligo-mix to detect virtually all known mutations for alpha and beta Thalassemia. The method includes sequencing of the HBB, HBA1 and HBA2 genes. We have also included primers for direct detection of the most common deletions in alpha and beta Thalassemia ("Gap-PCR"). In addition, we amplify regions upstream and downstream of the above-mentioned genes to find less common deletions using copy number variation (CNV) analysis. The upstream regions also include regulatory sequences, i.e. HS-40 (HBA) and LCRB (HBB). The protocol is user-friendly, requires less than one hour of hands-on time and only 10 ng of DNA is needed.
Results: Libraries have 100% uniformity at ≥ 0.3x mean coverage. We have analyzed 125 clinical samples with known mutations. All mutations were also detected with the Devyser Thalassemia kit. Interestingly, we could find additional mutations in 10% of the samples; mutations not previously detected or tested by the clinical laboratory. CNV analysis could successfully detect deletions and triplications (anti-3.7 triplication).
Summary/Conclusion: The Devyser Thalassemia kit is simple, user-friendly and efficiently detects mutations causing Thalassemia.

PS1513

PRE- AND POST-TRANSFUSION COMPLEMENT ACTIVATION IN TRANSFUSION-DEPENDENT B-THALASSAEMIA

E. Gavriilaki[1,*], I. Christodoulou[2], E.-E. Koravou[1], A. Paleta[1], M. Koutra[1], P. Zerva[1], T. Touloumenidou[1], A. Papalexandri[1], C. Apostolou[2], P. Klonizakis[2], A. Anagnostopoulos[1], E. Vlachaki[2]

[1]Hematology Department - BMT Unit, G. Papanicolaou Hospital, [2]Adults Thalassaemia Unit, [2]nd Department of Internal Medicine, Aristotle University of Thessaloniki, Hippokration General Hospital of Thessaloniki, Thessaloniki, Greece

Background: Introduction of safe transfusion and supportive practices has significantly improved survival of transfusion-dependent β-thalassaemia (TDT). Although regular RBC transfusions are the mainstay of treatment, their role in the pathophysiology of complications in TDT remains unclear. Transfusions may exacerbate the vicious cycle of thrombosis and complement activation. Nevertheless, limited data exist on complement activation in TDT.
Aims: We aimed to determine whether increased complement activation is evident in TDT; whether it is associated with disease characteristics and whether it is exacerbated post transfusion.
Methods: Consecutive TDT patients were enrolled prospectively from November to December 2017. Patient history, clinical and laboratory data were recorded. Sera were collected immediately before transfusion and 1 hour after completion of transfusion in each patient and stored at -80 °C. Complement activation was detected in patient sera measuring soluble human C5b-9 with a commercially available ELISA kit (AMSbio, Abingdon; United Kingdom). Normal human serum (NHS) from 10 age and gender-matched Caucasian healthy volunteers was used as a negative control.
Results: We studied 45 TDT patients (45.5±25.6 years of age, 21 male: 24 female), as shown in Table 1. Soluble C5b-9 levels before transfusion (median 68.2 ng/ml, interquartile range 62.1 ng/ml) were similar to C5b-9 detected in normal human serum (median 91.2 ng/ml, interquartile range 86.7 ng/ml, p=0.219). However, C5b-9 levels significantly increased 1 hour post transfusion (Figure 1, median 85.6 ng/ml, interquartile range 72.5 ng/ml, p=0.012). Then, we analysed clinical factors associated with increased C5b-9 levels post transfusion. Interestingly, C5b-9 levels post transfusion were significantly increased in patients traditionally classified as thalassaemia-intermedia (p=0.041) and patients post splenectomy (p=0.034). In addition, both patients with thalassaemia-intermedia and patients with splenectomy were older and had significantly higher platelet values compared to patients with thalassaemia-major (p=0.035 and p=0.021, respectively) and patients without splenectomy (p=0.008 and p<0.001, respectively). C5b-9 values did not correlate with other patient characteristics (iron overload, RBC volume/weight or development of red cell alloantibodies).

Table 1.

RBC volume / weight (ml/kg)	155.6±48.7
Platelets (10^9/L)	435.5±211.5
Ferritin (ng/mL)	1099.1±663.2
Deferasirox / Combined iron chelation (%)	17 (37.8%) / 19 (42.2%)
Hepatic / Cardiac T2* (ms)	14.7±10.6 / 31.4±6.1
Splenectomy (%)	28 (52.8%)
Red cell Alloantibodies (%)	5 (9.4%)

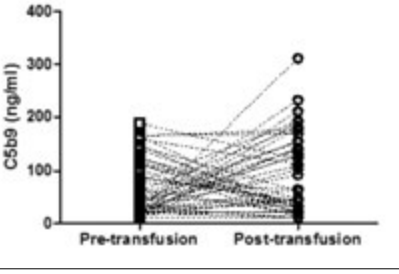


Figure 1.

Summary/Conclusion: Our study provides preliminary *in vitro* results of pre- and post-transfusion complement activation using a robust marker in a real-world patient population. We have found for the first time increased complement activation post transfusion in patients originally classified as thalassaemia-intermedia and in patients with splenectomy. These groups of patients have been traditionally considered to be of high thrombotic risk. The interplay between complement and thrombosis in these patients and the role of transfusions need to be further investigated in future studies.

PS1514

ASSOCIATION OF CLINICAL PHENOTYPE OF THALASSEMIA WITH COMMON FETAL HEMOGLOBIN VARIANTS IN LEBANESE PATIENTS BEARING THE CODON 29 BETA GENE MUTATION

V. Brancaleoni[1], H.M. Moukhadder[2], D. Consonni[3], E. Di Pierro[1], M.D. Cappellini[1,4], A. Taher[5,*]

[1]U.O.C. Medicina Generale, Fond. IRCCS Cà Granda Ospedale Maggiore Policlinico, Milano, Italy, [2]Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon, [3]U.O. Epidemiologia, Fond. IRCCS Cà Granda Ospedale Maggiore Policlinico, [4]Scienze cliniche e di comunità, Università degli Studi di Milano, Milano, [5]Internal Medicine, American University of Beirut Medical Center, Beirut, Italy

Background: Beta-thalassemia can present with a wide spectrum of phenotypes, ranging from almost absence of symptoms to severe anemia and regular transfusion dependence. The inheritance of α-thalassemia and hereditary persistence of fetal hemoglobin (HbF) have been identified among the genetic modifiers that can impart less severe phenotypes in β-thal. Other implicated influencers include polymorphic variants in the BCL11A, HMIP and HBB cluster, which have been shown to model the thalassemia phenotype through HbF induction. The codon 29 (Cod29) mutation in the beta gene (C/T) has been associated with a broad range of thalassemia phenotypes, possibly through genetic modifiers determining the genotype-phenotype relationship.
Aims: This study aimed to evaluate whether polymorphisms at known sites and/or coinheritance of α or βglobin gene deletions or duplication can influence HbF levels and eventually justify differences in the clinical presentation of β-thalassemia patients of Lebanese origin bearing the Cod29 mutation.
Methods: Twenty-one β-thal patients homozygous or compound heterozygous (with IVSI-1 or IVSII-1 mutations) for the Cod29 mutation and displaying heterogeneous clinical manifestations were recruited. Ten SNPs were selected based on previously published evidence of association with increased HbF levels (HBG2: rs7482144;HBBP: rs7482144;BCL11A: rs1427407, rs766432, rs11886868, rs4671393; HMIP: rs28384513, rs9399137, rs4895441, rs9402686). Genomic DNA was extracted from peripheral blood. HBG2 SNP was genotyped by PCR and direct sequencing. All other SNPs were genotyped by allelic discrimination using TaqMan assays. MLPA analysis on α and β clusters was performed using MRC Holland MLPA kits. Statistical analysis was performed with the Kruskal-Wallis and chi-square tests using Stata 15 software.
Results: Fourteen patients were homozygous for the Cod29 mutation (78.6% females), 3 were compound heterozygous for Cod29 and IVSI-1 (33.3% females) and 4 were compound heterozygous for Cod29 and IVSII-1 (75% females). HbF ranged from 4.9% to 66% and hemoglobin (Hb) levels from 5.6g/dL to 9.8g/dL. Our patients were divided according to transfusion frequency into two groups: patients who never or seldom did receive red blood cell transfusions during their lives (non-transfusion-dependent, NTD), and patients who were regularly transfused during their lives till the date of blood sampling for our study (transfusion-dependent, TD), with a transfusion frequency ranging from 15 to 30 days. None of the patients had β gene deletions and 2 patients co-inherited the $\alpha^{3.7}$ deletion. The minor allele frequencies (MAF) observed for the SNPs wil be presented. Hb, HbF and absolute HbF concentration were evaluated according to genotype. Heterozygotes or homozygotes for the effect allele in the BCL11A and HMIP SNPs had higher HbF percentages and absolute levels. Heterozygotes or homozygotes for rs1427407 and rs766432 on BCL11A had the highest HbF levels and least transfusion dependence (p=0.04). The proportions and absolute concentrations of HbF were found to be higher in the NTD than TD group (p=0.0002 and 0.0004). Hb levels did not differed much between the two groups.
Summary/Conclusion: Our preliminary results suggest that transfusion dependency in β-thal Lebanese patients bearing the Cod29 mutation correlates with HbF levels. Higher HbF levels were found to be associated with the presence of polymorphic bases in known loci, especially HMIP and BCL11A. Among these, rs1427407 and rs766432 were the most "favorable" SNPs, associated with better clinical outcomes in our patients.

PS1515

THALASSEMIA PREVENTION PROGRAM IN DEVELOPING WORLD SCENARIO: 13 YEARS EXPERIENCE

K. Perveen[1,*], G. Ahmed[1], S. H. Ansari[1], S. Siddiqui[1], S. Sharif[1], S. Ahmed[1], T. Shamsi[1]

[1]National Institute of Blood Disease and Bone Marrow Transplantation, Karachi, Pakistan

Background: β-thalassaemia is one of the most common monogenic disorders worldwide. In Pakistan around ten million people are carriers of β-thalassaemia. If both parents are thalassaemia carrier, expecting mothers must undergo prenatal diagnosis through Chorionic Villus Sampling (CVS) in each pregnancy. β-thalassaemia may exhaust existing health resources and there is a threat that it will become a major health issue in Pakistan as decline is observed in infection related deaths in children. Prevention is the only way to address the issue. This can be done through carrier screening, genetic counseling and prenatal diagnosis to control affected birth.
Aims: The aim of this study is to evaluate the outcomes of prenatal diagnosis for the establishment of thalassemia prevention program in Pakistan.
Methods: 2458 high risk mothers for β-thalassaemia with 12-16 weeks pregnancy were evaluated for CVS at National Institute of Blood Disease and Bone Marrow Transplantation, from January 2005 to January 2018. CVS results (fetuses with thalassemia major, minor or no mutation) were evaluated. Parents were counseled about the pattern of inheritance, the chances of having an affected child in the current or future pregnancy, the procedure of CVS, risk and complications of the procedure and the option of termination of pregnancy in case the fetus was detected to be homozygous for β-thalassaemia. Before the procedure an informed consent was obtained from both partners.
Results: 2458 procedures were performed. 624(25%) fetuses were diagnosed as major, 1253(51%) with trait, 2(0.08%) fetuses had undetected mutation, and 581(23.6%) were healthy. New mutations identified in our population were IVSI-30, IVSI-110, IVSII-1, Cd-39 and Fr 47-48. Placental site was posterior in 577(23.5%) pregnant mothers. Procedure related complications were seen in 20 cases (0.8%) which included bradycardia i.e <120pbm (0.08% fetuses) and bleeding (0.5%).Further complications included 2-3 attempts during performance of single procedure in 42(1.7%), missed abortions were observed in 6(0.24%). Mild, moderate and severe procedure related pain in 468(19%), 34(1.4%) and 2(0.08%) cases respectively. In 2458 procedures inadequate sampling was observed in 10(0.4%) cases. Seven (0.28%) couples refused abortion of β-thalassaemia major fetuses.
Summary/Conclusion: Recent acceptance of legal abortion with respect to Muslim rules has increased the effectiveness of the CVS procedure and made great advances in its application in Pakistan.Social knowledge has also improved but still there is a gap between the population at risk and the required prenatal diagnosis laboratories and sampling centers.

PS1516

ASSOCIATION OF VITAMIN D AND RETINOID×RECEPTORS EXPRESSION AND VITAMIN D BINDING PROTEIN VARIANTS WITH BONE DENSITY IN THALASSEMIA CHILDREN

S. Sahmoud[1,*], S. Elfiky[2], E. A. Toraih[34], M.S. Fawzy[5], N. Kamel[6], M.S. Ibrahim[7]

[1]Pediatric Hematology Department, Suez Canal University, [2]Pediatric Hematology Department, [3]Genetics Unit, Faculty of Medicine, Suez Canal University, [4]Center of Excellence in Molecular and Cellular Medicine, Suez Canal University, [5]Medical Biochemistry Department, [6]Clinical pathology Department, [7]Diagnostic Radiology Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt

Background: Studies previously conducted in 2 different cities in Egypt (Cairo, and Beni Suef) found that thalassemia (thal) patients were deficient for vitamin D and concluded an association with Bsm1, and Fok1 Vitamin D receptor (*VDR*) polymorphisms and rs7041 and rs4588 vitamin D binding protein (VDBP) polymorphisms.
Aims: This study aimed at evaluating vitamin D status in thal children in relation to *VDR* and retinoid×receptor (*RXRA*) expression as well as *VDBP* polymorphisms in Suez Canal area, Egypt.
Methods: 44 (24 male, 7.3±2.7 year old) thal children and 40 (19 male, 7.6±1.8 year old) healthy ones were subjected to bone chemistry profile, 25 (OH) vitamin D, dual energy X-ray absorptiometry (DEXA) scan, SNP identification of *VDBP* rs7041 and rs4588, and gene expression of *VDR* and *RXRA* genes.
Results: Vitamin D deficiency was significantly more frequent among healthy children (37, 92.5%) compared to children with thalassemia (16, 36.3%) (p=<0.001). All, and 40% of thalassemia and healthy children were regularly receiving the daily RDA of vitamin D and calcium (p=<0.01). Thal children

compared to controls had higher serum calcium (9±0.7, and 8.2±0.6, p=<0.001), and lower parathyroid hormone (22.2±13.1, and 38.4±21.2, p= 0.001). No significant difference in the bone mineral density (BMD) was found in both groups (p=0.54), but there was a negative correlation between BMD and age (r= - 0.51, p=<0.001). Serum *VDR* and *RXRA* mRNAs were significantly higher in thal children compared to controls (p=0.001 and <0.001). Expression levels of both genes were not associated with gender (p=0.786 and 0.548) nor bone density (p=0.208 and 0.176). Genotyping of the VDBP rs4701 and rs4588 polymorphisms revealed no significant difference between patients and controls (p=0.609). However, the TG genotype showed higher frequency of osteoporosis among thal children, while both homozygote carriers (TT and GG) were common among osteopenic patients (p=0.023).
Summary/Conclusion: Thalassemia children had higher expression levels of serum *VDR* and *RXRA*. VDBP polymorphisms were associated with bone osteoporosis in Thalassemia patients.

PS1517

NEW OCCURRENCES OF MACROSCOPIC MYOCARDIAL FIBROSIS IN THALASSEMIA AT LONG TERM BY MULTIPLE FOLLOW-UP

A. Meloni[1,*], L. Pistoia[1], A. Barone[2], A. Quarta[3], G. Roccamo[4], C. Paci[5], G. Colaci[6], N. Romano[7], M. Benni[8], F.M. Pasin[9], S. Bulgarelli[10], M. Missere[11], A. Vallone[12], V. Positano[1], A. Pepe[1]

[1]Fondazione G. Monasterio CNR-Regione Toscana, Pisa, [2]Azienda Ospedaliero-Universitaria di Parma, Parma, [3]Ospedale "A. Perrino", Brindisi, [4]Ospedale "Civile", S. Agata di Militello (ME), [5]Ospedale "S Maria alla Gruccia", Montevarchi (AR), [6]Presidio Ospedaliero N°2 "S. Giuseppe da Copertino", Copertino (LE), [7]Azienda Ospedaliera Arcispedale "S. Maria Nuova", Reggio Emilia, [8]Policlinico S. Orsola "L. e A. Seragnoli", Bologna, [9]Ospedale S. Pertini, Roma, [10]Ospedale Bufalini, Cesena, [11]Fondazione di Ricerca e Cura "Giovanni Paolo II", Campobasso, [12]Azienda Ospedaliera "Garibaldi" Presidio Ospedaliero Nesima, Catania, Italy

Background: To date in thalassemia patients it is recommended to repeat cardiac magnetic resonance (CMR) scans for iron quantification every 1 or 2 years based on the myocardial iron overload (MIO). Also in these patients, late gadolinium enhancement (LGE) has been demonstrated to be a strong predictor for cardiac events. However, many studies have shown an association between intravenous gadolinium based contrast agents (GBCA) exposure and neuronal tissue deposition. So, it appears prudent at this time to revisit institutional protocols for GBCA administration, in particular in the follow up (FU) studies.
Aims: We investigated the evolution of myocardial fibrosis in terms of new occurrences over a period of 6 years in thalassemia patients who underwent to multiple FU.
Methods: We considered 52 patients with thalassemia major (28.78±8.59 years; 28 females) consecutively enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network who underwent 5 LGE CMRs (baseline + 4 follow-up) using Gadobutrol (0.2 mmoli/kg). The time interval between two subsequent scans was 18±3 months.
Results: At the baseline CMR, 44 patients (84.6%) were LGE negative.
Eight new occurrences of myocardial fibrosis were detected at the first follow-up (FU). At the second FU, 2 out of the 36 previously LGE-negative patients had myocardial fibrosis. At the third FU, 9 new occurrences of myocardial fibrosis were detected. At the forth FU, 3 patients showed myocardial fibrosis for the first time. The Figure 1 shows a simplifying flow-chart. The 22 patients who developed myocardial fibrosis during the follow-up showed comparable frequency of diabetes and HCV infection and comparable baseline cardiac iron than patients who remained always LGE-negative.

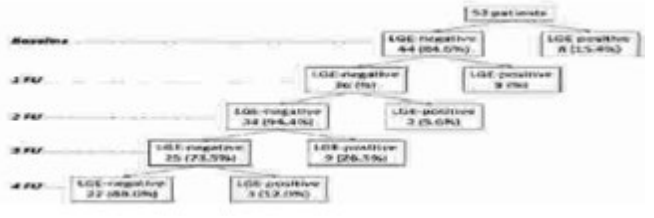


Figure 1.

Summary/Conclusion: A serial monitoring of thalassemia patients revealed an high number of new occurrences of myocardial fibrosis, suggesting the importance of repeating the LGE CMR over time using 'low risk' macrocyclic agents.

PS1518

COMMON LINK BETWEEN BONE AND HEART HEALTH IN THALASSEMIA MAJOR

A. Meloni[1,*], L. Pistoia[1], C. Vassalle[1], S. Maffei[1], T. Casini[2], A. Filosa[3], P. Keilberg[1], A. Olivi[1], L. Rigoli[4], R. Rosso[5], M. Rocca[6], A. Massa[7], C.C. De Bari[8], V. Positano[1], A. Pepe[1]

[1]Fondazione G. Monasterio CNR-Regione Toscana, Pisa, [2]Ospedale "Meyer", Firenze, [3]Azienda Ospedaliera di Rilievo Nazionale "A. Cardarelli, Napoli, [4]Policlinico "G. Martino", Messina, [5]Azienda Ospedaliero-Universitaria Policlinico "Vittorio Emanuele", Catania, [6]Presidio Ospedaliero ASL [5], Crotone, [7]Ospedale "Giovanni Paolo II", Olbia, [8]Ospedale "San Donato", Arezzo, Italy

Background: A common link between bone and heart health has been detected in the general population.
Aims: In the present study we evaluated the relationship between bone mineral density (BMD) and cardiac iron, cardiovascular complications, and cardiovascular risk factors in patients with thalassemia major (TM).
Methods: We considered 135 TM patients (68 M, mean age 37.79±7.74 years) enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) Network. BMDs of the lumbar vertebrae (L1-L4) and femoral neck were measured by dual X-ray absorptiometry (DEXA) and were expressed as T-scores. Myocardial iron overload (MIO) was quantified by the T2* Magnetic Resonance Imaging (MRI) technique.
Blood samples were drawn for the analysis of N-terminal pro-brain natriuretic peptide (NT-proBNP), the gold standard biomarker in determining the diagnosis and prognosis of heart failure.
Results: MIO (global heart t2*<20 ms) was detected in 20 (14.8%) patients. Patients with MIO showed significant lower femoral T-scores (-2.64±1.87 vs -1.96±0.99; p=0.045). Twelve patients showed cardiac complications (8 arrhythmias, 2 heart failure, 2 pulmonary hyperthension) and all of them had lumbar and femoral T-scores indicating osteopathy (osteopenia/osteoporosis). Patients with cardiac complications showed significant lower lumbar T-scores (-3.27±0.89 vs -2.43±1.29; p=0.031) and femoral T-scores (-2.96±2.02 vs -1.98±1.04; p=0.042). Patients with abnormal NTproBNP values (>157 ng/L) showed significantly lower femoral T-scores (-2.41±0.61 vs -1.79±1.16; p=0.048). Patients with diabetes (N=17) showed significant lower femoral T-scores (-2.45±0.79 vs -2.00±1.25; p=0.041).
Summary/Conclusion: Lower femoral BMD was associated with MIO, cardiac complications, elevated NTproBNP values, and diabetes in patients with thalassemia major. So, our data support and interaction between bone and cardiovascular diseases in TM, suggesting that prevention and treatment strategies targeted for one of the two diseases may be beneficial for the other one.

PS1519

Abstract withdrawn

PS1520

CEREBRAL IRON OVERLOAD IN BETA THALASSEMIA: DOES IT REALLY MATTER?

I. Tartaglione[1,*], S. Perrotta[1], M. Caiazza[1], M. Casale[1], R. Di Concilio[2], A. Ciancio[3], E. De Michele[4], G. Amendola[5], P. Gritti[6], P.A. Carafa[7], T. Ferrantino[8], A. Centanni[5], N. Ippolito[5], V. Caserta[9], T. Oliveto[5], I. Granato[5], G. Femina[10], F. Esposito[11], S. ponticorvo[12], A. Russo[11], C. Russo[13], M. Cirillo[14], A. Elefante[13], R. Manara[10]

[1]Dipartimento della Donna, del Bambino e della Chirurgia generale e specialistica, Università della Campania "Luigi Vanvitelli", napoli, [2]Dipartimento di Pediatria, Ospedale "Umberto I", Nocera Inferiore, [3]Unità Operativa Ematologia, Ospedale "Madonna Delle Grazie", Matera, [4]Medicina Trasfusionale, AOU "San Gioanni di Dio e Ruggi D'Aragona", Salerno, [5]Ospedale "Umberto I", Nocera Inferiore, [6]Dipartimento di Salute Mentale e Fisica e Medicina Preventiva, Università della campania "Luigi Vanvitelli", Napoli, [7]Dipartimento di Salute Mentale, Servizio Psichiatrico di Diagnosi e Cura, ASL Salerno, Salerno, [8]Università della Campania "Luigi Vanvitelli", [9]Università della Campania "L. Vanvitelli", Napoli, [10]Dipartimento di Medicina e Chirurgia, Scuola Medica Salernitana, Sezione di Neuroscienze, Università di Salerno, [11]Dipartimento di Medicina e Chirurgia, Scuola Medica Salernitana, Sezione di Neuroscienze, Università di Salerno Salerno, [12]Dipartimento di Medicina e Chirurgia, Scuola Medica Salernitana, Sezione di Neuroscienze, Università di Salerno Salerno,, salerno, [13]Neuroradiologia, Università degli Studi di Napoli "Federico II", [14]Dipartimento di Scienze Mediche, Chirurgiche, Neurologiche, Metaboliche e dell'Invecchiamento, Università della Campania "Luigi Vanvitelli, napoli, Italy

Background: Iron overload (IOL) in beta-thalassemia represents one of the

main cause of the complications of the disease, and it is invariably present both in transfusion dependent thalassemia (TDT), due to the transfusion burden, and in non transfusion dependent thalassemia (NTDT), due to the increased intestinal iron absorption. Heart, liver and endocrine glands are all known targets of IOL, while recent studies focus on kidneys and spleen, and ideally the non transferrin bound iron could accumulate in every body system. In the brain the presence of IOL has been rarely investigated, with uncertain results: IOL shown contemporarily in putamina, caudate nuclei and motor and temporal cortices, or only in some of these, or in none of the listed (Metafratzi *et al.* 2001, Akhlaghpoor *et al.* 2012, Qiu *et al.* 2014); moreover if cerebral IOL can affect cognitive functions has not been studied yet.
Aims: Aim of our study is to underline the presence, entity and distribution of IOL in cerebral structures, looking for any difference in the pattern of accumulation between TDT and NTDT and to clarify if it impacts on neurocognitive sphere.
Methods: We performed a cross-sectional multicenter study, enrolling patients with TDT, NTDT and healthy volunteers. After the signature of the informed consent, patients and controls underwent a 3 Tesla MRI with high resolution multi-echo 3D FLASH sequences for iron detection and quantification; Wechsler Adult Intelligence Scale-Fourth Edition (WAIS-IV) was administered to all study participants. Clinical chart review allowed the collection of clinical and laboratory data, with special regards to the disease history, the transfusion burden, ferritin, LIC and cardiac IOL by T2*.
Results: Seventy-one patients with beta thalassemia were enrolled in the study from 4 major Centers in the South of Italy: 48 with TDT (age: 36.9±10.3 years) and 23 NTDT (age: 29.2±11.7 years); fifty-seven age and sex-matched healthy controls (HC) (age: 33.9±10.8 years) were also enrolled. Comparing all patients (TD+NDT) to controls 4 clusters were found with R2* values higher in patients than in controls; these refer to the choroid plexuses in the left and right lateral ventricles and in proximity to the left and right Luschka foramina. Differences between beta-thalassemia patient subgroups were limited, anyway a regular blood transfusion treatment seemed to further accentuate brain iron content compared to controls; also, the R2* value was directly correlated with ferritin in the patients'group. Interestingly R2* did not correlate with the cognitive performance indices derived from the WAIS-IV test. Both in patients and in controls R2* values tend to increase with age.
Summary/Conclusion: Beta-thalassemia entails significant iron overload in small symmetric brain regions in correspondence of the choroid plexuses. Anyway these structures do not share any developmental, metabolic or functional characteristics, as proved also in our series by the lack of correlation with any degree of cognitive impairment. Moreover, the linear increase of iron in cerebral structures with age demonstrates a pattern of iron deposition in the brain for patients comparable to the general population. Based on our results routine MRI assessments in thalassemic patients to quantify cerebral iron overload would not be clinically indicated.

PS1521

LEPTIN IS ASSOCIATED WITH THE DEGREE OF ANEMIA AND THE ERYTHROPOIETIN LEVELS IN B THALASSEMIA PATIENTS

L. Mahajna[1], O. Tanous[2], T. Dujovny[3], H. Eitam[1], R. Masalha[1], R. Colodner[4], A. Koren[5], C. Levin[3,*]

[1]Endocrine Laboratory, [2]Ward A, [3]Pediatric Hematology Unit, [4]Yetzhak Rabin 20, 5Hematology Unit, Emek Medical Center, Afula, Israel

Background: Patients with thalassemia major often present with endocrine abnormalities due to dysfunction of the hypothalamic–pituitary axis (Poggi, 2016), and are frequently underweight with abnormally low body-fat percentage (Fung, 2010). Leptin plays a regulatory role in immunity and inflammation, and also seems to act synergistically with erythropoietin on mammalian hematopoiesis (Bennett, 1996). For example, a stimulatory effect of leptin on erythropoiesis among patients with end-stage renal disease was observed (Axelsson 2005). Previous studies on leptin in BT patients have shown lower levels than in healthy controls, and a negative correlation with the level of soluble transferrin receptor in transfused patients (Dedoussis, 2002). The reduced leptin levels were explained by a possible toxic effect of iron on adipocytes (Chaliasos, 2010).
Aims: In this study, we investigated the correlation between leptin level and anthropometric parameters in BT patients compared to heathy controls. We also explored the relationship between leptin and hematological and erythropoietic parameters, as well as iron-overload status in BT patients.
Methods: Transfusion-dependent BT patients (n = 33; 16 females, 17 males, mean age 23.5±8.6 [range 8–41] years), treated at the Pediatric Hematology Unit of Emek Medical Center, and 11 healthy controls (6 females, 5 males, mean age 26.4±10.4 [range 8–39] years) were studied. Patients were treated by regular blood transfusions and iron-chelation therapy. Anthropometric assessments included height, weight, fat percentage and BMI calculation. Blood samples were obtained at fasting and before blood transfusion. Serum leptin, complete blood count, hemoglobin and reticulocyte counts and serum erythropoietin were analyzed. These studies were performed twice, 3 months apart, and the mean values were utilized for the statistical analysis. Serum leptin levels were analyzed by radioimmunoassay, and leptin-normalized values were calculated (ng/dL leptin per% body fat).
Results: BT patients were found to have lower leptin levels than healthy controls (5.4±5.9 vs. 13±10.1 ng/dL; p=0.0006). Leptin levels were higher in females than in males (mean leptin 7.2±6.5 vs. 3.7±4.7 ng/dL; p=0.01), and increased with age (r = 0.4635, p=0.0066). A negative correlation was found between leptin and erythropoietin (r = -0.43473, p=0.0115), and a positive correlation between leptin and hemoglobin, as well as the mean pre-transfusion hemoglobin levels in the previous 5 years (r = 0.66884, p<0.001; r = 0.40261, p=0.0202, respectively). No correlation was observed between leptin levels and anthropometric parameters (weight, height, BMI), iron-overload parameters or reticulocyte count. Fasting and normalized leptin showed similar patterns.
Summary/Conclusion: This study confirms that BT patients are unable to maintain adequate leptin production, suggesting that adipose tissue dysfunction may be related to the chronic anemia. Our results correlate well with previous studies of lower leptin levels in BT patients. The positive correlation of leptin level with hemoglobin, together with the inverse correlation with erythropoietin provide further evidence of the effect of leptin on erythropoiesis. Additional studies are needed to examine the intricate interplay between adipose tissue, leptin and erythropoiesis in the environment of chronic anemia and iron overload present in BT patients.

PS1522

MUTATION SPECTRUM IN HBB, HBA1 AND HBA2 GENES IN PATIENTS FROM EUROPEAN PART OF RUSSIA

S. Mann[1,*], T. Varlamova[1], E. Raykina[1], E. Kazanets[2], E. Litvin[2], N. Smetanina[2]

[1]Laboratory of Molecular Biology, [2]Dmitry Rogachev National Research Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation

Background: Haemoglobinopathias as thalassaemia and abnormal haemoglobins are rare disorders for European part of Russia. Due to migration process from the North Caucasus and the other endemic regions of the post soviet republics the frequency of pediatric patients with haemoglobinopathias in European part of Russia has increased significantly.
Aims: The evaluation of mutation spectrum in HBB, HBA1 and HBA2 genes in patients from European part of Russia.
Methods: Allele-specific PCR was used for evaluation of the main α-thalassemia deletions (Y.T.Liu *et al.*, 2000), and Sanger sequencing - for detection of point mutations in exon 3 and polyA region of HBA1 or HBA2 genes and entire HBB gene.
Results: Totally 448 patients were tested during the 3-year period (2015-2017). Among 448 patients in 248 (55%) were found 280 causative genetic variants.β^0-thalassemia was the most frequent form of the disorder (60,7%), with CD8 -AA, IVS2-1 G>A, CD8/9 +G, IVS1-1 G>A, CD39 C>T, CD15 G>A, CD44 -C, CD41/42 -CTTT, IVS1-130 G>C, IVS1-2 T>C and few cases of CD36/37 -T, CD16 -C, CD5 -CT, CD37 G>A variants. β^+-thalassemia was less common (10%) (IVS1-110 G>A, IVS2-745 C>G, IVS2-654 C>T, CD26 G>A(HbE), IVS1-5 G>T and single cases of -30 T>A, IVS2-5 G>C , IVS1-128 T>G, IVS2-848 C>A variants). β^{++}- Thal was the most infrequent (1,4%), with only two variants found: -101 C>T promoter region, IVS1-6 T>C. Abnormal Hbs (7.8%) we found were Hb S (n=7), Hb Cheverly (n=3), Hb Southempton (n=2), Hb D-Los-Angeles (n=2), Hb Tacoma (n=3), single cases of Hb Louisville, Hb Tubingen, Hb Monroe, Hb Koln, Hb Mizuho. We identified two novel mutations in HBB gene: - 108 C>T in heterosygous state, leading to β^+-thal phenotype and CD70 G>A, in heterosygous state, leading to β^0–thal phenotype. For α-globin genes 56 genetic variants (20%) were revealed among all tested patients, most frequently were 3,7kb deletion (60.7%) and 20,5kb deletion (30%), and few cases of 4,2kb deletion, HBA2 PolyA (A>G) AATAAA>AATAAG and Hb Constant Spring.
Summary/Conclusion: The most prevalent HBB gene variants for European part of Russia can be divided in two groups: typical Mediterranean (CD8 -AA, IVS2-1 G>A, IVS1-1 G>A, CD39 C>T, IVS1-1 G>A, CD44-C, IVS2-745 C>G) and Chinese, Japan, Indian (CD8/9 +G, CD15 G>A, IVS2-654 C>T, CD 41/42-CTTT) variants. Novel mutations in HBB gene were found in patients Russian nationality. Among all β–thal variants the most common was CD8 -AA (33,6%), for α-thal variants the most common was deletion

3,7kb (60,7%) and 20,5kb (30%). Among abnormal Hbs the most frequent was HbS (28%).

PS1523

COMPREHENSIVE SCREENING FOR COEXISTING HETEROZYGOUS A0-THALASSEMIA IN HEMOGLOBIN E TRAIT

P. Wongprachar[1,*], P. Sutcharitchan[1], A. Suksusut[1], R. Setthaphiboon[1], N. Uaprasert[1], P. Rojnuckarin[1], C. Polprasert[1]

[1]Medicine, King Chulalongkorn Memorial Hospital, Bangkok, Thailand

Background: Detection of coexisting heterozygous α^0-thalassemia with hemoglobin E trait (HbE trait) is important to identify at-risk couples for Bart's hydrops fetalis. A very sensitive screening test is helpful to exclude cases that do not require DNA testing. A previous study showed that both low HbE levels by HPLC and low MCV were associated with α^0-thalassemia with 100% sensitivity. However, the data in patients with HbE ≥ 25% but MCV ≤ 75 fL are lacking. In this study, we aim to determine if we can omit DNA testing in this subgroup

Aims: To detect coexisting heterozygous α^0-thalassemia with hemoglobin E trait.

Methods: We collected 390 blood specimens from patients who were diagnosed as HbE trait by HPLC and/or IEF in King Chulalongkorn Memorial Hospital. The α^0-thalassemia ($--^{SEA}$, $--^{Thai}$, $--^{Philippine}$, $--^{Mediterranean}$ and $--^{20.5Kb}$ deletion) and α^+-thalassemia determinants (3.7 and 4.2 Kb deletion) were detected by multiplex gap polymerase chain reaction. The cut-off HbE level by HPLC from the previous study was validated (N = 274). The cut-off HbE level by HPLC was determined (N = 152) and then validated in the other set of patients (N=141). Finally, cases with HbE above the cut-off points by either techniques but MCV ≤75 fL were analyzed for the co-existing α-thalassemia (N=278).

Results: From 390 blood specimens, we found co-existing heterozygous α^0-thalassemia in HbE trait of 12.56% (12.31% in $-^{SEA}$ and 0.25% in $-^{Thai}$ deletion). Prevalence of α-thalassemia-thal-2 in HbE trait is 17.94% (15.38% in heterozygous 3.7 kb deletion, 1.53% in heterozygous 4.2 kb deletion and 1.02% in homozygous 3.7 kb deletion). Detection of HbE level by HPLC technique was done in 274 samples, by IEF technique in 290 samples and by both techniques in 174 samples. In specimen with HbE above the cut-off level (HbE levels 25-40% by HPLC technique), we could not detect co-inheritance of heterozygous α^0-thalassemia with low MCV (range 65-80 fL; 95%CI[0-1.5]). Upon further analysis, we found a suitable cut-off value for detection of co-inherited of heterozygous α^0-thalassemia in HbE trait are HbE level of < 21% by HPLC technique with 100% sensitivity, 91.2% specificity, 70.6% positive predictive value (PPV) and 100% negative predictive value (NPV). In IEF technique, HbE level of < 37.3% can achieve 100% sensitivity with 67% specificity, 29.8% PPV and NPV 100% to detect co-inherited of heterozygous α^0-thalassemia (Figure 1).

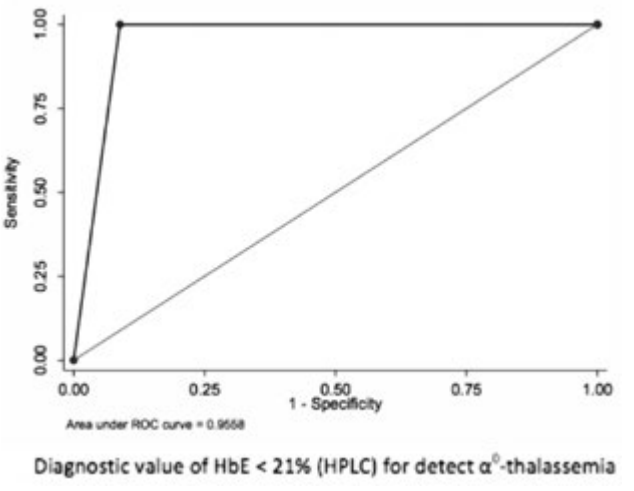


Figure 1.

Summary/Conclusion: In this study, HbE ≥25% by HPLC technique could be used for excluding co-inheritance of heterozygous α^0-thalassemia and Hb E trait despite of low MCV. For IEF technique, level of HbE >37.3% showed 100% sensitivity and 100% NPV in order to exclude heterozygous α^0-thalassemia.

Thrombosis and vascular biology & translational research

PS1524

INVOLVEMENT OF LEUCOCYTE DERIVED EXTRACELLULAR VESICLES IN THE PROCOAGULANT STATE FOUND IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA-RELATED CONDITION

A. Wannez[1,2,*], C. Bouvy[2], B. Devalet[1], H. Jacqmin[1], H. Haguet[1], B. Chatelain[1], C. Chatelain[2], J.-M. Dogné[2], F. Mullier[1]

[1]CHU UCL Namur, Université catholique de Louvain, Yvoir, [2]Pharmacy, University of Namur, Namur, Belgium

Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a disease characterized by the lack of complement regulatory proteins (CD55 and CD59) at the surface of hematological cells leading to complement-mediated hemolysis (Brodsky *et al.* Hematology, 2008). Complement can induce the production of extracellular vesicles (EVs) (Burnouf *et al.* Transfus Apher Sci, 2015). These EVs are cell-derived vesicles whose the size-range is around 50 and 1000nm. They can expose phosphatidylserine (PS-an anionic phospholipid) and tissue factor (TF), which explains their involvement in the coagulation cascade (Owens *et al.* Circ Res, 2011). Nowadays, the relevance of the EVs role in the pathophysiology of thrombosis in PNH is not fully understood even if there is some evidence about the contribution of EVs in the thromboembolic events.
Aims: This research aims to study the procoagulant activity of EVs potentially released during the complement attack by the cells who do not expose the glycophosphatidylinositol anchored proteins (PNH phenotype). The purpose is to better understand the thrombosis occurrence in PNH.
Methods: A cellular model of PNH was set up by removing glycophosphatidylinositol anchored proteins (GPI-AP) at the surface of endothelial cells and leucocytes with an enzyme in order to obtain PNH-like cells. Thereafter, these PNH-like cells and red blood cells (RBCs) derived from PNH patients were exposed to acidified serum to trigger the complement attack via the alternative pathway. Finally, thrombin generation assay with calibrated automated thrombogram was performed to study the procoagulant activity of EVs released by each cell type independently from other factors.
Results: This cell model was validated for all cell types. Indeed, endothelial cells and leucocytes treated with enzyme have a decrease in GPI-AP expression, which allows to obtain PNH-like cells. Thereafter, the complement attack has been checked with a calcein AM retention test for endothelial cells and leucocytes and by percentage of hemolysis measurement for the RBCs. These two experiments allowed to validate the complement attack. For the procoagulant activity, the results show that the EVs potentially released by PNH-like leucocytes have an increased procoagulant profile in comparison to healthy leucocytes (lag time for PNH-like leucocytes of 4.11 VS 6.94 for the healthy leucocytes, TTPeak of 7.22 VS 10.89, Peak of 1342 VS 1202 and ETP of 155.8 VS 99.78 $p<0.05$). Conversely, no difference in terms of procoagulant activity was observed between EVs isolated from GPI-AP positive and negative endothelial cells and between those from PNH RBCs and healthy RBCs.
Summary/Conclusion: This work enables to validate a model of PNH and to highlight a contribution of leucocyte-derived EVs in the hypercoagulable profile of PNH patients. Moreover, this PNH cellular model can be used to study other factors involved in this orphan disease, but also to test new treatment strategies.

PS1525

ARE ALL DIRECT ORAL ANTICOAGULANTS EQUAL TO SAFETY AND EFFICACY?

B. Navarro Almenzar[1,*], J.J. Cerezo Manchado[1], F. García Candel[1], V. Cabañas Perianes[1], M. Blanquer Blanquer[2], R. Pérez López[1], E. Fernández Poveda[1], A. Martínez Marín[1], M. Moya Arnao[1], F. Labbadia[2], M. Berenguer Piqueras[1], G. Elvira Ruiz[3], S. Manzano Fernández[3], C.S. Caro Martínez[4], J.M. Moraleda Jiménez[1]

[1]Hematology, Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, [2]Hematology, Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, [3]Cardiology, Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, [4]Cardiology, Hospital Vega Baja, Orihuela, Alicante, Spain

Background: Atrial fibrillation is the most common arrhythmia in our environment and the main cause of anticoagulation. Clinical trials and real-life records have shown that direct oral anticoagulants are at least as effective as non vitamin K oral anticoaulants in the prevention of thromboembolic stroke, becoming even safer. However, given their recent appearance, there is not a wide experience about theis use in real life, especially in the long term. Although safety and effectiveness have been well demonstrated in the context of clinical trials, there may be important differences in the selection, treatment and management of patients in actual clinical practice with respect to clinical trials.
Aims: To assess the clinical characteristics of patients with non-valvular atrial fibrillation (NVAF) that start direct oral anticoagulants (ACOD) and compare the effectiveness and safety of them in our environment.
Methods: From January 2013 to December 2014, patients with NVAF who started a direct-acting oral anticoagulant (Rivaroxaban, Apixaban and Dabigatran) for the first time were included. During the follow-up (646 [470-839] days), thromboembolic, hemorrhagic complications and mortality were recorded by reviewing electronic medical records and telephone contact (99.8% of patients).
Results: We recruited a total of 973 consecutive patients in three centres. We observed that patients who received Apixaban presented a higher frequency of chronic kidney disease, major global or digestive hemorrhage; and higher scores on thromboembolic and hemorrhagic risk scales. On the other hand, patients who received Dabigatran were the youngest and with the best renal function. The rate of thromboembolic events was higher in the Apixaban group (2.92/100 person-years) compared to Dabigatran and Rivaroxaban (1.91 and 1.53/100 person-years, $p<0.01$). Patients who showed lower rates of major bleeding were those treated with Dabigatran (1.92/100 person-years versus 2.90 / 100 person-years in Apixaban and 3.01/100 person-years in Rivaroxaban, $p<0,01$). Unadjusted mortality in the Apixaban group was 8.04/100 person-years, mainly at the expense of non-cardiovascular mortality, followed by those who received Dabigatran (5.94/100 person-years) and Rivaroxaban (4.96/100 people-year) ($p <0.01$). However, in multivariate Cox regression analysis, none of the ACODs were independent predictors of clinical events ($p>0.05$).
Summary/Conclusion: The prescription of direct anticoagulants differs according to the baseline clinical characteristics. In this way, elderly patients with comorbidities and, therefore, higher thromboembolic and hemorrhagic risk, receive Apixaban, while Dabigatran is prescribed in the youngest and with better renal function. In our population there were no differences in the effectiveness and safety of the different direct anticoagulants.

PS1526

INCREASED PLATELET RESPONSE TO ENDOGENOUS AGONISTS, IN PRE-DIABETIC C57BL/6 MICE

Z. Mkandla[1,*], B. Nkambule[1], P. Dludla[2]

[1]Human Physiology, University of KwaZulu-Natal, Durban, [2]Biomedical Research and Innovation Platform, South African Medical Research Council, Cape Town, South Africa

Background: Hyperinsulinemia, dyslipidaemia, hypertension and obesity are risk factors for the development of type 2 diabetes mellitus (T2DM). Obesity is associated with low-grade chronic inflammation characterized by pro-inflammatory signaling and abnormal adipokine production. Accelerated atherothrombotic conditions remain the main underlying factor contributing to the increased risk of atherosclerosis in diabetic patients. Activated platelets play a crucial role in the development of hypercoagulable states in type 2 diabetes.
Aims: To investigate platelet function and reactivity using a diet-induced pre-diabetes animal model.
Methods: A total of 18 male C57BL/6 mice were randomised into two diet groups; a 10 kcal% fat control diet group (diet code: D12450J) (n=5) and a 60 kcal% fat diet group (diet code: D12492) (n=13). The animals were kept on the diets for 3 weeks. Whole blood was collected from the lateral tail vein into 3.2% sodium citrate coated tubes. In order to assess platelet reactivity, twenty-five microliters (25ul) of citrated blood was stimulated with various endogenous agonists which included; 4µM and 20µM adenosine diphosphate (ADP), 0.19mg/mL collagen (COL) or 500µg/mL arachidonic acid (AA). These were then stained with 5ul of antibody (Ab) cocktail containing CD41-FITC (platelet marker), CD36-PE (platelet aggregation) and CD62P (platelet activation). Data acquisition was performed using the BD FACS Canto II flow cytometer within 1 hour of blood collection.
Results: At baseline, the levels of activated platelets were comparable between the two study groups p=0,5569. After stimulation with 4µM of ADP, the surface expression of CD62P was increased in the HFD group

compared to the control group;%CD62P control 0,91 [0,47-1,0] vs HFD 4,360[2,940-5,430], p=0,0028 and 20µM ADP%CD62P control 5,195[2,568-6,443] vs HFD%CD62P 9,880[8,270-11,23], p=0,0039. The control group showed increased levels of platelet expressing surface CD62P after stimulation with AA agonists compared to unstimulated controls,%CD62P 65,96[58,69-85,79] vs unstimulated%CD62P 6,430[3,883-9,863]; p=0,0286. In the HFD group, levels of platelet activation were increased after stimulation with 20µM ADP compared to 4µM ADP post stimulation. 4µM ADP%CD62P 4,370[2,940-8,005] vs 20µM ADP%CD62P 9,880[8,270-11,23], p<0,0001. In addition, the HFD group showed increased expression of CD36 compared to the control group after stimulation with AA; control group%CD36 44,02[41,40-57,44] vs HFD group%CD36 93,95[87,10-97,03], p=0,0039. The platelets showed biphasic response to stimulation with 20µM ADP compared to the 4µM ADP; 20µM ADP%CD36 1,460[1,355 -1,620] vs 4µM ADP%CD36 0,6500[0,4950 -1,000], p<0,0001. The platelet reactivity index (evaluated using CD36 expression), was increased in the HFD group compared to the control group after stimulation with 20 µM ADP; control group%CD36 median 12,13[8,337-18,07] vs HFD group%CD36 median 20,89[14,46-24,01], p=0,0362.

Summary/Conclusion: Platelets from pre-diabetic mice are hyper-responsive to endogenous agonists. This may indicate the hyperreactive nature of platelets in prediabetic individuals and an increased risk of CVD. We were able to demonstrate that all three major activation pathways; cyclooxygenase pathway, P_2Y_{12} receptor pathway and glycoprotein IV pathway, are hyperactivated in pre-diabetes. This may prompt the need for therapeutic strategies targeting these specific pathways in pre-diabetic individuals.

PS1527

ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA AND ACUTE PANCREATITIS: VARIABLE AND RECIPROCAL PATHOPHYSIOLOGIC RELATIONSHIPS

Q. Qin[1,*], X. Nan[1,2], C. Gentille Sanchez[1], L. Rice[34]

[1]Internal Medicine, Houston Methodist Hospital, Houston, [2]Hematology Oncology, University of California San Diego, San Diego, [3]Hematology, Houston Methodist Hospital, [4]Hematology, Weill Cornell Medical College, Houston, United States

Background: Acquired thrombotic thrombocytopenic purpura (TTP) is an aggressive thrombotic microangiopathy. Central to the pathophysiology is the loss of ADAMTS13 activity, due to inhibitors in the acquired form, allowing circulating ulVWF multimers to activate platelets and produce organ damage from microvascular thromboses. Autopsies uncover frequent pancreatic involvement, but clinically evident acute pancreatitis (AP) is uncommon. The relationship between TTP and AP may be variable and complex, with either capable of precipitating, exacerbating or mimicking the other.

Aims: In this observational, retrospective study, we review cases of TTP with AP (TTP-AP) and compare their clinical characteristics to those of TTP patients with severe ADAMTS13 deficiency (level <10%) but no clinical AP to highlight the pathophysiology associated with this unique organ.

Methods: We queried the Methodist Environmental for Translational Enhancement and Outcomes Research, a system that integrates all Houston Methodist Hospital patient data into a single, comprehensive data warehouse. Of the 149 identified encounters for TTP, 12 had TTP-AP and 41 had severe ADAMTS13 deficient TTP without AP. The rest of the encounters were excluded for no clinical pancreatitis or unknown/higher than 10% ADAMTS13 levels. In the TTP-AP group, the 7 cases where ADAMTS13 level was measured prior to plasma exchange (PEX) treatment also showed severe deficiency. Baseline demographics, variables associated with TTP, and complications were compared. Statistical analysis was performed using X2 test for dichotomous variables and the two-tailed Mann Whitney U test for the continuous parameters. Significance was defined as a p-value <0.05.

Results: In the TTP-AP group, 8 patients presented with concurrent TTP and AP with the other 4 having likely TTP induced AP with a time to AP between 3 to 28 days. The TTP-AP group had statistically higher initial median LDH (4610 U/L versus 1900 U/L, p=0.0038), gastrointestinal symptoms (92% versus 49%, p=0.0080), and renal involvement (81% versus 17%, p=0.0008, creatinine elevation at 3.3 mg/dL versus 1.0 mg/dL, p=0.0035) when compared to those in the TTP without AP group. No statistical differences were observed between the initial hemoglobin level and platelet counts. Statistical differences were also not observed for neurological and cardiac involvements, as well as percentage of refractory TTP, median PEX sessions, and overall mortality, although there was a trend toward higher incidence in the TTP-AP population. (Table 1).

Summary/Conclusion: We compared presenting features and clinical outcomes of 12 patients with TTP-AP to those of 41 patients with severe ADAMTS13 deficient TTP but no AP. Our results suggest a possible correlation between pancreatic involvement and more severe clinical outcomes. Pancreatic cytokine release observed in other studies may have contributed to ADAMTS13 depletion and the phenotypic severity (*Reiter et al.*, 2003; *Bernardo et al.*, 2004; *Mannucci et al.*, 2004; *Swisher et al.*, 2007; *Morioka et al.*, 2008). A review of literature also revealed the role of pancreatitis in nitric oxide depletion and complement activation, which may augment micro-thrombi formation and fuel TTP propagation. Whereas the relationship between TTP and other organ systems may be one of cause and effect, the relationship between AP and TTP may be one of reciprocal fueling. Early appreciation and treatment of pancreatitis might significantly decrease the severity of TTP and improve patient outcomes.

Table 1.

Clinical Features of Patients with TTP and Pancreatitis versus Those with Severe ADAMTS13 Deficiency but No Pancreatic Involvement

	TTP pancreatitis (n=12)	Severe TTP (n=41)*	P value (<0.05)
ADAMTS13 level <10%	7^	41	
Initial Hgb (Median)	9.6	8.7	0.45930
Hgb<7	2 (17%)	7 (17%)	0.97369
Initial platelet (median)	22.5	13	0.15272
Plt<=10	1 (8%)	12 (29%)	0.13921
Initial LDH (median)	4610	1900	**0.00350**
Creatinine (median)	3.3	1	**0.00386**
Neurological**, All	10 (83%)	31 (76%)	0.57392
Major	8 (67%)	18 (44%)	0.16532
Minor	2 (16%)	13(32%)	0.30901
Cardiac (acute coronary syndrome)	6 (50%)	14 (34%)	0.31901
GI (nausea, vomiting, abdominal pain, and/or ischemic colitis)	11 (92%)	20 (49%)	**0.00801**
Renal, All	9 (81%)	11 (27%)	**0.00087**
AKI	5 (42%)	9 (22%)	0.17304
ARF requiring HD^	4 (36%)	2 (5%)	**0.00621**
PEX (median)	13.5	9.5	0.12356
Refractory**	8 (67%)	19 (46%)	0.21544
Mortality	2 (17%)	2 (5%)	0.17391

Hgb: hemoglobin (k/µL); Plt: platelet count (k/µL); Cr: creatinine (mg/dL); LDH: lactate dehydrogenase (U/L); GI: gastrointestinal, AKI: acute kidney injury; ARF: acute renal failure; HD: hemodialysis; PEX: plasma exchange. *=number of patient encounters, ^all patients with ADAMTS13 measurements were <5%, others were not measured or inaccurate measurements post PEX. **major neurological symptoms are defined as coma, stroke, seizure, or transient focal abnormalities and minor neurological symptoms are defined as headache or confusion ^does not include the patient who had pre-existing ESRD; **defined as failure of platelet to double after four days of PEX, new neurological symptoms on PEX, or exacerbation of clinical or laboratory findings during or within 30 days of stopping PEX

PS1528

EVALUATION OF LUPUS ANTICOAGULANT TESTING: A THREE YEAR EXPERIENCE

S.F. Pinzon[1,*], M. Villalba Montaner[1], A.J. Garcia[1], J.M. Calvo Villas[1], N. Fernandez Mosteirin[1], E. Salvador Ruperez[1]

[1]Hematology, Hospital Universitario Miguel Servet, zaragoza, Spain

Background: The diagnosis of antiphospholipid syndrome (APS) requires, besides the clinical criteria, the demonstration of the presence of antiphospholipid antibodies which is constituted by cardiolipin, β -glycoprotein and lupus anticoagulant (LA) which is one of the three laboratory criteria to be persistently present. According to current guidelines it is sufficient to have one of the three laboratory criteria to classify a patient with thrombosis or pregnancy morbity as APS. Based upon consensus criteria from the International Society for Thrombosis and Haemostasis (ISTH), confirmation of LA requires that the following criteria are met; performing at last two phospholipid dependent clotting test, prolongation of at least one, evidence for inhibitory activity and the demonstration of phospholid dependence of the inhibitor on a confirmatory test.

Aims: The aim of our study was to evaluate retrospectively the frequency

testing of LA in our institution and the incidence of positive results and correct laboratory confirmation of them that lead to a diagnosis.
Methods: It was performed a cross-sectional study from the request for LA received over a period of three years, between 2015 and 2017. We examined which departments were requesting the test, the kind of results and the confirmation of the LA positive tests.
Results: From a total of 6344 of LA tests, 5837 (92%) were negative, 507 (8%) of the test demonstrated a positive LA. From these samples, 109 cases show a positive β -glycoprotein, 108 cardiolipin autoantibodies and 98 show both positive. After applying the confirmation tests criteria suggested by ISTH to all of the LA positive tests, 23 patients had a diagnosis of APS. The clinical specialities requesting the majority of tests were Internal Medicine (2075), Haematology (1158) and Neurology (606).
Summary/Conclusion: The results show a high frequency of LA requesting with a low of positive results (8%) and percentage of cases diagnosis for APS (0,36%), all the positive results was correctly confirmed. We consider the LA test is overclaimed leading to low diagnostic yield. Despite of using an adequate study protocol of the LA positive tests in our Laboratory based on ISTH recommendations, it is necessary to promote a better and more rational use of requesting LA test by clinical criteria, nevertheless, there is an adequate follow-up of international recommendations at the positive tests.

PS1529

THE INFLUENCE OF PGP AND PGR PEPTIDES ON ANTICOAGULANT CAPACITY AND ANTITHROMBOTIC POTENTIAL OF BLOOD IN HEMOSTATIC DYSFUNCTION OF AGING RATS

T. Obergan[1,*], T. Shubina[1]

[1]Lomonosov Moscow State University, Moscow, Russian Federation

Background: Clinical data and results of experimental studies indicate that age-related changes of hemostatic system are accompanied by dysfunction of the coagulation and anticlotting processes. This is manifested in the raising of platelet aggregation, increased the activity of coagulation factors and inhibition of fibrinolytic system due to the limitation of the appearance in the blood of enzymatic fibrinolysis activators. In these conditions, the risk of thrombosis and thrombotic complications increases. It is known that short peptides, which are composed of amino acids proline and glycine, are involved in hemostatic processes, inhibiting blood coagulation and impeding the formation of blood clots. Previously, it was shown that tripeptide Pro-Gly-Arg-treatment in animals with experimental metabolic syndrome accompanied by hemostatic dysfunction increases the antiplatelet and fibrinolytic properties of blood, impeding the thrombus formation.
Aims: The aim of this study was to demonstrate the anticoagulant and antithrombotic effects of Pro-Gly-Pro (PGP) and Pro-Gly-Arg (PGR) peptides in conditions of hemostasis dysfunction in aging.
Methods: Male albino rats of Wistar strain 3 («young») and 15 months of age («old») were used in the study in accordance with ethical principles of the Helsinki Declaration. Animals were divided into four groups (in each group $n = 10$) and were given following treatments: Group 1 – Control (3-month young rats were given 0.85% saline as vehicle for peptides); Group 2 – Untreated rats (15-month aging rats were given 0.85% saline); Group 3 – PGP-treated rats (15-month aging rats were treated with PGP in dose 1 mg/kg body weight); Group 4 – PGR-treated rats (15-month aging rats were treated with PGR in dose 1 mg/kg body weight). The peptides or saline were injected by intranasal way for 20 µl per rat once daily for 5 days. Blood samples were obtained 1 h after the last administration of drugs. ADF-induced platelet aggregation, enzymatic fibrinolytic activity (non-stabilized fibrin plate method), activity of tissue plasminogen activator (stabilized fibrin plate method) and anticoagulant activity (APTT test) were determined in blood plasma.
Results: In this study, aging rats were found to have decreased anticoagulant and fibrinolytic activity of blood plasma, as well as increased platelet aggregation, i.e. confirmed the fact of dysfunction of the hemostatic system. Thus, the formation time of the clot (APTT) in old rats (Group 2) accelerated by 25%, the enzymatic fibrinolytic activity of plasma (EFA) and the activity of the tissue plasminogen activator (t-PA) decreased by 35% in both cases, platelet aggregation increased by 45% compared to Control (young rats). Intranasal 5-fold treatment of animals with peptides PGP and PGR led to a significant increase in APTT by 30% and 24%, respectively, against untreated old animals (Group 2). This was accompanied by an enhancement in EFA by 36% (PGP-treated) and 40% (PGR-treated) due to rising t-PA activity by 2.3 and 3 times, respectively, compared to Group 2. Herewith, PGR-treatment caused a decrease in platelet aggregation by 24%, while PGP-treatment did not lead to a significant change in this parameter.
Summary/Conclusion: The study has shown a protective role of PGP and PGR peptides in their application during aging. Under these conditions, treatment with peptides contributes to the normalization of the hemostatic function, thereby reducing the possibility of thrombotic complications.

PS1530

APTT-BASED CLOT WAVEFORM ANALYSIS IN VARIOUS INFECTIONS

C.K. L. Ng[1,*], C.W. Tan[1], H.H.M. Cheen[2], W.H. Wong[1], M.H.Y. Chu[1], S.S. Lim[1], G.D.D. Yeo[3], G. Morvil[3], H.J. Ng[1]

[1]Haematology, [2]Pharmacy, Singapore General Hospital, [3]Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

Background: Infections cause varying degrees of haemostatic dysfunction. Clot waveform analysis (CWA), as a global haemostatic marker, might detect such changes. Biphasic waveform on CWA observed in sepsis has previously been demonstrated to precede disseminated intravascular coagulopathy (DIC) and to predict poor clinical outcomes. The effect of infection, in the absence of biphasic waveform and DIC, on CWA has not been established.
Aims: To evaluate the influences of different infections on CWA.
Methods: Data from patients admitted for infections and with an activated partial thromboplastin time (aPTT) were retrospectively analyzed. APTT-based CWA was performed on CS2100i automated analyser using Dade Actin FSL reagent. The CWA parameters were maximum velocity (min1), maximum acceleration (min2) and maximum deceleration (max2). Results from control patients were compared. Subjects who were on anticoagulant, with active thrombosis and cancer were excluded.
Results: The means aPTT (±SD) for dengue (n=36), bacterial (n=52), respiratory viral infection (n=13) and controls (n=112) were 37.99±7.93s, 33.96±7.33s, 29.98±3.92s and 27.80±1.59s, respectively without biphasic waveform. The means of all the CWA parameters (min1; min2; max2) were highest in bacterial infection, followed by respiratory viral infection, controls and dengue infection (Table 1). Multivariate analysis confirmed that bacterial and dengue infection remained as independent predictor for higher and lower CWA parameters, respectively (Table 2). This CWA changes were independent of their underlying aPTT result (Figure 1).

Table 1. Comparison of clot waveform parameters between infected and control blood samples.

					p-value (p-value after adjustment*)		
Mean CWA Parameter	Bacterial infection (n= 52)	Dengue infection (n= 36)	Other viral infection (n= 13)	Control (n= 112)	Bacterial vs. Control	Dengue vs. Control	Other virus vs. Control
Min1 (SD)	6.92 (1.60)	3.93 (1.02)	6.19 (1.32)	5.53 (1.16)	<0.001 (<0.001)	<0.001 (<0.001)	NS (NS)
Min2 (SD)	1.04 (0.28)	0.57 (0.17)	0.95 (0.21)	0.93 (0.46)	<0.001 (<0.001)	<0.001 (<0.001)	NS (NS)
Max2 (SD)	0.82 (0.24)	0.43 (0.14)	0.73 (0.18)	0.74 (0.16)	0.036 (0.030)	<0.001 (<0.001)	NS (NS)

SD – standard deviation; NS – Non significant ($p > 0.05$). *Adjusted for age, gender and ethnicity.

Table 2. Multivariate analysis of the subjects' variables influencing their CWA parameters.

Factor	Min1		Min2		Max2	
	b	p-value	b	p-value	b	p-value
Age	0.012	NS	0.003	0.043	0.002	0.029
Gender (Reference group: Female)	-0.258	NS	-0.048	NS	-0.045	NS
Ethnicity (Reference group: Non-Chinese)	-0.205	NS	-0.045	NS	-0.037	NS
Type of infection (Reference group: Control)						
Bacterial	1.453	<0.001	0.166	0.047	0.096	0.002
Dengue	-1.395	<0.001	-0.280	<0.001	-0.269	<0.001
Viral	0.443	NS	0.013	NS	-0.049	NS

Summary/Conclusion: Different types of infection modified CWA distinctively. CWA could provide information on the haemostatic milieu

triggered by infection beyond what the routine clotting time could offer and could be further evaluated as diagnostic and prognostic markers for infections.

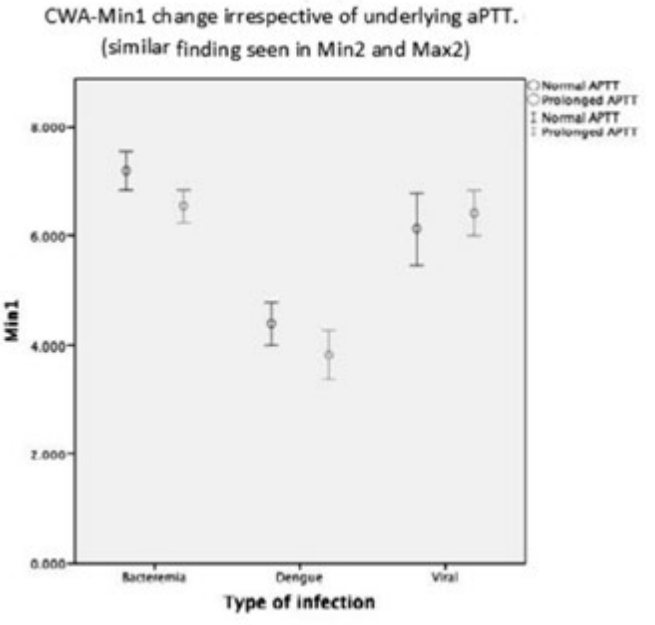


Figure 1.

PS1531

ANTITHROMBOTIC EFFECTS OF COMPLEX PREPARATIONS OF VEGETABLE HEPARINOID WITH LEUCINE

M. Lyapina[1,*], M. Kalugina[1]

[1]Moscow Lomonosov State University, Biological faculty, Moscow, Russian Federation

Background: In early studies, it was shown that complex preparations of high-molecular heparin(HMH) with amino acids (including leucine) have antiplatelet and anticoagulant action *in vitro* and with intravenous administration in animals [Pastorova *et al*., 1999]. The complex drugs geparinoida from the roots of Paeonia lactiflora with arginine showed anticoagulant and antiplatelet effect when administered orally to animals. In the heparinoid from the roots of this peony (Paeonia lactiflora), there were two fractions of heparin - low molecular weight heparin (LMH) by anti-factor Xa activity and HMH by anti-factor IIa activity. The correlation of these anti-IIa/anti-Xa activities averaged 1: 2,03 [Lyapina M. G. *et al*., 2017].
Aims: Creation of a complex preparation of vegetable heparinoid with leucine and testing of its antithrombotic effects in animals at oral administration in conditions thrombosis process.
Methods: A complex preparation of vegetable heparinoid from the roots of Paeonia lactiflora with leucine was obtained at a molar ratio of 1: 4, which was orally administered to an experimental group of rats at the dose of 1 mg/kg of body weight 1 h before the thrombosis process, caused by intravenous administration of threshold doses of thrombin (15 NIH) against the background of shutdown of the vegetatic nervous system by 1% aminazine (0.02 ml per 200 g of body weight). Aminazine was administered 15 minutes before the introduction of thrombin, which thus had a direct coagulating effect. Blood samples were taken 30 min after injection of thrombin. Control animals instead of the complex were injected physiological NaCl solution and the thrombin solution were also imposed on the background of 1% of chlorpromazine. In blood plasma we determined the anticoagulant activity test of activated partial thromboplastin time (APPT), platelet aggregation according to the method of Born, total fibrinolytic activity (SFA) (method idwell). Animal experiments were conducted in accordance with the ethical principles of the European Convention for the protection of vertebrates (Strasbourg, 15.06.2006).
Results: It was found that the complex preparation of vegetable heparinoid with leucine prevented the processes of thrombosis, as in the experimental group of rats platelet aggregation did not increase and remained at a normal level (100%), anticoagulant activity on the APTT test increased by 23%, SFA increased by 32%. All animals in the experiment after thrombin are alive. Another picture in the control group of rats: platelet aggregation increased by 35%, APTT and SFA decreased by 20-31% respectively. After the introduction of thrombin of from 10 control rat died three
Summary/Conclusion: Integrated drug heparinoid from the roots of Paeonia lactiflora with the amino acid leucine has in the organism of rats under conditions of high provocation of thrombosis antithrombotic effect, as evidenced by increased antiplatelet, anticoagulant and fibrinolytic activity in the plasma of experimental rats. In the control group of animals the introduced thrombin and, on the contrary, there was a suppression of platelet aggregation and a sharp decrease in anticoagulant and fibrinolytic properties of blood plasma.

PS1532

C-TERMINAL FRAGMENTS OF NEUROPEPTIDES CHANGE INTERACTION OF BLOOD CELLS IN THROMBOGENESIS

M. Golubeva[1,*]

[1]Biological Fac. Lab.of blood coagulation, Moscow State University, Moscow, Russian Federation

Background: In the process of thrombosis during therapy with antiplatelet agents, it should be borne in mind that red blood cells (RBC) can significantly increase platelet aggregation, thereby reducing the effectiveness of antiplatelet agents. The search for new drugs with a wide range of effects on the hemostatic system with minimal side effects made the researchers pay attention to the proteolysis products of neuropeptides, such as vasopressin and oxytocin. A significant effect of these peptides on platelet aggregation induced by ADP, fibrinolysis, tissue activator activity of plasminogen, etc. has been established. However, these studies do not affect all aspects the interaction of blood cells and effect of these peptides on the processes of thrombus formation with adrenaline-mediated aggregation, which was the purpose of this study.
Aims: The aim of this study was to compare the effect of small regulatory peptides, which are fragments of neurohormones, in *in vitro* experiments on the interaction of erythrocytes with platelets when they are aggregated under the influence of adrenaline.
Methods: Experiments were carried out on white mongrel male rats weighing 180-200 g body. We are using peptides representing the C-terminal fragments of vasopressin, oxytocin: - Pro-Arg-Gly-NH2.-Vasopressin fragment, (P1), - Pro-Leu-Gly-NH2 - fragment oxytocin(P2); - Platelet aggregation was measured in by adding epinephrin at a final concentration of 0.02 mmol. in platelet –rich plasma PRP, PRP+эритроциты, wash platelet + erythrocytes by addition P1 , P2 or 0,85% NaCl. Aggregation was recorded on an aggregometer (Moscow State University). The results were treated statistically. All experiments were conducted in accordance with the ethical principles and normative documents recommended by the European Science Foundation (ESF) and the Declaration on Humane Treatment of Animals.
Results: *In vitro*, peptides at a concentration of 10-4 -10-6M were added to either PRP or a mixture of PRP + RBC. It was shown that peptide P1 significantly reduced platelet aggregation (on 15% $p<0.001$). When adding red blood cells to PRP, there was an increase in platelet aggregation (on 17%). Addition of peptide P1 to this mixture caused a decrease in platelet aggregation on 24% ($p<0.001$), the decrease being stronger than in the former case. Peptide P2 was added to the PRP, there was no change in the platelet aggregation. Erythrocytes increased platelet aggregation by 8.5%. Against this background, the addition of the peptide also reduced platelet aggregation by 6.5%, ($p>0.1$), i.e. the aggregation is practically unchanged. When peptides were added to washed platelets and erythrocytes in the presence of calcium ions, P1 reduced platelet aggregation by 33.8%. The addition of erythrocytes to a mixture of erythrocytes and platelets led to an increase in aggregation of the latter by 74%. Against this background, the peptide caused a decrease in aggregation by 21%. P2 caused an increase in platelet aggregation by 52%, and the addition of the peptide to the cell mixture did not change the platelet aggregation.
Summary/Conclusion: C-terminal fragment of vasopressin has a more pronounced antiaggregant effect than the oxytocin fragment. The use of small regulatory peptides can reduce the aggregation effect of red blood cells, thereby improving therapy for thrombotic complications.

PS1533

COMPARATIVE STUDY OF DALTEPARIN SODIUM AND ACETYLSALICYLIC ACID IN PREVENTION OF ANTIPHOSPHOLID SYNDROM ASSOTIATED WITH RECURRENT PREGNANCY MISCARRIAGE

D. Marinitch[1,*]

[1]Consultative, Minsk Consultative Diagnostic Center, Minsk, Belarus

Background: Miscarriage or early pregnancy loss is the termination of pregnancy before 20 weeks' gestation or with a fetal weight of below 500 g. Recurrent pregnancy miscarriage (RPM) can be classified in women who had 2 or more episodes of pregnancy loss.
The presence of antiphospholipid antibodies (antiphospholipid syndrome - APS) has been associated with placental vascular thrombosis and hence placental infarctions and insufficiency which leads to pregnancy loss. Tests for APS, signaling the presence of the autoimmune disease, have reportedly been positive in approx. 20% of women with early pregnancy losses (RPM). Low-molecular weight heparin (LMWH) and acetyl-salicylic acid (ASA) has been recommended for women with APS and RPM as a prevention treatment. But this approach remains empiric regarding terms and dosage.
Aims: We compared anticoagulant (and partially anti-APS) treatment approaches with LMWH/ASA; LMWH alone and ASA in alone in increased live birth in women with consecutive RPM and APS.
Methods: 44 early pregnant women with RPM/APS were investigated and treated in Minsk Consultative and Diagnostic Center during last 3 years. Serologic criteria defined as at least **one** of the following tests found to be positive on 2 occasions within 3 months: anticardiolipin IgG (> 20 phospholipid units); antiglycoprotein (> 20 units); lupus anticoagulant (LAC) was measured as Russell's viper venom time; activated partial thromboplastin time and prothrombin time. The all cohort was divided to 3 groups: 1) 12 patients were given 75 mg daily of ASA; 2) 20 patients were prescribed LMWH (dalteparin sodium 2500 IU daily, subcutaneously) plus 75 mg of ASA and 3) 12 patients were received dalteparin sodium (2500 IU daily, subcutaneously) only. All medications were prescribed from the 2^{nd} – 3^{d} week of pregnancy till six weeks after the delivery.
Results: In the first group the live birth rate was 10 of 12 (83%); the second group showed 100% of safe births and the third one demonstrated 11 of 12 (91,6%) of successful deliveries. Besides, 2 pregnancies cases from the groups I and II were accompanied by moderate uterine bleeding; both episodes were occurred in the second trimester. The cases demanded no medical intervention; they were uncomplicated and ended by successful delivery. It is interesting to note, that in the groups included ASA the titer of antiphospholipid antibodies has incrementally decreased (20 to 40%) by the end of treatment.
Summary/Conclusion: According the results of our pilot investigation, the "golden standard" of RMC prevention seems to be the combination of ASA and LMWH. But both dalteparin sodium alone and aspirin alone also confers substantial benefit. In some circumstances the ASA treatment looks more attractive due to low price of medication. ASA treatment also seems positively influences on decreasing AA-concentration.

PS1534

LABORATORY CRITERIA FOR THE DIAGNOSTIC RELIABILITY OF TESTS FOR DETECTION OF LUPUS ANTICOAGULANT IN PATIENTS WITH SUSPECTED ANTIPHOSPHOLIPID SYNDROME

V. Krasivska[1,*], O. Stasyshyn[2]

[1]Laboratory of immunocytology and genetics of blood tumors, [2]Surgery and Clinical Transfusiology, State institution "Institute of Blood Pathology and Transfusion Medicine under the NAMS of Ukraine", Lviv, Ukraine

Background: Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by recurrent arterial or venous thrombosis and/or recurrent pregnancy morbidities in the presence of persistent positive antiphospholipid antibodies (aPL), which includes anticardiolipin antibodies (aCL), anti-b2 glycoprotein I (anti-b2GPI) and lupus anticoagulant (LA). Laboratory criteria of diagnostic reliability of the tests to detect LA haven't been researched profoundly, which is why the research of their informative value is a topical problem.
Aims: To find the most informative coagulologic tests for the detection of LA in patients with suspected APS by identifying diagnostic laboratory eligibility criteria.
Methods: In 233 patients with suspected APS the presence of LA was examined on the basis of a consistent implementation of a three-step test carried out in accordance with the current guidelines. We used clotting time with PL-dependent diluted viper venom time (DVVT), activated partial thromboplastin time APTT with LA -sensitive reagent (APTT) and prothrombin time with diluted to 50 and 500 times thromboplastin ($PT_{1:50}$, $PT_{1:500}$). Also we have calculated sensitivity (Se), specificity (Sp), accuracy (Ac) and likelihood ratio (LR+, LR -) for each of these tests.
Results: APS was diagnosed in 104 (44,6%) patients. Primary APS was detected in 89 (85,6%) patients, 15 (14,4%) were diagnosed with the secondary APS with the systemic lupus erythematosus alongside and other autoimmune diseases. LA was detected in 99 patients, which is 42,5% of the target group. In the group of patients with APS, LA was detected in 95,2% of cases. On the first stage of diagnostics a considerable Sp >75,0% (χ^2>60,0; <0,001) and LR+ (>10,0) of all PL-dependent coagulation tests indicates a high probability of LA presence if clotting time is prolonged. Ac of the first stage tests does not go <75,0% which indicates a fairly big part of true positive and true negative results of the all the examined patients. However, due to low Se (48,1%>56,6%) it's impossible to narrow the quantity of patients with the APC possibility. On the second stage, (mixing studies) we observed the low Se (33,3% - 44,6%) and high Sp (97,7% - 99,2%) of index of circulating anticoagulant (ICA) in all the tests (χ^2>44,74; <0,001). On the third stage (confirmatory tests) Se for LA ratio of all the tests was 61,5% - 74,4% and Sp LA ratio – 95,4% - 98,4% (χ^2 >87,42; <0,001). Ac of all the tests was >87,8%. The probability of LA and APS presence with the increase of LA ratio >1,15 was high (LR+ 16,9-38,1). With moderate possibility LA absence can be stated due to the negative results of all the tests for confirmation: LR - varying from 0,2 to 0,4.
Summary/Conclusion: None of the tests used to diagnose LA showed 100,0% Se and Sp. PL-dependent coagulation tests of the first stage of diagnostics LA have a high diagnostic reliability due to considerable Sp, Ac and LR+ if the coagulation time is prolonged. Low Se of the first stage tests does not give a considerable certainty of anticoagulant absence if the coagulation time is within the norm. On the second stage, with ICA >15,0%, LA can be suspected with high probability, but the low Se does not allow us to confidently differentiate the deficiency of clotting factor from the presence of pathological blood coagulation inhibitors. Statistically, the highest significant diagnostic efficacy was found in the 3-step tests to confirm the PL-dependent nature of the inhibitor, which can be considered sufficiently reliable for detecting LA activity and diagnosing APS.

PS1535

THE COAGULATION SYSTEM FUNCTIONING IN PATIENTS WITH MYELOFIBROSIS

E. Efremova[1,*], O. Matvienko[2], M. Fominykh[1], N. Korsakova[2], N. Silina[2], L. Papayan[2], V. Shuvaev[1], S. Voloshin[1]

[1]Clinical Department, [2]Russian Research Institute of Hematology and Transfusiology, Saint-Petersburg, Russian Federation

Background: Pathogenesis of myelofibrosis (MF) is associated with high risk of thromboembolism (arterial and venous). Age older than 60 years, leukocytosis, thrombocytosis and presence of *JAK2V617F* mutation are well-recognized risk factors for thrombotic complications. However, common coagulation tests in patients with MF frequently demonstrate not any or little abnormalities. The Calibrated Automated Thrombography (CAT) as integral hemostasis test could have of value in the coagulation system functional testing in MF patients.
Aims: To investigate functioning of the coagulation system in MF patients using thrombin generation test.
Methods: The study included 13 MF patients (26 – 71 years, median = 40) and 21 healthy controls (20 – 61 years, median = 31,5). There were 11 females and 2 males in the study group. Nine (69%) patients were *JAK2V617F* positive, 4 (31%) were *JAK2V617F* negative (3 were *CALR* positive, 1 had mutation in *MPL*). Thrombin generation was assessed by calibrated automated thrombinography (CAT) according to Hemker *et al.* Assessments were conducted in platelet poor plasma (PPP) with or without presence of thrombomodulin (PPP reagent +/– TM). The following parameters were evaluated: endogenous thrombin potential (ETP, nM*min), peak thrombin (Peak, nM). Sensitivity ETP and Peak for TM were calculated as percent of decreasing of these parameters after adding to assay of TM (S ETP,% and S Peak,% respectively). STATISTICA 6.0 package was used in data analysis. Results were presented as median (Me) with 95% confidence intervals (CI), $p<0.05$ was considered statistically significant for difference interpretation.
Results: Table 1.
Summary/Conclusion: ETP and Peak were lower in MF patients comparing to control group. However, MF patients have considerable decrease sensi-

tivity for TM, which indicates dysfunction of anticoagulant protein C system. The disability in anticoagulant protein C system can lead to offset hemostatic balance and hypercoagulability which serve as underlying conditions for thrombotic complications.

Table 1.

Parameters of CAT	Patients (n=13)	Controls (n=21)
ETP, nM*min	1781.26 (808.47-1759.19)*	1725.00 (1210.5-2179.5)
S ETP (%)	43 (3.67-67.31)*	51.53 (22.49-65.49)
Peak, nM	183.47 (103.37-292.58)*	288.61 (193.24-376.7)
S Peak, %	20.0 (0.26-46.99)*	35.49 (14.56-53.78)

*p<0,05 for difference between patients and controls.

PS1536

PLATELET MAPPING BY THROMBOELASTOGRAPHY- ASSESSMENT OF PLATELET INHIBITION SECONDARY TO ANTIPLATELET THERAPY AFTER PCI

D. De[1,*], S.S. Datta[2], N. Afroz[2]

[1]Dept of Hematology, [2]the Mission Hospital, Kolkata, India

Background: Platelet mapping by TEG is a modification of conventional TEG which measures platelet functions. Dual antiplatelet therapy are the gold standard to attenuate platelet function during PCI. However, it has been demonstrated that nearly 20% of patients undergoing PCI will experience recurrent thrombotic events.
Aims: To assess the extent of resistance to clopidogrel, prasugrel, and ticagrelor in a group of patients who underwent PCI due to coronary artery disease by platelet mapping by thrombelastography.

Results

	Clopidogrel (75 mg OD)	Prasugrel (10mg OD)	Ticagrelor (90mg BD)
Total Patient Tested	18	15	23
Resistant (<50%)	3	3	0
% of Resistance	16.67	20	0
Mean Platelet inhibition	69.228	64.113	82.257
SD	24.054	24.951	14.030
Std Error of Means	5.670	6.442	2.925
P value of means	0.0360	0.0068	-

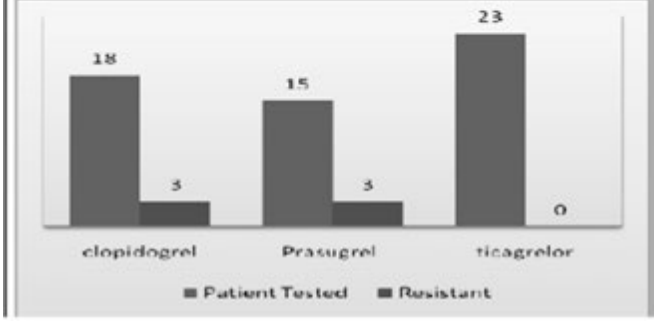


Figure 1.

Methods: This is a prospective, comparative, observational single-center cohort study. Included 56 patients who underwent a PCI from June 2016 to June2017. All patients were on aspirin and on any of the three drugs, namely, clopidogrel, prasugrel and ticagrelor, The average percentage platelet inhibition was assessed. Sensitivity and resistance to the specific drug was defined as >50% and <50% of mean platelet inhibition, respectively. The percentage of platelet aggregation to agonist can be calculated by: [(MA ADP/AA - MA Fibrin)/ (MA Thrombin – MA Fibrin)×100].
Results: The study found that the mean percentage platelet inhibition was significantly higher in patients with ticagrelor as compared with clopidogrel and with prasugrel (p value of 0.0360 and 0.0068, respectively) (Figure 1).
Summary/Conclusion: Platelet mapping by Thrombelastography can be successfully implemented in the patients undergoing PCI to assess its reliability as a monitoring tool for analyzing the response to antiplatelet therapy. Use of ticagrelor as dual therapy along with aspirin in patients with coronary artery disease (CAD) and undergoing PCI was associated with a significantly higher mean percentage platelet inhibition, higher sensitivity, and lower resistance as compared with the usage of clopidogrel or prasugrel.

Transfusion medicine

PS1537

IMMATURE PLATELET FRACTION: A USEFUL MARKER FOR IDENTIFYING THE CAUSE OF THROMBOCYTOPENIA AND PREDICTING PLATELET RECOVERY

E. Lee[1,*], K. Jeon[1], M. Kim[1], J. Lee[1], H.-S. Kim[1], H.J. Kang[1], Y.K. Lee[1]

[1]Laboratory medicine, Hallym Sacred Heart Hospital, Anyang, Korea, Republic Of

Background: We evaluated the discriminatory power of the immature platelet fraction (IPF%) along with plateletcrit (PCT), platelet distribution width (PDW), mean platelet volume (MPV), absolute immature platelet number (IPF#), and platlet-large cell ratio (P-LCR).
Aims: Aims of this study were to evalute the peformance of IPF% in differentiating hyperdestructive/consumptive thrombocytopenia from hypoproductive thrombocytopenia, and to evaluate its potential use as a predictive marker for platelet recovery.
Methods: The study included 105 healthy individuals (the control group), 31 patients with hyperdestructive/consumptive thrombocytopenia (14 with immune thrombocytopenic purpura [ITP] and 17 with liver cirrhosis [LC]), and 34 patients with hypoproductive thrombocytopenia (4 with aplastic anemia [AA] and 30 with cancer who were undergoing chemotherapy) by using a Sysmex XN-3000 hematology analyzer equipped with platelet specific channel (platelet-F).
Results: All the parameters analyzed in this study were significantly different in the 2 thromobocytopenia groups, but not always between the control group and the hypoproductive thrombocytopenia group. Compared to the control group, the hyperdestructive/consumptive thrombocytopenia group showed a 32.1% increase in the PDW, a 20.0% increase in the MPV, a 190.5% increase in the IPF%, a 25.6% decrease in the IPF#, a 45.7% increase in the P-LCR. Hypoproductive thrombocytopenia group showed a 6.1% decrease in PDW, a 6.5% decrease in MPV, a 9.5% decrease in the IPF%, a 69.8% decrease in the IPF# and a 4.9% increase in the P-LCR. In the hyperdestructive/consumptive group, the IPF% was 4.7%, 6.2%, and 11.4% in patients with platelet counts >90.0×10^3/μL, 40.0–90.0×10^3/μL and <40.0×10^3/μL, respectively (p=0.010). However, this phenomenon was not observed in the hypoproductive thrombocytopenia group. In a ROC analyses, the AUC was highest for IPF% (0.938), indicating that this parameter showed the best discriminatory ability between the two groups, followed by PDW (0.885), P-LCR (0.859), IPF# (0.827), and MPV (0.824) (all P-values <0.001). The IPF% showed an inverse relationship was observed between the platelet count and IPF%. The platelets increased to over 40.0×10^3/μL in 3–4 days after the IPF% decreased from its highest value in the 2 ITP patients with the baseline platelet counts under 10.0×10^3/μL. While thrombocytopenia occurred at each cycle of chemotherapy, an increase in the IPF% beyond the median value of IPF% in the control group (2.1%) coincided with a platelet count increase over 130.0×10^3/μL) in a median 5.5 days in 4 cancer patients under chemotherapy. The other patients (patient 11, 12 and 13) were excluded from the analysis because their platelet recovery seemed more dependent on platelet transfusion than on bone marrow thrombopoiesis during the study period.
Summary/Conclusion: We demonstrated for the first time that IPF% as reported by an XN-3000 instrument is an excellent marker for distinguishing hyperdestructive/consumptive thrombocytopenia from hypoproductive thrombocytopenia. Furthermore, by reflecting the BM's thrombopoietic activity, IPF% as reported by an XN-3000 is a robust and reliable marker for the prediction of platelet recovery in patients with ITP and those with cancer who are undergoing chemotherapy. This can assist in providing future therapy guidance and preventing unnecessary transfusions.

PS1538

PLATELETPHERESIS-ASSOCIATED LYMPHOPENIA IN FREQUENT PLATELET DONORS

J. Gansner[1,*], M. Rahmani[2], B. Fortin[1], I. Brimah[3], M. Ellis[2], R. Smeland-Wagman[2], D. Crown[2], J. Schenkel[2], N. Balan[1], R. Yefidoff-Freedman[1], S. Sloan[24], N. Berliner[1], N. Issa[1], L. Baden[1], D. Longo[1], D. Rao[1], D. Wesemann[1], D. Neuberg[5], R. Kaufman[2]

[1]Medicine, [2]Pathology, [3]Center for Clinical Investigation, Brigham and Women's Hospital, [4]Laboratory Medicine, Boston Children's Hospital, [5]Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, United States

Background: Platelet donors undergo plateletpheresis to collect platelet units that can be transfused to patients who are bleeding or at risk of bleeding. During plateletpheresis with the Trima Accel instrument, leukocytes are diverted into a leukoreduction system chamber, yielding leukoreduced platelet units. Based on an index case seen in our hematology clinic, we hypothesized that frequent plateletpheresis involving a leukoreduction system chamber would result in donor lymphopenia.
Aims: We investigated the blood counts (including CD4+ and CD8+ T-lymphocyte counts) of apheresis platelet donors who had undergone different numbers of plateletpheresis collections.
Methods: We conducted a prospective, observational study of platelet donors at one hospital-based donor center. A total of 60 adult donors were recruited, 20 in each of 3 pre-specified groups. These groups were defined by the number of successful plateletpheresis sessions in the prior 365 days, including the date of study participation: ≤2 sessions, 3-19 sessions, and ≥20 sessions. Subjects were excluded from participation if they had donated platelets at any other site in the past. All plateletpheresis procedures were performed using the Trima Accel instrument. Whole blood specimens were obtained before plateletpheresis, from the leukoreduction system chambers, and after plateletpheresis.
Results: The baseline white blood cell count, hematocrit, and platelet count were not significantly different among the three groups. Donors in the ≥20 sessions group exhibited reduced absolute lymphocyte counts compared to donors in the ≤2 sessions group, with striking reductions in both CD4+ and CD8+ T-lymphocyte counts. Six of 20 donors (30%) in the ≥20 sessions group had CD4+ T-lymphocyte counts below 200 cells/μL, compared with 2 of 20 donors (10%) in the 3-19 sessions group and 0 of 20 donors (0%) in the ≤2 sessions group, p=0.0189 (Image). CD8+ lymphocyte counts were below the reference range (<125 cells/μL) in 11 of 20 donors (55%) in the ≥20 sessions group, 4 of 20 donors (20%) in the 3-19 sessions group, and 0 of 20 donors (0%) in the ≤2 sessions group, p=0.0003. By examining donor history questionnaires, we confirmed the good health of all platelet donors, who are extensively screened for multiple pathogens; all are HIV negative. To investigate the mechanism of plateletpheresis-associated CD4+ and CD8+ T-cell lymphopenia in frequent platelet donors, we calculated the efficiency of blood cell extraction by the leukoreduction system chamber. Consistent with independent reports, these chambers contained high numbers of lymphocytes. The extraction efficiency of the chambers was ~15-20% for lymphocytes and monocytes, ~10-15% for basophils, and <1% for neutrophils and eosinophils. There was no clear selectivity in extraction efficiency with regards to lymphocyte subset (Figure 1).

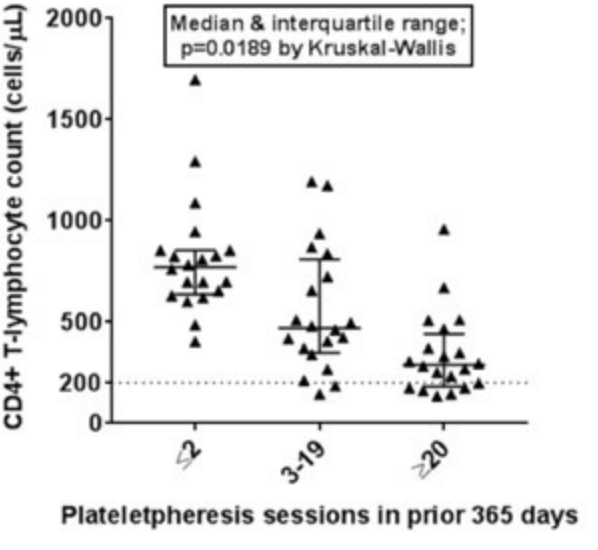


Figure 1.

Summary/Conclusion: Frequent plateletpheresis (≥20 plateletpheresis sessions in the prior 365-day period) involving a leukoreduction system chamber is associated with a surprising degree of lymphopenia in healthy platelet donors. We suspect that this may be due to repeated extraction of these cells by the chamber. A CD4+ T-lymphocyte count below 200 cells/μL has been used to define advanced immunodeficiency associated with HIV infec-

tion; however, our frequent platelet donors appear well. There is no evidence that the changes in cell counts are associated with increased risks. We are performing more extensive lymphocyte immunophenotyping as well as T-cell receptor repertoire sequencing to better characterize the immune systems of frequent platelet donors.

PS1539

INTRACRANIAL HEMORRHAGE IN LEUKEMIA PATIENTS: PRELIMINARY RESULTS OF AN ONGOING NESTED CASE CONTROL STUDY

L.L. Cornelissen[1,2,*], A. Kreuger[1,3], R. Middelburg[1,3], E. Beckers[4], P. von dem Borne[5], J. Kuball[6], K. de Vooght[7], J. Zwaginga[1,2], J. Kerkhoffs[1,8], J. van der Bom[1,9]

[1]Center for Clinical Transfusion Research, Sanquin Research, [2]Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, [3]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, Leiden, [4]Department of Hematology, Maastricht University Medical Center, Maastricht, [5]Department of Hematology, Leiden University Medical Center, Leiden, [6]Department of Hematology, [7]Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht, [8]Department of Hematology, Haga Hospital, Den Haag, [9]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands

Background: Intracranial hemorrhage is one of the most serious bleeding complications in thrombocytopenic hemato-oncology patients. To prevent such bleeding complications patients frequently receive prophylactic platelet transfusions based on the platelet count. However, the evidence for low platelet count as an independent risk factor is inconclusive. Other clinical risk factors are thought to also modify the bleeding risk.
Aims: To describe the association of platelet counts, transfusions and clinical risk factors like age, sex, infection and medication in the previous seven days with the risk of intracranial hemorrhage in patients with acute leukemia.
Methods: Nested case control study in a cohort of leukemia patients in four hospitals. Cases with intracranial hemorrhage were identified with a regression model previously designed to identify patients with major hemorrhages. Controls were matched on diagnosis, therapy and time. Cases were excluded in case of inability to identify suitable controls, uncertain date of intracranial hemorrhage or irretrievability of clinical data. Clinical risk factors were recorded from medical records for seven days preceding the bleeding for cases, or for the corresponding index period for controls. Univariate conditional logistic regression was performed to explore the association of bleeding with platelet count and potential risk factors.
Results: We identified 39 cases in four hospitals of which 25 were excluded for one of the exclusion criteria. Fourteen cases and 44 controls were included in the case control analysis. Most patients had acute myeloid leukemia (63.8%). 79.3% of the cases received remission induction for a new or relapsed leukemia, 3.5% received consolidation therapy, 3.5% allogeneic stem cell transplantation and 13.8% were admitted for disease or treatment related complications. Having a morning platelet count $<10x10^9$ was not associated with increased bleeding risk (OR 0.88, CI 0.10 to 8.13). Age was associated with an increased bleeding risk (OR 1.1/year, CI 1.01 to 1.18). Sex showed no relevant association (women: OR 1.21, CI 0.39 to 3.78). Compared to none, receiving one or two platelet transfusions in one week was associated with intracranial hemorrhage (OR 4.3, CI 0.70 to 26.28). For three or more platelet transfusions this risk increased (OR 6.4, CI 1.07 to 37.65. p-value for trend=0.044). Furthermore, presence of an infection proven by any positive culture or positive polymerase chain reaction (OR 2.58, CI 0.65 to 10.30) and usage of antihypertensive medication (OR 12.2, CI 1.36 to 110.36) were associated with an increased risk of intracranial hemorrhage.
Summary/Conclusion: Age, usage of antihypertensive medication and the presence of a culture or polymerase chain reaction proven infection were associated with an increased risk of intracranial hemorrhage. Also, the need for platelet transfusion was associated with an increased risk of intracranial hemorrhage. Although this does not imply causality, these conclusions could be relevant in platelet transfusion decision making.

PS1540

QUALITY CONTROL IN CRYOPRESERVED POOLED PLATELETS: 9-YEAR EXPERIENCE IN A TRANSFUSION CENTRE

A.M. Hernández Vidaña[1], J.A. García Bautista[1], P. Romero Garcia[2,*], C. Delgado Calvo-Flores[1], M.A. García Ruiz[3], M. Jurado Chacón[3], S. Oyonarte Gómez[1]

[1]Centro Regional de Transfusión Sanguínea, Granada, [2]UCI, Hospital de Santa Barbara, Soria, [3]Servicio de Hematología y Hemoterapia, Hospital Universitario Virgen de las Nieves, Granada, Spain

Background: The Centre for Blood Transfusion of Granada, Spain, offers as a part of its service portfolio, the production, storage and distribution of cryopreserved pooled paletelet concentrates for transfusional use. This procedure was implemented in 2009 to respond to potential situations of platelet shortage, as well as to supply platelet concentrates to hospital centers whose characteristics do not allow them to have a permanent stock of fresh platelets. Its main drawbacks are the loss of *in vivo* hemostatic capacity, and the cost and complexity of the manufacturing process.
Aims: Validate the efficacy of platelet cryopreservation to ensure transfusion requirements in any emergency in hospitals.
Methods: The production method used in our center is based on 5-unit pooled leucodepleted platelets, no older than 24 hours, which undergo successive steps of resuspension in citrate and mixing with cryopreservant solution composed of albumin and dimethylsulfoxide, which is finally removed by centrifugation before storage at -80°C. Pools with a platelet count above $1{,}000\times10^3/\mu l$ are selected, and the final volume is adjusted to 200 ml. This product can be issued frozen at -80 ° C for storage, or thawed and resuspended for use within 6 hours. We present the evolution of the production data in our center and the quality control parameters of cryopreserved pooled platelet units from 2009 to 2017. Annual and weighted average volume data, platelet recovery (calculated from platelet initial and final counts) and count of residual leukocytes are analyzed. In addition, a sterility control was performed prior to cryopreservation.
Results: In the reviewed period we have produced a total of 1,629 cryopreserved pooled platelet units. All of them have been quality-controlled before use, as described above, which allows us to verify compliance with standards regarding platelet recovery (>40% of baseline count) and residual leukocyte content ($<1x10^6$/unit).
Summary/Conclusion: The production and issuing of of cryopreserved pooled platelet units maintain an ascending line, which reflects their usefulness for Transfusion Services and the logistic advantages that it offers. Our experience throughout these 9 years in the elaboration and distribution of cryopreserved platelets has been very satisfactory, allowing the following advantages: 1) hospitals with a long distance to the Transfusion Center, where platelet transfusions are not usual, have seen a decline in the needs for urgent transport and expiration rate of fresh platelet pools, while allowing them to have a permanent stock of transfusion-ready platelets. 2) It has allowed to better adjust platelet unit stocks in all the Transfusion Services. 3) Ensuring that our center maintains a constant platelet safety stock on periods like holidays when it is difficult to guarantee a large enough number of donations. 4) Maintaining the availability of trnsfusion-ready platelets when facing unexpected peaks in consumption. Regarding production, we have achieved average values well above the standards in terms of platelet recovery (72.4%), platelet content ($3.15\ x10^{11}$ / unit), and residual leukocytes of $0.09x10^6$/unit. We must also note that these good results have remained constant over these years, despite the increase in production.

PS1541

BACTERIAL CONTAMINANTS AND BIOFILMS EFFECT PLATELET ACTIVATION AND FUNCTION: IMPLICATIONS IN PLATELET TRANSFUSIONS

J. Wilson-Nieuwenhuis[1,*], K. Whitehead[1], N. Dempsey-Hibbert[1]

[1]School of Healthcare Science, Manchester Metropolitan University, Manchester, United Kingdom

Background: Every year in the UK, approximately 250,000 platelet units are transfused and demand is increasing year on year. Due to their storage requirements pre-transfusion, platelet concentrates (PCs) are highly susceptible to bacterial growth if they have been contaminated during the donation or manufacturing process. Bacterial cells living freely within the blood unit may become attached to the surface, and form a biofilm. Biofilm formation can lead to increased virulence, and development of anti-microbial resistance. Much of the focus on bacterial contamination of PCs is on preventing the infusion of these bacteria in to the transfusion recipient, as transfusion-associated sepsis can cause major morbidity and mortality. And yet, identification of contaminated units within the blood bank still poses a problem and contaminated units are often released for transfusion, particularly if the bacteria have formed a biofilm. However, the effects of low levels of planktonic bacteria and biofilm formation on platelet activation and function has so far, been relatively understudied.
Aims: This work aimed to determine the effects of planktonic bacteria and bacterial biofilms on platelet activation and function in response to a com-

mon platelet agonist, with the purpose of establishing whether the efficacy of platelets within these contaminated units is also compromised.
Methods: Platelet rich plasma (PRP) was obtained by centrifugation of whole blood obtained from healthy participants after ethical approval and informed consent. PRP was incubated with either *Staphylococcus epidermidis* or *Serratia marcescens* (two species commonly implicated in platelet unit contamination) in either planktonic or biofilm forms prior to stimulation with ADP (10μM) and analysis of alpha granule secretion (CD62P expression) and GPIIbIIIa activation (PAC1 expression). Further, ADP-stimulated aggregation was assessed using light transmission aggregometry (LTA) after these treatments. Finally, RANTES release by platelets was determined via cytometric bead array.
Results: Platelet aggregation following contamination with planktonic cells showed no significant differences when compared with aggregation of uncontaminated platelets. However, incubation with biofilms (grown for 5 or 7 days prior to culture with platelets) was shown to significantly inhibit platelet aggregation ($p<0.05$). However, no significant changes in CD62P or PAC1 expression were determined following culture with planktonic bacteria or biofilms. RANTES release by platelets (ADP-stimulated or not) was significantly increased when platelets were incubated with a 7-day biofilm of either bacterial species, highlighting the platelets' role in host-defence.
Summary/Conclusion: The study demonstrates the inhibitory effects of low concentrations of commensal skin microflora on platelet activation and function, and highlights the need for strategies to prevent biofilm formation on platelet bag surfaces.

PS1542

ALLOIMMUNIZATION TO D AND NON-D RHESUS ANTIGENS INDUCED BY PLATELET TRANSFUSIONS

M. Daskalakis[1,*], J. Reckhaus[1], M. Jutzi[2], S. Fontana[2], U. Bacher[1], M. Vogt[1], B. Mansouri Taleghani[1]

[1]University Hospital of Hematology and Hematologic Central Laboratory, Inselspital, University of Bern, [2]Interregional Blood Transfusion, Swiss Red Cross, Bern, Switzerland

Background: Platelet concentrates (PC) contain residual contaminating red blood cells (RBC), being higher in pooled buffy coat PCs (BC-PC) than in apheresis units (AP-PC). Data about PC-induced alloimmunization against non-D Rhesus (Rh) antigens are limited.
Aims: Implementation of a systematic prospective assessment of every single newly identified Rhesus alloantibody for the putative triggering blood product transfused. With this hemovigilance-induced quality assurance measure we aimed to identify possible further cases in order to delineate the frequency, the antibody specificities, and the circumstances that may be associated with this phenomenon.
Methods: For all newly detected Rhesus D and non-D allo-antibodies between 08/2015 and 09/2017 we prospectively evaluated if they were triggered through PC by analysing for incompatible RBC and/or PC transfusions by compiling the patient s data (age, gender, AB0 blood group and Rhesus phenotype, main diagnoses, immunosuppressive drugs, transfusion history, and date of alloantibody detection) as well as transfusion history (included every RBC and PC unit). Recorded data of donors/PC/transfusions included: gender, age, AB0 blood group and Rhesus phenotype, AP-PC or BC-PC, date of transfusion. Routine quality control (QC) for residual RBC count was performed for 10 AP-PC and 10 BP-PC every month. Geometric mean and range was calculated including all QC RBC- counts for AP-PC and BC-PC from 01/2016 through 12/2016. Based on previous publications, we considered newly detected antibodies following a Rhesus incompatible PC transfusion until day 27 as secondary immune response (booster effect) and those found on day 28 or later as a primary immune response against Rh antigens.
Results: We identified 13 newly detectable Rh-antibodies through incompatible PCs in 11 patients: 6x anti-D, 4x anti-E, 2x anti-c, 1x anti-f. The patients received a total of 156 PC (83 BC-PC; 73 AP-PC): five patients received incompatible BC-PC only, one patient received incompatible AP-PC only, 5 patients received incompatible BC-PC and AP-PC. Quality control showed a mean (range) of 0.304 (0.152–1.662) and 0.014 (0.003–0.080)x10^9 RBC/L for BC-PC and AP-PC, respectively. Ten of the 11 patients received RBC-transfusions, all of them being antigen-negative for the alloantibodies identified.
Summary/Conclusion: PC transfusions may not only induce Rh D alloimmunization, but also immunization against further Rh antigens such as c, E, and f. The risk is higher for BC-PC than AP-PC. The results may have impact on future recommendations of PC transfusion with respect to Rh compatibility and upper limits of RBC contamination.

PS1543

MASSIVE TRANSFUSION PROTOCOLS - FRIEND OR FOE ?

S. Ching[1], G. Chen[1], M. L. Tung[1,2,*]

[1]Laboratory Medicine, Ng Teng Fong General Hospital, [2]Haematology-Oncology, National University Cancer Institute, National University Health System, Singapore, Singapore, Singapore

Background: Massive Transfusion Protocols (MTPs) are increasingly adopted by hospitals as the evidence shows that it is beneficial in bleeding patients. However, inappropriate or overactivation of MTPs can result in increased blood wastages.
Aims: The aim of this study was to review the effects of the implementation of a MTP in Ng Teng Fong General Hospital (NTFGH) and its impact on blood product wastage.
Methods: A retrospective review of all the MTPs activated in NTFGH from July 2016 to June 2017 was performed by the medical technologist from the transfusion laboratory. 3 categories of MTP was defined as per the hospital's protocol with the following blood product ratio: a) Pack 1: 4 units red blood cell (RBC), 4 units of platelet (PLT), 4 units of fresh frozen plasma (FFP) and tranexamic acid 1, b) Pack 2: 4 units RBC, 4 units PLT, 4 units FFP and 10 units cryoprecipitate (CRYO); c) Pack 3: 4 units RBC, 4 units PLT and 4 units FFP. The decision to activate MTP is made by the clinician based on impending or actual haemorhagic shock in a bleeding patient. The total number of blood products ordered, returned and wasted were recorded monthly.
Results: A total of 22 patients received MTPs during this period with 236 blood products ordered. Of these products ordered, 74 were returned and were made up of 28 units of RBC, 7 units of PLT and 39 units of FFP. FFP contributed to 97.1% of the blood products wasted. 33.3% of all the MTPs did not result in any blood products being returned or wasted (Table 1). Figure 1 shows that a total of 81 units of blood products were wasted during this period. 43.2% of those units were attributed to MTPs.

Table 1.

Month/ Year	MTP	Total Products Ordered	RBC	FFP	PLT	CRYO	Total Products Returned	RBC	FFP	PLT	CRYO	Total Products Wasted	RBC	FFP	PLT	CRYO
Jul-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aug-16	4	38	16	16	4	2	9	2	5	2	0	5	0	5	0	0
Sep-16	2	13	4	8	1	0	12	4	8	0	0	8	0	8	0	0
Oct-16	2	17	7	8	2	0	9	0	8	1	0	8	0	8	0	0
Nov-16	9	76	32	32	8	4	15	5	8	2	0	8	0	8	0	0
Dec-16	2	18	8	8	2	0	8	4	4	0	0	3	1	2	0	0
Jan-17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Feb-17	3	27	12	12	3	0	6	4	2	0	0	2	0	2	0	0
Mar-17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Apr-17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
May-17	4	40	18	16	4	2	11	6	4	1	0	1	0	1	0	0
Jun-17	1	7	4	2	1	0	4	3	0	1	0	0	0	0	0	0
Total	27	236	101	102	25	8	74	28	39	7	0	35	1	34	0	0

Figure 1.

Summary/Conclusion: Although MTPs are useful in bleeding patients, our data shows that it is a major contributor to blood product wastages, especially FFP. More research is required to help guide clinicians in the appropriate activation of MTPs.

PS1544

PLATELET-DERIVED MICROPARTICLES; THE EXPRESSION PROPERTIES AND POTENSIAL AS A SUBSTITUTE FOR PLATELETS

S. Azadpour[1,*], F. Yari[2]

[1]Hematology, Abadan school of medical sciences , Abadan, Iran., Abadan, [2]Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran, Islamic Republic Of

Background: Since the use of platelet products is limited due to the side effects of platelet transfusions, viral and bacterial contamination and short expiration date, many efforts have been made to create alternatives for platelets in transfusion medicine. one of the responses of activated platelets to certain stimuli is the shedding of microparticles *in vivo* and definitely *in vitro* upon aging under storage. Elevated MPs in various diseases indicate their diagnostic importance, particularly in vascular pathologies. It has been revealed that these microparticles had hemostatic activity. These activities originate from the surface markers of platelet-derived microparticles (PDM).
Aims: The aim of this study was to survey the size and expression of surface glycoproteins of PDM in platelet concentrate to characterize more these micropaticles.
Methods: 30 units of platele concentrate (PC) were prepared from Iranian Blood Transfusion Organization. PDM were isolated using centrifugation from PC during 7 days storage. The size of PDM were determined using a particle-sizing instrument. Besides, We determined MP adhesion capacity to annexin-V due their PS contents by using ELISA method. Additionally, the expression of gpIb and IIb/IIIa on the PDM during the storage time were also studied using flow cytometry method. For this purpose, FITC-conjugated mouse antiCD41 (HIP8), mouse anti-human CD42b, FITC-conjugated goat anti-mouse IgG (Fab') 2 were used and the analysis was carried out using flow cytometry technique. For all the experiments, nonspecific binding was analyzed as it was explained. for studying the vWF binding properties of MPs, MPs was added to the tubes containing human vWF in the presence of ristocetin as a modulator. After, the MPs was washed and incubated for 30 minutes with rabbit anti-human vWF. The MPs was washed again and incubated with fluorescein isothiocyanate–conjugated goat anti-rabbit IgG. To be sure of the specificity of results, nonspecific antibody background binding was determined with the appropriately labeled isotypic control immunoglobulin IgG. Finally, the results of the experiment were surveyed using flow cytometry technique.
Results: The size distribution of platelet-derived microparticles (PDM) was determined in the range of 146 to 797 nm (Z-Average=782 nm). Furthermore, the expression of gpIIb/IIIa and gpIba molecules was demonstrated on the surface of platelet-derived microparticles (PDM). Platelet-derived microparticles (PDM) as platelet had adhesion capacity to annexin-V due their phosphatidylserine (PS) contents. In addition, the data showed that MPs could bind to vWF at the days 2, 4 and 7 of storage. The difference was significant between the days 2 and 7 for the expression of gpIIb/IIIa and gpIba on the microparticles (P-value<0.05).
Summary/Conclusion: This study could imply the expression of the same surface markers of platelets on the platelet-derived microparticles. These markers could explain the hemostatic activities of these microparticles. The results can imply the potential capacity of the platelet-derived microparticles as a platelet substitute product. Inspired by this, It is hoped to be able to use of the nano techniques to design the platelet-derived nanomicroparticles that exhibit platelet-like coagulation functions. Additionally, the reduction of surface glycoproteins was revealed on the PDM during 7 days of storage.

SIMULTANEOUS SESSIONS IV

Aggressive B-NHL: 1st line chemotherapy and prognostic factors

S1545

RADIOTHERAPY TO BULKY AND EXTRALYMPHATIC DISEASE IN COMBINATION WITH 6XR-CHOP-14 OR R-CHOP-21 IN YOUNG GOOD-PROGNOSIS DLBCL PATIENTS: RESULTS OF THE 2X2 RANDOMIZED UNFOLDER TRIAL OF THE DSHNHL/GLA

M. Pfreundschuh[1,*], N. Murawski[1], M. Ziepert[2], B. Altmann[2], M. Dreyling[3], P. Borchmann[4], S. Luminari[5], M. Witzens-Harig[6], J. Dierlamm[7], M. Haenel[8], L. Truemper[9], B. Metzner[10], E. Lengfelder[11], U. Keller[12], C. Ruebe[13], C. Berdel[13], N. Schmitz[14], G. Held[15], V. Poeschel[1]

[1]Internal Medicine I, Saarland University Medical School, Homburg, [2]IMISE, Universität Leipzig, Leipzig, [3]Med. Klinik [3], LMU, München, [4]Internal Medicine I, Köln University, Köln, Germany, [5]Clinica Oncoematologica S.C. Ematologia, Arcispedale S. Maria Nuova, Reggio Emilia, Italy, [6]Heidelberg University, Heidelberg, [7]Oncology and Hematology, UKE Eppendorf, Hamburg, [8]Klinikum Chemnitz, Chemnitz, [9]University Goettingen, Goettingen, [10]Klinikum Oldenburg, Oldenburg, [11]University Mannheim, Mannheim, [12]TU München, München, [13]Clinic for Radiotherapy, Saarland University Medical School, Homburg, [14]University Muenster, Muenster, [15]Westpfalz-Klinikum, Kaiserslautern, Germany

Background: The role of RT to B and E for young patients with good-prognosis DLBCL is ill-defined.
Aims: *The aim of the study was to determine the role of radiotherapy to bulky and extranodal disease and compare the efficacy of R-CHOP-14 and R-CHOP-21 in young DLBCL patients.*
Methods: 18-60 year-old patients (aaIPI=0 with B [≥7.5 cm], aaIPI 1) qualifying for radiotherapy to B or E were randomized to 6xR-CHOP-14 or 6x-R-CHOP-21 followed by RT (39.6 Gy) to B and E sites or observation in a 2x2 factorial design. Primary endpoint was event-free survival.
Results: A planned interim analysis of the first 285 patients had revealed a significantly better EFS of patients assigned to RT (p=0.004) resulting in the pre-defined closing of the non-RT arms. 305 pts (R-CHOP-21: 155; R-CHOP-14: 150) assigned to RT and 162 (R-CHOP-21: 81, R-CHOP-14: 81) assigned to observation were evaluable for this final analysis. There were no relevant differences in protocol adherence and toxicity between the two chemotherapy regimens. EFS, PFS and OS after R-CHOP-14 and R-CHOP-21 were not different. After 66 months median observation 3-year EFS was worse in pts not assigned to RT (68% *vs* 84%; p=0.001), due to a higher rate of PR (11% *vs* 2%) triggering additional treatment (mostly RT) as an EFS event. 3-year PFS of pts assigned to RT was not significantly better (89% *vs* 81%; p=0.221) and 3-year OS (93% *vs* 93%, p=0.506) was not different, which was confirmed in a multivariate analysis adjusting for elevated LDH, stage III/IV, B and E involvement (HR_{EFS}=0.5 [95%CI: 0.4-0.8], p=0.001; HR_{PFS}=0.7 [0.5-11], p=0.174; HR_{OS}=1.2 [0.6-2.2], p=0.674). Results were not different when the analysis was restricted to patients with bulky disease only.
Summary/Conclusion: There were no differences in outcome between R-CHOP-14 and R-CHOP-21. Patients assigned to observation had a worse EFS because of more events largely due to a higher PR rate triggering additional treatment with no differences in PFS and OS. These results highlight the difficulties in interpreting residual masses in DLBCL without a PET which has been shown to identify (elderly) patients with B who can be spared from radiotherapy without compromising their outcome [Pfreundschuh et al., ASCO 2017, #7506]. S*upported by Deutsche Krebshilfe, Amgen and Roche*

S1546

SURVIVAL OF VERY ELDERLY PATIENTS WITH DLBCL ACCORDING TO TREATMENT INTENSITY IN THE RITUXIMAB ERA: A SWEDISH LYMPHOMA REGISTRY STUDY

K. Sonnevi[1,*], M. Christopher[2], H.R. Junlén[2], B.E. Wahlin[2]

[1]Hematology, [2]Medicine, Karolinska Institute, Stockholm, Sweden

Background: Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive lymphoma and its incidence rises with increasing age. Several studies have confirmed that rituximab combined with chemotherapy is the best treatment option, but few randomized trials included patients over 80 years of age. Anthracycline-based treatment in combination with rituximab offers a potential cure but comes with substantial risk of adverse events, especially in elderly patients. The treating doctor always has to consider the individual patient's co-morbidity and risk of complications.
Aims: To use data from the Swedish Lymphoma Registry to compare survival in DLBCL in the very elderly according to treatment intensity.
Methods: The Swedish Lymphoma Registry provided data on diagnosis, clinical factors and therapy and survival status on all patients ≥80 years diagnosed with DLBCL in Sweden 2007 through 2012. To reduce the selection bias that is inherent in retrospective analyses of different therapeutic algorithms, we also compared treatment differences in the Healthcare Regions. We investigated whether regional policy differences correlated with survival in all patients within the Regions (regardless of actual treatment given) using Cox proportional hazards model.
Results: In total 799 patients ≥80 years were identified from the SLR; 47% were male and 53% female. At diagnosis, age-adjusted international prognostic index (aaIPI) ≥2 was seen in 49% and bulky disease in 20%. Patients treated with anthracycline-based treatment with curative intention (R-CHOP-21, R-CHOP-14, R-CHOEP) showed significantly better survival with a hazard ratio (HR) of 2.5 (95% confidence interval [CI], 2.2-3.0) compared with patients treated with palliative regimens or no chemotherapy at all. In the Regional analysis, two Regions treated a relatively large proportion of their elderly patients with curative intent (58% in total) whereas three Regions showed a lower fraction (43% in total) and one Region deviated with a considerably low percentage (33%). These treatment differences were highly significant (p<0.001). The overall survival was also significantly better in the more intensive Regions compared with the less intensive and the least intensive Region, HR 1.3 (95% CI 1.1-1.6) and HR 1.5 (95% CI 1.2-1.9).
Summary/Conclusion: In this large, nationwide, population-based study we show that anthracycline-based treatment with curative intention is associated with improved survival also in the very elderly patients over 80 years of age. Furthermore, we can show that the proportion of patients treated with curative intention vary between different healthcare Regions in Sweden suggesting different routines when it comes to treating the very elderly. We used the Regional differences to validate our results by showing that Regions with more intensive treatment traditions have better overall survival. This analysis can be seen as a geographic randomization that is unaffected by response to therapy and co-morbidity since the intensity of treatment is significantly differs merely on the basis of region. We conclude that also patients over the age of 80 years benefit from anthracycline-based treatment with curative intention.

S1547

IMPACT OF CONCURRENT FOLLICULAR LYMPHOMA OR OTHER LOW-GRADE B-CELL LYMPHOMA ON THE CLINICAL OUTCOME OF DIFFUSE LARGE B-CELL LYMPHOMA

Y. Wang[1,*], B. Link[2], M. Maurer[1], C. Allmer[1], T. Witzig[1], T. Habermann[1], S. Ansell[1], R. King[1], A. Feldman[1], S. Slager[1], J. Cerhan[1], G. Nowakowski[1]

[1]Mayo Clinic, Rochester, [2]University of Iowa, Iowa, United States

Background: A significant percentage of patients with newly diagnosed diffuse large B-cell lymphoma (DLBCL) have concurrent follicular lymphoma (FL) or other low-grade B-cell lymphoma in tissue and/or bone marrow biopsies. Clinically these patients are usually treated in the same way as those with pure *de novo* DLBCL. However, it is unclear whether they also have identical outcomes compared with *de novo* DLBCL patients. As a result, whether to include these patients in clinical trials of DLBCL remains controversial.
Aims: To compare the clinical characteristics and outcomes of patients with *de novo* DLBCL, concurrent DLBCL and FL (DLBCL+FL), or concurrent DLBCL and other low-grade B-cell lymphoma (DLBCL+LG).
Methods: 1448 patients with newly diagnosed DLBCL from March 2002 to April 2015 were included in this study. Patients were enrolled in the Molecular Epidemiology Resource (MER) of the University of Iowa/Mayo Clinic lymphoma Specialized Program of Research Excellence (SPORE), treated with standard immunochemotherapy and followed prospectively. Event-free survival (EFS) was defined as time from diagnosis to progression or relapse, unplanned re-treatment after initial immunochemotherapy, or death from any cause. Overall survival (OS) was defined as time from diagnosis to death from any cause. Categorical data were analyzed by Chi-square test, and time-to-event data were analyzed using Kaplan-Meier method. Statistical analysis was done in SPSS (V22).
Results: Among the 1448 patients, 1262 (87.2%) had *de novo* DLBCL, 113 (7.8%) had DLBCL+FL, and 73 (5.0%) had DLBCL+LG. In the DLBCL+FL

cohort, 87 (77%) patients had DLBCL and FL at the same site (composite histology) while 26 (23%) had FL at other sites (discordant histology). In the DLBCL+LG cohort, 16 (21.9%) had composite histology and 57 (78.1%) had discordant histology, predominantly in the bone marrow (55). LG histology included marginal zone lymphoma (18), chronic lymphocytic leukemia/small lymphocytic lymphoma (14) and lymphoplasmacytic lymphoma (4). Baseline characteristics were summarized in Table 1. Compared with de *novo* DLBCL patients, DLBCL+FL patients had less elevations in LDH and lower IPI scores, while DLBCL+LG patients had more advanced stages (all *P*<0.001). Cell of origin by Hans was predominantly GCB in DLBCL+FL (92.1% *vs* 61.4% in *de novo* DLBCL, *P*<0.001). The proportion of GCB subtypes in DLBCL+LG was similar to that in *de novo* DLBCL (50.9% *vs* 61.4%, *P*=0.132). The median follow-up was 83.9 months. DLBCL+FL patients had superior EFS (median 145.9 *vs* 101.9 months, EFS rate at 24 months [EFS24] 74.5% *vs* 63.9%, *P*=0.049) and OS (median not reached *vs* 139.7 months, *P*=0.004) compared with de *novo* DLBCL patients (Figure 1). DLBCL+LG patients had similar EFS (median 63.0 *vs* 101.9 months, EFS24 65.8% *vs* 63.9%, *P*=0.269) and OS (median 120.7 *vs* 139.7 months, *P*=0.624) compared with de *novo* DLBCL patients. Relapses with DLBCL or FL/LG were equally common in DLBCL+FL (7 relapses with DLBCL, 7 with FL, 6 with both) and DLBCL+LG patients (12 relapses with DLBCL, 12 with LG, 3 with both).

Figure 1. (A) EFS and (B) OS of Patients with *de novo* DLBCL, DLBCL+FL and DLBCL+LG

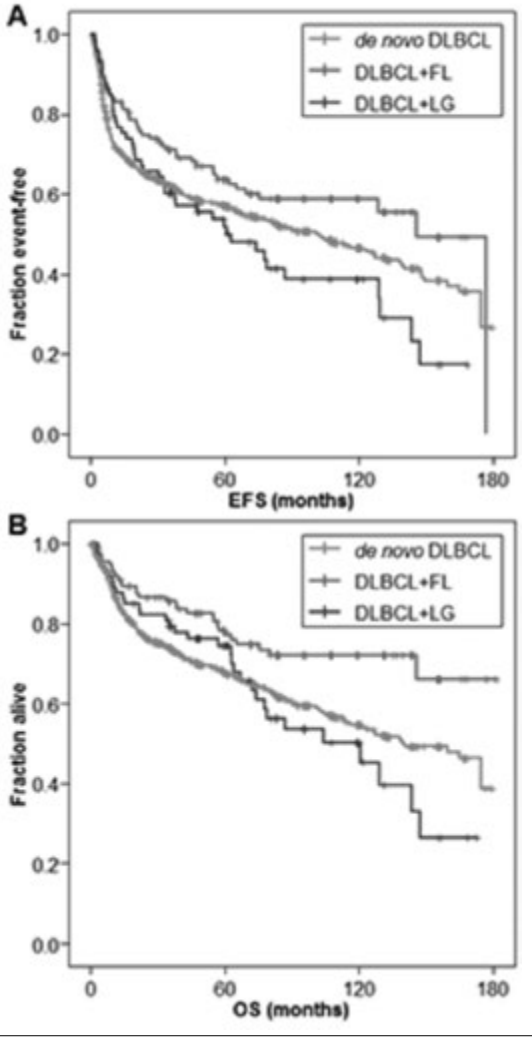


Figure 1.

Summary/Conclusion: Compared with *de novo* DLBCL patients, those with DLBCL+FL at diagnosis had predominantly GCB subtype of DLBCL, lower IPI scores, and improved EFS, EFS24 and OS, while those with DLBCL+LG had more advanced stages and similar outcomes. In DLBCL+FL and DLBCL+LG patients, relapses with DLBCL and FL or LG were equally common. DLBCL+FL likely behaves like GCB DLBCL, and this should be taken into consideration when including DLBCL+FL patients in DLBCL trials.

Table 1.

Table 1. Baseline Characteristics of Patients with *de novo* DLBCL, DLBCL+FL and DLBCL+LG

	DLBCL	%	DLBCL +FL	%	DLBCL +LG	%	DLBCL *vs* DLBCL +FL	DLBCL *vs* DLBCL +LG
Age							0.252	0.290
≤60	511	40.5	52	46.0	25	34.2		
>60	751	59.5	61	54.0	48	65.8		
Gender							0.163	0.457
Male	730	57.8	73	64.6	39	53.4		
Female	532	42.2	40	35.4	34	46.6		
ECOG PS							0.362	0.201
<2	1009	80.2	93	83.8	63	86.3		
≥2	249	19.8	18	16.2	10	13.7		
Missing	4		2					
Ann Arbor stage							0.115	<0.001
I-II	488	38.8	35	31.3	9	12.5		
III-IV	770	61.2	77	68.8	63	87.5		
Missing	4		1		1			
LDH							<0.001	0.190
Normal	485	42.6	63	64.9	34	50.7		
Elevated	654	57.4	34	35.1	33	49.3		
Missing	123		16		6			
Extranodal sites							0.536	0.224
≤1	1008	79.9	93	82.3	54	74.0		
>1	254	20.1	20	17.7	19	26.0		
IPI score							<0.001	0.193
0-1	411	32.6	41	36.3	15	20.5		
2	347	27.5	48	42.5	25	34.2		
3	334	26.5	15	13.3	22	30.1		
4-5	170	13.5	9	8.0	11	15.1		
Cell of origin							<0.001	0.132
GCB	524	61.4	82	92.1	27	50.9		
Non-GCB	330	38.6	7	7.9	26	49.1		
Missing	408		24		20			

S1548

FAVOURABLE OUTCOMES WITH R-CODOX-M/R-IVAC ACROSS ALL SUBGROUPS OF AGGRESSIVE HIGH GRADE B-CELL LYMPHOMA: PATHOLOGY AND UPDATED SURVIVAL RESULTS FROM A PHASE 2 UK NCRI/LLR TRIAL

E.H. Phillips[1,*], C. Burton[2], A. Kirkwood[1], S. Barrans[2], A. Lawrie[1], S. Rule[3], R. Patmore[4], R. Pettengell[5], K. Ardeshna[6], S. Montoto[7], S. Paneesha[8], L. Clifton-Hadley[1], D. Linch[9], A. McMillan[10]

[1]Cancer Research UK and UCL Cancer Trials Centre, University College London, London, [2]Haematological Malignancy Diagnostic Service, Leeds, [3]Derriford Hospital, Plymouth, [4]Haematology, Castle Hill Hospital, Hull, [5]Clinical Sciences, St George's, University of London, [6]Haematology, University College Hospital London, [7]Haemato-oncology, Barts Health NHS Trust, [8]Haematology, Heart of England NHS Trust, [9]Cancer Institute, University College London, London, [10]Haematology, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom

Background: Outcomes for patients (pts) with intermediate-high and high risk diffuse large B-cell lymphoma (DLBCL) after R-CHOP chemotherapy remain suboptimal but no other regimen has improved survival rates. Even less is known about the management of other forms of high grade B-cell lymphoma (HGBL) - double hit lymphoma (DHL) and HGBL not otherwise specified (NOS) - where outcomes are poor with no established standard of care. The phase 2 R-CODOX-M trial was designed to assess the efficacy of adding rituximab to the CODOX-M/IVAC regimen in high risk HGBL. Primary results have previously been presented (McMillan *et al.*, ICML 2015).
Aims: To present outcomes according to pathology subgroups and updated survival for both DLBCL/HGBL and Burkitt lymphoma (BL) cohorts.
Methods: Between 2009 and 2013, 150 pts were registered at 20 UK sites. Eligible pts were aged 18-65 years with stage II-IV untreated DLBCL/HGBL or BL and an International Prognostic Index (IPI) score ≥3. Pts received dose-modified CODOX-M and IVAC (Mead *et al.*, Blood 2008) with the addition of 8 doses of rituximab (375mg/m^2). Diagnostic pathology was centrally reviewed by the Leeds Haematological Malignancy Diagnostic

Service in 117 (78%) pts and diagnostic reports from central teaching hospitals were reviewed for the remainder. The original site diagnosis was changed from BL to DLBCL/HGBL in 11/36 (30.6%) pts and from DLBCL to BL in 4/114 (3.5%) following central review and FISH analysis. Six pts did not commence treatment and are excluded from further analyses.

Results: A total of 116 pts are included in the DLBCL/HGBL cohort. Median age was 50 years (range 18–65), IPI score was 3 (n=74; 64%), 4 (n=41; 35%) or 5 (n=1; 1%). Eleven pts (9.5%) had CNS involvement and 62 (53%) had a performance status (PS) ≥2. Cell of origin classification by Hans algorithm was available for 104 pts, of which 57 (55%) were germinal centre B-cell (GCB). FISH data was available for 57 pts, of which 7 (12%) were double/triple hit lymphomas. Five pts had HGBL-NOS (defined as a BL-like phenotype without *MYC*-R) and 4 pts were indeterminate between HGBL/BL due to failed FISH studies. With a median follow-up of 53 months for the whole DLBCL/HGBL cohort, 3-year progression-free survival (PFS) and overall survival (OS) were 68.4% (95% CI: 59-76.1; see Figure) and 76.2% (95% CI: 76.2–67.2), respectively. There was no difference in outcomes according to cell of origin (p=0.73), even excluding those with DHL, HGBL-NOS or indeterminate pathology. Three-year PFS rates were 57.1% for DHL (95% CI: 17.2–83.7) and 63.6% for those with HGBL-NOS/indeterminate histology (95% CI 29.7–84.5). Pts with secondary CNS lymphoma had a 3-year PFS of 72.7% (95% CI: 37.1–90.3). Treatment-related mortality was 4.3%; all were aged >50 years with a PS of 3. The BL cohort included 27 pts with a median age of 35 years (range 20–64). IPI score was 3 (n=13; 48%) or 4 (n=14; 52%); PS was ≥2 in 16 pts (59%) and 4 (15%) had CNS involvement. Three-year PFS and OS were 73.1% (95% CI: 51.7–86.2; Figure) and 76.4% (95% CI: 54.8–88.7), respectively.

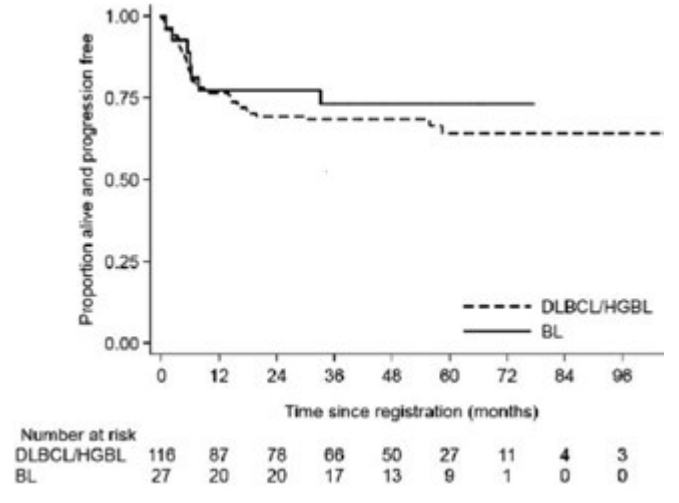


Figure 1.

Summary/Conclusion: These results demonstrate very good outcomes with R-CODOX-M/R-IVAC treatment in a high risk cohort of DLBCL/HGBL pts, similar to very high risk BL pts. For pts with aggressive HGBL, where urgent treatment is often necessary but accurate rapid diagnostic differentiation can be difficult, R-CODOX-M/R-IVAC is potentially a favourable treatment option offering improved outcomes for all diagnostic groups. Further trials are needed to assess this regimen against standard of care in high risk DLBCL/HGBL.

S1549

TP53 MUTATION HAD A NEGATIVE PROGNOSTIC IMPACT IN UNTREATED YOUNG PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA AT HIGH-RISK: A SUB-ANALYSIS OF FIL-DLCL04 STUDY

A. Chiappella[1,*], F. Diop[2], C. Agostinelli[3], A. Evangelista[4], L. Nassi[2], R. Bomben[5], G. Ciccone[4], A. Di Rocco[6], M. Fabbri[7], M. Martelli[6], F. Melle[7], G. Motta[7], A.G. Congiu[8], M. Gotti[9], V. Pavone[10], C. Stelitano[11], A. Tucci[12], F. Zaja[13], M. Ladetto[14], V. Gattei[5], S.A. Pileri[7], U. Vitolo[1], G. Gaidano[2]

[1]On behalf of Fondazione Italiana Linfomi, Hematology, Città della Salute e della Scienza Hospital and University, Torino, [2]On behalf of Fondazione Italiana Linfomi, Hematology Department of Translational Medicine Università del Piemonte Orientale Amedeo Avogadro Azienda Ospedaliero-Universitaria Maggiore della Carità, Novara, [3]On behalf of Fondazione Italiana Linfomi, Pathology Unit, Università degli Studi di Bologna, Bologna, [4]On behalf of Fondazione Italiana Linfomi, Unit of Clinical Epidemiology and CPO, Città della Salute Hospital and University, Torino, [5]On behalf of Fondazione Italiana Linfomi, Clinical and Experimental Onco-Hematology Unit, Centro di Riferimento Oncologico, IRCCS, Aviano, [6]On behalf of Fondazione Italiana Linfomi, Hematology, Università La Sapienza, Roma, [7]On behalf of Fondazione Italiana Linfomi, Unit of Hematopathology, European Institute of Oncology, Milano, [8]On behalf of Fondazione Italiana Linfomi, Division of Hematology and Bone Marrow Transplantation, Ematologia Ospedale Policlinico San Martino, Genova, [9]On behalf of Fondazione Italiana Linfomi, Department of Hematology Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia, [10]On behalf of Fondazione Italiana Linfomi, Ematologia e Centro Trapianti Midollo Osseo, Azienda Ospedaliera Cardinale G. Panico, Tricase, [11]On behalf of Fondazione Italiana Linfomi, Haematology, Grande Ospedale Metropolitano Bianchi Melacrino Morelli, Reggio Calabria, [12]On behalf of Fondazione Italiana Linfomi, Hematology, ASST Spedali Civili di Brescia, Brescia, [13]On behalf of Fondazione Italiana Linfomi, Clinica Ematologica Centro Trapianti e Terapie Cellulari "Carlo Melzi" Università degli Studi di Udine, Udine, [14]On behalf of Fondazione Italiana Linfomi, Hematology, Azienda Ospedaliera SS Antonio e Biagio e Cesare Arrigo, Alessandria, Italy

Background: The Italian phase III randomized study FIL-DLCL04 (Chiappella et al, Lancet Oncol 2017) showed that an abbreviated Rituximab-CHOP dose-dense chemotherapy followed by consolidation with Rituximab-high dose chemotherapy and autologous stem cell transplantation (R-HDC+ASCT) compared to a full course of R-CHOP dose-dense chemotherapy without consolidation, determined an advantage in term of failure-free survival (FFS) but no difference in overall survival (OS) in young patients with untreated diffuse-large B-cell lymphomas (DLBCL) at high-risk. The prognostic role of *TP53* is well-known in chronic lymphocytic leukaemia, but it has not yet been established in DLBCL.

Aims: Aim of this analysis was to correlate *TP53* mutations, cell of origin (COO) profile and the presence of biomarkers (MYC, BCL2), with OS and FFS.

Methods: From 2005 to 2010, 399 young untreated DLBCL at poor-risk, were enrolled in FIL-DLCL04 and randomized to receive R-HDC+ASCT in 199 and R-dose-dense in 200 (NCT00499018). *TP53* disruption by gene mutation was analyzed by Sanger DNA sequencing. COO classification as germinal center (GCB), activated B-cell (ABC) and unclassified was based on gene-expression profiling using the NanoString research use only lymphoma subtyping test. BCL2, MYC and TP53 were studied in immunohistochemistry (IHC); cases were deemed positive if at least 50%, 40% and 30% of lymphoma cells were stained with BCL2, c-MYC and TP53 antibodies, respectively. *Bcl2* and *Myc* translocations, copy gains and aberrations were tested by fluorescent *in situ* hybridization (FISH). OS and FFS were analyzed; a crude hazard ratio (HR) and an adjusted HR (aHR) for clinical characteristics (age, treatment, gender, age-adjusted International Prognostic Index, performance status, bone marrow involvement) and for biological factors (double-expressors, double-hit, COO profile) were calculated.

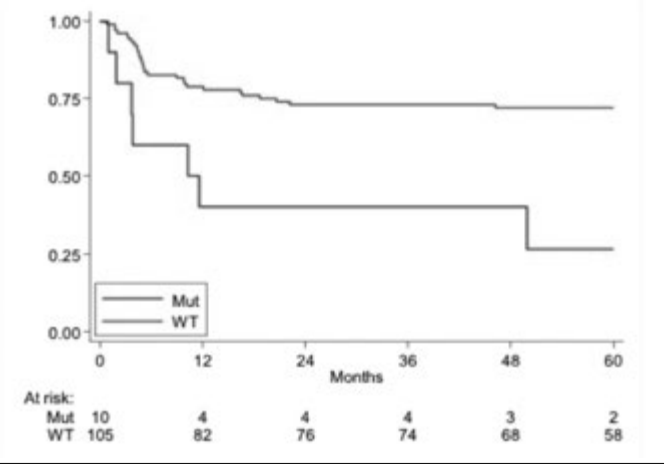


Figure 1.

Results: Of 399 DLBCL patients enrolled in FIL-DLCL04, 115 with tumor block available for subsequent analyses were analyzed for *TP53* mutation; no selection bias was observed between the 115 cases and the whole FIL-DLCL04 study population. Fifty-five of 115 patients (48%) received R-HDC+ASCT upfront, as for randomization arm; 22 (19%) had bone marrow involvement; 41 (53%) were GCB, 23 (29%) ABC and 14 (18%) unclassified; 20 (19%) were double-expressor (DEL) for MYC and BCL2 in IHC and 8 (8%) double-hit (DHL) for *Myc* and *Bcl2* in FISH. Regarding *TP53* status, 105 (91%) were wild type and 10 (9%) mutated; of 10 cases

with TP53 mutated, 5 were DEL, 2 DHL and 2 ABC. At a median follow-up of 72 months, 5-years FFS for TP53 mutated *versus* wild type were 27% (95% CI: 48-56) and 72% (95% CI: 62-80), respectively with a crude hazard ratio (HR) of 3.57 (95% CI: 1.56-8.17), p 0.003, an aHR (clinical) of 2.02 (95% CI: 0.75-5.44), p 0.165 and an aHR (biological) of 2.06 (0.70-6.11), p 0.190. (Figure 1). Five-years OS for TP53 mutated *versus* Wild type were 37% (95% CI: 10-66) and 84% (95% CI: 75-89), respectively; HR: 4.88 (95% CI: 1.91-12.42), p 0.001, aHR (clinical): 3.26 (95% CI: 0.98-10.9), p 0.055 and aHR (biological) 4.10 (0.89-18.86), p 0.070.
Summary/Conclusion: In this series of young patients with high risk DLBCL, *TP53* disruption by gene mutation identifies a very poor prognosis subgroup with a dismal FFS and OS, irrespective to IHC, FISH and COO profile. Likewise other hematological malignancies, *TP53* disruption is a negative prognostic factor also in DLBCL and these patients require different treatment with innovative drugs.

CML and MPN – Biology

S1550

THE TUMOR SUPPRESSOR MIR-300 PRESERVES CANCER STEM CELLS AND INHIBITS NK CELL ANTICANCER IMMUNITY

G. Silvestri[1], L. Stramucci[1], J. Ellis[2], J. Harb[3], P. Neviani[4], A.K. Eisfeld[5], B. Zhang[6], K. Srutova[7], C. Gambacorti-Passerini[8], G. Pineda[9], C. Jamieson[9], B. Calabretta[10], F. Stagno[11], P. Vigneri[12], G. Nteliopoulos[13], P. May[13], R. Garzon[5], D.-C. Roy[14], M. Guimond[14], P. Hokland[15], M. Deininger[16], G. Fitzgerald[17], C. Harman[17], F. Dazzi[18], D. Milojkovic[13], J. Apperley[13], G. Marcucci[6], J. Qi[1], K. Machova-Polakova[7], Y. Zou[1], X. Fan[1], M. Baer[1], R. Trotta[1], D. Perrotti[1,*]

[1]University of Maryland, Baltimore, [2]OSU, Ohio, [3]blood center of Wisconsin, Milwaukee, [4]Norris Comprehensive Cancer Center, Los Angeles, [5]OSU, Columbus, [6]City of Hope National Medical center, City of Hope, United States, [7]University of Prague, Prague, Czech Republic, [8]University of Milano-Bicocca, Monza, Italy, [9]University of California, San Diego, [10]Thomas Jefferson University, Philadelphia, United States, [11]policlinico Vittorio Emanuele, [12]University of Catania, Catania, Italy, [13]Imperial College, London, United Kingdom, [14]University of Montreal, Montreal, Canada, [15]Aarhus University Hospital, Aarhus, Denmark, [16]Huntsman Cancer Institute, Salt Lake City, [17]University of Maryland Medical Center, Baltimore, United States, [18]King's College London, London, United Kingdom

Background: Inhibition of protein phosphatase 2A tumor suppressor is essential for chronic myelogenous leukemia stem cell maintenance, evolution and innate anti-cancer immunity. In CML, persistance of quiescent LSCs, disease development and inhibition of NK cell growth and anti-cancer activity depend on cell-autonomous and bone marrow generated signals.
Aims: miR-300 interconnects CSCs, microenvironment and immune cells.
Methods: Exosomes purification, Cell Cycle analysis, LTC-IC and CFC assays, CFSE-mediated tracking of cell division, apoptosis assays, real time RT-PCR.
Results: We show that expression profiling of $CD34^+$ BM progenitors from healthy and CML patients CP and BC phase identified a number of miRs altered in CML and/or during BC transformation. We focused on miR-300 because it directly inhibited expression of multiple components of the PP2A inhibitory pathway and of other factors for LSC maintenance and disease progression, when ectopically expressed in CML but not in normal $CD34^+$ cells. MiR-300 acted as a potent tumor suppressor CML $CD34^+$ progenitors by inducing cell cycle exit and promoting spontaneous and (TKI)-induced apoptosis. In $CD34^+$ CML-BC progenitors miR-300 downregulation required the BCR-ABL1 kinase-dependent inhibition of C/EBPb, which was capable of binding and transcriptionally activating miR-300 promoter. Conversely, low O_2 levels and MSC-derived exosomes increased miR-300 expression in a TKI-insensitive manner to induce and/or preserve LSC quiescence and suppress NK cell growth. Indeed, lentiviral-mediated miR-300 inhibition in MSCs significantly prevented the MSC growth inhibitory activity on $CD34^+$ CML-BC and NK cells. Accordingly, miR-300 levels were found higher in $CD56^+CD3^-$ NK cells from CML patients at diagnosis compared to healthy individuals. Induction/maintenance of high miR-300 levels in LSCs required the hypoxia-induced inhibition of BCR-ABL1 activity and induction of C/EBPb expression/activity. LSCs escaped from the miR-300 apoptotic activity through an autocrine/paracrine mechanism that required the release by $CD34^+$ CML stem/progenitors of TGFb1 that, in turn, induced the expression of TUG1, a lncRNA acting as a miR-300 sponge. In fact, shRNA-mediated TUG1 suppression or Ab-dependent TGFb1 inhibition decreased the quiescent $CD34^+$ LSC number. By contrast, miR-300 inhibition did not alter LSC survival and self-renewal, further supporting a role for TUG1 as a miR-300 sponge. Accordingly, TUG1 was markedly induced in $CFSE^{MAX}$ but not dividing $CD34^+$ CML cells. In fact, low ectopic miR-300 expression induced growth arrest without affecting the number of $CFSE^{MAX}$ LSCs. High doses of miR-300 but not scramble oligonucleotides impaired LSC survival and self-renewal, induced a marked killing of quiescent LSCs and dividing $CD34^+$ CML progenitors, and impaired CML-BC engraftment in NRG-SGM3 mice.
Summary/Conclusion: In Summary, loss of miR-300 expression is essential for survival/proliferation of leukemic progenitors and, therefore, increased miR-300 expression is necessary for reduced NK cell number/activity and maintenance of the LSC reservoir. Induction of TUG1 may occur to preserve LSC survival after migration into the BM endosteal niche where quiescence is induced by MSCs and low O_2 levels through marked induction of intracellular miR-300 levels. Thus, disrupting the miR-300/TUG1 balance may represent a potential therapeutic approach for treatment/eradication of LSC-derived leukemias and restoration of innate immunity.

S1551

JAK1-STAT3 SIGNALING AXIS SUPPORTS LEUKEMIC STEM CELL PERSISTENCE IN CML

M.K. Kuepper[1], O. Herrmann[1], I.G. Costa[2], S. Koschmieder[1], T.H. Bruemmendorf[1], G. Mueller-Newen[3], M. Schemionek[1,*]

[1]Department of Hematology, Oncology, Hemostaseology and Stem Cell Transplantation, Faculty of Medicine, [2]Institute for Computational Genomics, [3]Institute of Biochemistry and Molecular Biology, RWTH Aachen University, Aachen, Germany

Background: Chronic myeloid leukemia (CML) is driven by the chromosomal translocation t(9;22) which transforms hematopoietic stem cells into leukemic stem cells (LCS). Blocking the oncogenic Bcr-Abl kinase by tyrosine kinase inhibitors (TKI) has greatly improved CML therapy, but is rarely curative as the disease-initiating LCS survive within the bone marrow (BM) niche independently of Bcr-Abl kinase activity. Recently, the signal transducer and activator of transcription 3 (STAT3) was shown to be highly activated in TKI-resistant CML cells that were supported by the BM microenvironment. However, while targeting STAT3 in combination with Bcr-Abl was potent to kill TKI-resistant LCS, the activating mechanism of persisting STAT3 activation remains unclear.
Aims: In this study, we first analyzed STAT3 activation in TKI treated leukemic cells, and how it is influenced by the BM microenvironment. Subsequently, we aimed to identify the mechanism allowing for TKI-persisting STAT3 activation and evaluated the therapeutic potential of combined Bcr-Abl and JAK1 inhibition using human cell lines, primary murine cells, as well as primary CML CD34$^+$ cells.
Methods: Gene Set Enrichment Analysis (GSEA) was performed using transgenic murine CML and control LSK cells. We generated conditioned medium (CM) by harvesting the supernatant of primary human mesenchymal stroma cells (MSC). qRT-PCR was performed to analyze target gene expression. Phosphorylation of STAT3 (pSTAT3^{Y705}) was evaluated by western blot upon Bcr-Abl (imatinib) and JAK1 (filgotinib/itacitinib) inhibition. Furthermore, an IL-6-blocking receptor fusion protein (RFP) was applied. Finally, colony forming unit (CFU) assay, CSFE staining and apoptosis analysis via flow cytometry of primitive CML CD34$^+$ cells were performed.
Results: GSEA pathway analysis revealed increased JAK/STAT signaling in transgenic CML LSK cells. We observed elevated STAT3 mRNA expression in KCL-22 (1.9-fold, p≤0.001) and CML MNC (1.6-fold, p≤0.05), as well as increased pSTAT3^{Y705} levels in murine Bcr-Abl BM, KCL-22 and CML-MNC upon imatinib treatment, exclusively in presence of MSC-derived CM. In the presence of JAK1- but not JAK2-specific inhibitors, persisting pSTAT3^{Y705} was decreased to basal levels in murine Bcr-Abl BM, KCL-22 and primary CML MNC. A similar effect was observed when applying an IL-6-blocking RFP. Combined inhibition of Bcr-Abl and JAK1 strongly reduced the CFU capacity of murine (2.2-fold, p≤0.001) and human CML cells (6.9-fold, p≤0.001) compared to IM treatment alone. Tracking the proliferation of CML CD34$^+$ cells by CSFE staining revealed that Bcr-Abl- and JAK1-inhibited cells showed increased quiescence (3.2-fold, p≤0.001) and decreased proliferation (3.6-fold, p≤0.001) compared to single treatments. Interestingly, combined Bcr-Abl and JAK1 inhibition strongly induced apoptosis even in quiescent LSC (2.4-fold, p≤0.001).
Summary/Conclusion: In the presence of BM microenvironment-derived CM, STAT3 is upregulated in Bcr-Abl leukemic cells upon oncogene inhibition. Here, we identified JAK1 as the STAT3-activating kinase in CML cells. JAK1 inhibition by TKIs decreases pSTAT3^{Y705} levels, blocks proliferation and reduces the CFU capacity. Upon Bcr-Abl and JAK1 inhibition, apoptosis is strongly induced compared to IM treatment alone in quiescent LCS. Our data demonstrate that persistent STAT3 activation is observed under IM treatment and supported by the microenvironment via JAK1 thus promoting LSC survival. As a consequence, JAK1 emerges as a potential therapeutic target for curative CML therapies.

S1552

MS4A3 REGULATES CELL SURFACE CYTOKINE RECEPTOR EXPRESSION, DIFFERENTIATION, AND DRUG RESISTANCE IN CHRONIC MYELOID LEUKEMIA

A. Eiring[1,*], J. Ahmann[1], J.-Y. Hwang[1], A. Senina[1], B. Helton[2], J. Khorashad[1], A. Pomicter[1], M. Zabriskie[1], A. Agarwal[3], R. Bell[1], C. Mason[1], H. Redwine[1], A. Bowler[1], P. Clair[1], S. Mc Weeney[3], V. Oehler[2], S. Varambally[4], J. Radich[2], K. Varley[1], T. O'Hare[1], M. Deininger[1]

[1]Huntsman Cancer Institute, The University of Utah, Salt Lake City, [2]Fred Hutchinson Cancer Research Center, Seattle, [3]Oregon Health & Science University, Portland, [4]University of Alabama Birmingham, Birmingham, United States

Background: We have previously demonstrated that the transcriptional profile of diagnostic CD34$^+$ cells from chronic phase chronic myeloid leukemia (CP-CML) patients exhibiting primary resistance to imatinib overlaps with that of patients with myeloid blast phase CML (BP-CML) (McWeeney *et al. Blood* 2010). These commonalities suggest that that primary TKI resistance and advanced disease are biologically related. Reduced expression of the hematopoietic cell cycle regulator, MS4A3, was identified as a principal component of the gene expression classifier predicting imatinib response.
Aims: The aim of this study was to identify the functional consequence of reduced MS4A3 expression in TKI resistance and blastic transformation of CML.
Methods: To assess the functional role of MS4A3 in CML and TKI response, we used ectopic MS4A3 expression and shRNA-mediated MS4A3 knockdown in CD34$^+$ cells from BP-CML and CP-CML patients, respectively. The phenotypic consequence of altered MS4A3 expression was measured using colony formation assays, apoptosis assays, long-term culture-initiating cell assays, and xenografts into NSG recipient mice.
Results: Low MS4A3 correlated not only with primary TKI resistance, but also with shorter overall survival in CP-CML (n=35). Microarray (n=19 CP-CML; n=16 BP-CML), qRT-PCR (n=22 CP-CML; n=17 BP-CML), and immunoblot (n=3 CP-CML; n=3 BP-CML) analyses demonstrated that MS4A3 mRNA and protein levels are reduced in CD34$^+$ progenitor cells from BP-CML *versus* CP-CML patients. Ectopic expression of MS4A3 in BP-CML CD34$^+$ progenitors (n=5) markedly reduced colony formation in the presence and absence of imatinib, consistent with a tumor suppressor role for MS4A3 in CML. While re-expression of MS4A3 alone did not increase apoptosis compared to controls, imatinib-induced apoptosis in BP-CML CD34$^+$ cells was increased by 62%, with no effect on normal CD34$^+$ cord blood cells (n=3). Conversely, shRNA-mediated MS4A3 knockdown (shMS4A3) in CP-CML CD34$^+$ cells (n=7) reduced the effects of imatinib in colony formation and apoptosis assays, with no effect on normal CD34$^+$ progenitors (n=4). These data suggest that MS4A3 positively regulates patient survival and imatinib response in CML progenitor cells. To evaluate MS4A3 in the leukemic stem cell compartment, qRT-PCR revealed that MS4A3 mRNA levels are markedly higher in committed CD34$^+$38$^+$ progenitors compared to more primitive CD34$^+$38$^-$ stem cells, suggesting a role for MS4A3 in differentiation. Flow cytometric analysis revealed that shMS4A3 in CP-CML CD34$^+$ cells reduced CD11b$^+$ cells by ~45% (n=3), which correlated with reduced cell surface expression of KIT, GM-CSFR, IL-3R, and FLT3R. To assess the function of MS4A3 in CML stem cells, we performed long-term culture-initiating cell (LTC-IC) assays and xenografts into NSG mice upon MS4A3 knockdown in CP-CML (n=3). shMS4A3 increased Ph$^+$ LTC-IC colony formation in the presence absence of imatinib. Consistent with these data, shMS4A3 enhanced engraftment of CD34$^+$CD45$^+$GFP$^+$ cells into the bone marrow of NSG recipient mice. DNA bisulfite conversion and patch PCR sequencing revealed that the MS4A3 promoter is highly methylated in CML compared to normal CD34$^+$ cells, but could not explain downregulation in BP-CML or TKI resistance. Rather, downregulation in the latter is due to loss of the myeloid-specific transcription factor, C/EBPbeta.
Summary/Conclusion: Altogether, these data suggest that MS4A3 plays a key role in 1) BCR-ABL1 kinase-independent resistance, 2) progression of CML from the chronic to the blastic phase of disease, and 3) in primitive CML stem cells *versus* progenitors.

S1553

THE COMBINATION OF THE MDM2 ANTAGONIST, IDASANUTLIN WITH NILOTINIB TARGETS PRIMITIVE CHRONIC MYELOID LEUKEMIA (CML) CELLS IN VITRO AND IN VIVO

M. Scott[1,*], C. Clarke[2], F. Warren[2], M. Drotar[2], H. Jorgensen[2], R. Hyde[2], C. Munje[2], R. Kinstrie[1], B. Higgins[3], L.-C. Chen[4], T. Holyoake[2], M. Copland[2], D. Vetrie[1]

[1]Institute of Cancer Sciences, [2]Paul O'Gorman Leukaemia Research Centre, University of Glasgow, Glasgow, United Kingdom, [3]Roche Innovation Centre New York, [4]Roche Innovation Center New York, Roche Pharma Research and Early Development, New York, United States

Background: Treatment with tyrosine kinase inhibitors (TKIs) fails to eradicate leukemia stem cells (LSC) in CML patients necessitating life-long therapy. Our recent work has shown that both p53 and c-Myc control the survival and proliferation of primitive CML cells (Abraham et al, Nature 2016), and that modulating both p53 and c-Myc can target CML LSC. Clinically, modulators of p53 and/or c-Myc are likely to be given in combination with TKI.
Aims: As our ultimate aim is to introduce novel therapies into the clinic that eliminate CML LSC, we examined the effect the MDM2 antagonist,

idasanutlin ('Idasa') has in combination with nilotinib ('Nil') on leukemia cell numbers and LSC *in vitro* and *in vivo*.
Methods: We performed cell counts, apoptosis and cell cycle assays to determine combination indices (CI) and therefore synergy for drug combinations; and confirmed effects on primitive cells by long-term culture-initiating cell (LTC-IC) assay *in vitro*. To further examine the clinical utility of these drugs in CML we utilised our preclinical murine models: a patient derived xenograft (PDX) model and our double transgenic (DTG) SCL-tTA/BCR-ABL model.
Results: At the empirically derived ratio of 30:1 (Nil:Idasa) in primary CML CD34+ cells, the combination was synergistic by both cell count and apoptosis. At this ratio, the Nil/Idasa combination resulted in a significant increase in apoptosis compared to no drug control (NDC) or TKI alone ($p<0.05$; n=3), while no significance was reached for Nil/Idasa against normal samples (n=3). Nil/Idasa also resulted in a significant decrease in colony forming cell (CFC) counts compared to NDC or Nil alone ($p<0.05$; n=3), while no significant difference was seen in normal CD34+ cells. Moreover, by LTC-IC assay, compared to NDC, Nil/Idasa resulted in a significant decrease in output ($p<0.05$, n=3), while no significance was reached with the individual treatments. Again, no significant effect was seen in normal cells. Following transplant and long-term engraftment (12 weeks) of CML CD34+ cells into immunocompromised mice (PDX model), treatment with Idasa and Nil, either alone or in combination, resulted in a decrease in the percentage of human cells engrafted compared to vehicle alone, including the more primitive CD34+38- cells. However, while the percentage of human cells engrafted in the Nil-treated animals increased following 4 weeks recovery without drug, the percentage in the Idasa and combination arms remained the same, or even decreased. This was particularly evident in the more primitive cells, where Nil/Idasa combination resulted in the percentage of CD34+38- cells being significantly lower following recovery than both the vehicle and nil alone arms ($p=0.029$ & $p=0.0107$ respectively; n=4 vehicle / combo, n=3 Nil). In addition, when we treated our DTG CML mouse model with MDM2 antagonist (RG7112) and nil for 4 weeks following leukemia induction, we saw a significant decrease in the percentage of LSK cells in both the bone marrow and spleen with the combination compared to Nil alone ($p<0.01$ for both).
Summary/Conclusion: Overall these data show that pharmacological modulation of p53 in combination with TKI can target the most primitive CML cells, potentially in a synergistic manner. Moreover, recovery data from our PDX model suggests that this combination may abrogate the stem cell expansion which is seen with TKI alone following drug withdrawal, giving hope for treatment-free survival in patients. A planned clinical trial will test the efficacy of idasanutlin in combination with TKI.

S1554

THE MUTATIONAL LANDSCAPE IN HYDROXYCARBAMIDE-RESISTANT/INTOLERANT ESSENTIAL THROMBOCYTHEMIA TREATED ON THE MAJIC-ET STUDY

J. O'Sullivan[1,2,*], A. Hamblin[3], A. Panchal[4], C. Yap[4], S. Fox[4], S. Alimam[5], H. Dreau[3], K. Howard[3], P. Ware[3], M.F. Mc Mullin[6], C.N. Harrison[7], A.J. Mead[8]

[1]Haematopoietic Stem Cell Biology Laboratory, MRC Weatherall Institute of Molecular Medicine, Oxford, [2] Department of Hematology, Guy's and St Thomas' NHS Foundation Trust, London, [3]Department of Haematology, Oxford University Hospitals NHS Foundation Trust, Oxford, [4]Cancer Research UK Clinical Trials Unit, University of Birmingham, Birmingham, [5]Department of Hematology, Guy's and St Thomas' NHS Foundation Trust, London, London, [6]Department of Haematology, Queen's University, Belfast, [7]Department of Haematology, Guy's and St Thomas' NHS Foundation Trust, London, [8]John Radcliffe Hospital, Weatherall Institute of Molecular Medicine, Oxford, United Kingdom

Background: MAJIC-ET a phase 2 study evaluated the role of ruxolitinib in hydroxycarbamide resistant/intolerant (HC-RES/INT) essential thrombocythaemia (ET). The impact of mutations in addition to recurrent driver mutations (*JAK2/CALR/MPL*) in RES/INT ET is currently unknown.
Aims: Here we expand the primary MAJIC-ET analysis to evaluate non-driver mutational profiles of MAJIC-ET patients (pts) using an ISO accredited Illumina TruSeq Custom Amplicon Panel, including 31 gene mutation hotspots & exons (~36,000 bp, 287 amplicons), followed by correlation with clinical outcomes.
Methods: Data was censored in January 2017 with 47.3% (n=52) randomised to best available therapy (BAT) & 52.7% (n=58) to ruxolitinib. Baseline clinical parameters including driver mutation status were matched.
Results: Overall, *JAK2*, *CALR* & *MPL* mutations were present in 48.6%, 30.3% & 4.6% of pts with 16.5% reported as "triple-negative". At one-year, responses were equivalent in BAT & ruxolitinib; 44.2% *vs* 46.6%, respectively, p=0.4. Molecular responses were rare, with complete molecular response (CMR) only in two *CALR*- & *JAK2*-mutated ruxolitinib & not BAT-treated pts. Additional mutations at baseline were detected in 29.1%. One additional mutation was present in 20% (n=22) & ≥2 mutations in 9.1% (n=10). The most frequently identified mutation was TET2 (n=14) followed by TP53 (n=8) & SF3B1 (n=7). The latter two detected at higher frequencies than previously reported in ET. TP53 mutations were more frequent in "triple-negative" (16.7%) than in *JAK2/CALR/MPL*-mutated pts (4.4%), p=0.087. Presence of additional mutations was not predictive of responses. Only one pt with a molecular response (partial) had a baseline additional mutation; *JAK2 V617F* co-mutated with *TP53*. Notably, one *CALR*-mutated pt with a CMR subsequently transformed to post-ET myelofibrosis with no additional mutation at baseline; mutational analysis at transformation is underway. Overall survival (OS) at 2 years was 95.7% in BAT & 93.6% in ruxolitinib-treated pts, p=0.264. OS was not affected by driver mutation status/allele burden or additional mutations. Transformation-free survival (TFS) at 2 years was similar in BAT (88.7%, 95% CI 77–94%) & ruxolitinib-treated pts (81%, 95% CI 70–88%), p=0.078. Presence of non-driver mutations was associated with increased risk of transformation (OR 8.1, 95% CI 1.9-33), p=0.003 & predictive of an inferior 2-year TFS of 67.2% (95% CI 54–77%) *vs* 94.2% (95% CI 86–97%) in pts with no additional mutations, p=0.003 (Figure 1). This remained significant on subgroup analysis of BAT-treated (p=0.016) but not for the ruxolitinib-treated pts.Presence of a TP53 mutation was associated with a poorer 2-year TFS: 54% (95% CI 31–72%) in TP53-mutated *vs* 88% (95% CI 80-92%) in TP53 non-mutated pts, p=0.042. TP53 mutations were not associated with prior busulfan/32P/Pipobroman. Presence of an SF3B1 mutation predicted a 2-year TFS of 57.1 (95% CI 34–74%) in SF3B1-mutated *vs* 89% (95% CI 82–93%) in SF3B1 non-mutated pts, p=0.001. High JAK2 allele burden (≥50%) did not predict transformation risk & neither JAK2 allele burden nor presence of additional mutations increased the risk of thrombotic events.

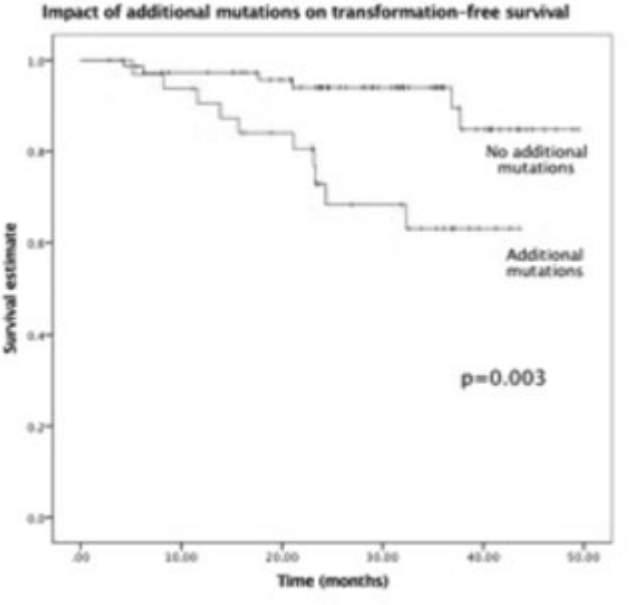


Figure 1.

Summary/Conclusion: We report for the first time a comprehensive mutational analysis of HC-RES/INT ET pts within the context of a clinical trial demonstrating a distinct mutational profile in this cohort. The presence of additional mutations was predictive for adverse TFS & we observed a high prevalence of TP53 & SF3B1 mutations. Our data highlight the clinical/prognostic utility of more extensive mutation screening in HC RES/INT ET.

Myelodysplastic syndromes – Clinical

S1555

A PHASE II CLINICAL TRIAL OF GUADECITABINE (SGI-110) FOR PATIENTS WITH PREVIOUSLY UNTREATED MYELODYSPLASTIC SYNDROME

G. Garcia-Manero[1,*], P. Bose[2], Y. Alvarado[2], G. Borthakur[2], N. Daver[2], F. Ravandi-Kashani[2], E. Jabbour[2], K. Takahashi[2], M. Andreeff[2], T. Kadia[2], C. DiNardo[2], J. Cortes[2], S. Kornblau[2], K. Naqvi[2], C. Benton[2], M. Ohanian[2], M. Konopleva[2], Z. Estrov[2], G. Montalban Bravo[2], S. Pierce[2], K. Bodden[2], H. Kantarjian[2]

[1]Leukemia, [2]MD Anderson Cancer Center, Houston, United States

Background: The hypomethylating agents (HMA) azacitidine and decitabine are the standard of care for most patients with higher risk myelodysplastic syndromes (MDS). Guadecitabine (SGI-110) is a next generation dinucleotide HMA with activity in patients with acute myelogenous leukemia and relapsed/refractory MDS. Here, we present results from a prospective ongoing single arm phase II trial for patients with previously untreated MDS.
Aims: The aim of this trial is to study the safety and clinical activity of guadecitabine in patients with previously untreated MDS.
Methods: Guadecitabine is administered at a dose of 60 mg/m2 subcutaneous daily x 5 days every 28 days. Supportive care measures such as transfusions and antibiotic therapy are allowed. Patients with int-2 or high risk MDS by IPSS (or more than 10% marrow blasts) not treated with prior chemotherapy are eligible. Patients could have received up to 1 cycle of prior HMA. Other inclusion criteria include age older than 17 years, adequate performance and renal and hepatic functions. Patients sign informed consent following institutional guidelines. The primary endpoint is achievement of a complete remission (CR). The study is designed with stopping rules for predetermined CR (stop if CR less than X in cohorts of 10 patients) or excess toxicity (cohorts of 5 patients). CR is defined as blasts less than 5% with complete peripheral blood recovery. Overall response rate is calculated using IWG 06 criteria. Toxicity is graded using CTCAE 4.0. Up to 100 patients are planned to be enrolled.
Results: In summary, 83 patients have been treated in this study. Median follow up is 8.6 months (range 1-38). Median age is 69 years (range 22 to 90) and 43% are older than 70 years. 49 patients (59%) are male. 13 (16%) have a diagnosis consistent with CMML. 66 (80%) patients have int-2 disease, 10 (12%) higher risk disease and 6 (7%) int-1 with more than 10% blasts. 46 (55%) patients had poor risk complex cytogenetics, 21 (25%) were diploid. An 81-gene NGS panel was used in all patients at baseline. Most frequent mutations by rank include: TET2 in 36 (43%) pts, TP53 in 25 (30%), ASXL1 in 22 (26%), RUNX1 in18 (21%), EZH2 in 17 (20%), DNMT3A in 13 (15%), IDH1 and NRAS in 10 (12%), GATA2 in 6 (7%). Median number of cycles administered is 5 (1 to 27). 70 patients (84%) are evaluable for response. CR was documented in 16 (23%), CRp in 3 (4%) and hematological improvement in 26 (37%) for an ORR of 64%. Complete marrow response with incomplete count recovery was considered as an HI. Median number of cycles to best response is 3 (1-12). Of note, 19 (23%) patients have received less than 3 cycles of therapy. The most common related toxicity has been infection. Only 2 (2%) patients have died during the first six weeks of therapy. Median overall survival is 14 months (1-38+). No association between cytogenetics or poor risk mutations and response was observed.
Summary/Conclusion: Guadecitabine (SGI-110) is clinically active and safe in this cohort of patients with higher risk MDS and poor risk features. Stopping rules have not been met and the study continues to accrue. Randomized trials will be required to compare guadecitabine with azacitidine or decitabine in the front line setting.

S1556

A RANDOMIZED PHASE II STUDY OF STANDARD DOSE AZACITIDINE ALONE OR IN COMBINATION WITH LENALIDOMIDE IN HIGH-RISK MDS WITH A KARYOTYPE INCLUDING DEL(5Q)

B. Rasmussen[1,*], L. Nilsson[2], M. Jädersten[3], M. Tobiasson[3], H. Garelius[4], J.M. Nørgaard[5], I. Dybedal[6], K. Grønbaek[7], A.O. Kittang[8], F. Lorenz[9], F. Eberling[10], M. Flogegård[11], C.W. Marcher[12], E. Ejerblad[13], A.Ö. Fernström[14], E. Bernard[15], G. Göhring[16], E. Papaemmanuil[15], L. Saft[17], E. Hellström-Lingberg[3], L. Möllgård[4]

[1]Department of Medicine, School of Medical Sciences, Örebro University, Örebro, [2]Department of Hematology, Oncology and Radiation Physics, Skåne University Hospital, Lund, [3]Center for Hematology and Regenerative Medicine, Department of Medicine Huddinge, Karolinska Institutet, Karolinska University Hospital, Stockholm, [4]Section for Haematology and Coagulation, Department of Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden, [5]Department of Haematology, Aarhus University Hospital, Aarhus, Denmark, [6]Department of Hematology, Oslo University Hospital, Oslo, Norway, [7]Department of Hematology, Rigshospitalet, Copenhagen, Denmark, [8]Division for Hematology, Department of Medicine, Haukeland University Hospital, Bergen, Norway, [9]Department of Medical Biosciences, Umeå University, Umeå, Sweden, [10]Deptartment of Medicine, Division of Haematology, Helsinki University Central Hospital, Helsinki, Finland, [11]Department of Medicine, Falu Lasarett, Falun, Sweden, [12]Odense University Hospital, Odense, Denmark, [13]Department of Medical Science, Section of Hematology, Uppsala University, Uppsala, [14]Department of Medicine Huddinge, Center for Hematology and Regenerative Medicine (HERM), Karolinska Institutet, Stockholm, Sweden, [15]Memorial Sloan Kettering Cancer Center, New York, United States, [16]Department of Human Genetics, Hannover Medical School, Hannover, Germany, [17]Department of Pathology, Karolinska University Hospital, Solna, and Karolinska Institutet, Stockholm, Sweden

Background: Patients with high-risk MDS with 5q deletion have a poor prognosis and there is a need for improvement of the current standard azacitidine (AZA) treatment. Lenalidomide (LEN) is an effective treatment for patients with lower-risk MDS with del(5q), and we previously showed that monotherapy with high-dose LEN may have antitumor effects also in high-risk del(5q) myeloid disease. We hypothesized that an upfront combination of AZA and high-dose LEN would be more effective than AZA alone for patients with del(5q) and an approved indication for azacitidine treatment according to the EMA label.
Aims: We designed a Nordic MDS group prospective multicentre randomized phase II trial and evaluated the efficacy and safety of AZA +/- LEN.
Methods: Consecutive patients with high-risk MDS (IPSS INT-2 and high) and AML with multilineage dysplasia and 20-29 % blasts (previous RAEB-t) with a karyotype including del(5q) were included. Patients were randomized to standard dose of AZA 5-2-2 (75 mg/m^2/ d sc.) q 4 weeks for 6 cycles, or the same schedule of AZA+LEN. The initial dose of LEN was 10 mg daily 21/28 days. If well tolerated the dose was increased to 25 mg daily during cycle 4-6. The primary end point was response according to international working group (IWG) criteria. Secondary endpoints encompassed safety, AZA cycle interval between groups and survival. Informed consent was obtained.
Results: Seventy-two patients, from 12 centers in Sweden, Denmark, Norway and Finland were included between March 2012 and Jan 2017. Thirty-six patients were randomized to each arm. Median age was 71.5 years (35-84 yrs.). Thirty patients (41%) were female. Fifty-two (75%) patients were diagnosed with MDS and 18 (25%) patients with AML. According to IPSS the cytogenetic risk group was good in 8 patients (11%), intermediate in 4 patients (6%) and poor in 60 patients (83%). Serious adverse events were similar in the two groups. 47 patients (65%) completed 3 cycles and 40 patients (56%) completed the total treatment period. There was no difference in AZA cycle interval between the two groups. In the AZA+LEN arm, 7 out of 36 patients (19%), increased the lenalidomide dose to 25 mg/day during cycle 4-6. The overall response rate (ORR) was 36% for patients receiving AZA alone and 28% for AZA+LEN (P=0.85) and the corresponding marrow CR rates were 28% and 36%, respectively (P=0.45). At follow-up at 7 months (range, 0 to 36 months) after the last patient completed the trial the median survival was 14 months for patients receiving AZA and 10 months for the AZA+LEN arm (P=0.18). Nine patients (25%) in the AZA arm and eight patients (22%) in the AZA+LEN arm had a hematological improvement (P=0.81). Cytogenetic response (karyotype showing CR and PR) was achieved in 17% in the AZA arm and 28% in the AZA+LEN arm (P=0.13). Corresponding FISH analysis showed 39% in each group. After 3 cycles there was a trend towards a better cytogenetic response rate (FISH) in the AZA+LEN arm, 50% and 36%, respectively (P=0.052).
Summary/Conclusion: This is the first prospective randomized clinical trial in higher-risk MDS with a defined cytogenetic lesion. In this study with high-risk MDS and AML patients with del(5q) and a dismal prognosis the addition of LEN to standard AZA treatment did not improve overall response or survival but may indicate a stronger antitumor response after three cycles.

S1557

IMETELSTAT IN RBC TRANSFUSION-DEPENDENT (TD) LOWER RISK MDS RELAPSED/REFRACTORY TO ERYTHROPOIESIS-STIMULATING AGENTS (ESA) (IMERGE): UPDATED EFFICACY AND SAFETY

P. Fenaux[1,*], A. Raza[2], E. Vellenga[3], U. Platzbecker[4], V. Santini[5],

I. Samarina[6], K. Van Eygen[7], M. Díez-Campelo[8], M.M. Patnaik[9], L.J. Sherman[10], L. Sun[11], H. Varsos[11], E. Rose[11], A. Rizo[11], D.P. Steensma[12]

[1]Hôpital St Louis, Paris, France, [2]Columbia Presbyterian, New York, NY, United States, [3]UMCG, Groningen, Netherlands, [4]Universitätsklinikum Carl Gustav Carus Dresden, Dresden, Germany, [5]AOU Careggi, University of Florence, Florence, Italy, [6]Emergency Hospital of Dzerzhinsk, Nizhny Novgorod, Russian Federation, [7]AZ Groeninge – Oncology Centre, Kortrijk, Belgium, [8]Hosp. Clinico Univ. De Salamanca, De Salamanca, Spain, [9]Mayo Clinic Rochester, Rochester, MN, [10]Janssen Research & Development, LLC, Spring House, PA, [11]Janssen Research & Development, LLC, Raritan, NJ, [12]Dana-Farber Cancer Institute, Boston, MA, United States

Background: Patients with lower-risk MDS that is relapsed/refractory to ESA have few treatment options. The first-in-class telomerase inhibitor imetelstat, which targets cells with short telomere lengths and active telomerase, has the potential to provide clinical benefit in this patient population.
Aims: Here we report updated safety and efficacy data from IMerge, an ongoing 2-part, global, phase 2/3 study of imetelstat in RBC TD patients with IPSS Low or Intermediate-1 (Int-1) risk MDS.
Methods: The IMerge population includes patients with MDS relapsed/refractory to ESA with a transfusion requirement of ≥4 units over 8 weeks prior to entry and ESA-naïve patients with sEPO >500 mU/mL. In Part 1 (open-label, single-arm), imetelstat 7.5 mg/kg was administered IV every 4 weeks (escalation to 9.4 mg/kg permitted after 3 cycles). The primary endpoint is the rate of RBC transfusion-independence (TI) lasting ≥8 weeks; secondary endpoints include safety, ≥24-week TI rate, time to and duration of TI, and hematologic improvement (HI) rate. Updated results for the first 32 patients enrolled in Part 1 (median follow up, 75.3 weeks) are reported here.
Results: Median age was 68.5 years. 59% of patients were IPSS Low and 41% Int-1. 13 patients (43%) had sEPO>500 mU/mL. 34% had a cytogenetic abnormality, including 22% with del(5q). Prior MDS treatments included ESAs (88%), lenalidomide (38%), and decitabine or azacitidine (HMAs) (25%); 41% were both lenalidomide and HMA naïve and non-del(5q). As of Jan 2018, RBC-TI ≥8-week was achieved in 38% of patients. Median time to onset of TI was 8 weeks with a median duration of TI of 23 weeks. 16% of patients achieved ≥24-week TI, and 63% achieved erythroid HI. Of 13 lenalidomide and HMA naïve and non-del(5q) patients, 54% achieved ≥8-week TI. Median time to onset of TI in the subset was 8 weeks with a median duration of TI of 43 weeks. 31% of these patients had ≥24-week TI, and 69% achieved erythroid HI. TI response did not differ based on the presence of ringed sideroblasts or baseline sEPO level (41% [7/17] ≤500 mU/L; 38% [5/13] >500 mU/L). Cytopenias, particularly neutropenia and thrombocytopenia, were the most frequently reported adverse events (AEs) overall and in the lenalidomide/HMA naive and non-del(5q) subset. The subset had a lower incidence of gr ≥3 neutropenia relative to the overall population (54% *vs* 66%) but a higher rate of gr ≥3 thrombocytopenia (62% *vs* 53%). Gr 4 neutropenia incidences were 41% overall and 38% for the subset. Overall, 28 patients (88%) had reversible LFT elevations by at least one grade. Four patients had gr 3 worsening of AST and/or ALT (1 also had gr 3 worsening of bilirubin), all of which were reversible. Eleven patients received growth factors during the study for treatment of an AE or ongoing medical history (n=10) or as prophylaxis (n=1).
Summary/Conclusion: Safety and efficacy reported here support continued investigation of imetelstat using the current dosing regimen of 7.5 mg/kg every 4 weeks in IPSS Low/Int-1 RBC TD MDS patients relapsed/refractory to ESA. AEs (mostly cytopenias) were predictable, manageable, and reversible. TI was observed in 38% and erythroid HI in 63% of patients with imetelstat therapy, with a 16% durable 24-week TI rate. Patients naïve to lenalidomide and HMAs and who lacked del(5q) had a 54% 8-week TI rate and responses were more durable (31% 24-week TI rate). To further validate these findings, additional patients meeting these criteria have been enrolled and are currently being followed.

S1558

PHASE 1B/2 COMBINATION STUDY OF APR-246 AND AZACITIDINE (AZA) IN PATIENTS WITH TP53 MUTANT MYELODYSPLASTIC SYNDROMES (MDS) AND ACUTE MYELOID LEUKEMIA (AML)

D. Sallman[1,*], A. Dezern[2], K. Sweet[1], D. Steensma[3], T. Cluzeau[4], M. Sekeres[5], G. Garcia-Manero[6], G. Roboz[7], A. Mc Lemore[1], K. Mc Graw[1], J. Puskas[1], L. Zhang[1], C. Bhagat[8], A. Graber[8], N. Al Ali[1], E. Padron[1], R. Tell[9], J. Lancet[1], P. Fenaux[10], A. List[1], R. Komrokji[1]

[1]Malignant Hematology Department, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, [2]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD, [3]Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, United States, [4]Hematology Department, Cote D'azur University, Nice Sophia Antipolis University, Nice, France, [5]Department of Hematology and Medical Oncology, Cleveland Clinic, Cleveland, OH, [6]Department of Leukemia, MD Anderson Cancer Center, Houston, TX, [7]Weill Cornell Medical College, New York, NY, [8]Medical Affairs, Genoptix, Inc., Carlsbad, CA, United States, [9]Aprea Therapeutics, Stockholm, Sweden, [10]Hospital St Louis, Paris [7] University, Paris, France

Background: *TP53* mutant (m*TP53*) MDS and AML represent a distinct molecular cohort with poor outcomes. Hypomethylating agents (HMA) have emerged as preferred treatment for these patients with CR rate of 20-30% and median OS of 6-12 months. APR-246 is a mutant p53 activator with single agent activity in m*TP53* AML. We report preliminary phase 1b results of APR-246 + AZA in m*TP53* MDS/AML.
Aims: To evaluate the safety, recommended Phase 2 dose, preliminary activity and minimal residual disease (MRD) status of APR-246 in combination with azacitidine in m*TP53* MDS/AML.
Methods: Eligible pts included HMA naïve m*TP53* MDS and oligoblastic AML (≤30% blasts) ≥18 years of age. Pts received APR-246 in a 3+3 dose escalation design (50, 75, 100 mg/kg lean body weight) IV daily over 4 days in a lead-in phase (days -14 to -10) followed by the same dose of APR-246 (days 1-4) + AZA 75 mg/m^2 SC/IV over 7 days (days 4-10 or 4-5 and 8-12) in 28 day cycles. Primary objective was safety with AEs graded by CTCAE v4.03 and DLT assessment over 6 weeks. Secondary endpoints included response by IWG 2006 criteria as well as serial next generation sequencing (NGS) and p53 IHC for evaluation of clonal suppression and remission depth. For MRD analysis, a custom target-capture NGS assay was developed using unique molecular Identifiers for error correction with a limit of detection defined as ≥5 mutant reads and ≥1000 read depth.
Results: As of Feb 12th, 2017, 9 pts (33% male; median age 65 years (39-73)) have enrolled with 3 pts per cohort. Three pts had AML-MRC and 6 had MDS; all pts had poor risk cytogenetics (11% poor, 89% very poor) and higher risk disease by IPSS-R (22% high, 78% very high). Median BM blasts were 18% (4-30). Seven pts (78%) remain on study: 2 pts in the 50mg/kg cohort discontinued treatment (Tx), 1 pt due to infection during C2 who later died of sepsis unrelated to Tx, and 1 pt electively discontinued in durable marrow CR (mCR) after 5 cycles of therapy. Median time on study is 133 days (41-248). Tx related AEs during the lead-in phase (all G1) included ataxia (n=1), dizziness (n=1), nausea (n=2) and neuropathy (n=2). AEs occurring in > 1 pt included dizziness (n=3), nausea (n=6), neutropenia (n=6), thrombocytopenia (n=3), infection (n=4), headache (n=2), pain (n=3), weakness (n=2), xerostomia (n=2), mucositis (n=2), falls (n=3), neuropathy (n=4), and ataxia (n=2); all G1/G2 except neutropenia/thrombocytopenia (G4). No Tx-related SAEs or DLTs have occurred to date. Five of six pts were response evaluable with 1 pt discontinuing tx prior to 1st disease assessment. ORR by IWG was 100% with 4 CR (80%, 3/3 in DL2) and 1 mCR. All CR patients achieved complete cytogenetic response. One CR patient achieved a mCR and partial cytogenetic response after APR-246 lead-in prior to combination therapy. All CR pts had high p53 positivity by IHC at baseline (55-70%) which normalized on serial assessment (<5%). Serial NGS with a variant allele frequency (VAF) cutoff of 5% was negative in 80% of patients (4/5). In pts negative on serial NGS, MRD analysis was performed with median *TP53* VAF at maximum clearance of 0.8% (not detectable-3.4%) with 1 pt achieving MRD negativity after cycle 6 and 1 pt with VAF of 0.2% after cycle 3. The remaining 3 pts entered the study in Dec 17/Jan 18 with no response data available at data cutoff.

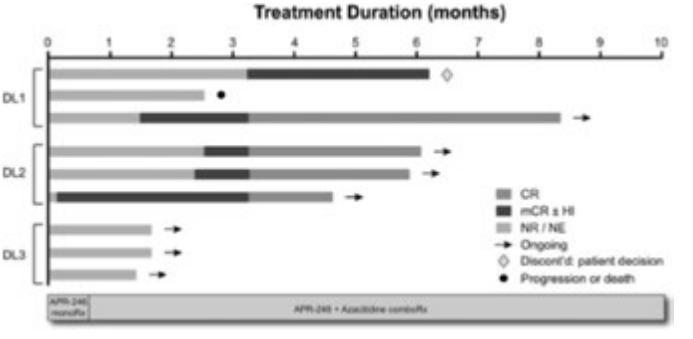


Figure 1.

Summary/Conclusion: APR-246 + AZA combination is well tolerated in m*TP53* MDS/AML. Responses have been achieved in all pts (80% CR) accompanied by deep molecular remissions. The maximum tolerated dose has not been reached and dose escalation is ongoing.

S1559

A MULTICENTER, RANDOMIZED, DOUBLE-BLIND PLACEBO-CONTROLLED STUDY OF DARBEPOETIN ALFA FOR THE TREATMENT OF ANEMIC PATIENTS WITH LOW OR INTERMEDIATE-1 RISK MYELODYSPLASTIC SYNDROME

U. Platzbecker[1,*], E.N. Oliva[2], A. Symeonidis[3], M. Delforge[4], J. Mayer[5], J.S. Goede[6], A. Guerci-Bresler[7], S. Badre[8], B.P. Mehta[8], C. De Oliveira Brandao[8]

[1]University Hospital Carl Gustav Carus Dresden, Medizinische Klinik und Poliklinik I, Dresden, Germany, [2]Azienda Ospedaliera Bianchi-Melacrino-Morelli, Reggio Calabria, Italy, [3]University of Patras Medical School, Patras, Greece, [4]University Hospital Leuven, Leuven, Belgium, [5]University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic, [6]University Hospital and University of Zürich, Zürich, Switzerland, [7]CHU Brabois Vandoeuvre, Nancy, France, [8]Amgen Inc., Thousand Oaks, United States

Background: Many patients (pts) with lower-risk myelodysplastic syndrome (MDS) experience anemia. Transfusion is a treatment option; however, it is often associated with iron overload and other adverse effects. Erythropoiesis-stimulating agents (ESAs) are recommended to treat anemia in pts with MDS; however, long-acting ESAs, such as darbepoetin alfa (DAR), have limited phase 3 data supporting this indication.
Aims: To assess long-term follow-up (LTFU) and survival in anemic pts with low or int-1–risk MDS who received DAR or placebo (PBO).
Methods: This is the final analysis from the LTFU of a phase 3, randomized (2:1), PBO-controlled trial (NCT01362140) that enrolled European pts with anemia and low/int-1 IPSS–risk MDS. Pts had not previously received ESAs or disease-modifying agents for MDS. Primary analysis results have been published (Platzbecker *et al.* Leukemia 2017). The study comprised a 3-wk screening period and a 24-wk double-blind (DB) treatment period; pts subsequently crossed over to a 48-wk open-label (OL) treatment phase and then LTFU for up to 3 years on study during which physicians could prescribe any therapy. Pts received DAR 500 µg Q3W, adjusted to maintain hemoglobin at 11–12 g/dL. Endpoints were incidence of RBC transfusion and IWG 2006 erythroid response in the DB and OL phases, and survival and disease progression to acute myeloid leukemia (AML) in LTFU.
Results: 98 pts received DAR and 48 received PBO. 55% were men and mean age was 72 years. In the DB phase, transfusion rates were 36% for DAR and 59% for PBO; erythroid response rates were 15% for DAR *vs* 0% for PBO (P=0.016). There were 41 deaths during the study; 27 pts (28%) in the DAR group and 14 pts (29%) in the PBO group. Most pts died during LTFU (DAR, 93% and PBO, 79%) and were int-1 at baseline (DAR, 56% and PBO, 71%). Survival curves are presented (**Fig 1A**). 10 pts progressed to AML (**Table 1.**). Median time to AML progression was not reached in either treatment group (**Fig 1B**).
Summary/Conclusion: In this study, there were no differences in rates of death (mostly reported in LTFU) or AML between DAR and PBO arms. Pts with int-1 risk MDS had worse prognosis. Findings are limited because the study was not powered to detect differences in survival and pts could receive any therapy of physician's choice in LTFU. In conclusion, DAR is a safe treatment and lowered transfusion rates associated with anemia of low/int-1 risk MDS.

Table 1. Pts who progressed to AML during the study.

Treatment group (Pt response per MDSAC)	IPSS at baseline	MDS duration at baseline, days	Highest DAR dose	Day of first progression to AML	Study period
PBO	int-1	82	0	46	DB
PBO	int-1	208	0	175	LTFU
PBO	int-1	91	Q3W	379	LTFU
DAR	int-1	61	Q3W	64	DB
DAR	low	247	Q3W	146	DB
DAR	int-1	85	Q3W	195	LTFU
DAR	int-1	76	Q3W	254	OL
DAR (pt had response from wks 37–45)	int-1	80	Q2W	489	OL
DAR	int-1	191	Q2W	788	LTFU
DAR (pt had response from wks 10–37)	low	296	Q2W	743	LTFU

Figure 1. Survival and Time to AML Progression

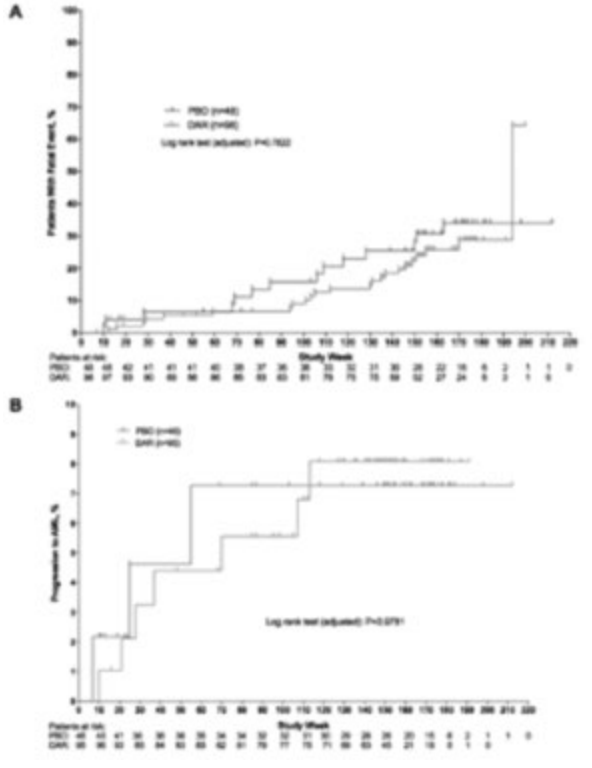


Figure 1.

IDHm and BCL2 inhibitors in AML

S1560

IVOSIDENIB (AG-120) IN MUTANT IDH1 RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA: RESULTS OF A PHASE 1 STUDY

D.A. Pollyea[1,*], C.D. Di Nardo[2], S. de Botton[3], E.M. Stein[4], G.J. Roboz[5], A.S. Mims[6], R.T. Swords[7], J.K. Altman[8], R.H. Collins[9], G.N. Mannis[10], G.L. Uy[11], W.B. Donnellan[12], A. Pigneux[13], A.T. Fathi[14], H. Liu[15], B. Wu[15], E.C. Attar[15], M.S. Tallman[4], R.M. Stone[16], H.M. Kantarjian[2]

[1]University of Colorado School of Medicine, Aurora, [2]University of Texas MD Anderson Cancer Center, Houston, United States, [3]INSERM U1170, Institut Gustave Roussy, Villejuif, France, [4]Memorial Sloan Kettering Cancer Center, [5]Weill Cornell Medical College, New York, [6]Ohio State University Wexner Medical Center, Columbus, [7]Sylvester Comprehensive Cancer Centre, Miami, [8]Northwestern University, Chicago, [9]University of Texas Southwestern Medical Centre, Dallas, [10]UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, [11]Washington University School of Medicine, St Louis, [12]Sarah Cannon Research Institute, Nashville, United States, [13]CHU de Bordeaux, Bordeaux, France, [14]Massachusetts General Hospital Cancer Center, Boston, [15]Agios Pharmaceuticals, Inc., Cambridge, [16]Dana-Farber Cancer Institute, Boston, United States

Background: Ivosidenib (IVO; AG-120) is an oral, targeted inhibitor of mutant isocitrate dehydrogenase 1 (mIDH1) that is being evaluated in a phase 1 dose escalation and expansion study of mIDH1 advanced hematologic malignancies (NCT02074839).
Aims: To report updated efficacy and safety data from all patients with relapsed/refractory acute myeloid leukemia (R/R AML) receiving IVO 500 mg once daily (QD).
Methods: All patients provided written informed consent. The primary efficacy endpoint was the CR+CRh rate (complete remission [CR] according to modified IWG 2003 criteria plus CR with partial hematologic recovery [CRh]). CRh was defined as absolute neutrophil count >0.5×10^9/L *and* platelet count >50×10^9/L. The overall response rate (ORR) comprised CR, CR with incomplete hematologic or platelet recovery, partial response, and morphologic leukemia-free state. The data cutoff date for this analysis was Nov 10, 2017.
Results: A total of 258 patients were treated with IVO. Among 179 R/R AML patients who received IVO 500 mg QD, 17 (9.5%) remained on treatment at data cutoff. In R/R AML patients, the CR+CRh rate was 31.8% (95% CI: 25.1%, 39.2%), including CR in 24.0% (95% CI: 18.0%, 31.0%). Median duration of CR+CRh was 8.2 months (95% CI: 5.6, 12.0), and median duration of CR was 10.1 months (95% CI: 6.5, 22.2). The ORR was 41.9% (95% CI: 34.6%, 49.5%). Treatment was well tolerated; the most common adverse events (AEs) of any grade, irrespective of causality and occurring in ≥25% of 179 R/R AML patients were diarrhea (33.5%), leukocytosis (31.3%), nausea (31.3%), febrile neutropenia (29.1%), fatigue (28.5%), and electrocardiogram QT prolonged (25.7%). The majority of these AEs were grade 1–2 and unrelated to treatment. IDH differentiation syndrome (IDH-DS) was reported in 19 of 179 (10.6%) patients, including grade ≥3 IDH-DS in 9 (5.0%); study drug was held owing to IDH-DS in 6 patients (3.4%), and no instances of IDH-DS led to dose reduction, permanent treatment discontinuation, or death. Updated mutation clearance results will be provided.
Summary/Conclusion: In a high-risk, molecularly defined R/R AML patient population, IVO induced durable remissions and was well tolerated. Studies in previously untreated AML populations are ongoing.

S1561

ENASIDENIB MONOTHERAPY IS EFFECTIVE AND WELL-TOLERATED IN PATIENTS WITH PREVIOUSLY UNTREATED MUTANT-IDH2 (MIDH2) ACUTE MYELOID LEUKEMIA (AML)

D.A. Pollyea[1,2,*], M.S. Tallman[3,4], S. de Botton[5,6], C.D. Di Nardo[7], H. Kantarjian[7], R. Collins[8], A.S. Stein[9], Q. Xu[10], A. Tosolini[10], J. Van Oostendorp[10], S.V. Agresta[11], E.M. Stein[3,4]

[1]University of Colorado School of Medicine, [2]University of Colorado Denver, Aurora, [3]Memorial Sloan Kettering Cancer Center, [4]Weill Cornell Medical College, New York, United States, [5]Gustave Roussy, Villejuif, [6]Univ Paris Sud, Université Paris-Saclay, Le Kremlin-Bicêtre, France, [7]The University of Texas MD Anderson Cancer Center, Houston, [8]University of Texas Southwestern Medical Center, Dallas, [9]City of Hope Comprehensive Cancer Center, Duarte, [10]Celgene Corporation, Summit, [11]Agios Pharmaceuticals, Inc., Cambridge, United States

Background: Enasidenib (AG-221) is an oral inhibitor of mIDH2 proteins. In the phase 1/2 AG221-C-001 trial, enasidenib was associated with an overall response rate (ORR) of 40.3% and median overall survival (OS) of 9.3 months in patients (pts) with relapsed/refractory m*IDH2*-AML (Stein, *Blood*, 2017). Older pts with untreated AML who are not candidates for standard induction chemotherapy due to advanced age, poor performance status, comorbidities, poor-risk cytogenetics, or other factors, pose a therapeutic challenge.
Aims: Determine clinical outcomes for older pts with previously untreated m*IDH2* AML receiving enasidenib monotherapy in the AG221-C-001 study (NCT01915498).
Methods: Pts aged ≥60 years with previously untreated AML who were not candidates for standard treatment (Tx), with ECOG performance status scores of 0-2 were eligible. Pts in the phase 1 dose-escalation received enasidenib doses of 50-650 mg/day, and all pts in the phase 1 expansion and phase 2 received enasidenib 100 mg/day, in continuous 28-day Tx cycles. Response was assessed per modified IWG response criteria for AML. Safety was assessed by Tx-emergent adverse event (TEAE) reporting.
Results: Of all 345 study pts, 39 (11%) had previously untreated m*IDH2* AML. At data cutoff (1 Sep 2017), 3 pts in complete remission (CR) continued to receive study drug, 2 pts in cycle 27 and 1 in cycle 35. At entry, median age was 77 years (range 58-87), 26% of pts had NCCN poor-risk cytogenetics, and 22 pts (56%) had an antecedent hematological disorder (AHD), including 17 with prior diagnosis of MDS. Median time from diagnosis was 1.0 month (range 0.1-4.7). Median number of enasidenib Tx cycles was 6.0 (range 1-35). Seven pts (18%) attained CR; estimated median durations of CR and of any response were not reached (NR) (**Table**). ORR was 30.8% (95%CI 17.0, 47.6). Times to first and best responses were 1.9 and 3.7 months, respectively. Three pts proceeded to transplant and were alive at data cutoff. At median follow-up of 8.4 months, median OS was 11.3 months (95%CI 5.7, 15.1) and event-free survival was 5.7 months (2.8, 16.0). Median OS in responding patients (n=12) was NR (95%CI 10.4, NR). The most frequent TEAEs (any grade or cause) were fatigue (44%), decreased appetite (41%), and nausea and constipation (38% each). The most frequent Tx-related TEAEs were hyperbilirubinemia (31%) and nausea (23%). The only serious Tx-related TEAEs reported for >1 pt were IDH differentiation syndrome (n=5, 13%) and tumor lysis syndrome (n=2, 5%). Tx-related TEAEs led to dose modifications for 3 pts (8%), dose interruptions for 9 pts (23%), and Tx discontinuation for 2 pts (5%).

Table 1.

Enasidenib Efficacy in Patients with Previously Untreated *mIDH2* AML (N=39)	
Overall response rate (ORR),* *n* (%)	**12 (30.8)**
ORR 95%CI	17.0, 47.6
Best response, *n* (%)	
CR	7 (18)
CRi/CRp	1 (3)
PR	2 (5)
MLFS	2 (5)
Stable Disease,† *n* (%)	19 (49)
Disease Progression, *n* (%)	1 (3)
Not evaluable,‡ *n* (%)	7 (18)
Overall survival, months, median (95% CI)	11.3 (5.7, 15.1)
Event-free survival, months, median (95% CI)	5.7 (2.8, 16.0)
Time to CR, months, median (range)	5.6 (3.4-12.9)
Duration of CR, months, median (95% CI)	NR (3.7, NR)
Time to first response, months, median (range)	1.9 (1.0-3.8)
Time to best response, months, median (range)	3.7 (1.0-12.9)
Duration of any response, months, median (95% CI)	NR (7.4, NR)

*Overall response rate included complete remission (CR), CR with incomplete count recovery (CRi/CRp), partial remission (PR), and morphologic leukemia-free state (MLFS), per modified IWG 2003 response criteria for AML
†Failure to achieve a response but not meeting criteria for progressive disease for a period of ≥8 weeks
‡Due to discontinuation before a response assessment
NR, not reached

Summary/Conclusion: Enasidenib induced hematologic responses in approximately one-third of these older pts with previously untreated m*IDH2* AML who were not candidates for standard Tx, and more than

one-half of whom had an AHD. Approximately 1 in 5 of these pts attained CR during enasidenib monotherapy. Responses were durable: median duration of any response was not reached. Median OS was also promising (11.3 months) in this cohort with median age 77 years; pts aged >65 have an estimated median OS of only ~5 months, even when treated (Medeiros, *Ann Hematol*, 2015). Tx-related TEAEs were infrequent and led to discontinuation for only 2 pts. These results suggest enasidenib may benefit older adults with m*IDH2* AML who are not fit to receive cytotoxic regimens. Enasidenib is under study in a similar AML patient population in the Beat AML Master Trial (NCT03013998).

S1562

MUTANT IDH (MIDH) INHIBITORS, IVOSIDENIB OR ENASIDENIB, WITH AZACITIDINE (AZA) IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)

C.D. Di Nardo[1,*], A.S. Stein[2], E.M. Stein[3,4], A.T. Fathi[5,6], A.C. Schuh[7], P. Montesinos[8], O. Odenike[9], H.M. Kantarjian[1], R.M. Stone[6,10], R. Collins[11], G. Martinelli[12,13], M. Arnan Sangerman[14], B. Wu[15], D.O. Koralek[15], J. Van Oostendorp[16], J. Gong[16], K.J. Mac Beth[16], P. Vyas[17]

[1]The University of Texas MD Anderson Cancer Center, Houston, [2]City of Hope Comprehensive Cancer Center, Duarte, [3]Memorial Sloan Kettering Cancer Center, [4]Weill Cornell Medical College, New York, [5]Massachusetts General Hospital Cancer Center, [6]Harvard Medical School, Boston, United States, [7]Princess Margaret Cancer Centre, Toronto, Canada, [8]Hospital Universitari i Politecnic La Fe, Valencia, Spain, [9]University of Chicago Medicine and the University of Chicago Comprehensive Cancer Center, Chicago, [10]Dana-Farber Cancer Institute, Boston, [11]University of Texas Southwestern Medical Center, Dallas, United States, [12]University of Bologna, Bologna, [13]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy, [14]ICO-Hospital Duran i Reynals, Barcelona, Spain, [15]Agios Pharmaceuticals, Inc., Cambridge, [16]Celgene Corporation, Summit, United States, [17]MRC Molecular Haematology Unit and Oxford Biomedical Research Centre; University of Oxford and Oxford University Hospitals, Oxford, United Kingdom

Background: *IDH1* and *IDH2* mutations occur in approximately 20% of patients with AML, with increasing frequency in older patients. Ivosidenib (AG-120) and enasidenib (AG-221) are oral small-molecule inhibitors of mIDH1 and mIDH2 proteins, respectively, both shown to promote myeloid differentiation. As monotherapies, enasidenib and ivosidenib induced clinical responses in patients (pts) with mIDH relapsed/refractory AML. AZA monotherapy prolonged survival *vs* conventional care in older pts with newly diagnosed (ND) AML. AZA reduces DNA methylation by inhibiting DNA methyltransferases, and mIDH inhibitors indirectly reduce DNA methylation by suppressing the oncometabolite, 2-HG, and restoring function to -ketoglutarate-dependent TET family enzymes. *In vitro*, mIDH inhibitor + AZA combinations enhanced cell differentiation and apoptosis.
Aims: Evaluate clinical outcomes with combination mIDH inhibitors + AZA in older pts with ND-AML in an ongoing phase 1b/2 study (NCT02677922).
Methods: Adult pts with m*IDH* ND-AML ineligible for intensive treatment (Tx) and ECOG PS scores ≤2 were eligible. Pts received ivosidenib (mIDH1) 500 mg QD or enasidenib (mIDH2) 100 or 200 mg QD, plus SC AZA 75 mg/m^2 x 7d, in continuous 28d cycles. Response was defined by modified IWG 2003 AML criteria; overall response rate (ORR) included complete remission (CR), CR with incomplete count recovery (CRi/CRp), partial remission (PR), and morphologic leukemia-free state (MLFS).

Table 1.

Grade 3-4 hematological AEs

	IVO 500 mg + AZA (n=11)	ENA 100 mg + AZA (n=3)	ENA 200 mg + AZA (n=3)
	n		
Thrombocytopenia	1	0	1
Anemia	2	0	1
Febrile neutropenia	2	0	1
Neutropenia	1	0	2
Lymphocyte decrease	0	0	1
WBC decrease	0	0	1

Results: At data cutoff (Sep 1, 2017) 17 pts had received ivosidenib 500 mg (n=11) or enasidenib 100 mg (n=3) or 200 mg (n=3) + AZA in the phase 1b portion of the study; 11 pts were ongoing. *Ivosidenib:* Median age was 76 yrs (range 74-82) and 82% of pts had an ECOG PS score of 1. Median number of Tx cycles was 3 (range 1-13). Three pts discontinued Tx, 2 due to progressive disease (PD). AEs in ≥4 pts (any grade) were nausea (n=8), constipation (6), fatigue (5) and diarrhea (4). Grade 3-4 hematological AEs (**Table 1**) occurred at similar frequency to what has been reported for AZA alone. Serious AEs in >1 pt were pneumonia and febrile neutropenia (n=2 each). Eight of 11 pts responded: 4 pts attained CR, 1 CRi, 1 PR, and 2 MLFS. *Enasidenib:* Median age was 68 yrs (range 65-76) and 83% of pts had an ECOG PS score of 1. Median number of Tx cycles was 9 (1-13). Three pts discontinued Tx, 2 due to PD. Most common AEs (any grade) were nausea and hyperbilirubinemia (n=4 each). Serious AEs in >1 pt were pyrexia, bilirubin increase, pneumonia (n=2 each). Four of 6 pts responded: 2 pts achieved CR, 1 PR, and 1 MLFS.
Summary/Conclusion: mIDH inhibitor + AZA regimens were generally well tolerated in these older pts with ND-AML; 65% of pts remained on-study at data cutoff. The most common AEs were grade 1-2 gastrointestinal events and enasidenib-related indirect bilirubin elevations, likely due to off-target inhibition of the UGT1A1 enzyme. Response rates are encouraging. Phase 1b enrollment completed in late 2017; updated data for all 23 ivosidenib and 6 enasidenib pts will be presented, as well as longitudinal changes in m*IDH* variant allele frequencies. Enrollment continues in the phase 2 portion of this study (enasidenib + AZA) and in the phase 3 AGILE study of ivosidenib+ AZA (NCT03173248).

S1563

DURABLE RESPONSE WITH VENETOCLAX IN COMBINATION WITH DECITABINE OR AZACITADINE IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)

C.D. Di Nardo[1,*], K. Pratz[2], J. Potluri[3], V. Pullarkat[4], B.A. Jonas[5], A.H. Wei[6], P.S. Becker[7], O. Frankfurt[8], T. Xu[3], W.-J. Hong[9], B. Chyla[3], D.A. Pollyea[10], A. Letai[11]

[1]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, [2]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, [3]AbbVie, Inc., North Chicago, [4]Department of Hematology and Hematopoietic Cell Transplantation and Gehr Family Center for Leukemia Research, City of Hope National Medical Center, Duarte, [5]University of California Davis Comprehensive Cancer Center, Sacramento, [6]The Alfred Hospital and Monash University, Melbourne, [7]Clinical Research Division, Fred Hutchinson Cancer Research Center and Division of Hematology, Department of Medicine, University of Washington School of Medicine, Seattle, [8]Northwestern University Feinberg School of Medicine, Chicago, [9]Genentech, Inc., South San Francisco, [10]University of Colorado School of Medicine, Aurora, [11]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, United States

Background: Venetoclax (VEN), an oral BCL-2 inhibitor, has synergistic activity when combined with hypomethylating agents.
Aims: This clinical study explores the optimal dose and efficacy of VEN in combination with decitabine (DEC) or azacitadine (AZA) in elderly AML.
Methods: This is an open-label, phase 1b, dose escalation and expansion study (NCT02203773) on the safety and efficacy of VEN, with DEC or AZA, in elderly patients (≥65 years) with untreated AML. VEN was coadministered daily with 20 mg/m^2 of DEC on days 1-5 or 75 mg/m^2 of AZA on days 1-7, each 28 day cycle. VEN was dosed at 400, 800, or 1200 mg in the escalation phase, and 400 or 800 mg in the expansion phase. Complete remission (CR), CR with incomplete blood count recovery (CRi), overall survival (OS) and adverse events (AEs) were evaluated.

Table 1.

Patient subgroup	n	CR/CRi n (%)	Duration of CR/CRi median months	OS
All VEN doses	145	97 (67)	11.3	17.5
Intermediate cytogenetic risk	74	55 (74)	12.9	NR
Poor cytogenetic risk	71	42 (59)	6.7	9.6
Secondary AML	36	24 (67)	NR	NR
Age ≥75 years	62	40 (65)	9.2	11.0
VEN 400 mg				
+ AZA	29	22 (76)	NR	NR
+ DEC	31	22 (71)	12.5	15.2
VEN 800 mg				
+ AZA	37	21 (57)	11.7	14.2
+ DEC	37	27 (73)	9.2	17.5

OS, overall survival; NR, not yet reached (if applicable)

Results: Data cutoff was July 7, 2017. Of 145 patients, 56% were male; the median age was 74 years (range: 65–86). Overall, 60, 74, and 11 patients received VEN at 400, 800, and 1200 mg, respectively. Key grade ≥3 AEs were febrile neutropenia (43%), thrombocytopenia (23%) and neutropenia (16%); pneumonia and bacteremia (all grades) occurred in 18% and 8% of patients, respectively. At 400mg of VEN, the rate of CR+CRi was 73% (76% with AZA and 71% with DEC); efficacy data are in the table. Minimal residual disease (MRD) assessment in marrow aspirates was performed at disease assessment in a central lab using multicolor flow cytometry assay;

overall, 37% (36/97) of patients with CR/CRi had MRD levels below the 10^{-3} cutoff. Median follow up was 15.1 months.
Summary/Conclusion: Preliminary data suggest that 400 mg of VEN has the optimal benefit-risk profile in combination with DEC or AZA, which demonstrated a tolerable safety profile with deep responses and durable outcomes in elderly patients with AML.

S1564

CHEMOTHERAPY AND VENETOCLAX IN ELDERLY AML TRIAL (CAVEAT): A PHASE 1B DOSE ESCALATION STUDY EXAMINING MODIFIED INTENSIVE CHEMOTHERAPY IN FIT ELDERLY PATIENTS

A.H. Wei[1,*], I.S. Tiong[1], A.W. Roberts[2], C.Y. Fong[3], C.C. Chua[1], S.B. Ting[4], J. Nguyen[1], F. Yuen[1], R. Hall[1], S. Fleming[1], J. Reynolds[5]

[1]The Alfred Hospital and Monash University , Melbourne, [2]Department of Haematology, Royal Melbourne Hospital, Parkville, [3]Department of Haematology, Austin Hospital, Heidelberg, [4]Department of Haematology, Box Hill Hospital, Victoria, [5]The Alfred and Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, Australia

Background: Venetoclax (VEN) is a potent, small molecule BH3-mimetic targeting pro-survival BCL-2 with an emerging role for treatment of elderly patients with Acute Myeloid Leukemia (AML) unfit for intensive chemotherapy in combination with low-dose cytarabine (LDAC)(Wei *et al*, ASH 2017) or hypomethylating agents (HMA)(Di Nardo *et al*, Lancet Oncol 2018). The optimal dose of VEN was 600mg and 400mg in the LDAC and HMA studies, respectively. The feasibility of combining VEN with more intensified chemotherapy regimens has yet to be reported.
Aims: To evaluate the optimal dose, safety and efficacy of VEN in combination with dose-modified intensive chemotherapy in older patients with AML.

Table 1.

Table 1. Baseline patient characteristics and treatment outcomes

	VEN 50mg (n=8)	VEN 100mg (n=8)	VEN 200mg (n=9)	VEN 400mg (n=8)	VEN 600mg (n=4)	Total (n=37)
Median age, years (range)	73 (68-80)	70 (65-77)	73 (63-80)	70 (64-78)	70 (67-72)	71 (63-80)
Males, n (%)	3 (38)	6 (75)	7 (78)	2 (25)	4 (100)	22 (59)
ECOG PS 1, n (%)	7 (88)	6 (75)	3 (33)	8 (100)	4 (100)	28/35 (80)
Secondary AML, n (%)[a]	5 (63)	3 (38)	3 (33)	3 (38)	2 (50)	16/37 (43)
Prior therapy, n (%)						
HMA	3 (38)	3 (38)	2 (22)	2 (25)	1 (25)	11/35 (31)
LDAC	-	1 (14)	-	-	-	1/32 (3)
ELN 2010 risk category, n (%)	(n=8)	(n=7)	(n=9)	(n=7)	(n=3)	(n=34)
Favorable	-	-	3 (33)	1 (14)	1 (33)	5 (15)
Intermediate-1	-	1 (14)	-	-	-	1 (3)
Intermediate-2	5 (62)	3 (43)	1 (11)	4 (57)	2 (67)	15 (44)
Adverse	3 (38)	3 (43)	5 (56)	2 (29)	-	13 (38)
Response among evaluable, n (%)	(n=8)	(n=8)	(n=9)	(n=7)	(n=3)	(n=35)
ORR (CR+CRi)	5 (63)	6 (75)	9 (100)	4 (57)	1 (33)	25 (71)
CR	1 (13)	4 (50)	7 (78)	4 (57)	1 (33)	17 (49)
CRi	4 (50)	2 (25)	2 (22)	-	-	8 (23)
MLFS	-	-	-	-	1 (33)	1 (3)
Treatment failure						
- Resistant disease	3 (38)	2 (25)	-	1 (14)	1 (33)	7 (20)
- Other[b]	-	-	-	2 (29)	-	2 (6)
Response not evaluable, n (%)[c]	-	-	-	1 (13)	1 (25)	2 (6)
Hematopoietic recovery after induction, median days (range)[d,e]	(n=7)	(n=6)	(n=9)	(n=4)	(n=1)	(n=27)
- Neutrophils ≥0.5x10⁹/L	32 (25-34)	25 (18-32)	25 (19-30)	24 (20-29)	18	25 (18-34)
- Neutrophils ≥1.0x10⁹/L	32 (26-35)	27 (21-33)	26 (20-35)	26 (21-36)	19	28 (19-36)
- Platelets ≥50x10⁹/L	31 (24-37)	28 (21-42)	22 (19-35)	22 (20-25)	19	23 (19-42)
- Platelets <25x10⁹/L by D42, n (%)	1 (20)	1 (17)	-	-	-	3 (12)
Hematopoietic recovery after first continuation, median days (range)[d,f]	(n=3)	(n=3)	(n=6)	(n=3)	-	(n=15)
- Neutrophils ≥0.5x10⁹/L	23 (23-23)	12 (0-20)	25 (18-32)	29 (29-35)	-	23 (0-35)
- Neutrophils ≥1.0x10⁹/L	23 (23-28)	12 (0-20)	29 (18-47)	35 (29-40)	-	28 (0-45)
- Platelets ≥50x10⁹/L	43 (37-48)	30 (26-34)	32 (21-40)	40 (33-46)	-	35 (21-48)
- Platelets <25x10⁹/L by D42, n (%)	1 (33)	1 (33)	1 (17)	1 (33)	-	4 (27)

[a] Secondary AML includes antecedent myelodysplastic syndrome (n=10), myeloproliferative neoplasm (n=3), therapy-related myeloid neoplasm (n=2), and chronic myelomonocytic leukemia (n=1).
[b] 2 subjects received ≥7 days of VEN but did not commence chemotherapy due to sepsis (n=1) or deterioration of ECOG (n=1).
[c] 1 subject each in cohorts D and E respectively were still receiving induction.
[d] Among patients who achieved CR/CRi. D1 is the first day of chemotherapy (i.e. day 8 of study protocol).
[e] After induction, 1 subject each in cohorts A and B respectively continued therapy without reaching platelets ≥50x10⁹/L.
[f] After first continuation, 1 subject in cohort D did not reach neutrophils ≥1.0x10⁹/L and platelets ≥50x10⁹/L, 1 subject in cohort B did not achieve nadir neutrophil <1.0x10⁹/L.
Abbreviations: AML, acute myeloid leukemia; CR, complete remission; CRp, complete remission without full platelet recovery; CRi, complete remission with incomplete hematologic recovery; ECOG PS, Eastern Cooperative Oncology Group Performance Score; ELN, European LeukemiaNet; HMA, hypomethylating agent; LDAC, low-dose cytarabine; MLFS, morphologic leukemia-free state; ORR, overall response rate; VEN, Venetoclax.

Methods: *Treatment*- Several factors were incorporated to mitigate the risk of hematologic toxicity and tumor lysis syndrome: 1) a 7-day VEN prephase incorporating dose ramp-up to achieve steady state prior to commencing chemotherapy; 2) dose escalation commenced at VEN 50mg (Cohort A), 100mg (B), 200mg (C), 400mg (D) and 600mg (E); 3) during induction, staggered and attenuated (Yoon *et al*, Am J Hematol 2013) addition of cytarabine (100mg/m^2/d continuous IVI d8-12) and idarubicin (12mg/m^2 IV d9-10); 4) a treatment-free VEN phase after d14 to allow hematopoietic recovery and 5) for patients in remission, further therapy with 4 cycles of "continuation" comprising 14 days of VEN at the cohort prescribed dose and bolus cytarabine 100mg/m^2 IV (days 8-9) and idarubicin 12mg/m^2 IV (day 8). After continuation, up to 7 cycles of VEN maintenance monotherapy were permitted. Antifungal azoles were prohibited during the period of VEN exposure in cohorts A-E. *Eligibility- De novo* or secondary AML (excluding APL), age ≥65 years (unless ≥60yo with monosomal karyotype), ECOG 0-1, adequate organ function, WCC <25x10^9/L, prior therapy for MDS/AML with HMA/LDAC was permitted after a 14-day washout. The first patient was enrolled on 17JUL2016. *Dose-limiting toxicity (DLT)*- Grade 4 neutropenia or thrombocytopenia lasting >42d after starting chemotherapy unrelated to residual AML/MDS and severe marrow hypocellularity <10%; other grade 4 toxicity related to VEN. *Response*- ELN 2010 response criteria.
Results: Data cut-off date was 13FEB2018. 37 patients were enrolled with 35 evaluable for response. Table 1 summarizes baseline characteristics of each cohort. Median age was 71 years (22% ≥75 years), 43% had secondary AML and 31% prior HMA failure. Grade ≥3 adverse events (≥10% of patients) during induction were thrombocytopenia (100%), neutropenia (100%), febrile neutropenia (70%), neutropenic sepsis (24%) and atrial fibrillation (14%). One hematologic DLT was reported in Cohort E (VEN 600 mg), with persistent neutropenia and thrombocytopenia beyond day 42 in a patient with transformed myelofibrosis. Maximum tolerated dose (MTD) has not yet been reached. Early mortality was 8% and 24% at 30 and 60 days, respectively. CR/CRi rate was 25/35 (71%) (Table 1). During the venetoclax pre-phase, the median relative bone marrow blast count change was -20% (range -70.5 to +14.5%). For those who achieved CR/CRi, the median time to both neutrophil (≥0.5x10^9/L) and platelet (≥50x10^9/L) recovery during induction was 25 days from day 1 of chemotherapy. Median follow-up of survivors was 9.2 months (range 0.3-17.9 months).
Summary/Conclusion: To date, the MTD has not been reached with VEN up to 600mg in combination with 5+2 chemotherapy in fit older patients with AML. The overall CR/CRi rate is 71%. Analyses for response duration and survival are ongoing.

Acute lymphoblastic leukemia – Clinical

S1565

OUTCOMES OF YOUNG ADULT (≥18-25 YEARS) AND PEDIATRIC (<18 YEARS) PATIENTS WITH RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA FOLLOWING TREATMENT WITH CHIMERIC ANTIGEN RECEPTOR (CAR) T-CELL THERAPY

S. Rives[1,*], M.W. Boyer[2], A. Baruchel[3], J. Buechner[4], H. Hiramatsu[5], P.L. Martin[6], F. Mechinaud[7], G.D. Myers[8], E. Nemecek[9], M.A. Pulsipher[10], M.R. Verneris[11], R. Mody[12], L.K. Elderjou[13], Y. Lalazar[13], L. Yi[13], S.A. Grupp[14,15]

[1]Department of Pediatric Hematology and Oncology, Hospital Sant Joan de Déu de Barcelona, Barcelona, Spain, [2]Department of Pediatrics and Internal Medicine, University of Utah, Salt Lake City, United States, [3]Pediatric Hematology-Immunology Department, University Hospital Robert Debré (APHP), Paris, France, [4]Department of Pediatric Hematology and Oncology, Oslo University Hospital, Oslo, Norway, [5]Department of Pediatrics, Graduate School of Medicine, Kyoto University, Kyoto, Japan, [6]Division of Pediatric Blood and Marrow Transplant, Duke University Medical Center, Durham, United States, [7]Children's Cancer Centre, The Royal Children's Hospital Melbourne, Melbourne, Australia, [8]Children's Mercy Hospital Kansas City, Kansas City, [9]Oregon Health & Science University, Portland, [10]Division of Hematology, Oncology, and Blood and Marrow Transplantation, Children's Hospital Los Angeles, Keck School of Medicine of USC, Los Angeles, [11]Division of Pediatric Blood and Marrow Transplant, University of Minnesota, Minneapolis, [12]Pediatric Phase-I and Experimental Therapeutics Program, Michigan Medicine, Ann Arbor, [13]Novartis Pharmaceuticals Corporation, East Hanover, [14]Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, [15]Division of Oncology, Center for Childhood Cancer Research and Cancer Immunotherapy Program, Children's Hospital of Philadelphia, Philadelphia, United States

Background: Tisagenlecleucel, anti-CD19 CAR T-cell therapy, produces high response rates with manageable safety in pediatric r/r B-cell ALL. However, limited data indicate that adult patients (pts) with ALL are at higher risk of adverse events (AEs), including cytokine release syndrome (CRS).
Aims: Characterize the safety and efficacy of young adult (YA) pts (≥18 y) with B-cell ALL treated with tisagenlecleucel in the ELIANA (N=75 [NCT02435849]) and ENSIGN (N=29 [NCT02228096]) trials.
Methods: 84 pts were <18 y and 20 were 18-25 y. Endpoints include overall remission rate (ORR) (complete remission [CR] + CR with incomplete blood count recovery [CRi]), minimal residual disease (MRD)–negative response, duration of response (DOR), overall survival (OS), and safety.
Results: Both cohorts had a median of 3 prior lines of therapy; rates of prior stem cell transplant (55% in YA; 62% in <18 y) were similar. At the time of enrollment, median (range) bone marrow blasts in YA pts was 84% (12%-98%) and 73% (5%-99%) in pts <18 y. Median age (range) at infusion was 20 y (18-25) in YA pts and 10 y (3-17) in pts <18 y. Median dose was $3.2x10^6$ CAR-positive viable T-cells/kg (range, $0.2\text{-}5.4x10^6$ cells/kg). ORR (95% CI) was 70% (46%-88%; 14/20 pts) in YA pts *vs* 80% (70%-88%; 67/84 pts) in pts <18 y. In the YA cohort, 13 of 14 responses were MRD negative, as were 66 of 67 in the <18 y cohort. Median DOR was not reached in either group. Relapse-free survival rates at months 6 and 12 were 83% and 52%, respectively, in YA pts and 75% and 60% in pts <18 y. OS rates at months 6 and 12 were 74% and 57%, respectively, in YA pts and 89% and 76% in pts <18 y. 8 YA pts (40%) died after infusion (all >30 d): 5 from ALL, 1 infection (systemic mycosis), 1 hepatobiliary disease, and 1 unknown (consent withdrawn). 21 pts (25%) <18 y died after infusion: 17 from ALL (2 ≤30 d), 2 infections (1 HHV-6 encephalitis, 1 lower respiratory infection), 1 cerebral hemorrhage (≤30 d), and 1 septic embolic stroke (≤30 d). The rate of grade 3/4 AEs within 8 weeks of infusion in YA pts was 90% *vs* 81% in pts <18 y; the rate of grade 3/4 AEs >8 weeks after infusion in YA pts was 41% *vs* 46% in pts <18 y. The rate of any-grade CRS was 90% in YA pts *vs* 79% in pts <18 y; rates of grade ≥3 CRS were similar (45% *vs* 44%). Median time to CRS onset was 3 d in both cohorts; median duration was 9 d in YA pts *vs* 8 d in pts <18 y. In pts with CRS, rates of intubation and dialysis were 28% and 22%, respectively, in YA pts *vs* 17% and 11% in pts <18 y. 44% of YA pts were admitted to the intensive care unit (ICU) *vs* 59% of pts <18 y; median duration (range) of ICU stay in YA pts was 16 d (3-27 d) *vs* 7 d (1-34 d) in pts <18 y. Of YA pts with CRS, 39% received anti-cytokine therapy, 17% received up to 3 doses of tocilizumab, and 28% received corticosteroids *vs* 42%, 5%, and 21%, respectively, in pts <18 y. Infection concurrent with CRS occurred in 33% of YA *vs* 18% of pts <18 y; no CRS-related fatalities or cerebral edema occurred in either cohort. Other grade ≥3 AEs of special interest included neurological events (YA, 15%; <18 y, 10%), prolonged cytopenias (30%; 29%), infections (35%; 16%), febrile neutropenia (20%; 38%), and tumor lysis syndrome (5%; 2%).

Table 1.

Table. Grade 3 and 4 AEs of Special Interest, Regardless of Relationship to Tisagenlecleucel Occurring ≤ 8 Weeks After Infusion

AEs of Special Interest, %	<18 Years (n = 84)		YA (≥18 Years) (n = 20)	
	Grade 3	Grade 4	Grade 3	Grade 4
Cytokine release syndrome*	21	23	15	30
Neurological events	10	0	15	0
Febrile neutropenia	36	2	20	0
Cytopenias not resolved by day 28	15	14	10	20
Infections	14	2	30	5
Tumor lysis syndrome	2	0	5	0

* CRS was graded using the Penn CRS grading scale.

Summary/Conclusion: Although sample size is small, tisagenlecleucel appears to induce high rate of durable responses in YA pts, with a safety and efficacy profile consistent with that in pts <18 y. No fatality related to CRS and no cerebral edema were reported. Tisagenlecleucel offers a potential new option for young adult pts with r/r B-ALL.

S1566

A NOVEL, INTEGRATED AND VALIDATED PROGNOSTIC INDEX FOR PREDICTING OUTCOME IN ACUTE LYMPHOBLASTIC LEUKAEMIA PROVIDES NEW APPROACH FOR RISK STRATIFICATION.

A. Enshaei[1], D. O'Connor[2], J. Bartram[2], J. Hancock[3], C. Harrison[1], R. Hough[4], S. Samarasinghe[2], M. den Boer[5], J. Boer[6], H. deGroot-Kruseman[7], H. Vibeke Marquart[8], U. Noren-Nystrom[9], K. Schmiegelow[10], M. Horstmann[11], G. Escherich[11], M. Heyman[12], R. Pieters[6], A. Vora[2], J. Moppett[13], A. Moorman[1,*]

[1]Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, [2]Department of Haematology, Great Ormond Street Hospital, London, [3]Bristol Genetics Laboratory, North Bristol NHS Trust, Bristol, [4]Department of Haematology, University College Hospital, London, United Kingdom, [5]Pediatric Oncology, [6]Princess Máxima Center for Pediatric Oncology, Utrecht, [7]Dutch Childhood Oncology Group, Den Haag, Netherlands, [8]Dept. Clinical Immunology, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark, [9] Pediatric Oncology Unit, Department of Pediatrics, Umeå University Hospital, UMEÅ, Sweden, [10]Department of Pediatrics and Adolescent Medicine, University Hospital Rigshospitalet, Copenhagen, Denmark, [11]Klinik für Pädiatrische Hämatologie und Onkologie, Universitätsklinikum Eppendorf, Hamburg, Germany, [12]Pediatric Oncology, Karolinska Institutet, Stockholm, Sweden, [13]Department of Haematology, Royal Hospital for Sick Children, Bristol, United Kingdom

Background: ALL is heterogeneous in terms of genetics, clinical presentation and therapy response. Risk stratification, based on diagnostic features and early treatment response, has improved outcome. However, current algorithms use risk factors as binary variables which reduces statistical power and fails to account for biological heterogeneity. Moreover, using thresholds results in fixed group sizes which reduces options when designing clinical trials.
Aims: To develop a prognostic index (PI) which (a) integrates genetic, clinical and response data; (b) utilises continuous data; and (c) can be used to define clinically meaningful risk groups.
Methods: The discovery cohort comprised 2,542 UKALL203 patients who achieved a morphological remission. The validation cohort comprised 2,470 patients treated on the COALL-03, DCOG-ALL10 or NOPHO-ALL2008 protocols. Patients were followed-up for a median of 7/6 years (discovery/validation). MRD values, $>1x10^{-5}$, were log transformed with undetectable MRD assigned a value one log below the minimum detection level. Age, MRD and WCC were examined as continuous variables. Good risk genetics (GR-GEN) comprised *ETV6-RUNX1* and high hyperdiploidy; while high risk abnormalities (HR-GEN) were *KMT2A*, haploidy, low hypodiploidy, *TCF3-HLF* and iAMP21.
Results: Univariate Cox regression analysis of the discovery cohort revealed 10 risk factors for EFS: sex, age, Down syndrome, WCC, CNS disease, T-ALL, MRD, slow early response, GR-GEN and HR-GEN (each p≤0.002). Boot-strapped multivariate modelling identified a prognostic model com-

prising MRD, WCC, GR-GEN and HR-GEN. The fit of this model was not improved by adding age nor diminshed in T-ALL. The robustness of the model was assessed using cross-validation techniques and Harrell's C index was 0.73. Coefficients were used to construct a linear model allowing patient specific PI scores to be calculated (Fig1); revealing a distribution with a mean and SD of -1.62 (0.80). The risk of relapse (RR) correlated with the PI and patients with a PI>0 had a RR>35%. Each unit increase in PI more than doubled RR: hazard ratio 2.74 (95% CI 2.38-3.16), p<0.001. The PI also correlated with time to relapse (p<0.0001). A variety of formal validation methods were used to evaluate the model. The distribution of PI in the validation cohort was similar (mean -1.59, SD 0.79), as was the hazard ratio and C-index (2.18 (1.89-2.51), p<0.001 and 0.69). The validation tests demonstrated that the model accurately predicted outcome for the vast majority of patients. Fig 2 compares the EFS in the two cohorts after segregation into risk groups comprising 50%, 40% and 10% patients. Thus, the model can discriminate risk groups in patient cohorts treated differently. Moreover, it demonstrates the flexibility of a numeric PI to define clinically relevant risk groups of any given size. To compare the prediction accuracy of the PI with traditionally defined risk groups, we assessed the area under the curve (AUC) for each clinical trial. For all 4 trials, the PI was better (higher AUC) at predicting EFS compared with standard risk groups: ALL2003 0.72 v 0.61, p<0.001; NOPHO-ALL2008 0.71 v 0.66, p=0.005; COALL-03 0.71 v 0.51, p<0.001; DOCG-ALL10 0.67 v 0.58, p=0.001.

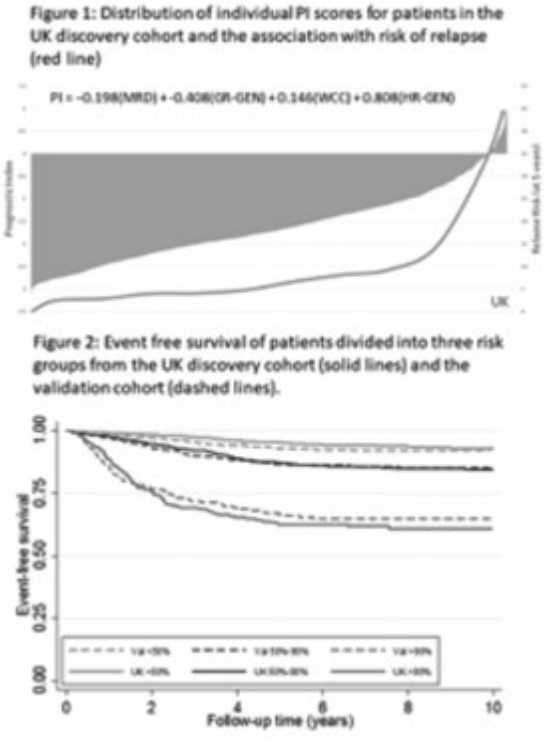


Figure 1.

Summary/Conclusion: We have developed and validated a novel PI for predicting outcome in paediatric ALL. The model integrates multiple risk factors using continuous data enabling individual numeric risk scores to be calculated. This model provides an accurate method for predicting outcome and allows greater flexibility for defining treatment group sizes.

S1567

GENOME-WIDE ASSOCIATION STUDY REVEALS ANKYRIN REPEAT AND SOCS BOX PROTEIN 3 (ASB3) AND INOSITOL POLYPHOSPHATE MULTIKINASE (IPMK) AS NEW RISK LOCI FOR RELAPSE IN B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

L. Hinze[1,*], S. Junk[1], N. Klein[1], M. Zimmermann[1], M. Schrappe[2], A. Moericke[2], G. Cario[2], R. Koehler[3], C.R. Bartram[3], R. Houlston[4], C. Kratz[1], M. Stanulla[5]

[1]Department of Pediatric Hematology/Oncology, Hannover Medical School, Hannover, [2]Department of Pediatric Hematology/Oncology, University Hospital Schleswig-Holstein, Kiel, Kiel, [3]Department of Pediatric Hematology/Oncology, Heidelberg University, Heidelberg, Germany, [4]Department of Pediatric Hematology/Oncology, Institute of Cancer Research, London, United Kingdom, [5]Hannover Medical School, Hannover, Germany

Background: Impressive improvements of survival rates in pediatric acute lymphoblastic leukemia (ALL) have been achieved during the last decades, but a significant proportion of patients still suffers from relapse and therapy-related toxicities. Further improvement of therapy can be achieved by better personalized adjustment of treatment to the risk of relapse.
Aims: While most research activities on genetic prognostic factors have focused on somatic features, relatively little is known on hereditary contributions to treatment response and outcome in pediatric ALL. Therefore, we conducted a genome-wide association study (GWAS) of relapse in B cell precursor (BCP) ALL.
Methods: Our study included 1152 patients from AIEOP-BFM ALL 2000 (Schrappe *et al.* Blood 2011). On this trial, patients were stratified into three risk groups, mainly by minimal residual disease (MRD) analyses. Treatment employed standard drugs. Complete remission (CR) was defined as the absence of physical signs of leukemia or detectable leukemia cells on blood smears, <5% leukemia blast cells present in the bone marrow, and normal cerebrospinal fluid (CSF). The diagnosis of relapse was only made after CR has been achieved before and was defined as isolated bone marrow, combined bone marrow, CNS or testicular relapse. Of the 1152 patients included, 145 relapsed (cases) and 1007 patients remained in long-term CR (controls). DNA isolated from remission samples was genotyped by Illumina Human1M-Duo BeadChips containing 1.048.711 single SNV markers. For GWAS analyses gPLINK v2.050, PLINK v.1.09 and RStatistics v3.3.1 were used. Replication analyses were conducted in a cohort of 73 relapsed and 615 non-relapsed patients treated on trial AIEOP-BFM ALL 2009.
Results: After quality control assessment, the analysis of the initial cohort included 751.432 SNPs among 130 relapsed compared to 889 control patients. Twenty candidate SNVs demonstrating promising associations with risk of relapse were selected for replication analysis, including intronic SNVs in the Ankyrin repeat and SOCS box protein 3 (*ASB3*, rs1549749, P=8.593×10^{-7}, odds ratio, OR, 2.48) and the inositol polyphosphate multikinase (*IPMK*, rs7919168, P=1.266×10^{-5}, OR 2.06). One additional SNV in *ASB3* and six additional SNVs in *IPMK* were in linkage disequilibrium with rs1549749 and rs7919168 and demonstrated significant levels of P ≤1.7×10^{-5}. Replication analyses in an independent cohort from AIEOP-BFM ALL 2009 confirmed the initial observations for risk of relapse conferred by rs1549749 and rs7919168 and - in combined analysis of the initial and replication cohort - revealed genome-wide significance (rs1549749, P=4.98×10^{-8} OR=2.09; rs7919168, P=1.3×10^{-8} OR=3.13). In further analysis of the initial cohort from AIEOP-BFM ALL 2000, rs1549749 and rs7919168 risk alleles were associated with a significantly lower 5-year-event-free survival (EFS) compared to wildtype (0.75±0.03 *vs* 0.9±0.01, P<0.0001; 0.79±0.09 *vs* 0.99±0.01, P=0.008). Multivariate Cox analyses of the above *ASB3* and *IPMK* SNVs demonstrated independence of the observed relapse risk associations (hazard ratio rs1549749: 1.55, 95% CI 1.15-2.10; hazard ratio rs7919168: 2.10, 95% CI 1.48-2.97).
Summary/Conclusion: We identified genome-wide significant associations of *ASB3* and *IPMK* germline variations with risk of relapse in BCP ALL patients treated on BFM protocols for pediatric ALL. These results suggest that germline genetic variation influences effectiveness of chemotherapy in pediatric ALL and represent an important additional step in defining the hereditary contribution to the risk of relapse in pediatric ALL.

S1568

PERSISTENT BCR-ABL1 CLONAL HEMATOPOIESIS AFTER BLAST CLEARANCE IDENTIFIES A CML-LIKE SUBGROUP OF PATIENTS WITH PHILADELPHIA CHROMOSOME-POSITIVE (PH+) ALL: INTERIM RESULTS FROM THE GRAAPH-2014 TRIAL

E. Clappier[1,2,*], R. Kim[1,2], J.-M. Cayuela[1,2], P. Rousselot[3], Y. Chalandon[4], M. Passet[1,2], X. Thomas[5], V. Havelange[6], P. Chevallier[7], F. Huguet[8], N. Boissel[1,2], C. Berthon[9], G. Stüssi[10], S. Maury[11], S. Chantepie[12], J.-B. Micol[13], I. Plantier[14], S. Chevret[1,2], J. Soulier[1,2], V. Lhéritier[15], N. Ifrah[16], H. Dombret[1,2]

[1]University Hospital Saint-Louis, AP-HP, [2]University Paris Diderot, Paris, [3]CH, Versailles, France, [4]HUG, Geneve, Switzerland, [5]CHU Lyon Sud, Lyon, France, [6]UC, Louvain, Belgium, [7]CHU, Nantes, [8]IUCT, Toulouse, [9]CHRU, Lille, France, [10]Oncology Institute, Bellizona, Switzerland, [11]University Hospital Henri Mondor, AP-HP, Creteil, [12]CHU, Caen, [13]IGR, Villejuif, [14]CH, Roubaix, [15]GRAALL, Lyon, [16]CHU, Angers, France

Background: Ig/TCR-based minimal residual disease (MRD) is a faithful marker of response to therapy and the strongest predictor of relapse in Ph-negative ALL. In adults with Ph+ ALL, MRD is commonly monitored by

BCR-ABL1 transcript quantification although its prognostic significance is less clear. It has been shown that *BCR-ABL1* rearrangement may be found in non-lymphoblastic cells in some of these patients.
Aims: We aimed to study the biological significance of BCR-ABL1 MRD in Ph+ ALL.
Methods: The study includes 57 adults with *de novo* Ph+ ALL enrolled in the ongoing GRAAPH-2014 trial. Bone marrow (BM) and peripheral blood (PB) follow-up (FU) samples were collected after each treatment cycle (first 4 time-points [TPs]) and during FU. MRD monitoring was performed by quantification of both *BCR-ABL1* transcripts and Ig/TCR clonal rearrangements. *BCR-ABL1* major molecular response (MMR) was defined as *BCR-ABL1/ABL1* ratio <0.1%. We sequenced genomic fusions using capture and NGS of *BCR* and *ABL1* introns and designed breakpoint-specific PCR systems to quantify BCR-ABL1 genomic fusion with the same methods as for Ig/TCR MRD. MRD levels between markers were compared using Spearman rank correlation (r). For a given FU sample, results were considered discrepant if more than one $\log_{10}$ difference or positivity/negativity discordance, taking account markers sensitivity.
Results: Quantification of genomic BCR-ABL1 and Ig/TCR MRD levels on 72 samples from 10 patients revealed poor correlation (r=0.51). This was related to discordant results from 2 patients who had repeatedly positive BCR-ABL1 samples while Ig/TCR MRD was undetectable, suggesting those patients had lymphoblast clearance but persistent clonal BCR-ABL1 hematopoiesis. By contrast, genomic and transcript BCR-ABL1 levels on the same FU samples were well correlated (r=0.80), showing that there was no disparity related to BCR-ABL1 transcriptional modulation. This prompted us to extend our study to the whole cohort by comparing *BCR-ABL1* transcript and Ig/TCR MRD levels. We obtained data for 360 FU samples (BM=152; PB=208). Patients with at least 2 samples with discrepant *BCR-ABL1* and Ig/TCR MRD levels were considered having divergent kinetics in relation with BCR-ABL1 clonal hematopoiesis. They represented a group of 27 patients (47%). As compared with remaining patients, this group showed unbalanced M/F sex ratio (2.9 *vs* 0.8, p=0.03), enrichment in major *BCR-ABL1* breakpoint (M-bcr, 33% *vs* 7%, p=0.02) and less frequent *IKZF1* intragenic deletions (48% *vs* 77%, p=0.03). As expected, those patients had less *BCR-ABL1* MMR at TP2 in BM (54% *vs* 100%, p<0.001) and PB (39% *vs* 96%, p<0.001). By contrast, the prevalence of poor early Ig/TCR MRD was similar in the two groups (35% and 29%). At later TPs, patients with divergent kinetics mostly reached MMR in BM but maintained relatively high *BCR-ABL1* levels in PB (MMR rate at TP4, 79% and 47% in BM and PB respectively).
Summary/Conclusion: A subgroup of adults with Ph+ ALL displays persistence of BCR-ABL1 detection during treatment which is not linked to poor lymphoblast clearance but to clonal hematopoiesis. This entity resembles CML, although mostly related to m-bcr breakpoint and without evidence of myeloproliferation. Its prognostic relevance will be evaluated in our ongoing GRAAPH-2014 trial. Yet, these observations have important implications as non-lymphoblastic persistent BCR-ABL1 hematopoiesis may not necessarily need treatment intensification nor indicate lymphoid antigen-directed therapies.

S1569

OUTCOMES OF PATIENTS WITH RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH PRIOR BLINATUMOMAB IN ZUMA-3, A STUDY OF KTE-C19, AN ANTI-CD19 CHIMERIC ANTIGEN RECEPTOR (CAR) T CELL THERAPY

O.O. Oluwole[1,*], B.D. Shah[2], M.R. Baer[3], M.R. Bishop[4], H. Holmes[5], G.J. Schiller[6], W. Donnellan[7], K.M. O'Dwyer[8], A. Mardiros[9], J.M. Rossi[9], T. Shen[9], A. Xue[9], R.K. Jain[9], R. Vezan[9], W.G. Wierda[10]

[1]Vanderbilt-Ingram Cancer Center, Nashville, [2]Moffitt Cancer Center, Tampa, [3]University of Maryland, Greenebaum Comprehensive Cancer Center, Baltimore, [4]The University of Chicago Medicine, Chicago, [5]Texas Oncology-Baylor Charles A. Sammons Cancer Center, Dallas, [6]David Geffen School of Medicine at UCLA, Los Angeles, [7]Sarah Cannon Research Institute, Nashville, [8]University of Rochester, Rochester, [9]Kite, a Gilead company, Santa Monica, [10]The University of Texas MD Anderson Cancer Center, Houston, United States

Background: KTE-C19 showed promising efficacy (71% complete response [CR] or CR with incomplete hematologic recovery [CRi]) and manageable safety for patients with relapsed/refractory acute lymphoblastic leukemia (R/R ALL) in Phase 1 of ZUMA-3 (Shah ASH 2017; NCT02614066). Blinatumomab (blin), a CD19/CD3 bispecific T cell engager, is US FDA-approved for R/R ALL. The impact of prior blin on KTE-C19 efficacy is unknown.
Aims: To report responses to KTE-C19 in patients who received prior blin.
Methods: All patients provided written informed consent. Eligible patients were aged ≥18 years with R/R ALL (Ph+ allowed), ≥5% bone marrow blasts (documented CD19+), and ECOG 0-1. Patients received 2, 1, or 0.5×10^6 CAR T cells/kg after low-dose conditioning chemotherapy. Patients who received the 2×10^6 dose could not have prior blin. Outcomes assessed included efficacy, safety, product characteristics, and levels of CAR T cells and cytokines in blood.
Results: As of July 31, 2017, 29 patients were evaluable for safety, 24 for efficacy. A total of 6, 14, and 9 safety-evaluable patients received 2, 1, and 0.5×10^6 CAR T cells/kg, respectively. Patients with (n=11) *vs* without (n=18) prior blin had worse performance status (ECOG 0: 27% *vs* 44%) and were more likely to be R/R to ≥2nd-line therapy (55% *vs* 28%). With ≥8 weeks of follow-up, 63% *vs* 75% of patients with *vs* without prior blin had CR or CRi, respectively. Overall, 88% (21/24) had minimal residual disease-negative remission (7/8 with *vs* 14/16 without prior blin). Of patients with *vs* without prior blin, 27% *vs* 28% had Grade ≥3 cytokine release syndrome and 36% *vs* 61% had Grade ≥3 neurologic events. KTE-C19 was manufactured successfully in both groups, with similar product characteristics (eg, CD4/CD8 ratio, % transduction). Patients with *vs* without prior blin had similar proportions of naïve (43% *vs* 36%) and effector memory (19% *vs* 20%) T cells. Postinfusion CAR T expansion was observed regardless of prior blin; peak CAR T cell levels occurred between Days 7 and 14 for both groups.
Summary/Conclusion: While potentially confounded by multiple factors, these data demonstrate that prior blin did not preclude manufacturing of efficacious products. KTE-C19 continues to show promising efficacy and manageable safety in patients with R/R CD19+ ALL independent of prior blin.

Genetics and epigenetics of CLL

S1570

LINKING ABERRANT CHROMATIN FEATURES IN CHRONIC LYMPHOCYTIC LEUKEMIA TO DEREGULATED TRANSCRIPTION FACTOR NETWORKS

D. Mertens[1,2,*], J.-P. Mallm[3], M. Iskar[4], N. Ishaque[5], S. Kugler[1], H. Döhner[1], P. Lichter[4], S. Stilgenbauer[1], K. Rippe[3]

[1]Internal Medicine III, University Ulm, Ulm, [2]Mechanisms of Leukemogenesis, [3]Chromatin Networks, [4]Molecular Genetics, [5]Theoretical Bioinformatics, DKFZ German Cancer Center, Heidelberg, Germany

Background: Though numerous recurrent epigenetic mutations have been identified in chronic lymphocytic leukemia (CLL), their role in the molecular and clinical pathomechanism is only partly understood.
Aims: To provide the missing links between the cancer epigenotype and phenotype, we profiled the chromatin landscape and the transcriptome in primary peripheral blood cells of 19 CD19 sorted samples of untreated chronic lymphocytic leukemia (CLL) patients in comparison to 9 pools of non-malignant CD19-sorted B cells from 65 age-matched healthy donors.
Methods: We characterized DNA methylation by whole genome bisulfite sequencing, the major histone modification (H3K4me1, H3K4me3, H3K9me3, H3K9ac, H3K27me3, H3K27ac and H3K36me3) by ChIP-seq, nucleosome occupancy from high coverage H3 ChIP-seq and open chromatin sites were identified by the assay for transposase-accessible chromatin (ATAC-seq) in both bulk and single cells. In addition, RNA transcription was analyzed by strand-specific RNA-seq of both long and short RNAs.
Results: Remarkably, the CLL DNA methylome contains a large genome fraction of ~50% partially methylated domains in comparison to non-malignant B cells (<1 %). Auto- and cross-correlation of histone modifications showed changes for the active promoter mark H3K4me3 and for the repressive H3K27me3 modification, leading to terminal inactivation of poised promoters and activation of alternative transcriptional start sites in CLL. Most significant changes were observed at enhancers, and 256/279 (92%) of enhancers with changed H3K27ac signal that overlapped with partially methylated domains were activated. ATACseq uncovered 24400 differentially open loci at promoters, enhancers and repressed regions in CLL cells. Single cell ATACseq correlations of loci were used to infer spatial enhancer-promoter contacts and their local enrichment. In total 17,122 promoter-enhancer pairs were identified, with 11,585 promoters being assigned to different enhancers in CLL and non-malignant B cells. Of these re-wired promoters 921 and 438 were linked to genes down- and upregulated in CLL, respectively. We selected the most relevant transcription factors (TFs) that displayed differential binding in two or more of the chromatin readouts (Figure). The aberrant regulatory epigenetic signals were detected at 83% of the transcriptionally deregulated of genes in CLL, explaining the large majority of transcriptional dysregulation in CLL. The connections present in our B-cell gene regulatory network together with direct protein-protein interactions listed in the String database defined TF regulatory modules and provided links to chromatin modifiers. A gene set enrichment analysis of targets of these TFs retrieved NFkB and BCR signaling as the top hits. The network also includes the induction of the TCF4 in CLL via a large-scale enhancer activation and its linkage to expression of the anti-apototic BCL2 factor.

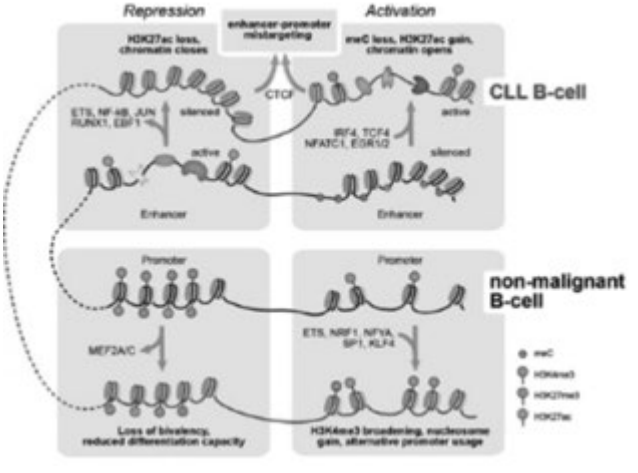


Figure 1.

Summary/Conclusion: The deregulated TF network identified here rationalizes how the epigenetic pathophenotype of CLL is linked to changes in TF activity. Understanding the transcriptional dysregulation of the molecular targets of novel therapies will help to personalize treatment, reduce serious adverse events and thereby optimize outcome of CLL patients.

S1571

CLINICAL IMPORTANCE OF DNA METHYLATION SIGNATURES IN CHRONIC LYMPHOCYTIC LEUKAEMIA PATIENTS TREATED WITH CHEMO-IMMUNOTHERAPY

J. Strefford[1,*], H. Amarasinghe[1], T. Wojdacz[1], A. Beattie[1], J. Forster[1], L. Kadalayil[2], S. Blakemore[1], H. Parker[1], M. Larrayoz[1], R. Clifford[3], Z. Davis[4], M. Else[5], D. Cohen[6], A. Steele[1], R. Rosenquist[7], A. Collins[8], A. Pettitt[9], P. Hillmen[6], C. Plass[10], A. Schuh[3], D. Catovsky[5], D. Oscier[4], C. Oakes[11]

[1]Cancer Sciences Unit, Faculty of Medicine, [24]Human Development and Health, University of Southampton, Southampton, [3]Oxford National Institute for Health Research Biomedical Research Centre and Department of Oncology, University of Oxford, Oxford, [4]Department of Pathology, Royal Bournemouth Hospital, Bournemouth, [5]The Institute of Cancer Research, The Institute of Cancer Research, Sutton, [6]Leeds Institute of Clinical Trials Research, University of Leeds, Leeds, United Kingdom, [7]Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden, [8]Human Development and Health, University of Southampton, Southampton, [9]Department of Molecular and Clinical Cancer Medicine, University of Liverpool, Liverpool, United Kingdom, [10]Division of Epigenomics and Cancer Risk Factors, The German Cancer Research Center, Heidelberg, Germany, [11]Division of Hematology, The Ohio State University, Columbus, United States

Background: Even in the era of the targeted therapies, there remains clinical value in exploring the impact of disease characteristics in chemo-immunotherapy (CIT) trials, namely to identify features that contribute most to long-term outcomes, thereby pinpointing patients destined to benefit from these therapies. One such feature is the CLL DNA methylome, that recapitulates normal B cell maturation, with IGHV mutated (M-CLL) and unmutated CLL (U-CLL) retaining an imprint of the DNA methylation signature of memory (m-CLL) and naive B cells (n-CLL), respectively, with a third intermediate epigenetic subgroup (i-CLL) with borderline mutation status. The pyrosequencing analysis of 5 CpG sites can divide CLL into these three subgroups, each with different clinico-biological features.
Aims: To validate the clinical utility of the epigenetic subgroups in patients entering clinical trials.
Methods: We classified 605 treatment-naïve patients randomized to one chemotherapy (CLL4) and two CIT (ARCTIC and ADMIRE) trials, into the three epigenetic subgroups using pyrosequencing and confirmatory Infinium HumanMethylation450 analysis (n=60, 96% concordance between technologies).

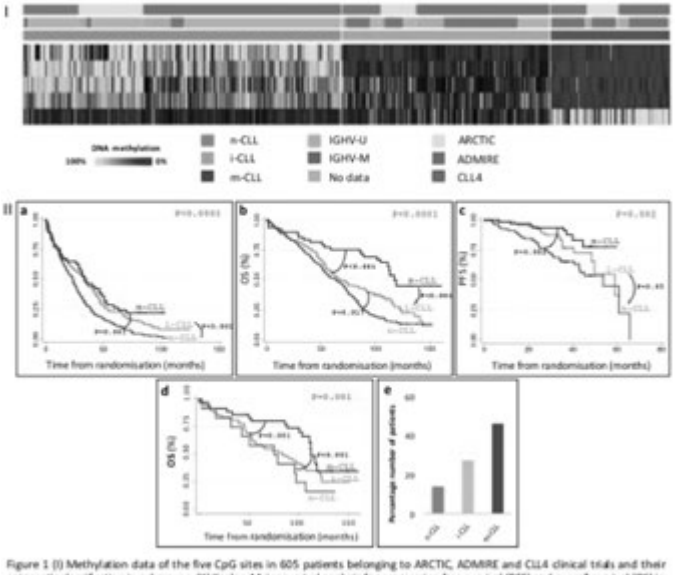


Figure 1 (i) Methylation data of the five CpG sites in 605 patients belonging to ARCTIC, ADMIRE and CLL4 clinical trials and their epigenetic classification in subgroups. (ii) Kaplan-Meier survival analysis for progression free survival (PFS) and overall survival (OS) in CLL4 (a and b) trial and PFS in combined ARCTIC and ADMIRE trials (c) for all patients. Kaplan-Meier survival analysis for OS in IGHV-M patients belonging to the CLL4 trial (d). Percentage long-term survivors (>10 years OS) in each of the three epigenetic subgroup (e).

Figure 1.

Results: We identified n-, i- and m-CLL in 49.5% (n=298), 32.0% (n=195) and 18.5% (n=112) of our patients, respectively (**Fig1I**). Fewer m-CLL patients were identified in our study compared to published data, reflecting the progressive nature of our cohort, with 80% (n=245/305, P<0.001) of U-CLL cases exhibited the n-CLL signature (i-CLL: 17% and m-CLL: 3%). For M-CLL cases 9%, 50% and 41% exhibited the n-, i- and m-CLL epigenetic signature, respectively. In 359 CLL4 patients, n-, i- and m-CLL patients exhibited a median PFS of 23, 35 and 33, and OS of 63, 66 and 106 months, respectively (**Fig1IIa and b**). n-CLL showed significantly shorter PFS than i-CLL (HR 0.64, p<0.001) and m-CLL (HR 0.52, p<0.001), and had the shortest OS, again compared to i-CLL (HR 0.73, p=0.01) and m-CLL (HR 0.33, p<0.001). The epigenetic signature was associated with long-term survival (>10 years OS), with n-CLL accounting for only 14% of this subgroup (P<0.001, **FigIIe**). In IGHV-M patients, the m-CLL subgroup showed 104 months of median OS compared to 79 and 84 months in n- and i-CLL subgroups (**FigIId**). Multivariate Cox proportional analysis, controlling for confounding variables (inc. clinical features, IGHV status, *TP53*, *NOTCH1* and *SF3B1*) in 278 patients showed that m-CLL was an independent prognostic factor for OS (HR 0.46, p<0.01), but not PFS. In univariate analysis, in 247 patients randomized to ARCTIC and ADMIRE, the i- (HR:0.57, p=0.05) and m-CLL (HR:0.3, p=0.002) subgroups displayed longer PFS (**Fig1IIc**). In a multivariate model, including *TP53* lesions and IGHV status (239 patients), the m-CLL subgroup retained its independent prognostic significance (HR:0.25, p<0.001).

Summary/Conclusion: In conclusion, we report the first analysis of the clinical utility of epigenetic subgroup signatures in patients entered into first-line chemotherapy and CIT trials and identified m-CLL as an independent marker of survival in both our CLL4 and ARCTIC/ADMIRE cohorts. This provides important evidence that DNA methylation analysis may aid in the identification of patients destined to demonstrate durable remissions when treated with these agents.

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INTEGRATED ATAC-SEQ AND SINGLE-CELL SYNERGISTIC CHEMOSENSITIVITY PROFILING IDENTIFIES RATIONAL DRUG COMBINATIONS IN IBRUTINIB-TREATED CLL PATIENTS

G. Vladimer[1,2,*], C. Schmidl[1], A. Renderio[1], S. Schnabl[3], C. Taubert[2], T. Pemovska[1], M. Araghi[3], T. Krausgruber[1], N. Krall[1,2], B. Snijder[1,4], R. Hubmann[3], A. Ringler[1,5], D. Demirtas[3], O. Lopez[1,2], K. Runggatscher[5], C. Skrabs[3], E. Porpaczy[3], M. Gruber[1,3], S. Kubicek[1,5], G. Hoermann[6], P. Staber[3], M. Shehata[3], G. Superti-Furga[1,7], C. Bock[1,8,9], U. Jäger[3]

[1]CeMM Center for Molecular Medicine, [2]Allcyte GmbH, [3]Department of Hematology, Medical University of Vienna, Vienna, Austria, [4]ETH Zurich, Zurich, Switzerland, [5]Christian Doppler Laboratory for Chemical Epigenetics and Anti-Infectives, CeMM Center for Molecular Medicine, [6]Department of Laboratory Medicine, Medical University of Vienna, [7]Center for Physiology and Pharmacology, [8]Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria, [9]Max Planck Institute for Informatics, Saarbrücken, Germany

Background: Chronic lymphocytic leukemia (CLL) is characterized by proliferation and accumulation of malignant B cells, where this process is associated with constitutively activated B cell receptor (BCR) signaling, and interference with BCR signaling provides therapeutic benefit. Specifically, the Bruton's Tyrosine Kinase (BTK) inhibitor ibrutinib prevents BTK tyrosine phosphorylation and thereby interferes with the pathway. It has shown high clinical response rates in patients with relapsed and refractory CLL, including patients with adverse cytogenetic profiles. Despite high responses achieved by ibrutinib, it has important limitations such as inducing CLL cell redistribution from protected niches to the periphery, and the clinical response to ibrutinib is slow and often incomplete. Further, there is no evidence that a cure can be achieved, even among patients that tolerate long-term treatment with ibrutinib, a considerable percentage develops drug resistance, *BTK* independent disease progression, or Richter's transformation, indicating drug synergies with ibrutinib may increase prognosis. Recent studies have explored combined use of ibrutinib with inhibitors for the proteasome (carfilzomib), BCL2i (venetoclax), and HDAC (abexinostat) in preclinical models, which has shown promising initial results. However, these approaches were largely empirical, and do not have systamatic rational.

Aims: We charted the ibrutinib-induced chromatin regulatory landscape of CLL, and in parallel mapped targetable pathways for synergistic combination therapies that could potentially improve disease control. Peripheral blood from 24 fully characterized CLL cases were collected before and during therapy with ibrutinib. Chromatin accessibility was measured by ATAC-seq to gather the genome-wide regulatory landscape, and *ex vivo* chemosensitivity to >140 drugs on paired CLL samples was measured using Pharmacoscopy, a translatable method for *ex vivo* single-cell drug cytotoxicity profiling in primary samples (Snijder *et al.* Lancet Haematology, 2017; NCT03096821).

Methods: We bioinformatically integrated these datasets, establishing a comprehensive picture of the cellular responses to ibrutinib, and to prioritize targets, we performed secondary differential synergy screening of 21 drugs in combination with Ibrutinib in 8 CLL patient samples taken prior to clinical Ibrutinib treatment, with the goal to visualize changes in the targeted sensitivity of key drugs with and without Ibrutinib treatment.

Results: ATACseq identified increases in chromatin accessibility and chemosensitivity in proteasome, inflammatory NF- B/TNF signaling, CoA biosynthesis, PI3K/Akt, pathways, along with changes affecting genes such as FOXO3 and I Ba, and both the ex vivo drug testing of samples from patients after clinical ibrutinb and *ex vivo* synergy screening revealed that ibrutinib treatment *in vivo* and *in vitro* sensitizes CLL cells to compounds such as the proteasome inhibitor bortezomib, the JAK inhibitor ruxolitinib, the bisphosphate zoledronate, and the aurora kinase inhibitor ZM447439.

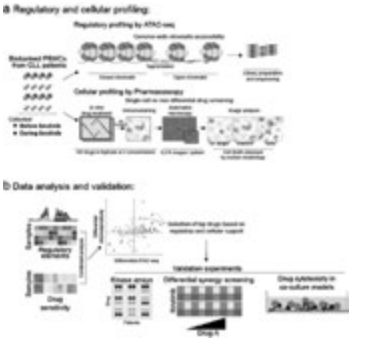

Figure 1.

Summary/Conclusion: Our results show that synergistic combination and informatics-integration of chromatin profiling with functional drug screening is a powerful tool to identify targetable pathways in CLL. This approach may be useful for designing personalized therapies as well as providing planning for clinical studies. Our approach is directly transferable to other leukemia where malignant cells can be obtained for chromatin profiling and drug sensitivity analysis, thus providing a widely applicable tool for the rational development of combination therapies.

S1573

GROWTH DYNAMICS IN NATURALLY PROGRESSING CHRONIC LYMPHOCYTIC LEUKEMIA

M. Gruber[1,2,3,4,*], I. Bozic[5], I. Leshchiner[3], D. Livitz[3], K. Stevenson[6], L. Rassenti[7], D. Rosebrock[3], O. Olive[4], J.G. Gribben[8], K.R. Rai[9], M.J. Keating[10], J.R. Brown[4,11,12], D. Neuberg[6], T.J. Kipps[7], M.A. Nowak[13,14], G. Getz[3,12,15,16], C.J. Wu[3,4,11,12]

[1]CeMM Research Center for Molecular Medicine, [2]Department of Internal Medicine I, Division of Hematology and Hemostaseology, Medical University of Vienna, Vienna, Austria, [3]Broad Institute of MIT and Harvard, Cambridge, [4]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, [5]Department of Applied Mathematics, University of Washington, Seattle, [6]Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, [7]Department of Medicine, University of California at San Diego Moores Cancer Center, La Jolla, United States, [8]Barts Cancer Institute, Queen Mary, University of London, London, United Kingdom, [9]Hofstra North Shore-LIJ School of Medicine, Lake Success, [10]Department of Leukemia, MD Anderson Cancer Center, Houston, [11]Department of Medicine, Brigham and Women's Hospital, [12]Harvard Medical School, Boston, [13]Program for Evolutionary Dynamics, [14]Department of Mathematics and Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, [15]Department of Pathology, [16]Center for Cancer Research, Massachusetts General Hospital, Boston, United States

Background: Exome-wide characterization of somatic mutations in large cohorts of chronic lymphocytic leukemia (CLL) patients provided a comprehensive catalogue of alterations that putatively drive the disease. But how the genomic features of a patient's CLL cells relate to individual disease kinetics remains poorly understood.

Aims: We leveraged the indolent growth dynamics of CLL to perform a time-resolved analysis of growth rates and genomic patterns of leukemia clones from 113 patients, spanning decades-long disease courses.

Methods: For high-resolution characterization we selected 21 CLL patients who progressed to need for treatment, through the CLL Research Consortium, housed at UCSD. We performed whole exome sequencing on 2-6 serial

samples spanning the period from diagnosis to start of chemoimmunotherapy. Using pre-treatment measurements of white blood cell counts (WBC), we determined growth kinetics over time. We validated findings in a cohort of pre-treatment samples from 92 CLL patients enrolled at the Dana-Farber Cancer Institute (DFCI, Boston, MA). For whole-exome sequencing (WES), we used Broad Institute sequencing and analysis protocols. Cancer cell fractions of mutations were estimated using the ABSOLUTE algorithm to calculate the purity, ploidy, and absolute DNA copy-numbers of each sample. To infer the clonal structure in serial samples, we employed a multi-sample phylogeny and clustering analysis tool PhylogicNDT. Growth rates per subclone were estimated based on a Monte Carlo Markov Chain algorithm integrating the uncertainty of the cluster assignment, given the proposed phylogeny and WBC measurements. Reference lists for SNVs and InDels in known putative CLL driver genes as well as for recurrent CNAs were concatenated based on previous sequencing studies of large CLL cohorts.

Results: We found that CLL commonly demonstrates not only exponential expansion but also logistic growth, which is sigmoidal and includes stabilization at a certain steady state level. In patients, whose serial samples underwent whole-exome sequencing, we found that dynamic changes in the CLL disease course were shaped by the activity of genetic events that were already present in earliest indolent stages. Each growth pattern was associated with marked differences in genetic composition, pace of disease progression and extent of clonal evolution. Finally, using an integrative analysis of somatic mutations and growth rates, we quantified the growth advantage putative CLL drivers confer *in vivo*. Accelerated growth of subclones was strongly enriched for presence of well-established statistically identified CLL drivers, for which we could also directly quantify the impact of specific mutations, such as second hits in the major tumor suppressor genes *TP53* and *ATM*.

Summary/Conclusion: By integrating information on tumor growth and genetic evolution information, we observed complex patterns even during the pre-treatment period, with various growth behavior and expansion rates amongst subclones over time. Our computational framework enabled us to assess the degree of growth acceleration of genetically defined subclones over their parental clones. This analysis provides clear evidence for the growth-accelerating role and potential synergies of subclonal driver mutations, while demonstrating the frequent existence of growth-neutral subclones that did not contain drivers and likely represent genetic drift. Our real-life *in vivo* data thus provide a conceptual framework for understanding the growth trajectories of individual populations of CLL cells.

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GENOMIC ALTERATIONS IN HIGH-RISK CHRONIC LYMPHOCYTIC LEUKEMIA FREQUENTLY AFFECT CELL CYCLE KEY REGULATORS AND NOTCH1 REGULATED TRANSCRIPTION

J. Edelmann[1,2,*], K. Holzmann[3], E. Tausch[1], B.M.C. Jebaraj[1], D. Steinbrecher[1], A. Dolnik[1], T. Blätte[1], D.A. Landau[4,5], J. Saub[1], S. Estenfelder[1], S. Ibach[6], F. Cymbalista[7], V. Leblond[8], A. Delmer[9], J. Bahlo[10], S. Robrecht[10], F. Kirsten[10], V. Goede[10], L. Bullinger[1,11], C.J. Wu[5], D. Mertens[1], J.G. Gribben[2], M. Hallek[10], H. Döhner[1], S. Stilgenbauer[1]

[1]Department of Internal Medicine III, Ulm University Hospital, Ulm, Germany, [2]Centre for Haemato-Oncology, Barts Cancer Institute, London, United Kingdom, [3]Genomics Core Facility, Ulm University, Ulm, Germany, [4]New York Genome Center, New York, [5]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, United States, [6]Wissenschaftlicher Service Pharma GmbH (WiSP), Langenfeld, Germany, [7]Service d'Hématologie Biologique, Hôpital Avicenne, Bobigny, [8]Service d'Hématologie, Hôpital Pitié-Salpêtrière, Paris, [9]Service d'Hématologie Clinique, Centre Hospitalier Universitaire de Reims, Reims, France, [10]Department of Internal Medicine I, University of Cologne, Cologne, [11]Department of Hematology, Oncology and Tumor Immunology, Charité University Medicine Berlin, Campus Virchow Klinikum, Berlin, Germany

Background: Beyond *TP53*, no genetic alteration has so far clearly been linked with the pathogenesis of primary high-risk or refractory chronic lymphocytic leukemia (CLL). TP53 dysfunction and defects in other DNA-damage response systems such as ATM lead to chromosomal instability with secondary events not necessarily associated with adverse prognosis. This constitutes the challenge, to differentiate between passenger alterations without survival advantage and driver alterations.

Aims: We aimed to identify pivotal genomic alterations contributing to high-risk CLL biology beyond *TP53* aberrations.

Methods: We performed high-resolution SNP-array profiling and targeted sequencing (*NOTCH1* and *SF3B1* among other candidates with potential functional relevance) on 75 relapsed/refractory CLL cases including 18 cases without TP53 dysfunction. Samples were derived from the CLL2O trial of the German / French CLL Study Groups (GCLLSG/FCLLSG). Our cohort was extended by 71 treatment-naïve *TP53*-deficient primary high-risk cases enrolled on the CLL2O trial or the CLL8 or CLL11 trial of the GCLLSG. All samples were CD19 tumor cell enriched. The GISTIC 2.0 algorithm was used to identify significantly enriched copy number alterations (CNAs). Expression of NOTCH1 target genes was quantified by TaqMan-probe-based quantitative PCR.

Results: Increased genomic complexity was a hallmark of relapsed/refractory and treatment-naïve high-risk CLL, and was associated with TP53 and ATM dysfunction. In relapsed/refractory cases previously exposed to the selective pressure of chemo(immuno)therapy, only gain(8)(q24.21) and del(9)(p21.3) were found particularly enriched beyond CNAs routinely assessed in CLL (17% and 11%, respectively). Both of these copy number alterations affected key regulators of cell cycle progression, namely *c-MYC* and *CDKN2A/B*. Gains in 8q24.21 were either focal gains in a *c-MYC* enhancer region or larger gains directly affecting the *c-MYC* locus, but only the latter type was highly enriched in relapsed/refractory CLL. Loss of *CDKN2A/B*, which never occurred in standard-risk CLL cases at treatment initiation, was found frequently to co-occur with gain of *c-MYC*. In this combination it was likely associated with Richter transformation, whereas monoallelic loss of *CDKN2A* in the absence of *c-MYC* gain was probably not. Mutations in *SF3B1* (23%) and *NOTCH1* (23%) were found enriched in high-risk CLL with a comparable distribution between relapsed/refractory and treatment-naïve cases. In addition to the high frequency of activating *NOTCH1* mutations, we found recurrent genetic alterations in *SPEN* (4% mutated), *RBPJ* (8% deleted) and *SNW1* (8% deleted), all affecting a protein complex that represses transcription of NOTCH1 target genes. We investigated the functional impact of these alterations on *HES1*, *DTX1* and *c-MYC* gene transcription. De-repression of these NOTCH1 target genes was particularly observed with *SPEN* mutations, but our results also suggested functional impact of *RBPJ* loss.

Summary/Conclusion: In summary, we show that highly complex genomic aberrations are a hallmark of high-risk CLL with *TP53* and *ATM* alteration. We provide a registry of significantly enriched CNAs, define novel recurrent CNAs and identify genomic alterations likely contributing to disease refractoriness. Enrichment of *c-MYC* gain and *CDKN2A/B* loss in relapsed/refractory CLL points to the significance of de-regulated cell cycle in high-risk biology and alterations in a protein complex repressing NOTCH1 target genes likely add to the pathogenic role of aberrant NOTCH1 signaling in CLL.

Biological insights and preclinical studies in multiple myeloma

S1575

THE CHRONOLOGICAL ORDER OF COPY NUMBER CHANGES IN HYPERDIPLOID MULTIPLE MYELOMA PATIENTS

F. Maura[1,2,*], N. Bolli[1,2,3], K. Dawson[1], N. Angelopoulos[1], R. Szalat[4], I. Martincorena[1], T. Mitchell[1], A. Fullam[1], S. Minvielle[5], S. Gonzales[6], D. Glodzik[1], D. Leongamornlert[1], M.K. Samur[4], M. Fulciniti[4], Y.T. Tai[4], F. Magrangeas[7], P. Moreau[7], P. Corradini[2,3], K. Anderson[4], M. Gerstung[6], H. Avet-Loiseau[5], P. Campbell[1], N. Munshi[4,8]

[1]Cancer Genome Project, Wellcome Trust Sanger Institute, Hinxton, United Kingdom, [2]Department of Oncology and Hemato-Oncology, University of Milan, [3]Department of Hematology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, [4]Jerome Lipper Multiple Myeloma Center, Dana–Farber Cancer Institute, Harvard Medical School, Boston, United States, [5]Unité de Genomique du Myelome, Centre Hospitalier Universitaire Rangueil, Toulouse, France, [6]European Bioinformatics Institute (EMBL-EBI), Hinxton, United Kingdom, [7]Université de Nantes, Nantes, France, [8]Veterans Administration Boston Healthcare System, West Roxbury, United States

Background: Multiple myeloma (MM) pathogenesis is characterized by a complex Darwinian evolution process where the progressive accumulation of genetic aberrations confers distinct proliferative advantage and clonal selection. Translocations between the immunoglobulin heavy chain (IGH) locus and known oncogenes, and multiple chromosome gains (Hyperdiploidy, HRD) are considered early drivers, being detectable also in premalignant stages of the disease. The IGH translocations are thought to arise from aberrant AID activity in the germinal centre; however, the pathogenesis of HRD and other aneuploidies remains unclear but are hypothesized to occur during a single failed mitotic event rather than through serial acquisitions over time.
Aims: To investigate the order of acquisition of aneuploidies in MM, we analysed whole genomes sequencing (WGS) data from 67 tumour samples, collected at different clinical time points from a cohort of 30 patients; 11 with smoldering MM (SMM) and 56 with MM at diagnosis or later stages (median of 2 samples per patients; range 1-4).
Methods: Aligned sequence files were analyzed using the published tools available at the Wellcome Trust Sanger Institute. Aneuploidies and their cancer cell fraction (CCF) was extracted by the previously published Battenberg approach.
Results: CCF analysis of the showed that 30% recurrent anueploidies were not fully clonal, suggesting their acquisition at different times during disease evolution. Ranking each event based on the distribution of its CCF, we observed that most chromosome gains in HRD, as well as amp1q, del1p and del13q occurred earlier compared to del17p, del14q and del8p. To increase the resolution of our analysis, we further developed a "molecular time" (MT) analysis of each clonal chromosome duplication greater than 1 Mb. The MT was calculated as the corrected ratio between the total number of clonal SNVs present on > 1 allele and all other clonal SNVs on one allele only. During a single allele duplication, all SNVs already present will be duplicated acquiring an allelic fraction ~66% and conversely all SNVs acquired on the non-duplicated allele (or on one of the two gained alleles after the duplication) will have an allelic fraction ~33%. In this analysis, we considered only those copy number alteration segments with a length > 1 Mb and with more than 50 clonal SNVs, extracted using the previously described dirichlet process. Integrating CCF and MT data we found that not all HRD gains were acquired at once, and in 11/18 (61%) cases the HRD karyotype was the result of multiple independent gains (MIG) acquired at different times. Interestingly we observed that in 12/18 (66%) patients the first HRD MIG event also involved other events, including amp1q that was therefore confirmed as an early event. Similarly, independent MIGs were also present in the MM (sub)clonal architecture evolution, with multiple CNAs gains lost and/or acquired during different cancer progression phases (from SMM to MM and from MM to relapsed MM). At the extreme end of the spectrum, we found 4 patients acquiring a whole genome duplication, whose occurrence only in relapsed samples suggests a potential role in relapsed/refractory stages.
Summary/Conclusion: In conclusion, in this study we described for the first time the pattern of acquisition of the HRD karyotype in MM. By integrating this analysis to the timing of acquisition of mutations, we have reconstructed the life history of the 30 MM cases, highlighting dynamic changes both spontaneous and after treatment, that will impact the way we manage the disease both in SMM and in active MM.

S1576

EXPRESSION OF PROTEINS IN CD138+ PLASMA CELLS PREDICTS PROGNOSIS IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS TREATED WITH VRD REGIMEN

I. Misiewicz-Krzeminska[1,2,3,*], L. Corchete[1,2], I. Isidro[2], T. Prieto[2], V. Gutierrez[2], E. Rojas[1,2], C. De Ramon[4], J. Martinez-Lopez[5,6,7,8], R. Garcia-Sanz[2,9,10], A. Oriol[11], J. Blade[12], J.J. Lahuerta[13], J. San Miguel[14], M.-V. Mateos[1,2,9], N. Gutierrez[1,2,9]

[1]Institute of Biomedical Research of Salamanca (IBSAL), [2]Centro de Investigacion del Cancer, Salamanca, Spain, [3]National Medicines Institute, Warsaw, Poland, [4]Hematology, University Hospital of Salamanca, Salamanca, [5]Hematology, Hospital [12] de Octubre, [6]CNIO, [7]Complutense University, [8]CIBERONC, Madrid, [9]University Hospital of Salamanca, Salamanca, Spain, [10]Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Poland, [11]Hospital Germans Trias i Pujol, [12]Hospital Clinic, Barcelona, [13]Hospital [12] de Octubre, Madrid, [14]Clínica Universidad de Navarra, Centro de Investigaciones Médicas Aplicadas (CIMA), IDISNA, CIBERONC, Pamplona, Spain

Background: The development of precision medicine requires powerful biomarkers mainly based on the mechanism of drug action to predict and improve response to treatment. Although genomic technologies have significantly increased the numbers of potential biomarkers in MM, most of them have not been subsequently validated at protein level. The low protein concentration obtained after CD138+ selection has limited the quantification of proteins in MM so far. We have recently reported the usefulness of a new technique based on capillary nanoimmunoassay (CNIA) for protein expression quantification from purified CD138+ plasma cells (PC).
Aims: To investigate the prognostic impact of the expression of proteins involved in the anti-myeloma mechanism of bortezomib (V), lenalidomide (R) and dexamethasone (D), together with the expression of cyclin D1 and D2 in a large cohort of CD138+ PC samples from MM patients treated with VRD.
Methods: Bone marrow aspirates from 213 newly diagnosed MM patients treated according to the Spanish clinical trial VRD-GEM followed by ASCT conditioned with Mel-200 *vs* BuMel, were included in the study. Plasma cells were purified by anti-CD138 magnetic microbeads using the AutoMACs separation system (purity was above 85%) and next, were stored in RLT+ buffer at -80^{0}C. Proteins were extracted by a method previously reported by our group. Total protein quantification and protein expression were analyzed by the CNIA methodology (ProteinSimple, WES™ system). Progression free survival (PFS) and overall survival (OS) were calculated for each protein. Survival curves were plotted by means of the Kaplan-Meier method and statistical significance was tested using the log-rank test. The Cutoff Finder software was used to obtain the optimal cutoff.

Table 1.

	PFS				OS			
Protein	**HR (CI)**	**Group**	**n**	**p-value**	**HR (CI)**	**Group**	**n**	**p-value**
Aiolos	1.44 (0.67-3.09)	High	105	*0.35*	0.37 (0.15-0.9)	High	156	***0.022***
		Low	78			Low	27	
Ikaros	3.14 (1.43-6.93)	High	28	***0.0027***	0.64 (0.28-1.49)	High	120	*0.3*
		Low	155			Low	63	
Cereblon	0.32 (0.12-0.84)	High	170	***0.014***	0.18 (0.07-0.46)	High	170	***0.00005***
		Low	13			Low	13	
IRF4	0.49 (0.24-1.02)	High	138	*0.053*	0.52 (0.22-1.26)	High	140	*0.14*
		Low	47			Low	45	
PSME1	27138793 (0-inf)	High	172	*0.13*	27063249 (0-inf)	High	172	*0.21*
		Low	11			Low	11	
Gankyrin (PSMD10)	3.33 (1.01-10.98)	High	136	***0.036***	3.43 (0.8-14.71)	High	136	*0.078*
		Low	47			Low	47	
GR	0.53 (0.25-1.12)	High	97	*0.093*	0.41 (0.17-0.97)	High	133	***0.036***
		Low	85			Low	50	
XPO1	0.51 (0.21-1.25)	High	163	*0.13*	0.33 (0.11-0.97)	High	80	***0.035***
		Low	22			Low	105	
Cyclin D1	0.4 (0.15-1.05)	High	63	*0.054*	0.2 (0.05-0.84)	High	63	***0.015***
		Low	121			Low	121	
Cyclin D2	6.19 (2.42-15.86)	High	15	***0.00004***	6.19 (2.42-15.86)	High	15	***0.00001***
		Low	168			Low	168	

Results: After the analysis of total protein content, 194 MM samples of 213 (91%) fulfilled the quantity and quality requirements. Among the 10 proteins analyzed the expression of aiolos, cereblon, ikaros, gankyrin, proteasome activator subunit 1 (PSME1), glucocorticoid receptor (GR), exportin 1 (XPO1) and interferon regulatory factor 4 (IRF4) were detected in about 90% of MM samples. On the contrary, cyclin D1 and cyclin D2 proteins were detected in only 43% and 25% of MM samples, respectively. Regard-

ing cytogenetic risk, IRF4, PSME1 GR and XPO1 proteins were significantly upregulated in low risk MM patients, while cyclin D2 was upregulated in high risk MM (p<0.05). At the time of study, the median follow-up for survivors was 25.5 months (range, 14.1-42.1). Kaplan-Meier survival analyses showed that high levels of ikaros and gankyrin were associated with shorter PFS ($p < 0.01$ and $p < 0.05$, respectively), while high level of cereblon was associated with longer PFS ($p < 0.05$). Regarding OS, high protein levels of aiolos, cereblon, GR and XPO1 were associated with better prognosis (see table below). Interestingly, patients with high cyclin D2 protein levels had a significantly shorter PFS and OS ($p < 0.0001$), while those with high level of cyclin D1 exhibited longer OS ($p < 0.05$).

Summary/Conclusion: The expression level of proteins involved in the mechanism of action of bortezomib, lenalidomide and dexamethasone, discriminate prognosis in MM patients. High level of cyclin D2 protein identified MM patients with poor outcome. The quantification of protein expression by CNIA platform can be a useful tool for biomarker identification in the era of precision medicine.

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S1577

EFFECTS OF DARATUMUMAB ON THE COMPOSITION AND ACTIVATION STATUS OF IMMUNE-CELL POPULATIONS IN CENTAURUS, A PHASE 2 RANDOMIZED STUDY OF SMOLDERING MULTIPLE MYELOMA (SMM) PATIENTS

H. AdamsIII[1,*], K. Vander Borght[2], Y. Abraham[2], G. Vanhoof[2], T. Smets[2], A. Axel[1], S. Gaj[3], F. Stevenaert[2], C. Chiu[1], C.O. Landgren[4], C. Hofmeister[5], H. Goldschmidt[6], T. Neff[1], M. Qi[1], T. Casneuf[2]

[1]Janssen Research & Development, LLC, Spring House, PA, United States, [2]Janssen Research & Development, LLC, Beerse, Belgium, [3]LabConnect, LLC, Seattle, WA, [4]Myeloma Service, Memorial Sloan Kettering Cancer Center, New York, NY, [5]Winship Cancer Institute, Atlanta, GA, United States, [6]University Medical Hospital and National Center of Tumor Diseases, Heidelberg, Germany

Background: Daratumumab (DARA) is a human monoclonal IgG1 CD38-targeted antibody that has several mechanisms of action, including complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity, antibody-dependent cellular phagocytosis, modulation of CD38 enzymatic activity, and induction of apoptosis with antibody cross-linking. DARA (16 mg/kg) single-agent, phase 1/2 translational studies (SIRIUS and GEN501) revealed an additional, novel immunomodulatory mechanism of action that increased the adaptive immune response in multiple myeloma (MM). Natural killer (NK) cells were reduced in these studies, with no effect on DARA efficacy or safety (Casneuf T et al, *Blood Adv*. 2017;1[23]:2105-2114; Adams HC 3rd et al, Presented at ASH 2016; Abstract 4521). Furthermore, subsequent combination studies showed DARA promotes robust pro-adaptive immunological changes when combined with lenalidomide and dexamethasone (van de Donk NWCJ et al, Presented at ASH 2017; Abstract 3124).

Aims: To explore the ability of DARA to promote adaptive T-cell responses and immune changes in asymptomatic SMM patients (pts) in CENTAURUS (**Figure;** NCT02316106) using cytometry by time-of-flight (CyTOF®).

Methods: Intermediate and high-risk SMM pt whole-blood samples from three treatment arms were analyzed: Long (L), Intermediate (I) and Short (S) at Cycle 1 Day 1 (L, n=34; I, n=39; S, n=30) and after a minimum of one cycle of DARA (16 mg/kg intravenous) monotherapy (L at Cycle 4 Day 1, n=33; I at Cycle 4 Day 1, n=36; S at End of Treatment, n=30). Samples were stained with a metal-conjugated antibody panel and evaluated by CyTOF®. Similar cellular events were clustered into nodes using the spanning tree progression of density normalized events (SPADE) algorithm and annotated into immune-cell populations via Cytobank® software. Differences in marker intensity and cell populations within subgroups were defined by *P* values derived from Student T-tests over time and at baseline. Results were visualized by SPADE-blend trees, where each cluster was colored using a combination of *P* values related to marker intensity and cell-population size changes, and using Radviz projections to monitor complex effects.

Results: Consistent with previous studies, we observed a reduction in circulating NK cells, along with CD38 downregulation predominantly in NK cells, basophils, and $CD38^+$ regulatory T cells ($CD3^+CD4^+CD25^+CD127^-$), in all treatment arms. The proportion of T cells increased across all arms and was correlated with a higher proportion of $CD8^+$ *versus* $CD4^+$ T cells. After DARA administration, the total T-cell populations shifted towards $CD8^+$ T cells with increased production of granzyme B, a cytolytic enzyme. Prominent increases in the $CD8^+$ T-cell effector memory compartment were observed in post-DARA/on-study samples. Likely driven by differences in time of collection between treatment arms, the increase in expression of the activation marker HLA-DR was more pronounced in $CD8^+$ central memory and effector memory T cells of pts in the S arm, while granzyme B increased independent of treatment arm.

Table 1.

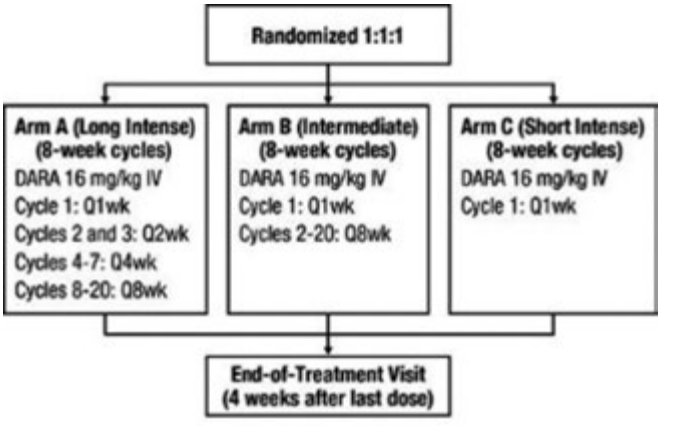

Figure. CENTAURUS Study Design.
DARA, daratumumab; IV, intravenous; Q1week, once weekly; Q2week, once every 2 weeks; Q4wk, once every 4 weeks; Q8wk, once every 8 weeks.

Summary/Conclusion: DARA administration across the three treatment arms induced clinical responses in pts and T-cell profile changes, including expansion of effector memory T cells and increased expression of activation markers. These changes are conducive to adaptive immunity. As SMM pts are relatively more immune-competent compared to MM pts, this study again demonstrates DARA's immunomodulatory activity in asymptomatic SMM which may play a part in delaying the progression to MM.

S1578

SIMULTANEOUS TARGETING OF MCL-1 (S63845) AND BCL-2 (VENETOCLAX) ANTI-APOPTOTIC PROTEINS IN MULTIPLE MYELOMA

E.M. Algarín[1,*], L. San-segundo[1], S. Hernández-García[1], L. González-Méndez[1], M. Martín-Sánchez[1], P. Mogollón[1], A. Díaz-Tejedor[1], M. Schoumacher[2], S. Banquet[2], L. Kraus-Berthier[2], I. Kloos[2], E. Halilovic[3], H. Maacke[4], N.C. Gutiérrez[1], M.-V. Mateos[1], T. Paíno[1], M. Garayoa[1], E.M. Ocio[1]

[1]Hospital Universitario de Salamanca (IBSAL). Centro de Investigación del Cáncer (IBMCC-CSIC-USAL), Salamanca, Spain, [2]Institut de Recherches Servier, Suresnes, France, [3]Novartis Institutes for Biomedical Research, Cambridge, MA, United States, [4]Novartis Institutes for Biomedical Research, Basel, Switzerland

Background: Drugs with novel mechanisms of action are essential to overcome resistance to current standards of care in MM. Recently, the BCL-2 inhibitor, venetoclax, has demonstrated to be effective in this disease, particularly in patients bearing the t(11;14) translocation. However, overexpression of MCL-1 seems to be more important in MM pathogenesis, which raises the possibility of using the new MCL-1 inhibitor, S63845, as a promising therapeutic agent for the treatment MM.

Aims: The aim of the present work was to evaluate the efficacy and mechanism of action of S63845 (MCL1i) alone and in combination with venetoclax (BCL2i) in preclinical *in vitro* and *in vivo* models of MM.

Methods: S63845 was provided by Servier. *In vitro* activity of MCL1i and BCL2i alone and in combination was evaluated on different myeloma cell lines. The combination index (CI) was calculated with Calcusyn software based on results from MTT assay. Four cell lines with different sensitivity to MCL1i (MM.1S, JJN3, KMS12-BM and NCI-H929) were chosen for the mechanistic studies. Effects on apoptosis and cell cycle were evaluated by flow cytometry. BCL-2 family protein levels were analysed by Western blot. The mechanism of action was assessed by immunoprecipitation assays. Finally, a plasmacytoma model in CB17-SCID mice and a disseminated model in BRG mice were used for *in vivo* studies.

Results: The MCL1i, S63845, showed a strong anti-tumor dose-dependent effect on nine multiple myeloma cell lines, with IC50 values at 48 hours ranging from 2.6 to 465 nM. The sensitivity to S63845 was independent of

the genetic alterations and the basal expression of the different BCL-2 family members. Although co-culture with stromal cells induced MCL-1 expression, S63845 remained active in these conditions. While no changes were observed in cell cycle, S63845 treatment induced apoptosis, triggered by mitochondrial outer membrane permeabilization. S63845 did not significantly modify the levels of MCL-1 or other BCL-2 family member in MM.1S or KMS12-BM, although immunoprecipitation assays in MM.1S, JJN3, NCI-H929 and KMS12-BM cell lines showed that S63845 impaired the MCL-1/BIM interaction. Interestingly, a compensatory increase in the BCL-2/BIM interaction in the two less sensitive cell lines tested, MM.1S and JJN3, was observed after treatment with S63845. On the other hand, the BCL-2 inhibitor displayed the opposite effect, with an impairment of the BCL-2/BIM interaction but a compensatory increase of the binding of MCL-1 to BIM. Accordingly, we hypothesized that the simultaneous inhibition of MCL-1/BIM and BCL-2/BIM interactions could result in significant synergistic induction of apoptosis. In this regard, the combination of MCL1i + BCL2i led to pronounced synergy, with CIs ranging from 0.1 to 0.4, in MM1S, JJN3, KMS-12.BM and NCI-H929 (Figure 1). Moreover, the triple combination MCL1i + BCL2i + dexamethasone showed an even stronger synergy than the double treatment of MM.1S and in JJN3 (CI<0.06 and CI<0.2, respectively). The efficacy of the triple combination MCL1i + BCL2i + dexamethasone is currently being evaluated in two murine models of human myeloma, a subcutaneous plasmacytoma and a disseminated one.

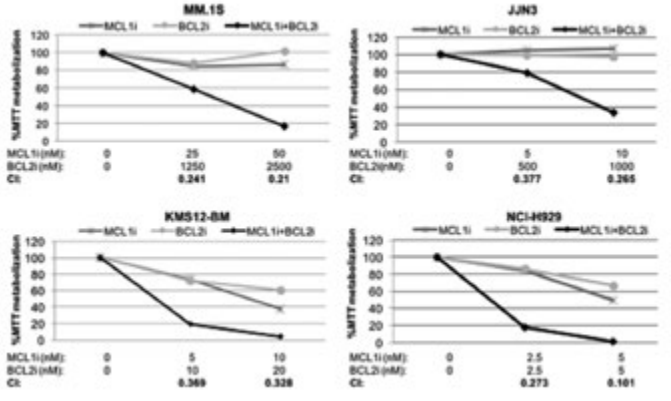

Figure 1.

Summary/Conclusion: Our preclinical data demonstrate the potent activity of the combination of MCL1 and BCL2 inhibitors (+/- dexamethasone) in MM, and provides the rationale for the clinical development of this combination in relapsed or refractory MM patients.

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S1579

PRECLINICAL EVALUATION OF THE NEW BCMAXCD3 BISPECIFIC ANTIBODY JNJ-957 FOR THE TREATMENT OF MULTIPLE MYELOMA

K. Frerichs[1,*], M. Broekmans[1], J. Marin Soto[1], B. van Kessel[1], A. Axel[2], C. Chiu[2], H. AdamsIII[2], S. Zweegman[1], T. Mutis[1], N. van de Donk[1]

[1]Hematology, VU University Medical Center, Amsterdam, Netherlands, [2]Research & Development, Janssen, Spring House PA , United States

Background: Multiple myeloma (MM) patients with disease refractory to all available treatments have a very poor outcome. B-cell maturation antigen (BCMA) is a tumor necrosis factor (TNF) family surface protein involved in differentiation of B-cells to plasma cells. The selective expression of BCMA on plasma cells renders it an attractive target for novel MM treatment strategies.

Aims: We evaluated the preclinical activity of a new BCMAxCD3 bispecific antibody (JNJ-957) for the treatment of MM. We also analyzed the biomarkers and T-cell subsets associated with the *in vitro* JNJ-957 response.

Methods: MM cell lysis by JNJ-957 was analyzed in MM cell lines and whole bone marrow (BM) samples from MM patients. In MM cell lines, peripheral blood mononuclear cells (PB MNC) from healthy donors or MM patients were used as effector cells. Serial dilutions of JNJ-957 (0.0064-4µg/ml) were incubated with the samples for 48 hours. The lysis of $CD138^{high}/CD38^{+}$ MM cells was assessed by flow cytometry. At baseline, the MNC were characterized for the composition of different T-cell subsets. JNJ-957 induced T-cell activation and degranulation were measured by the expression of CD25 and CD107a, respectively.

Results: JNJ-957 effectively killed $BCMA^{+}$ MM cell lines (NCI-H929 and RPMI8226) in a dose-dependent manner by healthy donor or MM patient derived PB MNCs . In newly diagnosed (ND)MM patient samples (n=8), the mean lysis of MM cells by JNJ-957 4.0µg/mL was 79% (range: 66-92%; figure 1A). Similar MM lysis, but with a larger variation, was achieved in lenalidomide (LEN) refractory patient samples (n=15; mean lysis at 4.0µg/mL: 69%; range: 24-98%; fig 1B), who were also bortezomib (73%), pomalidomide (82%) and carfilzomib (9%) refractory. JNJ-957 was also effective in samples from MM patients who were daratumumab (DARA) refractory (n=11; mean lysis at 4.0µg/mL: 83%; range: 52-99%; fig 1C). NK- and T-cell frequencies were not affected. JNJ-957-mediated MM cell lysis was associated with activation and degranulation of CD4+ and CD8+ T-cells.The heterogeneity in MM cell lysis by JNJ-957 could not be explained by BCMA or PD-L1 expression levels on MM cells, the presence of standard or high-risk cytogenetic abnormalities, or composition of T-cell subsets. Only at suboptimal JNJ-957 doses, the MM cell lysis was affected by high frequency of regulatory T-cells (Treg) and low frequency of effector memory (Tem) and terminally differentiated effector (TEMRA) T-cells. Since improvement in tumor reduction could be aided by the recently discovered immune stimulatory effects of DARA, we also analyzed sequential BM aspirates from MM patients before and after DARA treatment (n=5). Here we observed comparable BCMA expression, yet improved MM cell lysis by JNJ-957 in samples obtained after disease progression during DARA compared to samples before DARA initiation (mean lysis at 4.0µg/mL: 93 *vs* 74%; fig 1D).

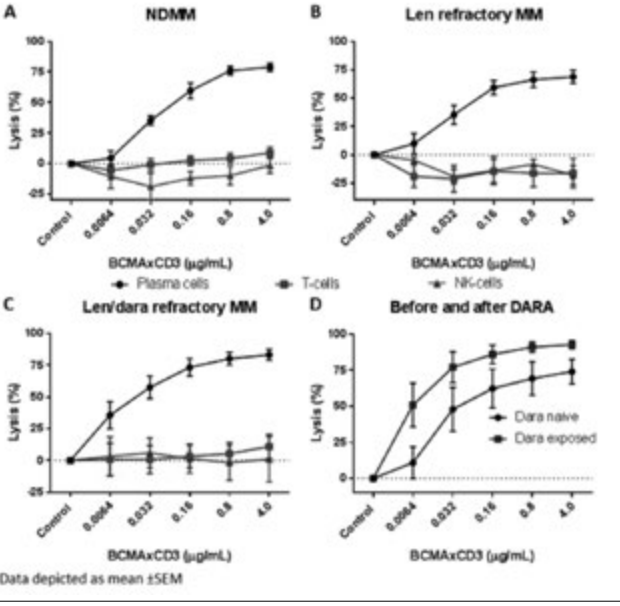

Figure 1.

Summary/Conclusion: The BCMAxCD3 bispecific antibody, JNJ-957, effectively lysed primary MM cells both in samples derived from heavily pretreated DARA-refractory patients and those with low BCMA surface expression. Optimal JNJ-957 dosing overcame the negative influence of high Treg and low Tem/TEMRA frequencies on the extent of MM cell killing. Our data also suggest that *in vivo* pretreatment with DARA improves the response to JNJ-957. Altogether, this strengthens the preclinical rationale for an ongoing phase 1 study with JNJ-957 in relapsed/refractory MM.

Inherited bone marrow failure including PNH

S1580

A COHORT OF 22 PATIENTS WITH SAMD9/SAMD9L DISORDER: BONE MARROW FAILURE AND MDS PREDISPOSITION WITH TRANSIENT MONOSOMY 7

M. Sébert[1,*], O. Bluteau[1], T. Leblanc[2], A. Raimbault[1], R. Peffault De Latour[3], S. Quentin[1], E. Lainey[4], L. Hernandez[1], J.-H. Dalle[5], F. Sicrede Fontebrune[3], E. Clappier[1], N. Boissel[6], C. Schmitt[7], M. Muller[7], N. Blin[8], B. Bruno[9], I. Pellier[10], A. Petit[11], S. Blanche[12], G. Leverger[11], G. Michel[13], Y. Bertrand[14], A. Baruchel[2], G. Socié[3], J. Soulier[1]

[1]Laboratoire d'hématologie, U944 , IUH (Hôpital St Louis), [2]Service d'Hématologie pédiatrique, Hôpital Robert-Debré, [3]Hématologie-greffe, Hôpital Saint-Louis (AP-HP), [4]Service d'hématologie biologique, [5]Service d'hématologie et immunologie pédiatrique, Hôpital Robert-Debré, [6]Hématologie AJA, Hôpital Saint-Louis, Paris, [7]Hématologie pédiatrique, C.H.U. de Nancy, Nancy, [8]Hématologie clinique, CHU Hôtel-Dieu, Place Alexis-Ricordeau, Nantes, [9]Hématologie pédiatrique, Hôpital Jeanne de Flandre, Lille, [10]Service des maladies du sang, C.H.U. d'Angers, [11]Service d'hématologie et oncologie pédiatrique, Hôpital d'enfants Armand Trousseau, [12]Service d'hématologie-immunologie et rhumatologie pédiatrique, Hôpital Universitaire Necker-Enfants Malades, Paris, [13]Service d'hématologie et oncologie pédiatrique, Hôpital de la Timone, Marseille, [14]Service d'hématologie et immunologie pédiatrique, Institut d'Hématologie et d'Oncologie Pédiatrique, Lyon, France

Background: *SAMD9L* and *SAMD9* are two genes located at chromosome 7q21 that encode for two large homologous proteins implicated in inflammatory and antiviral responses. While these genes can be lost somatically in myelodysplastic syndromes (MDS) (*Nagamachi, Cancer Cell 2013*), germline activating mutations have been recently associated to the autosomal dominant syndromes *Ataxia-Pancytopenia* (*SAMD9L gene; Chen, Am J Hum Genet 2016*) and *MIRAGE* (*SAMD9 gene; MDS, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy*; *Narumi, Nat Genet 2016*). We and other have found that *SAMD9* and *SAMD9L* germline heterozygous mutations further defined a spectrum of bone marrow failure (BMF) and MDS genetic predisposition in children (*Tesi, Blood 2017*; Bluteau, *Blood 2017; Schwartz, Nat com 2017*).
Aims: In order to delineate better the SAMD9/SAMD9L associated disorder, we analyzed a cohort of 22 patients (pts) with germline *SAMD9* or *SAMD9L* mutations originating from 16 families.
Methods: Patients and relatives were originally investigated in the laboratory of the French Aplasia Center to search for a familial origin to their BMF or MDS. All subjects gave informed consent for genetic investigation, tissue banking and research, and this study was IRB approved. We here report the natural clinical history of 22 patients, with a median FU of 6 years (7 months-37y). In 14 of them, we analysed hematopoietic somatic events using WES and Sanger re-sequencing on blood or bone marrow samples, in comparison with fibroblast DNA.
Results: Fifteen and 7 pts with *SAMD9L* and *SAMD9* germline mutations were identified, from 9 and 7 unrelated families, with a median age at first hematological signs of 13 months (8 months-46y) and 7 years (2-21y), respectively. Six out of 15 *SAMD9L* pts had neurological signs and 1/7 *SAMD9* pts had a MIRAGE syndrome. Immunoglobulin deficiency with recurrent infections was found in both SAMD9 (N=4/7) and in SAMD9L (N=3/15) pts. Regarding hematological features, 20 pts had central cytopenia, with BM dysplasia in 11 and monosomy 7 in 10. Except one with an additional TERC germline mutation, patients did not present excess of blasts or cytogenetic lesion in addition to monosomy 7 at any point of their natural history. Ten had a family history while 12 presented simplex. Strikingly, spontaneous improvement in blood cell counts was seen in 11 pts, and MDS and monosomy 7 disappearance in 5 pts, with a median follow-up of 4 y. HSCT that was planned for five of these pts was eventually cancelled and they are still alive without consistent cytopenia with a follow-up of four to 33 years. Finally, two carrier subjects had no overt hematological signs. We sequenced blood and/or marrow mononuclear cells in 14 pts and found somatic changes such as acquired 7q uniparental disomy (UPD) or additional *SAMD9* or *SAMD9L* cis-mutations in 7 of them, suggesting spontaneous genetic correction of the germline mutated allele.
Summary/Conclusion: Germline mutations of *SAMD9* and *SAMD9L* delineate an inherited BMF/MDS predisposition disorder in children and young adults with frequent transient monosomy 7. Patients may benefit from a careful watch-and-wait policy rather than upfront HSCT, even when presenting with dysplasia and monosomy 7, a practice-changing option that will have to be carefully evaluated.

S1581

CHARACTERIZATION OF ORALLY BIOAVAILABLE SMALL MOLECULE INHIBITORS OF COMPLEMENT C5

A. Ricardo[1,*], M. Hale[1], Z. Ma[1], J. Reyes[1], C. Sayegh[1], K. Sherry[1], Y. Sun[1], Y. Tan[1], D. Vadysirisack[1], N. Zu[1]

[1]Ra Pharmaceuticals, Inc., CAMBRIDGE, United States

Background: Abnormal activation of the complement cascade is implicated in the pathogenesis of many rare and common diseases. Complement C5 inhibition by intravenous administration of the monoclonal antibody eculizumab has demonstrated therapeutic benefit in paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS) and, most recently, in myasthenia gravis. Given the demonstrated and broad therapeutic utility of complement inhibition in these and other disorders, there is substantial interest in the development of an oral small molecule C5 inhibitor (C5SMi) as a treatment for autoimmune, hematological, renal, vascular and neurological diseases. We recently described the discovery of orally bioavailable small molecule inhibitors of complement C5. Here, we describe their pharmacology in human whole blood-based assay models, pharmacokinetics in several preclinical species, and their ex *vivo* PK/PD relationships.
Aims: -Characterize the pharmacology of orally available C5SMi in human whole blood-based models of disease; Determine the pharmacokinetic parameters of C5SMi in preclinical species (rodents and dog); Establish a PK/PD (ex vivo) relationship for orally available C5SMi in mice
Methods: Pharmacokinetic studies of C5SMi were conducted in rodents and dogs following intravenous and oral administration. Non-compartmental analyses were applied to the plasma concentration *versus* time data (PK analysis). Blood was drawn at multiple time points following administration to establish an ex-vivo PK/PD relationship by monitoring C5a formation after zymosan mediated complement activation (PD analysis). Potency in disease-relevant human whole blood studies was determined by ABO incompatibility reactions following mixing blood from a donor with serum from an incompatible recipient. Finally, a human whole blood hemolysis assay was performed using a polyclonal anti-human RBC antibody and hemolysis monitored using spectrophotometric methods.
Results: The pharmacokinetic data demonstrate that compounds within the lead series display dose proportional oral exposure and low clearance values in several preclinical species. *Ex vivo* and *in vitro* assays, including in human whole blood assays, demonstrate the selective engagement of complement C5, and the mouse PK/PD relationship following oral dosing informs the level of exposure required to achieve therapeutic efficacy in humans.
Summary/Conclusion: The results presented in here confirm the feasibility of inhibiting complement C5 using small molecule inhibitors via oral dosing. The excellent drug-like properties exhibited by these compounds, their favorable pharmacokinetic behavior in different preclinical species, and the understanding of the PK/PD relationship informs the required therapeutic drug levels that will be necessary to evaluate their pharmacology in human disease.

S1582

MANAGEMENT OF ELANE-NEUTROPENIA: LIFETIME G-CSF *VS* HAEMATOPOIETIC STEM CELL TRANSPLANTATION LEARNING LESSON FROM THE EXPERIENCE

F. Fioredda[1,*], F. Pierri[2], M. Lanciotti[2], S. Zanardi[3], B. Gaspar[4], P. Ancliff[5], M. Bierings[6], G. Ozturk[7], O. Smith[8], P. Veys[9], P. Ljungman[10], R. Peffaultdela Tour[11], R. Or[12], R. Wynn[13], K. Kalwak[14], G. Russo[15], F. Tucci[16], C. Knoll[17], B. Martire[18], C. Favre[19], C. Bellanné-Chantelot[20], B. Beaupain[21], J. Donadieu[21], C. Dufour[1]

[1]Haematology Unit, IRCCS Giannina Gaslini, [2]Haematology Unit, [3]Biostatistic and Epidemiology Unit, IRCCS Istituto Giannina Gaslini, Genova, Italy, [4]Infection, Immunity, Inflammation, Molecular and Cellular Immunology Section, University College London, [5]Department of Haematology, Great Ormond Street Hospital Children's Charity, London, United Kingdom, [6]Pediatric Blood and Marrow Transplantation Program, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, Netherlands, [7]Acıbadem University Atakent Hospital, Instanbul, Turkey, [8]Department of Pediatric Hematology, Our Lady's Children's Hospital, Dublin, Ireland, [9]Bone Marrow Transplant Unit, Great Ormond Street Hospital , London, United Kingdom, [10]Haematology Unit, Karolinska Institutet, Stockholm, Sweden, [11] Service d'hématologie greffe, Hôpital Saint-Louis , Paris, France, [12]Department of Bone Marrow Transplantation, Hadassah University Hospital, Jerusalem, Israel, [13]Department of Paediatric Haematology Bone Marrow Unit, Royal Manchester Children's Hospital, Manchester, United Kingdom, [14]Pediatric Hematology/Oncology and BMT, Medical University of Wroclaw,

Wroclaw, Poland, [15]Pediatric Hematology/Oncology Unit, Policlinico Vittorio-Emanuele, Università di Catania, Catania, [16]Pediatric Hematology/Oncology Unit, Meyer, Firenze, Italy, [17]Hematology/Oncology, Phoenix Children's Hospital, Phoenix, United States, [18]OncoEmatologia ed Immunologia Pediatrica, Policlinico Bari, Bari, [19]Oncoematologia Pediatrica, Meyer Hospital, Firenze, Italy, [20]Département de Génétique, , AP-HP Hôpital Pitié-Salpêtrière, UPMC Univ Pitiè-Salpetriere Hospital, [21]Service d'Hémato Oncologie Pédiatrique, Registre des neutropénies congénitales, AP-HP Hopital Trousseau, Paris, France

Background: Severe Congenital Neutropenia (SCN) due to ELANE mutations is usually managed with Granulocyte–Colony Stimulating Factor (G-CSF) but may also be amenable for treatment with Allogeneic Haematopoietic Stem Cell Transplantation (HSCT). According to the experts, HSCT is indicated in patients transformed into MDS/AL or who are refractory/poor responding to G-CSF (>20 mcg/kg/day). HSCT looks also appropriate for those subjects treated with G-CSF at doses between 10-20 mcg/kg/d, while the indications for those subjects managed with doses up to 10 mcg/kg are less clear.
Aims: To retrospectively compare the long term outcome of SCN-ELANE patients who underwent HCST *vs* those who did not, stratified according to G-CSF dose .
Methods: Data have been extracted from the Severe Chronic Neutropenia French registry (SCNFR), the Italian Neutropenia Registry (INR), from the database of the Severe Aplastic Anemia Working Group of the European Society for Blood and Marrow Transplantation (EBMT) and from Stem Cell Transplant for Immunodeficiencies in Europe (SCETIDE) in the years 1990-2017. The received dose of G-CSF was estimated by calculating the amount of drug that patients received for more than 75% of the treatment time (when daily dose was not available for the whole treatment period) or by calculating the mean dose (total amount/days of treatment) whenever daily dose was available for the full period. The cohort has been arbitrarly stratified in two subgroups according to the dose of G-CSF received: ≤10 mcg/kg/d, and >10 mcg/kg/d.
Results: 162 SCN ELANE patients were considered eligible for the study, 141 were treated with G-CSF and 52 received HSCT. Transplant indication was as per Center policy. Characteristics of the population are shown in Table 1. The 15 y-OS of the whole group was 91% (95% IC; 84-95). HSCT patients had worse survival *vs* those treated with G-CSF: 79% (95% IC; 52-92) *vs* 94% (95% IC 87-97) respectively (p=0.031). The 15y-OS of patients receiving G-CSF >11mcg/kg/d was 85% (95% IC; 51-96) *vs* 74% (95% IC 33-92) of HSCT patients (p 0.325). Transplanted patients receiving ≤10mcg/kg/d of G-CSF had significantly lower 15-yr OS if compared with the G-CSF treated subjects (74% -95% IC 24-94 *vs* 98% -95% IC 91-99-) (p=0.015). In the subgroup treated with G-CSF ≤10mcg/kg/d who underwent HSCT, 1 patients had AL, 1 had unmanageable infections and 5 had available an identical siblings. Comparison of OS in the two groups by additional "risk factors" other than CSF dose is ongoing.

Table 1.

TABLE 1 Characteristic of the cohort		Whole Cohort	Life time G-CSF pts N(%)	HSCT pts N(%)	P
No		162	141 (68%)	52(32%)	
Gender	M	72	49 (44%)	23 (44%)	ns
	F	90 (57%)	61 (66%)	29 (66%)	ns
Median age at last FUP (years)		12 (0.8-62)	14.4 (1.39-62)	8.8 (0.8-25)	<0.001
G-CSF µg/kg/day	**≤10**	**107**	**93**	**14**	**<0.001**
	>11	**41**	**13**	**28**	**<0.001**
	<5	**64 (60%)**	**28 (30%)**	**0 (0%)**	**0.01**
MDS/AL		5/6	0/2	4/5	<0.001
Deaths		13	7 (6%)	6 (11%)	ns
	GvHD	3	0	3	ns
	Infection	7	4	3	ns
	AL/other tumour	3	3	0	ns

Summary/Conclusion: This is the largest comparative analysis between HSCT and life-time G-CSF conducted in *ELANE*-SCN cohort . The OS of the whole cohort 91 % after 15 y of follow up. On the whole the HSCT group shows a significantly worse survival *vs* G-CSF treated one probably because a negative selection of the transplanted patients . However the OS of the group who received G-CSF ≤10mcg/kg/d and underwent HSCT was significantly inferior than that managed with G-CSF alone . This suggests that indication to transplant for patients receiving G-CSF dose ≤10mcg/kg/d needs to be carefully balanced. Definitive conclusion might be drawn at the completion of analysis including other factors that may affect the outcome like molecular characteristics, clonal evolution markers and infection load.

S1583

PROGNOSTIC FACTORS FOR HLH IN ADULTS – ANALYSIS FROM THE PALG HLH IN ADULTS DATABASE

R. Machowicz[1,*], A. Waszczuk-Gajda[1], M. Witkowska[2], A. Piekarska[3], P. Boguradzki[1], P. Smolewski[4], W. Knopinska-Posluszny[5], E. Cichocka[6], M. Razny[7], W. Sydor[8], M. Gorka[1], J. Drozd-Sokolowska[1], B. Garus[9], P. Mensah-Glanowska[10], K. Madry[1], R. Guzicka-Kazimierczak[11], S. Rejowski[12], P. Zielinska[13], D. Zdunczyk[1], P. Marszalek[14], A. Hajduk[15], K. Lis[1], A. Bogucka-Fedorczuk[16], L. Bolkun[17], K. Brzezniakiewicz-Janus[18], D. Bursa[19], M. Gasik[20], J. Gil[21], K. Kurowska[22], M. Paszkowska-Kowalewska[1], K. Romanowska-Prochnicka[23], E. Snarski[1], M. Swacha[24], A. Szymczyk[25], A. Swiderska[26], K. Torba[13], M. Ziarkiewicz[1], B. Kaczynski[27], J. Dwilewicz-Trojaczek[1], W. Wiktor-Jedrzejczak[1]

[1]Department of Hematology, Oncology and Internal Diseases, Medical University of Warsaw, Warsaw, [2]Department of Experimental Hematology, Medical University of Lodz, Lodz, [3]Department of Hematology and Transplantology, Medical University of Gdansk, Gdansk, [4]Department of Experimental Hematology, Medical University of Lodz, Lodz, [5]Hematology Department, [6]Oncology and Immunooncology Department, Independent Public Health Care Ministry of the Interior of Warmia and Mazury Oncology Center, Olsztyn, [7]Department of Hematology, Rydygier Specialistic Hospital, [8]The Second Chair and Department of Internal Medicine, Collegium Medicum, Jagiellonian University, , Krakow, [9]Department of Hematology, Holycross Cancer Center, Kielce, [10]Department of Hematology, Collegium Medicum, Jagiellonian University,, Krakow, [11]Department of Hematology, Pomeranian Medical University, Szczecin, [12]Department of Hepatology and Internal Medicine, Medical University of Warsaw, Warsaw, [13]Department of Hematology and Bone Marrow Transplantation, Medical University of Silesia, Katowice, [14]Collegium Medicum, Jagiellonian University,, Krakow, [15]Department of Internal Medicine, Connective Tissue Diseases and Geriatrics, Medical University of Gdansk, Gdansk, [16]Department of Hematology, Blood Neoplasms and Bone Marrow Transplantation, Wroclaw Medical University, Wroclaw, [17]Department of Hematology, , Medical University of Bialystok, Bialystok, [18]Department of Hematology, Multi-Specialist Hospital, Gorzow Wielkopolski, [19]Department of Infectious Diseases, Medical University of Warsaw, Warsaw, [20]Voivodship Integrated Hospital in Plock, Plock, [21]Department of Hematology, Markiewicz Memorial Oncology Center Brzozow, Brzozow, [22]Institute of Psychiatry and Neurology, Warsaw, [23]Department of General and Experimental Pathology, [2]nd Faculty of Medicine, Medical University of Warsaw, Lodz, [24]Department of Systemic Connective Tissue Diseases, Eleonora Reicher National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw, [25]Chair and Department of Haematooncology and Bone Marrow Transplantation, Medical University of Lublin, Lublin, [26]Department of Hematology, Karol Marcinkowski Hospital, Zielona Gora, [27]Department of Medical Informatics and Telemedicine, Medical University of Warsaw, Warsaw, Poland

Background: Hemophagocytic syndrome (HLH, hemophagocytic lymphohistiocytosis) is a rare syndrome of fatal hyperinflammation, where cytokine storm leads to bone marrow failure and death. Historically it was mostly diagnosed in pediatric population, but with raising awareness data form adults are emerging. Little is known about prognostic factors in HLH, especially in adult patients.
Aims: Aim of this study was search for prognostic factors in one of the largest cohorts of European adult HLH patients.

Table 1.

Table 1. Prognostic factors for OS in adult HLH – in univariate and multivariate analyses

Factor	n	Univariate			Multivariate		
		HR	HR 95% CI	p	HR	HR 95% CI	p
MAS	86	0.146	0.035-0.602	0.008	0.161	0.038-0.686	0.014
Hepatomegaly	83	0.574	0.324-1.017	0.057	0.515	0.283-0.937	0.03
RBC	86	0.591	0.366-0.954	0.031			0.284
Trig>265mg/dl	84	0.559	0.315-0.993	0.047			0.249
AT III	25	0.04	0.002-0.656	0.024			
ESR	21	0.974	0.95-0.999	0.04			

MAS: Macrophage Activation Syndrome; RBC: red blood cell count; Trig: triglycerides; AT III: Antithrombin III; ESR: erythrocyte sedimentation rate.

Methods: Data of 86 adult (≥ 18 years of age) patients form the HLH in Adults Database affiliated with PALG (Polish Adult Leukemia Group) were retrospectively analyzed. Risk factor analysis was made by Cox regression. Factors were analyzed in univariable analysis, and factors with $p < 0.06$ and $n > 82$ were retained in the multivariable model. Statistica 13 and MedCalc 18 softwares were used.
Results: A slight male predominance (62%; 53/86) was observed in the analyzed group. Median age was 38 (18-82). Median survival reached 144 days, 35% of patients died within one month. Among 29 patients who survived over one year, only two died (both due to HLH relapse). Longest observation

exceeded 10 years. There were 34 patients with malignancy-associated HLH, 20 with infection-associated HLH, 14 with HLH associated with autoimmune disease (MAS syndrome; Macrophage Activation Syndrome). In 18 patients triggering factor could not be determined. In univariate analysis (Table 1) MAS syndrome, red blood cell count (RBC) and hypertriglyceridemia (>265mg/dl – HLH-2004 criterion cutoff) were established as positive prognostic factors. Additionally, a trend for hepatomegaly was observed (p=0.057). Interestingly, antithrombin III and ESR were also significant in univariate analysis, but low number of available results precluded them from being tested in multivariate analysis. Among four analyzed parameters in multivariate analysis only MAS and hepatomegaly retained the prognostic factor status (Table 1).

Summary/Conclusion: Hemophagocytic syndrome in adults confers a poor prognosis, but patients who survive the first critical period may expect survival with a low relapse risk. MAS syndrome and hepatomegaly may be associated with a relatively better prognosis in newly-diagnosed adult HLH patients.

S1584

THE FREQUENCY OF THE ETHNIC NEUTROPENIA-ASSOCIATED RS2814778 SNP OF DUFFY ANTIGEN RECEPTOR FOR CHEMOKINES (DARC) GENE IN A COHORT OF EUROPEAN PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA

I. Mavroudi[1], I. Fragiadaki[1], K. Axarlis[1], S. Papadakis[1], A. Batsali[1], S. Mastrodemou[1], P. Kanellou[1], A. Damianaki[1], M. Ximeri[1], G. Goulielmos[2], K. Sfyridaki[3], A. Mörtberg[4], J. Palmblad[5], P. Höglund[4,6], H. Papadaki[1,*]

[1]Haemopoieseis Research Laboratory, School of Medicine, University of Crete, and Department of Haematology, University Hospital of Heraklion, [2]Department of Internal Medicine, Molecular Pathology and Human Genetics Section, School of Medicine, University of Crete, [3]Blood Bank, Venizeleion General Hospital, Heraklion, Greece, [4]Department of Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, [5]Departments of Medicine and Hematology, [6]Department of Medicine, Center for Hematology and Regenerative Medicine (HERM), Karolinska Institutet and Karolinska University Hospital, Huddinge, Sweden

Background: Chronic Idiopathic Neutropenia (CIN) is a mild bone marrow (BM) failure syndrome characterized by presence of myelosuppressive oligoclonal/monoclonal T-cell populations and usually benign course. The possible association of CIN with the Single Nucleotide Polymorphism (SNP) *GATA -67T>C* (rs2814778) of the Duffy Antigen Receptor for Chemokines (*DARC*), also known as Atypical Chemokine Receptor 1 *(ACKR1)* gene, associated with the benign Ethnic Neutropenia, has never been studied so far. This polymorphism is associated with the red blood cell Duffy Null phenotype; it is rare among individuals of European descent but common in Africans, African Americans and Yemenite Jews, populations characterized by the benign Ethnic Neutropenia. The mechanism of neutropenia implicates the Duffy-mediated regulation of chemokine gradients resulting in neutrophil influx from blood into the tissues.

Aims: The aim of the study was to evaluate the genotype and allele frequency of the rs2814778 SNP in patients with CIN living in the island of Crete, Greece.

Methods: We studied 50 patients (45 females, 5 males) fulfilling the previously defined diagnostic criteria of CIN. Specifically, the patients had absolute neutrophil counts (ANC) below $1800x10^6$/L (mean 1206 ± 477) at least for 3 months, no evidence of any underlying disease associated with neutropenia, no history of exposure to irradiation, use of chemical compounds or intake of drugs to which neutropenia might be ascribed, normal BM karyotype and negative antineutrophil antibodies. Two patients were of Balkan and one of English origin whereas the others originated from Crete. DNA was extracted from peripheral blood samples (Qiagen) and the genotyping for the rs2814778 SNP of the *DARC* gene was performed using the TaqMan SNP Genotyping Assay C__15769614_10 (Thermo Fisher Scientific).

Results: Four patients (one of Balkan and three of Cretan origin), representing a percentage of 8% of the CIN patients examined, were found to be homozygous for the C allele of rs2814778 (also designated as $FY*B^{ES}/FY*B^{ES}$); five patients (all of Cretan origin) *i.e.* a percentage of 10% were heterozygous (T/C or $FY*B/FY*B^{ES}$) for the *DARC* SNP under study. Red blood cell phenotyping was performed in one of the homozygous patients and was Duffy Null, as anticipated. No statistically significant differences were found between T/T CIN patients and the carriers of C allele (C/C or T/C) regarding gender, ANC, haemoglobin, lymphocytes and platelet counts. We also investigated the above groups for potential differences in parameters previously reported to characterize CIN. Specifically, we evaluated the serum immunoglobulin levels and the frequency of oligoclonal/monoclonal T-cell populations using flow-cytometric analysis but no significant differences were identified.

Summary/Conclusion: This is the first study demonstrating that the C/C ($FY*B^{ES}/FY*B^{ES}$) homozygosity of the rs2814778 SNP, implicated in Ethnic Neutropenia, is not rare among European patients with CIN. Therefore, the genotyping for rs2814778 SNP of *DARC* gene should be incorporated in the initial investigation of any patient with chronic unexplained neutropenia. We are currently investigating the correlation between the rs2814778 genotype and the red blood cell phenotype to simplify this initial screening and we are also organizing a large case-control study in Cretan population aiming to identify the genotype and allelic frequencies of the rs2814778 SNP and its potential association with the diagnosis of CIN.

Sickle cell disease and blood transfusion

S1585

RED CELL EFFECTS OF THE ANTI-CD47 MONOCLONAL ANTIBODY HU5F9-G4 IN A PHASE I STUDY FOR RELAPSED OR PRIMARY REFRACTORY ACUTE MYELOID LEUKEMIA

C. Brierley[1,*], J. Staves[1], C. Roberts[2], H. Johnson[3], P. Vyas[1,4], L. Goodnough[5], M. Murphy[1,6]

[1]Dept of Haematology, Oxford University Hospitals NHS Foundation Trust, [2]Centre for Statistics in Medicine, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, [3]Oncology Clinical Trials Office, Department of Oncology, University of Oxford, [4]MRC Molecular Haematology Unit, University of Oxford, Oxford, United Kingdom, [5]Departments of Pathology and Medicine, Stanford University, Stanford, United States, [6]National Health Service Blood and Transplant, Oxford, United Kingdom

Background: CD47 is a physiological 'don't-eat-me' signal to cells of the innate immune system, and its blockade results in phagocytic clearance of tumor cells in animal models. Downregulation of CD47 triggers clearance of ageing red blood cells (RBC). Hu5F9-G4 (5F9) is a first-in-class antibody targeting CD47 being explored clinically in multiple tumors. In non-human primate studies 5F9 induced an on-target anemia by CD47 blockade and removal of circulating senescent RBC. 5F9 binding to RBCs may also confound blood type and antibody testing.
Aims: Here we report the red cell effects, impact on blood bank testing and transfusion management in a Phase I trial of 5F9 in relapsed or primary refractory AML.
Methods: 13 patients recruited at five centers in the U.K. were allocated to one of four escalating dose cohorts of 5F9. For each patient we collected serial data on hemoglobin (Hb), markers of hemolysis, transfusion requirements and any blood compatibility testing issues. On the day of each treatment, blood samples were taken pre-dose and post-dose (4 hrs +/- 30mins post infusion). Doses of 5F9 ranged from 0.1mg/kg to 15.0 mg/kg twice weekly. The cut-off for data collection was June 6 2017.
Results: All patients (13/13; 100%) experienced a decline in Hb between the pre- and post-dose blood samples. The median Hb decline following any dose of 5F9 across the course of the study was 1.2 g/dL (range 0.4 to 1.6). In 3 patients (23%), the Hb decreased post- 5F9 by ≥3 g/dL, all after the first dose. The maximum observed Hb drop in any patient was 5.2 g/dL. There was no consistent laboratory evidence of hemolysis as evaluated by bilirubin, LDH or reticulocyte counts. 13/13 patients (100%) developed evidence of RBC agglutination on blood smear examination. No related clinically significant sequelae were observed. 12/13 (92%) of patients developed a positive direct antiglobulin test (DAT). DAT positivity was strongest after the first dose of 5F9 but remained detectable in 6/13 patients (48%) at end of treatment period. All patients (13/13; 100%) were transfused on-trial to a target Hb of 8-10g/dL. Transfusion requirements were higher on trial than pre-trial but this may be partially explained by a higher Hb threshold for transfusion after patients entered the trial. The median increment per unit of RBC was 1.0 g/dL which was maintained until the subsequent dose of 5F9. Discordant results from forward and reverse ABO typing were seen in 3/13 (23%), due to extra plasma reactivity in the reverse typing. 7/13 (54%) patients were found to have a positive antibody screen. In 6/13 (48%) patients this was a pan-agglutinin, and in 1/13 (8%) a known anti-E antibody. The median time to positive antibody screen was 2 days (range 2-114). Patients with a pan-agglutinin required matching of donor blood to the patient's genotype to provide RBC for transfusion. All patients were safely transfused with no clinical complications. The antibody effect could not be abolished with DTT, chloroquine, papain, ficin, or trypsin.
Summary/Conclusion: AML patients treated with 5F9 may experience a decline in Hb and increased transfusion requirements. The likely mechanism of anemia in AML patients is clearance of aged RBCs by CD47 blockade, combined with a failure to mount a compensatory reticulocytosis. Type-specific RBC based on baseline ABO blood group may be required where ABO typing interference occurs. Issues with compatibility testing because of newly positive antibody screens in ~50% of patients can be mitigated by pre-treatment geno/phenotype matching. Safe red cell transfusions were possible in all patients.

S1586

THE EFFECT OF COMPLEMENT INHIBITOR CP40 ON ERYTHROCYTE DESTRUCTION IN AIHA

I. Baas[1,*], P. Ligthart[2], C. Brouwer[2], C. Folman[2], E. Reis[3], D. Ricklin[3], J. Lambris[3], D. Wouters[1], M. de Haas[2], S. Zeerleder[1,4]

[1]Department of Immunopathology, [2]Sanquin Diagnostic Services, Sanquin, Amsterdam, Netherlands, [3]Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, United States, [4]Department of Hematology, Academic Medical Center, Amsterdam, Netherlands

Background: Autoimmune hemolytic anemia (AIHA) is a rare disease characterized by autoantibodies against erythrocytes. These autoantibodies may activate the classical complement pathway leading to opsonization by complement proteins C3b and C4b. This results in increased clearance of erythrocytes by phagocytes, called extravascular hemolysis. Occasionally, complement activation results in formation of the membrane attack complex (MAC), causing intravascular hemolysis. C3-inhibitor compstatin inhibits C3 activation and thus it is expected to reduce both, C3b deposition as well as well as formation of the MAC. Therefore, compstatin is a potentially efficient inhibitor of extra- and intravascular hemolysis in AIHA.
Aims: The current aim of this research is to investigate *in vitro* whether compstatin would be a suitable drug for AIHA treatment.
Methods: Healthy donor erythrocytes treated with bromelin were incubated with AIHA patient serum together with anti-C5 so that opsonization could be analyzed by FACS using anti-C3-FITC and anti-C4-APC antibodies. In the absence of eculizumab, hemolysis of RBCs was tested by measuring the extinction at 412/690 nm. The percentage hemolysis was calculated relative to the 100% water control. To assess the effect of compstatin cp40 on uptake of erythrocytes by phagocytes, erythrocytes were fluorescently labeled with PKH26 before opsonization. Monocytes isolated by leukapheresis were cultured for 8 or 9 days in the presence of 10 ng/mL GM-CSF to obtain M1-like macrophages or 50 ng/mL M-CSF to obtain M2-like macrophages. Opsonized erythrocytes were then incubated with healthy monocyte derived macrophages and phagocytosis of erythrocytes was measured with ImageStream after lysing the non-phagocytosed erythrocytes.
Results: Complement deposition and MAC formation was induced by AIHA serum via the classical pathway of complement, while this activation was efficiently inhibited by EDTA and other inhibitors, *e.g.* C1-inhibitors. In these systems, it was shown that compstatin completely inhibited C3 deposition on erythrocytes, while unexpectedly C4 deposition appeared to be increased. Regardless of the increase in C4, compstatin prevented formation of the MAC and RBC lysis. In addition, compstatin inhibited complement-mediated phagocytosis of erythrocytes by M1 macrophages while the effect on phagocytosis by M2 macrophages was less pronounced. When erythrocytes opsonized with both complement and IgG phagocytosis inhibition with compstatin was less efficient as compared to the inhibition of phagocytosis of erythrocytes opsonized with complement only.
Summary/Conclusion: We demonstrate that compstatin inhibits C3b deposition, C3-mediated phagocytosis of opsonized RBCs as well as MAC formation. Therefore, compstatin is a potential candidate for therapeutical application in AIHA patients to inhibit both intra- and extravascular hemolysis.

S1587

INTRAVASCULAR HEMOLYSIS INDUCES COMPLEMENT SYSTEM ACTIVATION MEDIATED BY HEME AND HEME-LOADED ERYTHROCYTE MICROVESICLES

L. Roumenina[1,*], N. Merle[1], V. Fremeaux-Bacchi[2], O. Blanc-Brude[3]

[1]INSERM UMRS[1138] Cordeliers Research Center, INSERM UMRS[1138] Cordeliers Research Center, [2]HEGP, APHP, [3]Paris Center for Cardiovascular Research, INSERM UMR_S [970], F-[75015], Paris, France, Paris Center for Cardiovascular Research, INSERM UMR_S [970], F-[75015], Paris, France, Paris, France

Background: In hemolytic diseases like sickle cell disease (SCD), intravascular hemolysis results in the release of hemoglobin, heme and heme-loaded membrane microvesicles in the bloodstream. Intravascular hemolysis is then associated with inflammation and organ injury. Our previous studies have demonstrated that the complement system can be activated by heme *in vitro.* However, the interrelation of intravascular hemolysis, complement activation and tissue damage remains unknown.
Aims: Our objective was to investigate the mechanisms by which hemolysis and red blood cells degradation products trigger complement activation *in vivo.*
Methods: We evaluated the deposits of C3 activation fragments and C5b-9 in kidneys of SCD patients (n=11) and two transgenic mouse models (SAD and Townes) by immunofluorescence and immunohistochemistry. Intravascular hemolysis was induced in C57Bl/6 mice by injection of phenylhydrazine in presence or not of hemopexin. The complement deposits in the kidneys were followed. RBC microvesicles were generated *in vitro* from erythrocytes of SCD patients or healthy donors. Complement activation (Ba, sC5b-9) in normal human serum was measured by ELISA. Complement deposits on cultured endothelial cells and their activation status was evaluated by flow cytometry.

Results: We analyzed kidney biopsies of patients with SCD nephropathy and model mice with SCD. We detected significant tissue deposits of complement C3 and C5b-9. Moreover, drug-induced intravascular hemolysis or injection of heme in mice triggered C3 deposition, primarily in the kidneys. Red blood cells degradation products like heme-loaded microvesicles and free heme induced alternative and terminal complement pathway activation in normal serum and on EC surfaces, contrary to hemoglobin. Heme triggered rapid P-selectin, C3aR and C5aR expression and down-regulated CD46 expression on EC. Importantly, complement deposition was attenuated *in vivo* and *in vitro* by hemopexin, the potent heme scavenger.
Summary/Conclusion: In conclusion, we describe for the first time that intravascular hemolysis triggers complement activation *in vivo*, and is associated with SCD nephropathy. Conversely, heme inhibition using hemopexin may provide a novel therapeutic opportunity to limit complement activation in hemolytic diseases.

S1588

GENOME WIDE ASSOCIATION STUDIES (GWAS) OF CEREBROVASCULAR EVENTS IN PATIENTS WITH SICKLE CELL ANAEMIA (SCA)

J. Brewin[1,*], K. Gardner[1], S.L. Thein[2], S. Menzel[1], D. Rees[1]

[1]Red Cell Biology, Kings College London, London, United Kingdom, [2]Sickle Cell Branch, National Heart, Lung and Blood Institute, Bethesda, United States

Background: Patients with SCA are at significantly increased risk of cerebrovascular disease (CVD). The role of genetics in the pathogenesis of CVD is supported by analysis of sibling pairs in both stroke outcomes and cerebrovasculopathy as detected by abnormal transcranial Doppler (TCD) studies. Another form of CVD in SCA are silent cerebral infarcts (SCI). These do not cause overt clinical signs, but are associated with reduction in IQ.
Aims: To perform genome wide association analysis of CVD outcomes in patients with SCA.
Methods: The south east London sickle cell disease genebank contains 832 African-Caribbean or West African heritage patients with datasets for 16.7 million non-monomorphic variants based on the Illumina Infinium MEGA chip plus imputation using the Michigan imputation server with 1000 genomes data. All available clinical neuroimaging reports (CT scans, MRI/A scans and TCDs) were reviewed to determine evidence of overt ischemic stroke (OIS), SCI and the highest TCD velocity recorded. Clinical notes were also reviewed to confirm OIS. We performed GWAS on OIS, SCI, max TCD, and a fourth composite "global" CVD outcome. Analysis used linear mixed modelling to account for genetic relatedness, with age and sex as fixed covariates. Duplicate samples, and one of a pair of genetically identified 1st or 2nd degree relatives, were removed. Each model was assessed with λ_{GC} and a QQ plot to evaluate genomic inflation.

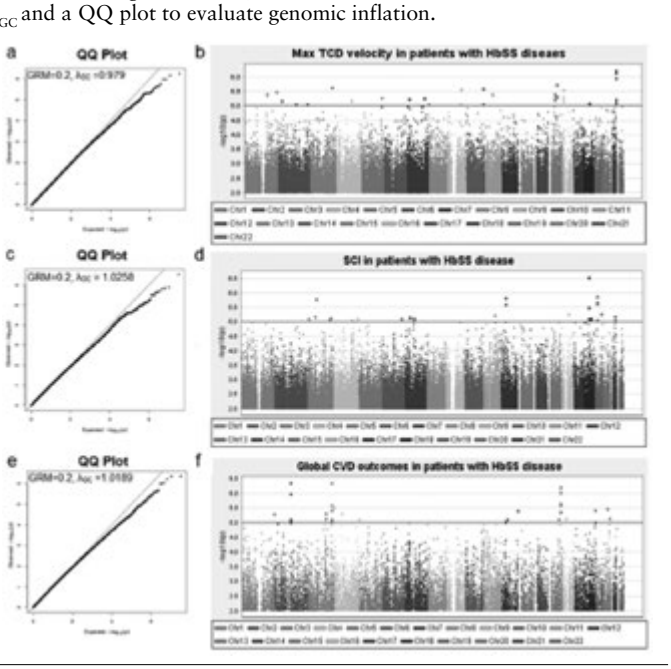

Figure 1.

Results: Although no variants achieved genome wide statistical significance, we investigated the most promising variant clusters seen on each Manhattan plot. A GWAS for TCD velocities was performed on 167 patients. The most significant peak was on chromosome 21 (rs2898354, Mean Allele Frequency (MAF)=0.43, p=5.69e^{-07}). These variants sit within intron 4 of the *ERG* gene, which has previously been associated with stroke in SCA. A cluster was also found on chromosome 9 (rs75829124, MAF=0.03, p=8.53e^{-06}) within 40kB of the *TGFBR1* gene. *TGFBR3* has previously been associated with stroke in SCA, and *TGFBR1* may have a role in the same pathway. A cluster (rs8032902, MAF=0.2, p=4.02e^{-06}) on chromosome 15 sits 40kB upstream of *fibrillin 1*, the major constitutive element of extracellular microfibrils in blood vessels. A GWAS for the SCI outcome was performed on 292 patients. The most promising cluster (rs879261, MAF=0.5, p=6.53e^{-06}) sits on chromosome 21 within intron 28 of the *intersectin1* gene. *Intersectin1* is involved in the endocytosis of Integrin beta-1 and transferrin. The GWAS model for OIS (n=317) showed evidence of genomic inflation. Modelling difficulties were likely due to the small number of patients with a positive event. Further analysis will be considered when more patients are recruited. A GWAS for global CVD was performed on 365 patients. A cluster of variants (rs150417193, MAF=0.2, p=7.47e^{-06}) on chromosome 15, sit within a copy number variant affecting a transcription factor binding site ~15kB upstream of *ADAMTS7*. The *ADAMTS* family have shown association with pediatric stroke and cardiovascular traits in the general population.
Summary/Conclusion: This is one of the largest studies looking at the genetics of CVD in SCD. We performed GWAS on a number of cerebrovascular outcomes in SCA. Although no result achieved genome-wide statistical significance, we have identified areas worthy of further validation. The most significant finding is a cluster of variants that fall within *ERG*, an erythroblast transformation-specific transcription factor that has a role in vascular cell remodelling, supporting previously published associations with this gene and CVD in SCA.

S1589

NOVEL INSIGHTS INTO SPLEEN INJURY DURING THE FIRST 2 YEARS OF LIFE IN SCA CHILDREN: A LONGITUDINAL STUDY

S. El Hoss[1,*], S. Cochet[1], M. Dussiot[2], M. Marin[1], C. Pereira[1], C. Lapoumeroulie[1], M. De Montalembert[3], C. Le Van Kim[1], Y. Colin Aronovicz[1], W. El Nemer[1], V. Brousse[1,3]

[1]Biologie Intégrée du Globule Rouge UMR_S[1134], Inserm, Univ. Paris Diderot, Sorbonne Paris Cité, Univ. de la Réunion, Univ. des Antilles, [2]INSERM UMR[1163], CNRS ERL[8254], Laboratoire d'excellence GR-Ex, Université René- Descartes, Imagine Institute, [3]Service de Pédiatrie Générale, Hôpital Necker-Enfants Malades, Centre de Référence de la Drépanocytose, AP-HP, Paris, France

Background: In sickle cell anemia (SCA), the spleen is the first organ to be injured notably because impaired deformability and/or adhesion of sickled RBCs promotes their sequestration. Moreover, acute splenic sequestration crisis (ASSC), a life-threatening complication, happens in 10-30% of SCA infants, with 75% of first cases occurring before the age of 2 years. Determinants, predictive factors and consequences of ASSC are not established.
Aims: Our study aims to evaluate the natural history of spleen dysfunction in a cohort of 47 SCA infants enrolled at 3-6 months and longitudinally followed-up to 24 months. Specifically, we analyzed RBC deformability and adhesion properties as potential determinants of ASSC and spleen injury.
Methods: ASSC were recorded prospectively and defined by the sudden enlargement of spleen (> 2 cm compared to basal) with a decrease of Hb level (> 2 g/dL compared to previous measurement). Blood samples were analyzed at enrollment and at 12, 18 and 24 months. Evaluation of spleen function was based on quantification of Howell Jolly Bodies (HJB) using an imaging flow cytometry (IFC) based method. Irreversibly sickled cells (ISCs) as a marker of RBC decreased deformability were quantified by IFC. Expression, activation and adhesion function of Lu/BCAM, a known erythroid marker, were quantified using flow cytometry, phosphorylation and dynamic adhesion assays.
Results: At enrollment, patients had HJB in circulation slightly higher than healthy controls (0.3% and 0.1%, respectively). Expectedly, a significant increase in HJB at 18 months (0.8%) was observed. At enrollment, there was no significant difference in HJB between infants who underwent ASSC (n=7) during the follow-up period, as compared to the group that remained both asymptomatic and ASSC-free (n=16). However, the ASSC group showed significantly higher HJB at 24 months as compared to the ASSC-free group (1.52% and 0.54%, respectively), a finding demonstrating that ASSC significantly decreases spleen function. Exploring the role of decreased deformability of RBCs in spleen injury, we observed the presence of ISCs at enrollment (0.96%) and a significant increase in ISCs at 18 months (1.61%). Moreover, the ASSC group showed significantly higher ISCs at enrollment than the ASSC-free group (1.61% and 0.54%). Thus, ISCs appear in the

circulation before the occurrence of an ASSC, indicating a potential role as a predictive marker of ASSC. Abnormal adhesion of RBCs to the spleen matrix in the open microcirculation might be another factor causing splenic injury. In particular, the spleen is lined with laminin, a known ligand for Lu/BCAM. We observed an increase in the expression and activation of Lu/BCAM with time. Moreover, we observed a significant increase in the number of adherent RBCs on laminin-coated channels between enrollment and 24 months (990 RBCs/mm^2 and 1787 RBCs/mm^2, respectively). However, there was no difference with respect to Lu/BCAM adhesion, expression and activation properties when comparing the ASSC group and the ASSC-free group.
Summary/Conclusion: Our study shows that spleen dysfunction is initiated as early as 4 months of age in SCA infants. ASSC greatly contributes to spleen loss of function. Changes in RBC properties including morphology, expression and activation of Lu-BCAM are initiated very early in infancy and increase with age. Importantly, we show that ISCs are a predictive marker of ASSC. Impairment of RBC deformability rather than adhesion plays a major role in spleen trapping and thereafter loss of function.

Hematopoiesis, stem cells and microenvironment

S1590

SPECIFICATION OF HEMATOPOIETIC STEM CELL FATE VIA MODULATION OF MITOCHONDRIAL ACTIVITY

M. Girotra[1,*], M. Rincon-Restrepo[1], C. Monnard[2], G. Coukos[1], S. Rezzi[2], N. Vannini[1]

[1]Ludwig Institute for Cancer Research, University of Lausanne, Epalinges, [2]Nestlé Institute of Health Sciences SA, Lausanne, Switzerland

Background: A tight control of hematopoietic stem cell (HSC) fate is crucial for lifelong blood production. Therefore, a fine balance of quiescence, self-renewal, and differentiation is key to maintain the HSC pool and blood homeostasis. In recent years cellular metabolism has emerged as a crucial regulator of HSC fate. HSCs differ from their committed progeny by relying primarily on anaerobic glycolysis rather than mitochondrial oxidative phosphorylation for energy production. However, whether this change in the metabolic program is the cause or a consequence of the unique function of HSCs remains unknown.
Aims: Here we asked if mitochondrial activity can be used as a reliable readout for functional HSCs and if modulation of mitochondrial activity results in enhancement of HSC function.
Methods: We used *in vivo* long term blood reconstitution assays as a readout of HSC functionality.
Results: We previously demonstrated that modulation of mitochondrial metabolism affects HSC fate, by chemically uncoupling the electron transport chain we were able to maintain HSC function in culture conditions that normally induce rapid differentiation (Vannini N, Girotra M. et al., Nat Comm 2016). Moreover, we demonstrated that modulation of mitochondrial activity in *ex-vivo* cultured human HSCs, via NAD+ boosting agent Nicotinamide Riboside (NR), results in better long-term blood production in serially transplanted humanized mice (*Under Revision*). Here we proceeded to carry out a mini screen, using mitochondrial activity as readout, to identify novel metabolic modulators that can be used to enhance HSC activity and function.
Summary/Conclusion: Our data thus reveal a causal relationship between mitochondrial metabolism and fate choice of HSCs, and also provide a valuable tool to identify optimal *ex vivo* conditions for HSC expansion and improve the outcome for patients suffering from bone marrow insufficiency.

S1591

P53 DEFICIENCY DRIVES LEUKEMIC TRANSFORMATION OF HEMATOPOIETIC STEM CELLS BY MAINTAINING QUIESCENCE IN RESPONSE TO DNA DAMAGE

A. Brown[1,*], M. Mulaw[2], J. Pospiech[1], K. Eiwen[1], M. Sacma[1], V. Sakk[1], B. Moehrle[1], K. Nattamai[3], K. Nalapareddy[3], H. Geiger[1,3]

[1]Ulm University, Institute for Molecular Medicine, [2]Ulm University, Institute for Experimental Cancer Research, Ulm, Germany, [3]Cincinnati Children's Hospital Medical Center and University of Cincinnati, Division of Experimental Hematology and Cancer Biology, Cincinnati, OH, United States

Background: The tumor suppressor gene *p53* is pivotal for DNA damage response pathways by forcing cell cycle arrest, DNA repair and apoptosis. Hence, *p53* is among the most frequently altered genes in cancer and its mutations are associated with large numbers of hematopoietic neoplasms, including MDS and AML. In hematopoietic stem cells (HSCs) p53 has been reported to be crucial for self-renewal, senescence and quiescence. Considering that very often leukemia emerges from stem and progenitor cells, it is remarkable that it still remains unclear how p53 activity prevents their transition to leukemia.
Aims: To investigate mechanisms that facilitate genomic and functional stability of the HSC pool and how p53 deficiency alters the functionality and the mutational landscape of HSCs in response to DNA damage.
Methods: We employed *P53*$^{-/-}$ mice together with wild type C57BL/6 mice to examine *in vivo* changes in cell cycle distribution and apoptosis levels of HSCs and less primitive progenitors in response to DNA damage. In this context, we also applied several assays to assess DNA repair pathways and changes in functionality of these cells. We visualized single HSCs within their bones by whole-mount immunofluorescence staining following confocal imaging. We performed transplantation experiments to study the consequences of HSC-associated DNA damage on the recipient mice. The detection of single point mutations and insertions/deletions was performed by sorting donor-derived HSCs following RNA-seq analysis and a bioinfor-

matic approach which extracts information about mutation frequencies out of these data.
Results: Here, we report a surprising new role of p53 in ensuring the integrity of HSCs: As previously reported, upon DNA damage HSCs from wild type mice enter the active cell cycle while simultaneously forcing apoptosis. HSCs from mice devoid of p53, however, preserve their quiescent state, protecting them from cell death. These HSCs exhibit a high abundance of 53BP1 foci suggesting a constitutive preference for the error prone non-homologues end joining pathway. These features are of cell-intrinsic and niche-independent nature, primarily stem cell specific and not present in more committed progenitors irrespective of p53. We demonstrate that *p53*$^{-/-}$ HSCs display persistently high P57(Kip2) levels after DNA damage offering a functional explanation for the observed phenotype. Indeed, *P53/P57* double deficient HSCs react similar to irradiation induced DNA damage in comparison to their wild type counterparts. Importantly, transplanted *p53*$^{-/-}$ HSCs engraft massively in the bone marrow several months after sublethal irradiation, severely lose their differentiation potential and the corresponding recipient mice develop early symptoms of leukemia, such as increased spleen sizes and high numbers of blast cells in the bone marrow. Strikingly, the single point mutation frequency of *P53*-deficient HSCs isolated from these mice is not notably high whereas we observe a global increase of insertions/deletions near splice sites and intron regions of protein coding genes. These observations imply that initial steps of transformation are not solely driven by the accumulation of mutations but specific changes in the mutational landscape stimulate the transformation process of HSCs.

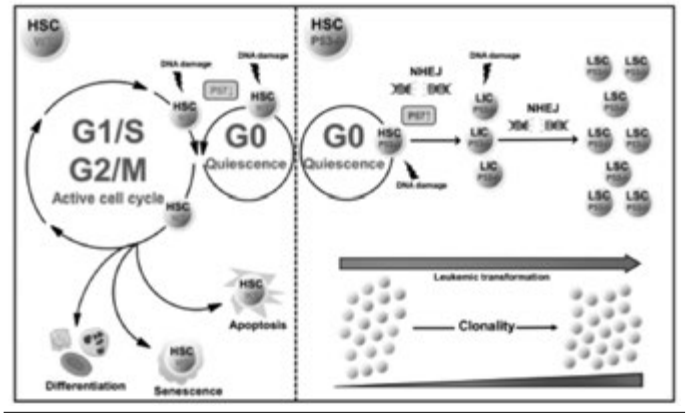

Figure 1.

Summary/Conclusion: In compliance with our previously published data we believe that we have found evidence for a novel p53-dependent pathway, forcing DNA-damaged HSCs out of quiescence to enable their removal. This proposed mechanism may turn out to play a crucial role in preventing the leukemic transformation of these cells.

S1592

OVEREXPRESSION OF THE TRANSCRIPTION FACTOR CDX2 IN HAEMATOPOIETIC STEM CELLS PREDISPOSES TO HAEMATOLOGICAL MALIGNANCIES

T. Vu[1,*], J. Straube[1], A. Song[1], V. Ling[1], C. Scholl[2,3], S. Fröhling[2,4], G. Magor[5], A. Perkins[5], S. Gröschel[2], J.-P. Mallm[3], S. Lane[1,6]

[1]QIMR Berghofer Medical Research Institute, Brisbane, Australia, [2]National Center for Tumor Diseases (NCT), [3]German Cancer Research Center (DKFZ), [4]Heidelberg University Hospital, Heidelberg, Germany, [5]Australian Centre for Blood Diseases, Melbourne, [6]Royal Brisbane and Women's Hospital, Brisbane, Australia

Background: The caudal-related homeobox gene *CDX2* is ectopically expressed in 90% of human acute myeloid leukaemia (AML), but not normal haematopoietic cells. Retroviral expression of Cdx2 causes AML *in vivo*, while CDX2 knockdown impairs growth of AML cell lines *in vitro*. These findings implicate Cdx2 overexpression as a clinically relevant event in leukaemogenesis, however existing studies have been limited by the requirement for *ex vivo* manipulation, retroviral overexpression and transplantation.
Aims: To understand the role of Cdx2 in *de novo* leukaemic transformation on a molecular and cellular level.
Methods: We generated an inducible transgenic mouse model whereby Cdx2 was specifically activated in haematopoietic stem cells (HSC) using the tamoxifen-inducible Scl-CreER^{T2}. We performed immunophenotyping, whole exome sequencing, RNA-sequencing, ATAC-sequencing, and *in vivo* treatment of leukaemic mice with the hypomethylating agent Azacitidine.
Results: Scl-CreER^{T2}:Cdx2 mice developed a dysplastic phenotype which progressed in some mice to either myelodysplastic syndrome (MDS, 35%), myeloproliferative/MDS overlap (20%) or acute leukaemia (25%) after a long latency. Competitive transplantation assays showed intrinsic loss of long-term HSC self-renewal. Transcriptional analysis revealed an anti-apoptotic, pro-proliferative gene signature, suggesting Cdx2 overexpression confers a pre-leukaemic phenotype permissive to transformation upon acquisition of secondary mutations. Mechanistically, Scl-CreER^{T2}:Cdx2 mice showed marked depletion of long-term HSC and abnormal cell cycle regulation. Whole exome sequencing indicated that Cdx2 acute leukaemia developed in the presence of pathogenic secondary mutations including Ikzf1, Notch1 and Kit. ATAC-sequencing for chromatin accessibility revealed widespread chromatin changes at haematopoietic-specific enhancers. These motifs include Runx1, Pu.1:Irf8, C/ebp and C/ebp , which are key lineage determinants in haematopoietic stem and progenitor cells. Intriguingly, we observed complete repression of *Hox* cluster genes, challenging the notion that HOX activation drives leukaemia transformation in CDX2-positive leukaemia. Scl-CreER^{T2}:Cdx2 leukaemias were treated with azacitidine, a hypomethylating agent approved for high-risk MDS and AML patients. Azacitidine treatment prolonged survival and decreased leukaemic burden, associated with *in vivo* differentiation and selective apoptosis of Kit-positive leukaemia stem cells.
Summary/Conclusion: Altogether, this work demonstrates that conditional Cdx2 expression in HSC is a novel, inducible model of *de novo* leukaemic transformation and reflects common genetic aberrations seen in human AML. This may help to identify tractable susceptibilities to target leukaemia cells and improve clinical outcomes.

S1593

PERIVASCULAR NICHE CELLS SENSE THROMBOCYTOPENIA AND ACTIVATE PLATELET-BIASED HSCS IN AN IL-1 DEPENDENT MANNER

T.C. Luis[1,2], *, N. Barkas[1,2], A. Giustacchini[1,2], B. Wu[1,2], T. Bouriez-Jones[1], I.C. Macaulay[1], C. Nerlov[2], S.E. Jacobsen[1,2,3]

[1]Haematopoietic Stem Cell Laboratory, MRC Weatherall Institute of Molecular Medicine, [2]MRC Molecular Haematology Unit, MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom, [3]Department of Cell and Molecular Biology and Department of Medicine Huddinge, Center for Hematology and Regenerative Medicine, Karolinska Institutet, Stockholm, Sweden

Background: Hematopoietic stem cells (HSC) are responsible for the on demand production of mature blood cells both in homeostasis and in response to stress situations such as injury and infection. HSCs reside in specialized niches in bone marrow (BM), which regulate their function. These niches are perceived as dynamic entities with the capacity to sense and respond to specific requirements in blood cell production, but the mechanisms underlying this dynamic regulation remain unclear. Accumulating evidence indicate that HSCs are highly heterogeneous, and different BM niches have been proposed, potentially supporting different subsets of HSCs. We recently identified a distinct subset of HSCs, which are molecularly and functionally primed for platelet replenishment (Carrelha J. *et al*, Nature 2018). However, the role of the niche in the regulation of platelet-biased HSC function is still unknown.
Aims: This work aims at investigating the role of the BM niche in the response of platelet-biased HSCs to platelet depletion.
Methods: Thrombocytopenia was induced in mice by the administration of an antibody against the platelet GPIb receptor, leading to fast and efficient platelet depletion. We used a RNA-sequencing approach to analyze different BM niche cell populations and HSCs in order to identify signals involved in the HSC response to platelet depletion.
Results: Platelet-biased HSCs are rapidly and selectively recruited into cell cycle in response to platelet depletion, through a feedback mechanism to replenish platelet numbers and homeostasis. Here we identified Interleukin-1 (IL-1) as a cytokine released upon acute platelet depletion and specifically sensed by niche LepR^{+} perivascular cells. Abrogation of IL-1 signaling specifically in LepR^{+} niche cells but not in hematopoietic cells impaired the platelet-biased HSC response to platelet depletion. This process was found to be dependent on platelet activation.
Summary/Conclusion: This work uncovers a molecular mechanism involving the pro-inflammatory signal IL-1 and the niche perivascular cell compartment in the rapid activation of platelet biased HSCs to thrombocytopenia, highlighting a mechanism by which a distinct subclass of HSCs senses and responds to the loss of the lineage for which it is intrinsically primed for.

SINGLE-CELL PROFILING REVEALS KEY DIFFERENCES IN THE CELLULAR ARCHITECTURE OF HUMAN HAEMATOPOIETIC STEM AND PROGENITOR CELLS THROUGHOUT FETAL AND ADULT LIFE

A. Roy[1,*], B. Psaila[2], S. Thongjuea[3], N. Ashley[4], G. Buck[1], S. O'Byrne[1], N. Elliott[1], E. Louka[2], N. Fordham[2], P. Hua[4], B. Povinelli[2], E.F. Heuston[5], D. Iskander[6], A. Karadimitris[6], D.M. Bodine[5], A. Mead[2], I. Roberts[1,2]

[1]Paediatrics, University of Oxford, [2]MRC Molecular Haematology Unit, MRC Weatherall Institute of Molecular Medicine, [3]RDM Clinical Laboratory Science, University of Oxford, [4]RDM Clinical Laboratory Science, MRC Weatherall Institute of Molecular Medicine, Oxford, United Kingdom, [5]National Human Genome Research Institute, National Institutes of Health, Bethesda, United States, [6]Centre for Haematology, Imperial College London, London, United Kingdom

Background: Mature blood cells of multiple lineages are constantly replenished throughout life. In humans, definitive haematopoiesis in the fetal liver (FL) commences at around 5 weeks of gestation, which remains the main site of haematopoiesis throughout fetal life. Haematopoiesis in the bone marrow (BM) starts around 11-12 weeks of gestation, but does not take over as the primary site of haematopoiesis until just after birth. Currently, we know very little about how haematopoietic stem/progenitor cell (HSPC) subsets change through ontogeny; and whether they do so in a site and stage-specific manner. Single cell RNA sequencing (scRNASeq) techniques now offer unprecedented opportunities to finely dissect the heterogeneity within specific HSPC populations and compare the cellular architecture of different sites of haematopoiesis.
Aims: To characterise the human HSPC compartment from early fetal life to adulthood using scRNAseq.
Methods: Lineage-negative, CD34+ cells from first trimester FL (n=2; gestation week 7-8); matched second trimester FL and fetal BM (FBM) (n=2 each; week 18-19); and healthy adult donor mobilised apheresis HSPC (n=2) were FACS-isolated for scRNAseq using the Chromium 10X platform. Data was processed using CellRanger, Seurat, Partek Flow® packages and in-house pipelines to perform dimensionality reduction, unsupervised clustering (tSNE) and to generate KNN graph differentiation trajectories.
Results: Data was obtained from 5000-9000 cells per sample. After filtering and quality control, data from around 49000 cells from the 8 samples was used for comparative analysis. The mean read count/cell was 47000 and the median gene count/cell was 2500 with 66-70% mapping. The composition of Lin-CD34+ cells was clearly distinct in different stages of ontogeny, with the early FL samples showing a substantially higher proportion of megakaryocyte-erythroid (MkE) progenitors, which decreased during development and in adult life (Fig 1). HSPC composition also varied in a site-specific manner as evidenced by differences seen in matched 2nd trimester FL and FBM samples from the same fetus. Second trimester FBM showed a B lymphoid bias compared to all other tissues including the matched FL samples. Further characterisation of normal and abnormal postnatal BM is currently underway.
Summary/Conclusion: We have used scRNAseq to analyse the CD34+ HSPC compartment throughout human ontogeny for the first time. There is clear evidence that this compartment varies in its composition and differentiation potential in a site and developmental stage-specific manner, as it may be dependant on the physiological processes/ demands of that particular developmental stage; and/ or in response to specific microenvironmental cues. Studying haematopoiesis throughout the human lifespan may be important not only to understand normal developmental processes, but also to understand the pathogenesis of postnatal haematological diseases that may have their origins in fetal life.

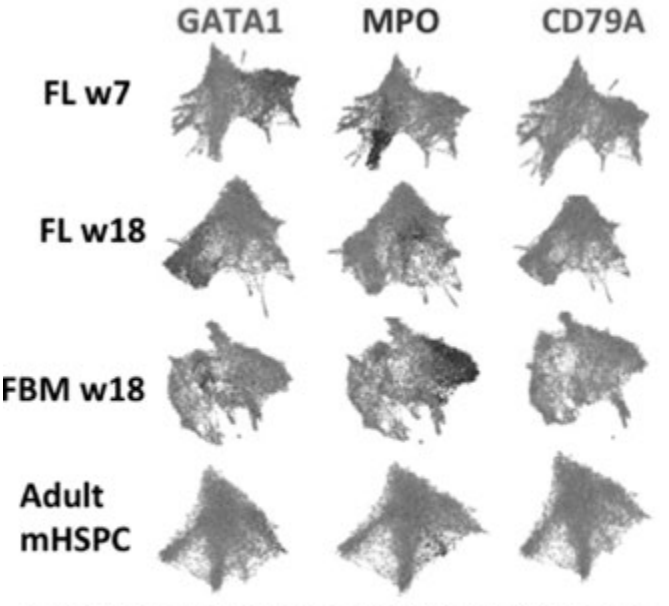

Figure 1. Striking differences in composition HSPC throughout ontogeny. Representative KNN graphs show differentiation trajectories of Lin-CD34+ cells from 1st trimester fetal liver (FL,week 7) week, matched 2nd trimester FL and fetal bone marrow (w18) and adult mobilised HSPC. *GATA1 (red); MPO* (blue) and *CD79A* (green) are shown to illustrate mega-erythroid, myeloid and B lymphoid differentiation trajectories.

Figure 1.

Acute lymphoblastic leukemia – Biology & Translational Research

PB1595

DEVELOPMENT OF A FLUORESCENCE IN-SITU HYBRIDIZATION PROBE FOR DETECTING IKZF1 DELETION MUTATIONS IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

J. Hashiguchi[1,*], M. Onozawa[1], S. Oguri[2], S. Fujisawa[2], M. Tsuji[3], K. Okada[1], M. Nakagawa[1], D. Hashimoto[1], K. Kahata[1], T. Kondo[1], C. Shimizu[2], T. Teshima[1]

[1]Department of Hematology, Hokkaido University Faculty of Medicine, [2]Division of Laboratory and Transfusion Medicine, Hokkaido University Hospital, [3]Chromosome science lab, Sapporo, Hokkaido, Japan

Background: Intragenic interstitial deletion of *IKZF1* is a recurrent genomic alteration in B-cell acute lymphoblastic leukemia (B-ALL). The deletions are mediated by illegitimate V(D)J recombination via cryptic recombination signal sequences (RSSs). There were 4 major types of *IKZF1* deletion (Δ4-7, Δ2-7, Δ4-8, Δ2-8). The presence of *IKZF1* mutation has been reported to be an independent risk factor of poor prognosis for patients with B-ALL. Despite the prognostic value, there are no suitable testing methods for detecting the mutation, and current clinical applications are therefore limited.
Aims: To detect various type of *IKZF1* deletion including the commonly deleted exon 4-7 region, we developed a fluorescence in-situ hybridization (FISH) probe set. We validated the probes using clinical samples.
Methods: The probe set consists of a designed probe for the commonly deleted region (Cy3, red) and a bacterial artificial chromosomes (BAC) clone probe for detecting the 3' flanking region (Spectrum Green; RP11-248J17). The *IKZF1* commonly deleted region was amplified using long PCR method. Normal cutoff was validated using 10 peripheral blood samples from normal individuals. Twenty-three clinical samples (9 Ph(+)ALL, 8 Ph(-)ALL, 3 CML lymphoid crisis, 3 AML) were analyzed using the probe. *IKZF1* deletion was also validated by multiplex PCR method and each deletion joint was sequence verified.

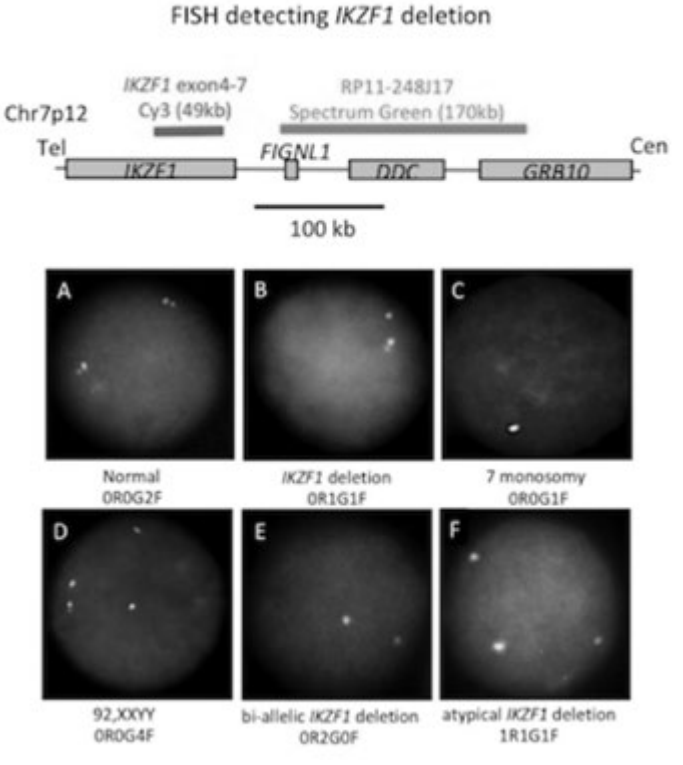

Figure 1.

Results: Normal cell showed 2 fusion signal reflecting intact *IKZF1* alleles (0R0G2F). In the cell with heterozygous *IKZF1* deletion, intact *IKZF1* allele showed a fusion signal, and the deleted allele showed loss of the red signal (0R1G1F). Normal cutoff value for *IKZF1* deletion was established to be 1.5% from 10 normal samples. Gain or loss of entire IKZF1 region was detected by gain or loss of fusion signal such as 0R0G4F or 0R0G1F. Intragenic *IKZF1* deletion was frequent in Ph(+)ALL (7/9, 77.8%) but relatively rare in Ph(-)ALL (3/8, 37.5%) and CML lymphoid crisis (1/3, 33.3%). On the other hand, *IKZF1* deletion was not detected in any of the AML samples. For the cases with *IKZF1* deletion, the exact deletion type could be identified by multiplex PCR in all cases. The joint sequence was unique to each patient showing a typical RAG1/2-mediated V(D)J recombination signature. One case showed 2 *IKZF1* deletion signals in each cell (0R2G0F). Multiplex PCR verified 2 independent deletion (Δ, 2-7 Δ4-8) in the case. One case showed an atypical break-apart signal (1R1G1F). Inverse PCR of the case revealed insertion of the deleted *IKZF1* fragment into a legitimate RSSs site at immunoglobulin kappa on chromosome 2. This is the first description showing that the insertion of a RAG1/2-mediated fragment can occur between genuine and cryptic RSSs in ALL. RAG1/2-mediated genome exchange could play a role in leukemogenesis by causing cancer-associated insertion that results in disruption of the expression of a tumor suppressor.
Summary/Conclusion: In this study, we established FISH probes detecting *IKZF1* deletion in a quick, quantitative and cost-effective manner. FISH based assay provided a novel insight into B-cell receptor editing by insertion of a cryptic RSS-mediated genomic fragment in ALL biology.

PB1596

PHOTOLUMINESCENT ZINC OXIDE NANORODS-A NEW TOOL FOR DETECTION OF HUMAN LEUKEMIC CELLS

Y. Harmaza[1,*], A. Tamashevski[1], R. Viter[2], E. Slobozhanina[1]

[1]Laboratory of medical biophysics, Institute of biophysics and cell engineering, Minsk, Belarus, [2]Institute of Atomic Physics and Spectroscopy, University of Latvia, Riga, Latvia

Background: Traditional methods of diagnostic of hematologic cancers (flow cytometer, immunohistochemistry analysis, etc.) have a high sensitivity and specificity, but they require expensive equipment and specially trained staff, which increase the costs of these analysis. Therefore, the actual task is to develop a new tool-diagnostic biosensors which are expanding beyond traditional clinical labs to point-of-care and home settings. Nowadays zinc oxide (ZnO)-based nanostructures owing to unique physical properties-high photoluminescence (PL), biocompatibility and other multifunctional characteristics attract attention as building blocks for biosensor development. These properties of ZnO help retain biological activity of the immobilized biomolecule and help in achieving enhanced sensing performance.
Aims: In current work, we demonstrate the possibility of a fluorescent detection of human leukemic cells: T- and B-lymphoblasts, using zinc oxide nanorod (ZnO NR) platforms and immobilized on them specialized monoclonal antibodies (MABs) against cluster of differentiation (CD) proteins on the surface of investigated cancer cells (CD5 and D19).
Methods: It was used human cell lines MOLT-4 derived from the patient with an acute lymphoblastic leukemia in relapse and IM-9 derived from the patient with a multiple myeloma and also healthy human's peripheral mononuclear cells as control samples where expression of CD5 and CD19 antigens were found in 8-12% and 5-9% of the cell population, respectively. For platform preparation, glass substrate was cleaned in ethanol and mQ and treated by the use of plasma technology; than ZnO NRs stock solution was dropped on it and annealed at 300°C.
Results: Firstly, we established the optimal concentrations of human anti-CD19 and anti-CD5 and their isotype controls (mouse anti-human IgG1 and IgG2a) for immune biosensor development, *i.e.* an appropriate amount of MABs to provide significant coverage of the ZnO NRs surface and maximum response of ZnO NRs photoluminescence. Next, it was shown that B- or T-lymphoblastoid cells bind to CD19 or CD5 targered ZnO NRs with high selectivity and PL signal increased on 50–70% in comparison with the signal from the control samples. Furthermore, rise of the ZnO NRs photoluminescence intensity correlated with the amount of CD19+ and CD5+ cells in the investigated populations (controlled using flow cytometry).
Summary/Conclusion: The outcomes of our study confirmed that ZnO NRs exhibit an optical property useful for effective monitoring of fluorescent signal from biological systems: human leukemic lymphocytes conjugated with CD19 or CD5 MABs, even at extremely low cell concentrations-from 5-10 till 250 cells per 1 mm^2 of ZnO NRs platform. We propose that MABs-targeted ZnO NRs can be used for the development of biosensors for detection of human leukemic cells.
This work has received funding from the European Union's Horizon 2020 research and innovation programme H2020-MSCA-RISE under grant agreement 777926.

PB1597

THE VR-ALL LINE: A NOTCH MUTANT MODEL FOR B-ALL

P. Takam Kamga[1,2,*], G. Dal Collo[2], G. Bassi[2], M. Midolo[2,] M. Delledonne[3], M. Chilosi[4], M. Bonifacio[2], M. Krampera[2]

[1]Academie de Versailles, Versailles, France, [2]Medicine, [3]Biotechnology, [4]Department of Diagnostics and Public Health, University of Verona, Verona, Italy

Background: Notch signaling contribution to B-cell acute lymphoblastic leukemia (B-ALL) development is still under investigation. The serendipitous onset of B-ALL in a patient affected by the germinal Notch mutation-dependent Alagille syndrome allowed us to establish a B-ALL cell line (VR-ALL) bearing a genetic loss of function in components of Notch signaling.
Aims: We used the VR-ALL cell line in comparison with two other B-ALL lines (RS4;11 and SUB-P15) and primary samples from B-ALL patients to analyze the contribution of defective notch signaling in B-ALL cell lines as well as cell line based-xenograft models of B-ALL.
Methods: B-ALL cell lines were obtained from ATCC, while B-ALL primary cells were obtained from bone marrow or peripheral blood of 30 B-ALL patients. Flow cytometry and western immunoblotting were used to study the expression of Notch receptors and ligands. Drugs used were Cytarabine, Dexamethasone and Doxorubicin alone or in combination with gamma secretase inhibitors (GSIs). Mice xenograft model of B-ALL were obtained by injecting the B-ALL line RS4;11 in NOD/Shi-scid/IL-2Rγnull mice (NOG). Cell viability was evaluated by Annexin-V/PI and MTT assay; proliferation was assessed through CFSE dilution.
Results: Flow cytometry analysis and May-Grünwald Giemsa staining revealed that the VR-ALL was a Pre-B-ALL cell line. Similarly, to RS4;11 and SUP-B15 cell lines, VR-ALL cells grew easily in RPMI or IMDM supplemented with 10% FBS. Western blot and flow cytometry analysis showed that VR-ALL cells as well as primary blast cells displayed a Notch expression pattern consisting in lower expression levels of Notch2 and Jagged1, high expression levels of Notch1, Notch3, Notch4, Jagged2, DLL3 and DLL4. But in contrary to SUP-B15 and RS4;11, the Notch target Hes1 was absent in VR-ALL cells. However, VR-ALL cells were still highly sensitive *in vitro* to GSI XII as shown by MTS and Annexin assays. Then, we successfully obtained a mice xenograft model of B-ALL with high bone marrow leukemic burden, by injecting the VR-ALL cell line in NOD/Shi-scid/IL-2Rγnull mice. Concordantly with *in vitro* observation, administration of GSI-XII to mice, significantly lowered the leukemic burden in the bone marrow. These activities of GSI-XII in cells with a defective Notch activity suggested a Notch-independent role of gamma secretase inhibitors. Seeking the influence of Notch status on drug response, we observed that the treatment of the cell lines *in vitro* with Cytarabine, Dexamethasone or Doxorubicin induced a dose-dependent decrease in VR-ALL cell viability. Noteworthy, VR-ALL cells were less sensitive to the treatment with glucocorticoids than the two other B-ALL cell lines. This reduction was abrogated when the glucocorticoid was associated to GSI-XII.
Summary/Conclusion: Through this study we propose a new cell line that has allowed us to understand B-ALL pathogenesis, representing an appropriate tool to better unravel the mechanistic role of Notch signaling in B-ALL. Moreover, VR-ALL could be a valuable tool to investigate the mechanisms of B-ALL relapse determined by glucocorticoid refractoriness.

PB1598

DOWNREGULATION OF CDKN2B EXPRESSION DUE TO DELETION OR HYPERMETHYLATION IN T-ACUTE LYMPHOBLASTIC LEUKEMIA

A. Kwon[1,*], H. Choi[1], J. Kim[1], M. Kim[1,2], Y. Kim[1,2]

[1]Catholic Genetic Laboratory Center, [2]Department of Laboratory Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic Of

Background: T-acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic malignancy that accounts for approximately 20% of all cases of ALL. It tends to be more common in adults than children.Understanding the disease pathogenesis and expected biological behavior of T-ALL on the basis of genetic profiles is essential if T-ALLis to be successfully treated.
Aims: In this study, we explored the association of pathogenesis and biology based on genetic aberrations in T-ALLbyintegrative genetic analyses using massive parallel sequencing as well as copy number analysis of T-ALL patients.
Methods: The study included 102 T-ALL patients (69 male and 33 females comprising 42 children and 60 adults. Massive parallel sequencing of the exons of 11 genes (*NOTCH1*, *DNMT3A*, *FBXW7*, *RUNX1*, *PHF6*, *PTEN*, *GATA3*, *KRAS*, *EZH2*, *NRAS*, and *SH2B3*) was performed to comprehensively characterize the patterns ofsomatic mutations in T-ALL. Multiplex ligation-dependent probe amplification (MLPA) was done for detection of commonly deleted genes in T-ALL.In addition, *CDKN2A* and *CDKN2B* mRNA expressionand promoter methylation were analyzed usingRT-qPCRand pyrosequencing, respectively.
Results: We identified principal genetic alterations in 97.1%(99/102) cases of T-ALL using integrativegenetic analyses including massive parallel sequencing and MLPA. A total of 134 mutations were found in descending order of genes: *NOTCH1*(66.7%), *FBXW7*(19.6%), *PHF6*(15.7%), *RUNX1*(12.7%), *NRAS*(10.8%), and *DNMT3A*(9.8%).Copy number alterations were most frequently detected *CDKN2B*,*CDKN2A* and genes on 9p21.3 in T-ALL(45.1%). Gene expression data demonstratedthe downregulation of *CDKN2B* in most cases of T-ALL, while *CDKN2A* downregulation was mainly restricted to deletions. Additional quantitative methylation analysis demonstrated that *CDKN2B* downregulation stemmed from deletion and hypermethylation. Analysis of 64 patients with *CDKN2B* hypermethylation indicated relationships with older age of onset and early T-cell precursor ALL, which involved very early arrest of T-cell differentiation. Genes associated with methylation and myeloid neoplasms including *DNMT3A* and *NRAS* were more commonly mutated in T-ALL with *CDKN2B*hypermethylation. Especially, *CDKN2B*biallelic deletion or high methylationlevel(≥45%) was a poor prognostic factor, as was age of onset, *GATA3* and *SH2B3* mutations.
Summary/Conclusion: This study clarifies one of the most important genetic events in T-ALL, *CDKN2B* downregulation, via the mechanisms of deletion and hypermethylation. Different susceptible genetic backgrounds exist according to *CDKN2B* downregulation mechanism.

PB1599

CRISPR/CAS9 SYSTEM IS ABLE TO TURN ETV6/RUNX1 EXPRESSION OFF IN ACUTE LYMPHOBLASTIC LEUKEMIA CELLS

A. Montaño[1,*], V. Alonso-Pérez[1], J.L. Ordoñez[1], A. Marin-Quilez[1,] M. Quijada[1], A. Martín-Martín[1], M. Herrero-García[2], M. Sánchez-Martín[2], R. Benito Sánchez[1], J.M. Hernández-Rivas[1], I. García-tuñon[1]

[1]Molecular genetics in onco-hematology Research Group, Institute for Biomedical Research of University of Salamanca (IBSAL)-University of Salamanca, [2]Servicio de Transgénesis, Nucleus, Universidad de Salamanca., Salamanca, Spain

Background: Acute lymphoblastic leukemia (ALL) is the most common cancer in childhood, around 75% of ALL occurs in children under 6 years. In spite of survival rate has increased over the last years, about 20% of patients continue to relapse. These cases are associated with a worse outcome, even in those genetic subtypes considered to have a good prognosis, such as the t (12.21) that fuses the *ETV6/RUNX1* (*TEL/AML1*) genes. *ETV6/RUNX1* gene fusion as even initiating in the acquisition of leukemic phenotype has been demonstrated. However, its function in the pathology development and maintenance is still unclear. Several studies suggest that *ETV6/RUNX1* promotes tumor survival through up- regulation of anti-apoptotic members of *BCL-2* family protein. In this study, we evaluated the ability of CRISPR/Cas9 genome editing system to eliminate the expression of *ETV6/RUNX1*, and therefore its effect on the expression of anti-apoptotic proteins.
Aims: To abrogate *ETV6/RUNX1* expression by CRISPR/Cas9 technology in the REH cell line.
Methods: The REH cell line (DSMZ), from LAL and expressing the *ETV6/RUNX1* fusion protein, was used. Based on the methodology of CRISPR/Cas9, sgRNAs directed towards the end of exon 5 of *ETV6*, were designed with the intention of producing indels that modify the ORF of the oncogene, and, therefore, the expression of the protein. These sgRNAs were cloned into a vector containing the Cas9 nuclease coding sequence and then were electroporated into the REH cells. The edition of the *ETV6/RUNX1* coding sequence was checked by PCR and Sanger sequencing. Single-edited cell derived cell lines were established to check *ETV6/RUNX1* mRNA expression and downstream targets expression such as *BCL-2* by qPCR and Western Blot. Two single-edited cell clones with wt *ETV6/RUNX1* sequence were used as controls.
Results: The *ETV6/RUNX1* coding sequence in the REH cell line through the CRISPR/Cas9 system was successfully edited. The edition resulted in several frameshift mutations as insertions and deletions that modified the ORF of the oncogene, as well as a deletion between both sgRNAs. Two single-edited cell clones carrying a deletion and another with an insertion were selected. *In silico* analysis of their sequence reveal the generation of KO alleles in all of them. All single-edited clones showed a reduction up to 99,9% of mRNA level compared with REH parental cell line, showing an efficient abrogation of *ETV6/RUNX1* expression. To evaluate the effects of lack of oncoprotein expression in REH cell, we assessed the expression level of *BCL-2* family member proteins. A decreased expression of *BCL-2* and *BCL-XL* in all *ETV6/RUNX1* KO clones compared with parental and control

cell lines we observed, which demonstrates the reduction of the oncogenic potential of these cells.
Summary/Conclusion: For the first time, the CRISPR/Cas9 system to edit the sequence of *ETV6/RUNX1* oncogene was using, inducing frameshift mutations, and leading to the eradication of the fusion product. The lack of *ETV6/RUNX1* expression prevents pro-tumoral effects, such apoptosis suppression through *BCL-2* family proteins overexpression. The use of CRISPR/Cas9 technology could be a promising new therapeutic option for ALL patients. Bone marrow leukemic stem cells could be edited by CRISPR-Cas9 technology to truncate *ETV6/RUNX1* fusions, and truncated cells specifically selected to transplantation treatment.

PB1600

THE EXPRESSION OF THE HISTONE METHYLTRANSFERASE G9A CORRELATES WITH VLA-4 INTEGRIN IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

E. Madrazo[1], D. Ruano[2], C. Sánchez-Valdepeñas[2], Á. González-Murillo[2], L. Abad[2], M. Ramírez[2], J. Redondo-Muñoz[1,*]

[1]Universidad Complutense, [2]Hospital Universitario Niño Jesús, Madrid, Spain

Background: Acute lymphoblastic leukemia (ALL) comprises a group of aggressive and heterogeneous malignancies, occurring in adults and representing the most common pediatric cancer. Up to 25% of patients are genetically unclassified or have an intermediate prognosis; therefore, identifying novel biomarkers and therapeutic targets, as genetic and epigenetic changes, is critical to accurately allocate patients in risk groups. Epigenetic enzymes represent novel therapeutic targets in acute leukemia; however, their connections with prognosis markers across children or adult ALL patients remain unclear
Aims: In this study, we described that the expression of G9A correlates with VLA-4 (very late antigen-4), an integrin cell receptor that serves as a central mediator for the dissemination of ALL cells and as prognostic predictor in pediatric ALL.
Methods: We analyzed the expression of G9A, VLA-4 and SUV39H1 in 51 children patients (range 1-14 years) by RT-qPCR. Then we evaluated the correlation between the expression levels and clinical characteristics of the cohort. We also analyzed the cell migration by using *in vitro* Transwell experiments.
Results: We demonstrated a positive correlation between VLA-4 and G9A levels but not between VLA-4 and SUV39H1. Remarkably, we did not observe any VLA-4/G9A correlation in normal lymphocytes (n=10), suggesting a fundamental connection in leukemia cells. To determine the role of G9A in ALL cell migration, we quantified the levels of H3K9me3 during ALL cell migration. Effective migration induced higher levels of H3K9me3 in moving cells compared to their counterpart in the upper chamber of the Transwell. Moreover, BIX01294 treatment (a G9A inhibitor) impaired the ALL cell migration, suggesting a critical role for this enzyme during leukemia dissemination.
Summary/Conclusion: In summary, our results suggested a clinical potential interest of G9A, as its correlation with VLA-4 might open future targeting approaches and innovative drugs for children ALL patients.

PB1601

ABNORMAL EXPRESSION OF RAS RELATED DEXAMETHASONE INDUCED 1 (RASD1) IN B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

R.-Q. Lu[1,*], L.-X. Wu[1], Y.-L. Zhou[1], J.-L. Li[1], H. Jiang[1], B. Jiang[1], Q. Jiang[1], X.-H. Zhang[1], L.-P. Xu[1], K.-Y. Liu[1], G.-R. Ruan[1], X.-J. Huang[1,2]

[1]Peking University People's Hospital, Peking University Institute of Hematology, [2]Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing, China

Background: Relapse remains the major cause of treatment failure in adults with B-cell acute lymphoblastic leukemia (ALL) achieving complete remission after induction therapy. Identifying new biomarkers in B-cell ALL and studying their clinical significance and biological function will be helpful for risk-stratification, treatment decision and targeted therapy. RASD1 (ras related dexamethasone induced 1) maps to chromosome sub-band 17p11.2, which encodes a member of the Ras superfamily of small GTPases. The role of RASD1 in cancer remains controversial. In our previously conducted bio-informatics analyses of transcriptomic data to identify mRNA transcripts aberrantly-expressed in B-cell ALL, RASD1 was identified as one of most differentially expressed genes which were up-regulated in B-cell ALL. However, the expression and biological function of RASD1 remain unknown in B-cell ALL.
Aims: To investigate the expression level of human RASD1 messenger RNA in B-cell ALL by real-time fluorescent quantitative reverse transcription-polymerase chain reaction assay.
Methods: A real-time quantitative RT-PCR based on TaqMan fluorescence methodology was used to examine RASD1 expression in 11 malignant hematological disorders cell lines and bone marrow samples from 158 adults with B-cell ALL and 40 healthy donors.
Results: Results showed that RASD1 gene expression was higher in cell lines from B-cell ALL (Sup-B15, BV-173), but nearly undetectable in cell lines derived from AML(KG-1, NB4, HL60, OCI-AML2), CML(K562), T-ALL(MOLT4, 6T-CEM), lympoma(Ramos, Raji) and Epstein-Barr virus-transformed lymphoblastoid cell lines (EBV-LCL)(Fig.1A). The relative levels of RASD1 gene expression in marrow from the 158 newly diagnosed B-cell ALL (median 80.12%; range 0.17-2822.33%) were significantly higher than those of marrow from the 40 healthy donors(median 7.59%; range 0.46-38.66%, $p<0.0001$, Fig.1B). The median level of RASD1 in 158 newly diagnosed patients was 80.12%, while the median level in 214 treated patients who achieved complete remission decreased to 3.71%. However, in 17 relapsed patients and 4 refractory patients, the median level was 47.29% and 29.01%, respectively(Fig.1B). In newly diagnosed B-cell ALL, 4 patients with MLL-AF4 translocation showed lower RASD1 expression compared with 68 patients without this translocation ($p=0.005$). RASD1 expression levels were not significantly associated with initial white blood cell counts, hemoglobin, and platelet counts in the peripheral blood, blast in the bone marrow, age, sex, prognosis grouping, immunophenotype, BCR-ABL fusion gene and IKZF1 deletion ($p>0.05$).

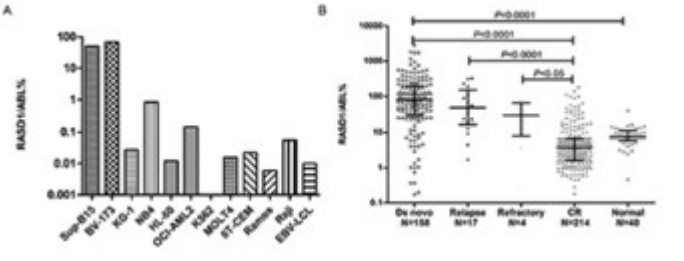

Figure 1. Levels of RASD1 expression in (A) leukemic cell lines; (B) de novo, relapsed, refractory and complete remission (CR) B-cell ALL and normal bone marrow.

Summary/Conclusion: These findings suggest that RASD1 was widely over-expressed in adults with B-cell ALL and that abnormal expression of RASD1 in leukemia may be involved in the pathomechanism of B-cell ALL.

PB1602

CD45 SURFACE EXPRESSION AS A PROGNOSTIC FACTOR IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

V. Antari[1,*], E. Vasileiou[1], A. Tragiannidis[1], E. Gounari[2], E. Hatzipantelis[1], V. Tsavdaridou[2], T. Anastasiou[3], T. Papageorgiou[1], A. Giannopoulos[1]

[1]Hematology Oncology Unit, Pediatric Department of Aristotle University of Thessaloniki, [2]Immunology Department, Microbiology Laboratory, AHEPA General Hospital, Thessaloniki, Greece, Thessaloniki, [3]Hematology Laboratory, Children's Hospital P. & A. Kyriakou, Athens, Greece

Background: CD45 antigen, also known as Protein Tyrosine Phosphatase, receptor type, C (PTPRC), is expressed in hematopoietic cells and its expression is commonly quantified at diagnostic immunophenotyping. Overexpression of CD45 has been previously linked to poor prognosis in children with B- and T-cell ALL.
Aims: Our goal is to study the possible use of CD45 expression as a prognostic factor.
Methods: Bone marrow samples of children with B-cell ALL were studied at the time of diagnosis before treatment initiation according to ALL-BFM 2009. All patients that were admitted to our department from April 2013 to October 2017 were enrolled in the study (44 patients in total). CD45 expression was measured both in normal lymphocytes and leukemic blasts and a relative ratio of its expression (leukemic *versus* normal) was calculated. Statistical analysis was conducted with the Statistical Package for the Social Science (SPSS) for Windows, version 23 (SPSS Inc., Chicago, IL).
Results: We divided the sample into two groups according to the cell line affected (B- and T-cell leukemia). In our study group 39 children were diagnosed with B-ALL and only 5 with T-ALL. The expression of CD45 was

assumed to be high when it exceeded the 75th percentile (relative ratio 12.4% for B-ALL). It was observed that the mean expression of CD45 in patients with B-ALL who had a relapse or died than in patients without an event was higher (Figure 1). We also found that there is a statistically important correlation between relapse or death and the expression of CD45 above the 75th percentile in patients with B-ALL, p<0.05 (p=0.011). Moreover, we calculated that the probability of an event (relapse or death) in patients with B-ALL was 18.67 times higher when the expression of CD45 was above the 75th percentile. Finally, patients with B-ALL and high expression of CD45 had a shorter 2-year event-free survival compared to patients with low expression (51.4% *versus* 96.6%, p<0.01). Due to the small sample of patients with T-ALL we could not calculate statistically important correlations in that group.

Summary/Conclusion: The high expression of CD45 could be used as a prognostic factor in patients with acute lymphoblastic leukemia.

PB1603

FXCYCLE BASED PLOIDY CORRELATES WITH CYTOGENETIC PLOIDY IN BCP-ALL AND IS ABLE TO DETECT THE HYPER-DIPLOID MRD CLONE

N. Gupta[1,*], N. Arora[2], M. Parihar[3], S. Banerjee[1], S. Brahma[1], R. Pawar[1], A. Rath[1], S. Shewale[1], S. Krishnan[4], M. Chandy[5], D.K. Mishra[2]

[1]Laboratory Hematology, [2]Molecular Pathology and Laboratory Hematology, [3]Cytogenetics and Laboratory Hematology, [4]Pediatric Oncology, [5]Clinical Hematology, Tata Medical Center Kolkata, Kolkata, India

Background: Flow cytometry (FCM) is a simple technique that can accurately determine DNA ploidy in B-cell precursor ALL (BCP-ALL).

Aims: We tried to analyze the applicability of FxCycle™ based DNA ploidy analysis in risk stratification of BCP ALLs at diagnosis. We also tried to assess the utility of FCM ploidy for monitoring Minimal Residual Disease (MRD) during follow-up.

Methods: A prospective FCM DNA ploidy analysis using FxCycle™ Violet dye was done in 125 consecutive new cases of BCP-ALL from May 2016 to July 2017. The FCM DNA Index (DI) was compared with karyotyping/FISH data wherever available. As a pilot study, in 16 MRD positive cases (n=6 with hyperdiploid diagnostic DI and n=10 diploid diagnostic DI), DNA ploidy based MRD analysis was also performed.

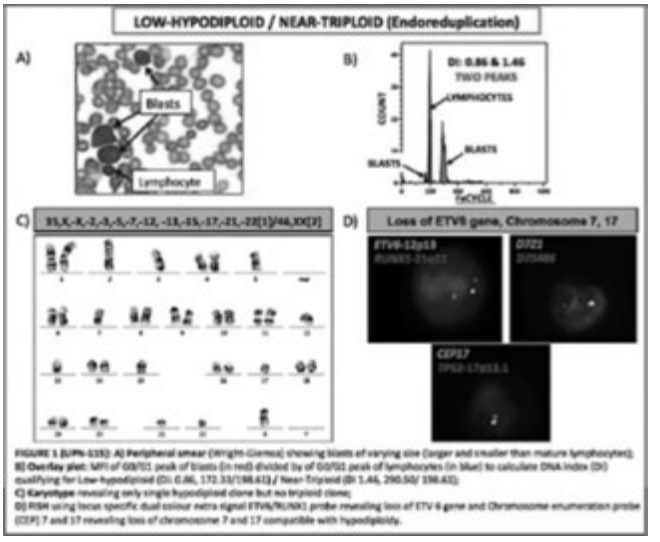

Figure 1.

Results: Of the total 125 cases (age range 10 months-66 years, M:F ratio 1.7:1), n=90 (72%) were pediatric (aged ≤15 years). Karyotype was available in n=119 cases (with 77.3% success rate). Overall cytogenetic ploidy was assessed by both karyotyping and FISH in n=81 cases, only karyotype in n=11 cases and only FISH in n=33 cases. Flow ploidy analysis revealed diploidy (DI 0.96-1.05) in 56/125 (44.8%), low-hyperdiploidy (DI 1.06 to 1.15) in 17/125 (13.6%), high-hyperdiploidy (DI 1.16-1.39) in 41/125 (32.8%) and near-tetraploidy (DI ≥1.80) in 3/125 (2.4%) cases. The high-risk sub-group (Figure 1) of Low-Hypodiploidy (DI 0.70 to 0.88) / Near-triploidy (DI 1.40 to 1.79) (*i.e.* endoreduplication) was seen in n=7 (5.6%) cases while one single case had near haploidy (DI 0.58) with modal chromosome number (MN) 25. Of the 56 cases with diploid DI, successful karyotype was available in n=39 cases of which 10/39 (25.6%) were classic diploids (46, XX or 46, XY) while 29/39 (74.4%) were pseudo-diploids having additional balanced / un-balanced translocations or structural chromosomal abnormalities. Of the n=17 low hyperdiploid DI cases, n=7 cases were concordant with cytogenetic ploidy (MN 47-50 and/ or by FISH) while n=7 cases had MN >50 fitting with description of low DNA index high hyperdiploid subtype (LDI-HHD). Of the n=41 high-hyperdiploid DI cases (karyotype available in n=29 cases), n=26 cases had MN 51-65 (FISH concordant), while in n=15 cases FISH alone confirmed high-hyperdiploidy. DNA ploidy (DI of hematogones 0.96-1.05) could confirm the residual hyperdiploid clone in n=6/6 hyperdiploid MRD positive cases tested.

Summary/Conclusion: FxCycle based DNA ploidy analysis is simple, sensitive and specific technique, which is complementary to cytogenetics in the diagnosis as well as follow-up of BCP-ALLs.

PB1604

CREB KNOCKDOWN INHIBITS GROWTH AND INDUCES APOPTOSIS IN PRE-B ACUTE LYMPHOBLASTIC LEUKEMIA CELLS WITHOUT HAVING A ROLE IN RESTORATION OF CAMP-INDUCED P53 DESTABILIZATION

R. Manafi[1,*], M. Safa[1], F. Alikarami[1], A. Kazemi[1], M. Banan[2]

[1]Iran University of Medical Science, [2]bGenetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran, Tehran, Iran, Islamic Republic Of

Background: The second messenger cAMP plays key roles in multiple intracellular signaling pathways. cAMP signaling pathway can impede drug-induced apoptosis in leukemic cells and elevated levels of this second messenger inhibit doxorubicin-induced apoptosis and p53 accumulation in acute lymphoblastic leukemia cells. In addition, elevated cAMP levels may activate a variety of signaling pathways through protein kinases. cAMP responsive element binding protein (CREB) is a transcription factor that can be activated by cAMP-dependent protein kinase A. CREB is overexpressed in majority of bone marrow samples from patients with ALL and AML and confers survival advantage to the cancer cells.

Aims: According to the fact that cAMP can activate CREB and the role of CREB in malignant cell growth, we sought to determine if CREB plays the mediator role in inhibitory effect of cAMP on doxorubicin-induced apoptosis and p53 accumulation in BCP-ALL cells or not. Moreover, we aimed to assess the effect of CREB on BCP-ALL, NALM-6 cells proliferation and survival.

Methods: In present study, CREB was knocked down using lentiviral CREB shRNA in NALM-6 cells. Knocked down cells were treated with doxorubicin in presence or absence of the cAMP-elevating agents. The expression levels of different apoptotic and anti-apoptotic proteins were assessed by western blot analysis. Gene expression levels were evaluated using qRT-PCR. The apoptosis was assessed using flowcytometry analysis.

Results: We demonstrated here that CREB knockdown induced apoptosis and impaired growth of BCP-ALL NALM-6 cells which was associated with caspase activation. The gene expression levels of prosurvival signals Bcl-2, Mcl-1, Bcl-xL, survivin and XIAP were down-regulated upon CREB suppression. However, the p53 and p21 levels remain unchanged in CREB knocked down cells. Amazingly, our results showed that CREB knockdown cannot eliminate the inhibitory effect of elevated cAMP on doxorubicin-induced apoptosis and p53 accumulation in BCP-ALL cells.

Summary/Conclusion: These findings indicate a critical role for CREB in proliferation, survival, and apoptosis of BCP-ALL cells. The data also suggest that targeting CREB alone could possibly serve as potential therapeutic approach in BCP-ALL but it cannot restore the doxorubicin-induced apoptosis and p53 accumulation in malignant cAMP-elevated cells.

PB1605

MINIMAL RESIDUAL DISEASE (MRD) MONITORING AND IKZF1/NRAS MUTATION STATUS IN ADULT PH-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) PATIENTS TREATED IN RUSSIAN MULTICENTER STUDY "RALL-2016"

O. Gavrilina[1,*], E. Parovichnikova[1], V. Troitskaya[1], G. Baskhaeva[1], K. Zarubina[1], B. Biderman[1], I. Galtseva[1], Y. Davydova[1], S. Kulikov[1], T. Obukhova[1], A. Sokolov[1], V. Savchenko[1]

[1]National Research Center for Hematology, Moscow, Russian Federation

Background: MRD-tailored therapy based on pediatric-inspired intensification is a back-bone of the majority of the European study groups in adult

ALL. Up to 50-70% of patients from the high risk group defined by specific molecular markers and MRD persistence are being now transplanted from allogeneic donors. The aim of the RALL-study group was to create the protocol that could be easily reproduced in any regional center, to reduce toxicity with the preserving high efficacy. The Russian multicenter ALL-2009 trial defined as non-intensive but non-interruptive approach was conducted since Apr 2009 till Dec 2016 and included 330 Ph-negative ALL pts. OS and disease-free survival (DFS) at 7-years constituted 54,7% and 57% for BCP-ALL and 61% and 68% for T-ALL, respectively. Since Dec 2016 were started new protocol RALL-2016 that based on results of previously ALL-2009 protocol.
Aims: To evaluate the frequency of MRD-persistence and impact IKZF1/NRAS mutation status in patients with d*e novo* acute lymphoblastic leukemia treated by RALL-2016 protocol.
Methods: Taking in consideration the major pitfalls of RALL-2009 (high CR death rate, early CNS relapses in T-ALL, selection in autoHSCT *vs* chemotherapy comparison, absence of MRD monitoring) we developed a new RALL-2016 protocol. One day high-dose MTX and high-dose ARA-C blocks were eliminated and substituted by 2 months of non-interruptive therapy, L-asparaginase was scheduled for 1 year of treatment instead of 2,5y, 15 intrathecal injections were increased up to 21 mostly while consolidation phase, CR T-ALL pts were brought to randomization after the informed consent: autoHSCT *vs* no autoHSCT, with the similar further maintenance. All primary bone samples are collected and tested for cytogenetics and molecular markers, all included pts are MRD monitored by flow cytometry in a centralized lab. Since Dec 2016 till Feb 2018 56 adult Ph-negative ALL pts from 5 centers were included in RALL-2016 protocol: median age 35 y (18-54) (BCP-ALL-34(62%) pts, T-ALL-19(34%), biphenotypic-2(4%).
Results: Induction results are evaluable in 53 pts: 44(83%) achieved CR, 5 died during induction (9%), 4(8%) was resistant. 15 patients with T-ALL after CR achievement were randomized for chemotherapy or autoHSCT: 7 and 8 pts, respectively. So far 5 of 8 T-ALL pts were transplanted at a median time of 6 months of CR. Long-term results will come in some years. MRD was detected in 14 (48%) out of 29 B ALL pts, in 1 (8%) out of 13 T-ALL pts and in 1 out of 2 biphenotypic pts on +70 protocol day (end of induction). MRD testing was performed in 27 B ALL patients and 13 T ALL patients on +133 and +190 protocol day. All T ALL patients were MRD negative. 8 (30%) and 7(26%) out of 27 B ALL patients were MRD positive on +133 and +190 protocol day, respectively.We estimated IKZF1 and NRAS mutation status in 17 pts with B-ALL. We detected IKZF1 and NRAS mutations in 3 (18%) different pts for both. All 3 pts with IKZF1 mutations were MRD-positive on day +70 (2 in CR and 1 with refractory disease). Only 1 pts (with refractory disease) was MRD-positive on day +70 with NRAS mutation, another 2 pts with NRAS mutation were MRD-negative.

Table 1.

B-ALL
MRD persistence and IKZF1, NRAS mutation status

Status	Pts with/without mutations		MRD status +70 day of protocol (end of induction)		
			MRD-positive	MRD-negative	Refractory disease
IKZF1	with mutation	3 из 17 (18%)	2 из 3 (67%)	0 из 3 (0%)	1 из 3 (33%)
	without mutation	14 из 17 (82%)	5 из 14 (36%)	7 из 14 (50%)	1 из 14 (7%)
NRAS	with mutation	3 из 17 (18%)	0 из 3 (0%)	1 из 3 (33%)	1 из 3 (33%)
	without mutation	14 из 17 (82%)	3 из 9 (33%)	5 из 9 (56%)	1 из 9 (11%)

Summary/Conclusion: MRD was detected more often in B ALL pts than T ALL pts on +70, +133 and +190 days of RALL 2016. Tumor clone decline was identified in B ALL MRD positive patients on +133 comparing to day +70 day. Pts with IKZF1 mutations were characterized MRD persistence after induction. Currently the group of analyzed pts is small and it seems to be reasonable to continue a study of MRD persistence and IKZF1 gene mutation status in pts with B-ALL.

PB1606

LEUKEMIA BY ASSOCIATION: A CASE SERIES OF MARITAL PARTNERS WITH BLOOD CANCERS

T. Nevill*, E. Nevill, M. Power, D. Sanford, M. Barnett, C. Toze
S. Nantel, J. White, Y. Abou Mourad, S. Narayanan, D. Hogge
K. Song, H. Sutherland, D. Forrest, A. Gerrie, R. Broady

Division of Hematology, Leukemia/BMT Program of BC, Vancouver, Canada

Background: Epidemiologic data suggests heritable factors play a minor role in leukemia causation and environmental factors are a major contributor (Lichtenstein, 2000). Exposure to radiation or toxins is linked to the pathogenesis of a variety of blood cancers and husbands and wives may experience similar environmental exposures. Leukemia in marital partners was first reported by Street in 1950 and rare reports since then have postulated a single causative agent including communicable infections (EBV, HTLV-I and HIV; Lynch, 1984). A larger group of affected couples would allow for the evaluation of commonalities and potential etiology.
Aims: To review cases where both marital partners were diagnosed with leukemia to determine patterns and identify common risk factors.
Methods: A retrospective review of patients (pts) presenting to our centre with leukemia between 2012 and 2017 was performed to determine if they previously had a spouse with a leukemia diagnosis. Data collected included basic demographics, diagnosis, order of onset, interval from marriage and between leukemia diagnoses, environmental exposures, comorbidities, family history of malignancy and treatment outcomes.
Results: Between 02/12 and 03/17, 5 pts presented with leukemia (2 ALL, 1 AML, 1 CML and 1 CLL) who had previously had a spouse with leukemia (2 ALL, 1 AML, 1 CML and 1 CLL). Three couples were Caucasian, 1 Asian and 1 South Asian; all marriages were non-consanguineous. None of the 10 pts had a family history of leukemia. Median age at time of first leukemia (L1) (3 wives, 2 husbands) was 44 years (y) (range 29-57) and of second leukemia (L2) was 62 y (range 45-72). None of the couples had the same leukemia subtype. Interval from L1 to L2 was a median of 14.5 y (range 0.8-21). Median duration of marriage at time of L1 was 16 y (range 7-35) and L2 was 27 y (range 17-45). Comorbidities included a prior malignancy in 2 pts (Hodgkin lymphoma and basal cell Ca). Seven pts were lifetime non-smokers; the remaining 3 pts had not smoked for 17-38 yrs. Excessive alcohol intake was seen in only 1 pt; 5 had never consumed alcohol. Five pts had potential occupational toxin exposures (2 health professionals, 1 marine engineer, 1 transportation industry worker and 1 construction worker); one other pt had previously received radiotherapy. Marital partners fell into 2 groups-either both had occupational toxin exposures (3 couples) or neither did (2 couples). The latter group included 2 wives with Ph+ CML whose husbands had ALL with common translocations [t(4;11), t(8;14)]. In both of these couples, the wife had a striking family history of solid tumours. Six of 10 pts are alive — 4 pts after allogeneic SCT, 1 pt with CML who received TKI for 10 y but is off therapy for 2 y without recurrence and 1 pt with CLL who has not yet required therapy. Four pts have died from leukemia progression (2 AML, 2 ALL).
Summary/Conclusion: In a short time period, 5 pts presented to our tertiary care centre with a leukemia having previously had a spouse with leukemia. Specific etiology was difficult to determine in most cases but certain patterns were identified. Leukemia typically developed many years after marriage, L1 at a median of 16 y and L2 at a median of 27 y. Potential occupational exposures were found in both marital partners in 3 cases; in the other 2 partners, the wife developed CML and the husband developed ALL with a classic translocation. This case series significantly adds to the sparse literature on spousal leukemia and suggests that the partner of a pt with leukemia is at increased risk for developing leukemia.

PB1607

MIR-21 REGULATE ADULT B-ALL BY TARGETING CELL CYCLE PROTEIN TLK2

X. Wang*, L. Xia

Union Hospital, Tongji Medical College, Huazhong University of Science & Technology, WuHan, China

Background: ALL remains one of the most challenging adult malignancies with a high recurrence rate and a low cure rate. The main causes of ALL relapse is a severe disturbance of apoptotic pathways occurs.It was reported that mir-21 is significantly increased in patients with B-ALL, however, the biological function of miR-21 in B-ALL remains poorly understood.Cell cycle disorders play an important role in the development of tumor, We used miRanda software to look for possible downstream target genes for miR-21, and found that the cell cycle protein TLK2 3 '-UTR region has a sequence that can be combined with the miR-21 seed region, as well as we found in the Oncomine database that TLK2 has differential expression in B-ALL patients and normal human bone marrow.
Aims: In this study, we detected miR-21 levels in B-ALL cell line (nalm-6) to investigate the mechanism of its oncogenic function.

Methods: First, we compared miR-21 levels of bone marrow mononuclear cells between patients (n=28) and normal control (n=15),Then, to investigate the miR-21expression in vitro, we detected the miR-21 levels in BCP-ALL cell lines Nalm-6 cells. To evaluate whether miR-21 influence the function of Nalm-6 cells, antagomir-21 was used to knockdown miR-21 and antagomir-NC was used as a control. After incubating antagomir-21or antagomir-NC with Nalm-6 cells for 24h, 48h and 72h, we detected the proliferation by Cell Counting Kit-8, and assessed the apoptosis and cell cycle by flow cytomete, western blot was performed to study the expression analysis of Bcl-2, Bax and active caspase-3 proteins.we used miRanda software looking for the downstream target genes of miR-21, and verified the relation of miR-21 and TLK-2, we detected the qPCR and western blot.
Results: The results showed that the relative expression of miR-21 3.328±0.5156 N=18 in the bone marrow of B-ALL patients was significantly higher than that in normal control (1.174+0.1641, N=15), and the difference was statistically significant (P<0.01). The expression of miR-21 in nalm-6 cells was about 3.2 times (P<0.01) the control. The proliferation ability of the nalm-6 transfected with antagomir-21 was significantly inhibited.Compared with the antagomir-nc negative control group, the proliferation ability of nalm-6 decreased in 48 hours (16.5+0.09287)%, and decreased in 72 hours (18.4+0.01479)% (P<0.05 and P<0.001), and the apoptosis rate of nalm-6 cells in the antagomir-21 group was about 2.44 times that of the negative control group (P<0.05). The expression level of bcl-2 protein was positively correlated with miR-21 expression, and miR-21 had no significant effect on Bax expression level.Compared with the control group, the proportion of G1 phase cells (55.75++3.748%) was significantly higher than that in the control group (44.635++1.266%) (P<0.05), while the proportion of S phase cells decreased significantly (41.88+2.630)% and (53.675+0.8132)% (P<0.05).PCR results showed no significantlly change on TLK2 mRNA levels after transfection with antagomir-21 (P<0.05), but western Blot results showed a significant decrease in TLK2 protein expression levels in transfected antagomir-21 group (P<0.05).

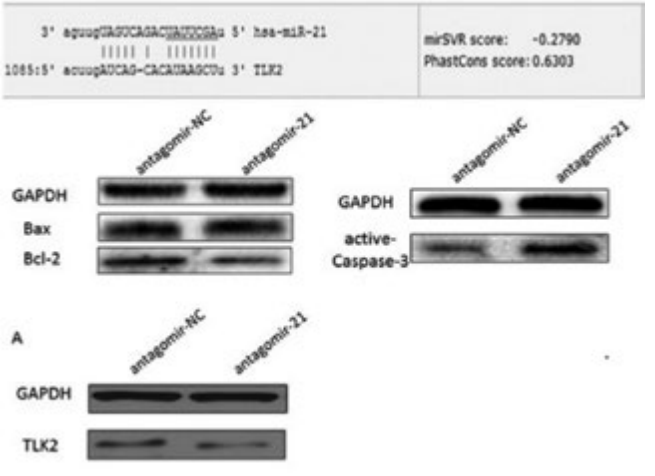

Figure 1.

Summary/Conclusion: Overall, in this work ,For the first time, the novel mechanism of miR-21 regulating cycle was explored in the b-all cell line nalm6, and an interesting relationship between mi21 and cell cycle protein TLK2 was introduced initially, providing new ideas for the oncogene role of miR-21 in B-ALL.

PB1608

PARTHENOLIDE, AN NF-KB INHIBITOR, INDUCE CELL DEATH AND SUPPRESS CELL PROLIFERATION IN LYMPHOID MALIGNANCIES

J. Neves[1], J. Jorge[2,3], R. Alves[2,3], A.C. Gonçalves[2,3], A.B. Sarmento Ribeiro[2,3,4,5,*]

[1]Department of Life Sciences, Faculty of Science and Technology, University of Coimbra, [2]Laboratory of Oncobiology and Hematology and University Clinic of Hematology, Faculty of Medicine, University of Coimbra (FMUC), [3]Coimbra Institute for Clinical and Biomedical Research (iCBR)-Group of Environment Genetics and Oncobiology (CIMAGO), FMUC, [4]Center for Neuroscience and Cell Biology (CNC), University of Coimbra, [5]Clinical Hematology Department, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal

Background: Acute Lymphoblastic Leukemias (ALLs) are hematologic malignancies characterized by dysregulated cell proliferation and differentiation arrest, resulting in the accumulation of immature lymphoid progenitors (B or T). Burkitt lymphoma (BL) represents a highly aggressive lymphoma, characterized by acute onset and rapid doubling time. The progress in new therapeutic approaches to treat patients with ALL are scarce, the survival rates have not increased substantially in recent years and relapses are frequent. Playing an important role in the regulation of diverse biological processes such as cell proliferation and survival, nuclear factor kappa B (NF-kB) is closely associated with various human malignancies. Parthenolide (PRT), a sesquiterpene lactone, was reported to inhibit the DNA binding of NF-KB. This compound induces apoptotic cell death by multiple pathways, including oxidative stress, endoplasmic reticulum stress, intracellular thiol depletion, caspase activation, and mitochondrial dysfunction, as well as sensitizes cancer cells to chemotherapeutic drugs.
Aims: In this context, this work aimed to evaluate the therapeutic potential of PRT on ALL and BL cell lines and characterize the type of cell death induced and its molecular mechanisms.
Methods: To this end, T-ALL (CEM, JURKAT, and MOLT-4), B-ALL (697 and KOPN-8), and BL cell lines (Raji) were incubated in the absence and presence of PRT in single and fractioned administration schemes. Metabolic activity was assessed by resazurin assay. Cell death was evaluated by optical microscopy (May-Grünwald-Giemsa staining) and by Flow Cytometry (FC; Annexin V/7-AAD staining). The expression of FAS, FAS-L, activated-caspase-3, phosphorylated NF-kB, mitochondrial membrane potential (Δ mit; JC-1) and oxidative stress [superoxide (O2-; DHE), hydrogen peroxide (H2O2, DCFH2DA), and reduced glutathione (GSH)] were also analyzed by FC. The results were statistically analyzed considering a level of significance of 95% (p<0.05).
Results: The results indicate that PRT reduced metabolic activity in a time-, dose- and cell line-dependent manner. KOPN-8, CEM, and RAJI (IC_{50} 50-75μM) were the most sensitive cells and MOLT-4 and JURKAT (IC_{50} 100μM) the less sensitive. Single and fractional administration regimens showed similar results. PRT induced cell death mainly by apoptosis associated with an increase in activated-caspases expression, decrease in Δ mit and an increase in oxidative stress levels (increased ROS and decreased GSH) in all cell lines. The morphological aspects observed in cells treated with PRT, such as blebbing and nuclear fragmentation, confirmed apoptosis induction. In addition, PRT also increased FAS and FAS-L expression levels in all KOPN8, 697 and MOLT4 cell lines. A cytostatic effect was observed in JURKAT (G_0/G_1 phase arrest) and MOLT-4 (G_2/M phase arrest) cells. Finally, PRT induced a decrease in phosphorylated p65 levels (a subunit of NF-KB) confirming the inhibition of NF-kB pathway.
Summary/Conclusion: In conclusion, these results suggest that PRT may represent a new potential therapeutic approach in ALL and BL. However, the therapeutic efficacy may depend on disease subtype.
This work was supported by CIMAGO and by the FCT through the fellowship SFRH/BD/51994/2012.

PB1609

BOTANICAL ALKYL HYDROQUINONE HQ17(3) INDUCES ENDOPLASMIC RETICULUM STRESS, MITOCHONDRIAL CALCIUM OVERLOADED AND MITOPHAGY TO EXERTS CYTOTOXICITY TO PHILADELPHIA CHROMOSOME(+) SUP-B15 ALL CELLS

Y.-C. Jou[1], C.-W. Chen[1], Y.-Y. Kuo[2], L.-I. Lin[1], C.-Y. Hu[1,*]

[1]Department of Clinical Laboratory Sciences and Medical Biotechnology, [2]Graduate Institute of Oncology, College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China

Background: Acute lymphoblastic leukemias (ALLs) harboring t(9;22)(Ph^+-ALL) is classified as very high risk (VHR) ALL due to poor clinical outcome irrespective of intensive chemotherapies and tyrosine kinase inhibitor treatment. Development of new adjuvant therapeutics will provide great value. HQ17(3)[10'(Z),13'(E),15'(E)-heptadecatrienyl hydroquinone] isolated from sap of the lacquer tree showed potent cytotoxic effect in 24 hours at micromolar concentration on several ALL cell lines, including Imatinib-refractory Ph^+-ALL SUP-B15 cells, spared normal PB leukocytes, and non-toxic in experimental rats. HQ17(3) presents as a potential anti-leukemic agents and a model for designing anti-leukemic regimen. We previously showed HQ17(3)-induced cell demise was characterized by oxidative stress, mitochondrial membrane potential (MMP) loss and nuclear DNA fragmentation. HQ17(3)-induced cell death is caspase-independent, and is different from the RIP1-mediated necroptosis or lysosomal permeabilization-mediated cell death since inhibitors to caspases, RIP-1K or lysosomal cathepsins/proteases failed to protect SUP-B15 cells from death. HQ17(3) enhanced autophagic flow and autophagy inhibitors attenuated the cell death. Interestingly, the ER stress markers (chaperon Grp78 and phosphorylated-eIF2a) were found upregulated as early as 5hr after HQ17(3) treatment.

Aims: To illustrate the characters of the HQ17(3)-induced non-classical death on Ph+-SUP-B15 cells, focus on ER stress and mitochondrial Ca^{2+} homeostasis.
Methods: Cell growth inhibition in response to HQ17(3) or inhibitors was analyzed by ACP assay. Annexin V/PI and flow cytometry analysis detected cell death. Autophagy was revealed by aggregation of ectopically expressed EGFP-LC3. MFN1/2, OPA1 (mitochondrial markers) were analyzed by western blot. Nuclear accumulation of apoptosis inducing factor (AIF), colocalization of mitochondrial COX-IV and LC3-II (mitophagy) were revealed by immunofluorescence stain and confocal microscopy. Mitochondrial Ca^{2+} [Ca^{2+}_m] accumulation was shown by fluorescent Rhod-2 probe, mitochondrial superoxide was measured after Mitosox stain.
Results: We showed [Ca^{2+}_m] accumulation at the time ER stress occurred, accompanied by mitochondrial superoxide elevation, followed by MMP loss and nuclear translocation of apoptosis-inducing factor (AIF). However, inhibition of AIF cleavage by calpain inhibitor PD150606 reduced the HQ17(3)-induced cell death slightly. Further, Ca^{2+} chelator Bapta-AM prevented [Ca^{2+}_m] overload and rescued cell death more effectively, indicating [Ca^{2+}_m] participated in other aspects in cell death. HQ17(3) treatment lead to decreased mitochondrial proteins MFN1/2 and OPA1, while Mitotracker green stain showed significant loss of mitochondrial mass preceded cell death, indicating damaged mitochondria succumbed to mitophagy, which was confirmed by the presence of COX IV and LC3B co-localization.
Summary/Conclusion: In Ph+-ALL SUP-B15 cells HQ17(3) induces ER stress by yet-defined mechanism, mobilizes Ca^{2+} to mitochondria, results in cleavage and release of AIF to mediate chromatin fragmentation, Ca^{2+}-overload leads to oxidative stress in turn and perturbs mitochondria integrity. Damaged mitochondria induce extensive mitophagy and cell death ensues. Therefore, agents that help elicit similar intricate effector network associated with ER/mitochondria stress will have potential as adjuvants controlling the VHR-ALL cells refractory to conventional chemotherapies and TKI regime.

PB1610

CELL CYCLE REGULATORY GENE (CDKN2A/2B) DELETIONS CORRELATE WITH POOR EVENT FREE SURVIVAL IN PEDIATRIC ALL

P. Bhatia[1,*], K.M.[2], M. Singh[3], A. Trehan[4], M. Sachdeva[5], D. Bansal[4], R. Jain[1]

[1]Asst Professor, Hematology Oncology Unit (Lab Hematology) Dept Of Pediatrics, [2]PGIMER, chandigarh, India, [3]Pediatric Hematology-oncology, [4]Professor Pediatric Hematology-oncology, [5]Additional Professor, Dept Of Hematology, PGIMER, chandigarh, India

Background: Studies have shown that approximately 30 to 35% of pediatric ALL cases are known to harbor genetic abnormalities in cell differentiation, cell cycle control and apoptosis related genes. These genetic abnormalities are prognostically important changes that can influence treatment outcome. Their early recognition is important to individualize treatment decisions and improve overall survival.
Aims: To note the frequency of cell cycle regulatory gene copy number abnormalities in pediatric Acute Lymphoblastic Leukemia (ALL) cases using MLPA assay and to correlate the copy number abnormality status with response to chemotherapy and outcome parameters.
Methods: 87 prospectively diagnosed and confirmed ALL cases were enrolled and cell cycle regulatory gene deletions (CDKN2A/2B and RB1) were noted by MLPA assay using ALL-P-335 kit and data analyzed by Coeffalyzer software. In addition clinical and outcome data was recorded as per treatment record files. Ethical clearance of study was obtained from Institute's Ethics Committee.
Results: Mean age in the study group was 5.87 years (6 months-12 years) with a M;F ratio of 2:1. There were 74 B-ALL and 13 T-ALL cases. The mean TLC in our study group was 1,08,499/cu mm and Good Prednisolone response at day 8 was noted in 70 (81.4%) cases. Day 35 post induction showed M1 response in 82(95.3%) children and M2+M3 response in a total of 4(4.7%) children, while data available in 73 B-ALL and 5 T-ALL cases showed MRD >0.01% in 21(28.7%) B-ALL and 1 T-ALL case respectively. 44.8% of children in the cohort had cell cycle regulatory gene copy number abnormalities of which frequency of CDKN2A/2B gene copy number abnormalities (both B and T cell ALL) was 43.6% and frequency of RB1 gene copy number abnormalities was 3.4% (p value-0.00). All the T cell ALL (100%) cases in the cohort had cell cycle regulatory gene copy number abnormalities, compared to 35.1% in B cell ALL cases (p value-0.00). In addition predominantly homozygous CDKN2A/2B gene deletions in T-ALL (92%) as compared to B-ALL (48%) were noted (p-0.00). On correlation of cell cycle regulatory gene deletional *versus* non deletional group with standard ALL risk factors and treatment outcome parameters, a significant correlation was noted of deletional group with high TLC (p -0.00), T-ALL phenotypes (p -0.00), events (p -0.28) and BCR-Abl gene positivity (p-0.00). The mean EFS duration was further decreased by 8.1 months in cases with only CDKN2A/2B gene deletions in comparison with those without cell cycle regulatory gene copy number abnormalities. (p value-0.00).
Summary/Conclusion: Our study results highlight that CDKN2A/2B deletions are common in B-ALL and seen in high frequency in T-ALL (100%) our study. Moreover CDKN2A/2B deletions in B-ALL confer a poor EFS in multivariate analysis suggesting their potential role as a poor prognostic marker in B-ALL.

PB1611

NOVEL COMBINATION OF RESVERATROL AND CERAMIDE METABOLIZING ENZYMES SYNERGISTICALLY ENHANCES THE ANTI-PROLIFERATIVE AND PRO-APOPTOTIC EFFECTS ON PH + ACUTE LYMPHOBLASTIC LEUKEMIA

A. Adan*, O. Oğuz

Molecular Biology and Genetics, Abdullah Gul University, Kayseri, Turkey

Background: Philadelphia chromosome positive acute lymphoblastic leukemia (Ph + ALL), expressing BCR/ABL oncoprotein, is the most common subtype with high mortality rate and poor prognosis. Overall survival rate can only be extented to a few years after treatment and complete remission can not be achieved. Therefore, it is quite important to investigate new therapeutic agents and define potential novel cellular targets for the treatment. The anticarcinogenic potential of resveratrol has not been *investigated in Ph +* ALL. Bioactive sphingolipids are a lipid family with important members including ceramide, sphingosine-1-phosphate (S1P) and glucosyl ceramide (GC), which have significant roles in the regulation of cell divison, growth, metastasis and apoptosis. Ceramide produced through *de novo* synthesis pathway (Serine Palmitoyl Transferase (SPT) is a key enzyme subjected to regulation) is a central molecule in sphingolipid metabolism playing significant roles in the induction of apoptosis. On the other hand, the conversion of ceramide into sphingosine-1-phosphate (S1P) by sphingosine kinase 1 (SK-1) or/and into glucosyl ceramide by glucosyl ceramide synthase (GCS) induces the proliferation of cancer cells. Therefore, ceramide metabolism and the regulation of enzymes such as SPT, SK-1 and GCS have a great therapeutic importance.
Aims: It is aimed to investigate *therapeutic potential of resveratrol on Ph + ALL cells and to identifiy potential mechanisms behind resveratol-mediated apoptosis in association with targeting of ceramide metabolism and regulation of BCR-ABL.*
Methods: The antiproliferative and apoptotic effects of resveratrol, SPT inhibitor (myriocin), SK-1 inhibitor (SKI II), GCS inhibitor (PDMP), resveratrol:myriocin, resveratrol:SKI-II and resveratrol: PDMP combinations on Ph + ALL cells, SD-1 and SUP-B15, are investigated by cell proliferation assay and flow cytometry (annexin-V/PI), respectively. Caspase-3 and PARP levels and cytochrome c release are checked by western blot to confirm apoptosis induction. The synergistic, antagonistic or additive effects of the mentioned combinations and the combination indexes are calculated using Calcusyn program. The cytostatic effects of each agent alone and combinations are determined by PI staining. BCR-ABL levels are determined by western blot after the cells are exposured to each agent alone and defined combinations.
Results: Proliferation of Ph + ALL cells were reduced dose and time dependently with treatment of either drug except myriocin. Drug interaction relationship were studied using Calcusyn software and effects of synergistic combinations (CI<1) involving lower doses of resveratrol and ceramide metabolism enzymes were selected for further studies.These combinations significantly induced apoptosis via enhanced PARP cleavage, caspase-3 activation and cytrochrome-c release. Combinations also caused ceell cycle arrest at different phases and modulated the expression of BCR-ABL.
Summary/Conclusion: The *anti-leukemic effect of resveratrol on Ph + ALL cells and mechanisms behind its action are enlightened by targeting critical enzymes involved in ceramide metabolism and regulating BCR-ABL for the first time.* Furthermore, resveratrol:SPT inhibitör, resveratrol:SK-1 inhibitor and resveratrol:GCS inhibitor combinations could be considered as a novel approach for the treatment of leukemia.

PB1612

INHIBITION OF DNA-PK ENHANCES CHEMOSENSITIVITY OF B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA CELLS TO DOXORUBICIN

F. Alikarami[1], M. Faranoush[2], P. Hayat[3], A. Kazemi[1], M. Safa[1,*]

[1]Iran University of Medical Science, [2]Pediatric Growth and Development Research Center, Institute of Endocrinology and Metabolism, Iran University of Medical

Science, [3]Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran, Islamic Republic Of

Background: DNA damage repair pathways greatly affect the response to genotoxic drugs in cancer cells, so inhibition of such pathways could be a potentially useful strategy to enhance chemosensitivity. DNA-dependent protein kinase (DNA-PK) plays a crucial role in the repair of DNA double-strand breaks (DSBs) that are probably one of the most detrimental types of DNA damage. It has been shown that DNA-PK is highly expressed in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) cells. Less well appreciated was the effect of DNA-PK inhibition on sensitivity of BCP-ALL cells to DNA-damaging agents.
Aims: Disruptions in DNA repair system is a way to induce cell death. So, we determine to evaluate the effect of DNA-PK inhibition on sensitivity of BCP-ALL cells to DNA-damaging agents. In the present study, we investigated the effects of DNA-PK inhibitor (NU7441) on doxorubicin-induced DSBs in BCP-ALL cells.
Methods: NALM-6 and SUP-B15 cells (BCP-ALL cell lines) were treated with doxorubicin in presence or absence of different concentration of DNA-PK inhibitor (NU7441). The apoptosis was assessed using Annexin V-PI staining followed by flow cytometry analysis. Phosphorylated H2AX was detected by immunofluorescence staining. The expression levels of a repertoire of apoptotic and anti-apoptotic proteins were assessed by western blot analysis. qRT-PCR were used to evaluate the gene expression levels.
Results: Here, we show that the DNA-PK inhibitor NU7441 increased doxorubicin-induced apoptosis in BCP-ALL cell lines, correlating with a reduction in DSB repair measured by γ-H2AX foci. NU7441 affected the cell cycle distribution and the cell cycle regulatory molecules in combination with doxorubicin treatment. Doxorubicin-induced DNA-PK phosphorylation was decreased in the presence of NU7441. Apoptosis induction by the combined treatment was associated with marked reduction of Bcl-2 and survivin and a significant increase of Bax mRNA expression levels.
Summary/Conclusion: our data indicate that inhibition of DNA-PK might be an effective approach to enhance the tumor-cell-killing effects of DNA-damaging agents such as doxorubicin in BCP-ALL and may deliver novel, targeted therapy into the clinic.

PB1613

EPIGENETIC MODULATORS SHOW *IN VITRO* THERAPEUTIC POTENTIAL TO TREAT B CELL ACUTE LYMPHOBLASTIC LEUKEMIA

B. Ribau[1], J. Jorge[2,3], R. Alves[2,3], I.P. Ribeiro[3,4], I. Marques Carreira[3,4], A.C. Gonçalves[2,3], A.B. Sarmento Ribeiro[2,3,5,6,*]

[1]Chemistry Department, Aveiro University, Aveiro, [2]Laboratory of Oncobiology and Hematology and University Clinic of Hematology, Faculty of Medicine, University of Coimbra (FMUC), [3]Coimbra Institute for Clinical and Biomedical Research (iCBR)-Group of Environment Genetics and Oncobiology (CIMAGO), FMUC, [4]Laboratory of Cytogenetics and Genomics, Faculty of Medicine, University of Coimbra (FMUC), [5]Center for Neuroscience and Cell Biology (CNC), University of Coimbra, [6]Clinical Hematology Department, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal

Background: B cell acute lymphoblastic leukemia (B-ALL) is the most frequent hematologic neoplasia in children and is characterized by the accumulation of blast cells that are phenotypically reminiscent of normal stages of B-cell differentiation. Epigenetic alterations, namely DNA methylation and histone modifications, are involved in B-ALL development and progression. Unlike chromosomal translocations or gene mutations, which induce permanent changes in the DNA sequence, hypermethylation of gene promoters is a reversible event that could be targeted with therapeutic agents designed to alter aberrant epigenetic events.
Aims: The aim of this study was to evaluate the therapeutic potential of hypomethylating agents ([DNMTi: Azacitidine (5-AC) and Decitabine (DAC)]) and histone deacetylase inhibitors [HDACi: Panobinostat (PAN) and Vorinostat (SAHA)], in monotherapy and in combination therapy, in *in vitro* models of B-ALL.
Methods: Two B-ALL cell lines, the KOPN-8 and 697 cells, were incubated in the absence and presence of DNMTis and/or HDACis, in monotherapy and in therapeutic combination. Cell viability was determined by the FMCA assay (Fluorometric Microculture Cytotoxicity Assay). Cell death was evaluated by optical microscopy (May-Grünwald-Giemsa staining) and by Flow Cytometry (FC; Annexin V/7-AAD double staining). The hypomethylating effect was evaluated through 5-methylcytosine (5-mC) levels analyzed by FC and the methylation status of 24 tumor suppressor genes was carried out by MS-MLPA. The results were analyzed statistically considering a significance level of 95% ($p<0.05$).
Results: The results showed that 697 cells have more tumor suppressor genes methylated (15/24) than KOPN8 cells (12/24). The studied drugs reduced cell viability in a time-, dose- and cell line-dependent manner, being the 697 cells more sensitive than KOPN-8 cells. B-ALL cells were found to be more sensitive to DAC (IC_{50} 697=10μM; IC_{50} KOPN-8 >15μM) than to 5-AC (IC_{50} 697=15μM; IC_{50} KOPN-8 >20μM), after 72h of treatment. They were also more sensitive to PAN (IC_{50} 697=7.5nM; IC_{50} KOPN-8 >20nM) than to SAHA (IC_{50} 697=750nM; IC_{50} KOPN-8=1000nM). In addition, therapeutic associations of DAC with PAN or SAHA reduced more effectively the cell viability when compared to monotherapy treatments. These drugs induced predominantly apoptosis in 697 cells and apoptosis and necrosis in KOPN-8 cells. Cell death by apoptosis was confirmed by morphological aspects such as blebbing, cellular contraction, and nuclear fragmentation. Moreover, SAHA induced cell cycle arrest in phase S and G_0/G_1, respectively in 697 and KOPN-8 cells. Finally, both DNMTi and their association with PAN induced a decrease in 5-mC levels and induced demethylation of at least two tumor suppressor genes (697: *TP73*, *KLLN*, *MGMT*, and *CD44*; KOPN8: *MGMT* and *STK11*).
Summary/Conclusion: Our results suggest the therapeutic potential of epigenetic modulators in the treatment of B-ALL in monotherapy and in the usefulness of the association of DNMTi with panobinostat. However, the therapeutic efficacy may depend on the methylation profile.
This work was supported by CIMAGO and by the FCT through the fellowship SFRH/BD/51994/2012.

PB1614

T(4;9)(Q21;P24), RECCURENT FOR THE CLASSICAL HODGKIN LYMPHOMA, IN A PATIENT WITH ETV6-RUNX1-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA

G. Balatzenko[1,*], N. Yurukova[2], M. Romanova[3], H. Burnusuzov[4], M. Guenova[5], D. Konstantinov[2]

[1]Laboratory of Cytogenetics and Molecular Biology, National Specialized Hospital for Active Treatment of Hematological Diseases, [2]Clinic of Hematology, Children's Oncohematology Clinic, University Hospital "Tsaritsa Yoanna-ISUL", [3]Laboratory of Cytogenetics and Molecular Biology, [1]. National Specialized Hospital for Active Treatment of Hematological Diseases, Sofia, [4]Clinical Immunology Department, Flow Cytometry Laboratory, Medical University Plovdiv, Department of Pediatrics and Medical Genetics, Oncohematology Unit; University Hospital "Sv. Georgi", Plovdiv, [5]Laboratory of Hematopathology & Immunology, National Specialized Hospital for Active Treatment of Hematological Diseases, Sofia, Bulgaria

Background: The cryptic translocation t(12;21)(p13;q22) and the respective *ETV6-RUNX1* fusion gene, is the most common genetic abnormality in childhood acute lymphoblastic leukemia (ALL), accounting for 20%>25% of the cases, while it is a very rare finding in adolescents and adults with ALL, with an incidence of ≤1%. Additional chromosome abnormalities are detected in more than two thirds of cases, however the t(4;9)(q21;p24) has not been reported so far. This abnormality that results in the formation of *SEC31A-JAK2* fusion gene has been previously reported as the first recurrent *JAK2*-associated translocation in classical Hodgkin lymphoma. To the best of our knowledge only two other cases of t(4;9)(q21;p24) have been reported so far-in a patient with ALL, with unknown *ETV6-RUNX1* status and in a patient with undifferentiated lung carcinoma.
Aims: To present for the first time a rare case of t(4;9)(q21;p24) in an adolescent with a molecularly defined *ETV6-RUNX1*-positive B-ALL.
Methods: A 19-old male was admitted to the hospital because of pancytopenia and elevated serum lactate dehydrogenase levels, tested in relation to complaints of acute thoracic back pain.
Results: On admission, the physical exam was unremarkable. The initial laboratory tests revealed white blood cell counts of $1.6x10^9$/l with no blasts; a hemoglobin level of 90 g/l and platelet counts of $10x10^9$/l. On aspirate smear, the bone marrow was markedly hypercellular with a subtotal infiltration with lymphoblasts. Flow cytometry of bone marrow detected a blast cells population accounting for 48.3% of all cells with the following phenotype: Syto41+/CD45dim+/CD19+/CD10+/ CD20-/CD34+/CD38+/ CD58+. Conventional cytogenetic analysis demonstrated an abnormal karyotype: 45,XY,-4, der(9)t(4;9)(q21;p24),14ps+[5]/46,XY,14ps+[15], while molecular analysis using qualitative reverse transcription polymerase chain reaction with two different sets of primers revealed the presence of *ETV6-RUNX1* (short type) transcripts. Based on these data a diagnosis of *ETV6-RUNX1*-positive B-cell precursor ALL was made and treatment was initiated according to the ALL-IC BFM 2009 protocol. At day 33 a complete hematological remission was registered with a minimal residual disease of 0,003% detected by flow cytometry. ETV6-RUNX1 transcripts gradually decreases up to undetectable levels. Currently, our patients is still in complete hematological and molecular remission 2 years after treatment discontinuation.

Summary/Conclusion: In this study we report on a t(4;9)(q21;p24) in a male adolescent patient with B-ALL, also positive for the short type of *ETV6-RUNX1* transcipts. The presence of this rare additional abnormality, that is recurrent for another hematological malignancy, was not associated with specific clinical, morphological or immunophenotypic features and it did not affect the characteristic favorable prognosis associated with *ETV6-RUNX1.*

PB1615

HIGH EXPRESSION OF PARTITION-DEFECTIVE 3 (PARD-3) IS ASSOCIATED WITH ADULT B CELL ACUTE LYMPHOBLASTIC LEUKEMIA

X. Yan*, J. Zhang, J.-M. Zhang, L.-X. Wu, X.-J. Huang, K.-Y. Liu, G.-R. Ruan

Institute of Hematology, Peking University People's hospital, Beijing, China

Background: The partition-defective 3 (PARD-3) gene is essential for the disassembly of the tight/ adhesive junctions and epithelial-mesenchymal transition (EMT) which is critical for tumor spreading. Previous research has shown that PARD-3 protein expression is frequently lost in primary esophageal squamous cell carcinoma(ESCC). In the progression of non-small-cell lung cancer (NSCLC), PARD-3 has also been verified to contribute to EMT, invasion, and chemoresistance in NSCLC. The effect of PARD-3 expression on adult B-cell acute lymphoblastic leukemia (ALL) has not been identified.
Aims: The purpose of this study was to investigate the transcriptional level of PARD-3 in adult B cell acute lymphoblastic leukemia.
Methods: A real-time quantitative reverse transcription-polymerase chain reaction assay based on Taq-Man fluorescence methodology was used to quantify the PARD-3 mRNA copy number in the bone marrow cells from patients with adult leukemia. Normal marrow samples from the allogeneic stem cell transplantation donors were served as control. Informed consent was obtained for every marrow sample.

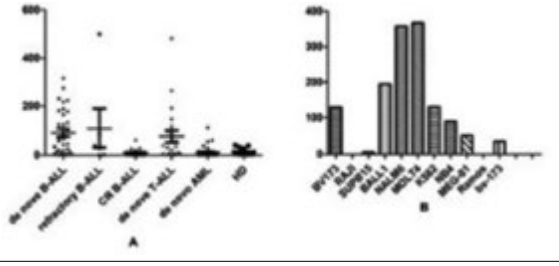

Figure 1.

Results: Expression levels of the PARD-3 in leukemia patients and normal donor marrow are shown in Figure 1. These results showed that the relative levels of PARD-3 gene expression in marrow from 32 newly diagnosed B-cell ALL (median 92.84%; range 0.002% 316.201%) was significantly higher than those of marrow from the 20 healthy donors (median 13.74%; range 2.93% 41.15%; *P*=0.004). Significant PARD-3 mRNA over expression was found in the *de novo* B-cell ALL patients compared with 32 treated B-cell ALL patients (median 10.46%; range 0.415% 64.489%) who achieved complete remission or 32 *de novo* AML (median 11.638%; range 0.021% 114.169%; *P*<0.0001). The expression levels of PARD-3 was higher in 6 refractory/relapsed B-cell ALL patients (median 110.315%; range 1.276% 499.772%) than that of newly diagnosed B-cell ALL, but no statistical significant difference was observed (*P*=0.72). Besides, no statistical significant difference was observed in 32 newly diagnosed AML (median 11.638%, range 0.021% 114.169%) and 20 healthy donors (*P*=0.72), but it was higher in BALL-1 and NALM6 cells from B cell ALL cell lines than in other cells from AML cell lines (Figure 1).
Summary/Conclusion: These observations suggest that PARD-3 is overexpressed in B-cell acute lymphoblastic leukemia and may play a role in the pathogenesis of B-cell acute lymphoblastic leukemia.

PB1616

NOTCH1 KNOCKDOWN PROMOTES THE GROWTH OF AND UPREGULATES NOTCH2 EXPRESSION IN JURKAT T-ALL CELLS

Y. Okuhashi[1,2,*], S. Tohda[2]

[1]Department of Medical technology, Tokyo University of Technology, [2]Department of Laboratory of Medicine, Tokyo Medical and Dental University, Tokyo, Japan

Background: NOTCH signaling is crucial for the growth of T-lymphoblastic leukemia (T-ALL) cells. We have previously reported that treatment with γ-secretase inhibitors (GSIs), which block NOTCH activation, suppresses the growth of several T-ALL cell lines. However, while GSI-XXI inhibited the proliferation of KOPT-K1 cellspromoted that of Jurkat cells, although both of these T-ALL cell lines harbor *NOTCH1*-activating mutations.
Aims: To elucidate the mechanism underlying the promotion of Jurkat cell growth by NOTCH inhibition.
Methods: KOPT-K1 and Jurkat cells were transfected with small interfering RNA (siRNA) targeting *NOTCH1* (siN1) or *NOTCH2* (siN2), or control siRNA (siCont) using the pipette tip chamber-based electroporation system. The effects of *NOTCH* knockdown on mRNA and protein expression were examined by quantitative RT-PCR and immunoblotting, respectively. Cell growth was assessed in three-day cultures by a colorimetric WST-8 assay, and the relative cell number was determined based on optical density measured using an ELISA plate reader and expressed as the percentage of the mean absorbance value normalized to that of siCont-transfected cells.
Results: Transfection with siN1 and siN2 selectively inhibited the expression of NOTCH1 and NOTCH2 (mRNA and protein), respectively, both in KOPT-K1 and Jurkat cells. *NOTCH1* and *NOTCH2* knockdown suppressed the growth of KOPT-K1 cells to 60% and 67%, respectively, but promoted that of Jurkat cells to 158% and 152%, respectively, of control. In Jurkat cells, *NOTCH1* knockdown abolished the expression of cleaved NOTCH1 fragment (active form of NOTCH1), downregulated HES1 and MYC, but slightly increased NOTCH2 levels, whereas *NOTCH2* knockdown slightly upregulated the levels of cleaved NOTCH1 fragment, HES1, MYC, and NOTCH1. In KOPT-K1 cells, *NOTCH1* knockdown decreased the expression of cleaved NOTCH1 fragment and HES1, while slightly increasing that of NOTCH2, whereas *NOTCH2* knockdown did not affect the expression of other proteins. The knockdown of *NOTCH1* and *2* did not affect the level of mTOR, Hedgehog, and Wnt signaling-related proteins in both cell lines.

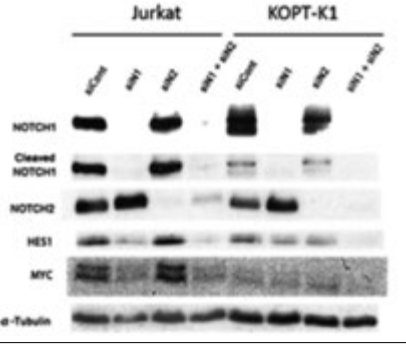

Figure 1.

Summary/Conclusion: In this study, we confirmed our previous findings that GSI-XXI treatment suppressed the growth of KOPT-K1 cells while promoting that of Jurkat cells using *NOTCH1*- and *NOTCH2*-specific siRNA. It is well known that NOTCH activation is oncogenic and promotes the growth of T-ALL cells. However, in Jurkat cells NOTCH obviously exerted the opposite effect. Interestingly, the knockdown of NOTCH2, similar to that of NOTCH1, affected the growth of the two cell lines, although they do not have *NOTCH2*-activating mutations. Furthermore, in Jurkat cells, we observed an interesting phenomenon that NOTCH1 knockdown upregulated NOTCH2 and vice versa, which might be related to the cell proliferation due to NOTCH suppression although this mechanism can not fully answer the question in the aim of this study. The underlying molecular mechanisms and the biological significance of the effect need to be further elucidated. Our results contribute to understanding of the role of NOTCH signaling in T-ALL cells and should promote the development of novel NOTCH-targeting therapies against T-ALL.

PB1617

C-MYC INHIBITION BY SMALL MOLECULE INHIBITOR 10058-F4 CAUSES APOPTOTIC CELL DEATH AND ENHANCESVINCRISTINE INDUCED CYTOTOXICITY IN PRE-B-ACUTE LYMPHOBLASTIC LEUKEMIA CELLS

N. Sheikh-Zeineddini*, N. Riyahi, M. Sayyadi, D. Bashash

Department of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic Of

Background: In spite of impressive advances in therapeutic approaches, some of the patients with acute lymphoblastic leukemia (ALL) experience a bone marrow relapse with the approximate morbidity and mortality rate of 60%. Since aberrant expression of c-Myc contributes to the unlimited proliferative potential of malignant lymphoblasts, and may confer resistance to chemotherapeutic drugs, inhibition of c-Myc proto-oncogene is proving to be a daunting task for clinicians.
Aims: To assess the anti-tumor effect of c-Myc inhibition in ALL, Nalm-6 cells were treated with the different concentrations of small molecule inhibitor of c-Myc, 10058-F4.
Methods: MTT, Trypan blue, annexin/PI staining, cell cycle analysis, caspase-3 activity, and BrdU cell proliferation assay coupled with analysis of gene expression by using quantitative real-time PCR were applied to examine the effects and molecular mechanisms of action of 10058-F4.
Results: Our results indicated that c-Myc inhibition using 10058-F4 resulted in a considerable growth suppressive effect in Nalm-6 cells mostly through p73-mediated G1 arrest. Moreover, we found that the cytotoxic effects induced in inhibitor-treated cells were likely due to the induction of a caspase 3-mediated apoptosis, as revealed by the increased percentage of Annexin-V and Annexin-V/PI double positive cells in the drug-treated group. Noteworthy, the resulting data showed that combination of 10058-F4 and vincristine produced synergistic anticancer effects and provided an enhanced therapeutic efficacy in pre-B ALL-derived Nalm-6 cells.
Summary/Conclusion: Our study not only indicated the potential application of single agent of 10058-F4 in Nalm-6 cells but also outlined the therapeutic efficacy of the inhibitor in combination with the common chemotherapeutic drugs in pre-B ALL.

PB1618

FOXP3 KNOCKDOWN INHIBITS THE PROLIFERATION AND REDUCES NOTCH1 EXPRESSION OF T- LYMPHOBLASTIC LEUKEMIA CELLS

S. Yonekura*, M. Itoh, E. Shiratori, M. Ohtaka, S. Tohda

Laboratory medicine, Tokyo Medical and Dental University, Tokyo, Japan

Background: Forkhead box P3 (FOXP3) is a master transcriptional factor of regulatory T-cells (Tregs). Recent studies have shown that FOXP3 expression is associated with growth inhibition of various solid tumor cells such as gastric cancer and ovarian cancer. However, the role of FOXP3 in T-cell acute lymphoblastic leukemia (T-ALL) cells is not known. It was also reported that NOTCH signaling promoted the expression of FOXP3 in Tregs. However, little is known about the effects of FOXP3 expression on NOTCH expression in T-ALL cells.
Aims: To elucidate the effect of *FOXP3* knockdown on the proliferation and NOTCH expression of T-ALL cells.
Methods: The two T-ALL cell lines Jurkat and KOPT-K1, harboring an activating *NOTCH1* mutation, were transfected with small interfering RNA (siRNA) against *FOXP3* or control siRNA using an electroporation system. Cell growth was assessed in five-day cultures with a colorimetric assay. The relative cell number was determined based on optical density measured using an ELISA plate reader and expressed as the percentage of the mean absorbance value normalized to that of control cells. Cell morphology was examined in cytospin preparations from the cells cultured for 6 hours. They were stained with Wright's stain and observed under a microscope. The effects of *FOXP3* knockdown on mRNA expression were examined by quantitative RT-PCR.

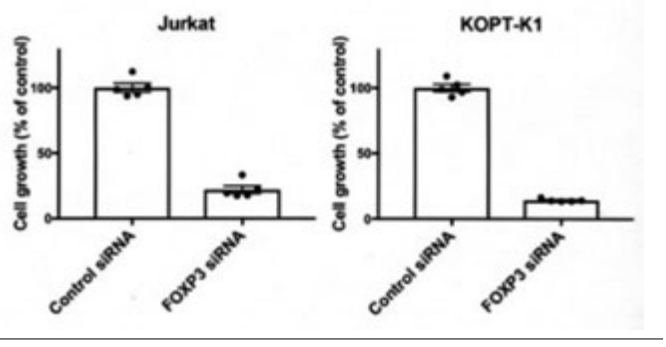

Figure 1.

Results: Transfection with *FOXP3* siRNA significantly reduced the *FOXP3* expression to 29% and 45% in Jurkat and KOPT-K1 cells, respectively. By *FOXP3* knockdown, cell growth was significantly suppressed to 22% and 14% in Jurkat and KOPT-K1 cells, respectively. Observation of cytospin preparations indicated that apoptotic cells with nuclear condensation and apoptotic bodies appeared in both cells. The expression of *NOTCH1* was significantly reduced to 56% and 60% compared with that of control in Jurkat and KOPT-K1 cells, respectively. *HES1* expression was significantly reduced to 52% and 34% in Jurkat and KOPT-K1 cells, respectively.
Summary/Conclusion: *FOXP3* knockdown suppressed the growth and induced apoptosis of the two T-ALL cell lines. This suggests that FOXP3 supports the growth of T-ALL cells although this can not be generalized in T-ALL because we just examined only two cell lines. We think that the role of FOXP3 on cell growth can diverge in different types of cancer. *FOXP3* knockdown downregulated *NOTCH1* expression in T-ALL cells. The observed growth suppression can be partly related to the downregulation of NOTCH1 signaling because NOTCH1 plays a role in the growth of some T-ALL cells. The underlying molecular mechanisms and the biological significance of the effect need to be further elucidated. Then, FOXP3 may be a potential therapeutic target in T-ALL.

PB1619

CENTAUREA ALBONITENS EXTRACT ENHANCES THE THERAPEUTIC EFFECTS OF VINCRISTINE IN LEUKEMIA CELLS BY INDUCING APOPTOSIS

F. Bahmani[1,*], A. Gharehbaghian[2], S. Esmaeili[3], D. Bashash[2,] N. Dehghan-Nayeri[2]

[1]Hematology and blood banking, Abadan school of medical science, Abadan, [2]hematology and blood banking, [3]Traditional Medicine and Materia Medica Research Center, shahid beheshti university of medical science, Tehran, Iran, Islamic Republic Of

Background: Drug-induced toxicities and dose-related side effects are the major challenges in the conventional cancer therapy by the chemo drugs. On the other hand, herbal derivatives have obtained a great research interest in the field of therapeutic applications because of their more favorable specifications including less toxicity, cost-effective and more physiologically compatible than the chemical drugs. *Centaurea* genus is one of the current medicinal plants, which has used in traditional medicine, However, there are rare studies to examine its anticancer properties against hematologic malignant cells.
Aims: For this purpose, we evaluated methanolic extract prepared from *Centaurea albonitens* Turrill extract (CAE) alone and in combination with Vincristine (VCR) for its potential cytotoxic effects in NALM-6, REH, NB4 and KMM-1 cell lines using the various approaches.
Methods: In this experimental study, to explore the effects of CAE and CAE/VCR on hematologic malignant cell lines, the cells were treated with increasing concentrations of extract and drug. Their cytotoxic and anti-proliferative effects were evaluated using trypan blue, MTT assay and DAPI staining. moreover, we performed annexin V/PI staining, cell cycle analysis, Caspase-3 activity assay and real-time RT-PCR to examine the mRNA expression level of BAX, BCL-2, p21 and c-MYC to further investigate how CAE and CAE/VCR exert their cytotoxic properties.
Results: In this study, we demonstrated *Centaurea albonitens* extract (CAE) induces cytotoxicity through G0/G1 phase arrest followed by apoptosis in a dose- and time- dependent manner, although with varying efficiency. Interestingly, MDBK normal cells didn't exhibit significant cytotoxicity after CAE treatment. Moreover, we found that low dose of CAE enhances anticancer effects of VCR in pre-B ALL cell lines (NALM-6 and REH). Further investigations validated synergistic anticancer activities of VCR and CAE through inducing apoptosis without significant cell cycle arrest.
Summary/Conclusion: Taken together, our results demonstrated for the first time that the methanolic extract of *Centaurea albonitens* can be considered as a potential anticancer agent and/or an enhancer of chemotherapeutic sensitivity of VCR.

PB1620

MINIMAL RESIDUAL DISEASE ANALYSIS BY FLOW CYTOMETRY IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

I. Frikha[1,*], Y. Fakhfakh[1], N. Louati[2], S. Hdiji[1], H. Mnif[2], M. Medhaffer[1], M. Elloumi[1]

[1]Hematology, Hedi Chaker Hospital, Sfax, Tunisia, [2]Regional transfusion center, Habib Bourguiba Hospital, Sfax, Tunisia, Sfax, Tunisia

Background: The prognosis of acute lymphoblastic leukemia (ALL) in children

is greatly improved by identification of prognostic factors and better stratification in risk group. Through this study we specify the interest of the minimal residual disease (MRD) by flow cytometry in patients treated for ALL.
Aims: In our study, we evaluated the response of treatment in acute lymphoblastic leukemia of child by analyzed of minimal residual disease with flow cytometry.
Methods: In our study we are interested at the results of MRD performed by flow cytometry (CMF) on two points: Day 36 (TP1) and Day 63 (TP2), in children treated for ALL according to the protocol EORTC 58951 from January 2015 to June 2017. MRD is considered positive when TP1>10^{-2} and TP2>10^{-3}. Then we observed the impact of the MRD on relapse.
Results: We collected 45 cases of ALL who received an MDR response assessment. These are 33 boys and 12 girls (sex ratio=2.75). The median age at diagnosis was 8 years (2- 29 years). There are 32 cases of B phenotype and 13 cases of T phenotype. The median rate of WBC was 11G / L. Two patients are classified LR, 18 AR1, 13 AR2 and 12 HR. 44 patients (98%) achieved a complete remission after induction and one patient was in therapeutic failure. TP1 was done for 40 patients (89%), TP2 achievement rate was 78% and 67% of patients have the 2 points (TP1 and TP2). For the patients who had TP1: 2 were positive and relapsed, 27 patients were negative of which 9 (24%) relapsed (p=0.061). Of the 35 patients who had TP2, 9 were positive, of which 3 (33%) relapsed and 26 had TP2 negative, 7 of them (27%) relapsed (p=0.033). Of the 38 TP1 negative patients, 28 had a second point among them 6 patients had TP2 positive, of which 4 patients (67%) relapsed.
Summary/Conclusion: The MRD study is currently an element determinant in the prognosis of ALL. In our series, this study was conducted by CMF for lack of molecular biology. It shows an interest in the occurrence of relapse as well as in the literature. We plan to expand this study and make it protocol for all our patients and especially the involved in the therapeutic attitude.

PB1621

ADDITIONAL CYTOGENETIC ABNORMALITIES IN ADULT PATIENTS WITH PHILADELPHIA CHROMOSOME POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA

O. Zotova[1,*], A. Lukianova[1], M. Valchuk[1], I. Vanko[1], Z. Maslyak[1], Y. Karol[2], Z. Misharina[3], V. Loginsky[1]

[1]SI «Institute of Blood Pathology and Transfusion Medicine, NAMS of Ukraine», [2]5th Clinical City Hospital, Lviv, [3]SI «National Centre for Radiation Medicine, NAMS of Ukraine», Kyiv, Ukraine

Background: Philadelphia chromosome (Ph), the result of a reciprocal translocation involving chromosomes 9 and 22: t(9;22)(q34;q11), is the most frequent genetic aberration in adult acute lymphoblastic leukemia (ALL) and is found in 15-30% patients. Additional chromosomal abnormalities are often found (~50%) in association with t(9;22)(q34;q11). The most common are: additional copy of Ph-chromosome (extra Ph), deletions del(22q) and del(7q), monosomy 7, trisomy 8 and others. Conventional chemotherapy programs that have been effective in other ALL cases have been unable to induce response in adults with Ph-positive ALL. Use of imatinib mesylate and other tyrosine kinase inhibitors as part of treatment have greatly improved the rates of complete hematologic and molecular remission in these patients. Imatinib interim therapy might improve the curative potential of allogeneic stem cell transplantation.
Aims: Aim of this study was to detect additional chromosomal abnormalities in adult patients with Philadelphia chromosome positive ALL and determine frequency of them.
Methods: Cytogenetic investigations of bone marrow and/or peripheral blood cells from 50 newly diagnosed adult patients with ALL were performed. The methods of conventional cytogenetics (GTG) and fluorescence *in situ* hybridization (FISH) were used.
Results: Cytogenetic investigations were performed in 50 newly diagnosed patients with ALL. Chromosomal aberrations of various kinds were found in 32 (64%) cases. According to the karyotype analysis patients were classified by risk groups: the group of patients with unfavorable cytogenetic markers (hypodiploidy, t(4;11)(q21;q23), t(9;22)(q34;q11), multiple changes (≥3)), the intermediate risk group without significant prognostic markers and the group with favorable prognostic factors (t(12;21)(p13;q22), high hyperdiploidy). Ph-chromosome was found in 8 (16%) cases. Five patients (62,5%), except for abnormal ones, had one or more normal metaphases in their karyotype. Of 8 patients with Ph-positive ALL, 3 (37,5%) had only Ph-chromosome, whereas other 5 (62,5%)-had additional cytogenetic abnormalities. Spectrum of additional chromosomal aberrations was as follows: extra Ph-chromosome and trisomy 8 (2 cases), extra Ph-chromosome and del(7)(q31) (1 case), trisomy 21 (1 case), high hyperdiploidy (57 chromosomes, extra Ph, trisomies X, 2, 4, 6, 8, 11, 14, 17, tetrasomy 21) (1 case). Cytogenetic investigations at relapse were performed in one case. Secondary chromosomal abnormalities in association with t(9;22)(q34;q11), both structural and numerical, were detected in this case, namely 1-2 additional copies of Ph, deletion del(1)(q24), trisomies 3, 6, 8 and marker chromosome. Presence of the additional/secondary chromosomal aberrations is the sign of clonal evolution and progression of disease and appears to have a significant deleterious effect on outcomes post allogeneic stem cell transplantation.
Summary/Conclusion: In our study frequency of Philadelphia chromosome in patients with ALL was 16%, which is comparable to the data reported in the literature. The most common additional chromosomal abnormalities in association with t(9;22)(q34;q11) were: extra Ph (80%) and trisomy 8 (60%). Presence of the additional/secondary chromosomal aberrations in Ph-positive cells is the sign of clonal evolution and disease progression.

PB1622

METHANOLIC EXTRACT FROM AERIAL PARTS OF ARTEMISIA ANNUA L. INDUCES CYTOTOXICITY AND ENHANCESVINCRISTINE INDUCED ANTICANCER EFFECT IN PRE-B ACUTE LYMPHOBLASTIC LEUKEMIA CELLS

P. Mashati*, S. Esmaeili, N. Dehghan-Nayeri, D. Bashash, M. Darvishi, A. Gharehbaghian

Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic Of

Background: Since the time immemorial, medicinal herbs have been broadly used as primary source for treatment of cancer, either applied individually or as adjuvant with conventional treatments. Nowadays, a remarkable attention has been drawn towards the effective therapeutic characteristic of natural products targeting cancerous cells.
Aims: This study aimed to investigate the anti-cancer effect of Artemisia annua extract (AAE), a medicinal Chinese herb alone and in combination with a microtubule binding agent used in ALL treatment, vincristine (VCR), in B-Acute lymphoblastic leukemia (ALL) Nalm-6 and Reh cells.
Methods: Cytotoxic activity of AAE and VCR was determined using MTT assay in Nalm-6 and Reh cell lines and synergism was evaluated using the CompuSyn software. Caspase 3 activity and Annexin/PI staining was performed for apoptosis assessment. The expression level of apoptosis-related genes, caspase 3, Bax and Bcl-2 were determined using real time-PCR. One-way ANOVA and post hoc Tukey multiple comparisons were used for statistical analysis.
Results: We found that treatment of the leukemic cells with increasing concentrations of this extract reduced (30 and 40 µg/ml) the viability and growth of the cells through caspase 3-dependent apoptosis after 48 h. Interestingly, the growth inhibitory activity of the extract after 48 h was more potentiated when combined with 0.1 and 1 nM (VCR), compared with either agent alone. Moreover, real-time PCR analysis showed that VCR-induced apoptosis was augmented by AAE through alteration of Bax and Bcl-2 mRNA expression, as the most substantial indicators of apoptosis.
Summary/Conclusion: Overall, owing to nontoxic nature of AAE and its explicit role in enhancing VCR effectiveness, our study provides a new insight into the development of novel combinatorial approach in ALL using natural herbs; however, more investigation, including clinical trial, is warranted to indicate the effectiveness of this extract in the treatment of acute lymphoblastic leukemia.

PB1623

THE ROLE OF PTEN AND ITS PSEUDOGENE PTENP1 IN THE PROLIFERATION OF PTEN-NULL JURKAT T-ALL CELLS

Y. Okuhashi[1,2,*], S. Tohda[2]

[1]Department of Medical technology, Tokyo University of Technology, [2]Department of Laboratory Medicine, Tokyo Medical and Dental University, Tokyo, Japan

Background: NOTCH/HES and AKT/mTOR pathways play important roles in the growth of T-lymphoblastic leukemia (T-ALL) cells. We have previously reported that treatment with γ-secretase inhibitor (GSI)-XXI, which prevents NOTCH activation, suppressed the growth of some T-ALL cell lines such as KOPT-K1, but promoted that of Jurkat cells, although both cell lines harbor activating *NOTCH1* mutations. Given that Jurkat cells are *PTEN*-null, whereas KOPT-K1 cells are *PTEN*-wild type and that a NOTCH target HES1 downregulates PTEN expression, we hypothesized that the interplay among PTEN/AKT, NOTCH and its related pathways may regulate the growth of Jurkat cells.

Aims: To elucidate the mechanism underlying the induction of Jurkat cell growth by GSI, we examined the role of *PTEN* and its pseudogene *PTENP1* reported to act as a tumor suppressor in some cancers.
Methods: Cells were transfected with small interfering RNAs targeting *PTEN* (siPTEN) and *PTENP1* (siPTENP1), or control siRNA (siCont) by electroporation and analyzed for mRNA and protein expression by quantitative RT-PCR and immunoblotting, respectively. Cell growth was examined by a colorimetric WST-8 assay in three-day cultures; optical density (OD) was measured using an ELISA plate reader and cell growth was calculated as the percentage of the OD value normalized to that of siCont-transfected cells. To inhibit NOTCH activation, cells were treated with GSI-XXI.
Results: PTEN mRNA and protein were detected in KOPT-K1 cells but not in Jurkat cells. In KOPT-K1 cells, transfection with siPTEN inhibited *PTEN* mRNA and protein expression but did not affect cell growth; however, the proliferation of siPTEN-treated cells was slightly promoted by GSI treatment. In Jurkat cells, both siPTEN and siPTENP1 suppressed cell growth to 74% and 69% of control, respectively. Furthermore, transfection with siPTEN increased the expression of NOTCH, mTOR, WNT, and HEDGEHOG signaling proteins such as NOTCH1, cleaved NOTCH1, NOTCH2, MYC, AKT, p-AKT, p-mTOR, S6K, p-S6K, CTNNB1, p-CTNNB1, and GLI1 in Jurkat cells, but not in KOPT-K1 cells as shown in Figure 1.

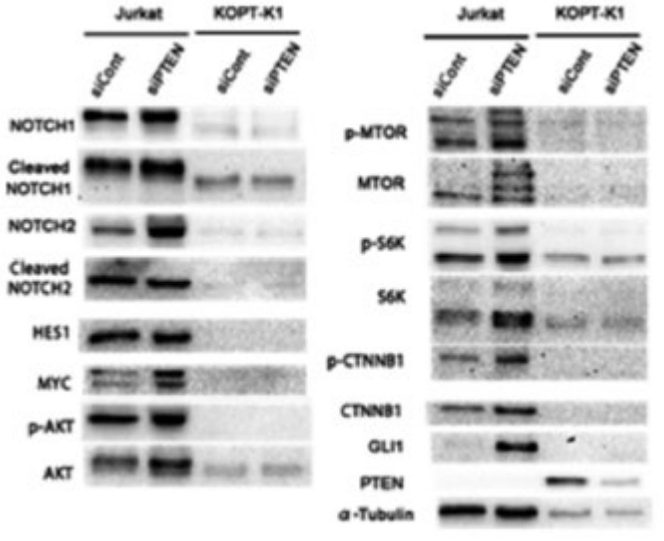

Figure 1.

Summary/Conclusion: Our study resulted in three interesting findings. First, siPTEN transfection suppressed the growth of PTEN-null Jurkat cells. One possible explanation is that siPTEN might bind to *PTENP1* mRNA which has high sequence homology to *PTEN* mRNA; this notion is supported by the fact that transfection with siPTENP1 also suppressed Jurkat cell proliferation. Second, the inhibition of Jurkat cell growth by siPTEN was accompanied by the upregulation of cell proliferation-inducing proteins in NOTCH, mTOR, WNT, and HEDGEHOG pathways. This seeming discrepancy may be related to our previous finding that GSI treatment promoted the growth of Jurkat cells, *i.e.*, that NOTCH activation apparently has suppressive effects on Jurkat cell proliferation. It is reported that *PTEN* expression is modulated by binding of some microRNAs and that *PTENP1* transcript acts as a decoy for microRNA binding; therefore, siPTEN might downregulate *PTENP1*, which might affect the level of these growth-related microRNAs and cell growth. Third, GSI treatment promoted the proliferation of *PTEN*-knockdown KOPT-K1 cells similar to that of *PTEN*-null Jurkat cells, suggesting that cell growth stimulation by GSI may be associated with PTEN deficiency. Although our findings are not consistent with well-known mechanisms of NOTCH signaling, elucidation of these phenomena should further development of effective NOTCH-targeting therapies against T-ALL.

PB1624

THE DETECTION OF P2RY8-CRLF2 FUSION IN ACUTE LYMPHOBLASTIC LEUKEMIA

D. Sae-Chua*, T. Siriboonpiputtana, N. Limsuwanachot, B. Rerkamnuaychoke, S. Pakakasama

Pathology, Ramathibodi Hospital, Bangkok, Thailand

Background: Acute lymphoblastic leukemia (ALL) is recognised as the most common cancers in childhood and a major cause of sickness and death in adults. Recently, *CRLF2* rearrangements were frequently identified in Ph-like acute lymphoblastic leukemia with the aggressive disease phenotype.
Aims: In this report, we performed a conventional RT-PCR method to detect *P2RY8-CRLF2* fusion which known as the most common *CRLF2* rearrangements in Ph-like ALL in childhood.
Methods: Conventional RT-PCR was performed to analyse *P2RY8-CRLF2* fusion in RNA isolated from bone marrow samples of 87 newly diagnosed childhood ALL patients. The *P2RY8-CRLF2* fusion sequence was subsequently confirmed by using Sanger sequencing technique.
Results: We found that 2 out of 87 patients (2.3%) are positive for *P2RY8-CRLF2* fusion. Additionally, one patient with *P2RY8-CRLF2* fusion was affected with Down syndrome (trisomy 21). Sequencing analysis on *P2RY8-CRLF2* fusion sequences in both two positive samples revealed that the fusion breakpoint is located at 111 nucleotides upper the start codon of *P2RY8* sequence (XM_011545631.2) and 2 nucleotides before the start codon of *CRLF2* sequence (NM_022148.3).
Summary/Conclusion: We could detect a *P2RY8-CRLF2* fusion by using a simple RT-PCR technique. Furthermore, *P2RY8-CRLF2* fusion sequence was conserved in our tested samples. In recent future, we are going to deeply investigate the function of *P2RY8-CRLF2* fusion in the initiation of Ph-like ALL in both *in vitro* and in mouse model.

PB1625

NOTCH PATHWAY AS A THERAPEUTIC TARGET IN ACUTE LYMPHOBLASTIC LEUKEMIA

J. Jorge[1,2], A. Pires[2], R.S. Alves[1,2], A.C. Gonçalves[1,2], A.B. Sarmento-Ribeiro[1,2,3,4,*]

[1]Coimbra Institute for Clinical and Biomedical Research (iCBR)-Group of Environment Genetics and Oncobiology (CIMAGO), [2]Laboratory of Oncobiology and Hematology, Faculty of Medicine, University of Coimbra (FMUC), [3]Center for Neuroscience and Cell Biology (CNC), University of Coimbra, [4]Clinical Hematology Department, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal

Background: Acute Lymphoblastic Leukaemia (ALL) is a malignant disease of hematopoietic system in which early lymphoid precursors proliferate and replace the normal hematopoietic cells, affecting both linages, B and T cells (B-ALL and T-ALL). NOTCH pathway is an evolutionary conserved signalling pathway that plays a significant role in cell fate decision during development, stem cell self-renewal and differentiation in haematopoiesis. Deregulation of NOTCH signalling was already reported in haematological disorders and various solid tumours, namely in T-ALL, were activating *NOTCH1* mutations are present in more than half patients, playing a significant role in its pathogenesis. Therefore, modulation of NOTCH signalling pathway, for example with gamma-secretase inhibitors, might provide a potential novel therapeutic approach in ALL.
Aims: In this context, the aim of this study was to evaluate the NOTCH pathway as a therapeutic target in ALL, using a -secretase inhibitor in two *in vitro* models of ALL.
Methods: For this purpose, we used two different ALL cell lines, CEM a T-ALL and KOPN-8 a B-ALL cell line. These cells were incubated in the absence and presence of increasing concentrations of the -secretase inhibitor, GSI-XXI (20μM-50μM). Cell viability and proliferation were assessed by trypan blue exclusion assay. Cell death was evaluated by optical microscopy and flow cytometry (FC) using annexin V/propidium iodide double staining and JC-1 probe to assess mitochondrial membrane potential. Apoptotic protein levels (BAX and BCL-2) and cell cycle distribution were also evaluated by FC. The expression levels of *CCND1*, *CCNB1*, *CCNE1* and *NF- B* genes were determined by RT-PCR. Results were considered statistically significant when $p<0,05$.
Results: Our results suggest that GSI-XXI reduced cell proliferation and viability in a dose- and cell type dependent manner with an IC_{50} at 24h of approximately 40μM for CEM and 30μM for KOPN-8 cells. This compound induced cell death mainly by apoptosis in both cell lines confirmed by morphological analysis, mediated by an increase in BAX/BCL-2 ratio and a decrease in mitochondrial membrane potential. The analysis of cell cycle also revealed a significant arrest in G0/G1 phase in CEM cells. This analysis also showed a sub-G1 peak in KOPN-8 treated cells, which correspond to DNA fragmentation a typical feature of apoptosis. Finally, GSI-XXI did not induce significant changes in the expression levels of *CCND1*, *CCNB1*, *CCNE1* and *NF- B* genes.
Summary/Conclusion: In conclusion, if these results can be translated to clinical practice, they suggest that NOTCH pathway and -secretase inhibitors, like GSI-XXI, might be a good therapeutic approach in acute lymphoblastic leukaemia patients.

Acute lymphoblastic leukemia – Clinical

PB1626

EXPERIENCE OF TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN OF WESTERN UKRAINIAN SPECIALIZED CHILDREN'S MEDICAL CENTRE, LVIV

O. Dorosh[1,*], I. Tsymbalyuk-Voloshyn[1], R. Polishchuk[1], O. Troyanovska[2], O. Vorobel[1], O. Kozlova[1], A. Stepanyuk[1], L. Skoropad[1], L. Dubey[3], K. Bodak[1]

[1]Department of Hematology and Intensive Chemotherapy, Lviv Regional Council Public Institution "Western Ukrainian Specialized Children's Medical Centre", [2]Assistant, Department of Pediatrics, Danylo Halytskyy Lviv National Medical University, [3]Professor, Department of Pediatrics and Neonatology, Faculty of Postgraduate Education, Danylo Halytskyy Lviv National Medical University, Lviv, Ukraine

Background: Acute lymphoblastic leukemia is a frequent disease in childhood. An analysis of the efficacy of standard programs chemotherapy for ALL treatment is an important part of introducing new methods for treatment in the future.
Aims: Analysis of the results of 25 years treatment period of acute lymphoblastic leukemia (ALL) in children.
Methods: The results of the treatment from February 1993 to January 2018 according to the standard chemotherapy programs ALL-BFM'90/95, ALL IC-BFM2002, ALL IC-BFM2009, INTERFANT'99/06 were analyzed in 370 patients with ALL at the age from 0 to 18 years. The program "Statistica for Windows 8.0" (Statsoft, USA) was used for the above mentioned analysis. The function event-free survival (EFS) and overall survival (OS) from the first diagnosis to death for any reason is calculated by the Kaplan-Mayer method. Comparison of survival between groups – by using the Cox's-F-test.

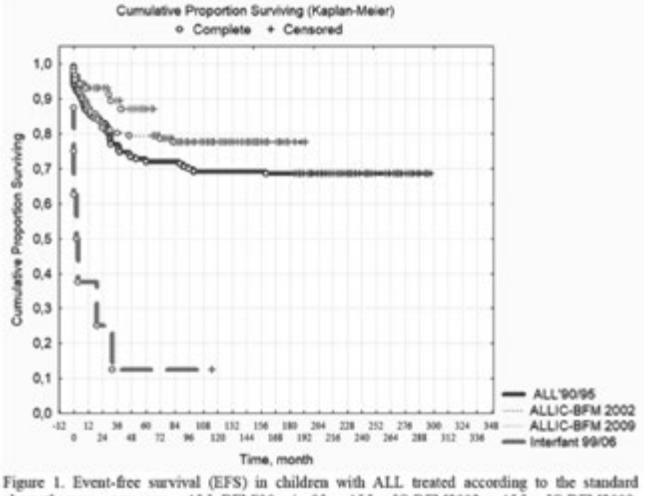

Figure 1. Event-free survival (EFS) in children with ALL treated according to the standard chemotherapy programs ALL-BFM'90 / 95, ALL IC-BFM2002, ALL IC-BFM2009, INTERFANT'99/06

Figure 1.

Results: From February 1993 to October 2002 143 (38,65%) patients received treatment based on programs ALL-BFM'90/95 (group 1); from November 2002 to June 2012-131 (35,41%) patients-ALLIC-BFM2002 (group 2); from June 2012-88 (23.78%) patients – ALLIC-BFM2009 (group 3) correspondingly. Children under the age of 1 year (8 persons) from August 2008 were treated according to INTERFANT'99/06 programs (group 4). EFS in group 1 is 68,0% with a median observation time (MO) for 219 months, in group 2-77,6% with MO 110 months, in group 3-85,2% with MO 38 months, in group 4-12,5% of MO 12,5 months (Figure 1). The sstatistically significant improvement of EFS indexes in group 3, the worst results in children under 1 y.o. ($p<0,05$) was found. The EFS for middle-risk group (MR) patients in the 1st group was 71,0%, in the 2nd group-83,7%, and in the 3rd group-90,5%. The EFS for the high-risk group (HR) patients in the 1st group was 50,0%, in the 2nd –55,6% and in the 3rd group-73,9%. The OS in the entire sample is 78,2%. The relapse of ALL was diagnosed in 53 (14,32%) persons. 34 (9,19%) children died, of which 11 patients (32,35%)-from septic complications before achieving remission of ALL, 19 children (55,88%)-from septic complications in the 1st remission during intensive chemotherapy, 1 HR-group patient-from post-transplant complications, 1 patient in long-lasting remission (160 months)-from the fulminant course of viral hepatitis B, two patients (5,88%) of secondary tumors. Secondary malignancy has been reported in 5 (1,35%) children. Astrocytoma, meningioma, secondary acute myeloid leukemia (AML) have been successfully treated, oligodendroglioma and secondary myelodysplastic syndrome with transformation in to AML was a reason of lethal consequence. 8 persons from the HR-group in the 1st remission had allo- (abroad), including 2 patients-for whom allo-BMT was a second therapeutic line for treatment of secondary AML.
Summary/Conclusion: The BFM international group's treatment programs of ALL show constantly increasing high effectiveness. Results of treatment in children of the first year of life are unsatisfactory and require improvement of therapeutic approaches. A rare, remote consequence of the treatment of ALL in children is secondary neoplasms, which should be kapt in mind by first-contact doctors and specialists.

PB1627

VENOUS THROMBOEMBOLISM IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) TREATED WITH ERYASPASE (L-ASPARAGINASE ENCAPSULATED IN RED BLOOD CELLS)

Y. Bertrand[1,*], E. Radu[2], I. El-Hariry[2], R. Larson[3], D. Douer[4]

[1]CHU, [2]Erytech Pharma, Lyon, France, [3]University of Chicago, Chicago, [4]University of Southern California, Los Angeles, United States

Background: L-asparaginase (ASNase) is a key drug in the treatment of ALL. Its toxicity includes venous thromboembolism (VTE) which can be serious and potentially fatal. The reported prevalence of VTE among children and adults with ALL ranges between 1% and 37%. The rate is dependent on cofounding factors such as higher age, presence of indwelling venous catheters, the use of prophylactic antithrombin replacement or anticoagulation, inclusion of asymptomatic cases, and other risk factors unrelated to the use of ASNase. ASNase leads to serum asparagine depletion, inhibiting the synthesis of anti-coagulation factors such antithrombin III (AT), protein C, and protein S, which leads to increase in thrombin generation and risk of TE. ASNase is derived from *E. coli* (native or the long acting pegylated forms) or *Erwinia chyrsanthemi* bacteria. Eryaspase is an investigational product under development that uses a novel system to deliver native *E. coli* ASNase encapsulated within donor-derived matched red blood cells (RBCs) by a proprietary osmotic-based method. The aim is to improve the tolerance and prolong the activity of this enzyme. After eryaspase infusion, plasma asparagine is actively transported into RBCs, where it is hydrolyzed. As a result, asparagine in the plasma is depleted. ASNase activity of eryaspase is longer that the native *E. coli*. RBC encapsulation is done at GMP facilities in a process that typically takes 24 hours from obtaining donor-matched RBC to shipment of the product to the investigator's center. Eryaspase is delivered directly to the Investigator for immediate transfusion over 1 hour.
Aims: We are reporting on the occurrence of VTE after eryaspase treatment in patients with ALL, given the use of RBC and its distinct manufacturing process.
Methods: Integrated safety data from 5 studies of eryaspase in ALL were retrospectively analyzed using MedDRA coding. Treatment with eryaspase was in combination with chemotherapy during induction and consolidation phases. The cut-off date for analysis was 28 Feb 2017.
Results: A total of 125 patients were included in this series, with median age of 20 years (range, 2-77). Treatment was generally well tolerated. 8 (6.4%) patients (7 adults; one child) had VTE; 6 (4.8%) were serious events. VTE was considered related to eryaspase in 6 (4.8%) patients. The most common event was reported as venous thrombosis (n=6 (4.8%)). None of the events had a fatal outcome. Decreased AT was found in 35 (28%) of the patients. In a subset of patients randomized to eryaspase (n=26) or to native ASNase (n=28), laboratory coagulation abnormalities were observed in 11.5% and 71.4% during induction, respectively.
Summary/Conclusion: The risk of VTE with eryaspase treatment in our ALL patients was low and seen mostly in adults. The low incidence of these events may be related to decreased incidence of impaired coagulation parameters. We conclude that eryaspase is not associated with an increased rate or severity of VTE toxicity.

PB1628

TIROSINE KINASE REARRANGEMENTS IN PH-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA-A FISH APPROACH

T. Garrido*, M.J. Soares, A. Barbosa, M.L. Amorim, P. Gomes, F. Trigo, J.E. Guimaraes

Hospital Sao Joao, Porto, Portugal

Background: "Ph-like" acute lymphoblastic leukemia (ALL) is a subtype of B-cell precursor ALL characterized by a poor outcome and various kinase-activating alterations. These include rearrangements of *CRLF2*, *JAK2* or *EPOR*, fusions involving *ABL*-class genes, mutations activating *JAK-STAT* or *Ras* signaling pathways, and other less common fusions. There are reports that suggest that the poor outcome of these patients (pts) may be improved with Tirosine Kinase (TK) inhibitors.
Aims: In this study we aimed to identify genomic rearrangements of some of the most frequent key tyrosine kinase genes in Philadelphia chromosome-negative (Ph-) and BCR-ABL1–negative (-) ALL adult patients.
Methods: We selected 39 consecutive B-ALL adult patients out of 93 diagnosed in our centre from 2009 to 2017, all of which were Ph(-) and BCR-ABL1(-), without translocations involving 11q23 (*MLL* gene) detectable by conventional cytogenetics. Fluorescent *in situ* hybridization (FISH) analysis was performed using break apart probes for *CRLF2*, *ABL1*, *ABL2*, *JAK2* and *CSF1R* genes on the diagnostic samples. Retrospective analysis of patients' records was performed.
Results: Twenty-two (56.4%) pts were female, with median age at diagnosis of 41 years old [17; 71]; 19 (48.7%) were young adults (<40 years old). Ten (25.6%) pts had hyperleukocytosis (>30x10^9/L) and three (7.7%) had central nervous system involvement. Conventional cytogenetic analysis was successful in 31 patients: 22 (56%) had normal karyotype, 3 (9.7%) complex karyotype, 2 (6.5%) hyperdiploidy and 2 (6.5%) hypodiploidy. TK rearrangements were observed in 7 (18%) pts: 4 (10%) *CRLF2*, 2 (5.1%) *ABL1* and 1 (2.6%) *CSF1R*. No patient had *ABL2* or *JAK2* rearrangements. Of the 39 cases, 10 (27%) had copy number gains of one or more genes, including ABL2 and JAK2. None of the patients with TK rearrangements had complex or hypo/hyperdiploid karyotype. All patients were eligible for intensive chemotherapy protocols (31 HOVON100; 4 HyperCVAD; 4 CLGC). Thirty-three (84.6%) obtained complete remission (CR) after induction, 4 (10.3%) were refractory, and 1 patient died during induction. Sixteen (41%) pts proceeded to allogeneic stem cell transplantation, 14 in first CR. Ten (25.6%) pts relapsed and 15 (38.5%) died (5 in CR). Those patients with TK rearrangements (7) showed inferior median time-to-relapse (9 *vs* 34 months; p=0.003), 5-year disease-free survival (0% *vs* 65.1%; p=0.016) and 5-year overall survival (0% *vs* 66.5%; p=0.012).
Summary/Conclusion: Our study demonstrated that, among the Ph(-) ALL patients, those with TK rearrangements had inferior outcome. In a real life perspective, and in the absence of gene-expression profiling or next-generation sequencing, FISH analysis seems like a reliable and cost-effective screening method to identify known rearrangements probably responsible for unfavorable "Ph-like" behavior and potentially targetable with TK inhibitors.

PB1629

MANAGEMENT OF ADULT ACUTE LYMPHOBLASTIC LEUKEMIA WITH A PEDIATRIC-BASED REGIMEN: A SINGLE CENTER EXPERIENCE

P. Kaloyannidis*, N. Al Mokhtar, A. Sayyed, E. Shaibani, K. Bakhit, S. Al Harbi, R. Alomeri, O. Abduljalil, M. Abdulbaqi, S. Kanfar, M. Kawari, A. Suhebeh, J. Apostolidis, H. Al Hashmi, K. Al Anezi

Adult Hematology and Stem Cell Translantation Department, King Fahad Specialist Hospital, Dammam, Saudi Arabia

Background: Adult patients (pts) diagnosed with acute lymphoblastic leukemia (ALL), are considered to have dismal outcome compare to pediatric/adolescent pts. The last two decades the incorporation of pediatric therapeutic protocols in adults–ALL treatment, resulted in promising response and survival rates, however their use still remains an experimental approach, not representing the standard of care for adult-ALL pts.
Aims: In the present study we retrospectively evaluated the outcome in terms of toxicity, complete remission (CR) achievement and overall (OS) and progression free survival (PFS), in 52 adult-ALL pts adult pts who treated with a pediatric-ALL protocol.
Methods: From January 2008 to December 2017, the Children Cancer Group-1961(CCG-1961) protocol which includes Doxorubicin, Vincristin, Asparaginase, Methotrexate and Cytarabine in induction remission (InRe) and consolidation phases, was applied in 34 males and 18 females, with a median age of 21 (16-54) years. Patients with concurrent malignancies or severe co-morbitities were excluded. In 42 the malignant cells were B- and in 10 T-origin. Two pts had CNS involvement, 8 had >50000/mm^3 WBCs in the peripheral blood, while 11 found to have poor risk cytogenetic or molecular abnormalities. As per protocol instructions, candidates for allogeneic stem cell transplantation (alloSCT) considered only pts with either minimal residual disease (MRD) post InRe phase or relapsed disease. All patients received antibacterial, antiviral, anti-PCP and antifungal prophylaxis Disease response was assessed at day +28 after treatment initiation. The Kaplan-Meir and log-rank tests were used for the statistical analysis.
Results: Currently, 52 pts completed the InRe and consolidation arm and 11 are in ongoing treatment. Two pts discontinued early the treatment (during the consolidation phase) because of either severe liver toxicity or intolerable mucositis (grade4). The grade 3 observed toxicities, which did not compromise the treatment plan, were febrile neutropenia in 40 (75%), liver dysfunction (elevation of liver enzymes by 3-fold) in 32 (60%), peripheral neuropathy in 4 (7%), mucositis in 7 (14%), thrombosis in 6 (11%) which was mostly venous catheter related, cardiac toxicity in 3 (6%) while 7(14%) required admission in intensive care unit. No pt experienced mortality related to the treatment protocol. In a total 46 pts were evaluated for disease response while in 6 the assessment was not avaiable; 43 (85%) were estimated to be in CR. Three (6%) had refractory disease while 11(25%) relapsed during or after protocol completion; 5/14 succumbed early due to disease refractoriness and 9 were finally able to undergo alloSCT and currently 4/9 (45%) are alive and disease free. The 2 pts who experienced severe toxicity and intolerability during consolidation treatment, underwent early alloSCT and currently are alive and well. The 11 pts with unfavorable cytogenetic/molecular abnormalities, based on protocol instructions, were not allografted in CR1 and continued with the as per protocol scheduled treatment. Five (45%) are currently alive without disease evidence. The 10-years OS and PFS (including the alloSCT treatment) are 60% and 40% respectively.
Summary/Conclusion: Our study showed that the application of the CCG-1961 pediatric protocol in adult pts is feasible, offering CR rates of 85% and long term survival of 60%. However, its toxicity seems to be considerable. Prospective well organized trials with large series of patients are needed to define the role of intensive pediatric protocol in the treatment of adults-ALL.

PB1630

FEASIBILITY AND RESULTS OF BONE MARROW TRANSPLANTATION IN HIGH RISK ACUTE LYMPHOBLASTIC LEUKEMIA FOR CHILDREN AND YOUNG ADULTS

I. Frikha[1,*], M. Medhaffer[1], N. Ben Abdeljalil[2], S. Hdiji[1], I. Ben Amor[1], S. Laddab[2], F. Kallel[1], O. Kassar[1], M. Ghorbel[1], T. Ben Othmen[2], M. Elloumi[1]

[1]Hematology, Hedi Chaker Hospital, Sfax, Tunisia, Sfax, [2]Hematology, Bone Marrow Transplantation center, Tunis, Tunisia, Tunis, Tunisia

Background: The majority of children with acute lymphoblastic leukemia (ALL) can be definitively cured through the use of risk-oriented chemotherapy protocols. However, there are still subsets of children with ALL in whom the probability of survival with chemotherapy alone remains unsatisfactory, because of the high chance of disease recurrence. In this study, we analyzed the feasibility and results of allogenic stem cell transplantation for high-risk ALL of children and young adults.
Aims: In this study, we analyzed the feasibility and results of allogenic stem cell transplantation for high-risk ALL of children and young adults.
Methods: Between January 2000 and December 2015, 72 patients (27%) aged less than 30 years, with high-risk ALL in first complete remission treated with a frontline EORTC 58951 pediatric protocol, underwent allogeneic stem cell transplantation (HSCT). For these patients, we studied the indication of the allograft, the search of HLA intra-familial sibling donor, the feasibility and the results of the allograft performed at the National Center for bone marrow transplantation in Tunis.
Results: Seventy patients with high risk ALL were eligible for allogenic SCT. Among them 57 patients (81%) who realized the HLA study and 34 (60%) had at least one HLA-identical siblings donor. The allograft has been performed for only 18 patients (32%): 2 patients had relapsed before allograft, five patients had a severe infection with impaired general condition and the others patients had refused allograft SCT. With a median follow-up of 6 years, 11 allografted patients (61%) are still alive in complete remission (RC), 4 patients have relapsed (at 7, 9, 10 and 12 months). The transplant-related mortality was 17%. Among the 45 patients who were not allografts, 18 were living in CR (40%), 8 had died in post induction chemotherapy, and 17 had relapsed (38%).
Summary/Conclusion: In our study, despite the presence of HLA-identical siblings donor in 50% of cases, bone marrow allograft was proposed for only 28% of patients less than to Western series 60% of cases (HLA-identical sibling, a matched unrelated donor or cord blood). Allogenic stem cell transplantation in high risk acute lymphoblastic leukemia of child give interesting responses with 2/3 of patients are alive without relapse in our study, comparable to the literature (60 to 70% at 5 years). To further improve the

results, it is necessary to expand donor sources such as matched unrelated donor and cord blood transplantation.

PB1631

SUCCESSFUL ALTERNATIVE TREATMENT OF RELAPSED ADULT ACUTE LYMPHOBLASTIC LEUKEMIA WITH DENDRITIC CELLS CYTOKINE-INDUCED KILLER COMBINED WITH RITUXIMAB-BASED REGIMEN

J. Huang*, X. Xiao, X. Ye

Hematology, the Fourth Affiliated Hospital, College of Medicine ,Zhejiang University, Yiwu, China

Background: Relapsed ALL in adult carries a dismal prognosis despite intensive treatment.
Aims: In this report, successful alternative treatment containing rituximab and infusion of Dendritic cells-CIK is described in a case of early relapsed B cell ALL patient who could not tolerate intensive chemotherapy.
Methods: A 52-year-old man first presented with persisting fever and weakness in May. 2014. A blood test showed decreased levels of white blood cell($1.8*10^9$/L) and platelets ($63*10^9$/L). Bone marrow aspirate revealed 72 percent primitive blast cells, and immunophenotypic analysis showed that the blast cells expressed CD10, CD19, CD22, CD79a and human leukocyte antigen D–related. No cytogenetic abnormalities were detected by G-banding analysis. Similarly, MLL gene rearrangement, BCR/ABL1, ETV5/RUNX1 were also negative. Diagnosis of B-lymphoblastic leukemia NOS was made on basis of 2016 update revision of WHO classification criteria. The patient received VDCLP regimen consisting of vindesine, idarubicin, cyclophosphamide, asparaginase and dexamethasone. Finally, he achieved complete remission (CR) with 3.5% blast cells in bone marrow. However, severe side effects induced by induction therapy including febrile neutropenia, hyperglycemia and hepatic toxicity were followed. After high-dose methotrexate combined with asparaginase and MA consolidation therapy, neutropenia lasted for 2 months, during which he suffered pulmonary infection, sepsis and liver dysfunction. He refused to receive any maintenance therapy for significant complications.
Results: Six months later, the patient presented with fatigue and petechiae with a short CR1. Bone marrow with 31% blast cells was consistent with relapsed B cell ALL (CD19+, CD20+, CD10+, CD22+) (Fig). Treatment approaches for this patient in poor performance status is limited. DC-CIK was chosen as therapeutic choice. Subsequently, rituximab combined VDLP regimen was adopted, and he got second remission. Thereafter, four times consolidation therapy including rituximab were processed, during which seven cycles of DC-CIK cells infusion (each (4–6) × 109 CIK cells) were also accomplished. After that he received rituximab alone every 3 months for a year, and took thioguanine and methotrexate tablets at intervals of immunotherapy until March 11, 2017. Impressively, the patient has been in bone marrow remission for 30 months since last relapse.

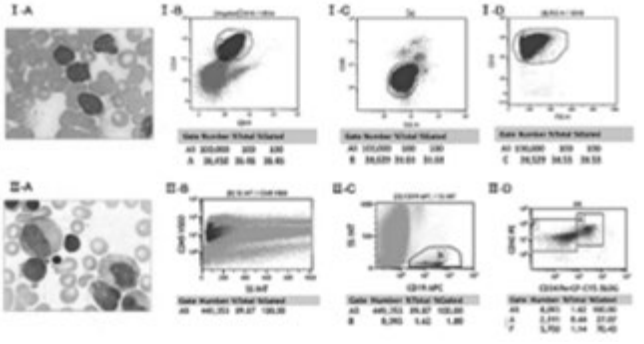

Figure 1.

Summary/Conclusion: High reinduction mortality, chemotherapy resistance and disease recurrent are impediments to successful management in relapsed ALL patients. The advantage of DC-CIK therapy is no adverse reactions, because CIK cells' anti-tumor activity is perforin mediated and various receptors were involved. Recent studies demonstrated that rituximab, anti-CD20 monoclonal antibody, may play a critical role in improvement of CIK-mediated cytotoxicity to leukemia cell. In this case, initiating DC-CIK therapy before chemotherapy help to improve the tolerability in patients with significant complications. To the best of our knowledge, it's the first report on DC-CIK combined with rituximab-based regimen for recurrent ALL. *Acknowledgment: The research was supported by funding of the Science and Technology Department of Zhejiang Province, China (2016C33160), and Yiwu public technology research projects, Zhejiang Province, China (2016-S-05). Correspondence to: Dr Jian Huang, E-mail: househuang@zju.edu.cn*

PB1632

SERUM LEVELS OF CYTOKINES AND ADHESION MOLECULES IN NEWLY DIAGNOSED B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA AND IN COMPLETE REMISSION, AND THEIR ASSOCIATION WITH OVERALL SURVIVAL

J.M. Horacek[1,2,*], T. Kupsa[1,2], J. Vanek[3], L. Jebavy[1,2], P. Zak[2]

[1]Department of Military Internal Medicine and Military Hygiene, University of Defence, Faculty of Military Health Sciences, [2]Department of Internal Medicine IV-Hematology, University Hospital and Charles University, Faculty of Medicine, [3]Department of Informatics and Quantitative Methods, University of Hradec Kralove, Faculty of Informatics and Management, Hradec Kralove, Czech Republic

Background: Dysregulated production of cytokines and adhesion molecules has been implicated in the onset and progression of various types of leukemia. Further knowledge gained from multiple cytokine and adhesion molecule evaluation could help to improve treatment outcomes.
Aims: The aim of this study was to evaluate serum levels of selected cytokines and adhesion molecules in newly diagnosed B-cell precursor acute lymphoblastic leukemia (B-ALL) and in complete remission, and their association with overall survival.
Methods: A total of 40 newly diagnosed B-ALL patients (median age 49, range 19–75 years, 27 males) were included in this study. Serum samples were taken at diagnosis and in complete remission. We evaluated serum levels of 12 cytokines and 5 adhesion molecules. From cytokines, we measured Interleukins (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10), Interferon-γ (IFN-γ), Tumour Necrosis Factor-α (TNF-α), Epidermal Growth Factor (EGF), Vascular Endothelial Growth Factor (VEGF) and Monocyte Chemotactic Protein-1 (MCP-1). From soluble adhesion molecules, we measured E-Selectin (E-SEL), L-Selectin (L-SEL), P-Selectin (P-SEL), Intercellular Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Molecule-1 (VCAM-1). All analytes were measured by biochip array technology on Evidence Investigator analyzer (Randox). Correlations between analytes and overall survival were evaluated separately in both clinical situations. Statistical evaluation was done by a professional statistician using software R 3.4.3 (R Core Team 2017). Probability values (p)<0.01 were considered statistically significant.
Results: At diagnosis of B-ALL, we found significantly higher levels of IL-6, IL-8, IL-10, TNF-α, E-SEL, L-SEL, ICAM-1, VCAM-1 ($p<0.01$) and significantly lower levels of EGF, P-SEL ($p<0.01$) in comparison with complete remission. Serum levels of other evaluated analytes were without significant differences. In complete remission, EGF correlated with P-SEL ($r=0.755$; $p<0.001$) and IL-1α with IL-4 ($r=0.612$; $p=0.007$). Other correlations between analytes did not reach statistical significance. Inferior overall survival was associated with higher IL-2 level at diagnosis ($r=0.448$; $p=0.003$) and higher L-SEL levels in complete remission ($r=0.410$; $p=0.001$).
Summary/Conclusion: Conclusion: Our results show that serum levels of some cytokines and adhesion molecules are significantly altered in newly diagnosed B-ALL, reflecting activity of the disease. In our cohort of B-ALL patients, we found statistically significant correlations between inferior overall survival and higher IL-2 levels at diagnosis and higher L-SEL levels in complete remission. Better understanding of leukemia microenvironment is essential for development of new treatment approaches. Further studies in this field are warranted. This work was supported by a long-term organization development plan 1011 (FMHS) and by program PROGRES Q40/08.

PB1633

EPIDEMIOLOGICAL EVALUATION, MANAGEMENT AND OUTCOME OF ACUTE LYMPHOBLASTIC LEUKAEMIA PATIENTS AGED MORE 65 YEARS IN A REAL LIFE SETTING. A SINGLE CENTER EXPERIENCE

E. Borlenghi[1,*], F. Schieppati[1], A. Passi[1], C. Cattaneo[1], E. Cerqui[1], V. Orlando[1], M. Sciumè[1], A. Sottini[2], G. Rossi[1]

[1]Hematology, [2]Laboratorio CREA, Spedali Civili, Brescia, Italy

Background: Acute Lymphoblastic Leukaemia (ALL) is considered rare in elderly patients (pts) but its frequency may be increasing, particularly due to the longer life expectancy of the general population. Management of

older pts is an unmet medical need. Significantly lower rates of complete remission, higher early mortality and poorer survival are observed in older compared with younger pts (Gokbuget, 2017). The potential benefit of using moderately intensive chemotherapy protocols for Ph/BCR-ABL-negative ALL and the combination of tyrosine kinase inhibitors (TKI) with chemotherapy in Ph/BCR-ABL positive ALL is debated.
Aims: To analyze the frequency, characteristics and outcome of older pts with ALL consecutively diagnosed at a single Institution receiving treatments of different intensity.

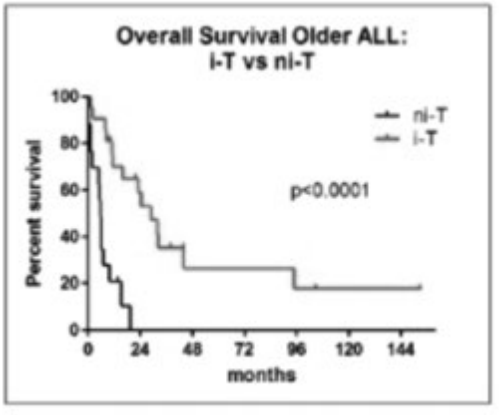

Figure 1.

Table 1.

	total	ni-T	i-T	P (Fisher exact test)
Pts number	38	17	21	
Age (median)	73 (65-88)	75 (71-88)	70 (65-79)	ns
Performance status ECOG > 2	16/38 (42%)	9/17	5/21	0.09
CR n° (%)	27/38 (71)	8/17 (47%)	19/21 (90)	0.0049
Relapse n° (%)	62.9%	6/8 (75%)	9*/19 (47.3%)	ns
Median survival mo (range)	11.1 (1-153)	5,7 (1-15)	29 (1-153)	<0.0001
			*2/9 molecular relapse	

Methods: Of 172 ALL pts diagnosed at our Institution between Jan 2000 to Dec 2017, 38 were ≥65 y old (22%). Median age was 73 y (65-88), F/M was 17/19, performance status according to ECOG was 2 in 13 pts and 3 in 14 pts. Molecular analysis, including AF4/MLL and BCR/ABL transcripts (Ph+), were performed in all pts and karyotype was available in 30 (78.9%). According to clinical judgement, age and molecular status pts received intensive treatment (iT) defined as dose-adjusted chemotherapy (da-CT), + TKI in Ph+ (NILG protocols) (Bassan 2015), or non intensive treatment (niT) *i.e.* steroids, best supportive care (BSC), + TKI alone in Ph+ pts.
Results: According to the year of diagnosis, the number of elderly ALL pts has increased. Between Jan 2000 and Dec 2008, a lower rate of ALL in pts aged ≥65 was observed, compared to the time period Jan 2009-Dec 2017 (6/66, 9% *vs* 32/106, 30.2%, respectively)(p0.001 chi-squared test). According to phenotype, 32 were B-ALL (84.2%), 1 mixed-phenotype (My-ALL) and 5 (12.8%) were T-ALL/lymphoblastic lymphoma. Two pts showed AF4/MLL rearrangements and 14 were BCR/ABL positive, with e1a2 or b3a2 transcript in 10 and 4 pts, respectively. Karyotype was normal in 7 pts, high hyperdiploidy (He) or near triploidy (Ho-Tr), chromosomal translocations [t(9;22) and t(4;11)] and chromosomal deletions were detected in 4, 16 and 3 pts, respectively. ECOG was >2 in 9/17 niT pts *vs* 5/21 iT pts (p0.09 Fisher's exact test). Complete remission and relapse rate were 71% (27/38) and 62.9% (17/27), respectively. Median follow-up of the whole elderly population was 30.5 months (mo) and median survival was 11.1 mo. The main cause of death was disease progression (22/28, 78.6%); other causes were: cardiac (2), myelodysplasia (1), strongyloides infection (1), hepatic failure (1), unknown (1). The characteristics and outcome of iT and niT are summarized in Table 1 and in Figure 1. In particular, median survival of ALL Ph- iT pts was 23,3 mo (1-105 mo) compared to ni-T pts (2 mo, 1-9) (p<0.0001, log-rank test) and median survival of ALL Ph+ iT pts (TKI+da-CT) was 32.4 mo (8-153) compared to ALL Ph+ niT (TKI alone) (6.8 mo, 2-15)(p 0.014 log-rank test). Autologous stem cell transplantation (ASCT) was performed in 7 pts, 4 Ph+ and 3 Ph- (1 MLL/AF4, 1 T-ALL, 1 with HoTr K). Five pts were in molecular CR at the time of ASCT (4 Ph+ and 1 MLL/AF4), two pts were in cytogenetic CR, not available molecular data.
Summary/Conclusion: A non-negligible number of pts is diagnosed with ALL over the age of 65, with an incidence that is progressively increasing (27.5% of ALL population). In our experience, intensive treatment including da-CT with TKI in Ph+ pts with or without ASCT was feasible and tolerated, also in a population-based setting, and improved survival both in Ph- but also in Ph+ ALL pts.

PB1634

SPECTRUM OF ASPARAGINASE-INDUCED ADVERSE REACTIONS IN PEDIATRIC PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

M. Elshinawy[1,2,*], A. Al Rawas[1], Y. Wali[1]

[1]Pediatric Hematology, Sultan Qaboos University Hospital, Muscat, Oman, [2]Pediatric Hematology, Faculty of Medicine, Alexandria University, Alexandria, Egypt

Background: Asparaginase is an essential drug for treatment of pediatric acute lymphoblastic leukemia (ALL). It acts by enzymatic cleavage of L-asparagine amino acid into aspartic acid and ammonia, thus depriving the leukemic cells from L-asparagine, resulting into cell death. Three preparations are available; the native asparaginase derived from *Escherichia coli (E. coli*-asparaginase), a pegylated form (PEG-asparaginase) and a product isolated from *Erwinia chrysanthemi, i.e.* Erwinia asparaginase. Owing to its easier administration and less frequent injections, our patients receive PEG-asparaginase as a standard of care.
Aims: To review the clinical presentations, radiological characteristics, management and prognosis of asparaginase-related adverse reactions in Omani children with ALL.
Methods: A retrospective cohort study of all children diagnosed with ALL who developed asparaginase-induced adverse effects while being on chemotherapy during the period January 2006 through January 2018.
Results: Out of 198 pediatric patients with ALL, 11 children (8 males and 3 females) developed severe asparaginase-related adverse effects, constituting 5.6% of the total number of patients. Severe full blown pancreatitis was encountered in only one patient (0.05%). He is an 11 year-old male which has survived this stormy mishap, but developed permanent diabetes mellitus that required lifelong insulin therapy. He has never been exposed to any further doses of asparaginase, and then went into complete remission. Two patients developed severe anaphylactic reaction, and then shifted to erwinia asparaginase with no recurrence. Notably, eight of our patients developed asparaginase-related cerebral venous sinus thrombosis, evidenced by contrast-enhanced cerebral MRI. The mean duration of stroke diagnosis was (6±2.1) days after the last asparaginase injection. All were managed conservatively with anticoagulation using unfractionated and/or low molecular weight heparin. Two of these patients have had a recurrence upon re-challenge, despite concomitant prophylactic heparin. Remarkably, seven patients (7/8) survived without any neurological sequelae. Unfortunately, a 30 month-old boy developed permanent right-sided hemiplegia secondary to extensive superior sagittal sinus thrombosis, which has been uniquely associated with massive left-sided fronto-parietal hemorrhage and midline shift (Figure). This patient required ICU admission and underwent a pressure-relieving craniectomy surgey. It has been planned not to expose him to any further asparaginase doses. In addition to these severe reactions, 23 patients developed mild to moderate asparaginase-related side effects in the form of transient hyperglycemia that required insulin therapy in 17 patients, local skin reaction at the site of injection in 3 patients and transient urticarial skin rash in 3 patients as well.

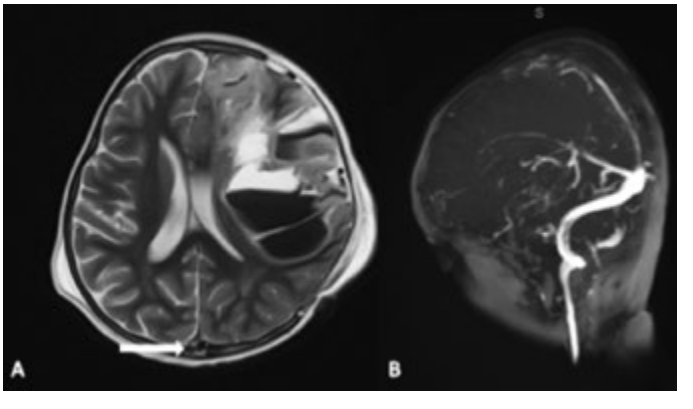

Figure 1.

Summary/Conclusion: Most of asparaginase-induced adverse reactions are transient and self-limited. However, a high index of suspicion is needed for early detection of intracranial thrombotic complications. Contrast-enhanced

magnetic resonance venography (MRV), or CT venography are the modalities of choice. Early diagnosis, discontinuation of asparaginase and prompt initiation of anticoagulation are mandatory steps to prevent permanent neurological deficits.

PB1635

FLOW CYTOMETRIC MINIMAL RESIDUAL DISEASE DETECTION IN B-ACUTE LYMPHOBLASTIC LEUKEMIA: EVALUATION OF TWO COMMERCIAL KITS

A. Ahn[1,*], C.-J. Park[1], Y.-U. Cho[1], S. Jang[1], E.-J. Seo[1], H. Kim[2], K.-N. Koh[2], H.-J. Im[2], J.-J. Seo[2]

[1]Department of Laboratory Medicine, [2]Department of Pediatrics, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea, Republic Of

Background: Minimal residual disease (MRD) is an important independent prognostic factor that can identify poor responders among patients with B-acute lymphoblastic leukemia (ALL). The fundamental principle of the detection of MRD by flow cytometry is that leukemic cells show altered patterns of antigen expression. There are ongoing efforts to standardize MRD quantification using flow cytometry to improve accuracy and reproducibility, because hematogones that may morphologically resemble the leukemic cells of B-ALL are often increased in regenerating marrow following chemotherapy or hematopoietic stem cell transplantation.
Aims: The aim of this study was to evaluate two commercial kits for flow cytometric MRD detection in B-ALL.
Methods: 50 bone marrow aspirates with less than 5% leukemic cells by conventional morphology from treated 30 pediatric patients with B-ALL were obtained. MRD was measured using two commercial kits for flow cytometeric MRD detection; DuraClone RE ALB Tube (Beckman Coulter, Miami, USA) and BCP-ALL-MRD (Cytognos SL, Salamanca, Spain). The main methodological approach for detecting MRD is to detect antigen over or under expression of DuraClone RE ALB Tube (CD58/CD34/CD10/CD19/CD38/CD20/CD45) and aberrant expression of BCP-ALL-MRD (tube1: CD20/CD45/CD81/CD66c+CD123/CD34/CD19/CD10/CD38, tube2: CD20/CD45/CD81/CD73+CD304/CD34/CD19/CD10/CD38). Acquisition cell numbers of DuraClone RE ALB Tube and BCP-ALL-MRD were $2.0x10^6$ and $5.0x10^6$, respectively. Flow cytometric analysis was performed by manual serial gating according to our protocol. All specimens for MRD detection were negative for by real-time quantitative polymerase chain reaction (RQ-PCR) or fluorescence *in situ* hybridization (FISH) according to genetic abnormalities at diagnosis except 4 bone marrow aspirates (minor *BCR-ABL1* RQ-PCR: positive, normalized copy number: 13.41, 1.25, and 0.25 and% cells with *MLL* deletion, *RUNX1* gain, and *IGH* gain by FISH: 0.50-0.75%).

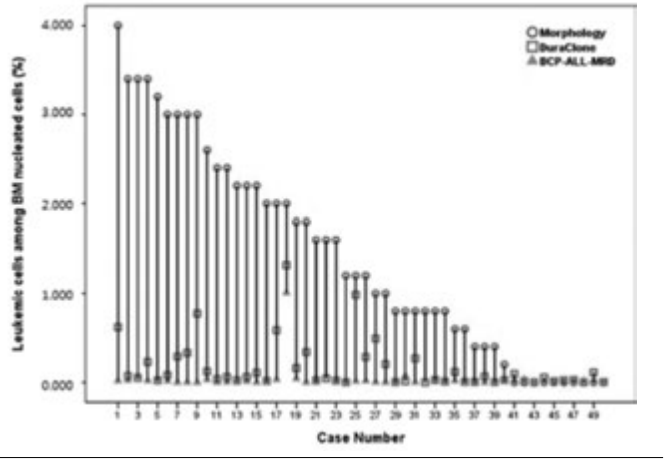

Figure 1.

Results: The median patient age was 7 years (range, 3-20) and the ratio of boy to girl was 1:1. The median time of sample collection was 153.5 days (14-3930) after diagnosis or relapse. The immunophenotype of leukemic cells at diagnosis in all patients showed CD10 positive (cutoff ≥20%) and variable CD34 and CD20 expression. Leukemic cells at diagnosis were all positive for CD58 but variable CD38 expression from negative to bright positive. Aberrant expression at diagnosis was CD66c (13/24, 54%), CD123 (12/24, 50%), CD73 (8/24, 33%), CD304 (1/24, 4%), CD13 (5/29, 17%), CD33 (3/29, 10%), and CD56 (1/29, 3%). The leukemic cell% (mean±SD) were 1.4±1.2% by conventional morphology, 0.166±0.268% by DuraClone RE ALB Tube and 0.034±0.143% by BCP-ALL-MRD (Fig.1, P=0.001 in difference). MRD% by DuraClone RE ALB Tube and BCP-ALL-MRD were well correlated ($P \leq 0.001$). However, the percentage of leukemic cells by conventional morphology show statistical significance with MRD% by DuraClone RE ALB Tube, but not with BCP-ALL-MRD (P=0.024 and P=0.521, respectively).
Summary/Conclusion: Detection of MRD in patients with B-ALL is limited by conventional morphology. For the flow cytometric detection of MRD in B-ALL, the kit detecting antigen over or under expression was adequate, but the kit detecting aberrant expression showed significantly lower level of MRD. It is very helpful for analysis of MRD to know the immunophenotype of leukemic cells at diagnosis.

PB1636

EFFECTS OF IRON OVERLOAD IN CHILDREN WITH ALL

K. Bruslova*, N. Tsvietkova, L. Honchar, S. Iatsemyrskii

Radiation Hematology for Children, SI "National Research Center for Radiation Medicine of NAMS of Ukraine", Kiev, Ukraine

Background: Iron overload of the body is associated with the risk of development of malignant hematologic pathology and frequency of complications in children with ALL and is one of the causes of patients death.
Aims: The goal is to determine the association of serum ferritin (SF) levels in the debut of ALL in children with the nature of complications, the development of relapses and the prognosis of disease course.
Methods: Serum ferritin (SF) has been determined and anemia has been identified in 146 children with ALL before chemotherapy, as well as in the treatment stages. The analysis of obtained data taking into account the ALL variant, presence of complications (bacterial infections, toxic hepatitis, etc.), number of RBC transfusions during treatment, development of resistance to chemotherapy and the number and nature of ALL relapses. Morphological evaluation of dyserythropoiesis manifestations in bone marrow elements was executed and parametrs of porphyrin metabolism (aminolevulinic acid and porphobilinogen) were studied.
Results: Distribution of 146 patients with ALL was performed by initial SF (before start of treatment) levels: up to 200ng/mL had 53 patients (36.3%); from 200 to 500ng/mL, normal-49 (33.6%); more than 500ng/mL-44 (30.1%). Anemia was not found in 15.7% of patients. Anemia with normal SF was found in 41.1% of patients, anemia with low SF in 15.9%, and with SF in 27.4%. More patients with iron excess had pro-B-ALL, smaller number of iros excess children suffered from common- ALL variant (rs=0.48). Bacterial infections were common for patients with SF higher than 200ng/ml, toxic hepatitis and chemotherapy resistance were found more frequently at SF levels higher than 500ng/ml (rs=0.48). The number of erythrocyte transfusions correlated with the development of relapse in children (rs=0.46). There is direct correlation between SF level and the presence of bone marrow relapse in patients (rs=0.44). In cases with SF levels above 500ng/mL, the percentage of sideroblasts and manifestations of dyserythropoiesis were higher in patients with ALL relapses or unfavorable disease course. Excess of iron may be the result of ineffective erythropoiesis, endogenous erythropoietin deficiency, reactive oxygen species levels incrising, and transfusion therapy.The removal of excess iron (reduction of serum ferritin) occurred gradually during the treatment and follow-up of patients, taking into account the established causes. The mean value of SF level before treatment was 784.4ng/mL, after-158ng/mL. Light microscopic examination of the bone marrow revealed increased iron granules in erythroblasts, dyseritropose phenomena.
Summary/Conclusion: The results of the studies have shown that iron overload is a severe complication of ALL. Particular attention is paid to the presence of high initial levels of SF in patients before treatment, the development of complications and the substitution of erythrocytic transfusions, which should be considered as a risk factor for development and unfavorable prediction of ALL course in children.

PB1637

THE TREATMENT OUTCOME OF CHILDREN WİTH ACUTE LYMPHOBLASTIC LEUKEMIA: 20 YEARS EXPERIENCE FROM A SINGLE CENTER IN TURKEY

A. Meral Güneş[1,*], M. Sezgin Evim[1], B. Baytan[1], M. Ertekin[1], S. Güler[2], U. Aygüneş[3]

[1]Departmant of Pediatric Hematology, Uludağ university, Bursa, [2]Pediatric

Hematology, Konya State Hospital, Konya, [3]Pediatric hematology, cumhuriyet University, Sivas, Turkey

Background: Acute lymphoblastic leukemia (ALL) is a malignant disease of the bone marrow in which early lymphoid precursors proliferate and replace the normal hematopoietic marrow cell.
Aims: We retrospectively evaluated the treatment results of children with de nova ALL treated with BFM ALL (1990-1995-2000-2009) protocols between the years 1993 and 2017.
Methods: The overall (OS) and event free (EFS) survivals according to age, initial leukocyte count, immunophenotype, chemotherapy responses (on days 8, 15, and 33), and risk groups were analyzed by Kaplan-Meier survival analysis by SPSS 2009. In total ,MRD(minimal residual disease) analysis was avaliable in 33% (n=141)of children either with flowcytometry or PCR.
Results: The data of 460 children diagnosed as de nova ALL were retrospectively evaluated. Male (291)/Female (169) ratio was: 1.7 and median age was 6.9 years (0.5-17.8). Median follow-up time was 7,7 years. (1-297 months). Children were classified as high risk (28%), medium risk(60.2%), and stantard risk(9.3%) groups.Relapse time was defined according to BFM definition. (Late relapse >30 months, early relaps 18-30 months and very early relapse <18 months from the initial time of diagnosis) Complete remission was achieved in 97% of children. EFS and OS at 5 years were found 78.8 and 82.3%, respectively. Children younger than 6 years old had better EFS and OS (83.7 and 85.2%) than children aged ≥6 years (71.4 and 72.8%)(p=0.23). Patients who had initial leukocyte counts of<20×10^9/L had better EFS and OS (80.6 and 84%) than children with higher initial leukocyte counts (76 and 80.2%)(p=0.22). The majority of the patients were B cell (80.2%) and the rest were T cell ALL(19.8%). EFS for B and T-cell ALL was 84.3 and 76.4%, respectively(p=0.128). Children with a prednisolone good response on day 8 (83,5%) achieved significantly better EFS and OS (80.3 and 84.6%) *vs* (71.2 and 71%)(p=0.025). n children whose bone marrow on day 15 m1 and m2 bone marrow (M1 and M2 bone marrow) had also higher EFS and OS (82.8 and 85.9% *vs* 71 and 77.5%) (p=0.001)than the childrem with m3. Children in the standard-risk and medium-risk groups obtained significantly higher EFS (88.4 and 81.5%) OS (90.7 and 86.6%) compared to the high-risk group (EFS 75.6%, OS 76.6%)(p=0.001). The relapse rate was found 18%. The median relapse time from diagnosis was 29 months.(3-129). Late relapses had higher OS (33.3%) than very early and early relapse cases (15%), respectively (p=0.003). Death occurred in 82 of 460 patients (17.8%). The major causes of death were neutropenic sepsis and relapse disease.
Summary/Conclusion: The high survival rate with BFM based ALL protocols were obtained in our center.These protocols can be succesfully applied in treatment of childhood leukemia in countries with limited sources.

PB1638

ALLOGENEIC STEM CELL TRANSPLANTATION FOR PHILADELPHIA POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA

H. Bouarab*, M. Benakli, S. Zerkout, K. Tarhi, F. Mehdid, N. Rahmoune, D. Ait Ouali, M. Baazizi, R. Belhadj, F. Harieche, R.-M. Hamladji, R. Ahmed Nacer

Hematology and Bone Marrow Transplantation, Pierre and Marie Curie Center, Algiers, Algeria

Background: Patients (pts) with Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL) in first complete remission (CR) are at risk because chemotherapy alone does not allow long-term survival. Hematopoietic stem cell transplantation (HSCT) is considered the most potent post-remission antileukemic therapy.
Aims: We report in this series results of this procedure in 31 pts with Ph+ ALL.
Methods: From January 2012 to December 2016, 31 pts with Ph+ ALL were treated with HSCT (24 sibling HLA-identical, 06 haplo-identical and 01 syngeneic). Twenty four pts (77%) received a tyrosine kinase inhibitor (TKI) during induction therapy. The disease status at transplant: first CR (23 pts), 2nd CR (07 pts), blast phase (01 pt) of whom 16 pts (51%) were in molecular remission. The median age is 29 years (06-49), of whom 08 (32%) are under 18 years of age. The sex ratio is 1.81. The average of last remission-transplant was 4 months (01-12). All pts received chemotherapy conditioning: BAM12 protocol (13 pts) associating Busilvex (dose according to weight in children, 12.8mg/kg in adults), Aracytine 12g/m^2 and Melphalan 140mg/m^2; the TUTSHKA-VP16 protocol (10 pts) associating Busilvex, Endoxan 120mg/kg and Etoposide 15mg/kg; SANTOS protocol (02 pts) associating Busilvex and Endoxan 200mg/kg. Six pts beneficied haplo-identical HSCT including 05 pts with a Chinese protocol combining Busilvex 9.6mg/kg, Aracytine 08g/m^2, Endoxan 3.6g/m^2 and Thymoglobulin 10mg/kg; one pt received a TBF protocole combining Busilvex (9.6mg/kg), Fludarabine (150mg/m^2) and Thiotepa (10mg/kg). All pts received a peripheral blood stem cell transplant obtained after mobilization with G-CSF alone with the average CD34+ cell count 9.88. 106/kg (4.1-41). An additional bone marrow transplant G-CSF primed was used in the 5 pts in haplo-identical HSCT with an average of nucleated cells 5.67. 108/kg (0.5-10.7). GVHD prophylaxis associated Cyclosporine-Methotrexate for sibling HLA-identical HSCT; Cyclosporine-Methotrexate-Cellcept for haplo-identical HSCT (Chinese protocole); Cyclosporine-Cellcept and post-transplantion cyclophosphamide (TBF protocol) and without prophylaxis (syngeneic HSCT). TKI was instituted as prevention for relapse from 3 months after transplant in 21 pts (76%). At December 2017 the minimal follow-up delay is 6 months and the maximal 60 months.
Results: The median duration of aplasia is 15 days (09-29). Neutrophils engraftment was obtained at day 15 (11-29). Transfusions were required with an average of red blood cells: 2,4 units/pt and Platelets concentrates: 1,8 units/pt. No pt showed rejection, VOD or haemorrhagic cystitis. Acute GVHD was observed in 08 pts (25%), including 07 grade III-IV, and chronic GVHD occurred in 07 pts (29%), of which 03 were extensive. CMV reactivation was observed in 08 pts (25%) on average day 46 (28-69). Relapse occurred in 12 pts (38%) within an average of 11 months (3-32) of which 10 pts was in TKI post-transplantation. At December 2017, 17 pts died, of which 08 related to the procedure (TRM: 25%, GVHA 07 pts, acute pancreatitis 01 pt) and 09 pts of relapse. Fourteen pts are alive in complete remission with a median follow-up of 28 months (06-50), including 13 in molecular remission. Overall survival and event-free survival at 57 months are 26.7% and 24.2% respectively.
Summary/Conclusion: Results of HSCT in pts with Ph+ ALL have to be improved. The addition of TKI as a preventive treatment for post-transplant relapses has not been beneficial in our series.

PB1639

THE OVERALL SURVIVAL (OS) OF PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL), DEPENDING ON THE INCLUSION IN CLINICAL TRIAL: THE ACUTE LEUKEMIA (AL) REGISTER AND THE CLINICAL STUDY "ALL-2009"

Z. Akhmerzaeva[1,*], S. Kulikov[1], Y. Chabaeva[1], E. Zotina[2], L. Gavrilova[3], A. Pristupa[4], N. Vopilina[5], E. Borisenkova[6], E. Parovichnikova[1]

[1]National Research Center for Hematology, Moscow, [2]Kirov clinic hospital, Kirov, [3]Mordovia republic hospital, Mordovia, [4]Ryazan regional clinical hospital, Ryazan, [5]Tambov regional clinical hospital, Tambov, [6]Kaluga regional clinical hospital, Kaluga, Russian Federation

Background: Several clinical and population studies have shown a significant impact of the inclusion of patients with AL in clinical studies on their overall and disease-free survival (DFS). In Russia, such data on the effect of inclusion in clinical trials on the effectiveness of therapy for patients with AL are not available yet.
Aims: Evaluation of OS patients with ALL depending on the inclusion in clinical trials in 5 regions of the Russian Federation according to the AL Register and the clinical study "ALL-2009" (NCT 01193933).
Methods: The AL Registry was conducted since Apr 2009 till Dec 2016. 333 AL cases from Kirov, Ryazan, Kaluga, Tambov regions and Mordovia republic were included into analysis. The Russian multicenter "ALL-2009" trial was conducted since Apr 2009 till Dec 2016 and included 330 Ph-negative ALL patients with a median age 28 y (15-55).
Results: The distribution by AL subtypes was following: AML-251 (75,3%), ALL-58 (17,4%), APL-19(5,7%), other-5 (1,5%). Median age was for AML-59y (17-85), ALL-38y (16-80), APL 51y (21-79) years. Gender female/male proportions 184/149. As the "ALL-2009" study had the inclusion criteria of age<55years, among 42 AL Registry patients under the age of 55 years, 20 were registered in the "ALL-2009" study. 22 patients, not included in the study due to comorbidity, were nontheless treated by the same protocol. 16 older patients received deescalated therapy. The 3-year OS of all ALL patients was 47% (median 27.6 months). The 3-year OS with ALL in the group of patients younger than 55 years was 51% (median 27 months); older than 55 years-27%; (median 5 months) (p=0.29). OS in patients from the AL Registry, included in "ALL-2009" study (n=22), constituted 58%. In patients not included in the "ALL-2009" study, but treated according to this protocol (n=20), the 3-year OS was slightly lower-47% than in the patients formally included in the study (p=0.68). OS of ALL patients, depending on the treatment center, differed significantly. OS in federal institutions and regional centers-61% *versus* 46%, respectively (p=0.003).
Summary/Conclusion: OS of ALL patients included and not included in the "ALL-2009" study, but treated according to the protocol, did not significantly differ. This fact demonstrates good reproducibility and effectiveness of treatment protocol. It was shown that the hematological hospital where

the treatment was administered became an independent significant prognostic factor for survival of AL patients.

PB1640

TOLERABILITY OF PEGYLATED L-ASPARAGINASE IN ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA: A SINGLE CENTRE EXPERIENCE

R. Lyne[1,*], J. Allan[2], M. Drummond[3], R. Soutar[4]

[1]Medical School, University of Glasgow, [2]Pharmacy, [3]Haematology, Beatson West of Scotland Cancer Centre, [4]Haematology, University of Glasgow, Glasgow, United Kingdom

Background: Overall survival in Adult Acute Lymphoblastic Leukaemia (ALL) has improved with time, the drug L-Asparaginase is an essential component in ALL treatment protocols, both in paediatrics and adults.
Aims: Compared to paediatric patients, adult patients tolerate intensive chemotherapy regimens less well. This audit investigated the use of PEGylated L-Asparaginase (PEG-L-ASP) in adult ALL chemotherapy regimens and its adverse effects, namely: hepatotoxicity, pancreatitis and hypertriglyceridemia.
Methods: Thirteen patients in the UKALL14 trial and nine in the UKALL2011 trial were studied by case note review retrospectively. These groups allowed age comparison: 30-55 years (UKALL14) and 17-24 years (UKALL2011). Toxicity gradings were calculated using the Common Terminology Criteria for Adverse Effects v4.0 (US Department of Health and Human Services, 2009).
Results: Overall the older patients had higher hepatotoxicity gradings than the younger patients (see Table). More critically, none of the older cohort could complete their proposed schedule because of PEG-L-ASP related toxicity compared to 78% of the younger cohort.

Table 1.

CTCAE Toxicity Gradings			
		Ages 17-24 (9 patients)	Ages 30-55 (13 patients)
Bilirubin Toxicity	Grades 1+2	6	9
	Grade 3	3	2
	Grade 4	0	2
ALT Toxicity	Grades 1+2	2	4
	Grade 3	6	8
	Grade 4	1	1
AST Toxicity	Grades 1+2	5	6
	Grade 3	4	6
	Grade 4	0	0
Mean Toxicity		2.2	2.4

Summary/Conclusion: This audit demonstrated significant toxicity of PEG-L-ASP in patients aged greater than 30 years. Toxicity of PEG-L-ASP has previously been noted in Ph positive adult ALL patients undergoing therapy. Treatment delays are very undesirable in the treatment of ALL. Alternative therapy with non-PEG-ylated L-Asparaginase allows for drug withdrawal if toxicity occurs because of its shorter half life. However, PEG-L-ASP has shown to be beneficial in paediatric and adolescent ALL protocols because of its faster asparagine depletion and more prolonged effects. While the optimum form of L-Asparaginase to use in older adult patients remains under investigation, our clinical practice in light of our findings, has been to revert to conventional asparaginase (Erwinia) in adult patients over 30 years old.

PB1641

A REPORT OF DIFFERENT PURPOSES: BLINATUMOMAB WITH PREVIOUS DEBULKING CHEMOTHERAPY TO REACH OR MAINTAIN RESPONSE

G. Ferrer Garrido[1,*], L. Costilla Barriga[1], B. De Rueda Ciller[1], C. Rodríguez-Vigil Iturrate[2], M. Villalba Montaner[1], E. Salvador Rupérez[1], P. Delgado Beltrán[1]

[1]Hematología clínica, [2]Oncopediatría, Hospital Universitario Miguel Servet, Zaragoza, Spain

Background: Blinatumomab is a bispecific T-cell antibody indicated for the treatment of adult and pediatric patients with Philadelphia chromosome-negative (Ph-) relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL).

Aims: We report 2 cases of response reach and maintenance to blinatumomab.
Methods: Case 1: A 21 years old man who presented peripheral blood (PB): leukocytes 2.4 x 10 3/µL, neutrophils 0.9 x 10 3/µL, hemoglobin 9.7 g/dL, platelets 22 x 10 3/µL. Lymphoblasts 1%. The bone marrow aspirate (BMA) with 35% of lymphoblasts. The immunophenotype (IF) (Table 1), cytogenetic and molecular biology analysis confirmed the diagnosis of B-II or common ALL Ph- (MLL negative). No central nervous system infiltration. He started induction therapy according to the PETHEMA ALL intermediate risk-2008 protocol with vincristine, daunorubicine and prednisone. BMA on day +14 without response, IF with 76% of blasts (Table 1), revealed diagnosis of more aggressive ALL: B-I or pro-B ALL Ph- (MLL negative). Due to it, he started reinduction of the PETHEMA ALL high risk-2011 protocol (Flag-IDA). Meantime an allogeneic stem cell transplant (ASCT) of unrelated donor was proposed. He presented as complications pulmonary fungal infection. On day +34 it showed lack of response, IF similar to the previous one (Table 1). A third line of treatment is proposed, starting blinatumomab after debulking with vincristine and dexamentasone. A Cytokine Release Syndrome (CRS) was reported after 3 weeks of treatment, therefore blinatumomab was delayed until CRS was controlled with conventional treatment. Reevaluation after the first cycle of blinatumomab, PB: leukocytes $2.7x10^3$/µL, neutrophils $1.5x10^3$/µL, hemoglobin 8,7g/dL, platelets $41x10^3$/µL and BMA showed morphological complete remission (CR) and minimal residual disease (MRD) + (Table 1). Finally, the patient died due to a new episode of pulmonary infection. **Case 2:** A 15 years old man, PB: leukocytes $7.8x10^3$/µL, neutrophils $1.2x10^3$/µL, hemoglobin 8.4g/dL, platelets $70x10^3$/µL, lymphoblast: 53%. BMA: 95% of lymphoblast. The IF (Table 1), cytogenetic and molecular biology analysis of the BMA confirmed the diagnosis of B-II or common ALL Ph-, t (1;19) positive. TCF3 positive and MLL negative. No central nervous system infiltration. He started treatment according to the SEHOP-PETHEMA ALL 2013 intermediate risk protocol. Early relapsed with 97% of lymphoblast in BMA. He was treated under the therapeutic recommendations for relapse SEHOP-PETHEMA ALL 2015 without response, with 33% infiltration. Rescue chemotherapy with clofarabine, cyclophosphamide and etoposide, obtaining CR with MRD: 0.01% (Table 1). As complications, febrile neutropenia with bacteremia secondary to E. coli and aspergillosis with orbital, paranasal sinus and pulmonary affectation. Due to the delay of the transplant by infections and MRD: 0.2%, blinatumomab was initiated as a bridging therapy to transplant, being well tolerated. He maintained MRD lower than 0.01% after the first cycle (Table 1). An ASCT of identical HLA brother was performed. Currently in remission and complete chimerism.
Results: The results are shown in Table 1.

Table 1.

PATIENT 1	Blasts % BM		
Time	M	FC	Immunophenotype (IF)
Diagnosis	35	35	CD45- CD34+ nTdT+ CD19+ CD22+ CD38-CD20- CD10+ CD13+ low CD15+ CD24+
Post PETHEMA IR 2008	16	76	CD45- CD34+ CD19+ CD22+ CD38- CD20- CD10- CD13- CD15+ (30%) CD24+
Post PETHEMA HR 2011	95	97	Similar IF
Post Vin, Dex and Blina 1 Cycle	1	1.05	CD45- CD34+ CD19+ CD22+ CD38- CD20- CD10- CD13- CD15- CD24 +
PATIENT 2	**Blasts % BM**		
Time	M	FC	IF
Diagnosis	95	95	CD45- CD34- nTdT+ CD19+ hetero CD22+ low CD38+ low CD20- CD73+ CD10 + CD13+ CD15- CD24 +
Post SEHOP-PETHEMA IR 2013	-	0.0003	Similar IF
Relapse	97	96.7	Similar IF
Post SEHOP-PETHEMA 15	33	64	Similar IF
Post Clofa, Cyclo and E	-	0.01	Similar IF
Pretransplant	-	0.2	Similar IF
Post Blina 1 Cycle	-	0.01	Similar IF

Table 1. Diagnosis and re-evaluation BMA studies. Blats % BM: percentage of lymphoblasts. M: % morphology. FC: % flow cytometry. Vin: vincristine. Dex: dexamentasone. Blina: blinatumomab Clofa: clofarabine. Cyclo: cyclophosphamide. E: etoposide.

Summary/Conclusion: According to the published studies, patients with a low tumor load (<50% infiltration) obtain better results than patient with highest tumor (>90%); with CR rate of 72,9% *vs* 21,6%. In the first case, a spectacular response was observed after treatment with blinatumomab with previous debulking therapy. This strategy can be an option for patients with high tumor burden.

PB1642

OSTEONECROSIS DURING ACUTE LYMPHOBLASTIC LEUKEMIA TREATMENT

I. Yaman Bajin[1,*], S. Aytac[2], B. Kuskonmaz[2], S. Unal[2], F. Gumruk[2], M. Cetin[2]

[1]Pediatric Hematology, [2]Hacettepe University, Ankara, Turkey

Background: Avascular necrosis (AVN), also called osteonecrosis or aseptic necrosis is a condition that occurs when there is loss of blood to the bone, an interruption to the blood supply causes bone to die. Approximately 90% of the acute lymphoblastic leukemia (ALL) patients in childhood can be cured with current intensive treatment protocols. But the morbidity associated with ALL treatment become more important everyday because of the increase in the number of long-term survivors. Osteonecrosis is one of the most common therapy-related debiliating side effects of ALL treatment. Incidence and risk factors vary between study groups and therapeutic regimens. Age at diagnosis >10 years and glucocorticoid cumulative doses are known as the most significant risk factors.
Aims: We aimed to analyse the clinical features of the ALL patients diagnosed with osteonecrosis in our department.
Methods: Patients diagnosed with ALL and treated with modified St. Jude Total XV protocol in Hacettepe University, Pediatric Hematology Department between 1 January 2008 and 31 December 2015 were analysed and the patients diagnosed with osteonecrosis during ALL treatment were included the study. The clinical features that may be associated with osteonecrosis among these patients were analysed retrospectively .
Results: There were 208 patients diagnosed with ALL. Nineteen of them (9%) were diagnosed with osteonecrosis. Nine of them were girls, 10 were boys. The median age of this 19 patients at the diagnosis was 11 years (2-18y) and 15 patients were >10 years of age at the diagnosis. The immunophenotyping was found to be T cell leukemia for 6 of the patients and CALLA+ B cell ALL for the others. Four of them were low risk patients while 6 patients were standart risk and 9 were high risk patients. According to Modified St. Jude Total XV protocol 7 days of high dose methlprednisolone (HDMP) added to protocol as an initial treatment and we randomized patients at doses of 10 mg/kg/d or 20mg/kg/d HDMP: not exceeding at maximum 1000 mg methylprednisolone. After the end of 7th day of steroid concomittant chemotherapy was given and the doses were tapered gradually to 5mg/kg/d and 10mg/kg/d in each group respectively. By the 3rd week of treatment steroid dose was tapered to 2mg/kg/d in both groups and continued with this dose till the end of 3rd week of induction phase. Six patients were found to start treatment with 20mg/kg/d HDMP while 7 started with 10 mg/kg/d and the other 6 patients could not receive HDMP because they had high WBC levels at the diagnosis.
Summary/Conclusion: Osteonecrosis is one of the most important morbidities among ALL survivors. According to our study most of the patients were found to be >10 years age and mostly standart or high risk patients compatible with the literature. More studies are needed to identify the therapy-related or non-therapy-related risk factors and determine which patients are under risk to prevent this sequelae.

PB1643

SEQUENTIAL USE OF IMMUNOTHERAPY IN REFRACTORY/RELAPSED B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: CLINICAL EXPERIENCE FROM A SINGLE-CENTRE

N.S. Fracchiolla*, M. Sciumè, G. Reda, R. Cassin, A. Freyrie, M.C. Goldaniga, F.I. Grifoni, E. Tagliaferri, A. Cortelezzi

Oncohematology Division, IRCCS Ca' Granda-Maggiore Policlinico Hospital Foundation and University of Milan, Milan, Italy

Background: Despite the significant advances in modern chemotherapy, it remains challenging to treat adult patients with acute lymphoblastic leukemia (ALL). The relapse rate continues to be high, and the outcome at the time of relapse is dismal. In this setting of patients, antibody-based therapies have demonstrated promising single-agent activity.
Aims: We aimed to describe our clinical experience in the sequential use of the bispecific T cell engager blinatumomab and the anti-CD22 antibody-drug conjugate inotuzumab ozoagamicin (IO) in relapsed/refractory B-cell precursor ALL (R/R B-ALL).
Methods: Adult patients with R/R B-ALL initially treated with blinatumomab and then moved to IO for disease reappearance were selected from the institutional database. Clinical data, diagnostic work-up, treatment modalities, and outcomes were extracted.
Results: We identified 3 R/R B-ALL patients who received blinatumomab for a bone marrow relapse after standard chemotherapy. At blinatumomab start, median age was 58 years, male/female ratio was 2:1 and median number of prior chemotherapy lines was 3 (2–4). At diagnosis all patients had a poor-risk disease: 2 patients were Philadelphia chromosome–positive (Ph^+) ALL, while for the remaining one hyperleukocytosis was present at ALL onset and cytogenetic analysis reported 47,XXX [20]. Prior chemotherapies included Hyper-CVAD regimen and pediatric-type therapeutic program proposed by the Northern Italy Leukemia Group; both patients with Ph^+ ALL had already been treated with all available tyrosine kinase inhibitors. Blinatumomab was obtained through an expanded access use and given as continuous IV infusion at a dose of 28μg/m^2/d for 4 weeks, with a 2-week break. The median administered cycle number was 4 (2-5). For all patients complete morphological remission and cytofluorimetric negative minimal residual disease were achieved since the first cycle. Unfortunately, after a median time of 63 days 2 patients had a bone marrow relapse, one of these with CD19- lymphoblasts, while the remaining patient had an extramedullary relapse with lymph-nodes involvement. In all 3 cases blast cells showed CD22s expression by flow cytometry. Only one patient experienced a G2 neurological toxicity which progressively improved with dexamethasone. Expanded access use of IO was started for relapse after blinatumomab treatment. Patients received a combination of vincristine and prednisone to reduce the amount of ALL before IO start. Median time from blinatumomab interruption and IO was 55 days. Patients received IO IV at a dose of 1.8mg/m^2 for the first cycle and then 1.5mg/m^2, divided in three weekly doses, every 3-4 weeks for a median number of 2 cycles (1-4). Again, all 3 patients received complete morphological remission and cytofluorimetric negativity after the first cycle; for the only patient with lymph-node involvement a PET analysis demonstrated absence of active disease. IO was generally well tolerated; 2 patients had a G2 transaminases elevation. Two patients proceeded to allogeneic hematopoietic stem cell transplantation (allo-HSCT); one of this died 4 months after allo-HSCT for ALL progression, while the other one is still alive and in ALL complete remission after 2 months from allo-HSCT. One patient was not candidate to allo-HSCT and we have planned to complete 6 IO cycles.
Summary/Conclusion: This monocentric survey underlined the feasibility and efficacy of sequential use of blinatumomab and IO in R/R B-ALL with the possibility to proceed safely to allo-HSCT.

PB1644

RELAPSES OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN ON PROGRAMMED THERAPY

E. Vaskina[1,*], E. Danilova[1], I. Tataurova[1], E. Butina[2], N. Minaeva[1], V. Rosin[3]

[1]Hematology, [2]Immunohaematology, [3]Pathomorphology, Federal State Institute of Science "Kirov research Institute of Hematology and Blood Transfusion of the Federal Medical and Biological Agency of Russia", Kirov, Russian Federation

Background: Acute lymphoblastic leukemia (ALL) occupies a leading position in the structure of childhood cancer.The use of long-term multimodal therapy allowed achieving high survival rates. However, 15-20% of patients have got the relapses of the disease, which are the cause of failure of treatment of children with ALL.
Aims: To study the frequency of development of relapses of acute lymphoblastic leukemia in children on programmed therapy.
Methods: 247 patients with newly diagnosed ALL were monitored in the children's hematology department in the period from 1991 to 2017. 36 (15,4%) of children at different stages of observation have relapses. The age of patients ranged from 2 till 18 years (median 8,0 years).
Results: In the first acute period 20 (55,5%) patients were treated with protocol ALL-BFM-90; ALL-MB-2002. ALL-MB-2008. ALL-MB-2015-16 (44,4%). The structure of recurrences was as follows: isolated bone marrow relapse-27 cases, the combined-4, isolated ekstramedullar-12 (33,3%) of the relapses classified as very early, 16 (44,4%) early and 8 (22,2%) late. Antireccurence therapy was conducted in 34 patients, the two did not receive treatment due to refusal of parents. Refractory to therapy was recorded in 6 patients, 4 patients died of various complications and progression of the disease before reaching remission. The second complete remission was obtained in 70% of cases (24 patients). Transplantation of hematopoietic stem cells from an related donor carried in 1 patient, ended lethally,1 patient died of various complications during remission, 9-developed a second relapse (3 patients are alive in one-third complete remission).16 (44%) patients are in long remission.
Summary/Conclusion: The frequency of relapses in patients of ALL was 15,4%(36). 44% patients are in long remission.

PB1645

EVALUATION OF DEMOGRAPHIC, CLINICAL AND LABORATORY FINDINGS OF PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA FOLLOWED UP IN OUR CLINIC BETWEEN 2010 AND 2015

M. Solmaz[1], M. Söker[1,*], K. Yılmaz[1], H. Uzel[1], K. Öncel[1], S. Söker[2]

[1]Pediatric Hematology and Oncology, [2]Histology and Embriology, Dicle University Medical Faculty, Diyarbakır, Turkey

Background: Acute Lymphoblastic Leukemia (ALL) is the most common type of childhood cancers with a survival rate up to 80% thanks to multiple chemotherapies.
Aims: This retrospective study aims to screen patients who had been diagnosed with ALL in the Pediatric Hematology-Oncology Department of the Dicle University Faculty of Medicine between 2010 and 2015; and to reveal the mortality rate by evaluating clinical and laboratory findings, immunophenotypic and cytogenetic characteristics, morphologic features of bone marrow, and evaluating chemotherapeutic response.
Methods: The files of 121 patients who had been diagnosed with Acute Lymphoblastic Leukemia (ALL) and who had been treated with TRALL-BFM 2000 and ALL IC-BFM 2009 protocol in the Hematology and Oncology clinic of Dicle University Faculty of Medicine between 2010 and 2015 were screened retrospectively and included in the study.
Results: Of the children included in the study, 70.2% was in the 1-6 age group and 47.9% was living in the rural area. The most common symptoms during admission were fever, fatigue, pallor and loss of appetite. The most common physical examination symptom was lymphadenopathy during admission. Of the patients, 41.3% was A Rh +, 34.7% was 0 Rh + and 13.2% was B Rh +. Of the patients, 65.3% had a white blood cell count of 0-20,000/mm^3, 71.1% had a hemoglobin value of 7 g/dl and above and 67.8% had a platelet count of 100,000/mm^3 and below. t(9,22) and t (4,11) were positive in 3.3% and 0.8% of the patients, respectively. Of the patients, 78.5% was found to be pB-ALL and 21.5% was found to be T-ALL. TRALL-BFM 2000 protocol had been applied in 57% of patients, whereas ALL IC-BFM 2009 protocol had been applied in 43% of patients. The relapse rate of patients was 5.8% and the overall mortality rate was 11.6%. There was no statistically significant difference in terms of gender, white blood cell count, hemoglobin level, platelet count, immunophenotype and mortality. There was a statistically significant difference between the risk group of the patients and the protocol applied and mortality ($p<0.05$). Of those who lost their lives, 57.1% was high risk group (HRG) patients. Of the relapses, 71.4% was T-ALL. And, TRALL-BFM 2000 protocol had been applied in 85.7% of them. There was a statistically significant difference between the immunophenotype and the protocol applied and relapse ($p<0.05$).
Summary/Conclusion: Considering the age, gender, clinical findings and laboratory results, results obtained were similar to the studies in the literature. We identified the factors affecting overall survival and relapse in patients. The mortality and relapse rates were higher in the patients in the HRG. We found that ALL immunophenotype was effective on mortality and relapse rate. The relapse rate of T-ALL patients was higher than that of pB-ALL patients. In addition, the relationship between mortality and relapse and treatment protocol was investigated. We found that mortality and relapse rates were higher in the patients receiving the TRALL-BFM 2000 treatment protocol compared to the ALL IC-BFM 2009 protocol.

PB1646

USE OF BLINATUMOMAB IN MOLECULAR RELAPSE OF AN INFANT, T(4;11) POSITIVE, ACUTE LYMPHOBLASTIC LEUKEMIA

F. Petruzziello[1,*], G. Giagnuolo[1], G. Beneduce[1], G. Cazzaniga[2], G. Muccioli Casadei[3], P. Stellato[1], B. Donnarumma[1], G. Maisto[1], N. Marra[1], G. Sepe[1], C. Montagnaro[1], F. Messina[1], G. Menna[1], R. Parasole[1]

[1]Pediatric Hemato-Oncology, Santobono-Pausilipon Hospital, Naples, Italy, Naples, [2]Department of Paediatrics, Centro RicercaTettamanti University of Milano Bicocca, Fondazione MBBM, Monza, [3]Research Institute SDN- Synlab Group, Naples, Italy

Background: At present, there is no consensus about how to treat infant with molecular relapse of Acute Lymphoblastic Leukemia (ALL) and t(4;11). "*Watch and wait*" strategy is the actual approach until evidence of hematological relapse for which there is no specific validate treatment.
Aims: We describe the case of a 2-year- and 6-month-old boy, with previous diagnosis (March 2016) of infant pro-B ALL, t(4;11) positive, treated according to the AIEOP INTERFANT 2006 Protocol. The patient resulted good prednisone responder on day +8. Minimal residual disease (MRD) was positive both on day +33 and +78. At the end of Induction, the MLL/AF4 rearrangement was negative. After Consolidation (MARMA cycle), MRD was still positive (Marker 1:<1x10^{-4}; Marker 2:<1x10^{-4}). According to protocol, the patient was not eligible for allogenic hematopoietic stem cell transplantation (HSCT) so, he continued therapy with Reiduction (OCTADAD); at the end of this cycle, MRD was still positive. On October 2016, the patient started Manteinance and MRD, at the first four controls resulted negative. On October 2017, it turned positive and MLL/AF4 rearrangement was again detected with negativity of Central nervous system (CNS). According to protocol, we had to monitor the disease course until a possible relapse. However, the new MRD positivity was pathognomonic of oncoming hematological relapse. The efficacy of Blinatumomab in MRD treatment of adult and pediatric ALL is well established. To avoid child's hematological relapse, at very poor prognosis, and to ensure a treatment option that allow the successful of allogenic HSCT, compassionate use of Blinatumomab was purposed.
Methods: In December 2017, written informed consent was obtained and Blinatumomab was started at the dose of 5mcg/m^2 daily for the first 7days of cycle 1. Then, the dose was increased to 15mcg/m^2 daily. To prevent neurological toxicity, prophylaxis with Levetiracetam was started. Two 28-days cycles of Blinatumomb were performed from December 2017 to February 2018. CNS prophylaxis was performed with 2 intrathecal administration of Methotrexate and Prednisone.
Results: MRD was already negative after 15 days of treatment, and remained negative until the end of the first cycle; at the same time, MLL/AF4 rearrangement resulted absent from day +15. The analysis of MRD and MLL/AF4 rearrangement persisted negative after the second cycle. The patient tolerated well the therapy, enjoying a good quality of life; the only side effect was fever during the first day of drug infusion. At the last clinical check, the child was in good general condition and shows no signs and/or symptoms of disease and he is waiting for allogenic HSCT.
Summary/Conclusion: In this case, the use of Blinatumomab alone allowed the patient to achieve complete molecular response. It is important to underline that, the child fastly obtained complete molecular remission with the use of immunotherapy without using chemotherapy. The use of Blinatumomab resulted active and well tolerated so, it was an excellent bridge to transplant. Blinatumomab in a molecular relapse of infant ALL could be an effective alternative to the current "*watch and wait*" strategy, whereas there is not specific validate treatment in case of hematological relapse.

PB1647

ACUTE LYMPHOBLASTIC LEUKEMIA IN PREGNANCY: A CASE REPORT

A. Zhumagaliyeva[1,*], D. Nurgaliyeva[2], L. Karazhanova[1]

[1]Internal Medicine, Semey State Medical University, [2]Hematology, Emergency hospital of Semey, Semey, Kazakhstan

Background: The frequency of cancer and pregnancy is relatively rare, occurring in about one in 1000 pregnancies. The most common tumors diagnosed during pregnancy are cervical and breast cancer followed by melanoma, leukemia and lymphoma. The conducting of a pregnant patient with malignancy is very challenging and requires a multidisciplinary approach.
Aims: Here, we present a case of acute lymphoblastic leukemia in pregnancy.
Methods: A 29-year-old female at 28 weeks gestation presented to the emergency department with a 7-day history of fatigue, fever, bruising along with nose and gum bleeding. Laboratory investigation revealed a white blood cell count of 77.3x10^3/mm^3, hemoglobin 3.8 (g/dl) and platelets 10x10^3/µl.
Results: A bone marrow biopsy revealed precursor B-cell ALL, 93% blasts with BCR-ABL rearrangement (Philadelphia chromosome) by fluorescent *in situ* hybridization (FISH) in 81% of cells and reverse transcriptase-polymerase chain reaction (RT-PCR) detecting BCR-ABL breakpoint fusion. Cytogenetics showed 46,XX,t(9;22)(q34;q11.2) and immunohistochemistry revealed the following: moderately positive for CD10, CD19, CD22, CD34, CD45, HLA-DR and cytoplasmic Tdt while negative for CD3, CD5, CD11b, CD15, CD20, CD33, CD38, CD56, CD71, CD117, kappa and lambda light chain surface antigen. Ultrasound revealed a single living fetus at 28 weeks with weight corresponding to the 39th percentile and normal amniotic fluid. The solution was made to wait until 30 weeks to deliver the baby via Caesarean section due to an operative risk of hemorrhage and sepsis to the mother if delivered while pancytopenic and to give more time for fetal maturity. The patient was started on intravenous HyperCVAD without any dose reduction. At 30 weeks gestation (2 weeks after initiation of induction therapy), a Caesarean section was performed with the delivery of a baby girl with a weight of 1346 grams corresponding to the 26th to 50th percentile for the gestational age. The baby required transient respiratory

assistance for 10 hours post delivery due to cyanosis and poor respiratory effort. On postpartum day 11 the mother was started on HyperCVAD combination chemotherapy and dasatinib at 50 mg twice a day for 14 days given with each cycle of chemotherapy. She was also given prophylactic intrathecal chemotherapy. Cerebrospinal fluid analysis done after delivery was negative for central nervous system implication. A bone marrow biopsy accomplished after completion of cycle one of high-dose cytarabine and methotrexate revealed normal cytogenetic. BCR-ABL was negative by FISH and PCR detected 0.005% residual BCR-ABL. No blasts in bone marrow biopsy were found and flow cytometry was negative. Currently she is awaiting allogenic bone marrow transplant. The baby continues to do well and has reached normal developmental milestones at 18 months of age.
Summary/Conclusion: Our case represents the feasibility of treating ALL with HyperCVAD during the third trimester of pregnancy with favorable outcomes.

PB1648

PRECURSOR B AND T CELL MALIGNANCIES IN ADOLESCENTS AND YOUNG ADULTS IN QATAR;EPIDEMIOLOGY AND CLINICAL FEATURES

R.Y. Taha*, A.Y. Ellahie, H. El Omri, O.M. Ismail, G. Pittari, H. Elsabah, I. Al Hijji

Hematology-BMT, Hamad Medical Corporation, Doha, Qatar

Background: Precursor B and T cell lymphoid malignancies represent heterogeneous disorders in terms of natural course and outcome especially when comparing pediatric and adult populations, such variation reflects the different biology between these age subtypes. The definition of adolescents and young adults (AYA) is itself controversial and varies in trials .It can be up to age of 40 or even 50. AYA patients with acute lymphoblastic leukemia (ALL) have outcomes that are historically inferior to those of children largely due to the increase frequency of adverse genetic alterations in adults; like the higher frequency of Philadelphia chromosome-positive or the recently described Philadelphia like ALL.
Aims: To report the clinical and prognestic factors of AYA patients diagnosed with precursor lymphoid malignancy in Qatar and establish the age cutoff at which adverse clinical and biological findings are observed.
Methods: This is a retrospective study in the National Centre for Cancer Care and Research which is the only cancer care provider for adults in Qatar. Patients diagnosed with B or T cell ALL or lymphoblastic lymphoma older than or equal 14 year old and who were diagnosed between January 2013 and December 2017 were included. We defined AYA as patients diagnosed at 39 year of age or younger (14-39 year old). we studied the occurence of adverse clinical and cytogenetic variables and compared them in AYA and non AYA groups. White blood cell count (WBC) cutoff at diagnosis of $>100x10^3$/mcl for T cell malignancy and $>30x10^3$/mcl for B cell subtype were considered adverse.
Results: A total of 83 patient were identified,74 males and 9 females (the significant gender variability is due to Qatar demographics were male industrial task force constitute most of the population). Median age at diagnosis was 30 (range15-71). 61 patients were in the AYA group (88% of the cohort) and 22 patients were non AYA. B cell lymphoblastic lineage contributed to 51% of ALL diagnosis in the AYA group and 48% were T cell malignancy. AYA patients with T cell lineage ALL had higher WBC at diagnosis when compared to non AYA (P=0.034).No significant difference in WBC between both groups in B cell subtype. Moreover,AYA patients had significantly lower frequency of (Ph+) ALL than non AYA.(P=0.004), other cytogenetic abnormalities were similar in both groups.
Summary/Conclusion: Our cohort identified 39 year of age as the cutoff at which prognosis differs. There was lack of favorable cytogenetics in all the cohort and predominence of (Ph+) ALL in patients 40 year old or older. Moreover AYA patients with T lymphoblastic malignancy presented with more bulky disease and higher risk features.

PB1649

NOSOCOMIAL AMPHOTERICIN B-RESISTANT OSTEOMYELITIS DUE TO ASPERGILLUS FLAVUS IN AN ADOLESCENT WIT ACUTE LYMPHOBLASTIC LEUKEMIA

N.A. Erdogmus[1], M.A. Atalay[2], A.N. Koç[3], A. Ozcan[1], S. Saracoglu[4], T. Patiroglu[5,*], M. Karakukcu[1], I. Kafadar[6], K. Deniz[7], E. Unal[1]

[1]Division of Pediatric Hematology, Oncology & HSCT Center, Department of Pediatrics, [2]Division of Mycology, Department of Medical Microbiology, Erciyes University, Faculty of Medicine, [3]Division of Mycology, Department of Medical Microbiology, Erciyes University, Faculty of Medicine, Kayseri, [4]Division of Pediatric Radiology, Department of Radiology, Erciyes University, Faculty of Medicine, [5]Division of Pediatric Hematology, Oncology & HSCT Center, Department of Pediatrics, Erciyes University, Faculty of Medicine, Kayseri, [6]Department of Orthopedics, [7]Department of Pathology, Erciyes University, Faculty of Medicine, Kayseri, Turkey

Background: Aspergillus flavus is a rare but increasingly common mold fungus. Especially in hematologic malignancies, it is seen as a factor in the neutropenic period when the immunity system is suppressed; and mortality is high in these patients.
Aims: We aimed to present a case of by Aspergillus flavus osteomyelitis with in an adolescent with acute lymphoblastic leukemia (ALL).
Methods: A 14-year-old male patient with ALL was hospitalized with neutropenia on the 30th day of treatment. On the 12th day of neutropenia, treatment with amikacin, meropenem and liposomal amphotericin B was initiated. In this period, the patient developed swelling of the left knee joint. Magnetic resonance imaging (MRI) showed that may be compatible with abscess and osteomyelitis in left femur distal metaphyseal. The patient was operated and intraarticulary washing was performed, the soft tissue / bone curettage materials were sent to pathology and microbiology laboratories. Pathology was again reported as fungal osteomyelitis. Aspergillus flavus was grew from the tissue samplesThe minimum inhibitor concentration (MIC) values for amphotericin B, caspofungin, voriconazole, anidulafungin, itraconazole and posaconazole were >2μg/ml, 0.125μg/ml, 0.064μg/ml, 0.002μg/ml, 0.25μg/ml, and 0.125μg/ml. Amphotericin B in the patient was discontinued and voriconazole was started because the A. flavus strain was resistant to amphothericin B. Progression was seen in the patient's knee MRI with complaints after the second week of voriconazole treatment. For the third time, the patient was treated with curettage and washing with voriconazole. Aspergillus-compatible fungi were seen in specimens taken prior to washing with voriconazole and sent to the mycology laboratory. Although galactomannan antigenemia tests in blood samples were negative during neutropenia, galactomannan antigenemia test was positive in the last joint fluid and tissue sample. However, there was no reproduction in culture. Caspofungin was added to the patient's treatment.
Results: Fungal infections and especially osteomyelitis related to Aspergillus species should be considered in children with ALL and neutropenia.
Summary/Conclusion: It should be kept in mind that A.flavus, which is seen more rarely, may also be resistant to the agent and amphotericin B as a Nosocomial fungal infecion

PB1650

VENA CAVA SUPERIOR SYNDROME IN CHILDREN WITH MEDIASTINAL TUMORS: SINGLE CENTER EXPERIENCE

T. Patiroglu[1,*], A. Ozcan[2], E. Unal[1], M. Karakukcu[1], A. Coskun[3]

[1]Pediatric Hematology, [2]Erciyes University Medical Faculty, [3]Pediatric Radiology, Erciyes University Medical Faculty, Kayseri, Turkey

Background: Vena cava superior syndrome (VCSS) comprises various symptoms of compression of vena cava superior. The resulting increased venous pressure in the upper body may cause edema of the head and neck associated with cyanosis, pletore and distended subcutaneous vessels.
Aims: VCSS is rare in childhood. Therefore, we planned this retrospective study.
Methods: The retrospective study was carried out on the children with mediastinal tumors in the department of pediatric hematology-oncology, Erciyes University Medical School, from january 2010 to december 2017. Diagnostic procedures included hematological investigations, chest radiography, thoracic computed tomography, echocardiography and lymph node or mediastinal biopsy.
Results: 19 (five were female) of 41 patients with mediastinal tumors had VCSS. Mean age of the patients with VCSS was 8.57 years (range:1-17 years). Diagnosis included Hodgkin's disease (HD) in 7,non-Hodgkin's lymphoma (NHL) in 6, acute T- lymphoblastic leukemia in 5, neuroblastoma and anaplastic round cell sarcoma in one each respectively. All the 19 patients had dyspnea, venous distention and mediastinal widening. Two patients with NHL had bilateral pleural effusion. All patients received intravenous corticosteroids (0.6 mg/kg dexamethasone). Furthermore, the patient with anaplastic round cell sarcoma recevied emergency radiotherapy. All patients received chemothepy and followed up in our PHO clinic. No patients died because of VCSS.
Summary/Conclusion: Compression of structures in the superior mediastinum is known as VCSS that it is a medical emergency requiring urgent treatment. In this retrospective study, we found that the most common cause of VCSS was HD as different from literature.

PB1651

BLASTS WITH ABUNDANT CYTOPLASMIC GRANULES: A CASE REPORT OF REFRACTORY GRANULAR ACUTE LYMPHOBLASTIC LEUKEMIA WITH HETEROZYGOUS DELETION OF IKZF1

L. Peng*, L. Ye, Y. Jiang

Department of Laboratory Medicine, West China Second University Hospital, Sichuan University, Chengdu, China

Background: The finding of cytoplasmic granules in blasts is one of main morpholoic features to distinguish AML from ALL. Rarely, significant and thick granules may be presented in cytoplasm of blasts in ALL, known as granular acute lymphoblastic leukemia, indicating a worse prognosis. IKZF1 deletion is an independent prognostic marker in childhood B-cell precursor acute lymphoblastic leukemia.
Aims: Morphology and cytochemical evaluation of peripheral blood or bone marrow aspirate smears are still main examinations to diagnose hematological diseases. To reduce the misdiagnosis and improve the prognosis of granular ALL, it is neccsary to understand its features differed to AML and classic ALL.
Methods: Morphology, flow cytometry, leukemia related fusion genes screen and karyotype analysis were applied in this 12-year-old boy whose white blood cell of peripheral blood rise obviously. csary to understand its features differed to AML and classic ALL.
Results: The peripheral blood and bone marrow blasts showed abundant of cytoplasmic granules(panel a and b; original magnification *1000, Wright-Giemsa stained) and were positive for CD10, CD19, CD20, CD34, HLA-DR, cCD79a, terminal deoxynucleotidyltransferase and CD22(partial). The heavily granulated lymphoblasts in the blastic population of bone marrow aspirate was 53.0%. These blasts were positive for Periodic Acid-Schiff reaction(panel c) but completely negative for myeloperoxidase(panel d) by cytochemical staining. His FAB subtype of morphology was L2. More than 30 leukemia fusion genes were screened and the results were negative. A heterozygous deletion of Exon 4-8 in IKZF1 gene was found. Cytogenetic analysis brought normal karyotype 46 XY. His prognosis was poor with VDLP chemotherapy (vincristine sulfate, daunorubicin, pamnidase, prednisone acetate, dexamethasone) induced chemotherapy was given successively.

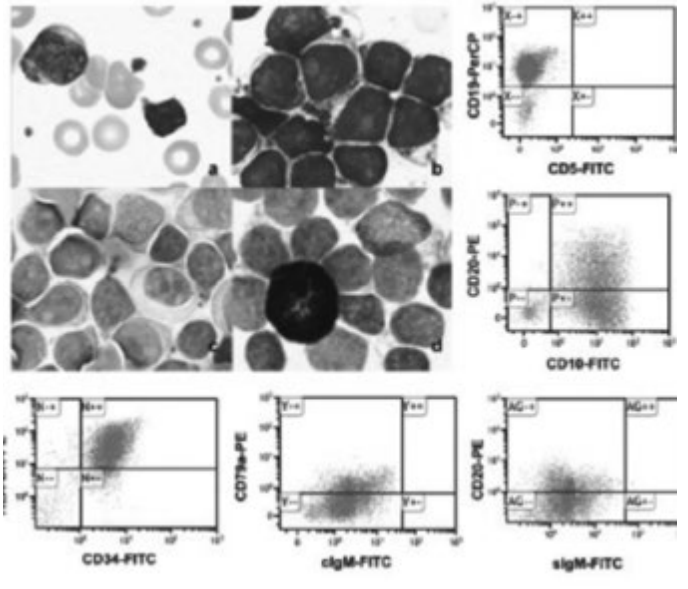

Figure 1.

Summary/Conclusion: Granular ALL is a rare morphologic subtype of ALL which was reported in just over 100 cases. Those large cytoplasmic granules closely mimicked heavily granulated myeloblasts in acute myeloid leukemia. The granules of granular ALL are thought to be the result of dysplastic organella formation, fusion, or degeneration, such as multivesicular bodies or Gall Bodies. Granular ALL may not only cause problematic distinction from myeloid differntiation but also associated with a worse prognosis when compared to other ALL patients. This patient was in a high risk of bad prognosis for granular ALL subtype and heterozygous deletion of IKZF1. We should keep in mind since this ALL subtype may easily be mistaken for acute myeloid leukemia during morphologic evaluation. This potentiality further emphasizes the importance of molecular diagnosis and immunophenotype in the characterization of leukemias.

PB1652

INTRAVENOUS IMMUNOGLOBULIN REPLACEMENT THERAPY IN ACUTE LEUKEMIA PATIENTS

A. Bahadır[1,*], E. Erduran[2], F. Orhan[3]

[1]Divison in Pediatric Hematology-Oncology, [2]Division in Pediatric Hematology-Oncology, [3]Division in Pediatric Allergy-Immunology, Karadeniz Technical Univercity, Trabzon, Turkey

Background: Intravenous Immunoglobulin (IVIG) is widely used in primary immune syndrome cases due to the high risk of infection associated with hypogammaglobulinemia. For the last two decades, immunoglobulin replacement therapy has been used to treat lymphoproliferative diseases which cause secondary antibody deficiency, and also to treat hematological diseases, such as multiple myeloma. In malignancies, iatrogenic hypogammaglobulinemia is associated with chemo-immunotherapy regimens (anti-CD20 therapy), immunosuppressive therapies (steroid, mycophenolate mofetil) and chemotherapeutic agents (cyclophosphamide, fludarabine), and in such patients, serious infection attacks may occur that are associated with the dysregulation of the immune system and hypogammaglobulinemia.
Aims: This study involves the initiation of IVIG prophylaxis in pediatric patients diagnosed with acute lymphoblastic leukemia (ALL) who were treated and followed-up by our team, who had frequent and life-threatening infections and who had decreased immunoglobulin levels when compared to their pre-treatment levels. This report contains the results of our preliminary study.
Methods: Pediatric hematology-oncology patients diagnosed with ALL between 2010 and 2017, presenting with no immunodeficiency at the time of diagnosis and receiving treatment based on the St. Jude Total XV chemotherapy protocol were enlisted in this study. Repetitive infection attacks, multiple neutropenic infection incidences per month, chemotherapy interruptions of longer than one month, age-adjusted drops in immunoglobulin levels below -2SD or by more than half of the immunoglobulin levels at the time of diagnosis were accepted as IVIG prophylaxis initiation criteria. Regular IVIG prophylaxis of 0.5 gr/kg at three-week intervals was initiated for these patients.
Results: Of the five patients, who were enrolled in the program, three were male and two were female. The patients were followed up with a high-risk ALL diagnosis, and that the findings showed that one patient had T-cell ALL and the others had B-cell ALL. The age range of patients was 10 months to six years. It was observed that incidences of neutropenic infection in patients that require hospitalization following prophylaxis decreased, and their treatment continued without interruption.
Summary/Conclusion: After IVIG prophylaxis, incidences of neutropenic infection in our patients were seen to decrease, and as a result of this decrease, our patients were provided with regular and continuous treatment. There are very few studies in the literature that involved IVIG prophylaxis in pediatric leukemia patients with secondary hypogammaglobulinemia. Being a preliminary study at its current stage, the collection of data related to this study is continuing.

PB1653

HEPATITIS C VIRUS INFECTION-ASSOCIATED MACROPHAGE ACTIVATION SYNDROME IN A PEDIATRIC PATIENT WHICH UNDERWENT HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ACUTE LYMPHOBLASTIC LEUKEMIA

G. Laccetta[1], M. Menconi[1,*], S. Bernasconi[1], G. Casazza[1], M. Brunetto[2]

[1]Pediatric Department, [2]Hepatology department, Azienda Ospedaliera Universitaria Pisana, Pisa, Italy

Background: Hepatitis C virus (HCV) infection causing Macrophage Activation Syndrome (MAS) in pediatric population has never been reported previously.
Aims: To describe a case of MAS associated with HCV infection in a pediatric patient.
Methods: We describe a case of MAS associated with HCV infection in a girl with acute lymphoblastic leukemia (ALL) which underwent hematopoietic stem cell transplantation (HSCT) and attained viral suppression with ledipasvir/sofosbuvir.
Results: A 13-year-old Ukranian girl with pro-B cell ALL t(4;11)(q21-q23), and HCV-genotype 1 infection (HCV-RNA 629.500 IU/mL) received chemotherapy according to the AIEOP ALL 2000 protocol and a myeloablative allogeneic HSCT from MUD; the engraftment was at day +19 after HSCT (neutrophils 580/μL). At day +22 the patient presented epigastric pain, icteric skin, fever (39°C), vomiting and diarrhea. Blood exams showed

progressive cytopenia (WBC 670/µL, N 120/µL, Hb 9.0 g/dL, PLT 22.000/µL), hypofibrinogenemia (fibrinogen 100 mg/dL), ALT 283U/L, AST 323U/L, hyperbilirubinemia (total bilirubin 11.0 mg/dL, direct bilirubin 10.4 mg/dL), hypertrigliceridemia (tryglicerides 460mg/dL), hyperferritinemia (ferritin 67.769 ng/mL). Blood culture was negative; CMV, EBV Adenovirus-PCR were negative; serology for hepatitis A and HBV-DNA were negative but HCV-RNA was >100.000.000 IU/mL. Clinical examination and abdominal ultrasound revealed hepatosplenomegaly and BOM showed CR for ALL but increased histiocytes and macrophages with hemophagocytosis; no signs of GvHD. MAS secondary to HCV infection was suspected and treatment with high-dose corticosteroids, cyclosporine-A and ledipasvir/sofosbuvir was initiated. At day +38 after HSCT fever disappeared, laboratory exams improved and quantitative PCR for HCV-RNA decreased (19.114.275 IU/mL); after 4 weeks of antiviral treatment HCV-RNA was negative. At day +82 the patient presented fever (39.1°C), headache, left facial droop, right convergent strabismus, aphasia, disequilibrium, gait unsteadiness, absence of deep tendon reflexes, clumsiness of both hands and drowsiness. Brain-MRI showed multiple supra and infratentorial areas of mass-like appearance with central necrosis surrounded by a hyperintense ring; a broad laboratory workup confirmed cerebral reactivation of MAS. Thus, the dose of intravenous corticosteroids was increased and intravenous mannitol was administered to reduce intracranial pressure; phenobarbital and carbamazepine were initiated. The antiviral therapy with ledipasvir/sofosbuvir was discontinued at day +200 (for a total of 6 months). At follow-up, ten months after HSCT the girl developed chronic kidney failure. Twelve months after HSCT the patient is alert, fully oriented, with fluent speech and normal language; she has a permanent left facial droop, a diffuse left lower extremity weakness, reduced deep tendon reflexes and steppage gait on her left. Brain-MRI shows neither new lesions nor an enlargement of the previously described areas; the last quantitative PCR for HCV-RNA is negative and BOM aspirate shows CR for ALL and MAS with full donor chimerism.

Summary/Conclusion: In our case MAS was probably triggered by HCV and the early treatment with ledipasvir/sofosbuvir revealed to be highly effective for HCV infection and well tolerated by the patient; despite viral clearance, HLH persisted over time with permanent neurologic impairment and chronic kidney failure.

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PB1654

A QUARTER OF NRAS POSITIVE PATIENTS CARRY MULTIPLE POINT MUTATIONS, EACH OF THEM LOCALISED SEPARATELY IN DIFFERENT ALLELES

A. Durinikova[1,2,*], I. Jeziskova[2], A. Folta[2], D. Al Tukmachi[1], Z. Kosarova[1], M. Culen[1,2], D. Dvorakova[1,2], N. Tom[1], P. Cetkovsky[3], P. Jindra[4], T. Szotkowski[5], P. Zak[6], J. Mayer[1,2], Z. Racil[1,2]

[1]Masaryk university, [2]University hospital Brno, Brno, Czech republic, Brno, [3]Institute of Hematology and Blood Transfusion, Prague, [4]University Hospital Pilsen, Pilsen, [5]University Hospital Olomouc, Olomouc, [6]University Hospital Hradec Kralove, Hradec Kralove, Czech Republic

Background: With frequency of 11-30% the *NRAS* mutations represent one of the most frequently reported mutations in patients with *de novo* acute myeloid leukemia (AML). Point mutations of the gene are heterozygous and exclusively affect glycine hotspot codons 12, 13 (exon 2) and 61 (exon 3). In some AML patients more than one *NRAS* mutation can be identified. The exact number of patients with multiple point mutations is unclear as well as it is not known whether the multiple point mutations are localised on the same allele of one clone or are carried by separate clones.
Aims: To describe the frequency and type of *NRAS* mutations and to analyse the polyclonality of mutations in selected patients.
Methods: NGS libraries were prepared from peripheral blood samples from the time of diagnosis of 258 consecutive *de novo* AML patients (median age 54 years; range 19-72) by ClearSeq AML panel (Agilent Technologies) and sequenced on NextSeq machines (Illumina). *NRAS* positive samples with a variant allele frequency (VAF) ≥1% according to NGS were verified by *NRAS* StripAssay (ViennaLab). The clonal background of multiple point *NRAS* mutations was analysed using TA Cloning Kit (Thermo Fisher Scientific). Sanger sequencing was used to analyse bacterial clones.
Results: In the studied cohort of AML patients the presence of *NRAS* mutation was identified in 58/258 (22.5%) patients analysed by NGS method. *NRAS* positivity was confirmed in all samples (58/58, 100%) by hybridization strips. One mutation was detected in 42/58 patients (72.4%), in remaining 16/58 (27.6%) patients multiple point mutations were revealed. Two point mutations were detected in 12/58 (20.7%), 3 mutations in 3/58 (5.2%) and 4 mutations in 1/58 (1.7%) patients. In total, 13 different gene aberrations were identified. The most frequent mutations were G12D (23/58 patients; 39.7%), G12S (14/58; 24.1%) and G13D (12/58; 20.7%). The polyclonality of *NRAS* mutations was analysed in two patients. First patient carried three mutations (G12D, G12S, G13D) in two different codons, second patient carried two mutations (G13D, Q61H) in two different exons. Analysis of 111 bacterial clones of the first patient and 112 clones of the second patient did not reveal any double-locus mutants with two mutations in the same allele. Each of the identified mutations was separate in different alleles indicating the presence of more clones.
Summary/Conclusion: More than one fifth of AML patients carry somatic missense mutation in *NRAS* gene and approximately a quarter of them has multiple point mutations. Our results show that each of the multiple point mutations is present separately in the allele and thus likely forms independent clone.

PB1655

ANTITUMOR ACTIVITY OF BIBR1532, A SELECTIVE SMALL MOLECULE INHIBITOR OF TELOMERASE, IRRESPECTIVE OF P53 STATUS OF TUMOR CELLS

A. Pourbagheri-Sigaroodi[1,*], D. Bashash[2], S.H. Ghaffari[3]

[1]Department of Biotechnology, faculty of Advanced Sciences and Technology, Pharmaceutical sciences branch, Islamic Azad University (IAUPS), [2]Department of Hematology and Blood banking, School of Allied Medicine sciences, Shahid Beheshti University of Medical Sciences, [3]Hematology, Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic Of

Background: The interweaving between telomerase and immortalization of tumor cells steered treatment strategies into an endless path of targeted therapies. For the nonce and among the overabundance of promising inhibitors, exploitation of potent molecules targeting telomerase turns to be intensively encouraging.

Aims: To assess the anti-tumor effect of telomerase inhibition in cancer, a panel of human tumor cells spanning from solid tumors to hematologic malignancies was treated with the different concentrations of small molecule inhibitor of hTERT, BIBR1532.
Methods: MTT, Trypan blue, annexin/PI staining, cell cycle analysis, caspase-3 activity, and cell-based NF-κB phosphorylation assays coupled with analysis of gene expression by using quantitative real-time PCR were applied to examine the effects and molecular mechanisms of action of BIBR1532.
Results: We found that BIBR1532 exerted a potent cytotoxic effect on all the tested human cancer cells; however, as compared with leukemic cells, solid tumor cells were more resistant to the inhibitor. By investigating the relative sensitivity of leukemic cells to BIBR1532, we failed to identify any relationship between p53 status and cell response to the inhibitor. Our results also indicated that telomerase inhibition using BIBR1532 resulted in a considerable growth suppressive effect in APL-derived NB4 cells, as the most sensitive cell line, mostly through p21-mediated G1 arrest coupled with a caspase-3-dependent apoptosis via suppression of NF-κB. Moreover, the resulting data showed that combination of BIBR1532 and ATRA produced synergistic anticancer effects in mutant p53-expressing NB4 cells.
Summary/Conclusion: Our study not only indicated the potential application of BIBR1532 in both wild-type and deficient p53-expressing cells but also outlined the therapeutic efficacy of the inhibitor as either single agent or in combination with ATRA in APL.

PB1656

INHIBITION OF DNA METHYLTRANSFERASES BY VOLASERTIB IN HMA RESISTANT CELL LINES

S.-H. Ko*, E.-H. Hur, B.-K. Goo, J. Moon, E.-J. Choi, H.-S. Park, K.-H. Lee, J.-H. Lee, J.-H. Lee

Department of Hematology, Asan Medical Center, Seoul, Korea, Republic Of

Background: Resistance to hypomethylating agents (HMA) in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) is a concerning problem. We established HMA resistant cell lines with overexpression of DNA methyltransferases (DNMTs), which were not suppressed by azacitidine or decitabine. Polo-like kinase 1 (PLK1) is a key cell cycle modulator and is associated with regulation of the PIK3/Akt pathway, which was reported to stabilize DNMTs.
Aims: We evaluated whether volasertib, a PLK inhibitor, might overcome HMA resistance through regulation of PIK3/Akt pathway and DNMTs.
Methods: Cell viability was determined in HMA resistant cell lines (MOLM/AZA-1 and MOLM/DEC-5) and MOLM-13. Specific antibodies including phosphorylated-histone H3 (PHH3), caspase 3, PARP, and XIAP were used to evaluate apoptosis or DNA damage. We also analyzed phosphorylated-Akt (p-Akt), Akt, and DNMTs (DNMT1, 3A, and 3B) by immunoblot assay.
Results: Volasertib effectively inhibit proliferation of MOLM/AZA-1 and MOLM/DEC-5 cells as well as MOLM-13 cells in a dose dependent manner. It also showed superior anti-leukemic effects compared to cytarabine, azicitidine or decitiabine. Volasertib showed a dose dependent increased expression of PHH3, caspase 3, and PARP, and decrease of XIAP. Treatment of volasertib caused decreased expression of p-AKT and all DNMTs (DNMT1, 3A, and 3B). GDC-0941, a PI3K inhibitor, suppressed DNMT1 and DNMT3A as well as p-Akt, but it could not decrease DNMT3B. A combination of volasertib and decitabine showed synergistic effects in MOLM/DEC-5. Anti-proliferative effects of volasertib were sustained in *ex vivo* AML or MDS samples.
Summary/Conclusion: In our HMA resistant models, volasertib effectively inhibited all DNMTs. DNMT1 and 3A seemed to be suppressed via regulation of PI3K/Akt, but suppression of DNMT3B by volasertib might have separate mechanisms. Our data suggest that volasertib with or without decitabine has potential role in overcoming the HMA resistance in patients with AML and MDS.

PB1657

CD44-HYALURONAN INTERACTION INDUCES VLA-4 CLUSTER FORMATION LEADING TO ENHANCED ADHESION OF AML CELLS ON VCAM-1

J.C. Gutjahr[1,2,*], E. Bayer[1,2], X. Yu[3], J.M. Laufer[4], A. Chigaev[5], D.F. Legler[4], R. Greil[1,2], L. Pleyer[1,2], V. Orian-Rousseau[3], T.N. Hartmann[1,2]

[1]Third Medical Department with Hematology, Medical Oncology, Hemostaseology, Infectious Diseases, and Rheumatology, Oncologic Center, Paracelsus Medical University, [2]Cancer Cluster Salzburg, Salzburg Cancer Research Institute-Laboratory for Immunological and Molecular Cancer Research, Salzburg, Austria, [3]Karlsruhe Institute of Technology, Institute of Toxicology and Genetics, Karlsruhe, Germany, [4]Biotechnology Institute Thurgau, University of Konstanz, Kreuzlingen, Switzerland, [5]Department of Pathology and Cancer Center, University of New Mexico, Albuquerque, NM, United States

Background: The bone marrow niche is critical for maintenance and retention of normal and malignant stem and progenitor cells. Key factors of this process are the adhesion molecule CD44 and the integrin VLA-4, a CD49d/CD29 heterodimer. CD44 comprises a glycoprotein family, which is tightly regulated by alternative splicing of up to 10 exons, thereby influencing the binding capacity of the ligands, particularly hyaluronan. In AML, CD44 has early been suggested as a marker for poor prognosis and leukemia initiating cells but how CD44 standard and variant forms interact with other adhesion molecules and shape the tumor environment is still not understood well.
Aims: Here we aimed to identify the functional interplay between the homing receptors CD44 and VLA-4 in AML primary patient samples as well as AML cell lines and elucidate the influence of therapeutic kinase inhibitors on the signaling cascades involved in the CD44/VLA-4 interaction, which may influence drug resistance and AML engraftment.
Methods: We employed flow cytometry for surface phenotyping of CD44, CD44v6, and CD49d on primary patient samples, and several AML cell lines. Composition of all CD44 variants was further screened by reverse transcription-PCR. Hyaluronan binding capacity was determined by flow cytometric assays and videomicroscopic adhesion assays under shear flow. VLA-4 binding capacity was determined in adhesion assays under shear flow on VCAM-1 substrates. VLA-4 activation was further studied by cytometrical assays determining integrin affinity in real-time and integrin clustering was analyzed by immunofluorescence and quantified using a confocal microscope. Signaling cascades were approached by using a range of src kinase, CD45, PI3K and other kinase inhibitors, among them midostaurin. Short-term adoptive transfer experiments of AML cells in immunodeficient donor mice were used to investigate the individual as well as synergistic role of CD44 and VLA-4.
Results: We demonstrate that CD44, CD44v6 and CD49d are expressed on primary AML cells and on AML cell lines OCI-AML3 and KG-1a at comparable amounts. Hyaluronan binding of AML cells was constitutive, suggesting an activated phenotype, dependent on functional CD44, which correlates with CD44v6 expression. Stimulation of CD44 via hyaluronan induced VLA-4 activation in a specific manner involving CD45, Src kinases, PI3-kinase, and could also be inhibited by midostaurin. CD44-induced inside-out activation of VLA-4 resulted in enhanced adhesion of AML cells on VCAM-1, which was mechanistically based on integrin clustering but not affinity regulation. Pathophysiological consequences are currently investigated using murine models engrafted with primary AML samples.
Summary/Conclusion: Based on our findings we propose that CD44-hyaluronan binding interactions promote clustering of VLA-4, which increases its adhesiveness to VCAM-1, resulting in supportive bone marrow lodgment of leukemia cells. Collectively, our investigations provide a mechanistical description of a novel CD44 function in acute myeloid leukemia, *i.e.* activation of VLA-4, required for leukemia cell recirculation and engraftment in the bone marrow environment.

PB1658

HISTONE DEACETYLASE 1 INDUCED BY NEDDYLATION INHIBITION CONTRIBUTES TO DRUG RESISTANCE IN ACUTE MYELOGENOUS LEUKEMIA

L. Wang[1,*], X.-W. Bi[2], Y.-Z. He[1], Q.-Y. Lai[1], J.-H. Wang[3]

[1]Hematology, ZhuJiang Hospital, Southern Medical University, [2]Sun Yat-sen University Cancer Center, [3]Hematology, GuangDong General Hospital, Guangzhou, China

Background: Neddylation, mediated by an ubiquitin-like molecule, Nedd8 (neural precursor cell expressed developmentally down-regulated 8), plays an important role in the regulation of protein function turnover. The role of neddylation of histone deacetylase 1 (HDAC1) in drug resistance of acute myelogenous leukemia (AML) cells remains unclear.
Aims: This study aimed to investigate the function and mechanism of neddylation of HDAC1 underlying drug resistance of AML cells.
Methods: The expression of HDACs (HDAC1–11) in five cases of refractory AML was compared to that of five cases of remission AML. In the attempt of identify the main role of HDAC1 in the drug resistance of AML, the nondrug-resistant AML cell lines (HL-60 and K562) and primary BMCs of remission AML patients were transfected with pcDNA-HDAC1 to stably express HDAC1. The effects of HDAC1 on the ADM resistance of AML

cells were assessed by the cell viability, apoptosis and ADM-releasing index. Next, we knocked down HDAC1 via the specific RNAi sequence for HDAC1 in HL-60/ADM, K562/A02, and primary BMCs of refractory AML patients and examined the effect of HDAC1 silencing on ADM resistance. To detect the potential mechanism underlying the higher level of HDAC1 in drug-resistant AML cells, we first compared the differential expression of HDAC1 in nondrug-resistant AML cells and drug-resistant AML cells incubated with cyclohexane (CHX), a protein synthesis inhibitor. We next sought to determine the mechanism in the degradation inhibition of HDAC1 protein in drug-resistant AML cells. Finally, an AML cell xenograft mouse model was established and used to evaluate the effect of HDAC1 on the tumor growth in the presence of ADM and Farydak treatment.
Results: The results showed that HDAC1 was significantly upregulated in refractory AML patients, as well as in drug-resistant AML cells (HL-60/ADM and K562/A02). Intracellular HDAC1 expression promoted adriamycin (ADM) resistance of HL-60, K562, and primary bone marrow cells (BMCs) of remission AML patients as shown by increasing cell viability and ADM-releasing index, inhibiting cell apoptosis. Moreover, HDAC1 protein level in AML cells was regulated by the Nedd8-mediated neddylation and ubiquitination, which further promoted HDAC1 degradation. In vivo, HDAC1 overexpression significantly increased ADM resistance; while HDACs inhibitor Farydak markedly improved the inhibitory effect of ADM on tumor growth. Furthermore, HDAC1 silencing by Farydak and/or lentivirus mediated RNA interference against HDAC1 effectively reduced ADM resistance, resulting in the inhibition of tumor growth in AML bearing mice.
Summary/Conclusion: In summary, our findings suggested that HDAC1 contributed to the multidrug resistance of AML and its function turnover was regulated, at least in part, by epigenetic modifications, including neddylation and ubiquitination.

PB1659

EARLY REDUCTION OF WT1 TRANSCRIPT LEVEL DURING INDUCTION CHEMOTHERAPY PREDICTS FOR LONGER RELAPSE-FREE AND OVERALL SURVIVAL IN ACUTE MYELOID LEUKEMIA

L. Girshova[1,*], I. Budaeva[2], E. Ovsiannikova[2], K. Bogdanov[3], T. Nikulina[3], J. Mirolubova[3], D. Motorin[1], A. Zaritskey[1]

[1]hematology, [2]Federal Almazov North-West Medical Research Centre, Saint Petersburg, Russian Federation, [3]Clinical diagnostic laboratory, Federal Almazov North-West Medical Research Centre, Saint Petersburg, Russian Federation

Background: Intensive chemotherapy allows to obtain complete remission (CR) rates ranging from 50 to 80% in adult patients with acute myelogenous leukemia (AML), but relapses still occur in 40–50% of cases. The cause of relapse is the persistence of tumor clones during therapy. The early response to the first course of chemotherapy is a marker of high chemosensitivity. It was shown by AMLCG that bone marrow blast level on day 14th of 1st chemotherapy cycle is predictive for remission rate, OS, RFS. RQ-PCR is much more sensitive than morphology for the identification and quantification of small blast populations in the bone marrow. The Wilms' tumor gene 1 (*WT1*), which is overexpressed in more than 90% of AML, is a useful marker for monitoring MRD. Quantification of *WT1* transcript level in bone marrow (BM) at day 14 induction chemotherapy could more precisely discriminates patients at different risks of relapse than the number of blast cells.
Aims: To determine the clinical significance of *WT1* overexpression level at the day 14th of the first chemotherapy cycle.
Methods: 51 *de novo* AML pts median age 42,7 (range from 16 to 70) with overexpression *WT1* were included in the study. «7+3» and «FLAG» were used for remission induction. Median of follow up was 18 mo. Bone marrow was aspirated prior to the start of chemotherapy and on the 14th day of induction treatment. *WT1* expression was evaluated by the *WT1* ProfileQuant (protocol EAC) kit (IPSOGEN) following the manufacturer's instructions. A value more than 250 *WT1*/10^4 copies ABL was considered abnormal after being compared with samples from healthy donors.
Results: 86,3% (44/51) pts had AML remission. Median of *WT1* level on the 14th day was 617copies/10^4 ABL (range from 3,7 to 19257,7). The remission rate did not depend on the achievement of normal expression of *WT1* on day 14 (p=0.11). However, there was a significant difference in values of *WT1* at day 14±3 in the patients with and without CR (740.0 *vs* 4616.21 copies/10^4 ABL, p=0.0051). Pts with early (less than 6 mo) relapse had overexpression *WT1* at day 14 (>250 WT1/10^4 copies ABL) (88,9% *vs* 0%, *p=0,001* Fisher exact test, two tailed, 100% sensitivity, 78% specificity). RFS and OS were more durable in pts with *WT1* less than 250 copies (42,8mo *vs* 6,3mo, p=0,0003 and not reached median *vs* 10,4mo, p=0,009). There was a correlation between the normalization of *WT1* at day 14 and the risk group (p=0,019). Intermediate risk group was divided into two: the pts with overexpression *WT1* had lower RFS 6,2 *vs* 10mo (p=0.026). 65,7% (23/35) the pts with <10% blasts on the 14th day had overexpression *WT1*. These pts had early (less than 6 mo) relapse: 84,6% (11/13) *vs* 0% (0/7) (p =0,002). The cut-off level of *WT1* transcript level decrease to predict early relapse is 1.1 log. In multivariate analysis including risk group, CR after the first induction course, blast percentage at day 14, level *WT1* at 14 and 28 days, independent prognostic factors for RFS were level *WT1* at day 14 and risk group (HR:8.66; 95% CI: 1,6-46.7; p=0.01 and HR:2.35; 95% CI: 1,0-5.3; p=0.04).

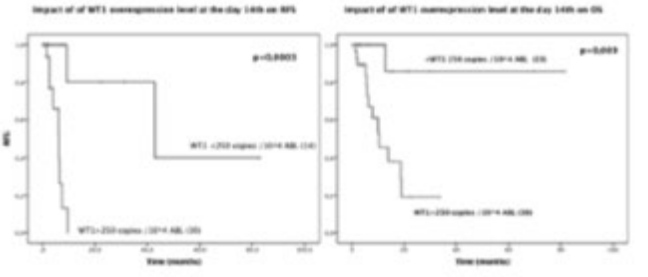

Figure 1.

Summary/Conclusion: *WT1* transcript level on day 14 of the first induction cycle is predictive for early relapse, RFS and OS. It is more sensitive that blast level. *WT1* transcript level can discriminate intermediate prognosis pts into better and poorer prognosis.

PB1660

ANTI-CD CELL-BINDING ANTIBODY MICROARRAY FOR INTEGRATION OF MORPHOLOGY AND IMMUNOPHENOTYPING IN ACUTE LEUKEMIA DIAGNOSIS

O. Fedyanina[1,2], A. Zakirova[1], A. Zadorozhnaya[3], S. Kuznetsova[1,2,*]

[1]Biophysics, National Research Centre for Pediatric Hematology, Oncology and Immunology, [2]Centre for Theoretical Problems of Physico-Chemical Pharmacology, [3]Faculty of Physics, Lomonosov Moscow State University, Moscow, Russian Federation

Background: Acute leukemia diagnosis requires the integration of morphology and flow cytometry data. However, it is not always straightforward due to the difference in the method for blast identification used in both cases.
Aims: To simplify the integration of morphology and immunophenotyping we have developed an anti-cluster-of-differentiation (anti-CD) antibody microarray on a transparent support for leukocyte sorting and a method for preparation of the microarray-bound cells for high-resolution morphology analysis.
Methods: The suspension of mononuclear cells separated by density gradient from bone marrow aspirate is incubated with the microarray in non-mixing conditions. After the unbound cells are washed away the bound cells are dried and flattened in a home-developed cytocentrifuge. The drying/flattening procedure makes the microarray-bound cells morphologically identical to the same cells in a smear and suitable for other standard smear-oriented techniques such as cytochemistry while the leukocytes on the microarray are grouped according to their surface CD antigens. Due to the non-mixing incubation the density of the cells bound to an anti-CD antibody permits to determine the fraction of cells positive for the corresponding CD antigen with high correlation with flow cytometry.
Results: We studied 66 patients with diagnosed acute leukemia of different subtypes and showed that the morphology of the microarray-bound blast cells was identical to the same cells in smears, the percentage of blast cells was in good correlation with the results obtained by flow cytometry and smear analysis and the blast immunophenotype determined as the list of anti-CD antibodies binding the leukocyte population with more than 2% of cells with blast morphology agreed well with flow cytometry results. Then we have compared the blast percentage among anti-CD45-bound bone marrow mononuclear cells for 23 nonneoplastic bone marrow aspirates, 17 samples from patients with chronic myelocytic or myelomonocytic leukemia and 90 bone marrow aspirates from patients with acute leukemias. The threshold for acute leukemia was found to be 25%. Finally we show that morphology and cytochemistry analysis of mononuclear bone marrow cells from patients with suspected acute leukemia bound by anti-CD antibody microarray including monoclonal antibodies against CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD22, CD33, CD41, CD45, CD61, CD64, CD117,

CD235, IgM, HLA-DR and negative control permits to arrive at preliminary diagnosis by FAB classification.

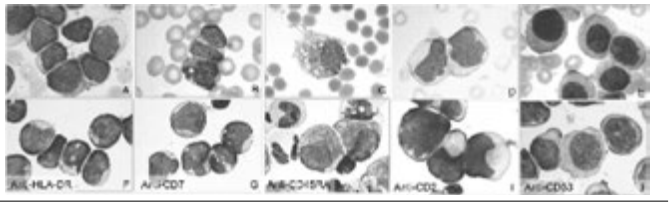

Figure 1.

Summary/Conclusion: The anti-CD morphology microarray can help in integration of morphology and flow cytometry data in differential diagnosis of acute leukemias and is a potentially useful diagnostic tool in resource-poor countries. The work was partially supported by gran #18-015-00272 from Russian Foundation for Basic Research.

PB1661

LOW-MYELOPEROXIDASE (MPO) MIXED-PHENOTYPE ACUTE LEUKEMIAS (MPALS) IDENTIFIED BY COMBINING CYTOCHEMISTRY AND FLOW CYTOMETRY

E. Kritikou-Griva[1], E. Goumakou[1], S. Papadhimitriou[1], G. Androutsos[1], D. Pavlidis[1], N. Tsagarakis[1], G. Paterakis[2,*]

[1]Laboratory Hematology, [2]Flow Cytometry Laboratory, Department of Immunology, General Hospital of Athens "G. Gennimatas", Athens, Greece

Background: Mixed-phenotype acute leukemias (MPALs) are rare leukemias often presenting with difficulties of classification and treatment. According to the revised WHO 2016 criteria, the identification of their myeloid component still relies on the expression of MPO, yet a new subgroup of otherwise typical B-ALLs with low levels of MPO has been recognized of unknown clinical significance up to now.
Aims: This study is a meta-analysis of our cohort of MPALs, the recent WHO revision taken in account.
Methods: In a total of 700 *de novo* adult acute leukemias 24 (3.4%) were classified as MPAL according to WHO 2008 by multi-color flow cytometry (FC), using CDs 19,79a, 10, 20 as B-lineage markers, CDs 3, 2, 5, 7 as T-lineage and cytoplasmicMPO (cut-off 10%), c13, s13, s33, 117 as myeloid markers. Cytochemical MPO (cut-off 3%) was also evaluated in all cases. Karyotype and FISH defined genetic groups.
Results: 16/24 MPALs were B+M and 8/24 T+M. Discordance of cytochemistry and FC for MPO determination was found at 10/24 MPAL cases (41.7%), that is 9 MPO-positive cases identified only by cytochemistry and 1 only by FC. All discordant cases had isolated low MPO expression (4-20% positive blast cells) and an immunophenotype of Pro-B (5), pre-B (1), common B (1), pre-T (2) and mature T(1) ALL, without any other markers of myeloid differentiation. Interestingly, 3 of these cases clearly co-expressed MPO and lymphoid markers at relapse by FC as well. According to cytogenetic data, 50% of the B+M MPALs were BCR/ABL- or MLL- associated and all but one of the "MPO- discordant" cases belonged to these genetic groups.
Summary/Conclusion: The determination of an isolated low-intesity MPO in MPALs may be challenging, due to the lack of an established threshold for FC and to technical reasons. The WHO 2016 revision draws attention to a group of acute leukemias with B-cell phenotype and low MPO expression of unknown clinical significance. In this study almost 50% of our B+M MPAL cases, all but one being MLL- or BCR/ABL- associated, matched this description and it was the combined cytochemical and FC approach for MPO that disclosed their myeloid component, as well as at a few T-ALLs. In our experience this combination is most helpful for the better identification of these rare MPALs with isolated low MPO.

PB1662

DETECTION OF CALR AND CBL MUTATIONS BY NEXT GENERATION SEQUENCING (NGS) IN ACUTE MYELOID LEUKEMIA (AML) AND MYELODISPLASIC SYNDROME (MDS)

C. Cuéllar[1,*], Y.P. Cánaves[2], E. Onecha de La Fuente[3], J.M. Sánchez[4], I. Rapado[4], J. Martínez López[4], R. Ayala[4]

[1]Hematology, Hospital Universitario de La Princesa, Madrid, Spain, [2]Hematology, Policlínico PAMI I, Rosario, Argentina, [3]Biología Molecular, [4]Hematology, Hospital [12] de Octubre, Madrid, Spain

Background: With the advent of NGS, an extended spectrum of molecular abnormalities has been discovered. However, the correlations between most somatic mutations and clinical characteristics and the underlying mechanisms still need further investigations. In AML, several mutations (FLT3-ITD, NMP1, DNMT3, CEBPA, TET2, IDH1/2) are already described with known implications in prognosis and clinical evolution. However, little is known about CALR and CBL mutations in AML and MDS. As known, Calreticulin (CALR) plays important roles in calcium homeostasis, protein folding, cell proliferation, apoptosis and inmune response. CALR is the second most frequently mutated gene in myeloproliferative neoplasms (MPNs). Casitas B-cell lymphoma (CBL) is an E3 ubiquitin ligase and promotes ubiquitination-directed degradation of target proteins (EGFR, FLT3, KIT, MPL and Src). Loss of E3 ligase activity together with additional gain-of-functions induced by these mutations promote malignant transformation.
Aims: To report and to describe the frequency of atypical mutations in AML ans MDS, focused on CBL and CALR detected by NGS myeloid panel.
Methods: We performed a custom-targeted sequencing panel of 32 genes (all coding regions) implicated in leukemia prognosis, including ASXL1, CBL, DNMT3A, EPOR, ETV6, EZH2, FLT3, HRAS, IDH1, IDH2, JAK2, KDM6A, KIT, KRAS, LNK, MLL, MPL, NRAS, PHF6, PRPF40B, PTEN, RUNX1, SF1, SF3A1, SF3B1, SRSF2, TET2, TP53, U2AF35, VHL, ZRSR2 and CALR, by Ion Torrent Proton System-Thermo Fisher. Kaplan- Meier survival curves and Long-rank test were used for estimation of survival and difference between groups.DNA samples collected between 2012 to 2018 years from a cohort of 335 patients with myeloid pathologies were screened by NGS. Among all studied patients, 190 (53,5%) were male and 165 (46,5%) female. 231 (65,1%) had AML diagnosis, 77 (21,7%) myelodisplasic syndrome (MDS), 42 (11,8%) chronic myeloproliferative syndrome (NMP), 3 (0,8%) medular aplasia and 2 (0,6%) other pathologies.
Results: Out of 77 patients with MDS, 8 (10%) were positive for CBL and 20 (26%) for CALR mutation. There were 20 deaths in the MDS group, none of them had CALR mutation and just one patient had CBL mutation. In NMPS, 2 (4%) patients were positive for CBL and 4 (10%) for CALR mutation. In AML cohort, 13 (5,6%) were CALR positive and 15 (6,5%) CBL positive. 114 (49,5%) of AML patients were deceased, of which 5 (4%) were CALR and 9 (8%) CBL positives These results were not statistically significant (p >0.05). We observed an unusual location of CALR mutation, mostly SNV in exon 5, instead of indels in exon 9, the typical location in NMPs. We detected a hotspot location of mutations in the ring finger domain of CBL similar to the ones described in previous publications. Median OS were 18 months for CALR negative AML group *vs* not reached median OS for CALR positive AML group (p=NS). Median OS were 71 months for CALR negative MDS group *vs* 108 months for CALR positive MDS group (p=NS)

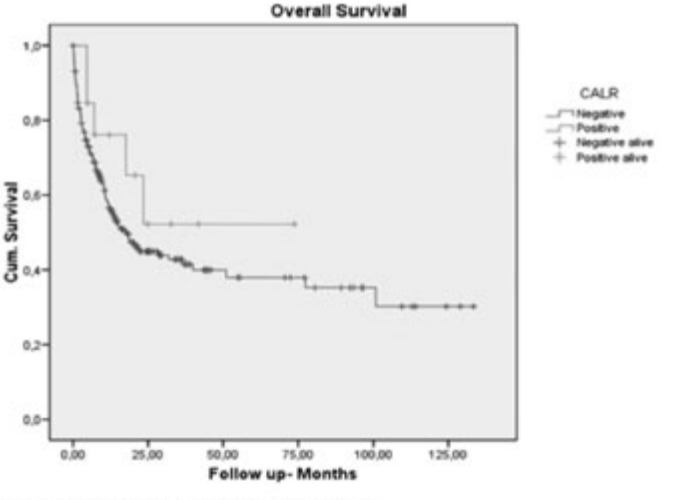

Figura 1. Kaplan Meier curve. OS CALR in AML.

Figure 1.

Summary/Conclusion: These results reveal a possible correlation between CALR gene mutation and prognosis features in AML or MDS patients. Despite of the lack of statistical significance, routine diagnostic approach of this gene could improve prognostic prediction. Moreover, it could be a therapeutic target, leading us to an individualized treatment. On the other hand, we could not find any correlation betweeen CBL mutation and MDS or AML and its prognosis. Therefore, larger controlled studies are necessary to confirm the findings.

PB1663

CLINICAL SIGNIFICANCE OF CONNEXIN 43 AND CONNEXIN 32 AS NEW PROGNOSTIC MARKERS IN ACUTE MYELOID LEUKEMIA

A. Shams[1], D. Gamil[1], M. Fateen[1], A. Seif[1], R. Salama[2,*]

[1]Clinical and Chemical Pathology Department, [2]Clinical Oncology Department, Cairo University, Cairo, Egypt

Background: Acute myeloid leukemia (AML) is the most common type of hematological malignancy. It seems likely that gap junctions are involved in the communication between AML and stromal cells during disease development. Gap junctions consist of arrays of intercellular channels composed of two hemichannels or connexons, one of which is formed by six protein subunits, termed connexins (Cxs). Cxs are a conserved family of transmembrane proteins which regulate the passage of biological molecules and allow the exchange of signaling molecules between the cytoplasm of two neighboring cells. Cx43 is the major component of hematopoietic tissue and Cx32 is found on bone marrow stromal cells.
Aims: The aim of this study was to evaluate the expression of Cx 43 and Cx 32 expression profiles and to correlate their expression with disease severity and other prognostic markers in patients with AML.
Methods: This study was carried out on samples from the peripheral blood of 60 patients with AML. Detection of Cx 43 and Cx 32 expression was performed using real time polymerase chain reaction (RT-PCR)
Results: The cases were divided into 39 males (57.4%) and 21 females (42.6%). Their age ranged between 14 and 82 years with a mean value of 49.68±16.1. Cx 32 expression showed a high median fold change along with a statistically significant difference between *de novo* AML patients and the control group (median Cx 32=18, p=0.009). On the other hand, Cx 43 expression showed a lower median fold in comparison to the control group (median Cx 43=0.6, p=0.013). A lower median fold change was observed in Cx 32 expression (median Cx 32=0.48), and Cx 43 expression (median Cx 43=0.1) in FAB subtype (M3) when compared with other FAB subtypes as (M1, M2, M4, M5).There was a statistically significant difference between the groups (Cx 32, p=0.009; Cx 43, p=0.002, respectively). In addition, CD34 positive AML patients showed a significantly higher level of Cx 32 (median Cx 32=20), and Cx 43 expression (median Cx 43=0.7) against CD34 negative AML patients (median Cx 32= 0.16; median Cx 43=0.7) (Cx 32 p=0.045; Cx 43 p=0.007). No statistically significant difference was found between favorable risk patients and those with intermediate/unfavourable risk in Cx 32 and Cx 43 fold change expression.
Summary/Conclusion: Our results indicate that Cx 43 and Cx 32 may have a role in AML pathogenesis.Further studies needed to evlauate their clinical significance as prognostic factors in AML.

PB1664

THE PROLIFERATION INDEX OF BONE MARROW CELLS FOR DIFFERENTIAL DIAGNOSIS OF CHRONIC MYELOMONOCYTIC LEUKEMIA *VS* ACUTE MONOBLASTIC/MONOCYTIC LEUKEMIAS

P. Leoz*, A. Berkovits, V. Guerri, C. Fernández, S. Barrena, A. López, J. Ciudad, M. Fierro, R. Rivas, M. Santos, A. Orfao, S. Matarraz

Centro de Investigación del Cáncer (IBMCC, University of Salamanca-CSIC), Salamanca, Spain

Background: Overlapping features often hamper the differential diagnosis of chronic myelomonocytic leukemia (CMML) *vs* acute monoblastic/monocytic leukemias (AMML).
Aims: We investigated the proliferation index (PI) of different bone marrow (BM) cell subsets by multiparameter flow cytometry as potentially useful for discrimination of both disease entities.
Methods: The PI (percentage of cycling S+G2/M cells) was studied using a 4-color (CD34/CD45/CD11b/CD13) antibody panel that included the DRAQ5 DNA stain. Thus, a total of 145 BM samples corresponding to healthy subjects (NBM; n=67), CMML (n=31; CMML-1, n=25 and CMML-2, n=6) and AMML (n=47; monoblastic leukemia, n=38 and monocytic leukemia, n=9) were studied for the PI among the more immature BM cell component in each case (*i.e.* CD34+ cells and/or leukemic cells) and also (residual) monocytic, neutrophil and erythroid lineage cells.
Results: As expected in NBN, the highest PI was shown by erythroid cells, followed by $CD34^+$ hematopoietic precursors, neutrophil and monocytic cells (PI of 28%, 15%, 5% and 4%, respectively). Conversely, the analysis of the more immature BM cell component from monocytic leukemias revealed that CD34+ cells from CMML patients had normal proliferation rates (PI of 16% *vs* 15% in NBM), while significantly decreasing in AMML (CD34+) leukemic cells (7% *vs* 15%; p<0.001). In detail, the PI of CD34+ cells from CMML-1 was significantly higher *vs* (CD34+) leukemic cells from monoblastic and monocytic leukemias (PI of 17% *vs* 7% and 4%, respectively; p<0.001), while a similar PI was found among CD34+ cells from CMML-2 *vs* leukemic cells from both groups of AMML patients (PI of 3%; p>0.05). As noted, a tendency to a lower PI of the (CD34+) blast cell compartment is observed from monoblastic to monocytic leukemia (7% *vs* 4%, respectively; p>0.05). In turn, AMML monocytic-lineage leukemic cells depicted a significantly increased proliferation *vs* monocytic cells from both NBM and CMML (PI of 7% *vs* 4% and 3%, respectively; p=0.001). Such overall increased PI was mostly related to the enhanced proliferation of monoblastic leukemia cells, which was almost twice the PI of monocytic cells from all the other groups (PI of 7% *vs* 3%, 3% and 4% for LMMC1, LMMC2 and monocytic leukemia, respectively; p=0.001). Most strikingly, the proliferation of the erythroid-lineage cells was found significantly decreased in AMML, as compared to NBM (PI of 25% *vs* 28%: p=0.02). Interestingly, this was at the expense of erythroid cells from monocytic leukemia, which showed a significantly lower proliferation *vs* all the other groups (PI:15% *vs* 27%, 30% and 23% for monoblastic leukemia, CMML-1 and CMML-2, respectively; p=0.005). No differences were found in the PI of any other residual cell lineage investigated.
Summary/Conclusion: The detection of decreased levels of proliferation, particularly among erythroid cells might help the differential diagnosis of CMML-2 *vs* monocytic leukemia, whereas an enhanced proliferation of leukemic cells is particularly recurrent in monoblastic leukemia *vs* the other monocytic lineage leukemias investigated.

PB1665

BONE MARROW LYMPHOCYTE PROFILE OF HLA-DR NEGATIVE NON-M3 ACUTE MYELOID LEUKEMIA

S. Cingelova[1,*], A. Mlcakova[2], E. Mikuskova[1], L. Demitrovicova[1], I. Oravcova[1,3], V. Mikudova[1], S. Kevicka[1], A. Slobodova[2], J. Gyarfas[1], M. Mego[3,4], L. Drgona[1,3]

[1]Oncohematology, [2]Laboratory Hematology, National Cancer Institute, [3]Faculty of Medicine, Comenius University, [4]Oncology, National Cancer Institute, Bratislava, Slovakia

Background: The impact of the bone marrow microenvironment on the behaviour of hematopoietic neoplasms is being increasingly studied. Human Leukocyte Antigen (HLA) Class II molecules play an essential role in presenting antigenic peptides to regulatory T-cells and in the generation of an immune response. HLA Class II molecules are expressed on acute myeloid leukemia (AML) blasts at diagnosis in most cases of non-M3 AML. The biological and clinical significance of HLA-DR antigen loss is not known.
Aims: We aimed to study a bone marrow lymphocyte profile (infiltrating lymphocytes) of HLA-DR negative non-M3 AML and possible association with bone marrow blast percentage and treatment outcome.
Methods: A total of 73 newly diagnosed patients with non-M3 AML admitted at National Cancer Institute Bratislava, Slovakia, between years 2012 and 2017 were included in this study. A diagnosis of AML was made based on the results of morphology, immunophenotype and genetics. An association between immunophenotypic characteristics of bone marrow blasts, bone marrow lymphocyte profile and blast percentage was evaluated by Kruskal-Wallis one-way ANOVA test, and for overall survival evaluation and response rate were used log-rank test and Fisher exact test, respectively, for multivariate analysis Cox regression and logistics regression models with AML genetic risk group, as appropriate.
Results: Patients were immunophenotypically characterized as HLA-DR positive (65 pts, 89%) or HLA-DR negative (8 pts, 11%). HLA-DR negativity was associated with CD11b negativity (100%) and CD34 negativity (62.5%). HLA-DR negative patients had significantly higher bone marrow blast percentages (median 92.2% *vs* 63.3%, *P*=0.005), lower bone marrow lymphocyte count (median 3.5% vs.8.0%, *P*=0.01) and lower bone marrow T-lymphocyte count (median 2.5% *vs* 6.8%, *P*=0.007), with no significant difference in B-lymphocytes or NK-cells. Infections rate, overall survival and treatment response rate showed no significant differences between the HLA-DR negative and the HLA-DR positive group.
Summary/Conclusion: HLA-DR antigen loss is not frequent in myeloid leukemogenesis, and may represent mechanism of immune escape. HLA-DR negative AML cases have bone marrow lymphocyte profile distinguishable from those of typical AML, which needs further investigation. Understanding the role of the immune microenvironment in the behaviour of AML is of great importance, especially with the success of immunomodulatory treatment.

PB1666

IN VITRO AND *IN VITRO* MODELS OF ACUTE MYELOID LEUKEMIA USING CRISPR/CAS9 SYSTEM

E. Gonzalez[1,*], A. Rosal-Vela[1], B. de Matteo[1], M. Ibañez[2,3], A. Liquori[1,4], A. SanJuan[5], C. Sargas[1], M. Boluda[1], C. Martínez[1], M. Llop[4,6], L. Cordón[4,7], E. Barragan[4,6], A. Neef[1], G. Sanz[2], M.Á. Sanz[2], J.M. Millán[8,9], R. Vázquez[8,9], J.V. Cervera[10]

[1]Research Group in Hematology, Instituto de Investigación Sanitaria La Fe, [2]Hematology Service, Hospital Universitario y Politécnico La Fe, Valencia, [3] CIBER de Oncología (CIBERONC), [4]CIBER de Oncología (CIBERONC), [5]Research Group in Hematology, Instituto de Investigación Sanitaria La Fe, Madrid, [6]Molecular Biology Service, Hospital Universitario y Politécnico La Fe, [7] Flow Cytometry Lab, Hematology Service, [8]Research Group in Molecular, Cellular and Genetic Biomedicine, Instituto de Investigación Sanitaria La Fe, Valencia, [9]CIBER de Enfermedades Raras (CIBERER), Madrid, [10]Hematology Service, Instituto de Investigación Sanitaria La Fe, Valencia, Spain

Background: Acute Myeloid Leukemia (AML) is a complex heterogeneous disease produced by clonal expansion of undifferentiated myeloid precursors, resulting in impaired hematopoiesis and bone marrow failure. Whole-genome sequencing and advances in next-generation sequencing have increased our knowledge of the molecular heterogeneity of AML, and have these insights been translated into improved disease classification. The *IDH2*^{R172} mutations have been proposed to constitute an independent subgroup into this classification, different from the rest of mutations in this gene. For understanding the role of these mutations in the leukemia progress and for the development of specific therapies is necessary to create new AML models. The CRISPR/Cas9 system has allowed a great advance in genome engineering due to its easy design and use in different model organisms. With this system is possible to develop AML models with single mutations or introduce them progressively to characterize the acquired driver mutations detected in patients.
Aims: We want sought to develop *in vitro* and *in vivo* models of AML using the CRISPR/Cas9 system to introduce *IDH2* mutations. Objectives: Development of an easy and fast method to produce guide sequences; Introduce *IDH2*^{R172K} mutation in leukemia cell lines; Introduce *IDH2*^{R172K} and *IDH2*^{R140Q} mutations in the nematode *Caenorhabditis elegans*. Due to the homology between the gene in model organism and humans this is feasible (the R172 and R140 amino acids are conserved).
Methods: To do the *in vitro* model, we have developed a fusion PCR system for generating constructs with the sgRNA sequences and the pU6 promoter, and optionally, adding the GFP reporter. These constructs were transfected in the broadly used HEK293 cells, and these constructs are able to cut the target genes. After a series of optimizations we have used these constructs in leukemia cell lines that express Cas9 to produce our model. Furthermore, we used ribonucleoprotein (RNP) complex in cells without Cas9 expression to compare the efficiency of the different procedures. We have also introduced the *IDH2*^{R172K} and *IDH2*^{R140Q} mutations in *C. elegans.* To do so we prepared a mix with RNP and an oligonucleotide, as an editing template carrying the desired mutation, were injected along with a gRNA against a gene which produces easy-to-see phenotypes, as a marker of edition. With a PCR with specific primers we isolated homozygote worms with each mutation.
Results: With the optimization in HEK293 cells we selected the best sgRNA combination to modify *IDH2* gene (20% of efficiency). Interestingly, we obtained the same efficiency in NB4 cell line which contained an insertion that induces constitutive expression of Cas9. The RNP complex was used in the NB4 cell line with 8% of efficiency. In future experiments we will introduce *IDH2* ^{R172K} using these two methodologies. On the other hand, the marker phenotype let us isolate those worms that were efficiently edited, among which we were able to isolate worms carrying *IDH2* ^{R172K} or *IDH2*^{R140Q} mutations.
Summary/Conclusion: The CRISPR/Cas9 technology represents a high biomedical revolution that has already been used in multiple fields. Specifically, this system can be used to elucidate the functional and cooperating effect of the mutations detected in patients with AML. We have used this technique to develop two AML models in which we can introduce mutations detected in patients in a sequential way, study the effect in the leukemic phenotype and could be a promising platform to test new drugs against this disease.

PB1667

APPLICATION OF THE GENEREADER NGS SYSTEM AND QIAACT MYELOID DNA UMI PANEL IN DETECTING RELEVANT MUTATIONS FOR MYELOID LEUKEMIA RESEARCH, INCLUDING FLT3 ITDS

V. Laloux[1,*], O. Biglia[2], A. Bona[2], S. Lafi[2], F. Charifi[2], L. Schauser[3], J. Larsen[3], D. Lueerssen[4], V. Gupta[3], J. Lauber[5], S. Hughes[4]

[1]QIAGEN, Courtaboeuf, [2]HalioDx, Marseille, France, [3]QIAGEN, Aarhus, Denmark, [4]QIAGEN, Manchester, United Kingdom, [5]QIAGEN, Hilden, Germany

Background: Myeloid malignancies comprise many different subtypes amongst which myeloproliferative neoplasms (MPN), myelodysplastic syndrome (MDS), myelodysplastic/myeloproliferative neoplasms (MDS/MPN) and acute myeloid leukemia (AML), are associated with somatic mutations acquired in around 20-30 key genes. To detect these relevant myeloid malignancy-related genetic variants, a targeted enrichment approach coupled to NGS analysis nicely complement the individual biomarker tests to provide a comprehensive mutational overview. The QIAact Myeloid DNA UMI Panel in combination with the QIAGEN GeneReader™ NGS System provides an integrated solution to simultaneously interrogate many candidate genes for actionable mutations, from as little as 40 ng input DNA, shortening test time and enabling simplification of lab operations. The QIAact Myeloid DNA UMI Panel is a 25-gene targeted sequencing panel for markers of known significance to clonal myeloid malignancies, allowing reliable and sensitive detection of single nucleotide variants (SNV) and large Insertion/Deletion (InDel) mutations.
Aims: To assess the QIAact Myeloid DNA UMI Panel performance in combination with the QIAGEN GeneReader NGS System.
Methods: Following the advice from key opinion leaders in the field of blood cancer disorders, the QIAact Myeloid DNA UMI Panel has been designed to detect relevant mutations throughout the most informative genes linked to myeloid disease. A key feature of the panel is the addition of a unique molecular index (UMI) to tag individual molecules prior to target enrichment by PCR. This enables sequencing and PCR bias correction, allowing sensitive detection of mutations (e.g. below 1% MAF for *JAK2* (exon 12, 13, 14 & 15) & *KIT* (exon 8, 9, 10, 11 & 17). To assess the assay performance, control samples and blood and bone marrow samples were used. Following target enrichment, libraries were sequenced on the GeneReader™ NGS system and mutations identified using the QIAGEN Clinical Insight (QCI™) Analyze software suite, adjusted to specifically support variant calling in this assay.
Results: Complex mutations like the 52 bp deletion CALR type 1 variant, FLT3 ITDs up to 121 bp insertion and NPM1 insertions were efficiently identified. Limit of detection testing using the power of UMIs demonstrated uniform amplification and sequencing coverage to consistently detect mutations with a 1% MAF and below for JAK2 and KIT (KIT D816V variant can be detected at 0.2%), and 5% for all the other genes covered by the panel. Overall mutational variants were correctly called, resulting in >99% concordance with an alternative testing technology.
Summary/Conclusion: The QIAact Myeloid DNA UMI Panel in combination with the QIAGEN GeneReader NGS System offer a fully integrated sample to insight solution. The optimized chemistry allows superior analytical sensitivity in accurately detecting highly relevant genetic alterations for myeloid malignancy research.

PB1668

ANTI-LEUKEMIC EFFECTS OF VOSAROXIN, AN ANTI-CANCER QUINOLONE DERIVATIVE, ON HYPOMETHYLATING AGENT-RESISTANT CELLS

H.-S. Park*, E.-H. Hur, B.-K. Goo, J. Moon, S.-H. Ko, E.-J. Choi, K.-H. Lee, J.-H. Lee, J.-H. Lee

Department of Hematology, Asan Medical Center, Seoul, Korea, Republic Of

Background: Although two hypomethylating agents (HMAs), azacitidine and decitabine, are widely used for treatment of patients with myelodysplastic syndrome (MDS) and elderly acute myeloid leukemia (AML), most patients eventually experience resistance to the agents. Vosaroxin, an anticancer quinolone derivative, acts as a topoisomerase II inhibitor, and improved survival in a phase 3 study of vosaroxin in combination with cytarabine in relapsed/refractory AML.
Aims: We investigated anti-leukemic effects and mechanism of action of vosaroxin in HMA resistant cells.
Methods: We established three HMA resistant cell lines: MOLM/AZA-1 and MOLM/DEC-5 from MOLM-13 (AML/MDS cell line [*Oncotarget* 2017;8:11748]), and THP/DEC-2 from THP-1 (AML cell line). Cell viability was performed using Celltiter-glo luminescent cell viability assay (Promega, WI) and Operetta high-content imaging system (PerkinElmer, MA). The cell cycle distribution was determined by propidium iodide staining and flow cytometry. The proteins of apoptosis and cell cycle regulator were detected with immunoblot assay using specific primary antibodies.

Results: Vosaroxin caused dose-dependent inhibition of HMA resistant cells as well as MOLM-13 and THP-1 cells, and IC50 values of vosaroxin were significantly lower than those of azacitidine or decitabine. Vosaroxin induced increase of nuclear size in all cell lines except MOLM-13, in which DNA fragmentation was induced. DNA flow cytometric analysis exhibit G2/M arrest by vosaroxin. The immunoblotting showed increase of p53 and p21 expression in MOLM-13, MOLM/AZA-1 and MOLM/DEC-5 cells, increase of CDK2 and CDK4 expression, and decrease of Rb expression. Synergistic effects with combination of vosaroxin with decitabine were found in THP-1 and THP/DEC-2. Vosaroxin exerted *in vitro* anti-leukemic effects in 20 of 26 bone marrow samples from MDS patients.
Summary/Conclusion: Our preclinical studies demonstrated excellent anti-leukemic activities of vosaroxin in our HMA resistant models, and the effects seem to be attributed to cell cycle regulation of vosaroxin.

PB1669

ASYNCHRONOUS MONOCYTIC MATURATION PATTERN IN AML WITH NPM1 MUTATION

M.Á. Álvarez Juárez[1,*], I. Carmona Zabala[1], I. Martín Rubio[1,]
J. Galán Vega[1], C. Escolano Escobar[1], F. Herrera[1], A. Yeguas Bermejo[2], N. Somolinos de Marcos[1], A.M. Hernández-Sánchez[3], L. Benito Parra[1], M.C. Monteserín[1], L. García Alonso[1], F. Oña Compán[1], J.A. García Vela[1]

[1]Hematology department, Hospital Universitario de Getafe, [2]Hematology department, Fundación Jiménez Díaz, Madrid, [3]Hematology department, Hospital Quirón Salud, Málaga, Spain

Background: Associations between immunophenotypic findings (IF) and genetic abnormalities have been proved for specific subtypes of AML harboring recurrent cytogenetic abnormalities. Mutation of the nucleophosmin gene (NPM1) is observed in approximately 30% of cases of AML, and is recognized as a distinct entity in the World Health Organization classification. It most commonly falls in the M1/M2 (myeloid) or M4/M5 (myelomonocytic/monocytic) FAB categories and it is associated with a favorable prognosis (although this effect is decreased in the context of concomitant FLT3-ITD mutations). There is evidence that some cases of NPM1-mutated AMLs are often negative for CD34 and HLA-Dr, showing an acute promyelocytic leukemia-like (APL-like) phenotype although there is limited information about the monocytic pattern of differentiation in these cases.
Aims: To describe the immunophenotypic pattern of the monocytic population in NPM1-mutated AMLs.
Methods: We included BM samples of 9 cases of NPM1-mutated AML (in the absence of the PML-RARA rearrangement). Staining of BM was performed as previously described using the next six-colour combinations of monoclonal antibodies: acute leukemia orientation tubes CD38/CD34/HLA-Dr/CD117/CD19/CD45 and cyMPO/cyCD79α/HLA-Dr/cyCD3/CD34/CD45, monocytic tube CD35/CD64/HLA-Dr/CD14/CD300e/CD45, granulocytic tube CD16/CD13/HLA-Dr/CD10/CD11b/CD45 and erythroid tube CD36/CD105/HLA-Dr/CD117/CD71/CD45. A minimum of 1 million of cells were acquired in a FACSCanto flow cytometer (Becton Dickinson, San Jose, CA, USA) using the FACSDiva software (BD). For data analysis the Infinicyt software (Cytognos, Salamanca, Spain) was used. Monocytic cells were identified by their expression of CD64. As previously described (Matarraz S, *et al.*, 2014) early monocytic cells (CD117low, HLA-Dr++, CD64+, CD14-, CD35- and CD300e-) sequentially acquire reactivity for CD14 and CD35 (promonocytes) and subsequently also for CD300e (mature monocytes). After selecting CD64++ cells (in monocytic cases and also in the residual monocytic cells in the myeloid cases) we identified mature monocytes by their expression of CD300e and in this target population we explored the expression of CD14 and CD35.

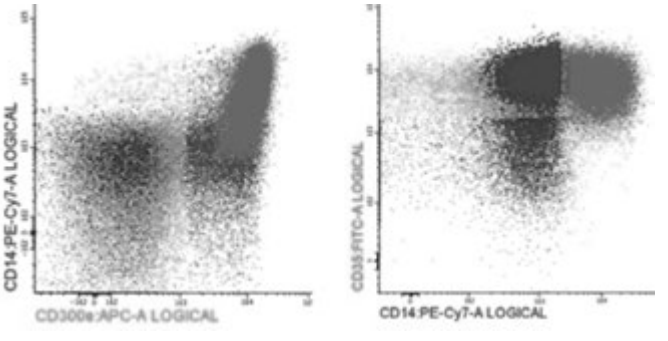

Figure 1.

Results: The cohort included 5 females and 4 males (F: M ratio=1.25), with a medium age at diagnosis of 66 years (range 42-87 years). 5 (55%) cases showed co-mutation of FLT3-ITD. 6 cases (66%) showed morphologic and immunophenotypic evidence of monocytic differentiation, while 3 (33%) of them showed ganulocytic differentiation. All cases were negative for CD34 and 2 were negative for HLA-Dr (22%) showing an "APL-like" phenotype. All cases studied showed an asynchronous maturation in the monocytic population and the expression of CD300e preceded that of CD14 and/or CD35. The average percentage of the studied population was 4.6%, with a range between 0.3% and 14% (violet population; figure 1).
Summary/Conclusion: Our results confirm previous observations regarding the asynchronous maturation in the monocytic population in NPM1-mutated AML (Matarraz S, *et al.* 2014). These preliminary results must be confirmed in a series with more cases. The expression of CD123, CD25 and CD99 should be included in future studies to explore whether their reactivity associated with the asynchronous pattern here described, can predict the association between NPM1 and FLT3 mutation.

PB1670

JQ1 TREATMENT OF U937 CELL LINES OF STAT-5 SIGNAL TRANSDUCTION PATHWAYS RESEARCH

M. Liangming*

Hematology, Shan Xi Da Yi Hospital, Taiyuan, China

Background: The purpose of study is to detect change of FAK and PAK1, thus to observe the regulation of the STAT-5 signal pathway in the acute myeloid leukemia cell line (U937) which is managed by the bromo domain inhibitors-JQ1.
Aims: The purpose of study is to detect change of FAK and PAK1, thus to observe the regulation of the STAT-5 signal pathway in the acute myeloid leukemia cell line (U937) which is managed by the bromo domain inhibitors-JQ1.
Methods: With the different concentration of JQ1 to treat U937 cells, the inhibition rate of cells was detected at different times by using tetramethazolazole blue experiment (MTT). The rate of Cell apoptosis was detected by flow cytometry. The mRNA expression of level about FAK and PAK1 were detected by real-time fluorescence quantitative (rt-PCR). And the expression of stat-5 protein which was analyzed by Western Blot in each group.
Results: JQ1 can effectively inhibit the proliferation of cells and induce cellular apoptosis, and the apoptosis rate of cells is significantly much more obvious in the high group than the low one. This feature show that a time-dose dependence. JQ1 can also reduce the expression of mRNA about FKA and PAK1 with U937 cell, whenever the concentration of it. The result of Western Blot show that the abnormal expression of STAT-5 in the control group was much more higher than that in the experimental group.
Summary/Conclusion: JQ1 can effectively inhibit the growth of U937 cells by influenting proliferation and cell apoptosis. Its effect mechanism may be through to influent the expression of FAK and PAK1, thus to influent the STAT-5 signal pathway (the pathway of cell proliferation).

PB1671

THE ROLE OF NA/H EXCHANGE I AS A THERAPEUTIC TARGET TO OVERCOME CYTARABINE RESISTANCE IN ACUTE MYELOID LEUKEMIA

J.-W. Cheong[1,*], S.Y. Hyun[2], E.J. Na[3], H. Chung[1], J.E. Jang[1,]
S.-J. Kim[1], J.S. Kim[1], Y.H. Min[1]

[1]Internal Medicine, Yonsei University College of Medicine, [2]Internal Medicine, Yonsei University Wonju College of Medicine, [3]Avison Biomedical Researcch Center, Yonsei University College of Medicine, Seoul, Korea, Republic Of

Background: Na/H exchanger 1 (NHE1), an important participant in the precise regulation system of intracellular pH (pHi), is known to be involved in pathological processes of the neoplastic disease.
Aims: In this study, we evaluated the intracellular (pHi) and NHE1 expression in acute myeloid leukemia (AML) cell lines, and whether it contributes the resistance to cytarabine (AraC).
Methods: We evaluated the pHi and NHE1 expression in AML cell lines AraC sensitive OCI-AML2 (AS-OCI) and AraC resistant OCI-AML2 (AR-OCI) cells. To modulate the NHE1 activity, the NHE1 inhibitor, 5-(N, N-hexamethylene) amiloride (HMA), and NHE1 activator, phorbol 12-myristate 13-acetate (PMA), were used. The level of apoptosis and proliferation induced by NHE1 inhibitor or activator with/without AraC was assessed at 24 hr after treatment by the annexin V assays and the CCK-8 assay.

Results: The pHi of AR-OCI cells was significantly higher than AS-OCI cells (7.839 *vs* 7.589, P=.045). Compared with AS-OCI cells, AR-OCI showed significantly higher NHE1 expression by western blot analysis, and NHE1 mRNA levels (1.565 *vs* 0.039, P<.01) by qRT-PCR. After 24hr treatment with HMA 10 ~ 30 μM, the proliferation of leukemic cells was inhibited, and the apoptosis was induced in a concentration-dependent manner, and higher concentration of HMA (30 μM) could induced apoptosis on most of AR-OCI cells. When treated by PMA with various concentration form 0.1 to 10 uM, proliferation of leukemic cells was inhibited in a concentration-dependent manner, but apoptosis was not induced. The addition of HMA 10 μM that concentration did not cause apoptosis of AR-OCI cells when treated alone to AraC treatment resulted in a significant increase in the fraction of apoptosis in AR-OCI cells (P<0.001) as compared with that of AraC treatment alone (26.33% *vs* 7.11%, P<.001). Also, the addition of PMA 1 μM to AraC treatment resulted in a significant increase in the level of apoptosis in AR-OCI cells as compared with that of AraC treatment alone (45.70% *vs* 7.11%, P<.001). These findings suggest that NHE1 plays an important role in resistance mechanism to AraC in AML cell line and NHE1 might be potential therapeutic target to overcome chemoresistance in AML. To elucidate the detailed signaling pathway after modulation of NHE1 activity, further experimental studies have been proceeding.

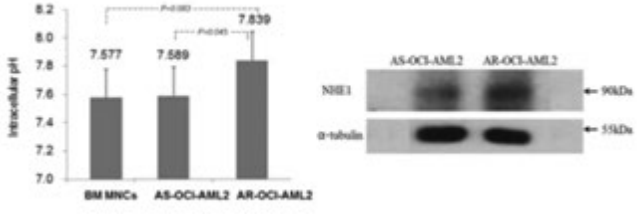

Figure 1.

Summary/Conclusion: These findings suggest that NHE1 plays an important role in resistance mechanism to AraC in AML cell line and NHE1 might be potential therapeutic target to overcome chemoresistance in AML. To elucidate the detailed signaling pathway after modulation of NHE1 activity, further experimental studies have been proceeding.

PB1672

THE EFFECT OF THE CRM1 INHIBITING COMPOUND KPT-330 ON P53 ISOFORM PROFILE

E. Hajjar[1,*], V. Andresen[1,2], A. Sulen[1], S.M. Hjelle[1,2], S. Knappskog[3], J.-C. Bourdon[4], B.T. Gjertsen[1,2]

[1]Center for Cancer Biomarkers (CCBIO), Department of Clinical Science, University of Bergen, [2]Department of Internal Medicine, Hematology Section, Haukeland University Hospital, [3]Department of Clinical Science, Oncology Section, University of Bergen, Bergen, Norway, [4]Division of Cancer Research, University of Dundee, Dundee, United Kingdom

Background: The tumor suppressor *TP53* is in average mutated in about 50% of solid cancers, however, over 90% of newly diagnosed AML patients express wild type *TP53*. This could indicate different levels of dysfunctionality of the p53 protein in AML, dependent on mutational status and genetics. At least 12 different protein isoforms of p53 have been described and we have previously reported that the p53 isoforms p53β and p53γ influence full-length p53 (FLp53) function and therapy response in AML. We found that *FLT3-ITD*, a poor prognostic marker for survival in AML, correlated with expression of FLp53. In contrast, mutated *NPM1*, a prognostic marker for longer survival, correlated with p53 isoforms β and γ expression. Chromosome region maintenance 1 (CRM1) is the sole nuclear exporter for several tumor suppressor proteins including p53. CRM1 is frequently overexpressed in AML associated with poor survival. Karyopharm Therapeutics has developed several selective inhibitors of nuclear export (SINE) of proteins dependent of CRM1 for nuclear export, including KPT-330, Selinexor; currently in clinical trials for treatment of AML.
Aims: To study the effect of KPT-330 on FLp53 and p53 isoforms in AML cells.
Methods: The effect of KPT-330 on p53 isoform protein expression was studied in p53 wild type AML cell lines. The p53 expression patterns were analysed using 2-dimensional gel electrophoresis (2DE) followed by image analysis using Gel2de software which apply pixel-by-pixel Spearman rank correlation between pixel intensity and external biological parameters. In addition 1-dimensional immunoblot (1D), quantitative PCR (qPCR), immunofluorescence and flow cytometry was performed.
Results: The OCI-AML3, MOLM-13 and MV4-11 p53 wild type cell lines were characterized for p53 mRNA isoform profile (FLp53, p53β, p53γ and p53Δ133) by qPCR with the p53 null cell line HL-60 as a negative control. KPT-330 treatment for 24h of OCI-AML3, MOLM-13 and MV4-11 increased total p53 protein expression as demonstrated by 1D immunoblotting. Two dimensional gel electrophoresis immunoblotting followed by Gel2de correlation analysis to increasing dosage of KPT-330 resulted in positive correlation to FLp53 expression and negative correlation to p53β/γ isoform expression. However, no significant changes were found on the FLp53 or p53 isoform mRNA levels by qPCR. We found that the p53β protein is degraded following KPT-330 treatment through the proteosomal pathway. We are currently investigating the effect of KPT-330 on the p53Δ133 isoforms and the p53 isoform profile following cellular differentiation caused by 72h KPT-330 treatment, including changes in subcellular localization. Finally, the p53 isoform profiles of primary AML cells before and after KPT-330 *in vitro* treatment will be studied and correlated towards mutational status and drug sensitivity.

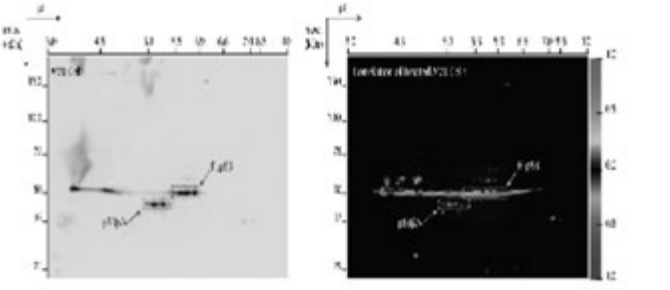

Figure 1.

Summary/Conclusion: Treatment with KPT-330 influences the p53 isoform expression profile at the protein level, where FLp53 increase and the p53β/γ decreases where p53β is degraded through the proteasome. The response of AML patients towards KPT-330 treatment could be dependent on the p53 isoform profile and be closely related to p53 dysfunction.

PB1673

PHARMACOLOGICAL INHIBITION OF WIP1 SENSITIZES ACUTE MYELOID LEUKEMIA CELLS TO MDM2 INHIBITORS

M.C. Fontana[1,*], J. Nanni[1], A. Ghelli Luserna di Rorà[1], A. Padella[1], S. Bruno[1], G. Marconi[1], C. Papayannidis[1], M. Abbenante[1], S. Soverini[1], M. Cavo[1], G. Simonetti[1], G. Martinelli[2]

[1]Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Institute of Hematology "L. e A. Seràgnoli", Bologna, [2]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy

Background: *PPM1D* (wild-type p53-inducible protein phosphatase, WIP1) is a serine\threonine phosphatase involved in negative regulation of cellular stress response pathways, leading to the suppression of p53. *PPM1D* mutations appeared to have oncogenic properties in many solid cancers, but their role is still unknown in acute myeloid leukemia (AML).
Aims: The aim of this study is to investigate whether the inhibition of WIP1 (GSK2830371, WIP1i) could increase the sensitivity to MDM2 inhibitor (Nutlin-3a) in order to restore p53 activity and obtain a novel therapeutic strategy for AML patients.
Methods: *in vitro* WST-1 cell-viability and Annexin V-PI apoptosis assays were performed on AML cell lines (MOLM-13, MV-4-11) and AML primary cells. The number of primary samples' viable cells was detected by Trypan Blue Exclusion Dye (Sigma-Aldrich). Whole Exome Sequencing (WES) and targeted Next Generation Sequencing (NGS) were performed to investigate the mutational state of *PPM1D* in newly diagnosed AML patients.
Results: WIP1i and Nut-3a reduced the cell viability as single agent in all the treated cells in a time and dosage-dependent manner. The IC50 values in TP53-wild type (wt) cells showed that MOLM-13 (10 mM) are more sensitive than MV-4-11 (20 mM) to WIP1i. Combination index analyses by WST1-proliferation assay confirmed that WIP1i and Nut-3a had a synergistic effect (C.I.<1) in both cell lines (Figure 1 A). Annexin V/PI staining performed after treatment with different concentration of Nut-3a (0.5 mM for MOLM-13, 15 mM for MV4-11) and WIP1i (10 mM for MOLM-13, 20 mM for MV4-11) demonstrated that the combination significantly increased the number of apoptotic cells in comparison to single agents. In particular, after 48 hours of incubation with Nut-3a and WIP1i as single agents, the percentage of apoptotic cells was 34.4% and 21.4% for MOLM-

13 and 17% and 18% for MV-4-11, respectively; while the combination induced 53.1% and 62.3% of apoptotic cells (Figure 1B). Moreover, we observed a decrease of G2/M phase cells induced by single agents, enhanced by the combination of drugs in both cell lines and, in particular, MV-4-11 were characterized by an increasing percentage of S phase cells allowing us to hypothesize that the apoptotic death induced by the combination of drugs occurs when cells are blocked in S phase. AML primary cells were also tested by Tripan Blue: we detected that the powerful combination of drugs on bone marrow AML samples at diagnosis was 5 mM of Nut3a plus 20 mM of Wip1i leading to life/death ratio of 0.77 *versus* 1.5 of single agents. Finally, by WES and targeted-NGS, 7 mutations have been detected in 2/37 and 4/96 patients at diagnosis. One patient harbored a truncating mutation on exon 6 (S468X), confirmed to be gain of function by Western Blot (WB), thus increasing the inhibition of p53; while mutations affecting exon 1 did not reveal any protein overexpression.

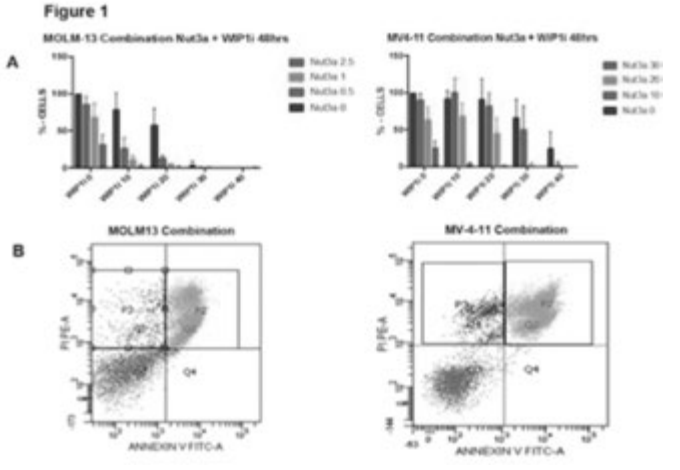

Figure 1.

Summary/Conclusion: We hypothesize for the first time that the pharmacological inhibition of WIP1 could potentiate the sensitivity to MDM2 inhibitors (Nut-3a, *e.g.* Idasanutlin) in AML patients, suggesting an important role of *PPM1D* in leukemia. Further studies will help to confirm these preliminary data: in particular, Gene Expression Profiling and WB will identify the proteins playing roles in the biological alterations induced by WIP1i alone and in cooperation with Nut-3a.

Supported by: ELN, AIL, AIRC, project Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project, HARMONY project, Fondazione del Monte BO e RA project.

PB1674

Abstract withdrawn.

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PB1676

STRATEGIES OF AFFECTING CELLULAR LOCALIZATION TO OVERCOME RESISTANCE IN FLT3-ITD-POSITIVE AML CELLS

U. Schnetzke*, M. Fleischmann, M. Fischer, A. Hochhaus, F. Heidel, S. Scholl

Klinik für Innere Medizin II, Abteilung für Hämatologie und Internistische Onkologie, Universitätsklinikum Jena, Jena, Germany

Background: FLT3-ITDs (internal tandem duplication) represent the most frequent molecular aberration in acute myeloid leukemia (AML) associated with an inferior prognosis. The pattern of downstream activation by this constitutively activated receptor tyrosine kinase is influenced by the localization of mutated FLT3 depending on its glycosylation status. Different pharmacological approaches can affect FLT3-driven oncogenic pathways by distinct compartmentalization of FLT3-ITD.
Aims: The objective of this study was to investigate the effects of either valproic acid (VPA) or tunicamycin on the localization of FLT3-ITD. We sought to investigate different susceptibility of mutated FLT3 towards combination treatment-*e.g.* with inhibitors of heat shock proteins (HSP)-dependent on both the localization of activated FLT3 and the subtype of FLT3-ITD variants.
Methods: Murine Ba/F3 leukemia cell lines were stably transfected with distinct FLT3-ITD variants resulting in IL-3-independent growth (Arreba-Tutusaus *et al.* Leukemia 2016). Signal transduction after exposing cells to VPA, tunicamycin and/ or the HSP inhibitor 17-AAG was characterized by Western blotting, MTS assay and flow cytometry analysis of apoptosis, cell cycle and surface expression of FLT3.
Results: Treatment of Ba/F3-FLT3-ITD cells with VPA is associated with a significant increase of FLT3-ITD surface expression that depends on FLT3 protein synthesis. In contrast, incubation with tunicamycin leads to intracellular retention of FLT3-ITD by inhibition of its glycosylation as an important mechanism of post-translational modification. Of note, allocation of FLT3 to different cellular compartments by VPA or tunicamycin is accompanied by distinct activation of oncogenic signaling pathways. In detail, VPA is associated with an increase of AKT and ERK phosphorylation while enhanced STAT5 activation can be observed following treatment with tunicamycin. Importantly, sequential combination of either VPA or tunicamycin with the HSP inhibitor 17-AAG demonstrates additive effects that are restricted to the treatment with tunicamycin and 17-AAG.
Summary/Conclusion: We can demonstrate the impact of VPA on cell surface stabilization of FLT3-ITD that results from FLT3 biosynthesis and is associated with an enhanced activation of the AKT and ERK pathway. Combination treatment suggests that the unglycosylated form of FLT3 is more susceptible to HSP inhibitor treatment. Thus, allocation of FLT3-ITD to different cellular compartments might represent a promising therapeutic strategy to overcome tyrosine kinase inhibitor resistance in FLT3-ITD-positive AML.

PB1677

LNCRNA BALR-2-CDK6 AXIS INFLUENCES METABOLIC STATUS AND CELL DIFFERENTIATION OF ACUTE MYELOID LEUKEMIA

V. Bisio[1,*], C. Tregnago[1], G. Borella[1], M. Benetton[1], T. Fernando[2], D. Rao[2], F. Locatelli[3], G. Basso[1], M. Pigazzi[1]

[1]SDB department, Università degli Studi di Padova, Padova, Italy, [2]Path & Lab Med, UCLA, Los Angeles, United States, [3]Department of Pediatric Hematology and Oncology, IRCCS Ospedale Pediatrico Bambino Gesù, Roma, Italy

Background: Non-coding RNA (ncRNAs) genes are at least as frequent as protein-coding genes in the human genome; however, our knowledge on their function is still preliminary. Their emerging role as new players in cancer development and therapy response is widely supported by several scientific reports.
Aims: Here, we aim to investigate BALR-2 involvement in pediatric acute myeloid leukemia (AML).
Methods: We retrospectively analyzed by RQ-PCR bone marrow samples of 132 children with *de novo* AML harboring different genetic abnormalities (CBF,MLL, and NUP98 rearrangements, FLT3-ITD, other rare translocations and patients without any recurrent molecular abnormality) diagnosed between 2002-2014 in one of the AIEOP centers and treated according to AML-2002/01 protocol. We compared patients' gene expression signatures (n=58, HTA affymetrix 2.0) with either high (4th quartile) or low expression (1st+2nd+3rd quartiles) of BALR-2, and used gene set and single sample enrichment analysis (GSEA, ssGSEA) to search biological differences. AML cell lines with different BALR-2 expression (high and low) were used to perform *in vitro* experiments.
Results: The expression of BALR-2 was found higher in all AML samples as compared with those collected from healthy volunteers. Moreover, we did not find any correlation between BALR-2 expression and any specific genetic subtype. We subdivided patients by using BALR-2 expression quartiles and found that patients with higher BALR-2 expression (4th quartile, n=32) had worse, although not statistically significant, EFS when compared to that of patients allocated to the 1st+2nd+3rd quartiles (n=100), butwe observed that the 4th quartile was enriched for patients who did not reach complete remission (CR) after induction therapy (28% *vs* 12%, p=0.03). Supervised clustering analyses showed that cases belonging to the 4th quartile (n=18) clustered separately from the remaining quartiles (n=40), irrespectively of the genetics. In particular, we found that patients with high BALR-2 (4th quartile) had 57 coding and 12 non-coding RNAs significantly differentially expressed (Fold Change|2|, p<0.01), and an upregulation of processes regulating mitochondrial mass and activity was found (p<0.05). We silenced BALR-2 in SHI-1 AML cell line (high BALR-2 expression) and revealed a decrease of mitochondrial mass by TOM20 staining (p<0.001), an enhanced mitochondrial depolarizion by JC1 staining, and an higher sensibility to FCCP after 6 and 24h of BALR-2 knockdown. We further

investigated CDK6, being chromosomally adjacent to BALR-2, and demonstrated a positive correlation of these genes expression levels (Pearson correlation>0.7, p<0.05). Silencing of BALR-2 *in vitro* reduced CDK6 mRNA and protein levels, as well as phospho-RB, its direct target. Noteworthy, we observed BALR-2 depletion increasing myelomonocytic differentiation, with CD11 and PU.1, both Runt-related transcription factor (RUNX1) targets, being upregulated. In agreement with these findings we showed that additional 81 RUNX1 target genes were down-regulated in patients with high BALR-2 (4th quartile) and-CDK6 expression.
Summary/Conclusion: Taken together, our data suggest that pediatric AML may have a broad heterogeneity in metabolic requirements and capacities as well as mitochondrial energetic through BALR-2 expression. We also highlight a cis-regulatory transcriptional relationship between BALR-2 and CDK6 linked to myeloid differentiation to be further dissected.

PB1678

GENOMIC-DRIVEN TRANSLATIONAL AND METABOLIC PERTURBATIONS IN ACUTE MYELOID LEUKEMIA CELLS UNDER THE SELECTIVE PRESSURE OF BROMODOMAIN INHIBITION IN THE HYPOXIC MICROENVIRONMENT

G. Simonetti[1,*], S. Bruno[1], C. Onofrillo[2], C. Papayannidis[1], G. Marconi[1], M. Cavo[1], L. Montanaro[2], G. Martinelli[3]

[1]Department of Experimental, Diagnostic and Specialty Medicine, "Seràgnoli" Institute of Hematology, [2]Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, [3]Istituto Scientifico Romagnolo per lo Studio e Cura dei Tumori (IRST) IRCCS, Meldola, Italy

Background: The hypoxic microenvironment plays a crucial role in survival and chemoresistance of leukemia stem cells (LSC) in acute myeloid leukemia (AML) and shapes cell metabolism. The inhibitor of bromodomain–containing (BRD) proteins (BETi), which is effective against AML cells in normoxia, targets the transcriptional program controlled by MYC, a crucial gene controlling LSC biology and metabolic functions under hypoxia.
Aims: To investigate the role of the hypoxic microenvironment on the selective pressure of BETi treatment on AML models with different genomic background.
Methods: AML cell lines (OCI-AML3: *NPM1* and *DNMT3A* mutated, Kasumi-1: t(8;21), H-60: MYC-amplified, MOLM-13, NOMO-1: MLL-driven, KG-1) were treated for 16 or 48h with the BETi GSK1215101A (250 nM or 500 nM) after 4h-adaptation to hypoxia. Downstream analyses were performed on Kasumi-1 and OCI-AML3 cells after 16h of treatment in order to allow cell adaption to the pharmacological pressure, while avoiding massive cell death. Gene expression profiling was carried out on actively translated mRNAs isolated by polysome profiling (Affymetrix) and enrichment analysis was performed by GSEA (Broad Insititute). The metabolic profile was obtained by Liquid Chromatography-Tandem Mass Spectroscopy (Metabolon).
Results: BETi induced a dose-dependent reduction of cell viability at 48h in AML cell lines (15%>35% decrease at 250 nM and 25%>65% decrease at 500 nM) except for HL60. Kasumi-1 was the most sensitive model. The treatment caused a significant arrest in the G_0/G_1 phase of the cell cycle in OCI-AML3, Kasumi-1, HL-60 and KG-1 cells and induction of apoptosis in NOMO-1 and Kasumi-1. Kasumi1 and OCI-AML3 showed a different translational profile, the latter being more active under steady state conditions. Hypoxia and BETi reduced the translational rate of both lines, as determined by a decrease of disome-polysome peaks height. This associated with BETi-mediated downregulation of a ribosome pathway signature (p<0.001). Downregulation of the MYC transcriptional program was enriched in the translatome of both lines under hypoxia (p<0.001). The two models shared a core translational program of 86 differentially expressed genes. Additional 881 and 168 genes were altered at translational level in Kasumi-1 and OCI-AML3 cells, respectively. Moreover, the cell type strongly influenced the metabolic profile in response to hypoxia and drug treatment. Hypoxic Kasumi-1 cells showed reduced lactate levels upon treatment, which may be due to a drug-dependent decrease in lactate dehydrogenase activity and decreased asparagine levels, along with downregulation of a gene signature of alanine, aspartate and glutamate metabolism (p<0.001), including asparagine synthetase (1.5-fold decrease, p<0.01). OCI-AML3 showed a significant increase of both reduced (3.9-fold) and oxidized (2.6-fold) forms of glutathione under hypoxia, which were confirmed by colorimetric assay.
Summary/Conclusion: AML cell lines are sensitive to BETi under hypoxia, which strengthens the drug effects on MYC expression and highlights novel potential dependencies. The consequences on leukemic cell metabolism suggest pathways to be exploited for combination therapies in genomic-driven approaches.

Supported by: EHA research fellowship award, ELN, AIL, AIRC, project Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project, HARMONY project, Fondazione del Monte BO e RA project.

PB1679

JAGGED-1 EXPRESSION IN MESENCHYMAL STROMAL CELLS HAS DISTINCT EFFECTS ON FLT3 MUTATED LEUKEMIA CELLS

M. Wobus*, M. Fusenig, K. Heidel, H. Altmann, M. Bornhäuser

Hematology/Oncology, Medical Faculty, University Hospital TU Dresden, Dresden, Germany

Background: Acute myeloid leukemia (AML) is a disease characterized by an increase in immature myeloid blasts in the bone marrow (BM) as a consequence of the loss of normal differentiation and uncontrolled proliferation of malignant hematopoietic stem and progenitor cells (HSPCs). As for normal HSPCs, the interaction with the surrounding BM microenvironment, *e.g.* with mesenchymal stromal cells (MSCs), mediated by cell surface proteins is important for leukemic cell fate and characteristics such as proliferation, survival and drug resistance. Amongst them, Notch pathways play an important role by regulating the crosstalk between leukemia cells and stromal microenvironment (Seke *et al.* 2012). Recently, we described the HSPC supportive effect of expression of Notch ligand Jagged-1 (Jag1) in MSCs (Duryagina *et al.* 2013).
Aims: Here, we aim to investigate specific effects of Jag1 expression in MSCs on leukemia cells.
Methods: MSCs were isolated by density gradient centrifugation and plastic adherence of mononuclear cells from healthy donors and AML patients (n=10) and were characterized by flow cytometry, Western blot and ELISA. The hTert immortalized MSC line SCP-1 with Jag1 over-expression or expression of dominant negative mastermind 1 (dnMAML1), in which Jag1 signaling of MSCs is blocked, has been described recently (Duryagina *et al.* 2013). AML cell lines MV4-11 (FLT-ITD positive) or OCI-AML3 (FLT3 wildtype) were co-cultured with the different SCP-1 cells and were analyzed by proliferation, adhesion and survival assays as well as by quantitative real-time PCR.
Results: MSCs of AML patients expressed common cell surface molecules, such as CD73, CD90, CD105, CD44, CD146 and CD166 but with higher variations than normal MSCs. Expression of the Notch ligand Jag1 was decreased in AML MSCs (detected in 3 out of 10 samples). The proliferation and adhesion capacity of MV4-11 cells was clearly decreased to about 35% on a SCP-1/Jag1 monolayer in comparison to wildtype and dnMAML1 cells. Addition of the g-secretase inhibitor DAPT partly rescued this effect confirming an involvement of Notch signaling. For OCI-AML3 cells no difference in proliferation and adhesion could be detected on the different SCP-1 layers. Treatment of MV4-11 cells with the tyrosine kinase inhibitor PKC412 (Midostaurin) or Ara-C increased the number of apoptotic and dead cells as detected by Annexin-V/PI staining in co-culture with SCP-1/Jag1 cells, suggesting that Jag1 over-expression can reverse the niche protective effect at least in part. Co-culture of MV4-11 cells on SCP-1/Jag1 cells caused a stronger induction of the Notch signaling pathway as shown by higher Hes-1 (20-fold) or Hey-1 (15-fold) mRNA expression levels than co-culture with SCP wildtype cells, whereas in OCI-AML3 this increase was significantly lower (2.5- and 1.5-fold, respectively). Whereas co-culture of AML cells caused inhibited secretion of SDF-1 by stromal cells, the co-culture of AML cells on SCP-1/Jag1 cells caused increased expression of CXCR4 mRNA in leukemia cells and accordingly 2- to 3-fold higher SDF-1 expression and secretion in the stromal cells in comparison to SCP-1 wildtype co-cultures. Again, these effects were much weaker in OCI-AML3 cells suggesting a role of FLT3 mutation.
Summary/Conclusion: In summary, we have demonstrated that the Jag1/Notch/Hes-Hey1 axis cause tumor inhibiting effects in FLT3 mutated AML cells. The different effects of Jag1 expression in MSCs on leukemia and normal hematopoietic cells may serve as prognostic marker and open a new therapeutic window.

PB1680

PRO-APOPTOTIC EFFECTS OF A METABOLIC-ORIENTED TREATMENT IN ACUTE MYELOID LEUKEMIA

C. Banella[1,2,*], G. Guerrera[3], G. Catalano[1], D.F. Angelini[3], M. Divona[4], S. Travaglini[1], L. Battistini[3], M.T. Voso[1], F. Lo Coco[1,2], N.I. Noguera[1,2]

[1]Biomedicine and Prevention, Tor Vergata University, [2]Neuro-Oncohematology, [3]Neuroimmunology, Santa Lucia Foundation, [4]Policlinico Tor Vergata, Roma, Italy

Background: Alteration of metabolic pathways induced by tumors may represent a potential target for therapeutic intervention. Acute myeloid leukemia (AML) mainly affects elderly subjects unfit for intensive chemotherapy in most cases. While AMLs have been extensively characterized in terms of genetic profile, little is known about their metabolic profile. Several agents currently tested in clinical trials interfere with metabolic pathways, including ascorbic acid (ASC), which is a potent pro-oxidant when used at high doses, and the anti-diabetic drug Buformin®.
Aims: To characterize metabolic pathways in different AML subsets and to assess the efficacy of Buformin® in combination with ASC in these tumors.
Methods: Using RQ-PCR and western Blot we analyzed in primary blasts from 50 AML patiens the expression levels of 7 metabolic enzymes: *HK1*, *HK2*, *PKM2* and *LDH,* active in glycolysis control; *PDH* and *PDK,* involved in the synthesis of acetyl-CoA; and the regulators of fatty acid oxidation (FAO) CPTA1 and CT2. We measured oxygen consumption (OCR) and media acidification (ECAR) in cell cultures, by using the Seahorse XF Analyzer. We then studied the effects of metabolic-interference treating with Buformin® and ASC in different tumor cell lines: PR9 which derive from U937 cells and are PML-RARA-inducible, U937 transfected with the RUNX1-RUNX1T1 oncogene, OciAML3 AML2 cells, all AMLs and U87MG cells (glioblastoma). Buformin® was tested at 100 μM and 500 μM and ASC at 1mM concentrations. Apoptosis was assessed by flow cytometry using annexin and propidium-iodide staining.
Results: In patients with AML overexpression of *PDH* and *CT2* mRNA was detected in primary Acute Promyelocytic Leukemia (APL) cells, as compared to other AML subtypes. *CT2* overexpression was confirmed at the protein level and in PR9 cells. Concerning the metabolic activity, we demonstrated a decrease in glycolysis and a clear increase of mitochondrial respiration in the presence of PML-RARA, concomitant with the increase of *PDH* levels. In addition, increased levels of FAO were observed in association with *CT2* overexpression. Conversely, expression of the RUNX1-RUNX1T1 transcript in inducible systems resulted in induction of glycolysis and mitochondrial respiration. In the presence of PML/RARA, Buformin® blocked the mitochondrial respiration and the consequent production of ATP in the Krebs cycle. ASC, alone or in combination with Buformin®, produced a slight increase in the cellular glycolytic capacity. Conversely, in RUNX1-RUNX1T1-positive cells, Buformin® shut down respiration, while in combination with ASC markedly decreased cellular glycolytic capacity. Treatment of both cell lines with Buformin® and ASC, blocked FAO, indicating that the cytotoxic effect could be partly due to the depletion of energy content. The pro-apoptotic effect was confirmed in primary blasts from seven AML patients by flow cytometry (p=0.01, Figure 1). The series also included one case characterized by the rare PLZF-RARa transcript, which is ATRA and ATO resistant.

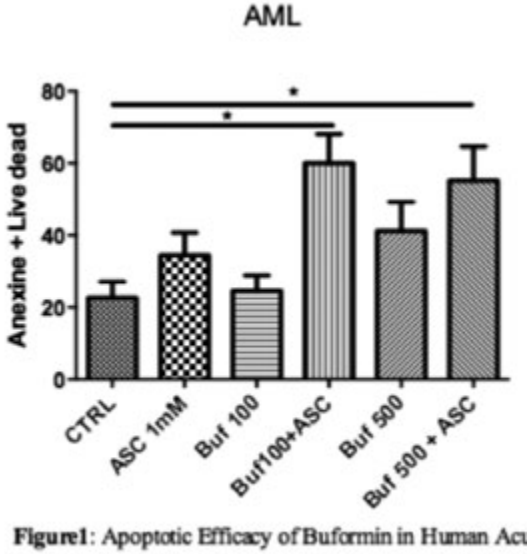

Figure1: Apoptotic Efficacy of Buformin in Human Acute Myeloid Leukemia Cells. Cooperation with Ascorbic Acid.

Figure 1.

Summary/Conclusion: Our data provide insights on metabolic changes in AML subtypes and indicates the possibility of a metabolically-oriented therapy in these diseases. Although further studies are required to confirm these observations, our data suggests that the Buformin®-ASC combination could be tested as an innovative and cost-effective therapeutic option in AML patients unfit for intensive chemotherapy.

PB1681

EXPLORATORY BIOMARKER ANALYSIS IN AML PATIENTS TREATED WITH ORAL SYK INHIBITOR ENTOSPLETINIB (GS-9973)

V. Munugalavadla[1,*], A. Joshi[1], A.R. Walker[2], T. Lin[3], M.D. Minden[4], H.E. Croswell[5], X. Tian[1], S.D. Patterson[1], A.V. Rao[1], Y. Pan[1]

[1]Gilead Sciences, Inc., Foster City, [2]The Ohio State University, Columbus, [3]University of Kansas Medical Center, Kansas City, United States, [4]Princess Margaret Cancer Centre, Toronto, Canada, [5]Bon Secours St. Francis Health System, Greenville, United States

Background: Acute myeloid leukemia (AML) is an aggressive hematopoietic malignancy characterized by diverse genetic and molecular abnormalities which contribute to poor response and survival rates. Spleen tyrosine kinase (SYK) signaling induces cell survival and proliferation by activating multiple pathways including chemokine regulation. Entospletinib (ENTO), an oral selective SYK inhibitor, is currently in clinical trials in AML. Previously we have shown that ENTO treatment inhibited BCR-mediated chemokines such as CCL3 and CCL4 in CLL patients (Sharman J *et al.*, 2015). This retrospective longitudinal systemic biomarker analysis aims to understand the effects of SYK inhibition by ENTO during lead-in monotherapy and induction chemotherapy (IC) in untreated AML patients (NCT02343939).
Aims: (1) Explore the pharmacodynamic (PD) effects of ENTO during monotherapy lead-in on systemic biomarkers related to SYK and or AML disease (2) Correlate baseline biomarkers with complete remission (CR) rates and various molecularly defined AML sub-groups [*NPM1*, *FLT3-ITD/TKD* and *KMT2A/*mixed lineage leukemia gene rearrangements (*MLL-R*)].
Methods: Longitudinal plasma samples [baseline, 6hr post dose on day 1, day 8, and day 14 during ENTO monotherapy and post induction chemotherapy] from AML patients treated with ENTO monotherapy for up to 14 days and subsequently in combination with induction chemotherapy (7+3; cytarabine 100mg/m^2 for 7 days plus daunorubicin 60mg/m^2 for 3 days) were analyzed. A panel of 64 cytokines/chemokines including markers known to be regulated by SYK and key markers implicated in driving AML disease was tested. We utilized validated electrochemiluminescence multiplex immune assays from Meso Scale Discovery (MSD). Biomarker fold changes at each time point were compared to baseline using a Wilcoxon signed rank test. Correlations of these cytokines/chemokines levels at baseline with response rates and with various molecular subgroups were assessed using Wilcoxon rank-sum test.
Results: Chemokines such as CCL3, CCL4, CCL17 and CXCL-10 known to be regulated by SYK were significantly decreased from baseline after ENTO monotherapy. Additionally, key markers that have a role in driving AML disease such as IL-12p40, IL-1RA and TNFα were also significantly reduced. Heatmap of changes over the time in various biomarkers are presented in Figure 1A. Higher baseline CCL1 were observed in patients that achieved CR compared to non-CR (Figure 1 B; p=0.027). Further molecular sub-set analysis showed that patients with *NPM1* mutation and with *FLT3-ITD/TKD* that were enriched in CR had high baseline CCL1 compared to wild type *NPM1* and *FLT3* patients. Baseline CCL1 levels were not significantly different between *MLL-R* patients and non-*MLL-R* patients even though most of *MLL-R* patients achieved CR suggesting underlying biology could be different in these sub-set of patients. Of note, in this study (NCT02343939) no ENTO exposure *versus* best overall response relationship was observed. Exploratory baseline *versus* post induction chemotherapy analysis is ongoing and updated results will be presented.

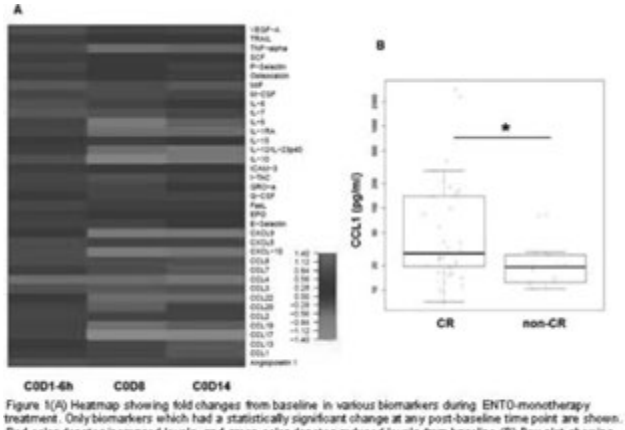

Figure 1(A) Heatmap showing fold changes from baseline in various biomarkers during ENTO-monotherapy treatment. Only biomarkers which had a statistically significant change at any post-baseline time point are shown. Red color denotes increased levels, and green color denotes reduced levels from baseline (B) Box plot showing baseline CCL1 levels in subjects that achieved complete remission (CR) compared with those that did not achieve complete remission (non-CR) in AML patients (NCT02343939); * Wilcoxon rank sum test p=0.027.

Figure 1.

Summary/Conclusion: This is the first study that has demonstrated a PD effect of the SYK inhibitor Entospletinib during lead-in monotherapy in AML patients. In addition, baseline biomarker CCL-1 was observed to be associated with clinical response. Conclusions from this study are limited given this exploratory analysis in this small study and considering that no adjustments for multiple testing were applied to *p*-values, hence, all observations should be considered hypothesis generating.

PB1682

SENSITIVE AND EARLY DETECTION OF THE PML-A216V MUTATION BY DROPLET DIGITAL PCR IN ARSENIC TRIOXIDE RESISTANT ACUTE PROMYELOCYTIC LEUKEMIA

V. Alfonso[1,*], L. Iaccarino[1], T. Ottone[1,2], L. Cicconi[1], S. Lavorgna[1], M. Divona[1], R. Cairoli[3], A. Cristiano[1], C. Ciardi[1], P. Chiusolo[4], A. Venditti[1], W. Arcese[1], M.T. Voso[1], F. Lo-Coco[1]

[1]Department of Biomedicine and Prevention, University of Rome Tor Vergata, [2]Neuroimmunology and Flow Cytometry Units, Santa Lucia Foundation, Rome, [3]Division of Hematology, ASST Grande Ospedale Metropolitano Niguarda, Milan, [4]Department of Hematology, Università Cattolica S. Cuore, Rome, Italy

Background: The use of ATRA combined with chemotherapy (CHT) and/or arsenic trioxide (ATO) can achieve long-term remission in the vast majority of patients with newly diagnosed APL. Despite this therapeutic success, 5-10% patients still relapse after modern treatment. Up to 30% of relapsed/refractory patients harbor point mutations within the ATO-binding domain (B2) of the PML moiety of the PML/RARA hybrid. These alterations affect the binding of ATO to the oncoprotein and thus impair PML/RARA degradation and clinical response to this agent. The most common mutation detected in APL refractory to ATO is the A216V, which is located within the PML-B2 domain.
Aims: In the present study, we developed and tested a droplet digital PCR (ddPCR) assay for the sensitive detection of *PML*-A216V mutation in relapsed APL patients as a tool to early predict ATO-resistance.
Methods: A total of 13 patients who relapsed after ATO treatment were analyzed. Table 1 shows the main patient characteristics at the time of APL diagnosis. Initial treatment included ATRA/CHT in 10 and ATO/ATRA in 3 cases. Eleven patients in the series underwent multiple relapses. Mutational analysis was performed at the time of first relapse in the 3 patients treated with front-line ATO and in ≥2nd relapse in patients receiving ATO as salvage treatment. Mutational analysis of *PML*-A216V by ddPCR assay (BioRad), was performed in duplicate, using 50 ng of DNA for each sample. The limit of detection (LOD) of ddPCR assay was determined by diluting mutant DNA in the DNA sample derived from a healthy donor at the following ratios: 1:10, 1:100, 1:250 and 1:500. The threshold for positive amplification was established within each reaction based on the results of *PML*-A216V negative template controls. The ddPCR assay was initially performed on DNA samples collected at the time of relapse where a high *PML/RARA* copy number had been detected by conventional RQ-PCR. We then investigated the mutation dynamics by backtracking the identified mutation in samples collected for routine PML/RARA monitoring prior to relapse. To confirm and compare the results obtained by ddPCR, all positive samples were also analyzed by Sanger sequencing.

Table 1.

Table 1. Clinical and biological characteristic of APL patients.

UPN	Age/Sex	Sono risk	PML/RARA isoform	First-line treatment	CR1 duration	Relapses (n)	Timing of ATO treatment	Response to ATO	PML mutational status by ddPCR
1	54/M	Standard	BCR1	ATRA-ATO	3 months	2	Front-line	mCR*	PML wt
2	32/M	Intermediate	BCR1	ATRA-ATO	23 months	1	Front-line	mCR*	PML wt
3	77/M	Intermediate	BCR3	ATRA-ATO	18 months	1	Front-line	mCR*	PML wt
4	29/F	Intermediate	BCR1	ATRA-CHT	35 months	4	Salvage	Refractory	PML A216V
5	77/M	Intermediate	BCR1	ATRA-CHT	17 months	2	Salvage	Refractory	PML A216V
6	40/F	Intermediate	BCR2	ATRA-CHT	3 months	1	Salvage	Refractory	PML A216V
7	68/M	Intermediate	BCR1	ATRA-CHT	24 months	2	Salvage	Refractory	PML A216V
8	38/M	Standard	BCR3	ATRA-CHT	8 months	2	Salvage	Refractory	PML wt
9	42/F	High	BCR3	ATRA-CHT	9 months	3	Salvage	Refractory	PML wt
10	42/F	Low	BCR3	ATRA-CHT	12 months	2	Salvage	mCR*	PML A216V
11	61/M	Intermediate	BCR3	ATRA-CHT	30 months	3	Salvage	Refractory	PML wt
12	66/F	Intermediate	BCR1	ATRA-CHT	26 months	3	Salvage	Refractory	PML wt
13	32/F	Standard	BCR1	ATRA-CHT	36 months	3	Salvage	mCR*	PML wt

*mCR=molecular Complete Remission

Results: The ddPCR test showed high reproducibility and sensitivity and was able to detect up to 0.4% *PML*-A216V-mutant allele fraction. After assessing the false positive rate (FPR), samples were considered positive if the mutation rate had ≥3 positive droplets above the threshold of the negative template controls. The A216V mutation was detected by ddPCR in 5/13 patients (38%) who relapsed after ATO. Sanger sequencing allowed to identify the *PML*-A216V mutation in 4/5 cases. The ddPCR assay carried out in follow-up DNAs of mutated patients (total of 44 samples, median 3 per patient, range:1-22), revealed the presence of *PML*-A216V mutation in 17 samples. Of these, 3 were collected in overt relapse while 14 were taken at molecular relapse in patients showing low *PML/RARA* transcript levels. Sanger sequencing confirmed the mutation in only 4/17 samples. In 3 mutated patients for whom several sequential samples were available, a positive-ddPCR test anticipated a positive Sanger sequencing result by 3, 4 and 24 months, respectively.
Summary/Conclusion: Our data show that a ddPCR assay can be efficiently employed in the screening of *PML*-A216V mutation in APL and is able to identify mutant cases earlier in the disease course as compared to conventional sequencing. This sensitive method may help to identify ATO-resistant APL patients who are candidate to alternative treatment strategies.

PB1683

GENE EXPRESSION PROFILING REVEALS A THREE-GENE ANTIAPOPTOTIC SIGNATURE USEFUL TO SWITCH-OFF ANTIAPOPTOTIC STIMULI IN ACUTE MYELOID LEUKEMIA

J. Nanni[1,*], G. Simonetti[1], G. Marconi[1], M.C. Fontana[1], S. Bruno[1], C. Papayannidis[1], M. Abbenante[1], S. De Polo[1], C. Sartor[1], S. Parisi[1], S. Paolini[1], A. Curti[1], M. Cavo[1], G. Martinelli[2]

[1]Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Institute of Hematology "L. e A. Seràgnoli", bologna, [2]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) s.r.l IRCCS, Meldola, Italy

Background: Apoptotic regulation involves several actors which can have a positive and negative effect and may represent a target for personalized therapy. Nowadays, BCL-2 inhibitors have successfully passed preliminary phases of clinical experimentation, however the involvement of other essential proteins could represent a resistance mechanism to BCL-2 inhibitors and reveal novel therapeutic target in acute myeloid leukemia (AML).
Aims: Our study aim to investigate the expression of anti-apoptotic genes in AML patients and to unravel if these differential expressions are associated to genomic alterations.
Methods: We performed Human Transcriptome Array 2.0 (Affymetrix) and SNP 6.0 or Cytoscan HD Array (Affymetrix) in cohorts of 59 and 270 newly diagnosed AML, respectively. K-means clustering has been used to categorize patients in different cluster of expression. A pool of 7 healthy donors was used to normalize expression data. Survival analysises were conducted with Kaplan-Meyer method and differences in survival were assessed with Log-Rank test. Fisher exact test has been performed to evaluate significant associations on Copy Numeber Data.

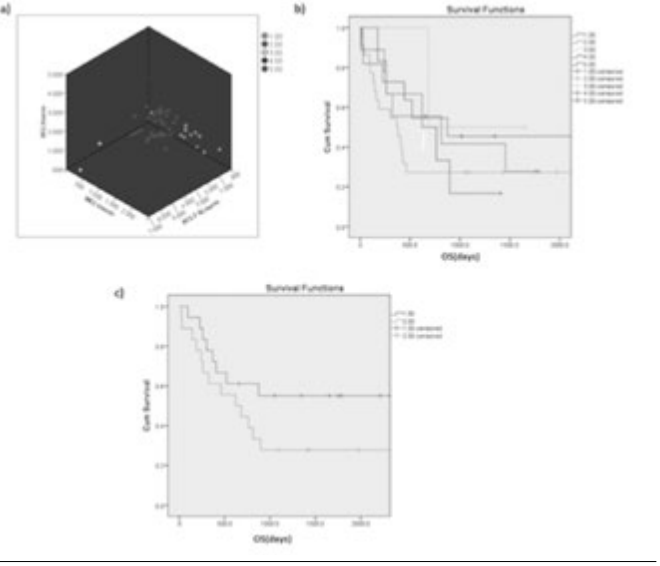

Figure 1.

Results: *BCL-2*, *MCL-1* and *BCL2L1* gene expression values in 59 AML patients allowed us to establish 5 clusters (Fig. 1a): cluster 1 with the overexpression of *MCL-1*, cluster 2 with low expression of all analyzed genes; cluster 3 with overexpression of *BCL2L1*, cluster 4 with overexpression of *BCL-2* and cluster 5 with high values of expression of all 3 genes. Clusters

2 and 5 included the majority of patients (40/59, 68%). Furthermore, the 3-D Scatter Plot (Fig. 1a) showed that the patients out of the central cloud (Clusters 2-5) always presented an overexpression of one out of the three anti-apoptotic genes. Moreover, when *BCL-2* level is low (Cluster 1-2-3), *MCL-1* or *BCL2L1* levels are high, respectively. In term of overall survival (OS), there were no statistically significant difference between Cluster 1-3-4, where one different anti-apoptotic gene is always overexpressed (Fig. 1b). Finally, analyzing the impact of *BCL-2* expression on OS in a cohort of 36 young patients treated with chemotherapy, those with high expression of *BCL2* gene had worse outcome (Fig. 1c, p=0.07). Subsequently, we screened a cohort of 270 AML patients for genomic copy number alterations by SNP array. We could not find significant CNA that could be rationally related to *BCL-2*, *MCL-1* and *BCL2L1* expression: *BCL2* was lost in 3.7% and gained 1.1% of cases; *MCL-1* was lost in 1.1% and gained in 0.37% of cases; and *BCL2L1* was lost 0.37% and gained in 1.85% of patients.

Summary/Conclusion: *BCL-2*, *MCL-1* and *BCL2L1* expressions are quite balanced in AML patients. One out of the three genes is often overexpressed, confirming the key role of anti-apoptotic genes in leukemogenesis. Even though *BCL-2* expression influences patients chemosensitivity, *MCL-1* and *BCL2L1* overexpression has been documented when *BCL-2* is normally or downregulated. The overexpression of these genes can not be explained only by genomic aberrations. Finally, a combined approach should be realized to switch-off leukemic anti-apoptotic mechanisms: gene expression profile could provide a synthetic lethal instrument to choose the proper BH3-mimetic drug or combination. Further studies are needed to confirm the post-traductional context and unveil other characters of this pathway regolation.

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PB1684

THE DNA DAMAGE REPAIR PATHWAY IS ALTERED IN POOR PROGNOSIS ACUTE MYELOID LEUKEMIA PATIENTS AND IDENTIFIES POTENTIAL TARGETS FOR SYNTHETIC LETHAL THERAPIES

A. Padella[1,*], G. Simonetti[1], M.C. Fontana[1], G. Marconi[1], A. Ferrari[1], C. Papayannidis[1], S. Bruno[1], E. Fonzi[1], M. Pazzaglia[1], E. Ottaviani[1], M. Cavo[1], S. Soverini[1], G. Martinelli[2]

[1]Department of Experimental, Diagnostic and Specialty Medicine, Seragnoli institute, Bologna, [2]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) Srl-IRCCS, Melodola (FC), Italy

Background: Partner and localizer of BRCA2 (*PALB2*) plays a key role in the DNA damage repair (DDR) and genomic alterations of DDR genes rarely occur in acute myeloid leukemia (AML), while their deregulation at transcriptional level is a known mechanism exploited by leukemic cells in order to sustain the high genetic instability and to continue proliferation.

Aims: We aimed to characterize the role of *PALB2* in AML by investigating its genomic alterations and its expression levels, in order to evaluate its potentiality as target of therapies based on a synthetic lethal approach.

Methods: We genotyped 270 AML samples by Single Nucleotide Polymorphism array (SNP 6.0 and Cytoscan HD, Affymetrix). We performed Whole Exome Sequencing (WES, Illumina) of 69 cases to detect single nucleotide variants (MuTect and Varscan 2.0). Gene expression profiling (GEP, Affymetrix) was performed on bone marrow cells of 7 healthy donors (HD) and 60 AML patients. K-means clustering of patients according to the expression of *PALB2* was performed.

Results: AML patients carried copy number alterations (CNA) in genes involved in the DDR pathway including *PALB2*, *BRCA1*, *BRCA2*, *FANCA* and *RAD50*. Among them, 11 patients (4%) carried a CN loss of *PALB2* with a minimal common region of 6.6 Kb, including ex 11-12 encoding for domain of interaction with other DDR related genes. Notably, these patients were also characterized by the co-occurrence of losses of 5q, 17q11, 16p13-p12 and gain of 21q22. Biologically relevant genes targeted by CNAs in PALB2-loss patients were *TP53*, *NF1*, *BRCA1*, *STAT3*, *FANCA*, *CREBBP*, *XPO* and *USP34*. Enrichment analysis of differentially altered genes (q<.001) revealed that GO biological processes affected by CNAs included protein folding, apoptotis, mitotic cell cycle, metaphase/anaphase transition, nucleic acid phosphodiester bond hydrolysis, double-strand break repair (p<1e-04). *PALB2* was not mutated in our WES dataset. However, DDR genes such as *TP53*, *BRCA1*, *BRCA2*, *CHEK2*, *FANCE* were found mutated at least once. Moreover, *PALB2* loss significantly associated with *TP53* mutations (p=0.015) while *KRAS*, *IDH1/2*, *TET2* mutations were mutually exclusive. Transcriptional analysis revealed variable *PALB2* levels in AML patients (range 52.90-244.37) and its median expression was higher compared with HD (129.26 *vs* 67.85, respectively; p=.019). We clustered patients according to *PALB2* expression and defined 2 clusters: H and L with high and low expression levels of *PALB2*, respectively (cluster centers 158.86 and 105.41, respectively). Enrichment analysis of differentially expressed genes revealed deregulations of the following GO pathways: mitochondrial translational, regulation of cell proliferation, negative regulation of myeloid cell differentiation and G1/S transition of mitotic cell cycle (p<.013).

Summary/Conclusion: We dissected the molecular and transcriptional landscape of AML and we identified alterations in *PALB2* and the DDR pathway in a small subgroup of patients. Our data open a new scenario in which *PALB2* may drive dependencies to be exploited by targeted therapies in AML: breast cancer patients carrying mutations in *PALB2* and *BRCA1/2* are candidate for PARP inhibitors treatment, while few clinical trials are available in AML. Therefore, we identified a group of AML patients which may benefit of personalized therapies based on synthetic lethal approaches targeting the DDR pathway.

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PB1685

ESTIMATING THE OPTIMAL THRESHOLD OF BAALC AND ERG AS PREDICTIVE BIOMARKERS IN ACUTE MYELOID LEUKEMIA

S.K. Hasan[1,*], N. Patkar[2], D. Vishwakarma[1], D. Rajamanickam[1], A. Bhattacharjee[3], A. Gokarn[1], S. Punatar[1], A. Bonda[1], L. Nayak[1], P. Tembhare[2], S. Kannan[4], B. Bagal[1], H. Jain[1], P. Amare[5], H. Menon[1], M. Sengar[1], S. Gujral[2], P. Subramanian[2], N. Khattry[1]

[1]Adult Hematolymphoid Disease Management Group, [2]Hematopathology Laboratory, Tata Memorial Centre, Mumbai, [3]Centre for Cancer Epidemiology, [4]Biostatistics, Advanced Centre For Treatment Research and Education in Cancer Mumbai, Navi Mumbai, [5]Cytogenetics Laboratory, Tata Memorial Centre, Mumbai, India

Background: The high expression of brain and acute leukemia, cytoplasmic (*BAALC)* and *ETS-related gene (ERG)* has been associated with poor prognosis in acute myeloid leukemia (AML) but due to limited prospective studies, their role as prognostic factors is still unclear. We had presented the *BAALC* & *ERG* expression data and its impact on survival during the ASH 2017 meeting at San Diego. In that study, the median value was taken as a cut-off to distinguish between high & low *BAALC/ERG* expressers. So far, there are no standard methods or tools to decide the optimal cut-off points. In this analysis of the same cohort of patients, we generated threshold values for *BAALC* & *ERG* cut-off points through P-chain procedure (Bhattacharjee *et al.* 2017). In brief, *BAALC* & *ERG* expression values were measured as continuous variable at baseline, post-induction and post-consolidation blood/marrow samples. Further, lists of p-values were generated from arbitrarily defined threshold values of *BAALC* & *ERG*. The lowest p-value was defined as maximum survival difference among these patients to decide the cut-off point for threshold values of *BAALC* & *ERG*.

Aims: To determine the optimal threshold values of *BAALC* & *ERG* expression as a cut-off point to predict the survival outcome.

Methods: All patients included in this prospective study (N=149) between March 2012 & February 2015 underwent following investigations: (i) Cytogenetic analyses- by standard techniques of chromosome banding & FISH (ii) Mutation profiling of *NPM1*, *FLT3*-ITD and *CEBPA* by capillary electrophoresis & direct DNA sequencing. Long-range PCR was done to establish *MLL*-PTD (iii) *BAALC* & *ERG* expressions by quantitative real-time PCR assays. Patients received standard induction chemotherapy with daunorubicin 60mg/m^2 for 3 days and cytosine arabinoside 100 mg/m^2 for 7 days. Marrow was done 21-28 days after start of chemotherapy. If marrow was in remission, then patients received 3 courses of consolidation therapy with 12-18gm/m^2 of cytosine arabinoside (HiDAC). The statistical analysis was done using R 3.4.0 version.

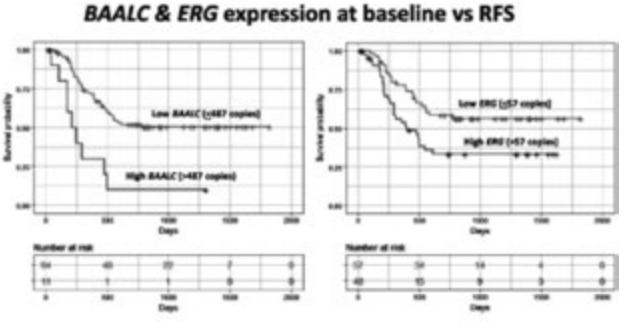

Figure 1.

Results: A total of 149 patients were accrued. Of 149 patients, 105 achieved CR after 1 or 2 cycles of chemotherapy. Twenty-seven patients were refractory of which 18 were lost to follow up and hence excluded from OS analysis. Seventeen patients died during induction. The follow-up samples included post induction (N=120) and post-consolidation (N=95) bone marrow. The OS of 132 patients was 36.5% at 2 years. The OS & RFS of 105 patients who achieved CR was 54% and 43.8% respectively. The threshold value (487 *BAALC* copies*/ABL [on OS and RFS]*, 34 *ERG* copies*/ABL[on OS] and* 57 *ERG* copies*/ABL[on RFS]*) was used to distinguish high expressers from low expressers. Initially, the baseline, post induction and post-consolidation expression values of *BAALC* & *ERG* were observed for their influence on OS & RFS using univariate Cox proportional hazards analyses & results are reported in Table 1. The data revealed that higher *BAALC* & *ERG* expression values were significantly hazardous for OS & RFS. Furthermore, threshold values of *BAALC* & *ERG* at baseline were calculated and results are reported in Table 2. The RFS of high *BAALC vs* low *BAALC* expressers at 2 years was 9% *vs* 51% (p=0.059), while RFS of high *ERG vs* low *ERG* expressers at 2 years was 31% *vs* 59% (p=0.013) (Figure 1).

Table 1.

Table1: Analysis to assess the impact of *BAALC* and *ERG* baseline, post induction and post consolidation expression on OS and RFS

Measurement	Parameter	Number of Patients	Number of Events	HR	se(coef)	95% CI	P-value
Overall Survival	ERG_Baseline	132	25	1.79	0.24	[1.11,2.87]	0.015
	ERG_PostInduction	120	18	2.11	0.25	[1.28,3.48]	0.003
	ERG_PostConsolidation	95	8	2.09	0.46	[0.84,5.20]	0.110
	BAALC_Baseline	132	25	1.07	0.17	[0.75,1.53]	0.676
	BAALC_PostInduction	120	18	1.66	0.19	[1.13,2.43]	0.009
	BAALC_PostConsolidation	95	8	1.75	0.23	[1.10,2.78]	0.016
Relapse Free Survival	ERG_Baseline	105	50	1.37	0.11	[1.11,1.74]	0.004
	ERG_PostInduction	98	50	1.07	0.10	[0.86,1.31]	0.567
	ERG_PostConsolidation	88	45	1.83	0.16	[1.30,2.51]	0.000
	BAALC_Baseline	105	50	1.62	0.09	[1.34,1.95]	0.000
	BAALC_PostInduction	98	50	1.51	0.09	[1.23,1.75]	0.000
	BAALC_PostConsolidation	88	45	1.61	0.08	[1.34,1.91]	0.000

Table 2.

Table2: Analysis to assess the impact of *BAALC* and *ERG* baseline expression on OS and RFS based on threshold values

Gene expression	Parameters	Parameter estimates	Error	Hazard ratio	P-value
BAALC baseline ≤ 487 .04 BAALC baseline > 487 .04	OS	0.917	0.505	2.503	0.0687
BAALC baseline ≤ 487 .04 BAALC baseline > 487 .04	RFS	0.828	0.431	2.291	0.0598
ERG baseline ≤ 34 .2 ERG baseline > 34 .2	OS	1.303	0.372	3.682	0.000
ERG baseline ≤ 57 .43 ERG baseline > 57 .43	RFS	0.705	0.284	2.204	0.013

Summary/Conclusion: This study established the optimal threshold values of *BAALC* & *ERG* expression as cut-off points to predict the outcome. The data suggest that high *BAALC* & *ERG* expression adversely affects prognosis of AML patients.

PB1686

THE ROLE OF DNA REPAIR MECHANISMS IN ACUTE MYELOID LEUKAEMIA

C. Crean*, K. Savage, K. Mills

Centre for Cancer Research, Queens University Belfast, Belfast, United Kingdom

Background: The therapeutic landscape for AML has changed little over the past four decades. Cytarabine, first approved in 1969, is still a standard of care treatment option for AML despite little improvement in survival rates over time. Whilst AML is a disease which can occur at any age, it predominantly affects people over the age of 65. With an increasing elderly population, the rate of incidence of AML is set to increase. Additionally, although some patients do respond to Cytarabine treatment, there is no signature or test available to determine which patients will, or will not, benefit from this type of treatment. A DNA Damage Repair Deficiency (DDRD) score was previously developed to identify breast cancers with an intrinsic DNA double strand break (DSB) repair deficiency and therefore may respond positively to DNA damaging chemotherapeutic agents such as cyclophosphamide. This assay can be adapted to different cancer types including AML.
Aims: The objective of this study was to examine if the DDRD assay can be applied to AML samples as a potential biomarker of response and/or survival.
Methods: To test the effectiveness of DDRD score as a biomarker the DDRD calculation was applied to RNA expression data from 645 AML patients. The score was also determined for a panel AML cell lines. Cell lines defined as DDRD positive or negative were tested for sensitivity to different DNA damaging agents using clonogenic survival assays. The DNA repair capacity of these cell lines was also examined by assessing their ability to repair ionising radiation induced DSBs.
Results: Approximately 39% of the patients scored as DDRD positive. This did not significantly correlate with any risk group, cytogenetic abnormality or mutation such as FLT3 or NPM1. Survival analysis showed that the DDRD positive patients had a significantly worse survival than the DDRD negative patients (10-year survival 27% in DDRD positive patients compared to 42% in DDRD negative (p-value=0.00047)) despite the prediction of responding better to DNA damaging agents as seen in the breast cancer studies. The response of the cell lines to the DNA damaging agents corresponded with the patient data with DDRD positive cell lines showing less sensitivity to cytarabine as DDRD negative cell lines. The foci analysis from the IR treated cells also showed that the DDRD positive cell lines do not repair DSBs as effectively as the DDRD negative cell lines.
Summary/Conclusion: These results suggest that determining the DDRD score could effectively be used to determine which patients would benefit from treatment with DNA damaging agents. Furthermore, the poor survival rate of the DDRD positive patients indicates that cytarabine is not the optimal treatment option for these. Further analysis of this unresponsive AML sub-group could highlight therapeutic pathways to target either as single agents or in combinations with for example PARP inhibitors.

PB1687

THE ROLE OF S100A4 IN ACUTE MYELOID LEUKEMIA

B. Alanazi[1,*], A. Pierce[2], A.D. Whetton[2], S. Knapper[1], C.R. Munje[3], R. Darley[1], A. Tonks[1]

[1]Department of Haematology, School of Medicine, Cardiff University, Cardiff, [2]Faculty of Medical and Human Sciences,Institute of Cancer Sciences, The University of Manchester, Manchester, [3]Paul O'Gorman Leukaemia Research Centre, University of Glasgow, Glasgow, United Kingdom

Background: Acute myeloid leukemia (AML) is a disorder arising from developmental arrest of cells of the myeloid lineage. To understand how regulators of transcription mediate this block, we previously analyzed the nuclear proteome of AML blasts against developmentally matched HSPCs and found nuclear mislocalization and differential expression of S100A4. Cytosolic overexpression of this calcium binding protein has been implicated in solid tumours and is associated with poor clinical outcomes; however, little is known about the role of aberrant nuclear expression of S100A4 in AML.
Aims: (i) To validate the overexpression of S100A4 in AML patients and cell lines; (ii) To determine the effects of overexpressed S100A4 on haematopoietic growth and development using normal human $CD34^+$ cells; (iii) To determine whether AML cell lines are dependent on S100A4 overexpression.
Methods: We analysed AML patient samples (n= 24) for S100A4 expression and subcellular localisation by western blot using normal $CD34^+$ cells as controls. Lentiviral vectors containing Nuclear Localisation Signals (NLS) were used to overexpress S100A4 into the nucleus of normal HPSCs and AML cell lines. shRNA was used to knock down S100A4 in normal HPSCs and in AML cell lines. Effects on growth and haematopoietic development were analysed by multiparameter flow cytometry.
Results: To confirm our initial studies, we analyzed a second cohort of AML patient blasts and identified S100A4 to be mislocalized to the nucleus in ~83% of AML cases (20/24). Interestingly, S100A4 protein was not expressed in the nucleus of normal HSPC or differentiated precursors in myeloid lineage (monocytes, erythrocytes, and granulocytes) throughout development. To determine the effect of nuclear-targeted S100A4 overexpression, we infected $CD34^+$ cells with lentivirus encoding nuclear-targeted S100A4. Whilst we could over-express S100A4 in the nucleus of some leukemic cell lines, we could not stably express it in HSPCs. We found that nuclear expression of S100A4 increased cell proliferation in TF-1 compared to uninfected controls. Conversely, knockdown of S100A4 in cell lines with elevated S100A4 expression using shRNA, induced cell death in THP-1, OCI-2 and TF-1.
Summary/Conclusion: We found that S100A4 is over-expressed and mislocalized to the nucleus in AML blasts. Whilst normal HSPCs express this protein in the cytosol of some myeloid differentiated lineages, they do not tolerate over-expression of this protein in the nucleus. In the context of transformed cells, S100A4 promotes proliferation and is essential for AML cell survival. These data suggest that S100A4 could be a target for therapy for AML.

PB1688

MOLECULAR CHARACTERIZATION OF PEDIATRIC ACUTE MYELOID LEUKEMIA: RESULTS OF A PROSPECTIVE MULTICENTRIC STUDY IN RUSSIA

A. Panfyorova*, M. Gaskova, N. Timofeeva, O. Soldatkina, A. Kazakova, I. Kalinina, G. Novichkova, M. Maschan, A. Maschan, Y. Olshanskaya

Dmitry Rogachev National Research Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation

Background: The genetic characterization of pediatric acute myeloid leukemia (AML) is an important outcome predictor. Genomic profiling studies have dramatically advanced our understanding of the origin, progression and clonal evolution of AML. High-Throughput Sequencing (HTS) provides a unique insight into mechanisms of disease initiation, risk stratification and treatment and multi-gene mutation panel is a part of diagnostic routine testing by now.
Aims: To perform expanded prospective molecular diagnosis in children with AML mainly concerning comprehensive risk assessment and stratification and to improve our understanding of mutational and clonal complexity in each patient because of the morphological and molecular heterogeneity of AML.
Methods: Genetic analyses of bone marrow (BM) samples from pediatric patients with newly diagnosed *de novo* AML recruited into the Russian National Prospective AML Study between September 2016 and February 2018 were performed by a combination of fragment analysis, Sanger sequencing for genes involved in risk stratification, and HTS with Human Myeloid Neoplasms Panel (HMNP) (Qiagen, Germany) for analysis of 141 genes most commonly mutated in myeloid neoplasm. Library was constructed for sequencing on a MiSeq using v3 chemistry with 2x150 bp read length.
Results: Diagnostic samples from 146 (75 male and 71 female) pediatric patients with *de novo* AML were analyzed. Age at diagnosis ranged from 0 to 17,6 years (median 6,6). An average depth of 978x was achieved. The total read fragments number was about 2 358 019 generated reads per sample with primer found on-target ~ 95.7%. Coding and splice site regions of *FLT3*, *NPM1*, *KIT*, *CEBPA* genes were fully covered, *GATA1* gene had almost full coverage with the exception of exon 4. All SNV mutations in risk stratification genes were confirmed by Sanger sequencing. However indel variants such as *FLT3*-ITD (18 cases, size range-from 20 to 115) we accurately identified in all cases just only by fragment analysis, HTS analysis algorithms could only find the smallest of them-20 and 22 bp. Overall, 15% (22/146) of analyzed patients had normal karyotype, they showed molecular mutations in risk-stratification genes: 1(4,6%) patient had isolated *FLT3*-ITD, 4 (18%) had isolated *NPM1*mut, in 5 (22,7%) cases combination of *NPM1*mut+*FLT3*mut was observed and only 1 (4,6%) patient had bi*CEBPA*. The numerous mutations were identified in additional genes: *RUNX1, NRAS, KRAS, PTPN11, WT1, IDH1, IDH2, DNMT3A, ASXL1, ASXL2, STAG2.* A highest mutational rate and their variable cooperation were observed in patients with normal karyotype and t(8;21). In 16 patients with t(8;21) *KIT* mutations were found in 10 (62,5%). AML with t(8;21) displayed also elevated mutational diversity: *NRAS, RUNX1, ASXL2, EZH2, STAG2, NOTCH1, CSF3R, KDM6A, TP53, RAD21, JAK3, U2AF2.*
Summary/Conclusion: Our results reflect the profile of pediatric AML cases in Russia. In our study the identification of subgroups defined by molecular aberrations, according to national diagnostic recommendations for specific treatment and assessment of molecular heterogeneity were performed. There were significant differences in gene mutations among morphological, cytogenetic and age groups. The nonrandom mutational cooperations were observed. Identification of genetic subgroups is important for the molecular epidemiology and biology of AML worldwide.

PB1689

ADAM28 AGGRAVATES THE LEUKEMIA BURDEN IN A MICE MODEL AND PREDICTED A HIGH RISK OF RELAPSE IN FAVORABLE-RISK AML PATIENTS

J.-M. Zhang[1,2,3,*], C.-C. Wang[1,2,3], Q. Jiang[1,2,3], H.-X. Fu[1,2,3], Q.-M. Wang[1,2,3], X.-L. Zhu[1,2,3], H. Jiang[1,2,3], Y. Wang[1,2,3], M. Lv[1,2,3], Y.-J. Chang[1,2,3], L.-P. Xu[1,2,3], K.-Y. Liu[1,2,3], X.-J. Huang[1,2,3], X.-H. Zhang[1,2,3]

[1]Peking University Institute of Hematology, Peking University People's Hospital, [2]Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, [3]Collaborative Innovation Center of Hematology, Peking University, Beijing, China

Background: We have reported that ADAM28 promoted the invasion and survival of leukemic cells; however, the precise prognostic relevance between ADAM28 and acute myeloid leukemia (AML) remain unknown.
Aims: To compare the outcome of AML depending on the expression of ADAM28.
Methods: A well-established NOD/SCID xenotransplantation model was used to test the effect of ADAM28 on the AML burden. Bone marrow samples were obtained from adults with AML (N=107) and normal individuals (N=24) recruited at the Hematology Department of Peking University People's Hospital between May 2013 and May 2014, among which 32 were part of the test group and 75 were prospectively enrolled to the validation group.
Results: The relative expression level of ADAM28 in the leukemic cells of the testing group was significantly higher than that in the control BM cells (0.98± 0.09 *vs* 0.49±0.04; p<0.001). Furthermore, the ADAM28 level in the CSF of patients with CNSL was significantly higher than that without CNSL (2.87±0.81 *vs* 0.50±0.08, p<0.0001). In the intrafemoral xenotransplantation experiments, peripheral leukocyte counts were significantly lower in mice receiving ADAM28-knock out (KO) cells compared with those in mice with control cells. A Kaplan-Meier plot demonstrates that mice bearing ADAM28-KO cells survived longer compared with control mice (P= 0.017). Slighter splenomegaly was observed in mice with ADAM28-KO cells. Additionally, knock out of ADAM28 decreases the incidence of CNS infiltration. In the *in vitro* experiment, the increased expression of ADAM28 led to more IGFBP-3 degradation and IGF-I-induced cell proliferation. To validate the effect of ADAM28 protein level on the prognosis of AML patients, we extended ADAM28 expression level detection to a validation group of 75 patients with AML. The ADAM28 expression level of the validation group in the bone marrow and CSF between patients with or without CR had the same trend as the testing group. Prognostic analysis revealed that in the enrolled 87 patients (patients with APL were excluded), the CIR and EFS was significantly higher in the ADAM28 high expression group (p=0.003, 0.021 and 0.017 respectively). However, the prognostic analysis in patients with M3 did not differ significantly. Notably, when separately analyzing the impact of the ADAM28 expression level on prognosis within clinically defined risk groups, patients with high ADAM28 expression levels had a significantly higher CIR and worse EFS in the favorable-risk group, and a significantly higher CIR in the intermediate-risk group, but this discrimination was not observed in the poor-risk group. Additionally, patients with high ADAM28 expression presented a significantly higher CIR than the low expression patients in the chemotherapy subgroup, whereas relapse did not differ significantly with the ADAM28 expression level in patients receiving transplantation. Interestingly, when the median or arithmetic average was taken as the cut-off value of ADAM28 expression, the result of the survival analyses had a similar trend in the favorable-risk group, which might indicate that ADAM28 was a valuable prognostic marker.
Summary/Conclusion: In summary, these data provide the first demonstration that high expression of ADAM28 correlates with a high risk of relapse in favorable risk AML patients. This correlation is seen in subjects receiving chemotherapy only but not in those also receiving an HSCT. However, whether these patients would benefit more from HSCT need further validation by prospective large-scale randomized clinical trials.

PB1690

FREQUENCY AND SIGNIFICANCE OF DICENTRIC CHROMOSOMES IN BONE MARROW CELLS OF PATIENTS WITH ACUTE MYELOID LEUKEMIA

S. Ransdorfova[1,*], I. Sarova[1], J. Brezinova[1], Z. Zemanova[2], L. Lizcova[2], L. Pavlistova[2], K. Svobodova[2], S. Izakova[2], K. Skipalova[2], L. Hodanova[2], A. Jonasova[3], C. Salek[4], K. Michalova[2]

[1]Cytogenetic Department, Institute of Hematology and Blood Transfusion, [2]Center of Oncocytogenetics, Institute of Medical Biochemistry and Laboratory Diagnostics, General University Hospital and First Faculty of Medicine, Charles University, [3]1st Medical Department, General University Hospital and First Faculty of Medicine, Charles University, [4]Clinical department, Institute of Hematology and Blood Transfusion, Prague, Czech Republic

Background: In 10-12% of patients with acute myeloid leukemia (AML) in bone marrow cells, complex karyotype (CK) is present in their initial cytogenetic evaluation. It is traditionally regarded as a poor prognostic factor, particularly in patients showing dicentric chromosomes.
Aims: The aim of the study was to review karyotypes of AML patients with CK and to verify the presence of dicentric chromosomes (DCs) by FISH with multi-centromeric probes. The frequency and prognostic significance of DCs in AML was evaluated as well.
Methods: During years 2006–2016, 607 adult patients with newly diagnosed AML (excluding the specific subtype of AML-M3) were examined by conventional cytogenetics. The complex aberrations were investigated using

multicolor fluorescence *in situ* hybridization (mFISH) and multicolor banding (mBAND). Monosomies were verified with the XCyting Centromere Multi-Color Probe Mix (MetaSystems), which is able to distinguish 18 chromosomal pairs in one test, and/or with a Vysis chromosome enumeration (CEP) probe (Abbott) and/or Satellite Enumeration (SE) FISH Probes SE 13/21 and SE 14/22 (Kreatech Diagnostics).
Results: CK was proved in 114 (19%) adult AML patients at diagnosis. Monosomy was detected in 63% of patients by conventional cytogenetics/mFISH, respectivly in 55% by centromeric FISH. Some of the losses were revised as the presence of a hidden DC. The use of centromeric FISH proved the increase of frequency of DCs in adult AML patients with CK from 27% (31/114) to 51% (58/114). In total, 79 dicentric, 2 tricentric, 1 quadricentric, and 12 isodicentric chromosomes were identified. DCs were often formed by chromosomes 17 and 20. Some DCs were observed repeatedly, however the breakpoints were not recurrent.
Summary/Conclusion: FISH analysis revealed high frequency of DCs in adult AML patients, particularly in bone marrow cells with complex karyotypes. Unbalanced aberrations leading to chromosomal losses indicate possible presence of hidden DC or its previous existence. DCs undergo variety of stabilization changes and can produce secondary monocentric derivative chromosome. DCs participate in raising of chromosomal instability (additional aberrations and new DCs) resulting in the clonal evolution of abnormal cells, which is associated with the adverse course of the disease and short survival of patients.

PB1691

A NGS-BASED APPROACH FOR MOLECULAR CLASSIFICATION OF ACUTE MYELOID LEUKEMIA (AML) WITH INTERMEDIATE PROGNOSTIC CYTOGENETIC RISK

L. Floreani[1], E. Toffoletti[1,*], A. Chiarvesio[2], S. Radovic[3], M. Morgante[3], D. Fabbro[4], G. Damante[4], A. Candoni[2], R. Fanin[1]

[1]Department of Experimental and Clinical Medical Sciences, Division of Hematology and BMT, University of Udine, [2]Hematological Division of Udine, Azienda Sanitaria Universitaria Integrata di Udine, [3]Istituto di Genomica Applicata, Istituto di Genomica Applicata, [4]Institution of Medical Genetics, Azienda Sanitaria Universitaria Integrata di Udine, Udine, Italy

Background: Acute Myeloid Leukemia (AML) cases presenting intermediate-prognostic cytogenetic features account for about 40% of adult AMLs and display heterogeneous clinical outcomes. Over the past 2 decades an increasing number of molecular lesions, having prognostic and therapeutic significance, have been described in AML. Mounting evidence is showing that deep genomic characterization might provide an important tool to predict pathology progression. In line with these findings, two recent works (Grimwade *et al*., 2015 and Pappaemmanuil *et al*.,2016) proposed a genomic classification of AML that relies upon the study of a number of recurrent mutations.
Aims: Validation of a specific gene panel, having prognostic and therapeutic relevance, on AML patients (preliminary results).
Methods: Taking advantage of Next Generation Sequencing (NGS) Illumina MiSeq platform, we performed deep sequencing analysis of the following genes: ABL (exons 4-9), ASXL1 (exons 9,11,12,14), BRAF (exon 15), CALR (exon 9), CBL (exons 8,9), CEBPA (all gene), CSF3R (all gene), DNMT3A (all gene), ETV6 (all gene), EZH2 (all gene), FLTR3 (exons 13-15,20), HRAS (exons 2,3), IDH1 (exon 4), IDH2 (exon 4), JAK2 (all gene), KIT (exons 2,8-11,13,17,18), KRAS (exons 2,3), MPL (exon 10), NPM1 (exons 10,11), NRAS (exons 2,3), PTPN11 (exons 3,7-13), RUNX1 (all gene), SETBP1 (exon 4), SF3B1 (exons 10-16), SRSF2 (exon 1), TET2 (all gene), TP53 (exons 2-11), U2AF1 (exons 2,6), WT1 (exons 6-10), ZRSR2 (all gene). In this study, AML cases at onset, that presented intermediate-prognostic cytogenetic risk were considered. We analyzed a total of 11 samples, 5 of which had normal karyotype, 5 patients harboured intermediate cytogenetic alterations, 1 case had complex karyotype.
Results: From our analysis we were able to collect all analyzed AMLs into molecularly-defined subsets, according to what reported by Grimwade and Pappaemmanuil and colleagues. In particular, we observed 36% of cases harbouring NPM1 as driver-mutation, of which 100% displayed co-occurrence of FLT3 mutation. On the other hand, 36% of cases were RUNX1-mutated. In this latter group, 75% of cases associated with lesions in ASXL1 gene and 50% with additional chromosome 8. Finally, 18% of cases did not meet criteria with classes presenting NPM1 or RUNX1 driver mutations, whereas the sample with complex karyotype displayed simultaneous TP53 mutation (see table 1). Importantly, we noticed that all AML samples, apart from the one with complex karyotype, had molecular alterations on epigenetic modifiers, mainly IDH1-2, DNMT3A or, alternatively, TET2, that displayed Variant Allele Frequencies (VAF) equal to or higher than 0.5, suggesting that aberrant epigenetic pathways affect the majority of blast cells, therefore representing an early event in AML pathogenesis.

Table 1.

Table 1. Proposed genomic classification of AML cases with relative sample distribution and concomitant mutations per each subgroup (adapted from Papaemmanuil et al.,2016)

Genomic subgroup of AML *as indicated by Papaemmanuil et al.,2016*	Frequency in the study cohort (N=11) *no. of patients (%)*	Most frequently molecular alterations *driver mutation (%), concomitant mutated genes or cytogenetic alterations (%)*
AML with NPM1 mutation	4 (36)	**NPM1 (100)**, FLT3 (100), CEBPA (50), ASXL1 (25), DNMT3A (25), IDH1 (25), TET2 (25), NRAS (25), del20q11 (25)
AML with mutated chromatin, RNA-splicing genes, or both	4 (36)	**RUNX1 (100)**, ASXL1 (75), +8 (50), DNMT3A (50), SETBP1 (50), IDH1 (50), SRSF2 (50), IDH2 (25),TET2 (25), +11 (25), t(1;9) (25)
AML with TP53 mutations, chromosomal aneuploidy, or both	1 (9)	**TP53 (100)**, complex karyotype (100), CBL (100), CEBPA (100)
AML with no detected driver mutations	2 (18)	TET2 (100), BRAF (50), FLT3 (50), WT1 (50), -Y (50), +8 (50)

Summary/Conclusion: Although the combinatorial effect of different molecular lesions remains to be uncovered, our data show that the emerging genomic classification system might represent a reproducible method for AML characterization based on molecular approaches.

PB1692

IDENTIFICATION OF A LONG NON-CODING RNA (LNCRNA) ASSOCIATED WITH FAVORABLE OUTCOME IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)

A. Neddermeyer[1,*], Y. Qu[2], K. Karlsson[3], M. Björklund[1], S. Mareschal[1,4], C. Nilsson[2], S. Bengtzén[2], A. Lennartsson[4], S. Lehmann[1,2]

[1]Medical Sciences, Uppsala University, Uppsala, [2]Medicine, [3]Medical Biochemistry and Biophysics, [4]Biosciences and Nutrition, Karolinska Institutet, Stockholm, Sweden

Background: Acute myeloid leukemia (AML) is the most frequently diagnosed type of acute leukemia in adults, with a dismal 5-year relative survival of only 25.9%. Efforts to improve prognosis focus on elucidating the mechanism behind AML development, to improve risk classification using novel biomarker and establishing new means for personalized treatment. Non-coding parts of the transcriptome, such as long non-coding RNAs (lncRNAs) exhibit crucial regulating functions and their dysfunction is often associated with cancer. However, the functional role of lncRNAs in the development and progression of AML is so far poorly understood.
Aims: In this study, we aimed to identify long non-coding RNAs (lncRNAs), which are differentially expressed in AML and study their role on leukemogenesis. We hypothesized that these non-coding transcripts could potentially play important biological roles in the development or progression of AML, serve as novel prognostic or predictive biomarkers or could indicate new treatment targets in AML.

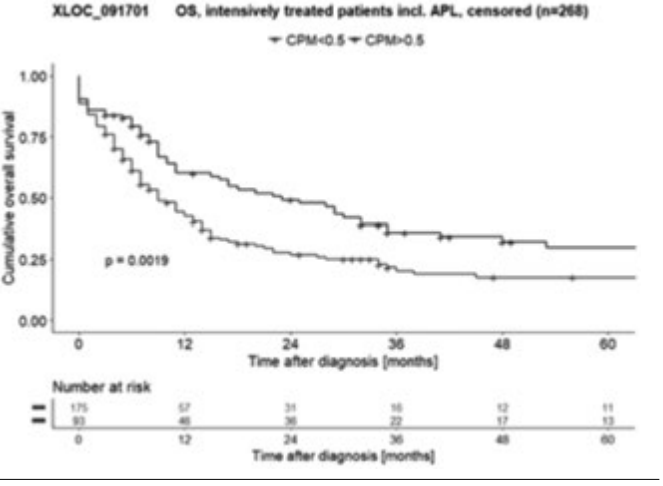

Figure 1.

Methods: We performed deep RNA-sequencing in a subset of AML samples and normal CD34+ bone marrow cells and identified, among others, lncRNA XLOC_091701 (internal reference), which is upregulated in AML. TSS peaks of XLOC_091701 were confirmed using FANTOM CAGE data

(RIKEN) and XLOC_091701 expression was validated in ten myeloid cell lines using RT-qPCR. Further, XLOC_091701 expression was validated using RNA expression data from our AML patient cohort (n=325, ClinSeq cohort) and confirmed by The Cancer Genome Atlas (TCGA)-LAML cohort (n=151). Next, multivariate cox regression analysis and clinical correlation analysis was done to identify significant correlations with clinical parameters and survival using available clinical data on intensively treated AML patients (n=268, ClinSeq-AML cohort).
Results: We found XLOC_091701 to be exclusively expressed in certain myeloid cell lines, especially in HL60 and U937 cells, and expressed in about a third of all tested AML patients in both ClinSeq-AML cohort (n=268) and TCGA-LAML cohort (n=117, cut-off: CPM>0.5). Next, multivariate cox regression revealed significant superior survival in patients with XLOC_091701 expression independent of other variates known for good prognosis (n=268, ClinSeq-AML cohort, p=0.002) and moreover associates with formerly used AML FAB classification M2 and M3 (APL) along with low genetic and low cytogenetic risk groups.
Summary/Conclusion: Here, we describe a long non-coding RNA transcript (XLOC_091701) whose expression is associated with better overall survival in AML patients. Our findings suggest that XLOC_091701 plays a biological role in AML and might serve as prognostic biomarker in clinical settings. Currently, we are creating a KO-model to characterize XLOC_091701 functionally to explore the regulatory mechanisms behind the observed favorable prognosis.

PB1693

ROLE OF CLEC12A IN THE LSC COMPARTMENT OF PEDIATRIC ACUTE MYELOID LEUKEMIA AT DIAGNOSIS *VERSUS* RELAPSE

B. Depreter[1,2,*], D. Hanekamp[3], T. Lammens[4], B. De Moerloose[4], E. Sonneveld[5], B. Denys[2], A. De Jong[5], G.J. Kaspers[6,7], G.J. Schuurhuis[7], J. Philippé[2], J. Cloos[3]

[1]Department of Paediatric Haematology-Oncology and Stem Cell Transplantation, [2]Department of Laboratory Medicine, Haematology, Ghent University Hospital, Ghent, Belgium, [3]Department of Haematology, VU University Medical Center, Amsterdam, Netherlands, [4]Paediatric Haematology-Oncology and Stem Cell Transplantation, Ghent university hospital, Ghent, Belgium, [5]Dutch Childhood Oncology Group, The Hague, [6]Princess Maxima Center for Paediatric Oncology, Utrecht, [7]Department of Paediatric Oncology/Haematology, VU University Medical Center, Amsterdam, Netherlands

Background: Despite high clinical remission rates (90%), pediatric acute myeloid leukemia (pAML) patients exhibit a high risk of relapse (30-40%). Recently, the leukemic stem cell (LSC) load at diagnosis, determined as $CD34^+/CD38^-/CD45^{low}$ cells expressing an aberrant marker, was shown to be an independent prognostic factor for relapse in pAML. Nevertheless, the flow cytometric aberrancies of LSCs remain poorly described. In adult AML, it was shown that the stem cell-associated antigen CLEC12A (CD371, CLL-1) strongly aids in discriminating normal hematopoietic stem cells (HSCs) from LSCs.
Aims: To evaluate the frequency and specificity of CLEC12A in the LSC fraction of pAML at diagnosis and relapse.
Methods: Bone marrow (BM, n=6) and/or peripheral blood (PB, n=4) of seven pAML patients were taken at diagnosis and relapse. For three patients, BM and PB was available at both time points. FCM data acquisition for CLEC12A (PE, clone 50C1) was performed together with backbone markers CD34 (PerCP-Cy5.5), CD38 (APC-H7) and CD45 (PacO or V500). All samples were analyzed on a 3-laser, 8-color FACSCanto II (BD) with instrument set-up performed according to EuroFlow guidelines. Within the $CD34^+/CD38^-$ (10^3 cut-off for CD38) stem cell compartment, expression of CLEC12A was analysed using the lymphocytes as negative control. In addition, $CD34^+/CD38^-$ cells from cord blood (CB, n=8) and normal pediatric bone marrow (NBM, n=6) were used to evaluate CLEC12A expression on normal HSC. Data analysis was performed using Infinicyt software v.1.8 (Cytognos, Salamanca, Spain) with a previously described gating strategy. Paired samples t-test was used to calculate statistical significance.
Results: The median blast percentage (% of white blood cells (WBC)), and CD34+ fraction within the blasts, was 40% and 64%, respectively, at diagnosis and 64% and 62%, respectively, at relapse. Total events measured ranged between 8575-1027340 (median 221579). Differences between the median $CD34^+CD38^-$ load within the WBC compartment at diagnosis *versus* relapse were not significant in our cohort (BM: 0.08% *versus* 0.13%, PB: 0.07% *versus* 0.27%; P>0.05). At diagnosis, five out of seven patients showed aberrant CLEC12A expression, with a median CLEC12A pos/CLEC12A neg ratio within the $CD34^+CD38^-$ compartment of 1.55 (range 0.46-20, BM=4, PB=3). The percentage of CLEC12A positive $CD34^+CD38^-$ cells of ranged between 4-95% (median 44%). At relapse, only one patient lost CLEC12A expression. CLEC12A pos/CLEC12A neg ratios were not significantly different at relapse (range 0-26, median 0.18) compared to diagnosis (range 0-20, median 0.49) in regard to the other six patients (BM=5, PB=3) (P>0.05). Analysis of PB was highly comparable to BM for the three patients with paired BM and PB samples. Importantly, no CLEC12A positive stem cell population (≥4 events and clustered on SSC/FSC) was observed within the $CD34^+/CD38^-$ compartment of pediatric NBM or CB.
Summary/Conclusion: This report describes the frequency of aberrant CLEC12A expression in LSC in pAML. These data suggest that CLEC12A is a valuable and specific marker in children to discriminate leukemic from normal HSC.

PB1694

SYNERGISTIC EFFECTS OF PI3K AND C-MYC CO-TARGETING IN ACUTE MYELOID LEUKEMIA: PROPOSING NOVEL THERAPEUTIC POTENTIAL FOR AML

N. Riyahi*, N. Sheikh-Zeineddini, M. Sayyadi, D. Bashash

Department of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic Of

Background: Interest into targeting PI3K pathway has elevated by the latest accomplishments which manifest that overexpression of PI3K not only is contributed to disease progression but also is associated with poor outcome in patients with leukemia. Despite the broad spectrum efficacies, drug resistance on account of multiple interfering factors, such as overexpression of c-Myc is known to be still a daunting challenge for the successful application of PI3K inhibitors.
Aims: To delve into whether c-Myc suppression could intensify the antitumoral effects of PI3K inhibitor in AML cells, U937 and KG1 cells were subjected to treatment with CAL-101 and 10058-F4.
Methods: Cell viability, growth kinetics, DNA synthesis rate, induction of apoptosis, and caspase-3 activity were assessed after exposing the cells with both individual and combination treatments of the drugs. Moreover, gene expression analyses by using quantitative real-time PCR were applied to examine the effects and molecular mechanisms of CAL-101 and 10058-F4 combination.
Results: Our results demonstrated that single agent of CAL-101, as the excelled member of PI3Kδ inhibitor, induced cytotoxic and growth suppressive effects through shifting the ratio of death promoters to death repressors via amendment of pro- and anti-apoptotic genes expression. Moreover, PI3K inhibition resulted in a concentration-dependent induction of apoptosis coupled with the enzymatic augmentation of caspase-3, as a substantial executioner of the apoptotic pathway. Noteworthy, the apoptotic effect was even more evident in combinational treatment, as the percentage of apoptotic dead cells was robustly higher in the cultures of AML cells co-treated with CAL-101 and 10058-F4. To investigate whether the augmentative impact of c-Myc inhibitor is a general feature in PI3K inhibition, we evaluated the effect of 10058-F4 in combination with pan-PI3K inhibitor BKM120. Of particular interest and in agreement with the results of CAL-101, it became evident that abrogation of c-Myc using 10058-F4 could enhance the tumor suppressive assets of the PI3K inhibition in acute myeloid leukemia.
Summary/Conclusion: Taken together, our findings not only highlighted that PI3K inhibition is a promising approach in AML but also illustrated the favorable therapeutic prospective for PI3K inhibitors in combination with 10058-F4.

PB1695

CHONDROITIN SULPHATE PROTEOGLYCAN 4 POTENTIAL MARKER OF MINIMAL RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIA AND BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM

M. Hisemová[1,*], V. Válková[1], I. Marinov[1], M. Marková[1], J. Polák[2]

[1]medical department, [2]department of genomics, Institute of Hematology and Blood Transfusion, Prague [2], Czech Republic

Background: A key prognostic factor in acute leukemia is the monitoring of minimal residual disease (MRD). Methods are based on flow cytometry, as well as molecular biology methods using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). The RT-qPCR methodology enables us to follow MRD-specific markers or non-specific markers such as WT1.
Aims: Assessment of suitable molecular-biological markers of MRD in patients with AML, and in patients with rare hematologic malignancy Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN).

Methods: Expression of the non-specific marker *WT1* was tested according to the European Leukemia Net protocol (ELN) using a WT1 ProfileQuant kit (Qiagen). To monitor the expression of *Chondroitin Sulphate Proteoglycan 4 (CSPG4),* one of the Leukemia Associated Antigens (LAA), we used a TaqMan Gene Expression Assay (ThermoFisher). The data were normalized to the expression of the *ABL* control gene TaqMan Gene Expression Assay with results in relative units (ru); upper normal limit is ru=1.00. Similarly, we measured the expression of the *XAGE1 gene,* another LAA. Additionally, an in-house methodology was established to obtain *CSPG4* absolute quantification, showing *CSPG4* copy number normalized to 10^4 copies of the *ABL* reference gene. Leukemia-Associated Immunophenotype (LAIP) measured by flow cytometer also monitored the MRD.
Results: In 170 patients with AML, *CSPG4* expression with a median expression of 0.517 was measured at diagnosis. Significantly increased expression of this marker was found in the AML M5 subtype (n=17, median=20.51, P 0.0001), in AML possessing a MLL translocation (n=12, median=59.15, P 0.0001), and in 4 patients with BPDCN (median=74.26, P=0.0001); all related to *CSPG4* values measured in peripheral blood of healthy donors (n=22, median=0.262). The median expression of the *CSPG4* in BPDCN patients was nearly 2 orders above the normal upper limit. No specific MRD marker is available in patients with BPDCN, and *WT1* expression at diagnosis is uniformly low. We validated the measurement of *CSPG4* expression in 4 patients by comparison with *WT1* and *XAGE1* expressions, reaching a high correlation (P=0.05, P=0.0006, P=0.0002, and P<0.0001). The MRD level was monitored in all patients at diagnosis, after induction therapy, after consolidation therapy, and after allogeneic stem cell transplantation (ASCT). The expression of this MRD marker correlated with the clinical status of the patients, as well as with flow cytometric data. Subsequently, a *CSPG4* absolute quantification methodology was developed and validated.
Summary/Conclusion: MRD monitoring in patients with AML has prognostic significance. By monitoring the expression of *CSPG4*, we found that it was overexpressed in patients with AML with *MLL* translocation, with AML M5 subtype, and in a rare BPDCN entity. Common features of the AML patients with *MLL* translocation and the BPDCN patients are poor prognosis and low *WT1* expression at diagnosis. We validated the methodology for monitoring of this alternative marker at the mRNA level instead of *WT1.* The *CSPG4* expression was in agreement with the clinical and flow cytometric data of the patients. Further prospective monitoring of this marker is necessary.Conclusion:*CSPG4* appears to be a potentially suitable molecular marker of MRD in AML M5, AML with *MLL* translocation and BPDCN. Supported by MH CZ-DRO UHKT 00023736

PB1696

DESCRIPTION OF ACUTE MYELOBLASTIC LEUKEMIA WITH ISOLATED TRISOMY 13. EXPERIENCE OF FIVE CENTERS

D. Cruz[1,*], R. Coll[2], M. Cabezón[3], L. Zamora[3], E. Tuset[1], N. Lloveras[1], N. Santos[1], R. Guàrdia[1], A. Cisneros[3], N. Ruiz-Xivillé[3], J. Grau[3], M. Xandri[3], S. Marcé[3], S. Vives[4], C. Talarn[5], M. Arnan[6], S. Brunet[7], C. Moret[2], D. Gallardo[2], I. Granada[8]

[1]Clinical Hematology Department, Institut Català d´Oncologia Girona. Hospital Universitari Doctor Josep Trueta, [2]Clinical Hematology Department, Institut Català d´Oncologia Girona. Hospital Universitari Josep Trueta de Girona., Girona, [3]Hematology Laboratory Institut Català d´Oncologia Badalona., Hospital Universitari Germans Trias i Pujol. Institut d'investigació contra la leucèmia Josep Carreras. Universitat Autònoma de Barcelona., [4]Clinical Hematology Department, Institut Català d´Oncologia Badalona. Hospital Universitari Germans Trias i Pujol., Badalona, [5]Clinical Hematology Department, Institut Català d´Oncologia Tarragona. Hospital Universitari Joan XIII., Tarragona, [6]Clinical Hematology Department, Institut Català d´Oncologia de l'Hospitalet., L'Hospitalet de Llobregat, [7]Clinical Hematology Department, Hospital de la Santa Creu i Sant Pau., Barcelona, [8]Hematology Laboratory, Institut Català d´Oncologia Badalona. Hospital Universitari Germans Trias i Pujol. Institut d'investigació contra la leucèmia Josep Carreras. Universitat Autònoma de Barcelona., Badalona, Spain

Background: Acute Myeloid Leukaemia with isolated 13 trisomy (AML +13), is classified as intermediate risk-II according with the European Leukaemia Net group (ELN), and has a worse relapse-free and overall survival compared to the other ELN intermediate-II patients, with a median overall survival of 9,3 months. AML +13 has an incidence of 0,7%, it's associated with undifferentiated morphology and immunophenotype features, and the hand-mirror blast cells are a characteristic finding. Previous works has suggested that AML +13, has a characteristic mutational profile with mutations of RUNX1, *SRSF2*, *ASXL1*, BCOR and CEBPZ.
Aims: To describe retrospectively the clinical, biological and morphological data from a cohort of 9 patients diagnosed of AML +13 and compare it with the AML +13 patients from Mehta *et al.*[1] and Harold *et al.*[2] cohorts.
Methods: We collected clinical, morphological and genetic data from 9 patients diagnosed of AML +13 in 5 Catalan hospitals, from September 2013 to May 2017.For the mutational analysis, we used next generation DNA sequencing with a panel of 32 genes often mutates in myeloid malignancies *(ABL1,ASXL1,BRAF,CARL,CBL,CEBPA,CSF3R,CSNK1A1,DNMT3A,ETV6,EZH2,FLT3,HRAS,IDH1 IDH2,JAK2,KIT,KRAS,KMT2A, MPL,NPM1,NRAS,PTPN11,RUNX1,SETBP1,SF3B1,SRSF2,TET2,TP53, U2AF1,WT1,ZRSR2*). We used MiSeq Illumina System and SOPHIA DDM software for data analysis.
Results: The most frequently mutated genes in our cohort were *RUNX1*(7/8, 88%), *ASXL1* and *SRSF2*(4/8, 50%), *DNMT3A*, *FLT3* and *TET2*(3/8, 38%). Other less frequent mutated genes were *IDH2, KMT2A, ZRSR2*(2/8, 25%) and *EZH2, IDH1, U2AF1, SETBP1, WT1* and *CBL*(1/8,13%). In Harold *et al.* cohort, the genes most frequently mutated were *SRSF2* 81%, *RUNX1* 75%, *ASXL1* 44%, *BCOR* 25%, *TET2* 19%, *IDH2* 19% among others, which is comparable with our cohort.In our cohort(n=9) the median age was 72 years-old [58-89], there was a male predominance(8:1), hand-mirror blast cells and small blast cells were present in 89% and 78% of cases, respectively and according FAB classification patients were diagnosed of M0 (n=4), M1(n=4) and M2(n=1), without dysplasia in any case. In the Metha's cohort(n=23), the median age was 66 years-old, a male predominance(17:6), hand-mirror blast cells and small blast cells were present in 52% and 71% of cases and FAB classification was M0(n=11), M1(n=6), M2(n=2), M4(n=2), M5(n=1), M6(n=1); very similar and comparable with our results. Three patients were treated with intensive cytarabine-based chemotherapy and one of them underwent allo-transplant, being alive after 6 years from the diagnosis. One patient was treated with hypomethylanting agents, one patient was included in a clinical trial and four patients aged >70 years old received only best supportive treatment. Four patients died due to sepsis (one of them during reinducction after relapse) and four patients died due to haemorrhage. The median overall survival was 8,1 months[0,16-33], being the results comparable with those of other series.

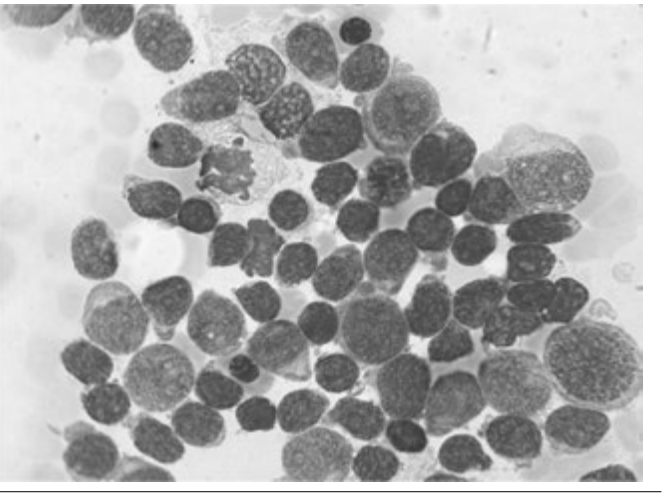

Figure 1.

Summary/Conclusion: The results of our series were comparable to those described in other series, which supports the different biological and therapeutic behavior from this AML group compared with other AML of the same risk group, according to the ELN.It's possible that the mutational profile, with frequent mutations in the *RUNX1, ASXL1, SRSF2*, and other genes related to resistance to treatment and less survival, plays a crucial role with the different behavior that AML +13 presents, requiring further studies with larger series to be able to confirm it, and to assess its inclusion as a differentiated entity within the WHO classification.

PB1697

PROGNOSTIC IMPACT OF DNMT3A MUTATIONS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) WITH NPM1MUTATION DEPENDING ON FLT3 MUTATIONAL STATUS

G. Oñate[1,*], A. Garrido[1], A. Aljarilla[1], J. Esteve[2], M. Arnan[3], R. Guardia[4], M. Tormo[5], S. Vives[6], O. Salamero[7], L. Escoda[8], A. Sampol[9], J. Bargay[10], M.P. Queipo de Llano[11], A. Garcia[12], S. Brunet[1], J. Sierra[1], J. Nomdedeu[1], M. Pratcorona[1]

[1]Hematology, Hospital Santa Creu i Sant Pau, [2]Hematology, Hospital Clinic,

Barcelona, [3]Hematology, Institut Catala d'Oncologia Bellvitge, Hospitalet de Llobregat, [4]Hematology, Hospital Josep Trueta, Girona, [5]Hematology, Hospital Clínico, Valencia, [6]Hematology, Hospital Germans Trias i Pujol, Badalona, [7]Hematology, Hospital Vall d'Hebron, Barcelona, [8]Hematology, Hospital Joan XXIII, Tarragona, [9]Hematology, Hospital Son Espases, [10]Hematology, Hospital Son Llatzer, Palma de Mallorca, [11]Hematology, Hospital Virgen de la Victoria, Málaga, [12]Hematology, Hospital Arnau de Vilanova, Lleida, Spain

Background: The association of *NPM1*mut and the internal tandem duplication of *FLT3* (*FLT3*-ITD) in *de novo* AML with intermediate-risk cytogenetics has different prognostic impact according to the *FLT3*-ITD/*FLT3*wt ratio, thus affecting clinical decisions about the indication of allogeneic hematopoietic stem cell transplantation (alloHSCT) in first complete remission (CR1). DNMT3A mutations (*DNMT3A*mut*)* have been suggested to have an adverse prognostic impact in this group of patients; however the effect of this triple mutation association is not well established.
Aims: To evaluate the prognostic value of additional *DNMT3A*mut in *de novo* AML with *NPM1*mut and *FLT3*-ITD, and its survival impact according to *FLT3* ratio.
Methods: Patients with *de novo* AML, intermediate-risk cytogenetics and *NPM1*mut were selected from CETLAM database, from our cooperative group protocols AML-2003 and AML-2012. Bone marrow samples from diagnosis were studied for *DNMT3A* mutations as previously described. The Kaplan-Meier test was used to estimate overall survival (OS) and leukemia-free survival (LFS), whereas risk of relapse (RR) was analysed by the cumulative incidence estimator with non-relapse mortality as the competing event.
Results: A total of 114 patients were selected from protocol AML-2003 (n=49) or AML-2012 (n=65). Median age was 51 years (18-71), with 54% females, median leucocyte count 42x10E9/L (1.2-408x10E9/L) and median bone marrow blasts 78% (20-100%). *FLT3* status was defined as wild type (n=34) or *FLT3*-ITD (n=76). High ratio (*FLT3*high) was considered when ≥0.50(n=43) and low ratio (*FLT3*low) when<0.5(n=33). *DNMT3A*mut was detected in 60 patients, the majority in amino acid R882(n=48). In patients with *NPM1*mut and *FLT3*-ITD, *DNMT3A*mut *vs DNMT3A*wt had no impact in 5 year OS (n=80): 40%±10% *vs* 56%±6% (p=0.28) or 5 year LFS (n=70): 27%±12.6% *vs* 55%±1% (p=0.105). Similarly, when divided according to *FLT3* status (*FLT3*wt *vs FLT3*low *vs FLT3*high) *DNMT3A*mut had no significant prognostic impact in 5 year OS (n=58) 70%±11% *vs* 58%±13% *vs* 28%±13% (p=0.104); 5 year LFS (n=54) 67%±12% *vs* 44%±13% *vs* 19%±14% (p=0.073); or RR (n=54) 20.4% (95%CI 2.6%>49.8%) *vs* 35.6% (95%CI, 11.4%>61.1%) *vs* 52.2% (95%CI, 24.7%>73.9%) p=0.117. Thirty-six patients of the entire cohort received alloHSCT in CR1. When censoring follow-up at the date of transplant, RR analysis confirmed an increasing risk in *DNMT3A*mut patients according to *FLT3* status: *FLT3*wt 23.4% (95% CI, 2.8%>55.4%; n=30) *vs FLT3*low 49.8% (95% CI, 13.8%>78.2%; n=28) and *FLT3*high 56.6% (95% CI, 23.2%>80.2%; n=32) p=0.0113. *DNMT3A*mut effect was analysed in *FLT3* subgroups, showing impact only in *FLT3*wt *vs FLT3*high 23.4% *vs* 56.6% (p=0.014), and a trend in *FLT3*low *vs FLT3*high 49.8% *vs* 56.6% (p=0.077). No significant differences were seen in *FLT3*wt *vs FLT3*low 23.4% *vs* 49.8% (p=0.178).
Summary/Conclusion: It has been established that AML with *NPM1*mut and *FLT3*low has better outcome than *FLT3*high. Overall, our study suggests that *DNMT3A* mutations do not have survival impact in AML with *NPM1*mut and *FLT3*-ITD. However, when RR is censored by date of alloHSCT in CR1, *DNMT3A*mut shows a trend to a higher relapse risk when associated to *FLT3*-ITD; this could be of clinical relevance in patients with a low *FLT3*-ITD ratio who are currently not considered for alloHCT in CR1 in our group. These results should be confirmed in future studies that gather a higher number of patients and may include the evaluation of measurable residual disease (MRD) that could anticipate patients with an inadequate MRD clearance who could benefit from alloHCT in an early phase.

PB1698

HAEMATOLOGICAL CANCERS & THE 100,000 GENOMES PROJECT-CREATING CLINICAL PATHWAYS TO ENABLE WHOLE GENOME SEQUENCING IN A DIAGNOSTIC SETTING

S. Henderson[1,*], A. Hamblin[2], A. Sosinsky[2], C. Turnbull[2], L. Jones[2,]
S. Hing[1], N. Murugaesu[2], J. Moorhead[1], T. Fowler[2], Z. Deans[1,]
A. Schuh[3], M. Caulfield[2], S. Hill[1]

[1]NHS England, [2]Genomics England, London, [3]Oncology, Oxford University, Oxford, United Kingdom

Background: The 100,000 Genomes Project in England is an initiative to sequence 100,000 genomes from patients with rare disease and cancer. One of the goals of the Cancer Programme is transformation with the aim of creating and embedding clinical pathways into National Health Service (NHS) care allowing cancer patients in the future, equitable and rapid access to Whole Genome Sequencing (WGS) which has the potential to simultaneously detect all types of variant and new predicative biomarkers such as mutational signatures. During 2017 eligibility for the Project was widened to include patients with haematological malignancies. Although there is a long track record of genetic analysis of both liquid and solid haematological tumours to aid diagnosis and therapy choice, significant clinical pathway adaptions are required to provide appropriate material for WGS.
Aims: For solid haematological tumours adaptions have mainly focused around the need to provide tumour DNA of sufficient quantity and quality for WGS which has driven the collection of fresh frozen samples from tumour types which would normally be processed in formalin (e.g. lymphoma). For liquid haematological cancers where it is usually possible to obtain good quality (unfixed) tumour DNA from blood or bone marrow, the challenge lies with the collection of suitable samples for germline (GL) DNA. It is usual practice in cancer WGS to sequence tumour and GL DNA samples to allow GL variants to be subtracted from the tumour sequence aiding the interpretation of somatic variants. For most cancer types a blood sample can be used as the source of GL DNA. However, collecting an equivalent GL sample from patients with many subtypes of haematological malignancy is difficult: if malignant cells are present in the blood or bone marrow, leucocyte DNA is unsuitable as it may contain tumour-specific acquired genetic changes.
Methods: Alternative GL DNA sources used in research include salvia, cultured fibroblasts from skin biopsies or post treatment blood taken once malignant cells have been cleared. The main disadvantages of these are potential tumour contamination and/or the delay between obtaining the tumour sample and the GL sample which would be problematic if WGS is to be used in diagnostic pathways.
Results: Work has been carried out to optimise the source of GL DNA for each type of haematological malignancy and appropriate solutions have been adopted. These include DNA extracted from saliva which has been collected post chemotherapy (once myeloid cells have been cleared from the blood) and cultured fibroblasts, often considered the 'gold standard' GL source. Measures to mitigate the effect of delayed GL collection include a two-step WGS analysis approach where the sequence is initially analysed using a reference GL sequence to identify urgent prognostic and diagnostic markers with subsequent more comprehensive analysis performed when the patient GL DNA is available. Additional studies have explored the practicality of using DNA extracted from sorted T cells for GL DNA which can be obtained at the time of diagnosis without incurring delays. This includes ensuring that sufficient T cell DNA of suitable purity can be robustly obtained and if the potential presence of genomic variants associated with clonal haematopoiesis of indeterminate potential (CHIP) would be compatible with current WGS analysis pipelines.
Summary/Conclusion: Due to this work we are now seeing the development of viable clinical pathways which will ensure that NHS patients with haematological malignancies can benefit from the diagnostic and therapeutic potential of WGS.

PB1699

HIGH-THROUGHPUT DRUG SCREENING TO IDENTIFY THERAPIES REVERSING ABERRANT CELL DIFFERENTIATION IN AML

E. Karjalainen[1,*], M. Wolf[1], G. Repasky[1], K. Wennerberg[1,2]

[1]Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland, [2]Biotech Research & Innovation Centre, University of Copenhagen, Copenhagen, Denmark

Background: Acute myeloid leukemia (AML) is a malignancy characterized by impaired cell differentiation and uncontrollable accumulation of immature myeloid progenitor cells in the bone marrow. Although most patients respond to the first line chemotherapeutic treatment, the majority of patients eventually relapse and therefore the overall survival from AML remains very poor. A therapy based on inducing the immature leukemic cells to differentiate may turn AML into a curable disease, as has been shown in the case of Acute Promyelocytic Leukemia (APL), the M3 subtype of AML. However, AML is a heterogeneous disease and we still lack knowledge of how different genetic alterations disturb normal hematopoiesis and how the cell differentiation blockade may be lifted in the various subtypes of AML.
Aims: Our aims are 1) to identify therapies reversing aberrant cell differentiation in AML by using flow cytometry-based high-throughput drug screening 2) to establish links between molecular subtypes of AML and responsiveness to identified cell differentiating therapies.
Methods: Our approach is to explore drug-induced cell differentiation in primary samples from AML patients and genetically engineered AML mouse

models using high-throughput flow cytometry. Specifically, we characterize samples by their expressed cell differentiation-related cell surface markers with and without drug treatment to identify compounds capable of inducing cell differentiation *ex vivo*. The primary AML patient samples are also profiled by exome- and RNA-sequencing.
Results: Preliminary results with a *Flt3/Npm1* –mutated mouse model suggest that several compounds elicit cell differentiation-state specific responses, some of which could be indicative of a dynamic shift towards cell differentiation. For example, treatment with mTOR-kinase inhibitors sapanisertib and vistusertib resulted in a concentration dependent increase of differentiating cells marked by CD11b expression and decrease of stem cells marked by CD34 expression.
Summary/Conclusion: We have established a high-throughput flow cytometric screening platform to identify drugs that are able to induce differentiation in primary AML samples and genetically engineered mouse models of AML. By integrating drug response data with genetic background information from samples we aim to reveal novel genotype-phenotype relationships in the level of cell differentiation that could eventually be translated into clinically relevant biomarkers and treatment options for AML patients.

PB1700

DETECTION OF FLT3-INTERNAL TANDEM DUPLICATION WITH NGS: A COMPARISON OF DIFFERENT ANALYTICAL TOOLS

M. Le Mercier[1,*], K. Vermeulen[1], C. Weyn[2], S. Van Lent[1], D. Lathauwers[1], C. De Beul[1], P. Holmgren[3], J. Van Den Bossche[1], R. Malfait[1], K. Deiteren[1], M.-B. Maes[1]

[1]Laboratory of Hematology, [2]Laboratory of Pathology, [3]ICT department, Antwerp University Hospital, Edegem, Belgium

Background: Internal tandem duplication (ITD) in the FLT3 tyrosine kinase gene is a somatic genetic alteration frequently found in acute myeloid leukemia (AML) and is associated with an aggressive phenotype. The accurate detection of FLT3-ITD together with evaluation of the mutant-to-wild-type allelic ratio are essential due to the strong prognostic and the potential therapeutic relevance of this marker in AML. The gold standard method for the detection of FLT3-ITD consists of PCR and electrophoresis-based product sizing. In the last years, as the number of relevant genetic alterations in myeloid neoplasm is increasing, next generation sequencing (NGS) has started to replace the conventional PCR methods in clinical diagnostic labs. However, NGS methods are usually not very performant for the detection of medium sized or long insertions or deletions.
Aims: The aim of this study was to evaluate the possibility to use NGS for accurate detection of FLT3-ITD in the daily practice.
Methods: We have developed a custom Haloplex NGS panel (Agilent) to sequence 29 genes related to myeloid neoplasm, among which FLT3 exons 14, 15 and 20. We used this panel on a MiSeq (Illumina) sequencing platform on 40 AML samples (23 FLT-ITD negative samples and 17 FLT-ITD positive samples) and compared the performance for FLT3-ITD detection of several analysis tools, both technical (different sequencing cartridges and analytical (Pindel, SOPHiA DDM and SeqNext).
Results: Best results for FLT3-ITD detection were obtained using 500v2 cartridge instead of 300v2. Regarding analysis software Pindel and *SOPHiA* DDM gave the best results in terms of detection (more than 88%). However, large differences were observed between NGS and fragment analysis regarding the mutant-to-wild-type ratio.
Summary/Conclusion: These results demonstrate that FLT3-ITD can be detected reliably with NGS. However, optimization is still needed to accurately determine the mutant-to-wild-type ratio since this ratio is an important aspect of the prognostic value of FLT3-ITD.

PB1701

RELATIONSHIP AMONG EXPRESSION LEVELS OF EPIGENETIC MODULATORS IN AML OF INTERMEDIATE RISK: BIOLOGICAL ASSOCIATIONS AND OUTCOME INFLUENCE

Y. Florido, C. Rodriguez Medina, S. Sánchez Sosa, M.T. Gómez Casares, G. Santana Santana, M.D.L.N. Saez Perdomo, R. Stuckey, E. Gonzalez Perez, T. Molero Labarta, M. Perera Álvarez, S. Jiménez Bravo de Laguna, S. de la Iglesia Íñigo, C. Bilbao*

Hematology, Hospital Universiatrio de Gran Canaria Dr. Negrin, Las Palmas de Gran Canaria, Spain

Background: Whole-genome sequencing has revealed a central role of epigenetic modulators in acute myeloid leukemia (AML) pathogenesis. Targeted therapy has also focused on epigenetics as seen by the use of IDH and BET-inhibitors. We believe that establishing the expression profile of epigenetic modulators in AML may help in the understanding of AML genesis, biology, evolution and treatment.
Aims: We wanted to get a better insight regarding the expression profile of epigenetic modulators in AML of intermediate risk by studying expression levels of EZH2, ASXL1, TET2, BRD4, c-myc and Bcl-2 in a consecutive series of AML patients at diagnosis and after induction and its association with clinical evolution.
Methods: Our series consisted of 120 intermediate-risk AML patients with a mean age of 56.04 years (range 15-79 years), diagnosed and homogenously treated between 2005 and 2017 at the Hospital Universitario de Gran Canaria Dr. Negrín with a median follow up of 14 months. Gene expression analyses were carried out by real time PCR in a LightCycler 480 Instrument II (Roche) using ABL as a control gene. Results were normalized with a cDNA pool from the bone marrow of 10 healthy donors, which was introduced as an internal control in each experiment. For statistical analyses, SPSS (v.15.0) software was used.
Results: ASXL1 levels were positively associated with EZH2 (Spearman's coefficient, SC=0.4, p<0.001). BRD4 expression was strongly correlated with ASXL1 (SC=0.56, p<0.001) and EZH2 (SC=0.31, p<0.001) and with c-myc expression (SC= 0.25, p=0.01). On the other hand, TET2 expression showed a positive relationship with BRD4 (SC=0.23, p=0.01), EZH2 (SC=0.24, p=0.01) and ASXL1 (SC=0.2, p=0.01). As shown in Fig.1 TET2 levels at diagnosis were marginally significantly lower in those cases that persisted or died compared to those that reached complete remission after induction (mean 0.19 *vs* 0.24, p=0.06). Measurement of BRD4 levels after induction showed that patients that displayed a reduction of more than 95% compared to levels at diagnosis exhibited worse overall survival (median overall survival, OS, 14 months *vs* 28 months, p=0.046).

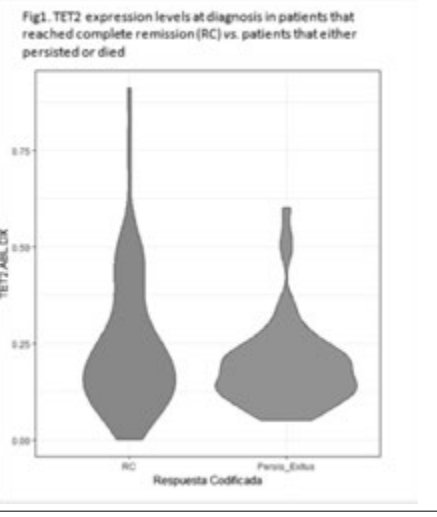

Figure 1.

Summary/Conclusion: The positive association observed between EZH2 and ASXL1 agrees with the fact that both cooperate in adding the epigenetic repressive H3K27 mark. In general, it seems that expression levels of genes that behave as tumor suppressor genes in AML, such us TET2, ASXL2 and EZH2, correlate well with BRD4 expression. BRD4 and c-myc levels were associated in accordance with what has previously been described, since BRD4 binds c-myc superenhancer regions, activating its expression. In contrast, lower levels of TET2 at diagnosis and a reduction of BRD4 levels after induction compared to diagnosis seemed to be related to worse prognosis. Further studies with a larger series are needed to confirm these preliminary results.

PB1702

BONE MARROW PLASMA LEVELS OF S100A8 ARE SIGNIFICANTLY INCREASED IN SOME SUBGROUPS OF AML PATIENTS WITH BETTER PROGNOSTIC

J. Mondet[1,2,*], M. Meunier[1,3], C. Lo Presti[1,4], E. Giraudon[5], M.-C. Jacob[6], C. Lefebvre[7], S. Berthier[8], A. Mc Leer-Florin[2], S. Park[1,3], P. Mossuz[4]

[1]UGA/INSERM U1209/CNRS 5309, Institute for Advanced Biosciences, [2]Pathologie moléculaire, [3]Department of clinical hematology, [4]Department of biological hematology, [5]CHU Grenoble Alpes, Grenoble, France, [6]Immunology

laboratory, [7]Oncohematology laboratory, [8]Flow cytometry platform, CHU Grenoble Alpes, Grenoble, France

Background: Increased intrablastic S100A8 has been associated with poor prognosis in AML. Moreover, its extracellular damage-associated molecular pattern activity regulates progression through genotoxic stress and myeloid differentiation independently of its frequently co-expressed protein S100A9. But extracellular S100A8 expression is not completely understood in AML.
Aims: In this context, we hypothesized that S100A8 can be secreted into BM niche and probably influences leukemic cell behavior and characterized its presence in AML.
Methods: Thus, we measured the level of S100A8 ([S100A8]) in 71 bone marrow plasma including 50 *de novo* AML, 12 myeloproliferative neoplasms (MPN), 7 myelodysplastic syndromes (MDS) and 9 healthy donors by ELISA technique. All patients gave their informed consent.
Results: Firstly, we found AML patients had significantly higher rates than healthy subjects but also than MDS or MPN patients. A significant correlation between bone marrow and blood plasma existed but bone marrow [S100A8] was 7 times higher than blood levels. We found a direct link between [S100A8] and the intensity of cell proliferation (peripheral and medullar leukocytosis). The linear correlation between [S100A8] and the monocyte count suggests that monocytes could be the main producers in the bone marrow microenvironment. No correlation was observed between [S100A8] and blasts nor with the neutrophil count. This hypothesis was reinforced by the particularly high rates of S100A8 observed in AML4 and AML5 of the French American British classification. S100A8 levels were significantly higher in AML with molecular abnormalities such as mutated *NPM1* or inv (16) of favorable prognosis. However, in the overall population, [S100A8] did not impact on remission rate, overall survival, or relapse rate. The cutpoint of 300µg/ml corresponding to the mean of S100A8 levels in AML was used to divide high and low expression of S100A8 in BM. The impact on prognosis was observed among mutated NPM1 and *FLT3*-ITD population for whom high levels of S100A8 were associated with significantly higher survival, compared to low S100A8 releasers.

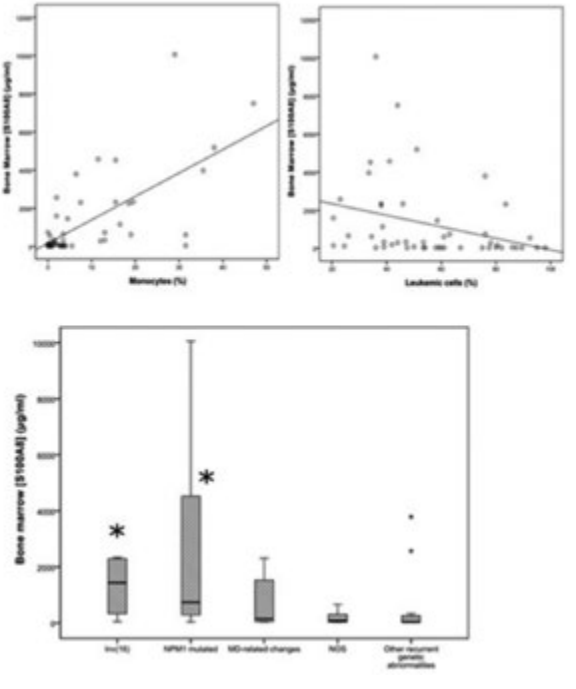

Figure 1.

Summary/Conclusion: In total, our study shows that there is a secretion of S100A8, probably of monocytic inflammatory origin, depending on the molecular status of patients that contributes to their prognosis.

PB1703

IMPROVING RISK STRATIFICATION IN ACUTE MYELOID LEUKEMIA WITH MRNA TECHNIQUES: BAALC AND WT1 LEVELS AFTER INDUCTION ARE BETTER THAN MORPHOLOGIC STATUS POST INDUCTION AND CYTOGENETIC AT DIAGNOSIS

C. Rodríguez Medina[1,*], Y. Florido Ortega[1], E. González Pérez[2,]
C. Bilbao Syero[1], R. Fernandez Martin[3], N. Cruz Cruz[1], M.D.L.N. Saenz Perdomo[1], S. Jimenez Bravo de Laguna[1], M.T. Gómez Casares[1,]
T. Molero Labarta[1]

[1]Hematology, Hospital Universitario de Gran Canaria Doctor Negrín, Las Palmas de Gran Canaria, [2]Hematology, HUGC Dr Negrin, Las Palmas, [3]Hematology, Hospital Insular de Gran Canaria, Las Palmas de Gran Canaria, Spain

Background: AML is a heterogeneous disease in whom cytogenetic (CG) and ages at the moment of diagnosis are the best prognostic variables and both of them are the basis for adapted therapy protocols. However, the morphologic response and MRD status by MFC after chemotherapy are too powerful variables.
Aims: To compare, retrospectively, the powerful of the CG at the moment of diagnosis, morphologic status after induction and changes in mRNA levels of genes WT1 and BAALC between diagnosis and post induction
Methods: We design a retrospective study with AML patients and intermediate CG (ELN 2010 criteria) treated with at least one cycle of intensive chemotherapy and morphologic status evaluated. Patients with all other CG or exitus in induction were excluded. Patients treated in Hospital Dr Negrín between 2004-17 and Hospital Insular Gran Canaria 2010-2016 were included. Morphological response was according to Cheson's criteria with a dichotomous approach: Complete response (CR) or not. Gene expression levels were studied in bone marrow specimen at the diagnosis and after first induction cycle. The study was performed with real time qPCR with Light Cycler 480. The changes in expression level was considered for each patient and was selected a cut-off point of 95% decrease between the two study moments.
Results: Our series consisted of 112 patients, 60.7% males and 39.3% females. Median age was 58 (R 16-77). The overall survival (OS) of the entire cohort hat a median of 26 months (CI95% 19.8-32.3). Distribution for intensification treatment approaches: Allogenic transplant (Alo) 35.8%, high dose chemotherapy, including auto transplant (Chemo) 35.8%, not receive or hipomethilating agents 28.4%, with a median OS: NR, 24 m and 12 m respectively with p<0.001. Global rate CR after first cycle was 62.5%. Median OS according to morphologic response was 38 m for CR (CI95% 4.8-71.1) and 16 m for non CR group (CI95% 8.4-23.5) with p=0.005. Total number of patients with gene expression study between diagnosis and post induction was 54 with 58 lost cases. Of valid cases, 60% achieved a decrease greater than 95% in their levels of expression. Median OS was 13 m (CI95% 6.2-19.7) and Non reached (NR) for those with better response with p<0.001. In patients without CR, the gene expression study was available for 14 cases. However 43% achieved a greater than 95% reduction in their levels. The median OS for those patients was NR and 7 m (CI95% 0.7-13.9) for low decliners with p=0.011. Considering intensification treatment, patients with greater decrease in expression, the median OS was NR for Alo and Chemo group, without significant difference, meanwhile in those with low decrease in gene expression the median OS for Alo group was 38 m (CI95% 17.1-58,8) and 11 m (CI95% 8.4-13.5) for chemo but without significance.

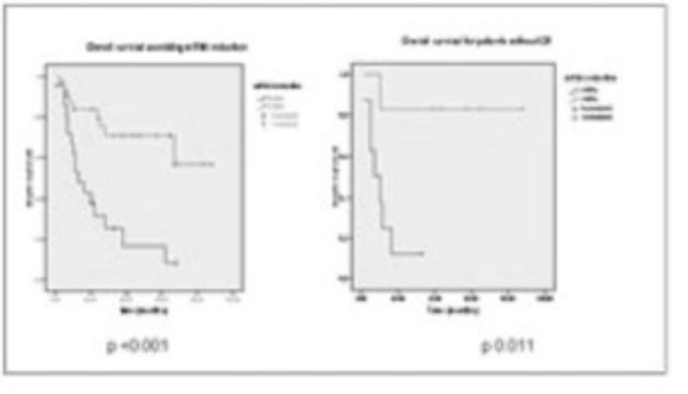

Figure 1.

Summary/Conclusion: 1) A decrease greater 95% in expression levels of genes WT1 or BAALC between diagnosis and post induction chemotherapy, was able to identify patients with greater probability of survival than the morphological response achieved after induction and the CG at diagnosis. Even in patients without CR, was able to separate patients into groups with significant survivals difference. 2) A decrease greater 95% in expression levels of genes WT1 or BAALC allowed identify patients with a good sensibility to chemotherapy and special good prognosis (>65% OS) in whom intensification with Alo would not provide benefit.

PB1704

FREQUENCY OF GENE MUTATIONS IN A COHORT OF COLOMBIAN PATIENTS WITH ACUTE MYELOID LEUKEMIA BEFORE AND AFTER INDUCTION TREATMENT

A. Cardona[1], J. Prada-Arismendy[1,*], E. Castillo[2], W. Castellanos[3], M.I. Hernández-Estrada[4], J. Machado[4]

[1]Grupo de Investigación e Innovación Biomédica, Instituto Tecnológico Metropolitano, Medellín, [2]Unidad de Oncología y Hematología Clínica, Hospital Manuel Uribe Ángel, Envigado, [3]Unidad de Hematología, Clínica Somer, Rionegro, [4]Facultad de ciencias exactas y aplicadas, Instituto Tecnológico Metropolitano, Medellín, Colombia

Background: Mutations that are associated with AML have a high impact on the prognostic of patients, and certain genetic profiles may guide an adequate therapeutic strategy. In Colombia, there are no data on the frequency of mutations associated with this neoplasm.
Aims: To describe the frequency of mutations in a cohort of patients with AML and how they can be related to the response to induction therapy.
Methods: Blood samples were collected from 27 AML patients before and after treatment induction during 2015-2017. Written informed consent was obtained at Hospital Manuel Uribe Angel and Clínica Somer. 500 ng of DNA were used to detect mutations in eight genes related to AML pathogenesis through a customized qBiomarker Somatic Mutation PCR array. For statistics analysis, *R* v.3.4.3 was used. Patient outcome was modeled using multinomial regression models including molecular and clinical data. Analyses on mutation associations were conducted with logistic regressions. Odds ratio (OR) were determined for regression coefficients and p-values with a Z-Wald test.

Table 1.

Gene	Domain	Mutation type and number (localization)	Patients carrying at least one mutation (Before n=27; after n=21)	
			Before (%)	After (%)
CEBPA	DNA binding and dimerization	3 insertions (p.K304, p.K313)	1 (3.7)	0
DMT3A	Methyltransferase	1 substitution (p.R882)	1 (3.7)	1 (3.7)
FLT3	Yuxtamembrane	2 substitutions, 5 ITDs	7 (25.9)	1 (3.7)
	2nd Tyrosine kinase	4 substitutions (p.D835)		
IDH1	IMDH conserved	5 substitutions (p.R132)	1 (3.7)	1 (3.7)
IDH2	IMDH conserved	2 substitutions (p.R140, p.R142)	6 (22.2)	6 (22.2)
NPM1	Nucleolar localization signal	7 frameshifts (p.W288)	1 (3.7)	1 (3.7)
NRAS	GTP binding	6 substitutions (p.G12, p.G13, p.Q61)	13 (48.1)	7(48.1)
KIT	2nd Tyrosine kinase	3 substitutions (p.D816,)	3 (11.1)	0

Results: The total number of patients was 27, which were followed for an average of 7 months. Thirteen patients (48,14%) were women and 14 were men (51.85%); age range was 18-78 y (mean 49.9 y). Twenty-one patients survived (77.7%), while 6 died during or after induction treatment (22.3%). Before treatment, the most frequent mutation was NRAS c.35G>A (22.22%), followed by IDH2 c.515G>A (14.81%). After treatment, IDH2 c.515G>A was the most frequent mutation (14.8%), while NRAS c.35G>A corresponded to 11.11%. In 8 patients (29.6%), we found 8 persistent mutations after treatment; curiously all patients (n=4) who had mutation in IDH2 c.515G>A persisted with it after treatment. The logistic regression analysis determined a higher relative risk trend of presenting AML-M1 in patients with FLT3, NRAS or IDH2 mutations. Mutated NRAS and IDH2 after treatment were highly associated with presenting the respective mutation at diagnosis. Multinomial models describing the outcome (death, refractoriness, partial response and complete response) based on molecular and clinical variables suggested -for this cohort of patients-, that mutated FLT3, IDH2 or NRAS after treatment confers a lower OR for death outcome *versus* complete response. Furthermore, age (OR: 0.56: p=0.0000025) and blast percentage at diagnosis (OR: 0.69: p=0.0002645) were inversely associated with complete response compared with patients who died. On the other hand, mutations before treatment in FLT3 were associated with a lower OR in refractoriness or partial response *vs* complete response. Finally, mutations in NRAS after treatment had a lower OR in relation to partial response *vs* complete response. Results regarding mutations are contradictory to what was previously published, nevertheless, the cohort size and a follow-up of only 7 months could have impacted on results reported here.
Summary/Conclusion: We are preliminarily describing the frequency of mutations of a cohort of AML patients in Colombia. Contrary to the literature, we found a high frequency of mutations in *IDH2* and *NRAS* genes, while *FLT3* remains as one of the most frequently mutated genes, being FLT3-ITD the most common in our cohort. It is interesting that mutations in IDH2 do not disappear after treatment. In sum, the present work describes gene mutations not previously described in AML Colombian patients. It is necessary to amplify the size of the cohort and to continue the follow-up of patients to observe adverse results that could be correlated with the presence of some mutations.

PB1705

SRSF2 GENE MUTATIONS ARE ASSOCIATED WITH TP53Γ EXPRESSION LEVELS AND OVERALL SURVIVAL IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)

K. Matiakowska*, A. Bartoszewska-Kubiak, E. Bielinska, M. Morgut-Klimkowska, O. Haus

Department of Clinical Genetics, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Bydgoszcz, Poland

Background: Acute myeloid leukemia (AML) is a heterogeneous clonal disorder with a presence of diverse genetic abnormalities in hematopoietic stem cells. *SRSF2* encodes serine/arginine-rich splicing factor 2, important for splice-site selection, spliceosome assembly, constitutive and alternative splicing. More than 90% of human genes undergo alternative splicing to make various protein isoforms. *TP53* gene, that encodes a tumor suppressor protein, may be translated into 13 different isoforms due to alternative splicing. Alternative splicing of its intron 9 can produce 2 different proteins: p53β and p53γ, without oligomerization domain (stop codon is localized in exon 9b).
Aims: The aim of the study was to assess mutational status of *SRSF2* as well as *TP53β* and *TP53γ* expression levels in association with hematological and clinical features of patients with AML.
Methods: 49 AML patients with normal karyotype were included in the study. *SRSF2* gene mutations in codon 95 were analyzed by direct sequencing. *TP53*β and *TP53γ* expression levels were assessed with real time PCR. Expression levels were analyzed with ΔΔCt method, with *ABL* as a control gene and K562 cell line as a calibrator.
Results: *TP53β* and *TP53γ* transcripts were detected in all patients. 5 patients carried mutation in *SRSF2*. Mutation status of *SRSF2* had no statistical significance to overall survival (p=0,36). Assessed median expression level was much higher for *TP53β* than *TP53γ* (ΔΔCt 44,04 *vs* 9,42, respectively; p<0,05). Furthermore, expression level of *TP53γ* was significantly associated with *SRSF2* mutations (p=0,021); patients with mutation had lower expression level. Patients were also classified into two groups, according to median expression value of *TP53*: with overexpression or with low expression of *TP53β/γ*. Kaplan-Meier estimation showed statistically significant difference in overall survival between the groups-longer for patients with overexpression of *TP53γ* isoform (p=0,03).
Summary/Conclusion: The above results may suggest a clinical importance of simultaneous analysis of *TP53* isoform expression and *SRSF2* gene mutations. It may be hypothesized that a changed sequence of the *SRSF2* gene regulates *TP53γ* expression and, as a consequence, regulates the cell cycle and overall survival.

PB1706

ACUTE MYELOID LEUKAEMIA (AML) PATIENTS WITH CO-EXISTING T(8;21) AND TRISOMY 4 ABNORMALITIES

L.C. Lau[1,*], Y.C. Linn[2], S.T.A. Lim[1], S.L. Tien[2]

[1]Molecular Pathology, [2]Hematology, Singapore General Hospital, Singapore, Singapore

Background: The t(8;21) rearrangement is closely associated with AML-M2 and is often co-detected with deletion 9q, and loss of the sex chromosomes. This abnormality is associated with a favourable prognosis. On the other hand, its coexistence with trisomy 4 is uncommon and the clinical significance is unclear.
Aims: To highlight the disease course of two patients with AML harbouring a co-existing t(8;21)(q22q22) and trisomy 4 abnormality.

Methods: Bone marrow cells were cultured at 37^{O}C for 24 and 48hrs and cells from both samples were harvested as per standard protocol. Cytogenetic analysis was carried out on G-banded metaphase cells.
Results: Both patients had trisomy 4 in addition to a t(8;21) rearrangement in the first patient and a t(8;10;21)(q22;q24;q22) rearrangement in the second patient. c-KIT exon 17 mutation was detected in both patients. The first patient achieved complete remission after 35 days of induction. However, the patient relapsed after 5 months of myeloablative haemotopoietic stem cell transplant (HSCT). A more complex 47,XX,t(3;10)(p21;q24),+4,t(8;21)(q22;q22) karyotype was obtained. Disease progression was evidenced by the additional abnormality. The patient achieved complete remission in the marrow after a complicated course of chemotherapy. After nine months of HSCT, the second patient developed granulocytic sarcoma in the sternum. He achieved remission and the marrow showed a normal karyotype and all cells were of donor origin after one year of treatment.
Summary/Conclusion: To our knowledge, there are only 4 reported cases of AML with co-existing t(8;21) and trisomy 4 in the Asian population. No detailed cytogenetic investigation and treatment regimens were reported by these authors. Our first patient had a slow response to treatment and a rapid relapse with disease progression despite early ablative HSCT at first remission. The second patient developed granulocytic sarcoma after the HSCT. From the observation of these two patients, the presence of the trisomy 4 may define a unique subtype of AML with t(8;21)(q22;q22). Such patients should be monitored closely for risk of relapse. c-KIT mutation has been shown to confer drug resistance. Treatment with novel therapy inhibiting KIT tyrosine kinase activity may be of potential value.

PB1707

PECULIARITIES OF IMMUNOLOGICAL INDICATORS IN ACUTE MYELOBLASTIC LEUKEMIA IN DYNAMICS

O. Smirnova*, E. Kasparov

Research Institute of Medical Problems of the North FIC KSC SB RAS, Krasnoyarsk, Russian Federation

Background: Acute myeloblastic leukemia is a severe onco-hematologic disease. Reducing the reactivity of the patient's immune system is one of the provocative mechanisms in its progression.
Aims: The purpose of this study was to study the dynamics of the subpopulation composition of lymphocytes in the blood in patients with acute myeloblastic leukemia (AML).
Methods: In the study group there were 100 AML patients, in the acute debut stage-30 patients, in the stage of complete remission after the treatment-49 patients, in the stage of repeated relapse-21 people. For the stage of acute debut, the first manifestation of the disease with the number of blasts in the bone marrow is 25% or more. Complete remission after treatment in patients was diagnosed with detecting in the myelogram of not more than 5% of blast cells with normal cellularity, in the cerebrospinal fluid there were no leukemia cells. Repeated relapse was detected when more than 25% of the blasts were detected in the bone marrow indices after the remission achieved before. The control group consisted of 125 practically healthy volunteers. BD MultiTEST test systems with a 4-color antibody panel: CD3 / CD8 / CD45 / CD4 and CD3 / CD16 + CD56 / CD19, labeled with fluorochromes FITC, PE, PerCP and APC, were used to estimate the relative content of lymphocyte subpopulations. To calculate the absolute content of subpopulations, the absolute number of lymphocytes obtained from the blood test on the hematological analyzer Cell Dyn1800 (Abbott, USA) was used. Student t-test, non-parametric Mann-Whitney criterion U, and linear correlation coefficient r were used for statistical data processing.
Results: The state of the cell link of immunity in patients with the M2 variant of AML during primary attack is characterized by a decrease in the relative number of pan-markers of T-lymphocytes and T-helpers, which indicates not only the development of an immunodeficiency state, but also the inferiority of the T-helper link, leading to inefficiency and cellular, and humoral units of immunity. At the stage of complete remission, leukopenia, a decrease in the relative and absolute indices of the T-cell link of immunity, NK cells, and B-lymphocytes with violation of activation processes are revealed. With repeated relapse of AML, the state of cellular immunity manifests itself in the form of leukopenia, lymphopenia, a decrease in the relative and absolute indices of the T-cell link of immunity, NK cells, and B-lymphocytes with disruption of activation processes.
Summary/Conclusion: At the stage of acute debut and remission of AML, T-cell immunodeficiency develops, at the stage of repeated relapse-combined immunodeficiency with defeat of T- and B-systems of immunity. A feature of AML is the depletion of NK cells, which contributes to the progression of the disease and the development of relapse.

PB1708

DYNAMICS OF METABOLIC INDICATORS OF ACUTE NON-LYMPHOBLASTIC LEUKEMIA

O. Smirnova*, E. Kasparov

Research Institute of Medical Problems of the North FIC KSC SB RAS, Krasnoyarsk, Russian Federation

Background: Acute myeloblastic leukemia is a severe onco-hematologic disease. The functioning of the immune system depends on the characteristics and severity of metabolic processes in its cells. Enzymes of oxidation-reduction reactions are used to evaluate biochemical processes in lymphocytes, because they realize the basic reactions of intracellular metabolism and are responsible for combining all metabolic exchanges.
Aims: The purpose of this study was to study the dynamics of the mechanisms of metabolic status of lymphocytes in the blood in patients with acute myeloblastic leukemia (AML).
Methods: In the study group there were 100 AML patients, in the acute debut stage-30 patients, in the stage of complete remission after the treatment-49 patients, in the stage of repeated relapse-21 people. The control group consisted of 125 practically healthy volunteers. Bioluminescent determination of the activity of NAD (P) -dependent dehydrogenases was carried out on the biochemiluminometer BLM-3607. This method was used to determine the activity of the following enzymes: glucose-6-phosphate dehydrogenase, glycerol-3-phosphate dehydrogenase, malic enzyme, NAD- and NADH-dependent lactate dehydrogenase reaction, NAD- and NADH-dependent reaction of malate dehydrogenase, NADP- and NADPH-dependent glutamate dehydrogenase, NAD-and NADH-dependent glutamate dehydrogenase, NAD- and NADP-dependent isocitrate dehydrogenases and glutathione reductase. Activity of dehydrogenases in blood lymphocytes was expressed in enzymatic units (1 E=1μmol / min) per 104 cells). Based on the results of the research, a database was created in MS Excel 2000 spreadsheets, on the basis of which statistical analysis was carried out using descriptive statistics methods with Student's t-criterion, nonparametric using exact Mann-Whitney criteria using the Statistica 8.0 application software package.
Results: When studying the features of the metabolic status of blood lymphocytes in patients with a primary AML attack, a decrease in the activity of LDH aerobic reaction is revealed. The decrease in the intensity of terminal glycolysis reactions and the level of concentration of intermediates for the Krebs cycle is proved by the revealed decreased activity of LDH and NADH-LDH. The level of reaction of the Krebs cycle of NADP-dependent isocitrate dehydrogenase in patients at the acute debut stage relative to control is decreased. In patients in the stage of complete remission of AML, the activity of the NADH-LDH enzyme is restored, while the activity of the LDH enzyme remains reduced. When studying the metabolic status of patients with the M2-variant AML in a repeated relapse, a decrease in the transfer of lipid catabolism products to the redox-oxidative reactions of glycolysis is revealed, due to a decrease in the activity of G3PDH. The decrease in glycolysis activity leads to its inhibition, which is confirmed by the decreased activity of the anaerobic LDH reaction. The activity of NADH-LDH is decreased. The activity of aerobic LDH reaction in lymphocytes was reduced. However, in patients with AML at the stage of recurrence, the activity of glutathione reductase is restored.
Summary/Conclusion: At all stages of AML, a marked decrease in the intensity of intracellular metabolic processes of lymphocytes is observed, mitochondrial transport, glycolysis, and metabolism in the lemon cycle decrease. A feature of acute debut and remission of AML is the reduction of peroxide processes in lymphocytes. With repeated relapse, peroxide processes in lymphocytes are restored; however, intracellular lipid catabolism decreases.

PB1709

TO IDENTIFY PROGNOSTIC BIOMARKER AND GENE REGULATION BY TCGA DATASET: ALDH1A1 IN ACUTE MYELOID LEUKEMIA

H. Yi[1,*], X. Su[2], R. Deng[1], F. Fan[1], H. Sun[1], G. He[1], S. Lai[1], Y. Su[1]

[1]Hematology, Chengdu General Hospital, Chengdu, [2]Children's Heart Center, Institute of Cardiovascular Development and Translational Medicine, Wenzhou, China

Background: Identify prognostic biomarker is benefit to acute myeloid leukemia (AML) stratification and making individual therapeutic strategies.
Aims: We try to demonstrated the prognostic biomarker of acute myeloid leukemia (AML) and gene regulation pattern.
Methods: We analyzed general clinical information of AML, detected prognostic impact and gene regulation pattern of ALDH1A1 from The Cancer Genome Atlas (TCGA) dataset.

Results: The most highly incident rate of AML patients was at sixties'. More than half of patients were intermediate risk by NCCN stratification. Fms-like tyrosine kinase-3 (FLT3) was the most frequent mutation gene. Patients less than 60 years old and M3 type in French-American-British (FAB) classification were independent favorable prognostic factors, but not the gender, peripheral or bone marrow blast count, white blood cell count, hemoglobin concentration or platelet count. The expression of ALDH1A1 was associated with risk stratification and could be as a prognostic impact biomarker in AML. Knocking down ALDH1A1 affected leukemia cell survival and self-renewal property. The expression of ALDH1A1 was correlated to the expression of ITGB3 and could be regulated by integrin αvβ3.
Summary/Conclusion: Our study demonstrated TCGA is a high fidelity dataset for AML research and ALDH1A1 is a prognostic factor which could be regulated by integrin αvβ3.

PB1710

CHROMOSOMAL ABNORMALITIES IN PAKISTANI PATIENTS WITH ACUTE MYELOID LEUKEMIA

M.S. Shaikh*, Z. Ahmed

Aga Khan University, Karachi, Pakistan

Background: The heterogenous response to treatment in acute myeloid leukemia (AML) can be attributed largely to the difference in cytogenetic features identified in between cases. Cytogenetic analysis in acute leukemia is now routinely used to assist patient management, particularly in terms of diagnosis, disease monitoring, prognosis and risk stratification. Knowing about cytogenetic profile at the time of diagnosis is important in order to take critical decisions in management of these patients.
Aims: The study was conducted to determine the distribution of cytogenetic abnormalities in Pakistani adult patients with AML in order to have insights regarding behavior of the disease.
Methods: A retrospective analysis of all the cases of AML (≥15years old) diagnosed at Aga Khan University from January 2011 to December 2016 was performed. Cytogenetic analysis was made for all cases using the trypsin-Giemsa banding technique. Karyotypes were interpreted using the International System for Human Cytogenetic Nomenclature (ISCN) criteria.
Results: A total of 321 patients were diagnosed with AML during the study period, of which 288 samples successfully yielded metaphase chromosomes. The male to female ratio was 1.7:1. A normal karyotype was present in 61% (n=176) of the cases whereas, 39% (n=112) had an abnormal karyotype. Of the abnormal cases, t(8;21)(q22;q22) and t(15;17)(q22;q12) were identified in 8.3% and 4.9% cases respectively. Adverse prognostic cytogenetic subgroups including complex karyotype, monosomy 7 and t(6;9)(p23;q34) were identified in 9%, 1% and 0.7% patients respectively.
Summary/Conclusion: This largest cytogenetic data in adult AML from Pakistan showed comparable prevalence of favorable prognostic karyotype to international data. The prevalence of specific adverse prognostic karyotype was low.

PB1711

SUCCESSFUL COVERAGE OF DIFFICULT TO SEQUENCE GENES (CALR, CEBPA, AND FLT3) ASSOCIATED WITH MYELOID DISORDERS USING A HYBRIDISATION-BASED ENRICHMENT APPROACH PRIOR TO NEXT-GENERATION SEQUENCING (NGS)

L. Georgieva*, E. Uddin, J. Chan, G. Speight

Oxford Gene Technology, Begbroke, United Kingdom

Background: The application of short read NGS for research into myeloid disorders such as myeloproliferative neoplasms (MPNs) and acute myeloid leukaemia (AML) has been hampered by the inability to sequence certain genes. These genes can harbour key mutations so it is desirable to ensure suitable sequencing coverage is obtained. These genes amongst others include: *CALR* exon 9 insertions and deletions (up to 52 bp), *CEBPA* single nucleotide variants (SNVs) and *FLT3* Internal Tandem Duplications (ITDs) and SNVs. Each of these regions contain certain challenging DNA sequences that can impact the quality of the data generated, *e.g.* large indels and low complexity regions (*CALR*), high GC content (75% on average for the whole gene with specific regions at 100%) and repetitive regions (*CEBPA*), and complex repetitive elements (*FLT3*).
Aims: To determine whether a hybridisation-based enrichment approach overcomes the difficulties associated with these genes, and permits the generation of high quality (sufficient de-duplicated depth) data to allow these targets to be accurately interrogated.
Methods: We utilised a hybridisation-based enrichment approach for library preparation in combination with a SureSeq myPanel™ NGS Custom AML panel. The library was then sequenced using a 2x150 bp read length protocol on an Illumina MiSeq®.
Results: Here we present the coverage and variants generated from numerous research samples for each of these difficult to sequence genes. The results clearly show that this approach can reliably detect and accurately size (including low allele frequency) insertions and deletions of up 52 bp in *CALR* (exon 9), SNVs and deletions in *CEBPA* with a de-duplicated depth in excess of 2000x as well as ITDs of between 24 and 201 bp in *FLT3*.
Summary/Conclusion: This approach is suitable for the analysis by NGS of these difficult genes and therefore removes the requirements for supplementary approaches to analyse these difficult genes, such as Sanger sequencing (*CEBPA*) and fragment analysis (*CALR* and *FLT3*).

PB1712

MUTATIONAL LANDSCAPE OF ACUTE MYELOID LEUKEMIA WITH NORMAL CYTOGENETICS IN ROMANIAN PATIENTS-A TARGETED NEXT GENERATION SEQUENCING PRELIMINARY REPORT

A. Arghir[1,2,*], S.M. Papuc[2], D.-S. Soare[1,3], E. Radu[1,3], I. Ciocănea-Teodorescu[2], V. Popov[4], M. Găman[1,3], C. Marinescu[1,3], A. Nicolescu[3], R. Colesniuc[2], M. Murat[4], M. Popescu[4], O. Patrinoiu[4], M. Andreescu[4], N. Berbec[1,5], S. Angelescu[1,5], D. Cîşleanu[1,3], A.-M. Vlădăreanu[1,3], H. Bumbea[1,3]

[1]Carol Davila University of Medicine and Pharmacy, [2]Victor Babes National Institute of Pathology, [3]Emergency University Hospital, [4]Colentina Clinical Hospital, [5]Coltea Clinical Hospital, Bucharest, Romania

Background: Acute myeloid leukemia (AML) is a complex and dynamic disorder characterized by a wide range of recurrent driver genetic defects. The introduction of next generation sequencing (NGS) in AML genome investigation led to a better understanding of the mutational spectrum intricacy. Characterization of the patterns of mutation co-occurrence has become equally significant, as most AML patients bear more than one driver mutation. The functional consequences and clinical relevance of mutations and patterns of co-mutation are not fully understood, thus further studies are needed in order to inform clinical practice.
Aims: We report on the results of targeted NGS and fragment analysis for characterization of the mutational landscape in a group of 12 Romanian AML patients with normal cytogenetics (NC-AML).
Methods: Targeted NGS testing with Ion AmpliSeq™ AML Research Panel (ThermoFisher Scientific) was performed on genomic DNA extracted from diagnostic bone marrow samples according to manufacturer's recommendations. Nineteen genes were targeted for entire coding regions (*CEPBA, DNMT3A, GATA2, TET2, TP53*) or mutational hotspots (*ASXL1, BRAF, CBL, FLT3, IDH1/2, JAK2, KIT, KRAS, NRAS, NPM1, PTPN11, RUNX1, WT1*). Ion PGM System (ThermoFisher Scientific) and NextGENe v.2.4.2.1 (SoftGenetics) were used for sequencing and data analysis. Internal tandem duplication of FLT3 gene (FLT3-ITD) were assessed by PCR amplification of exons 13 and 14 and the spanning intronic region of the *FLT3* gene. The amplified products were analysed by automated electrophoresis on the 2100 Bioanalyzer system (Agilent Technologies).
Results: A total of 38 mutations, with a median of 3 mutations per patient (ranging from 2 to 5 mutations/patient), were identified in our patient group. Among these, 31 were driver mutations recurrent in myeloid malignancies, while 7 mutations, with *in silico* predicted deleterious effects, were not previously reported in cancer databases (COSMIC, ClinVar). The highest mutation frequency was observed for *NPM1*, as previously reported in NC-AML, followed by *DNMT3A* gene. The mutation co-occurrence patterns in our patient group followed the previously reported ones, however some uncommon association were observed, such as between *NPM1* and *NRAS*Q61. None of the investigated patients showed *FLT3-ITD*. However, two patients presented *FLT3-TKD* point mutations in association with *NPM1* mutations.
Summary/Conclusion: Targeted NGS combined with fragment analysis proved a successful approach for molecular profiling of NC-AML highlighting the complexity of the molecular profiles in this AML subgroup. The study represent one of the first NGS analyses of Romanian patients with NC-AML. The mutational data obtained in our patients are consistent with previously published data, while also revealing new insights, such as previously unreported variants or new mutational associations. The data contribute to a better understanding of the mutational landscape of AML and, ultimately, to an improved patient care.

PB1713

INVESTIGATING THE EFFECT OF C-MYC PROTO-ONCOGENE INHIBITION IN ACUTE PROMYELOCYTIC LEUKEMIA

M. Sayyadi*, N. Riyahi, N. Sheikh-Zeineddini, D. Bashash

Department of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic Of

Background: The c-Myc proto-oncogene, an important regulator of cell proliferation, is amplified in many tumor types and has been linked to both the development and progression of a wide range of human cancers spanning from solid tumors to leukemia. Interest into c.Myc targeted therapy has elevated by the intertwining between acquisition of chemoresistant phenotype and the aberrant expression of c.Myc in leukemia patients.
Aims: This study was aimed to investigate the anti-cancer effects of 10058-F4, a potent small-molecule inhibitor of c-Myc, in acute promyelocytic leukemia (APL).
Methods: APL-derived NB4 cells were subjected to the increasing concentrations of 10058-F4 and then, the cytotoxic effect of the inhibitor was studied using trypan blue, Annexin/PI, MTT, caspase-3 activity, and BrdU cell proliferation assays. Moreover, gene expression analysis by using quantitative real-time PCR was applied to investigate the molecular mechanisms of action of 10058-F4.
Results: We found that the cytotoxic effects of the inhibitor, as revealed by the decreased cell viability and metabolic activity, were mediated through induction of apoptotic pathway in NB4 cells. The resulting data also outlined that inhibitor-induced apoptosis was accompanied by the elevated Bax/Bcl-2 molecular ratio and the enhanced augmentation of caspase-3 activity. Moreover, we found that abrogation of c.Myc using 10058-F4 resulted in a considerable increase in the mRNA expression levels of cyclin-dependent kinase inhibitors p21 and p27, which was in agreement with the induction of G1 cell cycle arrest in APL-derived NB4 cells.
Summary/Conclusion: The present study revealed that c-Myc inhibition using a potent small-molecule inhibitor affects multiple cellular activities in NB4 cells and represents a novel promising anti-leukemic approach in APL treatment.

PB1714

KARYOTYPE COMPLEXITY AND CHARACTERIZATION OF CHILDHOOD ACUTE MYELOID LEUKEMIA (AML) IN PAKISTAN

Z. Ahmed[1,*], M.S. Shaikh[2], A. Nasir[2], S. Alatf[3], Z. Fadoo[3], T. Moatter[2]

[1]Pathology and Laboratory Medicine, Aga Khan University, [2]Pathology and Laboratory Medicine, [3]Pediatric Oncology, Aga Khan University Hospital, Karachi, Pakistan

Background: Acute myeloid leukemia (AML) is a heterogeneous disease. Based on cytogenetics findings, AML patients are stratified into three major risk categories: favorable, intermediate and unfavorable. For the intermediate risk category, it has been difficult to stratify prognostically due to the clinical heterogeneity and scarce knowledge of the molecular alterations underlying in childhood AML subgroup. Knowing about cytogenetic profile at the time of diagnosis is important in order to take critical decisions in management of these patients.
Aims: To determine and characterize cytogenetic abnormalities in Pakistani pediatrics patients with AML
Methods: A retrospective review of all the cases of AML (≤15years old) diagnosed at Aga Khan University from January 2011 to December 2016 was performed. Cytogenetic analysis was made for all cases using the trypsin-Giemsa banding technique. Karyotypes were interpreted using the International System for Human Cytogenetic Nomenclature (ISCN) criteria.
Results: A total of 67 patients were diagnosed as AML during the study period with male to female ratio of 1.5:1. A normal karyotype was present in 45% (n=30) of the cases whereas, 55% (n=37) had an abnormal karyotype. Of the abnormal cases, t(8;21)(q22;q22) was identified in 15% cases. In poor prognostic group complex karyotype was 19%. Intermediate prognostic cytogenetic subgroups including structural anomalies (partial deletions and additions and translocations except deletion 7), trisomies were identified in 10% and 06% patients respectively
Summary/Conclusion: This study showed recurrent cytogenetic abnormalities in 55% Pakistani Children with AML. Favorable karyotypes, t(8;21)(q22;q22.1) was identified as the most prevalent specific chromosomal abnormality; the cumulative prevalence however was not significantly different in various age groups. This is in agreement with observations in international literature. Our data shows that the structural anomalies and complex karyotypes constituted the predominant unfavorable karyotype. To the best of our knowledge, this observation has not been reported before in Pakistani pediatrics patients with AML

PB1715

BRIVANIB ALANINATE KILLS LEUKEMIC STEM CELL BY INHIBITING VASCULAR ENDOTHELIAL GROWTH FACTOR-2 AND FIBROBLAST GROWTH FACTOR RECEPTORS

S. Alikhani[1,*], S. Kaviani[2], S. Mohammadi[3], S. Alikhani[4]

[1]Hematology department, Faculty of medical sciences, Tarbiat modares university, [2]Hematology department, Faculty of medical sciences,Tarbiat modares university, [3]Hematology/oncology and stem cell transplantation research center, Shariati Hospital, [4]Biochemistry department, Pharmaceutical sciences branch, Islamic Azad University, Tehran, Iran, Islamic Republic Of

Background: Acute Myeloid Leukemia (AML) is a heterogenous, clonal hematopoietic malignancy with overproduction of abnormal myeloid progenitors in bone marrow. Although the 7+3 regimen for patients with AML has increased the survival rate, common chemotherapy is not very efficient especially for older patients and the possibility of relapsed and refractory is high which is due to malignant cell called leukemic stem cell. Hence, targeting overexpressed receptors like VEGFR-2 and its related signaling pathway in leukemic blasts is needed.
Aims: So, we aimed to evaluate the cytotoxicity of brivanib alaninate on AML cell lines by inhibiting VEGFR-2 and FGFR signaling pathways.
Methods: In this study, KG1a which has been considered as a drug resistance AML cell line and a leukemic stem cell model, was used for cell culture. Cells were treated with 5 different doses of Brivanib Alaninate (Apexbio company, USA) for 48 and 72 hours and then MTT assay (sigma-Aldrich) was performed to evaluate the viability of these cell lines. Optical densities of each wells were measured at 540 nm wavelength. Finally, annexin V FITC/PI staining to investigate apoptosis effects of Brivanib Alaninate.
Results: Viability of the cells at doses of 0.01, 0.1, 1, 10 and 100 M were 87, 82, 80, 73 and 71% after 48 hours and 75, 70, 65, 56 and 50% after 72 hours, respectively. The data showed that IC-50 of the drug is 100 M which was then confirmed with Flowcytometry. The flowcytometry results showed that 31.2% of cell are in late apoptosis and 20.4% of cells in early apoptosis state.
Summary/Conclusion: The effective dose of brivanib alaninate for treatment of KG1a cell line is 100 M with 50% cell death after 72 hours treatment. So, targeting two or more dominant signaling pathway and is suggested for target therapy and the use of brivanib alaninate in clinical trials for patients with AML especially in relapse state in recommended.

PB1716

MDR1 GENE DETECTED *in vitro* HAVE A PROGNOSTIC SIGNIFICANCE IN LEUKEMIA PATIENTS

M. Kolesnikova[1,*], A. Sen'kova[2], O. Berezina[1], M. Zenkova[2,]
T. Pospelova[1]

[11]Department for therapy, hematology and transfusiology of Novosibirsk State Medical University, Novosibirsk State Medical University, [22]Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russian Federation

Background: Actual prognostic factors for testing acute leukemia are formed on detectable cytogenetic and molecular-genetic disorders, as well as initial data including the patient's age, leukocytosis, amount blast in the bone marrow. However, the study of genes of primary drugresistance or factors contributing to its development is not mandatory. Expression of the MDR1 gene by tumor cells is considered as one of the most common mechanisms of drugresistance.
Aims: The aim of this study was to assess the risk of developing drugresistance with acute leukemia patients based on MDR1 gene expression determination prior to initiation of therapy.
Methods: The study included 12 patients with acute lymphoblastic leukemia (ALL) and 25 patients with acute myeloblastic leukemia (AML). The average age of the patients was 47.9±15.7 years. Leukemia cells were taken from peripheral blood and bone marrow of patients. Assessment of multidrug resistance in tumor cells of leukemia patients was performed on bone marrow and/or peripheral blood samples using quantitative real-time reverse transcription-polymerase chain reaction (real-time RT-qPCR). RT-qPCR was used to evaluate the expression of the MDR1 gene. The results were normalized to GAPDH used as an internal standard. All statistical analyses were done with computer software MS Excel, OriginPro 7.5, Statistica 10.0.
Results: All leukemia patients were divided into groups with low, medium and high levels of expression based on the level of expression of MDR1. All leukemia patients were divided into groups based on their response to chemotherapy treatment (effectiveness of chemotherapy). For examination

of the chemotherapy effectiveness for acute leukemia patients was determined the number of blasts in the bone marrow after 1-2 chemotherapy courses. The intensive the expression level of the MDR1 gene is, the higher the risk of developing drugresistance is. Patients with AML have a moderate direct correlation ($r=0.66$, $p<0.05$) between the response to therapy and the expression of the MDR1 gene. In patients with ALL, a strong direct correlation was found ($r=0.77$, $p<0.05$) between response to therapy and expression of the MDR1 gene. Thus, patients with expressed expression of this gene should be classified as high risk, low level of expression and its absence-to a low risk group, and with moderate expression-to an intermediate risk group. In patients with high and moderate expression of the MDR1 gene, individual sensitivity to cytostatic drugs should be assessed for personalized correction of therapy regimens.

Summary/Conclusion: The data obtained demonstrate the importance of studying the expression level of the MDR1 gene with acute leukemia patients before initiating therapy to assess the prognosis of the disease.

PB1717

DIFFERENTIAL GENE EXPRESSION PROFILE ALTERATIONS IN CHEMORESISTANT AML CELLS FOLLOWING THEIR EXPOSURE TO IDARUBICIN AND CYTARABINE

F. Kontsioti, E. Maratou, V. Mpakou, A. Bouhla, G. Stavroulaki, I. Glezou, E. Bazani, S. Papageorgiou, G. Dimitriadis, V. Pappa*

Second Department of Internal Medicine and Research Institute, Attikon University General Hospital, Athens, Greece

Background: Acute myelogenous leukemia (AML) is the most common malignant myeloid disorder in adults. Relapses are initiated by chemoresistant leukemic cells. DNA damage and repair mechanisms influence not only the genetic predisposition to leukemia but are also very important for refractoriness to treatment.

Aims: The aim of this study was to investigate the possible alterations in the gene expression profile in DNA damage signaling pathways and apoptosis in two leukemic cell lines following their exposure to chemotherapeutic agents, idarubicin and cytarabine.

Methods: Kasumi-1 and MV4-11 AML cells were treated with either idarubicin (0.1μM) for 6h or cytarabine (1μM) for 48h. Apoptosis was determined through FACS(Annexin 5/7AAD). Statistics were performed through One Way Anova analysis. Dead cells were eliminated from drug- treated cells through the appropriate commercial kit. Gene expression profiling through PCR arrays analysis (RT^2Profiler,Qiagen)was performed after RNA extraction from untreated, total drug-treated and chemoresistant (live)cells. Human DNA Damage Signaling pathway related genes' expression was evaluated and analyzed through RT^2Profiler PCR Array data analysis tool.

Results: Kasumi-1 and MV4-11, idarubicin- and cytarabine- treated cells presented with enhanced apoptosis compared to untreated cells. PCR Array analysis after idarubicin treatment of total Kasumi-1 cells revealed a significant up-regulation of genes involved in apoptosis(BBC3), cell cycle(CDKN1A, PPP1R15A), DNA damage and repair(PNP), and ATM/ATR signaling (RBBP8) while Cytarabine treatment led to the up-regulation of an even greater number of genes (involved mostly of the DSB repair pathway *i.e.* HUS1,MLH1,NBN,XRCC1,XRCC2). Interestingly, significant differences in their gene expression patterns were observed between total cytarabine-treated Kasumi-1 cells and chemoresistant ones. HUS-1 gene (DSB) was up-regulated in cytarabine-treated cells and down-regulated in chemoresistant cells, while MLH1 and NBN genes presented the opposite pattern. Cytarabine treatment of total MV4-11 cells led to an up-regulation of genes involved in cell cycle(CDKN1A,TP73), DNA damage repair(GADD45A), including DSB repair(H2AFX) and NER(PCNA). Idarubicin also led to the up-regulation of a significant different panel of examined genes. Most importantly, PPP1R15A gene's expression in both cytarabine and idarubicin chemoresistant MV4-11 cells was significantly up-regulated compared to drug treated cells.

Summary/Conclusion: Our data demonstrate that both idarubicin and cytarabine result in enhanced cell death of Kasumi-1 and MV4-11 cells. In terms of DNA damage related genes' expression, both agents seem to affect a large number of genes involved in various mechanisms, mostly leading to their up regulation, in both cell lines. Most importantly, our data suggest differences in the gene expression pattern between chemoresistant cells and drug-treated cells, indicating the significance of DNA damage and repair pathways involved in chemoresistance. Specifically, the up-regulation of PPP1R15A gene in chemoresistant MV4-11 cells after treatment with both agents is of great importance since this gene participates in cell cycle and its transcript levels are increased following stressful growth arrest conditions and treatment with DNA-damaging agents. Considering that MV4-11 cell line is an AML chemoresistant one, up-regulation of the expression of PPP1R15A gene which facilitates recovery of cells from stress is totally compatible. Our results need to be further confirmed in AML patients with the view to design more effective treatments.

Acute myeloid leukemia – Clinical

PB1718

Abstract withdrawn.

PB1719

A SUBSET OF AML PATIENTS SHOWS DIFFERENT QUIMIOSENSITIVY *EX VIVO* PROFILES TO ANTHRACYCLINES AND ITS COMBINATION WITH CYTARABINE; COULD PRECISION MEDICINE BE THE KEY SELECTION CRITERION?

D. Martínez Cuadrón[1], J. Ballesteros[2,*], J. Martínez López[3], P. Hernández[2], D. Primo[2], J. Gorrochategui[2], J.L. Rojas[2], J.M. Bergua[4], M. Tormo[5], J. Serrano[6], A. Gonzalez[7], J. Pérez de Oteyza[8], S. Vives[9], B. Vidriales[10], P. Herrera[11], J.A. Vera[12], A. López[13], A. de la Fuente[14], M.L. Amador[15], J.A. Hernández Rivas[16], M.A. Fernández[17], C.J. Cerveró[18], D. Morillo[19], J.E. Megías[1], M. Sanz[1], P. Montesinos[1]

[1]Hospital Universitari i Politècnic La Fe, Valencia, [2]Vivia Biotech, Tres Cantos, [3]Hospital Universitario [12] de Octubre, Madrid, [4]Hospital San Pedro de Alcantara, Cáceres, [5]Hospital Clínico de Valencia, Valencia, [6]Hospital Universitario Reina Sofía, Cordoba, [7]Hospital Universitario Clínico San Carlos, [8]Hospital Universitario Madrid Norte Sanchinarro, Madrid, [9]Hospital Universitari Germans Trias i Pujol, Badalona, [10]Hospita Clinico Universitario de Salamanca, Salamanca, [11]Hospital Universitario Ramon y Cajal, Madrid, [12]Hospital Universitario Virgen Macarena, Sevilla, [13]Hospital Arnau de Vilanova, Valencia, [14]MD Anderson Cancer Center, Madrid, [15]Hospital de Montecelo, Pontevedra, [16]Hospital Infanta Leonor, Madrid, [17]Hospital Xeral Cies, Vigo, [18]Hospital Virgen de la Luz, Cuenca, [19]Hospital General de Villalba, Madrid, Spain

Background: Induction treatments for acute myeloid leukemia (AML) patients are based on the combination of Cytarabine (CYT) and one anthracycline, mainly Idarubicin (IDA), Daunorubicin (DNR), or Mitoxantrone (MIT). The choice of the anthracycline employed has been widely studied in multiple clinical trials showing similar CR rates, with some exceptions in which IDA reported higher CR. A new Personalized Medicine (PM) test developed by Vivia Biotech, based on an actionable native environment method which enables the establishment of pharmacological responses in *ex vivo* patient samples, is uncovering individual responses to these treatments.
Aims: Our objective is to explore whether a significant percentage of individual patients may respond differently to IDA *vs* DNR *vs* MIT treatments, in spite that of their "on average" similar response shown by clinical trials.
Methods: Bone marrow (BM) samples were collected at diagnosis from AML patients. Samples were incubated for 48 hours in 96 well plates, each well containing different drugs or drug combinations at 8 different concentrations, enabling calculation of dose-response (DR) curves for each single drug (CYT, IDA, DNR, MIT) and combination used in treatments (CYT-IDA, CYT-DNR, CYT-MIT). *ex vivo* drug sensitivity analysis was made using the PharmaFlow platform maintaining the BM microenvironment. Drug response was evaluated as depletion of AML blast cells in each well after incubation. Annexin V-FITC was used to quantify the ability of the drugs to induce apoptosis, and pharmacological responses were calculated using pharmacokinetic population models.

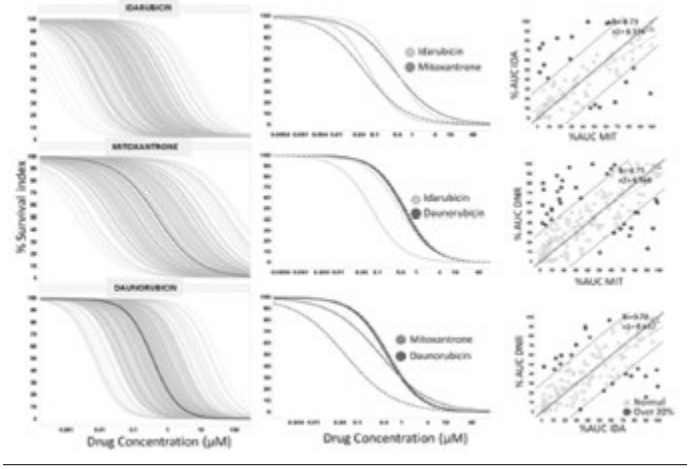

Figure 1.

Results: DR graphs were generated for each anthracycline (IDA, DNR and MIT) using PD models. Left panel of figure 1 shows the individual (grey lines) and average DR for IDA (yellow), MIT (blue) and DNR (red) from 289, 274 and 333 AML patients, respectively. As we expected, the average dose responses of the four anthracyclines were similar, with a slight increase in survival index with IDA. However, the interpatient variability of either drug is quite large, which could explain the differences in anthracycline sensitivity reported in some patients. As an example, middle panel figure 1 shows a patient sample that is resistant to IDA and DNR but sensitive to MIT. To identify those cases of selective sensitivity to anthracyclines, we compared the potency, in terms of AUC, between IDA *vs* MIT, DNR *vs* MIT and DNR *vs* IDA (right panel figure 1). Most dots tend to line up, but red dots represent patient samples with a difference in potency between these drugs greater than 30%. Red dots from 3 pairwise comparisons identify 28.3% of patient samples with this different potency between single anthracyclines. Similar DR graphs were generated for CYT-IDA, CYT-DNR and CYT-MIT combinations. The pairwise comparison of these combination treatments obtained different sensitivity in 8.2% of patients. Overall differences in sensitivity combining both the monotherapies and the CYT combinations were 30.6%.
Summary/Conclusion: These results show that this PM test seems able to identify a subset of AML patients whose *ex vivo* pharmacological response to anthracyclines is significantly different. A fraction of these patients may benefit if treatment selection among these alternatives were to be aided by this PM *ex vivo* test. To identify which fraction would benefit we would need a trial specifically designed.

PB1720

Abstract withdrawn.

PB1721

IMPACT OF DIFFERENT BY INTENSITY CONSOLIDATION REGIMENS ON MINIMAL RESIDUAL DISEASE REDUCTION AND RELAPSE INCIDENCE IN ADULTS WITH ACUTE MYELOID LEUKEMIA

T. Lobanova*, E. Parovichnikova, I. Galtseva, Y. Davydova, N. Kapranov, V. Troitskaya, A. Sokolov, I. Lukianova, V. Savchenko

Hematological Oncology and BMT, National Research Center for Hematology, Moscow, Russian Federation

Background: As low minimal residual disease (MRD) value at any time point, assessed by multicolor flow cytometry (MFC) correlates with the better outcome in AML patients (pts), it seems very important to evaluate the impact of treatment intensification on MRD level reduction.
Aims: The aim of the study was to compare the MRD clearance after standard and after intensive consolidation cycles in AML patients and to reveal risk factors of the relapse.
Methods: From March 2016 to February 2018, 60 pts with *de novo* AML (median age 37, 18-60 yy) were treated in the National Research Center for Hematology, Moscow, with 7+3 (Ara-c 200 mg/m^2 c.i, 1-7 days, Daunorubicin 60 mg/m^2 1-3 days) as the 1st induction cycle. 39 pts in complete remission (CR) after induction completion were included in the MRD study, comparing different consolidation intensity approaches: (A) 26 pts received 3 «7+3» consolidation cycles with anthracyclines alternation (Daunorubicin 60 mg/m^2 in 1st cycle, Idarubicin 12 mg/m^2 in 2nd cycle and Mitoxantrone 10 mg/m^2 in 3rd 1-3 days with Ara-c 100 mg/m^2 bid 1-7 days in each cycle); (B) 13 pts received consolidation with 2 FLARIDA courses (Fludarabine 30 mg/m^2 1-4 days, Ara-c 1000 mg/m^2 1-4 days, Idarubicin 8 mg/m^2 1,3 days). The (A) and (B) cohorts were similar by the age and ELN risk groups. Allogeneic HSCT was performed in 19 pts. MRD was measured in the bone marrow after induction and consolidation by 6 color-MFC on FACS Canto II with a standard antibody panel and 4 tubes, including anti-CD34, CD33, CD117, CD45, CD13, CD11b, CD66b, HLA-DR, CD2, CD4, CD7, CD56, CD38, CD14, CD15, CD16, CD19, CD65, CD99 antibodies. Any detectable MRD value was estimated as MRD positivity; sensitivity level was 0.01%.
Results: CR after 1st induction 7+3 in the whole cohort was achieved in 41 pts (68%) with MRD-negativity in 27 (65,8%) of them. 26 CR pts after 7+3 consolidation in (A) group have demonstrated much less duration of critical neutropenia (<0,5*10^9/l) in comparison with 13 CR pts after FLAR-IDA in (B): 16(1-47) days and 18(0-35), respectively (p=0,029); median intervals between consolidation courses constituted 32 (30-67) days in (A) *vs* 38 (29-57) in (B) (p=0,005). The frequency of severe septic complications did not differ in both cohorts: 23.1% (A) *vs* 46.1%, (B) (p=0,16). MRD-positivity after the 2nd cycle was 3/26 (11,5%) in (A) and 5/13 (38,4%) in

(B) cohorts (p=0,08). The same results were obtained after the whole consolidation program-MRD-positivity in 4/21(19%) in (A) and in 2/11 (18%) in the (B) groups (p=0,95). The pts who were MRD-positive after 1st induction had significantly higher relapse risk than MRD-negative: 6/15 (40%) *vs* 1/27 (3,7%) (p=0,005). Relapse frequency in pts who were MRD-negative after the 2nd course was 26% (6/23) in (A) cohort and 12,5% (1/8) in the (B). The incidence of relapse during 12 months after CR achievement constituted 30,7% in (A) and 23% in (B) cohorts with no significant difference (pic.1).

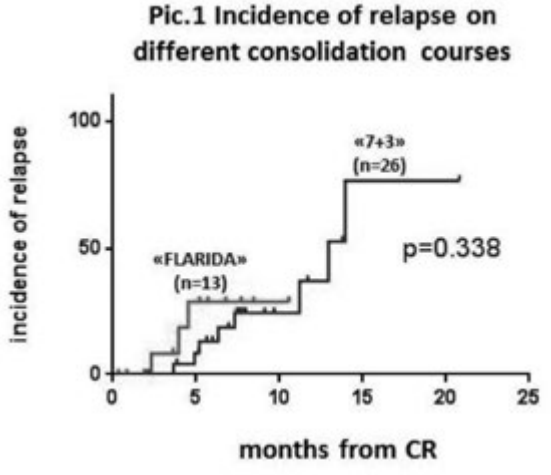

Figure 1.

Summary/Conclusion: Though the studied groups are small, we can assume that intensification of consolidation treatment was not translated into better MRD clearance. Moreover, escalation of the chemotherapy induced more profound and prolonged neutropenia with the higher risk of severe infections and more durable intervals between consolidation cycles. The incidence of relapse was comparable in the different by intensity consolidation groups. It's worth to note that the achievement of MRD negativity after the first induction cycle is associated with better outcome.

PB1722

AN EFFECTIVE TOOL FOR COMPREHENSIVE GERIATRIC ASSESSMENT IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA

C.-L. Zhang*, H. Liu, R. Feng, J.-T. Li, Y. Tian, T. Wang

Department of Hematology, Beijing Hospital, Beijing, China

Background: Acute myeloid leukemia (AML) is one of the common types of hematological malignancies in elderly people. Thus, how to select patients who are suitable to receive intensive induction chemotherapy is critical to improve the clinical outcomes of elderly patients with AML. Over the last few decades, geriatricians have developed comprehensive geriatric assessment (CGA) methods for elderly patients, which are useful for the evaluation of cancer patients. however, the tools used for CGA varied and not uniform in AML.
Aims: We aimed to validate the instrumental activities of daily living (IADL) scales, age, comorbidities, and albumin (IACA) index in elderly patients with acute myeloid leukemia (AML) and identify whether this index can serve as a guide for optimal personalized therapy for elderly patients with AML.
Methods: Patients aged ≥60 years, who had been diagnosed with AML in the Department of Hematology, Beijing Hospital, were screened for eligibility (n=61).
Results: A total of 21, 34, and 6 patients were categorized as IACA low-risk, intermediate-risk, and high-risk groups, respectively. Thirty-nine patients received induction chemotherapy (IC, n=35) or decitabine (n=4) after diagnosis, 19 (48.7%) achieved complete remission (CR), and the rate of CR was significantly higher in the IACA low-risk group than that of intermediate-risk group (68.4% *vs* 30.0%, P=0.016).The rates of grade ≥3 toxicities and treatment-related mortality were comparable between low- and intermediate-risk groups. The rate of relapse/progression-related mortality was 23.8%, 58.8%, and 100.0% in the IACA low-, intermediate-, and high-risk groups, respectively (P<0.001). The 2-year probability of overall survival (OS) was 47.7% and 20.2% in the IACA low- and intermediate-risk groups, respectively, which were both significantly higher than that of the high-risk group (0.0%). In the IACA low-risk groups, the 2-year probability of OS in patients receiving IC was significantly higher than in those receiving best supportive care (50.8% *vs* 0.0%, P<0.001) .
Summary/Conclusion: We observed that the IACA index could predict the clinical outcomes of elderly patients with AML, and the IACA low-risk patients may benefit more from IC.

PB1723

ALTERATIONS IN SERUM BILIRUBIN DURING TREATMENT WITH ENASIDENIB IN PATIENTS WITH OR WITHOUT UGT1A1 MUTATIONS

A.T. Fathi[1,2,*], C.D. DiNardo[3], I. Kline[4], N. Tu[4], E.C. Attar[5], E.M. Stein[6,7], S. de Botton[8]

[1]Massachusetts General Hospital Cancer Center, [2]Harvard Medical School, Boston, [3]The University of Texas MD Anderson Cancer Center, Houston, [4]Celgene Corporation, Summit, [5]Agios Pharmaceuticals, Inc., Cambridge, [6]Memorial Sloan Kettering Cancer Center, [7]Weill Cornell Medical College, New York, United States, [8]Gustave Roussy, Villejuif, France

Background: Enasidenib is an oral, potent inhibitor of mutant IDH2 (mIDH2) proteins. In a phase 1/2 study (NCT01915498) in patients with m*IDH2* myeloid malignancies, 38% of patients developed bilirubin elevations, likely due to off-target inhibition of the UGT1A1 enzyme, which metabolizes bilirubin in humans. Homozygous *UGT1A1* mutations (*UGT1A1*$^{mut/mut}$) are associated with inherited Gilbert Syndrome. A protocol amendment was added to include *UGT1A1* gene testing at patient screening.
Aims: To evaluate bilirubin changes associated with *UGT1A1* mutational status of patients with m*IDH2* hematologic malignancies in the phase 1/2 study of enasidenib monotherapy.
Methods: Enasidenib doses ranged from 50 to 650 mg/day. Total blood bilirubin (TBili) was assessed in all patients at baseline and at each site visit. Normal TBili ranges varied among reference labs (~3-25 µmol/L).
Results: As of Sept 2017, *UGT1A1* data were available for 65 patients: 16 patients had homozygous m*UGT1A1* (*UGT1A1*$^{m/m}$), 27 had heterozygous m*UGT1A1* (*UGT1A1*$^{mut/wt}$), and 22 had wild type *UGT1A1* (*UGT1A1*^{wt}). 60 patients received enasidenib 100 mg/d and 5 patients (*UGT1A1*$^{mut/mut}$ n=2; *UGT1A1*$^{mut/wt}$ n=3) received >100 mg/d enasidenib. Mean TBili at baseline was similar among the 3 genotypes (**Table**). Patients with *UGT1A1*$^{mut/mut}$ had more rapid TBili increases during early treatment than the 2 other genotypes, followed by stabilization of TBili levels; mean increases from baseline during treatment in patients with *UGT1A1*$^{mut/mut}$ indicated mild hyperbilirubinemia (~1.5 x ULN). Mean TBili levels during treatment in patients with *UGT1A1*$^{mut/wt}$ or *UGT1A1*^{wt} were within normal range (**Table**). Indirect bilirubin increases followed similar patterns to those of TBili. AST or ALT increases to ≥3 x ULN during Tx were not more frequent in *UGT1A1*$^{mut/mut}$ patients (6%) than in *UGT1A1*$^{mut/wt}$ (15%) or *UGT1A1*^{wt} (14%) patients. Incidence of treatment-emergent adverse events of hyperbilirubinemia were similar among genotypes although more patients with *UGT1A1*$^{mut/mut}$ (13%) or *UGT1A1*$^{mut/wt}$ (11%) had grade 3 bilirubin events than patients with *UGT1A1*^{wt} (0%). Dose interruptions or reductions due to increased TBili were infrequent in patients with m*UGT1A1* and none were reported for *UGT1A1*^{wt} patients.

Table 1.

Mean ±SD Total Bilirubin

	UGT1A1$^{mut/mut}$ N=16	*UGT1A1*$^{mut/wt}$ N=27	*UGT1A1*^{wt} N=22
	µmol/L		
BL	10.9 ± 7.1	9.5 ± 4.2	9.7 ± 4.0
C1D15	27.9 ± 21.0	18.4 ± 9.0	16.1 ± 6.7
C2D1	31.8 ± 26.7	18.2 ± 6.7	18.7 ± 8.4
C2D15	28.4 ± 14.0	22.9 ± 10.4	20.0 ± 8.8
C3D1	28.8 ± 14.1	21.6 ± 13.3	20.8 ± 8.0
C4D1	29.3 ± 14.3	21.9 ± 16.2	22.3 ± 9.1

C, cycle; D, day

Summary/Conclusion: These data suggest initial enasidenib dose restrictions are not necessary for patients with *UGT1A1* mutations; however, if dose reductions for hyperbilirubinemia are made, they should be informed by the enasidenib prescribing information.

PB1724

EFFECT OF A HIGH-FAT AND HIGH-CALORIE MEAL ON THE PHARMACOKINETICS (PK) OF QUIZARTINIB (Q) AND ITS ACTIVE METABOLITE

J. Li[1,*], M. Holmes[1], M. Kankam[2], D. Trone[3], J. Mendell[4], G. Gammon[3]

[1]Daiichi Sankyo, Inc., San Diego, [2]Vince & Associates, Overland Park, [3]Formerly Daiichi Sanyko, Inc., San Diego, [4]Daiichi Sankyo, Inc., Basking Ridge, United States

Background: Quizartinib (Q) is an oral, highly potent and selective, next-generation FMS-like tyrosine kinase 3 (FLT3) inhibitor. Q has demonstrated promising activity in early clinical studies for treatment of AML and is currently in phase 3 studies for treatment of *FLT3*-internal tandem duplication (ITD) mutated AML.
Aims: In preclinical studies in rats, food increased oral absorption of Q. Therefore, this study aimed to characterize the effect of a high-fat, high-calorie meal on plasma PK parameters of Q in healthy subjects after a single administration of Q. An additional aim was to evaluate tolerability and safety of Q when administered with or without food.
Methods: Healthy subjects, ages 18-55, were randomized in parallel groups to receive a single 30-mg tablet (formulation used in phase 3 trials) of Q (administered as Q dihydrochloride and equivalent to 26.5 mg free-base) under fasted conditions or after a high-fat, high-calorie meal (fed condition). Plasma levels of Q and its active metabolite, AC886, were measured immediately before Q dosing through 504h post dose using a validated method. PK parameters of Q, AC886, and Q+AC886 were determined using non-compartmental modeling. Ratios (fed/fasted) of geometric least squares means (Geo LSM) and 2-sided 90% confidence intervals (CI) of C_{max}, AUC_{last}, and AUC_{inf} of Q were calculated to assess effect of food on PK. A 90% CI entirely within an 80% >125% limit indicated no food effect. Safety and tolerability were assessed.
Results: 66 subjects were enrolled; 64 received study treatment, 30 under fed conditions and 34 under fasted conditions. Overall, 75% of treated subjects were male and the median age of subjects was 34 years. Mean PK profiles of Q administered under fasted and fed conditions were similar. Administration of Q in the fed condition led to ~8% decrease in C_{max}, and increases of ~5% and 8% to AUC_{last} and AUC_{inf}, respectively, *vs* the fasted condition. The 90%CI for the ratio of the fed *versus* fasted condition fell within the 80%>125% limits for C_{max} and AUC_{last}, and the upper bound of the 90%CI for AUC_{inf} was slightly outside the 125% limit (~128%). Similar trends were also observed for C_{max}, AUC_{last}, and AUC_{inf} for AC886 and Q+AC886. Presence of food delayed T_{max} of Q by 2h (T_{max} was 4h in fasted *vs* 6h in fed subjects). $T_{1/2}$ of Q and AC886 was comparable in fasted and fed conditions. All TEAEs reported were mild or moderate; no serious AEs or discontinuations due to AE occurred. Overall, 6 subjects (9.4%) had a TEAE considered related to Q: 2 subjects in the fed condition and 4 subjects in the fasted condition. No significant ECG abnormalities were observed.

Table 1. Statistical Analysis of Q PK Parameters.

PK Parameter	Fed State Geo LSM (N)	Fasted State Geo LSM (N)	Ratio of Geo LSM (fed/fasted), %	90% CI for Ratio of Geo LSM
C_{max}, ng/mL	90.94 (29)	99.31 (34)	91.58	82.15, 102.08
AUC_{last}, ng*h/mL	8788.07 (29)	8338.27 (34)	105.39	90.79, 122.35
AUC_{inf}, ng*h/mL	9459.56 (27)	8727.51 (30)	108.39	91.54, 128.34

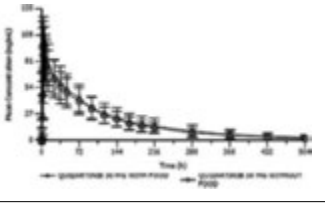

Figure 1.

Summary/Conclusion: Administration of food resulted in slight increase in Q exposure, but is not clinically relevant. TEAEs were similar following Q administration in the fed and fasted conditions. Therefore, Q can be administered with or without food.

PB1725

A DRUG-DRUG INTERACTION STUDY TO ASSESS THE EFFECT OF ACID REDUCING AGENT, LANSOPRAZOLE, ON QUIZARTINIB PHARMACOKINETICS

J. Li[1,*], D. Trone[2], J. Mendell[3], P. O'Donnell[1], N. Cook[1]

[1]Daiichi Sankyo, Inc., [2]Formerly Daiichi Sankyo, Inc., San Diego, [3]Daiichi Sankyo, Inc., Basking Ridge, United States

Background: Quizartinib is a highly potent, selective FLT3 inhibitor currently being investigated in phase3 studies in patients with AML with *FLT3* internal tandem duplication mutations. Acid reducing agents (ARA) such as proton pump inhibitors are frequently used during AML treatment.
Aims: To evaluate the effect of gastric pH on bioavailability of quizartinib, using *in vitro* data describing pH-dependent solubility profile of quizartinib dihydrochloride as a drug substance, and data from a phase 1 study in healthy volunteers to assess the effect of ARA (the proton pump inhibitor lansoprazole) coadministered with quizartinib as a tablet formulation, on pharmacokinetics (PK) of quizartinib.
Methods: pH-dependent solubility of quizartinib dihydrochloride as a drug substance was evaluated in aqueous buffers at pH 1.1-8.0 at 37°C. Healthy adult subjects were randomized in an open-label, parallel-group study to receive quizartinib alone (single dose of 30 mg quizartinib dihydrochloride tablet formulation [equivalent to 26.5 mg free base]) or lansoprazole + quizartinib (lansoprazole 2 × 30-mg oral delayed-release capsule once daily from Days 1 to 5; single dose of 30 mg quizartinib on Day 5). Plasma concentrations of quizartinib and its active metabolite, AC886, were measured to 504 hours post dose. PK parameters included maximum observed plasma concentration (C_{max}) and area under the concentration-time curve to infinity (AUC_{inf}). Effect of lansoprazole on quizartinib PK was assessed by analysis of variance. Safety and tolerability were also assessed.
Results: The solubility of quizartinib dihydrochloride as a drug substance decreased sharply with pH ≥2 (Figure). In the phase 1 study (enrolled N=64), 63 subjects received quizartinib and 59 completed the study. Coadministration of lansoprazole resulted in a lower quizartinib C_{max} (~14%) and AUC_{inf} (~5%; Table). The 90% CI for the ratio of AUC_{inf} fell within the 80% to 125% limits. The lower bound of the 90% CI for AUC_{last} and C_{max} were slightly below the limit at 79.6% and 78.4%, respectively. However, this decrease is not considered a clinically significant effect. A similar trend was observed with AC886. All treatment-emergent adverse events were mild (n=19) or moderate (n=1; headache).

Table 1. Statistical ANOVA Comparisons of Quizartinib Pharmacokinetic Parameters.

PK Parameter	Q+L		Q Alone		Ratio of Geo LSM ((Q+L)/Q), %	90% CI for Ratio of Geo LSM
	n	Geo LSM	n	Geo LSM		
C_{max}, ng/mL	32	90.3	30	104.8	86.11	78.36-94.64
AUC_{last}, ng*h/mL	32	7825.6	30	8328.9	93.96	79.63-110.86
AUC_{inf}, ng*h/mL	31	8257.4	30	8664.7	95.30	80.16-113.30

ANOVA, analysis of variance; CI, confidence interval; Geo LSM, geometric least squares mean; L, lansoprazole; Q, quizartinib.

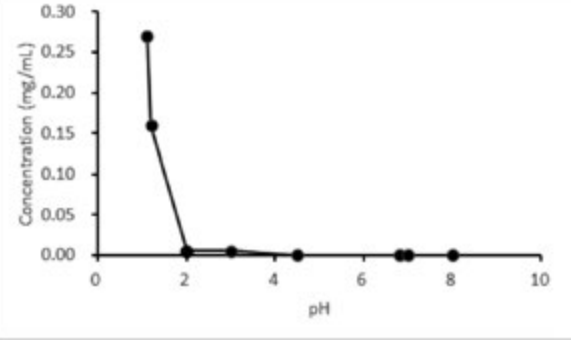

Figure 1.

Summary/Conclusion: Although quizartinib dihydrochloride demonstrated pH-dependent solubility *in vitro* as a drug substance, the proton pump inhibitor lansoprazole had no clinically significant effect on quizartinib PK. Quizartinib as a formulated tablet can be coadministered with and without acid reducing agents.

PB1726

PHARMACOKINETICS OF HU5F9-G4 A FIRST IN CLASS ANTI-CD47 ANTIBODY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

B. Agoram[1,*], B. Wang[2], P. Vyas[3], M. Dennis[4], R. Kelly[5], S. Knapper[6], R. Salim[7], H. Johnson[8], C. Roberts[9], J. Liu[10], R. Secreto[11], C. Takimoto[12], M. Chao[12]

[1]Clinical Pharmacology, FortySeven Inc, Menlo Park, [2]Clinical Pharmacology, LucidCPS, Pleasanton, United States, [3]Clinical, University of Oxford and Oxford University Hospitals, Oxford, [4]Clinical, Christie NHS Foundation Trust, Manchester, [5]Clinical, Leeds Teaching Hospitals NHS Trust,, Leeds, [6]Clinical, Cardiff University School of Medicine, Cardiff, [7]Clinical, Royal Liverpool University Hospital, Liverpool, [8]Clinical, [9]Centre for Statistics in Medicine, University of Oxford, Oxford, United Kingdom, [10]Research and Development, FortySevenInc, [11]Clinical Operations, [12]Clinical development, FortySeven Inc, Menlo Park, United States

Background: Hu5F9-G4 (5F9) is a humanized monoclonal antibody targeting CD47, a protective "don't eat me" signal on cancer cells. Blocking CD47 stimulates tumor cell phagocytosis and activates an anti-tumor T-cell response. Pre-clinically, 5F9 eliminates leukemic disease and induces durable remissions in patient-derived xenograft mouse models.
Aims: The objectives of this analysis were to characterize the pharmacokinetics (PK) and anti-drug antibody (ADA) incidence of 5F9 after single and multiple doses in patients with acute myeloid leukemia (AML).
Methods: Data from the Phase 1 CAMELLIA study (MRC grant: MR/L008963/1) were used for this analysis. Multiple intravenous (IV) doses of Hu5F9 in the range 0.1-30 mg/kg were given at twice weekly frequency. PK samples were drawn in all patients after single and multiple doses. A total of 13 AML patients provided PK and ADA data. PK data were analyzed by a noncompartmental approach using the PKNCA package in R language. Population modelling using NONMEM software was performed by combining PK data in AML patients with data from patients with solid tumours and lymphomas. Model-estimated individual PK parameters (linear and nonlinear clearance, and volume of distribution) were used to compare PK parameters in AML *vs* solid tumour patients. Simulations using the model were used to identify optimal Phase 2 dose in AML patients. Presence of ADA was analyzed using a 3-tiered approach consisting of screening, confirmatory, and titre determination.
Results: Increases in maximum serum concentration (C_{max}) of 5F9 were greater than dose-proportional in the dose range 0.1-10 mg/kg indicating nonlinearity in PK. At doses ≥10 mg/kg, increases in C_{max} were linear. The PK parameters were consistent with the presence of a CD47 antigen sink, which was saturated at doses ≥10 mg/kg. Population PK modelling indicated that PK parameters in AML patients were similar to those estimated in solid tumour patients. Simulations with the model predicted that a maintenance dosing regimen of 30 mg/kg every week after week 2 would result in serum concentrations ≥200μg/mL, a level where near-maximal CD47 receptor occupancy on blood and bone marrow cells was observed in this study.
ADA was confirmed in 1 subject (7.7%) in this study but had no observable impact on the PK parameters.
Summary/Conclusion: 5F9 exhibits nonlinear PK in AML patients, typical of a receptor-targeted antibody. The PK in AML patients is similar to that in patients with solid tumours. The immunogenic potential of 5F9 in AML patients is low. Overall, the PK profile of 5F9 is suitable for once weekly dosing in AML patients. Ongoing studies in AML have implemented this dosing regimen (NCT03248479).

PB1727

EFFICACY AND SAFETY OF C-CAG REGIMEN (CLADRIBINE,G-CSF, LOW-DOSE CYTARABINE AND ACLARUBICIN) IN PATIENTS WITH REFRACTORY/RELAPSED ACUTE MYELOID LEUKEMIA: A PHASE 2, SINGLE CENTER, SINGLE ARM STUDY

H. Wang, Y. lu*, Y. wang

Hematological Oncology, Sun Yat-Sen University Cancer Center, Guangzhou, China

Background: Although the considerable advances have been made in the treatment of acute myeloid leukemia (AML) in recent decades, only one third of patients (except for acute promyelocytic leukemia) can be cured, and most suffer relapse or primary refractory (R/R). However, there is no standard regimen for R/R AML patients after conventional chemotherapy.A regimen consisting of granulocyte colony-stimulating factor (G-CSF) priming the combination of low-dose cytarabine (Ara-C) and aclarubicin, termed the CAG regimen,was first reported by a Japanese group in 1995. It has been proved that cladribine is a attractive drug in AML because of their significant synergy with other chemotherapeutic agents and favorable toxicity profile. Efforts were made to improve the efficacy of the CAG regimen among institutions, including combining it with other chemotherapy drugs.
Aims: Therefore, the purpose of this study is to prospectively evaluate efficacy and safety of C-CAG regimen (cladribine in combination with granulocyte colony-stimulating factor, low-dose cytarabine and aclarubicin) in patients with R/R AML (**This study was registered at www.chictr.org, the Clinical Trial Registration Number was ChiCTR-OPC-16010166**).

Table 1.

Patients Characteristics.

Characteristics	n	(%)
Age [median (range), years]	34	
< 60 years	10	90.9
≥60 years	1	9.1
Sex		
Male	5	45.5
Female	6	54.5
FAB subtypes		
M2	5	45.5
M4	1	9.1
M5	5	45.5
Blood cell count before re-induction		
WBC (×10^9/L, mean±SD)	19.6±4.75	
PLT (×10^9/L, mean±SD)	72.8±7.54	
HB (×g/L, mean±SD)	97.0±2.61	
Cytogenetic risk group,		
Low risk	3	27.2
Intermediate risk	4	36.4
High risk	4	36.4
Disease status		
Relapse	9	81.8
Refractory	2	18.2
Duration of first complete remission, months (range)	10	

Methods: Enrolment began in August 2016 for a Phase II single-center clinical trial. The patients in this arm will receive C-CAG regimen for salvage treatment,detailed as following:Cladribine 5mg/ ,d1-5 G-CSF 300ug,d0-9; aclarubicin 10mg,d3-6;cytarabine 10mg/ q12h, SC, d3-9;4 weeks a cycle.The patients are permitted to quit the study if complete remission(CR) is not achieved after 2 course of chemotherapy. If conditions were right,the patients achieving CR were recommended to receive allogeneic hematopoietic stem cell transplantation(HSCT). Otherwise, the patients were given for a total of six cycles unless there was disease progression or unacceptable side effects, or withdrawal of patient consent.
Results: Until December 2, 2017, we have completed the enrolment of 11 patients with R/R AML. The main clinical characteristics of the 11 patients are presented in Table 1. The median age was 34 years and ranged from 18 to 72 years. The majority of patients presented with FAB subtype of M2 or M5. All patients(n=11)have begun treatment and are evaluable for response. Of the 11 patients, complete remission rates is 72.7% to C-CAG regimen. No patients received allogeneic transplantation due to scarcity of bone marrow donors or inability of the patients to afford the expensive medical treatment.At a median follow-up of 8 months (3–18), the 1-year PFS and OS rates for all 11 patients were 43.6 and 62.3%,respectively .The most common adverse effect was myelosuppression. The incidence of grade 3 or 4 hematological toxicity was 90.9%.The respective median duration of neutropenia (neutrophils<0.5×10^9/L) was 7 days. The median time of platelet recovery over 50 × 10^9/L was 12 days.All patients presented an incidence of 4.1% for Grade 3 or 4 infectious toxicity. No treatment-related deaths occurred. Non-hematological side effects were mild. Grade 3 or 4 gastrointestinal side effects were rarely observed, owing to the prophylactic antiemetic drug administration.
Summary/Conclusion: Preliminary data indicate that the C-CAG regimen chemotherapy is significantly effective against R/R AML with a high remission rate and a low hematological toxicity, thus serves as an alternative treatment for R/R AML.

PB1728

HEALTH RELATED QUALITY OF LIFE (HRQOL) IN ACUTE MYELOID LEUKEMIA (AML) PATIENTS NOT ELIGIBLE FOR INTENSIVE CHEMOTHERAPY (NIC AML): RESULTS OF A SYSTEMATIC LITERATURE REVIEW

A. Forsythe[1,*], C.S. Kwon[1], T. Bell[2], T.A. Smith[3], B. Arondekar[2], A.P.J. Rabe[1]

[1]Purple Squirrel Economics, [2]Pfizer Inc, New York, United States, [3]Pfizer Ltd, Walton Oaks, United Kingdom

Background: AML is diagnosed at a median age of 67; once over 60 years of age, the 5-years survival rates for patients with AML fall substantially because they are often not eligible for intensive chemotherapy (NIC). Less intensive chemotherapeutic agents and best supportive care are potential treatment options in this population. There is scant published literature on

the impact of disease and treatment on the health-related quality of life (HRQoL) in NIC AML patients.
Aims: We aimed to determine the reported quality of life among NIC AML patients.
Methods: We conducted a systematic literature review (SLR) of evidence on HRQoL reported in patients with NIC AML. MEDLINE, Cochrane database, and conference abstracts (EHA, ASCO, ESMO, and ASH) were searched using matches on pre-specified population, interventions, comparators, outcomes and study designs (PICOS approach) from January 2000 through November 2017 for relevant studies that reported HRQoL and patient preference utilities in NIC AML. Based on the WHO AML criteria, studies on patients with RAEB-t myelodysplastic syndrome (MDS) (≥20% bone marrow blast) were also included. Randomized clinical trials (RCTs), prospective observational studies and patient surveys were included. Systematic reviews and meta-analyses were used for bibliographic search. Two researchers independently selected trials, assessed trial quality, and extracted and analysed data.
Results: A total of 13 records from 12 original studies were identified. These included 5 records from 4 original RCTs, 3 prospective studies, 4 patient survey studies, and 1 cost-effectiveness analysis reporting utility values. Ten studies utilized the EORTC QLQ-C30 questionnaire, 5 reported EQ-5D values. Other scales used included QOL-E, QOL Cancer Survivor, FACT-Leukemia, FACT-Fatigue, Global Fatigue Scale, FACIT Fatigue, Activities of Daily Living index and Hospital Anxiety and Depression Scale. Four QLQ-C30 domains were considered most relevant: fatigue, physical function (PF), Global Health Status (GHS) and dyspnea. A 10-point minimally important difference (MID) threshold on a 100-point scale was assumed by a majority of studies to represent meaningful change. At baseline, NIC AML patients had poor HRQoL scores especially in fatigue (33) and GHS (50) on a 0-100 scale, with higher scores indicating better health. Low baseline HRQoL scores, especially PF and fatigue (<50) were shown to be significant independent predictors of poor survival. Clinical responders demonstrated meaningful improvements in QLQ-C30 physical, role, cognitive and social functioning, GHS, fatigue and EQ-5D scores from baseline after being treated with chemotherapy. Clinically meaningful and significant improvements in fatigue and PF were observed with non-intensive chemotherapeutic agents across several studies.
Summary/Conclusion: Although HRQoL is highly subjective, it plays a crucial role in the treatment of AML patients. Fatigue and physical function at baseline have been identified as independent prognostic factors for overall survival with several studies showing improvement in both domains with treatment. Randomized controlled studies should incorporate evaluation of treatment impact on patient's physical function and fatigue as important measures of effectiveness.

PB1729

IMPORTANCE OF CLINICAL RISK IN CYTOGENETIC-MOLECULAR FAVOURABLE RISK AML WITH NPM MUTATED, FLT3-ITD UNMUTATED AND NORMAL KARYOTYPE

M.V. Dubbini*, A. Candoni, M.E. Zannier, C. Filì, D. Lazzarotto, N. Rabassi, S. Frisoli, R. De Marchi, D. Schiavo, R. Stella, E. Simeone, R. Fanin

Division of Hematology and Stem Cell Trasplantation, Azienda sanitaria Universitaria Integrata di Udine, Udine, Italy

Background: Acute Myeloid Leukemia (AML) with NPM mutated (NPM+), FLT3-ITD unmutated (FLT3-) and Normal Karyotype (NK) are included in the favourable cytogenetic-molecular risk class according to the 2010 and 2017 classifications. Frequently, in clinical practice, we focus only on this cytogenetic-molecular risk to define the AML prognosis regardless of clinical risk profile (particularly secondary AML and high blast cells count-BC in peripheral blood-PB, at onset).
Aims: We analyse clinical characteristics, response to chemotherapy (Complete Remission-CR), relapse rate (RR) and outcome of 29 AML NPM+/FLT3-/KN that were homogeneusly treated at our Center in the least 10 years.
Methods: There were 15 female and 14 male with a median age of 53,5 yrs (range 20-68) and 21% of cases had more than 65 yrs. Four (14%) AML were secondary to a previous Myelodysplastic Syndrome and 41% (12/29) had more than 30.000/mmc BC in PB at onset. Overall 52% of case are at High Clinical Risk (HCR) at diagnosis. All patients (pts) were treated with FLAI scheme (Fludarabine, Cytarabin-Ara-C, Idarubicin) as induction, followed by Intermediate dose Ara-C+Idarubicin and High Dose Ara-C, as consolidation therapy.

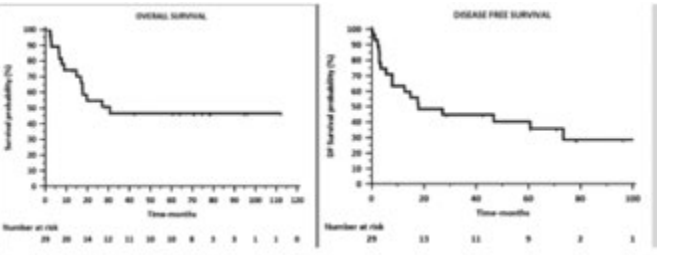

Figure 1.

Results: The CR after FLAI was 90% (26/29 cases) and only 7% (2/29) of pts were resistant. DDI was 0%. After a median follow up of 20 months (2-112), 52% of pts (15/29) are alive and 48% (14/29) died. The RR was 41% (12/29) and 8/12 relapsed pts (67%) had CHR at diagnosis. Allo-SCT rate was 41% (12/29) and Allo SCT was performed mainly in CHR and after relapse. The probability of OS at 12, 24 and 36 mths, was 74%, 55% and 47%, respectively (FIGURE 1). The probability of DFS at 12, 24 and 36 mths, was 60%, 48% and 45%, respectively (FIGURE 1). The OS and DFS did not differ between CHR and other pts but we underline that a significant higher proportion of CHR pts received Allo-SCT (10/15 CHR cases *vs* 2/14 other cases, P<0,05) mainly after their relapse.
Summary/Conclusion: In our experience the AML with NPM+/FLT3-/KN have a RR not negligible (41%) with a probability of DFS at 24 mths of 48%. The relapse occured mainly (67%) in pts with CHR (high PB-BC and secondary AML). For this CHR population early and close monitoring of minimal residual disease (quantitative NPM-today available) should be performed in all cases in order to avoid cytologic relapse and to decide if it is worth consolidating their cytologic CR with Allo SCT.

PB1730

IMPACT OF INFECTION PRIOR TO THE INITIATION OF INDUCTION CHEMOTHERAPY ON INDUCTION OUTCOMES IN PATIENTS WITH NEWLY DIAGNOSED ACUTE LEUKEMIA IN A TERTIARY CENTRE HOSPITAL IN INDIA

R.R.[1,*], S.T.[1], M.M.[1], S.R.[1], J.V.[2]

[1]Dept of Hematology, AIIMS Delhi India, New delhi, [2]Radiology, AIIMS, Delhi, India

Background: There is paucity of published data regarding the treatment strategy of patients infected at baseline prior to initiation of induction chemotherapy.
Aims: To determine the impact of infection at baseline(prior to the initiation of chemotherapy) in acute leukemia patients undergoing induction chemotherapy with respect to mortality and CR rates.
Methods: Ambispectively (from 1-7-2015 to 31-12-2017) we included newly diagnosed patients with acute leukemia undergoing induction chemotherapy.
Results: A total of 268 admitted in-patients of acute leukemia were screened in the Dept of Hematology at All india Institute of Medical sciences(AIIMS), New Delhi, India. Out of these 124 admitted patients were found to have infections at baseline prior to initiation of induction chemotherapy. A large proportion of admitted infected acute leukemia patients died before the initiation of induction chemotherapy (54.83%).The median duration of illness prior to admission in the group of infected patients who later proceeded on to receive induction chemotherapy was longer in comparison to patients without infection and this difference was statistically significant (12 weeks *versus* 6 weeks, p<0.00001). Ninety-six percentage of the patients with baseline infection prior to initiation of induction chemotherapy were treated with IV antibiotics and antifungals each. The mean duration of therapeutic antibiotic therapy and antifungal therapy prior to induction chemotherapy was 16.08 days and 13.6 days respectively. Prior to the initiation of induction chemotherapy it was determined that clinically 78% of patients had improvement in the status of their infection, twelve percentage had complete resolution of the documented baseline infection.In the patients with baseline infection, after initiation of induction chemotherapy, the majority (82%) developed either new infections and/or worsening of pre-existing infections in comparison to the group without infection at baseline (53.73%), and this difference was statistically significant (p=0.0005). Lesser proportion of the uninfected patients at baseline developed febrile neutropenia (53.73%) in comparison to the group with baseline infections (80%) upon induction chemotherapy initiation, and this difference was statistically significant(p=0.0012). After the initiation of chemotherapy the occurrence of septic shock in the group of patients with infection at baseline (42%) was more frequent than in the group without infection at baseline

(22.388%) and this difference was statistically significant (p=0.0084). The difference in the induction mortality rates in both groups was not significant statistically (32% in baseline infected group *versus* 23.13% in the uninfected group, p=0.1968), even on subset analysis comparing AML/ALL of either groups individually; AML (40% *versus* 29.16%, p= 0.3262) /ALL(22.2% *versus* 18.18%, p=0.7019).The CR rates in the baseline uninfected group *versus* in the baseline infected group was 78.78% *versus* 61.11% respectively in ALL and was 39.58% *versus* 36.66% in AML and neither of the difference was statistically significant.

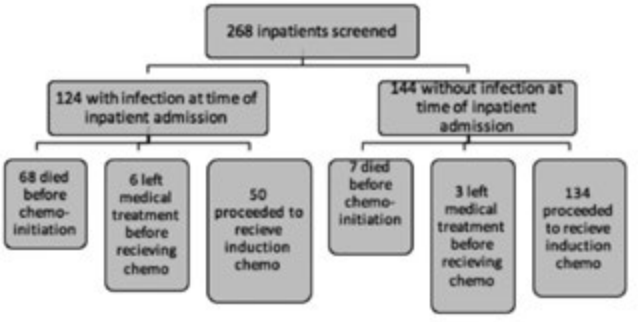

Figure 1.

Summary/Conclusion: Patients with infection at baseline demonstrating clinical improvement upon treatment with antibiotics/antifungals can be considered for induction chemotherapy. There is need for studies using newer therapeutic agents with potentially lower disruption of muco-cutaneous barriers, lower myelotoxicity and potential infliction of lesser physiological stress as a bridge to subsequent full dose induction chemotherapy in these patients infected at baseline even prior to the initiation of chemotherapy.

PB1731

OUTCOME PREDICTION OF AML PATIENTS SUBMITTED TO ALLOGENEIC STEM CELL TRANSPLANTATION IN RECENT YEARS. A SINGLE CENTER EXPERIENCE ON 101 PATIENTS

N. Polverelli[1,*], V. Cancelli[1], M. Malagola[1], A. Turra[1], E. Morello[1], F. Cattina[1], B. Rambaldi[1], L. Gandolfi[1], S. Bernardi[2], F. Re[2], C. Zanaglio[2], E. Dereli[2], D. Russo[1]

[1]Unit of Blood Diseases and Stem Cells Transplantation, Department of Clinical and Experimental Sciences, University of Brescia, ASST Spedali Civili di Brescia, [2]Laboratorio CREA (Centro di Ricerca Emato-Oncologica AIL), ASST Spedali Civili di Brescia, Brescia, Italy

Background: Allogeneic hematopoietic cell transplantation (allo-HCT) represents the most effective therapy for high risk acute myeloid leukemia (AML) patients. Due to its morbidity and mortality, patients' selection is crucial. Several prognostic score are used to define the pre-HCT risk for AML patients, however most of them are dated or difficult to be applied in clinical practice.
Aims: To evaluate the clinical outcome of AML pts submitted to allo-HCT.
Methods: Clinical and laboratory data of 101 AML patients submitted to allo-HCT from October 2006 to June 2017 at the Stem Cells Translantation Unit of Spedali Civili Hospital in Brescia were retrospectively collected. A multivariable model was used to weight parameters associated to lower survival post-HCT, in order to build a prognostic score.
Results: The clinical features of the cohort were the following (median):age 52y (18-67), ≥55y, 47%; male, 56%; AML with myelodysplasia related changes (7%); therapy-related (9%). Karyotype was normal in 39%, unfavorable in 54% and favorable in 7% of cases. Seventeen patients (17%) had FLT3-ITD, 20 (20%) NPM1 and 5 (5%) CEBPA mutations. WT1 gene was overexpressed in 56% of patients. ELN risk category was low/intermediate 1 in 41 (40%), intermediate-2/unfavourable in 60 (60%) patients, respectively.Twenty-one patients (21%)presented chemo-refractoriness to induction treatment; 81(80%) were submitted to allo-HCT in complete hematological remission. Allo-HCT was performed after a median time from diagnosis of 9 months (range, 3-118), conditioning regimen was myeloablative in 55% of cases; matched-related donor was employed in 43 cases (43%), matched-unrelated in 44 (44%), and alternative in 14 (13%) cases. Peripheral blood was the most frequent source of stem cells (73%). According to Sorror HTC-CI, 6 (6%) patients presented 0, 20 (20%) 1, 10 (10%) 2 and 65 (64%) 3 or higher score. Seventy-two patients had a Karnofsky (KPS) equal to 90-100, 29 (28%) pts<90. After a median follow-up of 31 months (range, 6-133) from allo-HCT, acute and chronic graft *versus* host disease (GVHD) were encountered in 38% and 20% of patients, respectively. At last follow-up, 55 patients (54%) died, for a 2-year overall survival (OS) of 51%. Causes of death were related to: allo-HCT complications (TRM) (30%), disease relapse (66%), other unrelated in 4% of cases, respectively. Among baseline features, age ≥55 years (p=0.03), disease-persistence at transplantation (p<0.001), induction chemotherapy refractoriness (p=0.001), and KPS<90 (p<0.001) significantly correlated with higher mortality. Notably, no differences were observed according to HCT-CI (p=0.096). In multivariate analysis, age ≥55 (HR 2.3 CI95% 1.3-4), disease status at transplantation (HR 2.2 CI95% 1.04-4.8) and lower performance status (HR 2.6 CI95% 1.2-5.4) confirmed their negative prognostic association. Based on these HR, a weighted score was developed; accordingly, age received 2 points, disease-persistence 2 points and KPS 3 points. Low (0 points), intermediate (2-3 points) and high risk (>3 points) categories were so projected to an OS of 74%, 54% and 10% at 2 years, respectively (p<0.0001)(figure 1).

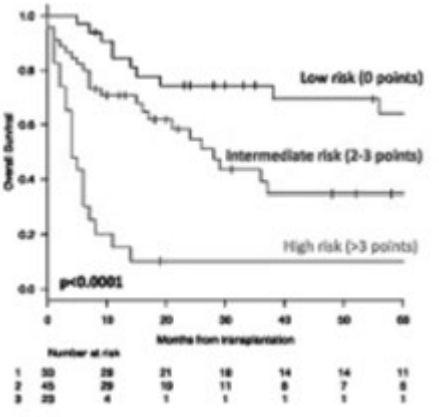

Figure 1.

Summary/Conclusion: In our experience, advanced disease and performance status were the most important prognostic factors for allo-HTC. With the limitations of the retrospective nature of the study and the number of enrolled patients, our simple clinical score seems to predict allo-HCT outcome in AML patients. Further larger studies are needed to confirm these preliminary data.

PB1732

FEASIBILITY AND EFFICACY OF INTENSIVE SALVAGE TREATMENTS FOR ELDERLY PATIENTS WITH RELAPSED ACUTE MYELOID LEUKEMIA

B. Massimo[1,*], M. Carrabba[1], C. Messina[1], F. Pavesi[1], L. Vago[1], B. Gentner[1], R. Milani[2], S. Mastaglio[1], F. Lorentino[1], G. Casirati[1], F. Ciceri[1]

[1]Haematology and BMT Unit, [2]Flow-cytometry and Cell-manipulation lab, San Raffaele Scientific Institute, Milan, Italy

Background: outcome of AML patients (pts) older than 60 years is poor because of unfavourable disease characteristics and of comorbidities, frequently tailoring under-powered treatment. The complete remission (CR) rate after intensive treatments is lower than in younger pts, with relapse incidence and mortality associated to treatment (TRM) being higher. Rarely, elderly pts who relapse after initial remission receive salvage treatments aiming to obtain further disease remission.
Aims: to evaluate the feasibility and efficacy of intensive treatments in our elderly pts with relapsed AML.
Methods: retrospective analysis of data from 50 pts with AML in CR relapsed between 2/2002-1/2018. Criteria for pts selection to receive salvage treatments: PS (ECOG) ≤2, renal and hepatic parameters within normal ranges or<2 times normal values, no active infections and cardiac ejection fraction >50%.
Results: median pts age at relapse was 70 (61-81). Cytogenetics and molecular data at relapse were available only for a minority of pts and were not analyzed. Median time to relapse was 227 days (range 42-3369). Overall, 30 pts (60%) received at least one reinduction treatment: chemotherapy (CHT) in 23 cases, upfront allogeneic transplantation (alloSCT) in 3 cases, alloSCT after no response to CHT in 4 cases. Eleven pts had previously received an autologous SCT, 19 pts CHT with intermediate or high dose cytarabine, as consolidation of first CR. Twenty-one pts (68%) obtained

second CR (CR2), 15 after CHT alone, 3 after upfront alloSCT, 3 after CHT and alloSCT. TRM was 27% (8 pts), overall, 17% after CHT alone, 75% after upfront alloSCT, 50% after CHT and alloSCT. Of the 15 pts in CR2 after CHT alone, 10 did not receive further treatments, 5 received an alloSCT. At last follow up 4 pts out of 30 (13%) are alive in CR2, with a median survival from relapse of 421 days (255-4906). Median OS from relapse of all pts who received any reinduction treatment was 276 days (33-4904). Median OS from relapse of pts who obtained the CR2 750 days (137-4904). Median DFS from CR2 was 429 days (29-4848).

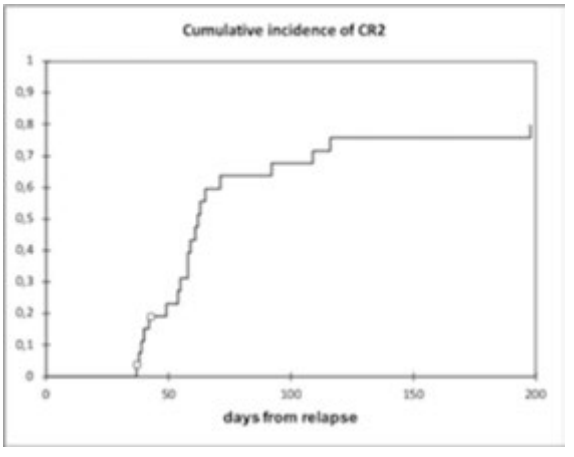

Figure 1.

Summary/Conclusion: intensive reinduction approaches proved feasible in our elderly pts with relapsed AML. The CR rate was similar to that obtained at diagnosis. Actually, TRM was high, in particular after alloSCT (57% overall). Of note, 4 pts (57%) had active disease at time of alloSCT. Moreover, median age of the treated population was high. Anyway, several pts obtained prolonged overall and disease free survival. We conclude that elderly pts with relapsed AML, fit according to PS and general criteria, could benefit from an intensive curative approach. Recent introduction of new strategies to prevent and treat potentially fatal complications after alloSCT, in particular infections and graft *versus* host disease, should prospectively reduce TRM and improve survival.

PB1733

RISK FACTORS AND OUTCOMES OF PATIENTS WITH RELAPSED CORE-BINDING FACTOR-POSITIVE ACUTE MYELOID LEUKEMIA

M. Virijevic[1,2,*], M. Mitrovic[1,2], Z. Pravdic[2], A. Novkovic[3], V. Djordjevic[2], N. Suvajdzic[1,2], A. Vidovic[1,2], D. Tomin[1,2]

[1]Faculty of Medicine, University of Belgrade, [2]Clinic for Hematology, Clinical Center of Serbia, [3]Clinical Hospital Center Zemun, Belgrade, Serbia

Background: Core-binding factor-positive acute myeloid leukemia (CBF-AML) (t(8;21) or inv(16)/t(16;16)) is associated with favorable prognosis. However, first relapse of AML-CBF occurs in 30% to 40% patients and can still be cured in half of patients. A number of studies identify predictive factors of outcome in relapsed CBF-AML which may help to improve post-relapse therapy strategies.
Aims: to determine risk factors for relapse in CBF-AML and to report clinical outcome of relapsed CBF-AML.
Methods: his single-center study involved 74 patients with *de novo* AML with rearrangement of CBF genes during the period between January 2006 and December 2017. We assessed clinical, biological, histological and cytogenetic characteristics for relapse and outcome of relapsed CBF-AML. Risk factors were identified using univariate and multivariate analysis. Kaplan-Meier method is used to estimate overall survival (OS).
Results: Among the 74 CBF-AML patients, 21 (28.4%) patients relapsed. Median age at relapse was 52 years (range, 28-64 years); inv(16) had 5/21 (23.8%) and t(8;21) 16/21 (76.2%) of patients) The median duration for CR1 was 20 months (range, 1-132 months) for inv(16) and 12 months (range, 1-120 months) for t(8;21) (p=0.051). Univariate analysis detected the following significant risk factors for relapse in CBF-AML: age ≥50 years (p=0.039), CD56$^+$ (p<0.001). Multivariate analysis identified CD56$^+$ as the most important risk factor for relapse in CBF-AML (p=0.001; relative risk (RR)=0.59; 95% confidental interval (CI)=0.011-0.308). The median OS for all the patients after relapse was 24 months (2-132 months); 25 months (range 2-132 months) for those with inv(16) and 18 months (range, 3-132 months) for those with t(8;21) (p=0.551). The CR2 rate for the inv(16) and the t(8;21) was 80% and 53.3%, respectively (p=0.630). Univariate analysis detected the following significant risk factors for poor OS in relapsed CBF-AML patients: elevated LDH (p=0.05), CD56$^+$ (p=0.05) and t(8;21) (p= 0.045). Multivariate analysis identified t(8;21) as the most important risk factor for poor OS in relapsed CBF-AML (p=0.045; relative risk (RR)=3.171; 95% confidental interval (CI)=0.891-11.283).
Summary/Conclusion: According to the results obtained from the analysis, CBF-AML with CD56$^+$ have a higher risk of relapse. Among patients with relapsed CBF-AML, the outcome was worse in patients with t(8;21).

PB1734

FEASIBILITY AND SAFETY OF AN EARLY INTENSIFICATION APPROACH WITH FLAG-IDA IN NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA (AML) PATIENTS WITH MORPHOLOGICAL RESIDUAL LEUKEMIA AT DAY 14 BONE MARROW AFTER 3+7

F. Pavesi[1,*], M.G. Carrabba[1], C. Messina[1], F. Lorentino[1], E. Sala[1], G. Casirati[1], C. Liberatore[1], L. Vago[1], R. Milani[2], P. Ronchi[3], B. Gentner[1], F. Ciceri[1], M. Bernardi[1]

[1]Hematology and BMTUnit, [2]Flow Cytometry and Cell manipulation lab, [3]Immunohematology and Transfusion Medicine, San Raffaele Scientific Institute, Milan, Italy

Background: 3+7 chemotherapy (CT) is considered reference treatment for AML patients (pts) treated with a curative intent although the rate of complete remission are lower than those that can be obtained with more intense regimens (eg FLAG-ida). Early assessment of response in AML by day 14 bone marrow (day 14 BM) has been proposed as a tool to optimize the response rate by the introduction of an early second induction in patients with >10% residual blasts. Recent data and reviews criticize this approach since day 14 BM specificity might be suboptimal and an early re-treatment be too toxic. In this context we reviewed our experience of an early intensification with FLAG-ida in pts obtaining a suboptimal response at day 14 BM after 3+7.
Aims: To evaluate the safety, the feasibility and the outcome of FLAG-ida as early re-induction for newly diagnosed AML pts treated at our hospital between february 2009 to june 2017.
Methods: Retrospective analysis of 19 consecutive newly diagnosed AML pts who were candidate to early re-induction due to residual blasts >10% (12-90%) at day 14 BM morphological examination and who were considered fit (without active infection, PS ECOG<2, without renal and/or hepatic impairments) to receive FLAG-ida. As outcomes we defined day 30 and 60 not relapsed mortality, complete remission (CR) rate at day 30 post FLAG-Ida, percentage of patient completing their intention to treat (ITT), event free survival (EFS) and overall survival (OS).
Results: Median age of our series was 60 years (26-78 years). 84% pts were de novoAML. 2 pts were ELN favorable risk, 13 intermediate and 4 adverse. Median follow-up was 669 days (248-1479 days). Median time from the induction start to FLAG-Ida was 17 days (15-23 days). After FLAG-ida median time to neutrophil recovery was 20 days (16-26 days), we recorded 2 cases of bacterial pneumonia and 2 possible invasive fungal infection which recovered with appropriate therapy. Non relapse mortality rates at 30 and 60 days from FLAG-ida were 0% and 10.5% (2/19). One pt died in CR for an adenovirus hepatitis, the other for a septic shock (from MDR Pseudomonas aeruginosa) after receiving high dose cytarabine as consolidation. Seventeen of 19 pts (89.5%) obtained the CR after FLAG-ida (median time 29 days). Two pts (10,5%) were refractory. Sixteen of these 17 (94%) pts completed treatment according to the ITT: 12 pts received allogeneic stem cell transplantation, 1 pt autologous transplantation and 3 pts consolidation chemotherapy. Twelve pts were alive when we analyzed the data, 9 of them in a durable CR. Two year EFS and OS rates were 45.6% (95% CI: 20-68.2) and 78.9% (95% CI: 53.2-91.5) respectively. (Figure 1).

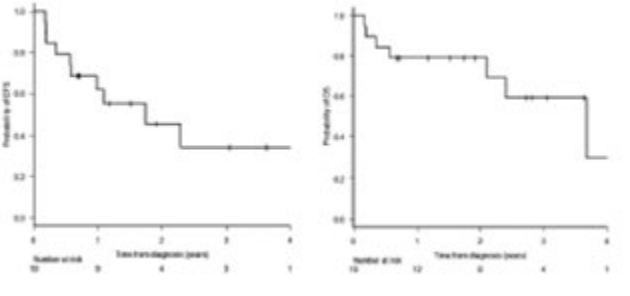

Figure 1.

Summary/Conclusion: Our findings suggest that FLAG-ida as early reinduction is feasibile and safe at least in a subgroup of AML pts selected on the basis of fitness criteria. Although recent data raise questions on day 14 BM morphology specificity we believe that our results are of relevance for designing strategies based on early residual disease (eg multi parametric flow cytometry based) assessment in AML to be tested in prospective study. The number of pts which completed the treatment in respect to the ITT and the persistent CR in spite of the prognostic value of day 14 BM suggest that a strategy of early intensification on the top of 3+7 can be considered in future clinical trials.

PB1735

REGISTRY FOR THE OFF-LABEL USE OF VENETOCLAX IN PATIENTS WITH RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA

R. Shahswar[1,*], I. Hamwi[1], C. Lueck[1], M. Toepperwien[1], C. Koenecke[1], W. Fiedler[2], R. Madanchi[2], J. Krauter[3], A. Trummer[3], M. Eder[1], G. Beutel[1], M. Stadler[1], S. Ehrlich[1], L. Hambach[1], A. Ganser[1], F. Thol[1], M. Heuser[1]

[1]Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Medical School Hannover, Hannover, [2]Department of Medicine II, Oncological Center, Hubertus Wald University Cancer Center, University Hospital Hamburg-Eppendorf, Hamburg, [3]Department of Hematology and Oncology, Klinikum Braunschweig, Braunschweig, Germany

Background: Previous data have shown promising safety and efficacy of venetoclax (VEN) in combination with hypomethylating agents (HMAs) or low-dose cytarabine (LDAC) in untreated AML patients (pts). VEN is a potent oral small molecule inhibitor of the anti-apoptotic protein BCL-2 and is currently approved for use in refractory chronic lymphoid leukemia pts.
Aims: To establish a registry of AML patients treated with VEN off-label and to report initial results.
Methods: Patients with refractory or relapsed (R/R) AML who were treated with VEN at three German medical centers were included. Patients received VEN for salvage treatment in combination with HMAs or LDAC outside of a clinical trial. We retrospectively collected data regarding clinical, genetic and treatment characteristics. All patients had given informed consent to the off-label use of VEN, genetic analysis and use of clinical data according to the Declaration of Helsinki.
Results: Eight patients with R/R AML who received salvage treatment with VEN in combination with HMAs or LDAC were eligible for analysis. Median age was 68 years (range 41-78). The patient cohort had an unfavorable risk with 5 pts (63%) having secondary or therapy-related AML, 7 (88%) pts being treated in second or higher salvage therapy (range 1-4), 3 (38%) pts having adverse ELN risk, 7 (88%) pts having received prior HMA or LDAC therapy and 1 (12%) patient having received prior allogeneic stem cell transplantation (SCT). In 6 of 8 pts (75%) VEN was combined with azacitidine (n=5) or decitabine (n=1), in 2 pts (25%) it was combined with LDAC. The VEN dose ranged from 50 to 600 mg per day with dose reduction related to concomitant CYP3A4 inhibitor treatment, primarily azole antifungals, in 6 pts (75%). At data cutoff pts received a median of 2.5 cycles (range 1-7, one patient ongoing). Of the 7 patients who discontinued VEN, reasons included progressive disease (n=3), death in aplasia due to sepsis (n=1) and transition to SCT (n=3). The overall response rate (ORR), defined as complete response (CR) or CR with incomplete blood count recovery (CRi) or partial response (PR) per IWG criteria was 6/8 (75%, 1 CR, 3 CRi, 2 PR with peripheral blood count recovery). All 6 patients treated with dose-reduced VEN because of concomitant azole use achieved a response. Three pts were successfully bridged to transplant. Median follow up was 9.4 months. Median survival was 6.6 months. Five patients died (63%), 3 from progressive disease and 2 from infectious complications. Transfusion-dependent anemia (n=8) and thrombocytopenia (n=6) were common prior to the start of therapy. Four of 8 patients (50%) achieved red blood cell transfusion independence. Three of 6 patients (50%) achieved platelet transfusion independence with recovery of platelet counts to >50/nl after a median of 36 days (range 29-47). One pancytopenic patient only received VEN on days 1-14 of each cycle in combination with LDAC and achieved complete blood count recovery after the second cycle (PR).
Summary/Conclusion: We established a clinical registry for R/R AML patients who were treated with VEN. Results are comparable to other reports of off-label use in R/R AML patients. Efficacy of VEN was maintained after dose reduction for concomitant azole treatment. Continuous VEN dosing may result in long lasting cytopenias, which may require dose interruptions. Including patients treated with off-label VEN in a registry can help us to better understand this novel treatment.

PB1736

OUTPATIENT-BASED HIGH DOSE CYTARABINE FOR PATIENTS WITH ACUTE MYELOID LEUKEMIA: SAFE, FEASIBLE AND COST EFFECTIVE APPROACH

P. Kaloyannidis*, E. Shaibani, F. Aburahma, A. Ferrer, J. Abiera, S. Kanfar, O. Abduljalil, K. Bakhit, A. Suhebeh, R. Khalili, A. Abualruz, O. Qariesh, J. Apostolidis, K. Al Anezi, H. Al Hashmi

Adult Hematology and Stem Cell Translantation Department, King Fahad Specialist Hospital, Dammam, Saudi Arabia

Background: Effective treatment of acute myeloid leukemia (AML) includes the induction remission therapy which is followed by consolidation chemotherapy. Regimens for consolidation are as intensive as induction therapy, and in the most centers are usually given in an inpatient basis, for 2-3 cycles, lasting approximately for a total of 3–4 months. Given the practical considerations such as healthcare costs and limited inpatient resources and the current advantages in the supportive care post chemotherapy, the administration of consolidation treatment in an outpatient basis emerges as an appealing approach.
Aims: In this study we evaluated the feasibility and safety of high dose Cytarabine (HiDAC) as consolidation treatment in an outpatients basis
Methods: Sixteen patients of a median age 48,5 (17-64) years who met the eligibility criteria (good compliance, general health status WHO: 0-1, no severe preceding or residual infections, timely reach the hospital services) received in a total 20 cycles of HiDAC (2g/m^2 intravenously over 3 hours infusion, twice daily for 3 days); 4 patient received 2 cycles of HiDAC. All patients received orally prophylaxis against bacterial, viral and fungal infection started from the 1st day of chemotherapy infusion till blood-counts recovery. GCSF was not given routinely for neutrophils recovery. Chemotherapy and supportive care were given in an allocated room in our department. Patients and their relatives had been previously informed in details regarding the consequences and the potential risks of chemotherapy. Patients were closely monitored (every 1-2 days) in the outpatient clinic
Results: All the 20 cycles successfully completed and no severe side effects occurred during chemotherapy infusion; patients mainly complained for nausea and mild vomiting which fully controlled with the appropriate treatment. The median day for neutrophils (>500/mm^3) and platelets (>20000/mm^3) recovery were +21 (19-24) and +23 (19-26) after HiDAC treatment. Only 3 /16 patients were admitted for a total of 13 days (5, 5 and 3 days respectively), for neutropenic fever of unknown origin. All cases promptly responded to the treatment and no patient admitted to intensive care unit.
Summary/Conclusion: Our study demonstrated that HiDAC can be safely given in an outpatient basis, provided that the aforementioned eligibility criteria are met. The extremely short hospitalization period (13 days for the total of 20 HiDAC cycles) resulted in lower exposure to nosocomial pathogens, saving also significant inpatients beds. Keeping also in mind that the inpatient administration of HiDAc usually requires at least 20 hospitalization-days per cycle, we can easily conclude that the outpatient approach of HiDAC chemotherapy offers also significant cost effectiveness.

PB1737

DOES THE ICU ADMISSION OF THE *de novo* AML PATIENTS DURING REMISSION INDUCTION HAVE INFLUENCE ON THE LONG-TERM OUTCOME?

A. Bazhenov*, G. Galstyan, E. Parovichnikova, V. Troitskaya, M. Drokov, V. Savchenko

National Research Center for Hematology, Moscow, Russian Federation

Background: Treatment of acute myeloid leukemia (AML) is associated with high rate of the life-threating complications requiring intensive care unit (ICU) admission. The long-term outcomes of AML pts discharged from ICU are widely discussable and generally unknown.
Aims: To compare the overall and the disease free survival in *de novo* AML pts who did not require and those pts who required admission to ICU during remission induction.
Methods: All *de novo* AML pts, younger than 60 yo, who were treated in NRC from 2013 to 2015 yy, were enrolled into the study evaluating the impact of ICU admission on the long-term outcomes. Median age was-34 yo (20-60 yo). Patients had advanced disease: LDH activity median 767u/l (263-5152u/l); 76% of *de novo* AML pts had ECOG score 3-4 [1]. Patients were divided into 2 groups: 1st (n=24)-pts who were required ICU admission during induction chemotherapy due to life-threating complications and emergency events and 2nd (n=33)-pts who did not require ICU admission

during induction chemotherapy. 3 years overall survival (OS) and disease free survival (DFS) were assessed by the Kaplan-Meier method, log rank value $p<0.05$ consider as significant. Univariate analysis was performed with $\chi 2$ tests or Fisher's exact tests for categorical variables to find an independent ICU mortality predictor. All calculations and graphics were performed on IBM SPSS Statistics.

Results: 42% (24 of 57 pts) were admitted to the ICU due to life-threating complications and emergency events during induction chemotherapy. All *de novo* AML pts admitted to ICU were characterized by very advanced disease: LDH activity median 1056 u/l; 25% pts with WBC more than $100x10^9$/l; 35% pts of adverse cytogenetic group,-in comparison with pts who did not require admission to ICU: LDH activity median 689 u/l; 15,8% pts with WBC more than $100x10^9$/l; 18% pts of adverse cytogenetic risk. Reasons for ICU admissions were the following: acute respiratory failure (ARF) - 50%; acute neurological event-19,2%; septic shock (SS)-11,5%; urgent caesarian section-11,5%; arrhythmia-7,8%. ICU survival rate was 75%. 5 pts died due to the SS and 1 due to the ARF. Need for mechanical ventilation, need for vasopressors, 2 and more organ dysfunction were independent significant predictors of ICU mortality ($p<0.05$). A landmark analysis for OS was performed for patients who survived the first 30 days of treatment (1st induction chemotherapy cycle). OS and DFS were the similar in 1st and 2nd groups; p (log rank) =0.946 and p (log rank)=0.339 n.s. (picture 1 a, b).

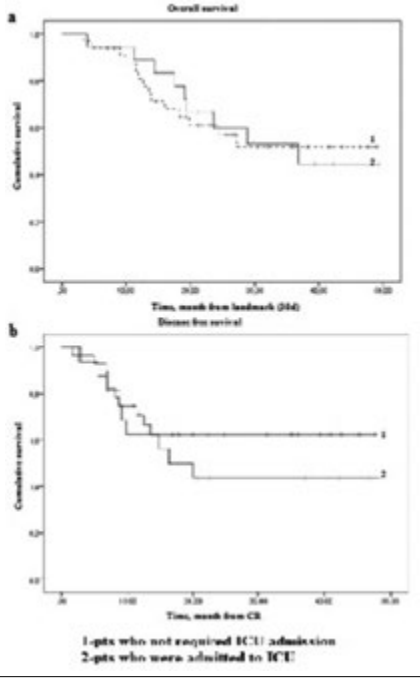

Figure 1.

Summary/Conclusion: There is no significant difference between 2 groups (ICU requires and non-requires) in OS and DFS. The successfully treated life-threating complications had no influence on the long-term outcomes.

Reference

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PB1738

REFRACTORY/RELAPSED ACUTE MYELOID LEUKEMIA PATIENTS WITH HIGH CD117 EXPRESSION BUT WITHOUT FLT3-ITD MUTATION COULD BE A SPECIAL GROUP WITH GOOD RESPONSES TO SORAFENIB TREATMENT ALONE

J. Xu[1,*], B. Wu[2], X. Liu[3], Y. Zhong Yuxia[4]

[1]Hematology Department, Zhujiang Hospital, Southern Medical University, Guangzhou, [2]Hematology Department, Shunde Hospital of Southern Medical Universtiy, Shunde, [3]Hematology Department, the Trusted Hospital of Panyu, Panyu district, Guangzhou, [4]Clinical Laboratory Department, Zhujiang Hospital, Southern Medical University, Guangzhou, China

Background: There are great unmet needs to explore more efficient and low-cytotoxic treatment for refractory or relapsed acute myeloid leukemia (R/R AML) patients. CD117 high expression was reported to be related with higher relapse of AML. But its inhibitors, such as imatinib or dasatinib, were not reported to successfully treat R/R AML by now. Because sorafenib targets multiple tyrosine kinases including CD117, and it has been reported to successfully treat R/R AML patients with FLT3/ITD mutation as a single agent. Here we retrospectively investigated the outcomes of seven R/R patients with high CD117 expression but without FLT3/ITD and C-kit mutation treated with single sorafenib.

Aims: The aims of this study is to see if sorafenib treatment alone could benefit this special group of patients with high CD117 expression but without FLT3/ITD and C-kit mutation.

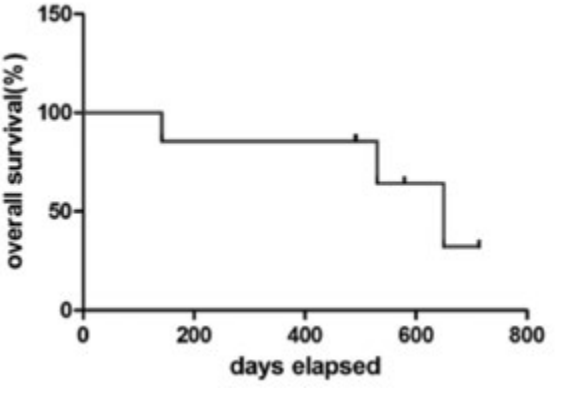


Figure 1.

Methods: Seven R/R AML patients without FLT3-ITD or C-kit mutations who were treated by single sorafenib, 0.4 gram twice a day, were sequentially enrolled in our center from May 2015 to August 2016. CD117 were positively expressed on more than 60% of bone marrow blast cells. Diagnosis was made according to the category criterion of WHO 2008. The risk status assessments and standards of patients' responses were referred to the NCCN guideline. Side effect grade was named according to common terminology criteria for adverse events v 4.0 by US National Institutes of Health. The outcomes of patients were followed up till September 2017.

Results: Four of the 7 patients achieved CR or CRi with single sorafenib. The time required to remission ranged from 31 to 100 days variably. In other three NR patients, two could also have a temporary decrease of bone marrow blast cells in the first week, but all had increment of blast cells in the latter two to three weeks and prescription of sorafenib went to cease. During sorafenib induction, all patients experienced grade 3 febrile neutropenia but had no unendurable infections. One patient developed grade 3 palmar-plantar erythrodysesthesia at the day 54. But it tapered in one week after stopping the drug. The median follow-up for the whole cohort have been more than 17 months. Three patients passed away for relapse of AML and their event free survival with sorafenib ranged from 2 to 20 months. All four patients who accepted stem cell transplantation were still alive at the end of follow-up no matter they responded to sorafinib or not. The median survival time initiating from sorafenib usage has been 650 days.

Summary/Conclusion: The preliminary study on single sorafenib treatment responses of R/R AML with high CD117 expression and no FLT3-ITD mutation gave an encouraging result. Sorafinib, at least as a bridge to transplantation, should be considered in such special R/R AML patients in further study.

PB1739

DO LEUKAPHERESIS IMPROVE EARLY DEATH RATES IN ACUTE MYELOID LEUKEMIA PATIENTS WITH HYPERLEUKOCYTOSIS?

O. Ilhan*, G. Cengiz Seval, A. Uslu, S. Civriz Bozdag, S.K. Toprak, M. Kurt Yuksel, P. Topcuoglu, O. Arslan, M. Ozcan, T. Demirer, H. Akan, M. Beksac, G. Gurman

Hematology, Ankara University School of Medicine, Ankara, Turkey

Background: Hyperleukocytosis (HL) is defined as the clinical condition when the white blood cell (WBC) count is above 100 000/mm^3 in peripheral blood and the reported incidence of HL is between 5 and 20% in acute myeloid leukemia (AML). The effect of leukapheresis on early mortality data is scarce.

Aims: The aim of this study was to investigate the effect of leukapheresis on early mortality of AML patients with HL
Methods: From January 2005 through October 2017, data from 70 patients with AML who were eligible for leukapheresis were evaluated. All these data were obtained from the Ankara University Faculty of Medicine Center for Therapeutic Apheresis and written informed consent was signed according to our institution regulations. All leukapheresis procedures were performed according to the institutional standard operating procedures after informed consent. Leukapheresis was performed with a continuous-flow blood cell separator (COBE Spectra; TerumoBCT, software version 7.0) via central venous access. The leukapheresis procedures were continued on a daily basis until clinical improvement was determined. Early mortality was defined as death within the first 15 days of leukapheresis.

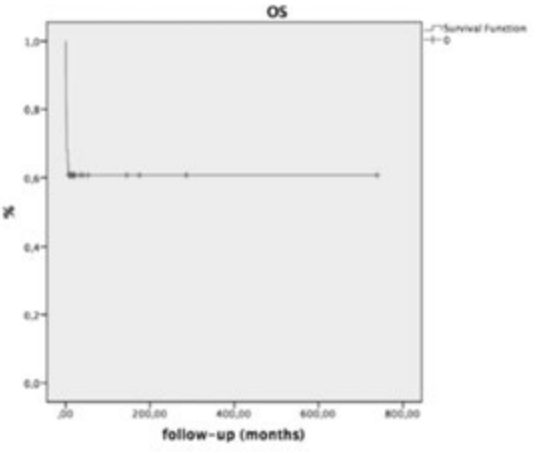


Figure 1.

Results: The study cohort consisted of 70 (36 male/33 female) newly diagnosed AML patients who had presented with HL and/or symptoms of leukostasis and underwent leukapheresis. The median age was 52 years (range, 4–86 years). The median WBC counts at diagnosis was 179.2x10^9/L (range, 56.5-558.0x10^9/L). The majority of patients, 88.6% (n=62) had WBC count ≥100x10^9/L. The majority of patients had symptoms of pulmonary leukostasis. A total of 140 leukapheresis cycles were performed among the 70 AML patients. The median number of leukapheresis cycles was 2 (range, 1–7). The median initial WBC was 179.2×10^9/L, which reduced to 121.7x10^9/L after the first leukapheresis. Eleven of the seventy patients had died by the time of analysis. Seven patients (10%) died within two weeks after leukapheresis commenced. Among the 7 patients, one patient was treated with induction chemotherapy and the remaining received palliative and supportive care. The main cause of early death was respiratory failure. The mean overall survival for all patients was 112±17 months (95% CI 78–145 months) (Figure). The median overall survival for patients who achieved complete all-cause 2-week mortality rate was 10% (7/70 patients) and the all-cause 4-week mortality rate was 12.8% (9/70 patients).
Summary/Conclusion: Leukapheresis is effective and safe procedure in reducing the peripheral blood leukocytes and leukemia blasts. Furthermore, high initial response rates in a subgroup of newly diagnosed AML patients fit to receive intensive chemotherapy suggest that leukapheresis could be beneficial in reducing the complications associated with hyperleukocytosis until systemic intensive chemotherapy commences.

PB1740

DETECTION OF PML-RARA IN APL: COMPARING FISH, PCR AND CONVENTIONAL KARYOTYPING

M. Pereira[1,*], G. Marques[2], R. Reis[2], A. Sousa[2], S. Pereira[2], N. Jerónimo[2], R. Tomé[2], F. Martins[2], L. Jorge[2], L. Ribeiro[1], F. Rodrigues[2]

[1]Clinical Hematology Department, [2]Clinical Pathology Department, Coimbra University Hospital, Coimbra, Portugal

Background: "Acute promyelocytic leukemia (APL) with PML-RARA" (WHO, 2016) is characterized by the presence of the aforementioned fusion gene, classically secondary to the t(15;17)(q24.1;q21.2) translocation, although cases of variant or cryptic translocations have been described. The presence of the translocation and its fusion product can be identified by conventional karyotyping (CK), interphase FISH analysis using adequate probes, and molecular biology (MolB) through polymerase chain reaction (PCR), three techniques with different sensitivities, advantages, disadvantages and limitations, that have been well described in the literature.
Aims: We aim to compare the outcome of the simultaneous application of the three techniques in a real-world setting.
Methods: We reviewed all peripheral blood (PB) or bone marrow aspirate (BM) samples that were analysed in our lab over a five-year period (from January 1st 2013 to February 14th 2018) for a suspected diagnosis of APL at presentation or relapse, and that were submitted for near simultaneous testing using at least two of the three techniques. Samples that were submitted for analysis but that were not processed due to a limitation of the sample (such as insufficient volume), were counted as not analysed. Results were classified as "positive" or "negative" for the translocation. Abnormal karyotypes that nevertheless did not reveal the classical t(15;17) translocation or one of its variants were considered "negative" in this context.
Results: Over the timeframe under consideration, 55 patients underwent a combination of two or more of the techniques at diagnosis or suspected relapse: 87.3% had FISH analysis (14.6% in a full unsorted mixed-cellularity BM or PB sample, and 85.4% in fluorescence-activated cell sorting (FACS)-separated APL blast cells); 65.4% had MolB testing; and 67.3% underwent conventional karyotyping. Overall, 22.9% of patients had all three tests, 37.5% had FISH + MolB and 39.6% had FISH + CK. Only two samples were negative for all three tests. All MolB and CK testing results were fully concordant. Likewise, all negative MolB results were also negative by FISH; however, 11.1% of positive MolB results were negative by FISH, k=(-1.0), p=1.00; half of these samples were submitted for conventional karyotyping and were positive for the translocation. All samples with a normal karyotype had a negative FISH test; all abnormal karyotypes without a t(15;17) translocation were negative by FISH; however, 12.5% of abnormal karyotypes with a detectable t(15;17) translocation were also negative by FISH (and positive on MolB), k=(-0.3), p=0.88. In all the above discordant cases, both morphology and cellular immunophenotyping by flow cytometry were not diagnostic for APL, and FISH probe hybridization had been performed on FACS-sorted cells; all cases were suspected relapses.
Summary/Conclusion: In our cohort, molecular biology and conventional karyotyping results were fully concordant, with a lower rate of detection for FISH analysis, where approximately 10% positive samples by MolB and CK were undetected, always in the context of a low disease burden, as evidenced by a normal BM smear and immunophenotype, and in a suspected relapse. FACS-separation of blast cells, especially when the immunophenotype failed to identify these cells as pathologic APL blasts, does not appear to be of benefit and may, in fact, have hindered the detection of the fusion gene by FISH.

PB1741

AZACITIDINE OR DECITABINE FRONTLINE THERAPY FOR ACUTE MYELOID LEUKEMIA IN ELDERLY PATIENTS?

S. Imbergamo*, A. Visentin, I. Kostic, S. Pravato, E. Scomazzon, M. Laurino, G. Binotto, F. Lessi, T. Berno, C. Gurrieri, G. Semenzato, L. Trentin

Department of Medicine, Hematology and Clinical Immunology Unit, Padua University School of Medicine, Padova, Italy

Background: Acute myeloid leukemia (AML) is a rare aggressive hematologic disease that occurs most often in the elderly and treatments for these patients are limited, particularly in those with poor performance status (PS) and comorbidities. Poor prognosis of elderly AML patients is due to several factors, including comorbidities, decreased organ function, poor performance status and a higher incidence of adverse karyotype. Although intensive chemotherapy can bring a high rate of complete remission (CR) in elderly AML patients, the toxicity and fatal side effects limit its extensive application in elderly unfit patients. Recent updated on AML treatment recommendations include hypomethylating agents 5-azacytidine (AZA) and decitabine.
Aims: The aims of this study were the comparison the two hypomethylating agents and the evaluation of the overall survival (OS) of Azacitidine and Decitabine in patients with AML, not eligible for intensive chemotherapy.
Methods: A retrospective analysis was performed on 64 patients with AML, 39 treated with Azacitidine and 25 treated with Decitabine, followed at Padua University Hospital from April 2012 to February 2018. The diagnosis was made according to 2016 WHO criteria. Thirty-nine patients received s.c. 5-Azacitidine 75mg/m^2 for 7 days every 4 weeks until disease progression, and twenty-five patients received e.v. Decitabine 20mg/m^2 for 5 days every 4 weeks until disease progression.
Results: The median age at diagnosis was 70 years (range 49-83). Eighteen patients (46%) were considered at high, 19 (48%) intermediate and 2 (6%) low-cytogenetic risk in the AZA group, while twelve (80%) patients were

considered at high, 3 (12%) intermediate and 2 (8%) low-cytogenetic risk in the Decitabine group. The complete remission was reached in 37% patients in AZA group, and in 30% in Decitabine group. The median OS for the AZA cohort was 10.7 months, while the median OS for the Decitabine cohort was 6.9 months, without any difference (p=0.9980). The median PFS for the AZA cohort was 4.4 months, while 11 months for Decitabine cohort, without any difference (p=0.2469). In univariate analysis, the variable associated with increased OS was adverse cytogenetic-risk (p=0.0367) in AZA cohort, not reaching in Decitabine cohort. No significant differences comparing the two treatments in patients with and without unfavorable cytogenetics-risk were found. Myelosuppression was the most common toxicity observed in decitabine treated patients. Infection-related complications occurred in 35 patients, 21 (54%) patients in azacitidine cohort and 18 (72%) patients in decitabine cohort. Pneumonia (62%) and sepsis (9%) were the most frequent infectious complications. These latters may occur during any cycle of therapy.
Summary/Conclusion: In conclusion, in our study there were no significant differences between azacitidine and decitabine. These agents are an effective and well-tolerated therapeutic alternative with acceptable side effects in elderly AML patients.

PB1742

THE ROLE OF MUTATIONS OF THE GENE FLT3 IN ACUTE MYELOID LEUKEMIA: THE EFFECT ON THE COURSE OF THE DISEASE AND THE RESULTS OF THE THERAPY

A. Radzhabova[1,*], S. Voloshin[1], I. Martynkevich[2], A. Kuzyaeva[3], V. Shuvaev[3], E. Motyko[3], L. Polushkina[3], M. Buckeye[3], N. Potikhonova[3], A.R.S. Kudryashova[3], V. Balashova[3], J. Chubukina[3], O. Uspenskaya[4], A. Chechetkin[3], M. Zenina[3]

[1]Russian Scientific and Research Institute of Hematology and Transfusiology of Federal Medical Biological Agency, Federal State Budget Institution, [2]Federal State Budget Institution, Russian Scientific and Research Institute of Hematology and Transfusiology of Federal Medical Biological Agency, [3]Federal State Budget Institution "Russian Scientific and Research Institute of Hematology and Transfusiology of Federal Medical Biological Agency", Federal State Budget Institution "Russian Scientific and Research Institute of Hematology and Transfusiology of Federal Medical Biological Agency", [4]Leningrad regional clinical hospital, Saint-Petersburg, Leningrad regional clinical hospital, Saint-Petersburg, St-Petersburg, Russian Federation

Background: Acute myeloid leukemia (AML) is an aggressive disease with extremely poor prognosis. Radical treatment like conventional chemotherapy (CT) +/– allogenic hematopoietic stem cell transportation (allo-HSCT), is rarely considered in elderly patients due to age, comorbidities and poor somatic state. The presence of mutations of the *FLT3* gene (*ITD* or *TKD*) in AML is recognized as an adverse prognosis affecting the course of the disease, the development of relapses with decrease in overall survival (OS) and disease-free survival (DFS) of patients. Despite the high incidence of mutations *FLT3-ITD* and *FLT3-TKD*, the role of the allelic load of the mutant gene *FLT3* has not been completely identified. Up to date we have no clinical studies with treatment individualized to elderly AML patients. The introduction of target therapy in AML gives hope for diminishing treatment side effects ang prolongation of life for elderly frail patients with AML.
Aims: To assess the frequency of *FLT3* gene mutations and their influence on clinical manifestations, relapse rate, OS and DFS of patients with AML.
Methods: A retrospective study of the incidence of *FLT3* gene mutation in blood or bone marrow and clinical outcomes in 199 AML patients (83 males) was conducted. Median age at the time of diagnosis was 52 years (20-86 years). The polymerase chain reaction (PCR) method with further restriction and determination of 2 main mutation types-internal tandem duplication (*FLT3-ITD*) and point mutation in "A-loop" (*FLT3-TKD*) was used to evaluate the *FLT3* status.
Results: The incidences of *FLT3* gene mutations were as follows: *FLT3-ITD*-22,6% (45/199), *FLT3-TKD*-5,5% (11/199), *FLT3-ITD* and *FLT3–TKD* in combination-1,0% (2/199); the rest-70.8% (141/199) patients did not have any mutation in the FLT3 gene (FLT3–). Complete blood counts have statistical significant differences in the white blood cells (p=0.0009) and the blast percent (p=0.01) between FLT3(-) and FLT3(+) patients. Chromosomal abnormalities were revealed in 38% (17/45) patients of *FLT3-ITD+* group, 64% (7/11)-*FLT3-TKD+* and in51% (72/141) of *FLT3–* cases. Both *FLT3-ITD/TKD+* patients had normal karyotype,. No significant (p=0.2990) differences have been yielded in the duration of OS between groups of patients with normal karyotype and chromosomal aberrations, regardless of the presence or absence *FLT3* gene mutations. The presence of *FLT3-ITD+* mutation was associated with higher risk of AML relapse (p=0.00006) than *FLT3-TKD+* or *FLT3(-)* patients. All patients received various CT regimens depending on age, performance status and comorbidity (7+3, 5+2, HAM). We observed significant (p=0.00024) differences in the OS between *FLT3*-ITD+, *FLT3-TKD+* and *FLT3–* patients. Median OS were: 5.1 months for *FLT3-ITD+*; 7.1 months for *FLT3-TKD+* and 13.0 months for *FLT3–* patients. Univariate analysis showed the next adverse factors for OS: any *FLT3* gene mutation (p=0.00007) and fail to achieve remission (p=0.000001). Factors influencing DFS were as following: karyotype at disease onset (favorable, intermediate and unfavorable) (p=0.013), the presence of *FLT3* mutations (p=0.0000013) and type of mutation (p=0.0001).
Summary/Conclusion: *FLT3* mutations in AML patients give adverse prognosis for OS and DFS. There is urgent need to have molecular genetic markers screening in the AML workup at initial diagnosis and risk-adapted therapy choice.

PB1743

OUTCOME OF ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA IN A SINGLE INSTITUTION

A. García-Noblejas*, E. Jiménez-Barral, A. Arriero, J. Cannata-Ortiz, A. Figuera, R. De La Cámara

Hematology, Hospital La Princesa, Madrid, Spain

Background: In order to cure Acute Myeloid Leukemia (AML) intensive chemotherapy +/- Stem Cell Transplant (SCT) is require. Nevertheless, the median age of these patients is around 70 years. Elderly patients, defined in the AML literature as aged ≥60 years, historically have lower complete remission (CR) and relapse-free survival (RFS) rates than their younger counterparts and, in practice, only a minority of them can receive intensive treatments. For the majority of patients, the options are demethylating agents or supportive care.
Aims: To analyze the outcome of AML patients ≥60 years old according to the different therapeutic approach in a single institution. ts, defined in the AML literature as aged ≥60 years, historically have lower complete remission (CR) and relapse-free survival (RFS) rates than their younger counterparts and, in practice, only a minority of them can receive intensive treatments. For the majority of patients, the options are demethylating agents or supportive care.
Methods: Unicentric, retrospective analysis of AML patients ≥60 years old, not treated previously and diagnosed from 2011 to 2016. Statistical analysis was performed using SPSS v.15.0.

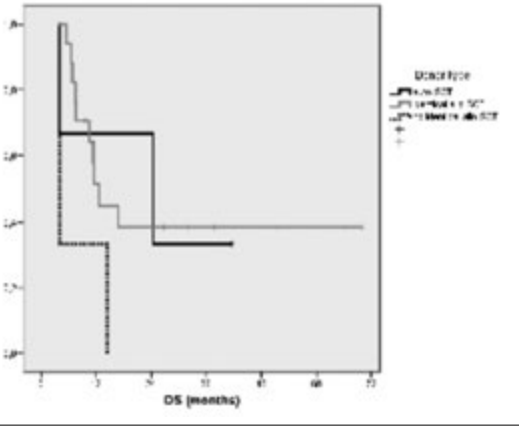


Figure 1.

Results: Patients' characteristics: Sixty-nine patients were registered: 51% male, median age 69 years old (range 60-90), 87% ECOG ≤1, 52% *de novo* and 48% secondary, and 22% with monosomal or complex karyotype. 49 patients (71%) received induction chemotherapy, 6 patients (9%) demethylating agents, only one patient (1%) received chemotherapy plus demethylating agents and 13 (19%) supportive care. Among patients treated with chemotherapy (n=49), 23 patients (47%) received consolidation with SCT (19 allogenic and 4 autologous) and 13 (26%) received demethylating maintenance. **Survival:** Median follow-up for alive patients was 42 months (range 4-81 months). Median overall survival (OS) from diagnosis according to the initial treatment was: 17 months (CI95% 10-23) for patients treated with chemotherapy (n= 49), 9 months for patients treated with demethylating agents (n=6) (CI95% 0-25), 8 months for the only patient who received chemotherapy plus demethylating agents and less than 1 month for those that received supportive care (n=13) (p<0.001). For patients treated in induction with chemotherapy, neither age (60-69 *vs* 70-79), type AML (de novo

vs secondary), FLT-3 mutation, or SCT as consolidation (allogenic or autologous) have an impact in overall survival. Nevertheless, no complex/monosomic karyotype (p=0.01) and receiving demethylating maintenance (p=0.02) were associated with a better outcome. Patients treated in induction with chemotherapy plus maintenance with demethylating agents compared with those that received SCT, were older (p<0.001) but showed no differences in the main characteristics of their leukemia (secondary, karyotype and FLT3). Median overall survival in transplanted patients (n=23) was as follows: 24 months for auto-SCT (n=3), 12 months in HLA-identical related/unrelated SCT (n=17) and 4 months in haploidentical or mismatched HLA unrelated SCT (n=3) (p=0.1). Both auto-SCT and identical allo-SCT observed plateau in survival graphics (**Figure 1**).
Summary/Conclusion: Our study shows the outcome of 69 older patients with AML in our current practical clinical. If patients are fit induction chemotherapy seems to be the best option to achieve longer outcomes but the post-induction isn't clear and have to be personalised.

PB1744

REAL LIFE SINGLE CENTER EXPERIENCE WITH TREATMENT REGIMENS IN ADULT PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA-EARLY DEATH RATES (2002-2018)

I. Oravcova[1,2,*], E. Mikuskova[1], L. Demitrovicova[1], S. Cingelova[1], V. Mikudova[1], S. Kevicka[1], A. Slobodova[1], A. Mlcakova[3], J. Gyarfas[3], M. Mego[1,2], L. Drgona[1,2]

[1]Oncohematology, National cancer Institute, Bratislava, Slovakia, [2]Oncohematology, Faculty of Medicine Comenius University, [3]Laboratory Hematology, National cancer Institute, Bratislava, Slovakia, Bratislava, Slovakia

Background: An integral part of the initial treatment of acute promyelocytic leukemia (APL) is a supportive treatment of hemorrhagic diathesis. Early death (ED) either before or during induction treatment remains the most frequent cause of failure in the treatment of APL. The induction death rate within the first month of ATRA treatment ranged between 5%–10% in clinical trials, however in real-life non-selected patients the ED rate ranged between 17%–30%. The most frequent reason of ED is fatal intracranial (57%–62%) or alveolar hemorrhage or both.
Aims: Some adverse prognostic factors are known being in correlation with ED and some are still being studied. We analyzed biologic and laboratory characteristics of patients with APL in relation to ED.
Methods: Eligible patients were adults, at least 18 years of age with *de novo* APL. Risk group was set according to Sanz *et al.* predicting model. ED was the death from any cause during induction. Patients were treated progressively over the years by the Spanish treatment protocol (LPA96, LPA99, LPA2005) and from the June 2017 by the Intergroup Study Protocol (GIMEMA + DSIL, APL 0406), which means that the induction treatment till June 2017 consists of ATRA and anthracycline for all risk groups and thereafter consists of ATRA and ATO for low and intermediate risk group, while high risk group continues the induction ATRA + anthracycline + cytarabine. All patients were analyzed for: sex, age, leukocytes, thrombocytes, blasts in peripheral blood (PB) and bone marrow (BM), lactate dehydrogenase (LDH), HLA-DR and CD56 expression, fibrinogen, PT and APTT, additional chromosomal abnormalities and PML/RARA type of transcript in relation to ED. For survival evaluation were used log-rank test (Kaplan-Meier) and Fisher exact test was used for early death risk factor analysis.
Results: 24 patients with APL were diagnosed and treated from 10/2002 to 1/2018. Two patients died till 12 hours after admission to the hospital (46 years old and 20 years old woman with initial clinical signs of intracerebral bleeding at the time of initial investigation, both started treatment of APL) and they were excluded from the survival assessment. We observed totally six ED (25%), representing 66.7% of total APL deaths. Five (25%) of them due to hemorrhagic event, four (16.7%) patients had intracerebral hemorrhage and 1 patient had intrapulmonary hemorrhage. One patient died of tumor lysis syndrome. 17 (70.8%) patients achieved first complete molecular remission, two patients died in relapse and one woman had primary myelosarcoma of the breasts, APL subtype, she didn t achieved complete remission and refused any treatment after induction as well and died. At median time of follow-up 46 month (0.16–159 months) the estimated 3-year OS and RFS were 77.0% CI 95% (59.3%–94.7%) and 71.9% CI 95% (52.7%–91.1%) respectively. In univariate analysis the prognostic factor that most influenced survival were the number of leukocytes ≥$10x10^9$/l (p=0.018), blasts in PB ≥70% (p=0.007), PT-INR >1.5 (p=0.001), APTT ≥35s (p=0.014). Risk factors associated with early death were: Le ≥$10x10^9$/l (p=0.015), blasts in PB ≥70% (p=0.001), PT-INR >1.5 (p=0.001), LDH >4μkat/l (p=0.002) and presence of bcr3 transcript (p=0.033).
Summary/Conclusion: Hemorrhagic events are the main cause of ED. Quick transport to hematologic center treating APL, recognizing high risk patients and supportive treatment are crucial for favorable outcome. Outcome of APL patients in our center is comparable to the results reported as real life data or from cancer registries.

PB1745

EARLY MORTALITY IN ACUTE MYELOID LEUKEMIA WITH HYPERLEUKOCYTOSIS

A. Moicean*, R. Billström

Hematology, Dept of Medicine M[1], Skaraborgs Hospital, Skövde, Sweden

Background: Age and cytogenetic/mutational leukemia cell profile are the most important determining factors for long-term survival after therapy in acute myeloid leukemia (AML), whereas the adverse factor elevated white blood cell count (WBC) at diagnosis is often linked to high early mortality.
Aims: Assessment of early mortality
Methods: In a community hospital covering a population of 255000, 67 out of 116 adult patients with newly diagnosed non-APL AML received combination chemotherapy aiming at complete remission (CR) during the time period 2007-2016. We assessed 30-day mortality in the subgroups with WBC 51-100x10E9/l (N=5, 8%) and >100x10E9/l (N=7, 10%, range 126-402x10E9/l), respectively, at diagnosis.
Results: No early deaths were observed in the WBC 50-100 group. In contrast, 2 out of 7 with WBC >100 died day 16 (WBC 126, liver failure) and day 18 (WBC 312, septicemia), respectively. Remarkably, two of the 7 patients with WBC>100 including a 28-year old male with a spectacular WBC 402x10E9/l, are among the long term survivors at 42+ mo and 90+ mo.
Summary/Conclusion: Leukocytosis >50,000 is not defining for early mortality (in the first 30 days) in non-APL AML. Extreme leukocytosis (WBC >100) does not affect survival if applied with a therapeutic strategy with a curating but protective purpose to avoid acute complications of treatment (such as leukostasis, progressive disseminated intravascular coagulation or acute tumor lysis syndrome). We discuss strategies to avoid complications such as the late insertion of the central venous catheter or another cytostatic sequence during induction for AML with extreme leukocytosis. This strategy replaces leukopheresis that was previously recommended for these cases and allows for an effective and rapid reduction in leukemic tumor mass.

PB1746

RARE REPORT OF PROMYELOCYTIC SARCOMA AFTER RENAL TRANSPLANT

L.-J. Shen*, F.-Y. Chen, J. Hou, H.-H. Huang, X. Gao

Hematology, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

Background: Promyelocytic sarcoma (PS) following renal transplantation is an extremely rare phenomenon. Here we report a 26-year-old male suffered with PS at the site of transplanted ureter six months after renal transplant. Bone marrow aspiration and biopsy were normal, but Bcr1 subtype (intron 6) of the PML/RARα rearrangement gene was showed positive with 0.005 through common myeloid leukemia associated mutation genes and fusion genes screening. Chromosome was 46, XY. Flow cytometry detection of bone marrow cells had no special found. Then, fluorescence *in situ* hybridization (FISH) for PML/RARα rearrangement was performed on the ureteral tumor and it suggested positive at 15q22/17q21.
Aims: As the lesion seemed to be limited in the transplanted tissue, we sought to determine whether the tumor was a transmission of cancer from organ donor to the recipient.
Methods: In order to address this hypothesis, DNA was extracted from the excised ureteral mass and compared with DNA from the patient's blood. Just like as we suspected, DNA by short tandem repeat analysis revealed that ureteral mass was not of recipient origin. The young donor was died from cerebral hemorrhage. We proposed that she may be suffered from disseminated intravascular coagulation, which is the severe and common complication of acute promyelocytic leukemia.
Results: Chemotherapy (arsenic trioxide 0.15mg/kg/d on d1–28, and ATRA 40mg/d for 28 days) was administered to induce remission. Immunosuppression therapy was changed into rapamycin single agent. One month later, PML/RARα fusion gene from bone marrow sample was negative. Thereafter he received arsenic trioxide five days a week with 0.15mg/kg/d for 4 weeks every 8 weeks, and ATRA 40mg/d on d1-14 every 28 days for 28 weeks as the post-remission therapy recommended by Lo-Coco F published in New England Journal of Medicin. This patient maintains complete remission for nearly 2 years. Now his renal function sustains on a normal level and the

urinary tract is unobstructed. Till now, the other recipient of this deceased donor's kidney hasn't caught by tumor.

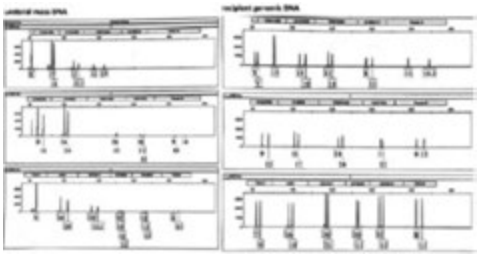


Figure 1.

Summary/Conclusion: Malignancy, as a major complication of renal transplant, accounts for 20% of the exitus of renal transplant patients every year and accounts for 30% of the death causes of the renal transplant recipients with a follow-up greater than 20 years. PS in the complete absence of bone marrow disease is an extremely rare phenomenon. To our knowledge, there had been only 2 patients presenting with promyelocytic sarcoma following renal transplant in the past three decades. They both suffered kidney failure again and subsequently had an allograft nephrectomy during the following chemotherapy. Management of recipients with *de novo* cancers after transplantation is complex and difficult. Recommendations for cancer screening in the renal transplant population (including donors and recipients) were mostly extrapolated from the general population. It's better to reduce the intensity of the combined chemotherapy and adjust immunosuppression therapy to protect the function of fragile allograft once *de novo* tumor occurred.

PB1747

INTENSIVE INDUCTION TREATMENT OF ACUTE MYELOID LEUKEMIA IN PATIENTS AGED ABOVE 60 IN SHATHD BETWEEN 1.01.2010 AND 1.01.2018, PROGNOSTIC FACTORS FOR INDUCTION MORTALITY

S. Lozenov[1,*], V. Stoeva[2], Y. Petrov[1], S. Mishkarova[1], Z. Popova[1], G. Arnaudov[1], B. Spassov[1]

[1]NSHATHD, [2]NSHATHD, Sofia, Bulgaria

Background: Acute myeloid leukaemia is an oncohaematological disease, with an incidence rate increasing with age. The patients aged above 60 years represent more than 60% of all cases, but the results from the treatment in this age group are worse and the therapeutic approaches available are more limited. With induction mortality rates with standard 7+3 based regimens reaching above 20% in some series one of the most difficult questions in this subset of patients is the proper assessment of the "fitness" of the patients.
Aims: We aimed to assess the therapeutic results from the intensive treatment of AML patients 60 years and older, concerning their overall survival and induction mortality rates and also to identify prognostic factors related to the induction mortality rates in the population of patients which was considered "fit" for intensive 7+3 based induction regimen.

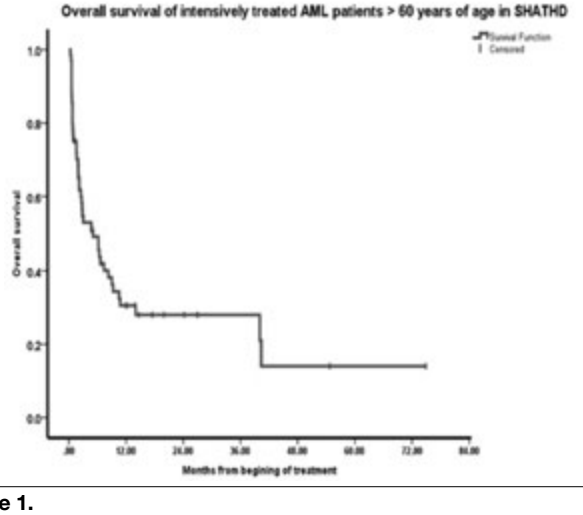


Figure 1.

Methods: 70 patients aged above 60 years with AML, diagnosed and treated intensively for AML in the period from 1.01.2010 to 1.01.2018 were evaluated in the SHATHD (Specialized hospital for active treatment of haematological diseases), with a median of the age of the analysed population of 66 +- 4.08. The treatment consisted of 7+3 based regimens including at least 5 days of administration of Ara-C at a dose above 100 mg per day and at least two administrations of an anthracicline at a standard dose (Epirubicin, Mitoxantron, Idarubicin). We evaluated the overall survival of the patient population, some of the conventional prognostic factors at diagnosis-serum albumin, creatinine, bilirubin, leukocyte count, platelet count, cytogenetic and molecular anomalities, percentage of blast infiltration in marrow, underlining MDS/MPP, some comorbidity indexes (Charlson Comorbidity Index, HCT-CI) and the levels of inductions mortality (IM) rate for the intensively treated patients, defined as mortality within 28 days from the commencing of treatment. The statistical methods used include the methods of Kaplan-Meier, Chi-Square, Fisher's exact test and Non-parametrical analyses between categorical and continuous variables conducted on the software product SPSS version 22.0.
Results: The OS within the study population is 28% at the second year and 14% at he fifth year. The level of the induction mortality as defined above stands at 21.42%. The analysis of the factors with prognostic significance for induction mortality in patients treated with 7+3 based regimens revealed strongly significant impact of platelet count on induction mortality (p 0.002), pretreatment levels of albumin and creatinine were also significantly affecting prognosis for induction mortality (p 0.028, p.0.030). The lassociation of AML with MDS/MPP also exposed the patients to a significantly higher risk of IM (p. 0.002), while the presence of cytogenetic or molecular aberration correlated with lower risk of IM (p 0.025). Interestingly in our studied population CCI and HCT-CI did not have statistically significant impact on IM.
Summary/Conclusion: The increase of the age for intensive treatment of elderly AML patients and the increasing transplant activity in these patients is a global trend now. In order to achieve significantly better results in terms of overall survival, new prognostic systems with a better potential for controlling the treatment related mortality and better selection of the "fitness" of patients for intensive treatment, are needed. This will probably require the development of expected IM risk calculators specific for the elderly patients with AML and their specific risk factors.

PB1748

IMPACT OF BONE MARROW ASPIRATE TREGS ON THE RESPONSE RATE OF YOUNGER NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA PATIENTS

M. Delia*, P. Carluccio, A. Mestice, C. Brunetti, F. Albano, G. Specchia

University of Bari-Bari, Italy, Hematology and Bone Marrow Transplantation Unit, Bari, Italy

Background: Acute myeloid leukemia (AML) is widely considered as a distinct clinical entity with a well-defined molecular and genetics-based prognosis. Particularly, in the younger patient the therapeutic approach depends largely on diagnostic risk stratification, which has an impact on the outcome after therapy.
Aims: We have added T regs evaluation to the usual molecular and cytogenetics profile in the AML younger patients diagnostic bone marrow aspirate (dBMA) in order to look for any correlation between Tregs and overall response (OR) as well as survival (OS) rates.
Methods: The study included 19 AML patients (12 males and 7 females, median age 57 years, range 20-65) all treated with "3+7" regimen. According to cytogenetic-molecular risk stratification: 2 (11%), 9 (47%) and 8 (42%) patients were assigned to the favorable, intermediate and adverse prognosis groups, respectively. Molecular evaluation (*i.e.* NPM; FLT3; CEBPA) was performed in all cases: NPM1 (A or B mutation) and FLT3 mutations (ITD or D835) were positive in 3 (16%) and 3 (16%) patients, respectively. There were no CEBPA positive cases. Median values of white blood cells (WBC) were 10400/uL and of dBMA Tregs 21/uL.
Results: OR (Complete remission (CR) + CR incomplete (CRi)) was documented in 7 of 19 patients (37%); there were two partial responderpatients. The optimal dBMA-Tregs cut-off value for predicting response to treatment (>21/uL) was obtained by ROC curve analysis. Apart from the expected impact of the molecular/cytogenetic group (p=0.05) and the NPM mutation (p=0.036), OR was also correlated with dBMA Tregs >21/uL (p=0.020). Furthermore, the same Tregs value seemed to correlate with a better median OS (21 *vs* 4 months, Log-Rank test, p=0.028).
Summary/Conclusion: Obviously, the possible prognostic value in terms of OR and survival rates of such an immunological player as BMA Tregs should be confirmed in larger patients numbers

PB1749

CORRELATION OF INTERLEUKIN-6, INTERLEUKIN-10, AND TRANSFORMING GROWTH FACTOR BETA SERUM LEVELS WITH THE RESPONSE TO INDUCTION CHEMOTHERAPY IN NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA PATIENTS

T. Salah[1,*], Z.A. Abdelhafez[2], M.A. Abdou[2], M.M. Salah Eldeen[2,] S.M. Mansour[3], S. Mabrouk[4]

[1]Clinical Oncology Department, [2]Clinical Pathology Department, Assiut faculty of medicine, Assiut university, [3]Clinical pathology department, South Egypt cancer institute, Assiut university, [4]medical oncology department, South Egypt cancer institute, Assiut university, assiut, Egypt

Background: Cytokines are involved in the pathogenesis of acute myeloid leukemia (AML) but their prognostic significance in these diseases is unknown. In this study, We assessed the association between changes in serum levels of interleukin-6, interleukin-10, and transforming growth factor beta cytokines baseline and after induction chemotherapy and the response to induction chemotherapy in patients with recently diagnosed previously untreated Acute Myeloid Leukemia (AML) patients.
Aims: To Assess the serum levels of IL-6, IL-10 and TGF beta in different types of newly diagnosed acute myeloid leukemia patients (FAB classifications) and the relationship between these cytokines and the laboratory findings and To determine the pre-and post-induction levels of these cytokines and their relationship to bone marrow blast cells.
Methods: This is a prospective case control study carried out on patients from Hematological Diseases Unit in internal Medicine Department, Assiut University Hospital and Medical Oncology Department, South Egypt Cancer Institute and clinical oncology and nuclear medicine department, Faculty of medicine, Assiut university. This study recruited 30 newly diagnosed patients with AML diagnosed by WHO classification and sub-classified (M1-M6) by FAB classification from February 2016 to May 2017. Mean age of the patients was 44 years (20-68). 30 patients (12 males and 18 females) as well as 20 age and sex matched healthy controls were also enrolled. Blood samples and estimation of serum levels of studied cytokines were collected from patients before induction and recollected again 10-14 days after induction and results were compared to its serum levels in 20 healthy control group (20 healthy individuals).The study was approved by the ethical committee of Faculty of Medicine, Assiut University. Informed Consents were obtained from the patients before enrollment in this study.
Results: Baseline serum levels of IL-6 and IL-10 levels were significantly higher in newly diagnosed AML patients than in control group (P-value=0.000*) suggesting their role in pathogenesis of the disease and their levels decreased when patients underwent remission (bone marrow blast cells count<5%),with significant decrease in both responders and non-responders for IL-6 but regarding IL-10 there was a significant decrease only in responders (P-value=0.012*) and insignificant decrease in non-responders (P-value=0.2) .On the other hand, baseline TGF-β level was significantly lower in newly diagnosed AML patients (before induction chemotherapy) than control group and its level significantly increased in patients responded to induction chemotherapy (P-value=0.012*) and insignificant increase in non-responders(P-value= 0.227).
Summary/Conclusion: Persistant Increased Serum level of IL-10 and persistant decreased TGF-β level may be used as a prognostic marker for AML indicating poor response to induction chemotherapy and unfavorable prognosis.

Aggressive Non-Hodgkin lymphoma – Clinical

PB1750

IS DA-EPOCH -/+ RITUXIMAB FOR AGGRESSIVE LYMPHOMA FEASIBLE AND EFFECTIVE IN CLINICAL PRACTICE? A RETROSPECTIVE STUDY

S. Matsuda[1,*], Y. Suehiro[2], N. Tomita[3], K. Izutsu[4], N. Fukuhara[5,] Y. Imaizumi[6], K. Shimada[7], T. Nakazato[8], I. Yoshida[9], T. Takahashi[1,] R. Suzuki[1], M. Yamaguchi[10], J. Suzumiya[1]

[1]Cancer center, Shimane University Hospital, Izumo, [2]Hematology, National Hospital Organization Kyushu Cancer Center, Fukuoka, [3]Hematology, St. Marianna University School of Medicine, Kawasaki, [4]Hematology, Toranomon Hospital, Tokyo, [5]Hematology, Tohoku University Hospital, Sendai, [6]Hematology, Nagasaki University Hospital, Nagasaki, [7]Hematology, Nagoya University Hospital, Nagoya, [8]Hematology, Yokohama Municipal Citizen's Hospital, Yokohama, [9]Hematologic Oncology, National Hospital Organization Shikoku Cancer Center, Matsuyama, [10]Hematology and Oncology, Mie University Graduate School of Medicine, Tsu, Japan

Background: CHOP is the standard chemotherapeutic regimen for aggressive lymphomas. CHOP with rituximab (R) therapy is the standard of care for newly diagnosed diffuse large B-cell lymphoma (DLBCL), but it is not sufficient for DLBCL with adverse prognostic factors. Excellent efficacy of dose-adjusted (DA)-EPOCH -/+ R therapy has been reported for primary mediastinal large B-cell lymphoma (med DL), Burkitt lymphoma, and peripheral T-cell lymphomas (PTCLs). A phase II study on DA-EPOCH-R/high-dose methotrexate therapy for untreated stage II-IV CD5+ DLBCL also demonstrated promising efficacy (Miyazaki *et al.* ASH2016). However, the feasibility and efficacy of DA-EPOCH -/+ R in clinical practice has not been fully evaluated.
Aims: To evaluate the feasibility and efficacy of DA-EPOCH -/+ R for aggressive lymphomas in clinical practice, we conducted a retrospective study.
Methods: We retrospectively analyzed clinical features and outcomes of 149 patients with aggressive lymphoma who received DA-EPOCH -/+ R between 2007 and 2015 in 17 institutes in Japan.
Results: The patients' characteristics were as follows: male, 55%; median age, 62 years (range, 17-87); >65 y.o, 44%; no prior treatment, 60%; DLBCL, 54%; ECOG PS >1, 35%; and stage III/IV, 78%. All patients were hospitalized, and a central venous catheter was used in 96% of patients. The maximum dose level of DA-EPOCH was<1 in 13% of patients, level 1 in 57%, level 2 in 11%, level 3 in 13%, and >3 in 7%. The median number of cycles was 4 (range, 1-8). There was no treatment-related death. Grade 3/4 febrile neutropenia, infection, and constipation were documented in 54%, 28%, and 6% of patients, respectively. In elderly patients (≥65 y.o), these were 60%, 32%, and 9%, respectively. Only constipation (all grades) and neutropenia (grade 3/4) occurred more frequently in elderly patients than in younger patients. Patients with newly diagnosed DLBCL-NOS (*n*=46) exhibited the following clinical features: median age: 65 years (range, 28-87); >65 y.o, 50%; male, 50%; ECOG PS >1, 46%; and stage III/IV, 83%; IPI high/high intermediate, 73%; CD5+, 49%. The median follow-up duration was 23.9 months (range, 2.2-79.9). CR and estimated 2-year OS rates were 80% and 81%, respectively. There was no difference in OS according to dose levels of DA-EPOCH-R. The estimated 2-year OS rate for med DL (*n*=14) was 100%.
Summary/Conclusion: These results suggest that DA-EPOCH -/+ R is feasible in clinical practice and safe for elderly patients. Promising efficacy of DA-EPOCH -/+ R for aggressive lymphomas was also observed in this study, warranting further evaluation.

PB1751

THE AVERAGE RELATIVE DOSE INTENSITY OF R-CHOP IS AN INDEPENDENT PROGNOSTIC FACTOR DETERMINING OVERALL SURVIVAL IN DIFFUSE LARGE B CELL LYMPHOMA PATIENTS

M. Długosz-Danecka[1,*], S. Szmit[2], T. Ogórka[1], K. Krawczyk[1], E. Łątka[1], A. Skotnicki[1], W. Jurczak[1]

[1]Department of Hematology, Jagiellonian University, Krakow, [2]Department of Pulmonary Circulation, Thromboembolic Diseases and Cardiology, Centre of Postgraduate Medical Education, European Health Centre, Otwock, Poland

Background: Prognosis of diffuse large B cell lymphoma (DLBCL) patients depends on lymphoma and patient-related risk factors, best summarized in

IPI (International Prognostic Index). The aim of the study was to determine whether the average relative dose intensity (ARDI) of anthracycline containing regimen could be an IPI-independent prognostic factor.T

Aims: The aim of the analysis was to assess the relationship between the calculated ARDI and PFS and OS in DLBCL patients treated with R-CHOP chemotherapy.

Methods: We analysed 223 white Caucasian DLBCL patients, who completed at least four cycles of first-line R-CHOP immunochemotherapy (Rituximab, Doxorubicin, Cyclophosphamide, Vincristine, Prednisone). ARDI was calculated by specially developed software, in each individual patient, simultaneously with chemotherapy prescription. It allowed to address instantly all revealed causes of decreased ARDI. Importance of ARDI for progression-free/overall survival (PFS/OS) was evaluated.

Results: ARDI was decreased due to prolonged interval between immunochemotherapy cycles caused by neutropenia and infections (absolute neutrophil count<1,0x10^9/l) in 49.32% (110/223) or reduction of cytostatic doses in 19.73% (44/223) patients mainly as the consequence of cardiotoxicity or neutropenia (85,18% and 14,81% respectively, estimating in the group of patients with reduced doses of cytostatics). Progression free and overall survival (PFS and OS) varied significantly for ARDI >90%, 89-80% and<80% respectively (p<0.00001). Multivariate analysis confirmed that ARDI>90% was an IPI independent predictor of prolonged PFS (Hazard Ratio (HR), Confidence Interval (CI): HR=0.31; 95%CI: 0.20-0.47; p<0.000001) and OS (HR=0.32; 95%CI: 0.21-0.48; p<0.000001). Even with real time ARDI analysis it was possible to maintain it above 90% in 161 of 223 patients (72%). Further improvement may be possible only after implementing primary neutropenia prophylaxis and primary cardioprotection.

Table 1. Favorable PFS and OS depending on high ARDI and low IPI (Cox proportional risk model).

Survival		ARDI >90%	low IPI (0 or 1)
multivariate analysis	PFS	HR=0.31 95%CI: 0.20 - 0.47 p<0.000001	HR=0.43 95%CI: 0.24 - 0.76 p=0.004
	OS	HR=0.32 95%CI: 0.21 - 0.48 p<0.000001	HR=0.48 95%CI: 0.27 - 0.85 p=0.01

Summary/Conclusion: DLBCL patients with ARDI >90% have significantly better outcome regardless of IPI, therefore we postulate to regard adequate dose density as an official recommendation.

PB1752

CLINICO-PATHOLOGICAL ANALYSIS OF LIMITED-STAGE DIFFUSE LARGE B-CELL LYMPHOMA RELAPSING MORE THAN FIVE YEARS AFTER INITIAL DIAGNOSIS: POSSIBLE MECHANISM OF LATE RELAPSE

T. Suzuki[1,*], S. Fukuhara[1], A. Maeshima Miyagi[2], J. Nomoto[1], K. Tajima[1], Y. Ito[1], S. Hatta[1], S. Yuda[1], S. Makita[1], W. Munakata[1], T. Suzuki[1], D. Maruyama[1], H. Taniguchi[2], K. Izutsu[1], K. Tobinai[1], Y. Kobayashi[1,3]

[1]Hematology, [2]Pathology, National cancer center hospital, [3]Hematology, International University of Health and Welfare, Mita Hospital, Tokyo, Japan

Background: A continued risk of relapse in limited-stage diffuse large B-cell lymphoma (DLBCL) has been reported (Stephens *et al. J Clin Oncol* 2016). However, the characteristics of patients (pts) who develop late relapse (LR) are not well known.

Aims: To investigate the clinico-pathological features of pts with DLBCL who develop LR and its possible mechanisms.

Methods: Of 339 pts with limited-stage (Ann Arbor stage I/II) *de novo* DLBCL diagnosed between 1997 and 2012, 21 pts who had achieved a complete response to initial therapy but relapsed after >5 years since the initial diagnosis (*i.e.* LR) underwent clinico-pathological analyses. Immunohistological analyses were performed on pts who relapsed with a DLBCL histology and whose samples were available. Cell of origin (COO) subtypes were classified by Hans algorithm. Programmed death–ligand 1 (PD–L1) expression on tumor cells and tumor-infiltrating cells (TICs) were evaluated by double staining for PAX5 and PD–L1. Cut-off values for the PD–L1(+) stain on tumor cells and TICs were 30% and 20%, respectively (Kiyasu *et al. Blood* 2015). Fluorescence *in situ* hybridization (FISH) analysis using probes mapping to 9p24.1 and centromere 9 was performed on PD–L1(+) tumor cells. The cut-off value for MYC(+) was 40%. CD163 or PD–1(+) TIC numbers were counted manually in three representative fields (0.14 mm^2 area); the average number of positive cells per unit area in samples at initial diagnosis and LR were then compared.

Results: The characteristics of the 21 pts at initial diagnosis were as follows: 60 years median age (range, 32–77); 13 (62%) had stage I disease; 12 (57%) had extranodal disease; and frequent diseases in gingival, sinonasal/nasal cavities and testes (Table). The majority of pts (n=14, 70%) had a non-germinal–center B-cell–like (non-GCB) subtype. As an initial treatment, CHOP (median, 5 cycles; range, 3–8), with (n=18) or without (n=2) radiation therapy (RT), was performed. One pt received other intensive chemotherapy. Rituximab was administered to eight pts. The median duration from initial diagnosis to LR was 7 years (range, 5–18) and the median follow-up time for survivors was 11 years. Systemic relapses (n=15, 71%) were more common than local ones. Nine pts (43%) had more than one extranodal disease; skin/subcutaneous and bone/bone marrow diseases were frequent. Of 18 pts who had received RT, 12 pts relapsed outside of the RT field only, and six relapsed both within and outside the RT field. One pt relapsed showing a composite histology of DLBCL and a low-grade B-cell lymphoma (LGBCL), and two pts relapsed with only a LGBCL histology. Four pts had a different COO subtype from that at initial diagnosis. At initial diagnosis, MYC was positive in six pts and negative in eight pts; at LR, it turned positive in five pts, all of whom relapsed with stage IV disease, although it turned negative in another five pts. Interestingly, two pts showed PD–L1(+) tumor cells at LR despite being negative at initial diagnosis. FISH analysis revealed a 9p24.1 gain in one of the two pts. More PD–1(+) TICs tended to be observed at LR than at initial diagnosis, although this was not statistically significant (p=0.13). No clear trend in the number of PD–L1(+) or CD163(+) TICs was found.

Table 1.

Table. Characteristics of 21 patients with DLBCL who developed late relapse

	At initial diagnosis	At late relapse	*P* value
Median age, years (range)	60 (32–77)	71 (45–84)	
Gender, male : female, n	12 : 9		
Ann Arbor clinical stage, I / II / III / IV, n	13 / 8 / 0 / 0	5 / 1 / 1 / 14	<0.001[1)]
Number of extranodal diseases, 0 / 1 / ≥2, n	9 / 12 / 0	2 / 10 / 9	<0.001[1)]
Site of extranodal disease, n			
Gingival	3	0	
Nasal or paranasal cavity	3	2	
Testis	3	3	
Skin or subcutaneous	0	7	
Bone or bone marrow	0	6	
Intestine or colon	0	3	
Liver	0	2	
Other sites	3	7	
Immunohistochemistry			
Cell of origin, GCB / non–GCB / NA / non–DLBCL, n	6 / 14 / 1 / 0	6 / 12 / 1 / 2	
PD–L1+ on tumor cell, + / - / NA, n*	0 / 18 / 1	2 / 16 / 1	
PD–L1+ on TICs, + / - / NA, n*	4 / 14 / 1	2 / 16 / 1	0.48[2)]
MYC expression + / - / NA, n*	6 / 8 / 5	6 / 8 / 5	
PD–1+ TIC count, median (range)*	9 (0–36.7)	13.1 (0–63.3)	0.13[1)]
CD163+ TIC count, median (range)*	12.5 (0–28.7)	9.3 (1.3–20)	0.56[1)]

*Two patients who relapsed with only a low grade B-cell lymphoma histology were excluded from the analyses. 1):by Wilcox signed-rank test, 2): by McNemar's test; DLBCL, diffuse large B-cell lymphoma; GCB, non-germinal–center B-cell–like; TIC, tumor-infiltrating cell; PD–L1, programmed death–ligand 1; NA, not applicable

Summary/Conclusion: LR patterns of advanced-stage disease, multiple extranodal diseases and non-GCB subtype were frequently seen in limited-stage DLBCL. This study suggests heterogeneity as a LR mechanism, in which, in addition to the well-known mechanism of relapse with LGBCL components, we speculate functions associated with immune evasion or cell proliferation are acquired.

PB1753

COMPARISON BETWEEN CONTRAST-ENHANCED MAGNETIC RESONANCE IMAGING AND 18F-FDG POSITRON EMISSION TOMOGRAPHY-COMPUTED TOMOGRAPHY FOR THE DIAGNOSIS OF NEUROLYMPHOMATOSIS

Y. Abe*, K. Narita, H. Kobayashi, A. Kitadate, M. Takeuchi, K. Matsue

Division of Hematology/Oncology, Department of Internal Medicine, Kameda Medical Center, Kamogawa, Japan

Background: Neurolymphomatosis (NL) is an extremely rare clinical condition and its optimal diagnostic approach remains unclear. Although pathological confirmation is the gold standard for NL diagnosis, it is often unfeasible due to the anatomical difficulty in approaching the culprit lesions and the potential risk of irreversible nerve damage caused by nerve biopsy. Contrast-enhanced magnetic resonance imaging (MRI) and fluorine-18 fluorodeoxyglucose (18-FDG) positron emission tomography-computed tomography (PET-CT) have been identified as useful modalities for the NL diag-

nosis. However, the sensitivities of contrast-enhanced (MRI) and 18F-FDG PET-CT have not been compared thus far.
Aims: We aimed to compare the usefulness and detection sensitivities of contrast-enhanced MRI and PET-CT for the diagnosis of NL.
Methods: We enrolled 18 consecutive patients diagnosed with NL between 2007 and 2018. Criteria for diagnosis of NL were as follows: (1) clinical symptoms and/or neurological findings related to the peripheral nerves, and (2) histological confirmation of lymphoma cells within the peripheral nerves or imaging findings including enlargement and/or enhancement of these nerves detected by contrast-enhanced MRI and/or abnormal accumulation of 18F-FDG in these nerves detected by PET-CT. Imaging findings of all patients were confirmed by two experienced radiologists. Detection sensitivities were compared using the McNemar test.
Results: A total of 18 patients were treated at our institution and evaluated using both pretreatment contrast-enhanced MRI and PET-CT. Of these patients, contrast-enhanced MRI identified abnormal findings in 17 (94.4%) patients, while PET-CT identified them in 12 (66.7%) patients, although the difference was slightly insignificant (P=0.074). Among the 53 involved PN, 52 (98.1%) were deemed positive for NL by contrast-enhanced MRI, whereas only 21 (39.6%) were deemed positive by PET-CT (P<0.001). Detection sensitivity of PET-CT for the cauda equine (11.1%) and lumbosacral nerves (31.0%) was lower than that of contrast-enhanced MRI. However, PET-CT and contrast-enhanced MRI showed equivalent sensitivity for detection of abnormal findings in cranial nerves (81.8%). Levels of soluble interleukin-2 receptor in patients with NL who were deemed negative for NL by PET-CT were significantly higher than in those deemed positive for NL (P=0.049). The group deemed negative for NL by PET-CT included more patients with systemic presentation of lymphoma than those deemed positive for NL by PET-CT, although the difference was not statistically significant (50.0% *vs* 33.3%, respectively; P=0.627). Additionally, patients deemed negative for NL by PET-CT had a more favorable overall survival than those deemed positive for NL (median, 37.2 *vs* 7.5 months, respectively; P=0.234). All abnormal findings for PN identified by contrast-enhanced and PET-CT in patients who responded to therapy and had follow-up imaging studies showed significant improvement, suggesting that these findings were the manifestations of neural involvements of NL.

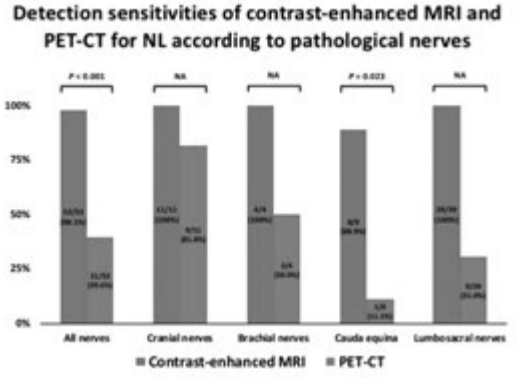


Figure 1.

Summary/Conclusion: To our knowledge, this is the first study to investigate the difference between detection sensitivities of contrast-enhancement MRI and PET-CT for the diagnosis of NL. Contrast-enhanced MRI has superior sensitivity to PET-CT for the diagnosis of NL, particularly in the cauda equine and lumbosacral nerves. Thus, contrast-enhanced MRI should be performed in combination with PET-CT for the diagnosis of NL.

PB1754

EXPRESSION AND CLINICAL RELEVANCE OF PROGRAMMED CELL DEATH-1 AND PROGRAMMED CELL DEATH LIGANDS IN EXTRANODAL NATURAL KILLER/T CELL LYMPHOMA

H. Muhamad[1,*], N. Sukswai[2], K. Wudhikarn[1]

[1]Medicine, [2]Pathology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Background: Programmed cell death-1 (PD-1)/Programmed cell death-ligands (PD-Ls) signal plays a vital role in regulating T-cell response. Several cancers especially EBV-associated malignancy aberrantly express PD-1/PD-Ls leading to immunologic escape of tumors contributing to disease progression. In lymphoma, PD-1/PD-Ls expression was mostly described in Hodgkin lymphoma. Extranodal Natural Killer/T cell lymphoma (ENKTL) is one of the EBV-associated lymphomas more commonly found in Asia. Assessment of PD-1/PD-Ls expression in ENKTL is clinically relevant as a prognostic factor and biomarker for immune checkpoint based therapy.
Aims: To characterize PD-1/PD-Ls expression and its association with clinical characteristics and prognosis of ENKTL.
Methods: Thirty-nine ENKTL patients diagnosed at the King Chulalongkorn Memorial hospital between January 2008 and December 2017. Detailed clinical data was abstracted from medical record and immunohistochemical staining was performed on available formalin fixed paraffin embedded tissue archives to identify PD-1/PD-Ls expression within lymphoma and stromal cells. PD-L1 IHC was stained using the PD-L1 IHC E1L3N rabbit XP monoclonal primary antibody (1:400; Cell Signaling Technology, Danvers, MA, USA). PD-L2 IHC was stained using the rabbit anti-PD-L2 polyclonal antibodies (Sigma-Aldrich, St. Louis, MO, USA; catalogue# SAB3500395-100UG, used at a dilution of 1:1000). IHC staining was evaluated in agreement by two independent pathologists and two hematologists. The 5% cut-off was adopted for the positivity criteria. Clinical, pathological data and outcome were reported.
Results: Of 39 cases, median age at diagnosis was 51 years old. About half of patients presented with advanced stage and high-risk Korean ENKTL Prognostic Index (KPI). Among 33 chemotherapy treated patients, 21 (63.6%) received asparaginase based regimen. PD-L1 and PD-L2 expression were frequently presented within lymphoma cells (n=27; 69.2% for PD-L1 and n=29; 74.4% for PD-L2) with median expression of 15% and 10% respectively. Expression of PD-L1 and PD-L2 were noted on non-tumor stroma of 32 (82.0%) and 0 (0%) patients. Median stromal PD-L1 expression was 5%. PD-1 positivity within stroma was seen in 8 cases (20.5%) with median positive cells of 5% whereas there was no PD-1 expression within lymphoma cells. There was no difference in clinical features between positive and negative PD-1/PD-Ls expression except higher proportion of impaired performance status (PS) among cases with negative PD-L1 within lymphoma cells (P=0.03). There was no correlation between EBV viremia level and PD-1/PD-Ls expression. Positive PD-Ls expression within tumor cells was not associated with different EFS (2-year EFS 45.1 *vs* 54.7%; P=0.50 for PD-L1 and 42.8 *vs* 60%; P=0.31 for PD-L2) or OS (2-year OS 48.4 *vs* 74.1%; P=0.29 for PD-L1 and 51.7 *vs* 70%; P=0.36 for PD-L2). Neither PD-Ls nor PD-1 expression within stroma was associated with outcome, however, patients with positive stromal PD-1 had trend toward inferior EFS (P=0.08) (Figure 1). Univariable analysis revealed that stage, PS, KPI and asparaginase based treatment as prognostic determining factors.

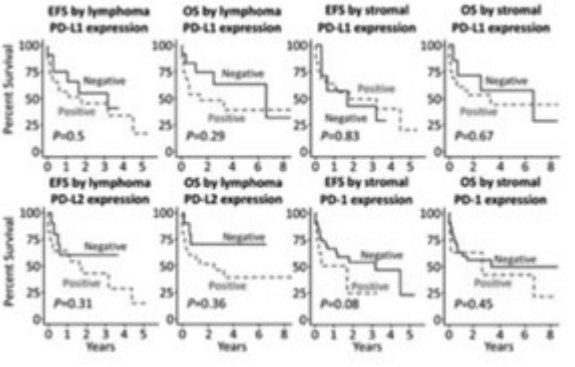


Figure 1.

Summary/Conclusion: In ENKTL, PD-Ls were aberrantly expressed within lymphoma cells at high prevalence whereas PD-1/PD-Ls were variably expressed within stroma. Our study did not demonstrate association between PD-1/PD-Ls expression and outcome, however, there was a trend toward worse EFS in patients with positive stromal PD-1. Further studies are warranted to explore significance of PD-1/PD-Ls expression in ENKTL.

PB1755

COMBINATION OF SERUM ALBUMIN AND PLATELET-LYMPHOCYTE RATIO (COA-PLR) AS A PROGNOSTIC MARKER IN PATIENTS WITH RELAPSED/REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA

Y.H. Park[1,*], J.H. Lim[2]

[1]Department of Hematology, St.Vincent's Hospital, College of Medicine, The Catholic University of Korea, Suwon, [2]Hemato-oncology, Inha University Hospital and School of Medicine, Incheon, Korea, Republic Of

Background: Recently, several inflammation-based parameters, such as lactate dehydrogenase (LDH), the neutrophil/lymphocyte ratio, the platelet/lymphocyte ratio (PLR), the absolute lymphocyte count, and serum albumin, were established for determining clinical outcomes in diffuse large B-cell lymphoma (DLBCL). However, these inflammatory parameters have been studied mainly in newly diagnosed DLBCL patients and there are few inflammation-based markers for predicting clinical outcomes of relapsed/refractory patients.
Aims: Therefore, we evaluated the usefulness of combination of serum albumin concentration and PLR (COA-PLR), as an inflammatory marker, in predicting response to salvage chemotherapy and prognosis for patients with relapsed/refractory DLBCL after R-CHOP chemotherapy.
Methods: We retrospectively reviewed data from 113 patients with relapsed/refractory DLBCL who received front-line R-CHOP chemotherapy between January 2000 and December 2016. PLR was calculated from differential count dividing the platelet measurement by the lymphocyte measurement and was recorded at first relapse or refractoriness after R-CHOP chemotherapy. A PLR of 270 was identified as the most discriminative cutoff value for overall survival (OS) and progression-free survival (PFS) from relapse or refractoriness by receiver operating characteristic curve analysis. The COA-PLR was calculated as follows: patients with both hypoalbuminemia (<3.5 g/dL) and an elevated PLR (>270) were allocated a score of 2, and patients with one or neither were allocated a score of 1 or 0, respectively.
Results: Overall, this study included 68 males and 45 females with a median age at diagnosis of 58 years (range, 23–76). Of the patients, 30 (26.5%) had refractory disease and 83 (73.5%) had relapsed disease. Of the relapsed patients, 36 (43.4%) and 47 (56.6%) showed early (≤1 year) and late relapse (>1 year) after achieving complete response (CR) or partial response (PR) at the end of R-CHOP chemotherapy, respectively. The COA-PLR score was 0 in 22 (19.4%), 1 in 55 (48.8%), and 2 in 36 (31.8%). The proportion of old patients (age >60 years) was significantly higher in the COA-PLR 2 group than in COA-PLR 1 and 0 group (61.4% *vs* 38.2% *vs* 39.5%, p<0.001). Poor Eastern Cooperative Oncology Group performance status, presence of B symptoms and high-risk international prognostic index were also observed frequently in the COA-PLR 2 group. ICE (ifosfamide, carboplatin, and etoposide) and DHAP (dexamethasone, cytarabine, and cisplatin) were the two most commonly used salvage chemotherapies resulting in overall response rate (ORR, CR+PR) of 68.1% with 46% of CR patients (n=53). The ORR after salvage chemotherapy decreased according to COA-PLR score: 82.1% at COA-PLR 0, 61.2% at COA-PLR 1, and 41.2% at COA-PLR 2 (p<0.001). Among the responders, 47 (62%) received autologous stem cell transplantation after salvage chemotherapy. Of a median follow-up of 42 months (range, 14-72), the 3-year PFS and OS rates were 32.5% and 44.2%, respectively. In multivariate analysis, early relapse (hazard ratio [HR] 2.108, 95% confidence interval [CI] 1.277-3.579), high LDH (HR 1.536, 95% CI 1.195-3.158), and COA-PLR 2 (HR 1.902, 95% CI 1.270-3.468) were independent poor prognostic factors for PFS. The independent poor prognostic factors for OS were early relapse (HR 2.104, 95% CI 1.178-3.768) and COA-PLR 2 (HR 1.976, 95% CI 1.370-2.852).
Summary/Conclusion: This study suggests that the COA-PLR maybe a potentially useful and easily available tool for predicting clinical outcomes in relapsed/refractory DLBCL patients.

PB1756

RETROSPECTIVE ANALYSIS OF METHOTREXATE-ASSOCIATED LYMPHOPROLIFERATIVE DISORDERS

K. Yoshinaga*, Y. Kato, Y. Iizuka, A. Watanabe, N. Tanaka, M. Ishiyama, A. Shinohara, H. Kazama, S. Hagiwara, N. Mori, M. Shiseki, J. Tanaka

Hematology, Tokyo Women's Medical University, Tokyo, Japan

Background: Methotrexate-associated lymphoproliferative disorders (MTX-LPD) are included in category of other iatrogenic immunodeficiency-associated LPD in WHO 2016 classification. Although tumor regression by MTX discontinuation without any additional treatment is often observed, proportion of these phenomenon remains unclear. Risk factors for MTX-LPD development, and appropriate treatment strategy have not been well-known.
Aims: We conducted a retrospective observational study to characterize MTX-LPD and to evaluate prognostic factors.
Methods: We reviewed laboratory data and medical record of the pathologically confirmed MTX-LPD patients between Jan 2012 and Dec 2016 in our hospital.
Results: A total of 31 patients (23 females and 8 males; median age 71 years old, ranging 41-87 years old) were included in this study. Median observation period was 27.4 months (range 1.0-62 months). Histological diagnoses were as follows: 21 diffuse large B-cell lymphoma, 4 follicular lymphoma, 3 T-cell lymphoma, 2 Marginal zone lymphoma, one NK/T cell lymphoma. Spontaneous tumor regression within 3 months after MTX discontinuation was observed in 16 (51%) patients. Nine of them maintained remission without any additional therapy for MTX-LPD. Remaining seven patients received chemotherapy because of regrowth of LPD. Totally, 21 patients received chemotherapy. Median duration from the diagnosis to chemotherapy initiation was one month (range 3 days-38.6 months). Complete response and partial response was obtained in 11 (61%) and 5 (28%) patients. Therefore, overall response rate was 16/18(88%). Estimated 1-year overall survival (OS) rate was 93% and 3-yr OS rate was 69%. Kaplan-Meier plots demonstrated that following clinical variables were associated with worse OS: older age (65 years or older) (3-years OS, 52% *vs* 93%, log rank test, P=0.0019), higher serum LDH (>upper limit of normal range) (3-year OS, 62% *vs* 100%, log rank test, P=0.034), lower serum albumin (<3.8g/dL) (3-year OS, 42% *vs* 85%, log rank test, P=0.063), higher IPI risk categories (High-Int, and High risk groups) (3-year OS, 70% *vs* 80%, log rank test, P=0.024), and poorer performance status (PS score, 2 or higher) (3-year OS, 25% *vs* 80%, log rank test, P=0.012).
Summary/Conclusion: In conclusion, 10 (32%) patients were alive without chemotherapy after MTX discontinuation. Older age, higher serum LDH, higher IPI risk categories, and poorer PS predict survival of patients with MTX-LPD.

PB1757

PROGNOSTIC SIGNIFICANCE OF TRYPTOPHAN CATABOLISM IN NEWLY DIAGNOSED DIFFUSE LARGE B-CELL LYMPHOMA

A. Masaki[1,*], S. Kusumoto[1], Y. Maeda[2], T. Narita[1], A. Ito[1], S. Kinoshita[1], T. Yoshida[1], M. Ri[1], H. Komatsu[1], T. Ishida[3], H. Inagaki[1], R. Ueda[4], S. Iida[1]

[1]Hematology and Oncology, Nagoya City University Graduate School of Medical Sciences, [2]Laboratory of Hospital Pharmacy, Nagoya City University Graduate School of Pharmaceutical Sciences, Nagoya, [3]Division of Hematology and Oncology, Internal Medicine, School of Medicine, Iwate Medical University, Morioka, [4]Tumor Immunology, Aichi Medical University School of Medicine, Nagakute, Japan

Background: Indoleamine 2,3-dioxygenase 1 (IDO1, IDO) is an enzyme catabolizing tryptophan (Trp) into the kynurenine (Kyn), and is a tumor microenvironment factor that suppresses antitumor immune responses. IDO is an attractive target for malignant tumor immunotherapy.
Aims: This study determined the prognostic significance of tryptophan (Trp) catabolism in patients with newly diagnosed diffuse large B-cell lymphoma (DLBCL) and primary mediastinal large B-cell lymphoma (PMBCL).
Methods: A total of 133 DLBCL and 5 PMBCL patients who received at least 1 cycles of rituximab-CHOP like regimen were enrolled. Baseline serum Trp and Kyn levels in 138 lymphoma patients and 50 healthy volunteers were measured using an ultra-performance liquid chromatography–tandem mass spectrometry system, and associations with various clinical parameters analyzed. IDO expression was histologically evaluated within affected tumor sites of 103 patients.
Results: Enrolled patients comprised 77 males and 61 females (age range 24-90, median 69 years), with estimated overall survival (OS) at 5 years of 74.0%. The serum Kyn concentration was significantly higher in the patients than in the healthy volunteers (median: 1.63 μM, 1.09 μM, respectively, *P*<0.001). The serum Trp concentration was significantly lower in the patients than in the healthy volunteers (median: 50.9 μM, 63.4 μM, respectively, *P*<0.001). Therefore, the serum Kyn/Trp ratio was significantly higher in the patients than in the healthy volunteers (median: 31.2, 16.5, respectively, *P*<0.001). Patients were divided into two groups according to their serum Kyn/Trp ratio (Kyn [μmol/L)/Trp [μmol/L] x10^3) since high IDO activity results in increased Kyn and decreased Trp concentrations, meaning a high serum Kyn/Trp ratio. Of four factors determining the international prognostic index for DLBCL, a high serum Kyn/Trp ratio (>27.5) was significantly associated with advanced Ann Arbor stage (III-IV), high LDH (>upper normal limit), high age (≥61), poor performance status (2-4), but was not associated with extranodal sites (>1). Interestingly, hypoalbuminemia (Alb<4) was also significantly associated with high serum Kyn/Trp ratio. OS was significantly shorter in patients with a high serum Kyn/Trp ratio compared to those with a low ratio (OS rate at 5 years, 62.8 *vs* 92.7%, *P*=0.0025; **Figure**), however, high serum Kyn/Trp ratio was not an independent risk factor for OS in multivariate analysis. In immunostaining analyses, lymphoma cells were negative, but macrophages and dendritic cells in the microenvironment were positive for IDO. No significant correlation existed between the serum Kyn/Trp ratio and the degree of histologically IDO positive cells in the tumor microenvironment. There were no significant differences in OS according to the degree of histologically IDO positive cells in the tumor microenvironment.

Figure: Overall survival of DLBCL patients according to the serum Kyn/Trp ratio

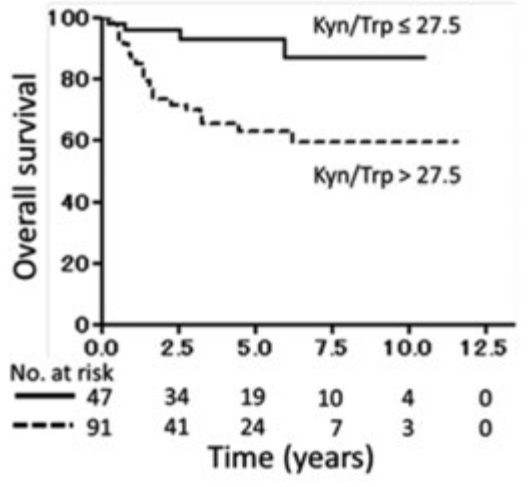


Figure 1.

Summary/Conclusion: Baseline high serum Kyn/Trp ratio was associated with poor prognosis in DLBCL patients, however the immunohistological evaluation of IDO expression in the tumor sites was associated with neither serum Kyn/Trp ratio nor prognosis. Furthermore, high serum Kyn/Trp ratio was significantly associated with known poor prognostic factors of DLBCL. Wherefore, novel treatment strategy targeting IDO is attractive for DLBCL patients with high serum Kyn/Trp ratio.

PB1758

EARLY MORTALITY IN PATIENTS WITH NEWLY DIAGNOSED PRIMARY CNS LYMPHOMA

C.-J. Liu[1,*], C.-H. Lin[2], Y.-C. Liu[1], C.-J. Teng[3], C.-M. Yeh[1], Y.-C. Hong[4], Y.-B. Yu[3], P.-S. Ko[1], L.-T. Hsiao[1], T.-J. Chiou[5], J.-H. Liu[1], J.-P. Gau[1], H.-Y. Wang[1]

[1]Division of Hematology, [2]Department of Medical Education, Taipei Veterans General Hospital, Taipei, [3]Division of Oncology and Hematology, Far Eastern Memorial Hospital, New Taipei, [4]Division of Hematology and Oncology, Kaohsiung Taipei Veterans General Hospital, Kaohsiung, [5]Division of Transfusion Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, Republic of China

Background: Primary central nervous system lymphoma (PCNSL) is a relatively rare non Hodgkin lymphoma (NHL) and counts for approximate 3% of all CNS neoplasms. Despite advances in treatment, patients still encounter early mortality. The previous reports focused on long-term outcomes (Abrey LE *et al*., *J Clin Oncol*, 2006; Ferreri AJ *et al*., *J Clin Oncol*, 2003), in which the risk factors included age, performance status, CSF protein level, involvement of deep brain, serum lactate dehydrogenase, and intraocular involvement.
Aims: In this study, we would like to investigate the risk factors of early mortality in patients with PCNSL.
Methods: We enrolled newly diagnosed PCNSL patients at Taipei Veterans General Hospital, a national medical center in Taiwan, between January 1, 2002 and October 31, 2017. Patients without histopathological confirmations, those diagnosed with secondary CNS lymphoma, or those with AIDS were excluded. The cohort was followed up until the end of February 2018. The risk factors for mortality within 180 days were identified using univariate and multivariate Cox proportional hazards models. The factors with $p<0.1$ in the univariate analysis were included in the multivariate analysis. All treatments were estimated with the use of time-dependent covariates to prevent immortal time bias.
Results: The study cohort consisted of 129 PCNSL patients, with a median follow-up of 8.4 years. The median age of the patients was 64 years and 73 (56.6%) of them were male. The probability of early mortality (≤180 days) was 13.4% (95% confidence interval [CI] 8.4–20.9%). In the univariate analysis, age ≥80 (hazards ratio [HR] 4.19), involvement of basal ganglia (HR 4.56) and ECOG performance status ≥2 (HR 2.93), hemoglobin<11 g/dl (HR 2.69), platelets<150,000/ml (HR 2.53), albumin ≥3.9 g/dl (HR 0.23), receiving steroid (HR 0.40), methotrexate (HR 0.33), and immunotherapy with rituximab (HR 0.28) were associated with early mortality. Age ≥80 (adjusted HR 5.08, 95% CI 1.21–21.25, p=0.026), involvement of basal ganglia (adjusted HR 7.46, 95% CI 2.08–26.78, p=0.002), and receiving steroid (adjusted HR 0.18, 95% CI 0.04–0.88, p=0.034) were identified as independent predictors for early mortality in the multivariate analysis.

Table 1.

Table Risk factors for early mortality in patients with Primary CNS Lymphoma

	Univariate analysis		Multivariate analysis[a]	
Predictive variables	**HR (95% CI)**	***P* value**	**HR (95% CI)**	***P* value**
Age ≥ 80	4.19 (1.45–12.09)	0.008	5.08 (1.21–21.25)	0.026
Sex (male)	0.82 (0.31–2.18)	0.688		
Site				
Frontal lobe	0.55 (0.18–1.70)	0.297		
Parietal lobe	0.56 (0.16–1.97)	0.367		
Temporal lobe	1.04 (0.38–2.87)	0.938		
Occipital lobe	1.56 (0.50–4.83)	0.443		
Basal ganglia	4.56 (1.58–13.14)	0.005	7.46 (2.08–26.78)	0.002
Brain stem	0.58 (0.08–4.38)	0.596		
Cerebellum	1.10 (0.25–4.82)	0.904		
ECOG ≥ 2	2.93 (0.95–9.08)	0.063	0.78 (0.21–2.89)	0.704
Hemoglobin < 11 g/dl	2.69 (0.98–7.40)	0.056	3.92 (0.96–15.93)	0.056
Platelets < 150,000/μl	2.53 (0.92–6.95)	0.073	2.29 (0.71–7.36)	0.165
Albumin ≥ 3.0 g/dl	0.23 (0.08–0.66)	0.007	0.43 (0.12–1.58)	0.205
Lactate dehydrogenase ≥ 250 U/L	1.34 (0.49–3.69)	0.574		
CSF protein ≥ 100 mg/dl	1.05 (0.11–10.13)	0.964		
Treatment[b]				
Steroid	0.40 (0.14–1.14)	0.087	0.18 (0.04–0.88)	0.034
Methotrexate	0.33 (0.12–0.90)	0.030	0.54 (0.13–2.20)	0.386
Rituximab	0.28 (0.09–0.88)	0.030	0.40 (0.08–1.96)	0.260

HR, hazard ratio; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group performance
[a]All factors with $p < 0.1$ in the univariate analysis were included in the Cox multivariate analysis.
[b]Tuberculosis was analyzed as a time-dependent covariate in the Cox regression model.

Summary/Conclusion: We identified age ≥80 and involvement of basal ganglia as independent risk factors for early mortality in the patients with PCNSL. Of note, steroid treatment could prevent the patients from the early death. Identifying patients with risks of early mortality may help clinicians initiate appropriate management and early treatment. Further validation of our findings in other cohorts is warranted.

PB1759

DESCRIPTION AND ANALYSIS OF 36 CENTRAL NERVOUS SYSTEM RELAPSE IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA WITHIN LYSA STUDIES

S. Bernard[1,*], L. Oberic[2], J. Lazarovici[3], A. Perrot[4], K. Van Eygen[5], C. Sarkozy[6], C. Sebban[7], E. Van Den Neste[8], O. Fitoussi[9], B. Slama[10], Y. Arkam[11], C. Thieblemont[1]

[1]Hemato-oncology, Hôpital Saint Louis-APHP, PARIS, [2]Hematology, IUCT Oncopole, Toulouse, [3]Hematology, Institut Gustave Roussy IGR, Villejuif, [4]Hematology, CHU Brabois, Vandoeuvre les Nancy, France, [5]Hematology, Kortrijk, Kortrijk, Belgium, [6]Hematology, CHU Lyon Sud Pierre Benite, [7]Hematology, Centre Leon Berard, Lyon, France, [8]hematology, UCL, Louvains Saint Luc, Belgium, [9]Hematology, Polyclinique Bordeaux Nord, Bordeaux, [10]CH Avignon, Avignon, [11]hematology, CH Emile Muller, Mulhouse, France

Background: Central nervous system (CNS) relapse in patients with diffuse large B-cell lymphoma (DLBCL) is an uncommon event (2-5%) associated with a very poor prognosis. We present a descriptive analysis of characteristics and outcome of 36 patients who presented CNS relapse following their 1st line of treatment for a DLBCL treated prospectively in 7 LYSA Phase III trials (LNH03 / LNH07)/LNH09).
Aims: CNS relapse in the rituximab era is a rare event but with still a poor prognois. We proposed in this study to collect datas of patients with a CNS relapse and treated with RCHOP regimen to analyse pertinent clinical findings to improve diagnosis and therapeutic options in this population.
Methods: We reviewed the records of 1885 patients with *de novo* DLBCL and included in 7 phase III LYSA studies between 2003 and 2009. 1615 patients younger than 80 were treated with CHOP +/- Rituximab and ACVBP +/- Rituximab, and 270 older than 80 were treated with mini-CHOP-Rituximab. All patients with a documented CNS relapses (CSF analysis, MRI, CT-Scan) were collected and analysed.
Results: Median age of patients with CNS relapse was 71 (20-89).28 patients were under 80 years of age and were treated for 4 patients with R-ACVBP, and for 24 patients with RCHOP21/14. 8 patients were older than 80 years and were treated with RminiCHOP. At the initial diagnosis, 2 patients had a low risk CNS-IPI score (0-1), 15 an intermediate risk (2-3) and 17 patients presented a high risk (4-6) (2 unassessed patients). The

median time to relapse was 247.5 days (93-781). At CNS relapse, 18 patients (50%) had parenchymal involvement, 16 had meningeal involvement (44%), 6 had epidural disease (17%), 1 had ocular involvement (2.8%), and 7 had two associated CNS lesions (19%). Treatment of this CNS relapse included methotrexate and / or aracytin high dose-based chemotherapy in 20 cases (56%) with 3 intensifications (8.3%), local treatment in 20 cases [intrathecal chemotherapy in 12 cases (33%); radiotherapy in 8 cases (22%)], and palliative treatment in 10 cases (28%). The median survival after CNS relapse was 86 days (2-3172), or 2.9 months. 2 patients (5.4%) had a survival greater than 2 years; in both cases patients had benefited of an autologous stem cell tranplantation (ASCT).

Summary/Conclusion: We confirm the poor prognosis of CNS relapses in DLBCL patients, as well as their early onset. An optimization of CNS relapses prophylaxis is essential in agressive lymphoma. The therapeutic management of CNS relapses is disparate and non-consensual, requiring the establishment of multicenter relapse therapy protocols adapted to these rare events.

PB1760

OXALIPLATIN-BASED REGIMEN AS SALVAGE AND STEM-CELL MOBILIZING THERAPY IN RELAPSED REFRACTORY LYMPHOMAS

L. Manconi*, E. Coviello, F. Guolo, L. Giannoni, P. Minetto, F. Canale, M. Gambella, F. Ballerini, R.M. Lemoli, M. Gobbi

Clinic of Hematology, Department of Internal Medicine (DiMI), University of Genoa, Policlinico San Martino, IRCCS per l'Oncologia, Genoa, Italy

Background: Cisplatin containing regimens showed promising results as salvage chemotherapy (CT) in relapsed or refractory (R/R) lymphomas, mainly as bridge to autologous stem cells transplant (ASCT). Tolerability is mainly impaired by mucositis and cisplatin-associated renal and neurological toxicity. Oxaliplatin shows a better toxicity profile, but at the present time few data are available in R/R lymphomas setting.

Aims: To investigate the feasibility and efficacy of oxaliplatin, cytarabine and dexamethasone (DHAOx) as salvage and mobilizing regimen for peripheral blood stem cells (PBSC).

Methods: We retrospectively analyzed 83 R/R lymphoma patients diagnosed between October 2004 and October 2014, who received DHAOx as salvage CT. Forty-one had Diffuse Large B-cell Lymphoma (DLBCL, 49%), 10 Hodgkin Lymphoma (HL, 12%), 13 Mantle Cell Lymphoma (15%), 15 other Indolent Lymphomas (18%) and 4 Peripheral T-cell Lymphoma (5%). Median age was 59 (range 22-79). DHAOx schedule consists inq21 days-administration of dexamethasone 40 mg/die on days 1-4, oxaliplatin 130 mg/m^2 on day 1, cytarabine 2 g/m^2 bid on day 2.In B-cells lymphomas, Rituximab 375 mg/m^{2}was added on day 2. G-CSF was administered from day 7 until absolute neutrophil count recovery or from day 5 to 10 (5 mcg/Kg/die) if PBSC collection was scheduled. We analyzed overall response rate(ORR), progression free survival (PFS), hematological and non-hematological toxicities and success rate of BPSC collection.

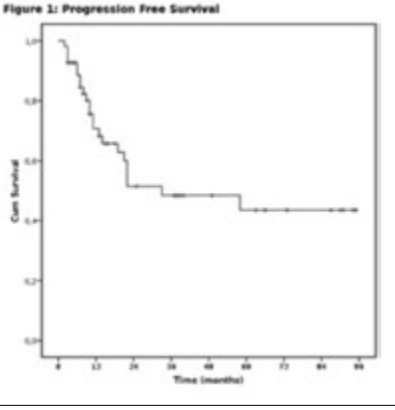


Figure 1.

Results: ORR was 64%, Complete remission (CR) was achieved in 48 patients (55%), partial remission (PR) in 8 patients (9%), 32 patients did not respond (36%). Patients affected by DLBCL had significantly higher probability of achieving CR if compared to HL, MCL and indolent lymphomas ($p<0.05$). Median PFS was 33 months (fig.1). The projected OS at 36 months was 54.5%.%. Survival was better in patients with DLBCL and HL ($p<0.05$). Grade >2 non hematological toxicity was observed in 27 patients (33%),most frequently oral mucositis and diarrhea (6 cases, 8%), FUO (17 cases, 21%), sepsis (3 cases, 4%), paresthesia (5 patients, 6%). No patients experienced acute renal impairment. Among DLBCL patients, 81% were treated in second line,19% in third or subsequent line. ORR was 71%. CR was obtained in 60%, PR in 11%. Eleven patients did not respond 29%. The median PFS was not reached and the projected OS at 36 months was 45.5. Following DHAOx and G-CSF priming, stem cell harvest for ASCT was planned in 40 patients (48%), all of them successfully performed the procedure and reached the target of 4x10^6 CD34+/Kg. Only 1 heavily pretreated patient needed plerixafor administration. All patients performing stem cell collection were able to proceed to ASCT.

Summary/Conclusion: Our experience shows that the use of oxaliplatin instead of cisplatin is feasible, alongside showing similar efficacy compared to conventional cisplatin containing regimens for R/R lymphomas. Low hematological and non-hematological toxicity as well as good capability of PBSC mobilization were observed. Notably, no renal toxicity was observed. The very good toxicity profile allowed all eligible responding patients to proceed with planned ASCT consolidation. Oxaliplatin-high dose cytarabine salvage therapy is therefore a reasonable option, expecially for R/R DLBCL patients considered eligible for ASCT.

PB1761

SECOND TUMORS IN PATIENTS WITH B-CELL NON-HODGKIN'S LYMPHOMAS (BNHL): WITH HEPATITIS C(HCV) AND WITHOUT HEPATITIS C

S. Lepkov[1,*], I. Subortceva[2], J. Ribuchina[3], G. Tumian[4], O. Kolomeitsev[4], N. Kokosadze[4], P. Zeynalova[4], I. Komarov[4], S. Borisovckay[5], N. Kuprishina[4], O. Zaharov[6], R. Ivashenko[7], Y. Kemih[7], I. Lazarev[6], O. Ettinger[1], I. Nikitin[1]

[1]therapy, National Research Medical University named by N.I. Pirogov, [2]haematology, National Medical Research Center for Hematology, [3]oncology, [1] N.N. Blokhin National Cancer Research Center, Moscow,, [4]oncology, N.N. Blokhin National Cancer Research Center, Moscow,, [5]therapy, City Clinical Hospital. V.M. Buyanova, [6]haematology, Hematologic Moscow City Center at the State Clinical Hospital named by S. Botkin, [7]radiology, City Clinical Hospital. V.M. Buyanova, moscow, Russian Federation

Background: Information about the second tumors that arise after recovery from BNHL is not so extensive. Information about the second tumor after BNHL with HCV is scarce.

Aims: The purpose of our study was to study which secondary tumors, with what frequency and at what time develop in these groups of patients. The study included patients with BNHL treated from 2001 to 2017.The first group was patients with BNHL+HCV, there were 213. In the second group was patients with BNHL without HCV there were 835. The median age was in the first group-48 years, in the second group-60 years. The ratio of men and women in the first group was 1: 1, in the second 1: 2.5

Methods: During the observation in the first group, the second tumors developed in 23 patients (11%), in the second group-in 18 patients (2%). In the first group of the onset of the second tumor ranged from 16 to 96 months, the median was 44 months after the end of treatment BNHL. In the second group, the second tumor occurred from 47 to 144 months, the median was 60 months.

Results: During the observation in the first group, the second tumors developed in 23 patients (11%), in the second group-in 18 patients (2%). In the first group of the onset of the second tumor ranged from 16 to 96 months, the median was 44 months after the end of treatment BNHL. In the second group, the second tumor occurred from 47 to 144 months, the median was 60 months. In the first group, 5 of 23 patients developed MDS / AML. Another 17 patients developed solid tumors. Among the solid tumors were 3 primary liver cancer, 5 renal cancer, 5 colon cancer. According to one type of cancer, the following occurred: uterine body cancer, brain tumor, soft tissue sarcoma, lung cancer, breast cancer. In all patients during the diagnosis of lymphoma, the viral load was high. The median viral load was 2.7x10^6 copies / ml. The genotype of hepatitis C was determined in 18 of 23 patients. The genotype 1b was in 14 of 18 patients, 2 genotype in 2 patients, and the 3 genotype was in 2 patients. Of the 23 cases, the second tumors were diagnosed in 18 patients who did not receive antiviral therapy and only 5 patients who received antiviral therapy with interferon + ribavirin. All patients who developed MDS / AML received only polychemotherapy. In the first group with IHC, the study of the expression of HCV proteins was carried out in 13 cases. Expression of HCV proteins was detected in 6 cases: liver cancer-3, kidney cancer-3. In the second group of patients, of the 18 second tumors were breast cancer-3 patients, uterine body cancer-4 patients, thyroid cancer was in 2 patients. In one case: stomach cancer, colon cancer, prostate cancer, melanoma, kidney cancer, laryngeal cancer, hepatocellular cancer, lung cancer, skin cancer were identified.

Summary/Conclusion: When comparing these two groups of patients with BNHL + HCV and BNHL without HCV, the frequency of diagnosis of the second tumor was higher than in the BNHL group-11% *versus* 2%. The first group of patients was younger, the second tumors occurred earlier than 44 months compared to 60 months. In patients receiving antiviral therapy, the second tumor was diagnosed reliably less frequently than in a group of patients who did not receive antiviral therapy. HCV is an additional risk factor for developing second tumors.

PB1762

CLINICAL VALUE OF ABNORMAL FINDINGS ON BRAIN MAGNETIC RESONANCE IMAGING IN PATIENTS WITH INTRAVASCULAR LARGE B-CELL LYMPHOMA

Y. Abe*, K. Narita, H. Kobayashi, A. Kitadate, M. Takeuchi, K. Matsue

Hematology/Oncology, Kameda Medical Center, Kamogawa, Japan

Background: Neurological symptoms related to the involvement of the central nervous system have been commonly seen at diagnosis and at relapse in intravascular large B-cell lymphoma (IVLBCL). Although various patterns of abnormal findings on brain magnetic resonance imaging (MRI) in patients with IVLBCL have been reported, most of them were from case reports or small case series in selected patients.
Aims: We aimed to investigate the clinical value of abnormal findings detected by brain MRI in patients with IVLBCL with regard to diagnosis and prognosis.
Methods: Thirty-three consecutive patients pathologically diagnosed and treated at Kameda Medical Center between 1998 and 2017 were identified. The baseline clinical characteristics, treatments, and outcomes of these 33 patients had been retrospectively reviewed. Brain MRI was performed as previously reported, and the abnormalities were classified into the following four patterns by two neuroradiologists (Figure): (1) hyperintense lesion in the pons on T2-weighted imaging (T2WI), (2) nonspecific white matter lesions, (3) infarct-like lesions, and (4) meningeal thickening and/or enhancement.

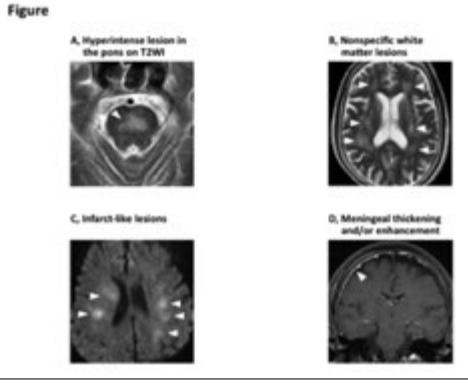


Figure 1.

Results: We identified 18 patients with NL with a total of 53 involved peripheral nerves (PN) diagnosed and treated at our institution who were evaluated with both pretreatment contrast-enhanced MRI and PET-CT. Among the 18 patients with NL, contrast-enhanced MRI described abnormal findings on PN in 17 (94.4%) patients, while PET-CT described them in 12 (66.7%) patients, although the difference was slightly insignificant (P=0.074). Among the 53 involved PN, 52 (98.1%) were positive for contrast-enhanced MRI, while only 21 (39.6%) were positive for PET-CT (P<0.001). Detection sensitivities of PET-CT for cauda equine (11.1%) and lumbosacral nerves (31.0%) were lower compared to contrast-enhanced MRI, whereas PET-CT detected abnormal findings on cranial nerves with as high detection sensitivity (81.8%) as contrast-enhanced MRI. Patients who were negative for PET-CT included more patients with systemic presentation of lymphoma compared to those who were positive for PET-CT, although the difference was not statistically significant (50.0% *vs* 33.3%, respectively; P=0.627), and soluble interleukin-2 receptor in patients with NL negative for PET-CT was significantly higher than those positive for PET-CT (P=0.049). Patients negative for PET-CT had relatively favorable overall survival than those positive for PET-CT (median, 37.2 *vs* 7.5 months, respectively; P=0.234). All abnormal findings on PN described by contrast-enhanced MRI and PET-CT in patients who responded to therapy and had follow-up imaging studies showed significant improvement, suggesting that these findings were the manifestations of neural involvement of NL.

Summary/Conclusion: Our findings revealed that most patients with IVLBCL presented abnormal findings on pretreatment brain MRI even if they had no neurological symptom. In particular, hyperintense lesion in the pons on T2WI was frequently observed in patients with IVLBCL irrespective of presence or absence of impaired consciousness, suggesting that this pattern may be pathognomonic and valuable for timely diagnosis of IVLBCL. Improvement in all types of abnormal findings on follow-up brain MRI indicated that all these findings might reflect structural changes associated with IVLBCL and might be useful for confirmation of the therapeutic effect.

PB1763

NEW TRICKS FOR OLD DRUGS: COMBINATION OF ALBUMIN-BOUND PACLITAXEL AND PEGYLATED LIPOSOMAL DOXORUBICIN IN THE TREATMENT OF RELAPSED/REFRACTORY DIFFUSE LARGE B CELL LYMPHOMA

L. Wang[1,*], Z.-J. Xia[2], X.-Q. Chen[2], W.-D. Wang[2]

[1]Hematology, ZhuJiang Hospital, Southern Medical University, [2]Sun Yat-sen University Cancer Center, Guangzhou, China

Background: There is great unmet need in improving the prognosis of patients with relapsed/refractory diffuse large B cell lymphoma (DLBCL).
Aims: How to get those patients into remission and enable them to receive autologous stem cell transplantation (ASCT) is a great challenge.
Methods: A phase 2 clinical trial was conducted to evaluate the efficacy and safety of rituximab in combination with **two nanoparticle-delivered chemotherapy drugs, albumin-bound paclitaxel and pegylated liposomal doxorubicin** (RAD) in patients with relapsed/refractory DLBCL who received at least two prior lines of immunochemotherapy.
Results: 13 patients were enrolled with a median age of 40 years. All patients received a median of 3 prior lines of therapy. 12 patients received at least two cycles of RAD. After 2 cycles of RAD, the complete response rate was 38.5%, partial response rate was 46.2%, and overall response rate was 84.6%. Three patients successfully received subsequent ASCT, and were still alive without disease after a follow-up time of 4 months, 15 months, and 29 months, respectively. Three patients died of disease progression when waiting for ASCT, and another three patients failed collection of stem cells due to bone marrow dysfunction. This RAD regimen was well tolerated with mostly reported adverse events being grade 2 or 3 hematologic events.

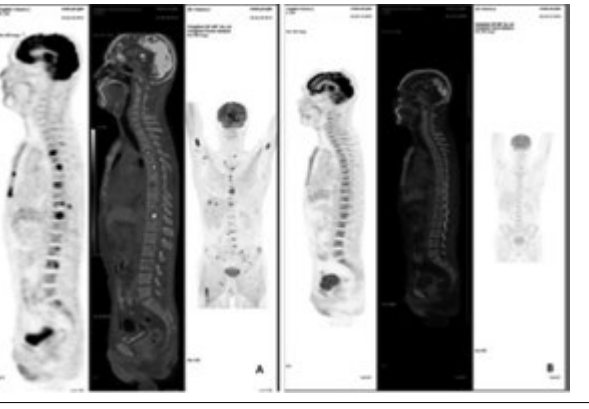


Figure 1.

Summary/Conclusion: In conclusion, we demonstrated that rituximab in combination with albumin-bound paclitaxel and pegylated liposomal doxorubicin was highly active in patients with relapsed/refractory DLBCL, and this regimen should be considered as an effective salvage therapy to bridge subsequent ASCT.

PB1764

"REAL WORLD" PRESENTATIONS OF DIFFUSE LARGE B CELL LYMPHOMA: A RETROSPECTIVE AUDIT OF HISTOPATHOLOGICAL & CLINICAL DIVERSITY, & EARLY MORTALITY

A. Thavarajah[1,*], W. Chinnery[2], L. Thomas[3], U. Igbokwe[4], P. Greaves[3]

[1]Barts and the London School of Medicine and Dentistry, Queen Mary University of London, [2]Department of Cancer & Clinical Support Services, [3]Department of Haematology, [4]Department of Histopathology, Barking Havering and Redbridge University Hospitals NHS Trust, London, United Kingdom

Background: Diffuse large B cell lymphoma (DLBCL) is diagnosed in 3-5/100,000 people annually, with RCHOP (Rituximab, Cyclophosphamide, Hydroxydaunorubicin, Oncovin, and Prednisolone) chemotherapy remaining the standard & central nervous system (CNS) prophylaxis delivered based on an International Prognostic Index (IPI) of 4/5, or having a high risk anatomical site. Extended molecular histopathology with Ki67 proliferative index, cell of origin immunohistochemistry (COO-IHC: germinal centre/GCB or activated B cell/ABC), MYC expression, & genetic rearrangements are now routinely undertaken to evaluate prognosis. Local audit of practice is essential to capture histopathological diversity, early response, & mortality rates to ensure that we can adapt and provide the best care possible.
Aims: This retrospective audit scrutinises diagnostics, initial therapy, & mortality of DLBCL treated at a busy general hospital serving 800,000 people in a socio-economically challenged area of London, UK.
Methods: 100 patients diagnosed in 2016 & 2017 were retrospectively assessed using electronic health records. The Swedish Lymphoma Registry (SLR) was chosen as a comparator dataset.
Results: To obtain a snapshot of diagnostics and initial care, median follow-up was short (150 days). Median age was 72 years (27-90, where 33% of patients were over 80). 65% presented with stage 3/4 disease (comparable with SLR). IPI was 4 or 5 in 43% of patients (16% in SLR). 60% of patients were initiated on RCHOP chemotherapy and 20% on RCVP or R-GemCVP due to comorbidities. 2 received RCODOX-M due to high risk features. 15% received only palliative steroids or radiotherapy. CNS prophylaxis criteria were met in 50% of cases: 90% due to high IPI; 10% due to anatomical site. Prophylaxis was only administered in 15% of these cases (IT methotrexate alone in 50% & systemic methotrexate in 50%). Omissions were due to patients being unfit for treatment. 4 CNS relapses occured with 2 deemed high risk at diagnosis. Of the 85% of biopsies reporting Ki-67, 60% had a Ki-67>90% (10-30% reported elsewhere). COO-IHC was ABC in 41% of cases, GCB in 30%, & unclassifiable in 35% (comparable to literature). Of the 90% of cases reporting MYC, 44% showed overexpression where 85% were double or triple expresser with BCL2/6. Rearrangement of MYC/BCL2/BCL6 genes were assessed by FISH in only 45% of cases (45% omitted due to low IHC MYC expression, 30% due to inadequate biopsy material). In the 37 cases assessed, 5% showed only MYC rearrangement & 15% had a double or triple hit, greater than the rates of 5-10% reported elsewhere. Around 80% of patients under 60 years of age(yoa), 70% of patients 60-70yoa, & 60% of patients 70-80yoa survive 2 years, comparable to the SLR. 23 patients died within 100 days of diagnosis of whom 13 were over 80yoa & 3 under 60yoa. 62% of patients diagnosed over 80yoa died within 100 days; 50% of these deaths occurred in palliative patients & 50% received chemotherapy.

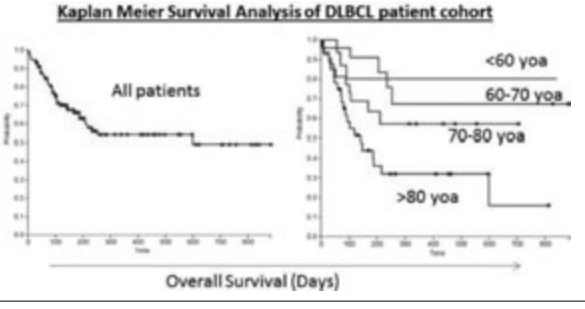


Figure 1.

Summary/Conclusion: This audit has revealed survival rates largely comparable to the registry data, but with high early death rates requiring scrutiny. High IPI scores account for a greater proportion than expected (43% *vs* 16% in the SLR). This may reflect late presentation of disease, or more molecular adverse features, with Ki67>90% & 'double-hit' disease apparently over-represented in this cohort. We emphasise that audit of 'real-world' practice is essential to elicit trends & regional differences, ensure equitable high quality care & delivery, enabling tailored service provision to its population.

PB1765

LIMITED IMPACT OF MYC, BCL2 AND BCL6 BREAKS IN THERAPEUTIC DECISIONS FOR DIFFUSE LARGE B CELL LYMPHOMAS (DLBCL) IN A REAL WORLD SETTING-A SINGLE CENTER EXPERIENCE

F. Rigotti[1], M. Gomes da Silva[2,*], S. Esteves[3], J. Cabeçadas[4]

[1]Facoltà di Medicina e Chirurgia, Università di Bologna, Bologna, Italy, [2]Hematology, [3]Clinical Trial Office, [4]Pathology, Instituto Portugues de Oncologia de Lisboa, Lisboa, Portugal

Background: Diagnosis of lymphomas with DLBCL morphology and *MYC, BCL2* and/or *BCL6* breaks (double hit, DH) is rare but relevant given the poor outcome with conventional treatment and possible treatment-escalation benefits suggested by retrospective studies.
Aims: To characterize the impact of *MYC*, *BCL2* and *BCL6* breaks in treatment decisions in DLBCL patients (pts) enriched for immunophenotypically defined germinal center type (GC) and to evaluate the relationship between breaks and MYC and BCL2 expression in a single center.
Methods: All DLBCL biopsies tested by Fluorescence *in situ* hybridization (FISH) for *MYC*, *BCL2* and *BCL6* breaks between 2010-2017 were reviewed. Cell of origin was defined according to Hans algorithm. Positivity cut-offs for MYC and BCL2 were ≥40% and ≥50%, respectively. FISH was mostly done in pre-selected GC cases and in additional pts with unusual morphological and/or clinical features. Pts characteristics and outcomes were reviewed and groups compared by Fisher exact test. The impact of FISH on clinical decisions was assessed as the number of pts needed to screen (NNS) in order to intensify treatment in one, calculated in two scenarios using the prevalence of DH and the number of treatment changes informed by FISH. The NNS to avoid one progression/death was calculated based on published data in DH pts comparing PFS with aggressive treatments *versus* RCHOP according to Altman *et al.* (B Med J 1999)
Results: 76 biopsies (73 diagnosis, 3 relapse) (9% of all DLBCL) underwent FISH; the proportion of pts analyzed increased over time, the majority (58%) after the publication of the revised WHO classification draft. The population (49% male, median age 57, 66% stage III/IV, 39% intermediate-high and high-risk IPI) included 43% pts older than 60 years, 16% transformed lymphomas and 30% pts with significant co-morbidities, including 9 previously exposed to anthracyclines. 85% were GC and 16% were double MYC/BCL2 expressors (DE). 16% (95%CI 8%>26%) had DH (*6 MYC/BCL2*, *5 MYC/BCL6* and 1 *MYC/BCL2/BCL6),* more frequently in the DE group (45% *versus* 9% in non-DE, p=0.01). 75% pts received RCHOP and 12% had aggressive regimens (mostly LMB96) based on clinical risk factors, age, fitness and FISH. Out of 12 DH cases 3 had aggressive regimens, only in one due to the FISH result; reasons for not escalating treatment in others included age, comorbidities and prior anthracyclines. Only 35/76 pts were fit for aggressive regimens; of those 3 were DH (9%, 95%CI 2-23%) and treatment was escalated in 2. Assuming a 9% prevalence of DH in fit pts and that 2/3 would change treatment based on FISH, the NNS for one therapeutic change would decrease from 76 to 17. For a median 8-month PFS with RCHOP (Petrich *et al*., Blood 2014), a 47% reduction of PFS events with aggressive regimens (HR=0.53; 95% CI 0.29-0.98) (Howlett *et al*., Br J Haematol 2015), a 16% prevalence of DH and 8% therapeutic changes, 469 pts (95% CI 313-11250) would need testing to avoid one progression/death at 8 months. If selection criteria for FISH included only fit pts, this would decrease to 102 (95% CI 68-2425).
Summary/Conclusion: This retrospective single center study suggests that from the therapeutic perspective DLBCL candidates for FISH can be restricted considering clinical information added to cell-of-origin and/or protein expression. This impacts on costs and feasibility of large scale testing while new targeted therapies with acceptable toxicity profiles for a predominantly elderly/co-morbid population are unavailable.

PB1766

R-VACOP-B+RT *versus* DOSE-ADJUSTED R-EPOCH IN FRONTLINE MANAGEMENT OF PRIMARY MEDIASTINAL B-CELL LYMPHOMA: SIMILAR EFFICACY WITH DIFFERENT TOXICITY PROFILE

E. Scomazzon[1,*], S. Imbergamo[1], A. Visentin[1], M. Riva[1], F. Lessi[1], A. Branca[1], T. Berno[1], G. Scapinello[1], M. Laurino[1], S. Pravato[1], S. Vio[2], M. Gregianin[3], C. Boso[4], F. Adami[1], G. Binotto[1], R. Zambello[1], G. Semenzato[1], F. Vianello[1], F. Piazza[1], C. Gurrieri[1], L. Trentin[1]

[1]Haematology and Clinical Immunology Unit, University of Padua, [2]Radiology Unit, University of Padua, Padua, [3]Nuclear Medicine Unit, S.Giacomo Hospital, Castelfranco Veneto, [4]Radiotherapy and Nuclear Medicine Unit, IOV-IRCCS, Padua, Italy

Background: Primary mediastinal large B-cell lymphoma (PMBCL) is a subtype of aggressive B-cell non-Hodgkin lymphoma, with a usual presentation being a bulky anterior mediastinal mass. It affects predominantly females in their third-fourth decade of life, who present signs and symptoms related to the mediastinal mass. Historically, R-CHOP (Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) has been the standard treatment for PMBCL, in combination with consolidative radiotherapy. An Italian retrospective study (Zinzani PL *et al.* 2009) showed that regimens R-

MACOP(Rituximab/methotrexate/leukovorin/cyclophosphamide/doxorubicin/vincristine/prednisone/bleomycin)/R-VACOP-B (Rituximab, etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin) with radiotherapy is an effective therapy for the treatment of PMBCL; recently, a comparison study demonstrates a superiority of Dose Adjust (DA)-R-EPOCH (rituximab, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin) towards R-CHOP. In literature there are no comparison study between DA-R-EPOCH and R-VACOP-B.
Aims: The aim of this study was to compare the long-term efficacy and the most relevant side effects of the R-VACOP-B and DA-R-EPOCH regimens in PMBCL patients from a single institution.
Methods: We conducted a retrospective study on 49 patients (30 females, 19 males) affected by PMBCL, based on WHO criteria 2008, referred to the Hematology Division of Padua University Hospital. Median age at the diagnosis was 33 years (18-58). Fifteen patients (30.6%) were treated with DA-R-EPOCH with a median of 6 cycles (6-8), 34 patients (69.3%) were treated with R-VACOP-B followed by consolidative radiotherapy (RT) (30 Gy in 17 fractions).
Results: During a median follow up of 63 months, 8 patients out of 49 relapsed and 4 died (3 for progression, 1 for heart attack). The estimated 5-year progression free survival (PFS) and OS for the whole population were 81.8% and 92.5%, respectively. In the R-VACOP-B group with a follow up of 95 months, we found a 5-years PFS of 82% and a OS of 97%, for the DA-R-EPOCH group the median follow-up is shorter (13 months) with a 1-year PFS and a OS of 89% and 84%, respectively. Regarding the serious adverse events, we found an increase of haematological toxicities in the DA-R-EPOCH group ($p<0.00001$) without a significant incidence of major infections. We found a similar rate of cardiological events between the two groups, but a higher pulmonary toxicity in the R-VACOP-B + RT group ($p<0.0006$).
Summary/Conclusion: With the limitations of a retrospective survey with a small number of patients, our data suggest that both regimens are equally effective for the treatment of PMBCL. Regarding the toxicities, DA-R-EPOCH causes more G3/G4 neutropenia than R-VACOP-B regimen, with no increase in major infections. Likewise, there is no difference in the cardiological toxicity between the two regimens probably due to the presence of a comparable cumulative dose of anthracyclines in both treatment schemes. On the contrary, Bleomycin and consolidative mediastinal radiotherapy in the R-VACOP-B regimen may account for the significant difference in pulmonary side-effect observed. In conclusion, our study provide evidence that both DA-R-EPOCH and R-VACOP-B regimens are very effective for the treatment of PMBCL, with response rates higher than those reported in the literature for R-CHOP+RT, DA-R-EPOCH showed a worse though manageable toxicity profile than R-VACOP-B + RT, however, in the latter regimen the role of consolidative RT is still controversial.

PB1767

A PHASE IB, OPEN LABEL, MULTICENTER TRIAL OF PIXANTRONE, ETOPOSIDE, BENDAMUSTINE AND, IN CD20-POSITIVE TUMORS, RITUXIMAB (PREBEN) IN RELAPSED AGGRESSIVE LYMPHOMAS OF B- OR T-CELL PHENOTYPE

J. Jørgensen[1], S. Leppä[2], T. Relander[3], T.S. Larsen[4], G.F. Lauritzsen[5], P. Brown[6], S. Mannisto[2], G. Minotti[7], P. Menna[7], H. Holte[5], K. Liestol[8], H. Toldbod[1], F. Damore[1,*]

[1]Dept. of Hematology, Aarhus University Hospital, Aarhus, Denmark, [2]Dept. of Oncology, Helsinki University Hospital, Helsinki, Finland, [3]Dept. of Oncology, Skane University Hospital, Lund, Sweden, [4]Dept. of Hematology, Odense University Hospital, Odense, Denmark, [5]Dept. of Oncology, Oslo University Hospital, Oslo, Norway, [6]Dept. of Hematology, Copenhagen University Hospital, Copenhagen, Denmark, [7]Clinical Pharmacology Laboratory, University Hospital Campus Bio-Medico, Rome, Italy, [8]Dept. of Informatics, University of Oslo, Oslo, Norway

Background: Aggressive non Hodgkin lymphomas (aNHL) relapsing after high-dose therapy or, in not transplant-eligible patients (pts), after 1st line chemotherapy, represent an unmet clinical need. Therefore, we developed a salvage combination regimen based on pixantrone, an azaanthracenadione recently approved in Europe for pts with multiply relapsed or refractory aNHL, etoposide, bendamustine and, in CD20+ tumors, rituximab (PREBEN/PEBEN). A preliminary pre-trial experience on heavily pretreated aNHL pts showed good feasibility and efficacy and was previously reported. On this background, the Nordic Lymphoma Group launched an open label phase 1b/2 study (EudraCTno.201500075839) testing the feasibility and efficacy of the P[R]EBEN regimen in relapsed aNHL of B-or T-cell phenotype.
Aims: Here, we present the final results of the dose finding phase 1 part of the P[R]EBEN study.
Methods: According to predefined criteria, pts were subdivided in 'fit' and 'frail'. 'Fit' pts entered the phase 1 part of the study at baseline dose level. This consisted of 4 to 6 cycles of Pixantrone 50 mg/m2 i.v. day 1+8, Etoposide 100 mg i.v. day 1, Bendamustine 90 mg i.v. day 1 with or without the addition of Rituximab 375 mg/m2 i.v. day 1. 'Frail' pts entered directly the phase 2 part of the study at the same baseline dose level. Dose escalation was only applied to 'fit' pts and done according to a Bayesian model. Maximum tolerated dose (MTD) was based on the occurrence, within the first two cycles of therapy, of at least two DLT events (uncomplicated Grade 4 neutropenia lasting more than 5 days in patients without marrow involvement; grade 3-4 febrile neutropenia, or neutropenic infection; grade 4 thrombocytopenia; grade 3 drug-related non-hematopoietic toxicity with the exception of nausea, vomiting and alopecia). Primary end-points were identification of the MTD in phase 1 and overall response rate (ORR) in phase2. MTD was defined as the Response evaluation was performed by PET/CT after cycle 2 and at the end of therapy (EOT).
Results: In total, five pts were treated in the phase 1 part of the study. They had a median age of 60 years (range 39-68 years). Four of the pts (3 males and 1 female) had diffuse large cell B-cell lymphoma (DLBCL) and one (male) peripheral T-cell lymphoma (PTCL). All had relapsed disease after autologous transplant. The median time from last treatment to relapse was 20 months (range 8-86 months). All the chemotherapy courses were administered in an out-patient setting. No toxic deaths occurred. A total of 6 SAEs were reported among 4 pts: one grade 1 (transitory asymptomatic troponin T increase after cycle 3), one grade 2 (respiratory tract infection with non-neutropenic fever at cycle 6), three grade 3 (1 non-neutropenic fever at cycle 4, 1 neutropenic fever with anemia at cycle 4, 1 pneumonia/sinusitis at cycle 2), and one grade 4 (sepsis at cycle 3). All SAEs resolved without premature treatment interruption. No overt cardiac toxicity was recorded. Two DLT-defining events occurred within cycle 2 at baseline level, fulfilling MTD criteria. The two DLT defining events were: (i) neutropenic infection and (ii) post-therapeutic neutropenia (<0.5 mia/l) of >5 days in a pt without marrow involvement. PK measurements for pixantrone are ongoing. ORR was 60% after 2 cycles (1CR, 2 PRs) and at EOT (1 PR converted to CR: 2CR, 1PR).
Summary/Conclusion: The MTD of the PREBEN regimen was Pixantrone 50mg/m^2 i.v. day 1+8, Etoposide 100 mg i.v. day 1, Bendamustine 90 mg i.v. day 1 and Rituximab 375mg/m2 i.v. day 1. The schedule was feasible and the preliminary efficacy data are promising.

PB1768

A COMPREHENSIVE REVIEW OF PUBLISHED DATA ON THE TREATMENT OF RELAPSED/REFRACTORY (R/R) PERIPHERAL T-CELL LYMPHOMA (PTCL): AN EVIDENCE-BASED ASSESSMENT

E. Marchi[1,*], K. Tobinai[2], D. Maruyama[3], H. Nagai[4], O. O'Connor[1]

[1]Medicine, Columbia University Medical Center, New York, United States, [2]Geriatric Health Services Facility Rehabilitation Care Funabashi, Chiba, [3]Department of Hematology, National Cancer Center Hospital, Tokyo, [4]Department head of Hematology/Oncology Research, National Hospital Organization Nagoya Medical Center, Nagoya, Japan

Background: Peripheral T-cell lymphomas (PTCL) are a group of rare clinically aggressive heterogeneous diseases associated with a poor prognosis. Standard first-line chemotherapy achieves complete response (CR) rates ranging from 30% to 60% and long-term survival rates of only 15% to 25% at 5 years. Patients with relapsed/refractory (R/R) disease have an even worse prognosis with overall survival (OS) and progression-free survival (PFS) of 5.5 and 3.1 months, respectively. There is no consensus on the management of relapsed disease, primarily because the evidence supporting most treatment approaches is modest at best. Many approaches, deemed 'standard-of-care,' are often not well supported by the literature, and broad categorizations are commonly made regarding efficacy and toxicity without attention to differences between studies. Furthermore, treatments that have recently achieved regulatory approval with stringent independent assessment of pathology response are viewed as less established, or inferior to combination therapy.
Aims: In the effort to take a critical and comprehensive evidence-based approach to available standards in R/R PTCL we developed an objective scoring system for all types of studies published in the literature (*e.g.*, randomized phase 3, case match control, phase 2, phase 1, case reports and small series) to aid decision-making based on an assessment of all the available data.
Methods: We performed an extensive review on PubMed of the clinical trials published in the literature that included patients with R/R PTCL. We proposed a rigorous scoring system based on a survey from nearly 100 authorities in the field to assess the scientific impact of each study based on the agreed study characteristics, including type of study (*i.e.*, randomized

phase 3, case match control analysis, phase 2 weighted based on number of PTCL patients [≥100 *vs* <100 patients], phase 1 with ≥5 or <5 PTCL patients enrolled, and retrospective) weighted based on percent of PTCL patients included; weighting for inclusion of central pathology or response review; weighting for detailed reporting of study metrics (ORR, CR, DoR, PFS). The scoring system included a penalty of -0.5 each for ORR, DoR, or PFS not being reported and -0.2 for omitting previous lines of therapy. Studies that ranked 0 or below were all grouped under the minimum score of 0. The proposed scoring system was evaluated by a panel of experts belonging to different academic institutions in three different continents, actively involved in T-cell lymphoma clinical research. The scoring system was modified accordingly based on recommendations made by 2 or more of the panel members.

Results: We identified 58 original publications between 2004 and 2018 that involved clinical studies enrolling patients with R/R PTCL. The scoring system spanned from 0 to 9. Interestingly only 12 of the 58 studies had a score above 5; 15 out of 58 studies had a score between 1 and 5; and the remaining publications all scored between 0 to 1.

Summary/Conclusion: When compared to standard recommendations reported by various societies, our analysis suggests most practice patterns are based on studies with low priority scores, and underweight more robust clinical experiences. This analysis aims to guide physicians in critically evaluating the published literature when choosing salvage therapy for patients with R/R PTCL.

PB1769

MANAGEMENT OF RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA (MCL) IN ROUTINE CLINICAL PRACTICE IN SPAIN (IMORS STUDY). DESCRIPTIVE DATA AND EFFICACY RESULTS

R. Arranz[1,*], J.L. Bello[2], M.Á. Canales[3], J. Cannata-Ortiz[1], F.J. Capote[4], A. Cladera[5], J.Á. Hernández Rivas[6], A. Marín[7], A. Martín[8], M.J. Rodriguez Salazar[9], A. Salar[10], M.J. Terol[11], M.J. Villanueva[12], C. Castillo[12]

[1]S° Hematología, H.U. La Princesa, Madrid, [2]S° Hematología, C.H.U. Santiago, Santiago, [3]S° Hematología, H.U. La Paz, Madrid, [4]S° Hematología, H. U. Puerta del Mar, Cádiz, [5]S° Hematología, Son Llatzer, Manacor, [6]S° Hematología, H.U. Infanta Leonor, Madrid, [7]S° Hematología, H.U. Vall d'Hebron, Barcelona, [8]S° Hematología, H.C.U. Salamanca, Salamanca, [9]S° Hematología, H.U. Canarias, Tenerife, [10]S° Hematología, Hospital del Mar, Barcelona, [11]S° Hematología, Hospital Clínico Valencia, Valencia, [12]Medical Department, Janssen, Madrid, Spain

Background: Most of the patients with MCL have an unfavorable prognosis. It is a heterogenous disease that remains incurable despite progress in therapies developed with quite heterogeneous approaches in frontline and relapse scenarios.

Aims: This retrospective, multicentre, observational study describes and analyses the therapeutic strategies and their results in MCL from first line and beyond in routine clinical practice in Spain.

Methods: Patients included were diagnosed since 2005, older than 18 years with relapsed/refractory MCL and followed during a minimum of 6 months after the last treatment received. Quantitative variables were described by measures of central tendency and dispersion and qualitative variables by absolute and relative frequencies. Survival analysis were made by Kaplan-Meier Method. Analysis done with SPSS v18.0

Results: Primary endpoint: efficacy of treatments in terms of response, progression free and overall survival. **Secondary endpoints:** Description of demographic characteristics, morbidities, treatments and safety profile. 67 patients were analysed: 43 not candidates and 24 candidates to intensive therapy at diagnosis. Median age was 71 (range, 42-90 yo), blastoid histology 40%, 93% extranodal disease (86% bone marrow, 48% gastrointestinal) and 40% high-MIPI. Comorbidities: hypertension (43%), cardiovascular disease (40%), AF/Flutter (18%), thrombosis (12%) and liver disease (12%). Most concomitant treatments were: antihypertensive therapy 45%, antidepressants 38%, anticoagulants 34% and 24% antiplatelet agents. Treatment decisions were made by: routine clinical practice (2/3), clinical protocol (1/3). None treatment was discarded due to economic issues. *Main treatments in first line were*: R-CHOP (54%), R-HyperCVAD or R-CHOP/R-DHAP (39%), R-CVP (5%) and R-Bendamustine (3%), 19% received rituximab maintenance and 18% ASCT. For *second line*: R-Bendamustine (30%) and R-GemOX (19%), 21% patients received rituximab maintenance, 5% ASCT and 5% alloSCT. The rest were highly variable. For *third line*: R-Bendamustine (29%), ibrutinib (16%), temsirolimus (11%), radiotherapy (11%), 2% ASCT and 4% alloSCT. *In 4th line*, R-Bendamustine (33%), bortezomib combinations (25%) and ibrutinib (13%). Survival results with every line according to non-candidates /candidates to intensive therapy are shown in the table below.

Table 1.

Non candidates	N	Treatment duration (m, median [min-max])	Response (%)	CR (%)	PFS (m, median [95%CI])	Overall survival (m, median [95%CI])
First line	43	4,3 (0,7 – 30,9)*	79,1*	44,2*	19,5 (14,6 – 24,5)*	74,6 (41,1 – 108,2)
Second line	43	4,6 (0,2 – 41,9)	67,4	39,5	22,5 (15,6 – 29,4)	93,1 (1,3 – 64,8)
Third line	20	4,6 (0 – 15,3)	48,2	24,1	9,2 (6,6 – 11,8)	10,4 (2,9 – 17,9)
Fourth line	10	3,1 (0,1 – 14,9)	25,1	6,3	3,4 (0,4 – 6,4)	5,0 (0 – 20,6)
Fifth line	8	0,7 (0 – 5,7)	25	0	1,5 (1,5 – 1,6)	5,5 (0 – 11,1)
Sixth line	3	5,5 (1,9 -9)	1 patient	0	2 patients progression/exitus	2 patients exitus

Candidates	N	Treatment duration (m, median [min-max])	Response (%)	CR (%)	PFS (m, median [95%CI])	Overall survival (m, median [95%CI])
First line	24	7,0 (2,4 – 27,5)	91,7	79,2	35,9 (22,2 – 49,5)	NR
Second line	24	4,2 (0,3 – 47,7)	70,9	66,7	27,3 (11,1 – 43,6)	63,4 (33,5 – 93,4)
Third line	10	2,3 (0,1 – 16,9)	50,3	43,8	16,0 (4,3 – 28,9)	32,5 (20,4 – 44,6)
Fourth line	8	4,5 (1,8 – 6,5)	37,5	37,5	12,8 (2 – 23,7)	20,1 (9,2 – 31,1)
Fifth line	5	4,7 (0,5 – 8,9)	27	20	22*	27,9 (12,4 – 43,5)
Sixth line	3	6,9 (0,7 – 13,2)	2 patients	0	2 patients progression/exitus	2 patients exitus

*p=0,05 candidates vs. non candidates for intensive treatment; NR: not reached. *Standard error not available due to low N of patients

Summary/Conclusion: This study shows the highly heterogenous treatments in MCL in a sanitary scenario without economic restrictions. Overall survival differs according to intensive *versus* non intensive treatment in first line and, it markedly decreases from the second line onwards, especially in older patients. We need new therapeutic targets to improve the current outcomes.

PB1770

A PHASE 3 STUDY COMPARING POLATUZUMAB VEDOTIN PLUS R-CHP *versus* R-CHOP IN PATIENTS WITH DLBCL (POLARIX)

H. Tilly[1,*], C. Flowers[2], J.W. Friedberg[3], C. Herbaux[4], F. Morschhauser[4], L.H. Sehn[5], J. Sharman[6], M. Trněný[7], L. Wang[8], C. Lee[8], G. Salles[9]

[1]Centre Henri Becquerel, ROUEN CEDEX, France, [2]Winship Cancer Institute of Emory University, Atlanta, [3]University of Rochester Medical Center, Rochester, United States, [4]University of Lille, CHU Lille, Lille Cedex, France, [5]BC Cancer Agency, Vancouver, Canada, [6]US Oncology and Willamette Valley Cancer Institute, Springfield, United States, [7]Charles University, Prague, Czech Republic, [8]Genentech, Inc., South San Francisco, United States, [9]Hospices Civils de Lyon, Université Lyon-1, Lyon, France

Background: R-CHOP remains the standard of care in patients with previously untreated diffuse large B-cell lymphoma (DLBCL). However, patients with high-risk disease have poorer outcomes with R-CHOP. Polatuzumab vedotin (pola) is an antibody-drug conjugate targeting CD79b; it delivers the antimitotic agent MMAE and is being evaluated as a replacement strategy for vincristine within the R-CHOP regimen. In a phase Ib/II study in higher risk DLBCL patients, pola + R-CHP produced promising efficacy across different subtypes of DLBCL and a safety profile similar to that observed in the R-CHOP arm of the GOYA study (Tilly H, *et al.* Hematol Oncol 2017; Vitolo U, *et al.* J Clin Oncol 2017).

Aims: To evaluate the efficacy and safety of pola plus R-CHP compared with R-CHOP in patients with previously untreated DLBCL to see whether pola can improve outcomes in patients with low to high-risk disease.

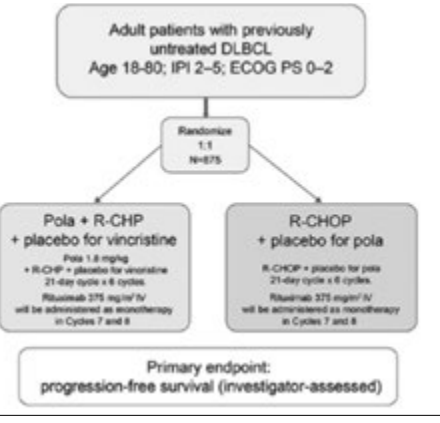


Figure 1.

Methods: This is a multicenter, randomized, double-blind, placebo-controlled, phase 3 study in patients with previously untreated DLBCL. Patients (planned N=875) aged 18-80 years with CD20-positive DLBCL (including DLBCL not otherwise specified [NOS], germinal center B-cell like [GCB],

and activated B-cell like [ABC] subtypes), ECOG performance status 0-2, and IPI score 2-5, will be randomized 1:1 to one of two treatment groups, stratified by IPI score (2 *versus* 3-5), bulky disease and geographical region (Figure). Arm A will receive pola 1.8 mg/kg on Day 1 plus R-CHP (standard dosing schedule) plus vincristine placebo for 6 cycles; Arm B will receive R-CHOP (standard dosing schedule) with pola placebo for 6 cycles. In both arms, R will be administered as monotherapy in cycles 7 and 8. The primary endpoint is progression-free survival (PFS), as assessed by the investigator, using the Lugano classification (Cheson B, *et al.* J Clin Oncol 2014). Secondary endpoints include PET-CT complete response rate at end of treatment assessed by an independent review committee, event-free survival due to efficacy reason, 2-year PFS rate, and overall survival. PET-CT and CT scans will be obtained at screening, after 4 cycles (planned interim assessment), and 6–8 weeks after end of study treatment. Patient follow-up will continue for 5 years after end of treatment.
Results: Enrolment began November 2017.
Summary/Conclusion: This phase 3 study, POLARIX, will evaluate clinical outcomes with pola plus R-CHP compared with R-CHOP in patients with previously untreated DLBCL. Clinical trial information: the study is funded by F. Hoffmann-La Roche Ltd; NCT03274492.

PB1771

CUTANEOUS MANIFESTATIONS OF ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA

N. Chernova[1,*], M. Sinitcina[2], Y. Sidorova[3], N. Soboleva[4], A. Sudarikov[3], A. Kovrigina[2], E. Zvonkov[1]

[1]Department Chemotherapy of Lymphomas, [2]Department of Pathology, [3]Department of Molecular Hematology, [4]Clinical diagnostic laboratory, National Research Center for Hematology, Moscow, Russian Federation

Background: Angioimmunoblastic T-cell lymphoma (AITL) is a rare form of non-Hodgkin's lymphoma, characterized by generalized lymphadenopathy and frequent extra nodal sites. Cutaneous lesions of AITL are observed in 38-58% of pts and often precede lymphadenopathy.
Aims: The aim of our study is to identify the genesis of cutaneous lesions of AITL.
Methods: Clinical data of 54 patients with newly diagnosed AITL we analyzed. Diagnosis was based on standard WHO criteria. The male/female ratio was 30/24; median age was 61 (29-81) years. Histological, molecular studies of skin biopsies were performed. To evaluate T-cell clonality TCRG and TCRB gene rearrangements were PCR-amplified according to BIOMED-2 standardized protocol and analyzed by capillary electrophoresis on ABI PRISM 3130 (Applied Biosystems). Sensitivity of T-cell clonality assay was limited to 10% of clonal -cells of the total T lymphocytes in the sample. Gly17Val RHOA mutation was analyzed by quantitative allele-specific (qAS) TaqMan Real-Time PCR assay. The detection level of this method was 1% of mutated cells in the total cell population.
Results: Cutaneous lesions were observed in 24 (44.4%) of 54 pts, they were more common in males (75%) than females (25%). Maculopapular rash was noted in 22 (91.7%) of 24 cases. Morphological and molecular studies of skin biopsies with maculopapular rash demonstrated reactive changes. The level of polyclonal IgE was elevated in patients with maculopapular rash. Tumor cutaneous lesions were detected in 8 (14.8%) of 54 pts and were represented by "livedo reticularis" in 3 cases, focal hyperpigmentation-in 2, erythroderma-in 1, tumor node-in 1 and plaques-in 1. Tumor and reactive cutaneous lesions were presented in 6 pts, in 4 (7.4%) of 54 cases they were noted at the same time. The type of cutaneous elements changed during of the disease in 3 (5.6%) pts.
Summary/Conclusion: Maculopapular rash is the most frequent cutaneous manifestation of AITL and it has a reactive genesis. Tumor lesion of the skin is rarely and can manifest by various cutaneous elements. In some cases AITL tumor and reactive cutaneous lesions can be observed simultaneously or sequentially.

PB1772

DA -EPOCH- R CAN CAUSE ADRENAL INSUFFICIENCY IN NON-HODGKIN'S LYMPHOMA PATIENTS

I. Vaxman[1,2,*], O. Katz-Beyar[3,4], A. Gafter Gvilli[1,5], A. Grossman[1,6], A. Schlesinger[1,7], N. Horowitz[3], O. Pasvolsky[1,2], E. Joffe[1,2], P. Raanani[1,2], U. Rozovski[1,2], M. Lahav[1]

[1]Sackler School of Medicine, Tel Aviv University, Tel Aviv, [2]Institute of Hematology, Beilinson, Petah tikva, [3]Rappaport Faculty of Medicine, [4]Department of Hematology and Bone Marrow Transplantation, Technion, Haifa, [5]Medicine A, [6]Medicine B, Rabin Medical Center, Petah tikva, [7]Home care unit of sharon shomron, Sharon Shomron, Tel Aviv, Israel

Background: Adrenal insufficiency (AI) is caused by insufficient production or action of glucocorticoids. The most frequent cause of AI is high dose and prolonged use of corticosteroids. However, there is evidence that even shorter periods of corticosteroid therapy may affect adrenal function. The standard protocol for diffuse large B cell lymphoma (DLBCL) is 6 to 8 cycles of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone), which includes five days of prednisone at a dose of 100 mg/d. DA- EPOCH- R (rituximab, etoposide, doxorubicin, vincristine, cyclophosphamide and prednisone) is an alternative protocol used in patients with aggressive lymphomas and especially for primary mediastinal B cell lymphoma (PMBCL). The protocol consists of higher doses of prednisone compared to R-CHOP (five days of prednisone 60 mg/m^2 twice daily). In both regimens, there is no recommendation for tapering down of corticosteroids or assessment of potential AI. Because the DA -EPOCH-R protocol includes higher dose of corticosteroids we hypothesized that it may induces higher rates of AI.
Aims: To determine the incidence of AI in non-Hodgkin's lymphoma (NHL) patients treated with R-CHOP or DA- EPOCH- R.
Methods: We prospectively enrolled patients with newly diagnosed NHL that received either DA-EPOCH- R (study group) or R- CHOP (control group) protocols. We excluded patients with known adrenal disorders, patients receiving corticosteroid treatment at any time during the past six months, known adrenal involvement with lymphoma and treatment with drugs that are known to cause a false positive ACTH test. We evaluated adrenal function by 250 microgram ACTH tests at 5 time points: prior to first treatment cycle, on day 5 or 6 of the cycle and 21 after the first cycle, at the completion of the 5th cycle and on the first day of the 6th cycle. A positive ACTH test was defined as a cortisol level of less than 500 nmol/liter at least in one of these test.
Results: Between May 2015 and May 2017, ten patients who received DA-R-EPOCH and 17 patients who received R-CHOP were enrolled. The median age at time of diagnosis was 60 years (range: 21-89) and 52% (14 patients) were females. The diagnosis was DLBCL in 19 patients and other (PMBCL, follicular lymphoma grade 3a, primary effusion lymphoma) in 8 patients. There was no difference between the two groups with regards to baseline characteristics in terms of blood pressure, hemoglobin or platelet levels, white blood cells, eosinophils, glucose, sodium or potassium levels. However, patients in the control group that received R-CHOP were significantly older than those in the DA- EPOCH- R arm (the median age was 63 in R-CHOP group and 34 in DA-EPOCH-R, p=0.001). Out of the 27 patients included in the trial 2 died at the time of data collection, both were in the R-CHOP arm. ACTH test was positive at least once during the study follow-up in 9 patients -7 patients who received DA -EPOCH-R and 2 patients who received R-CHOP (P=0.04). Tests were mostly positive on day 5 (8 patients. Patients with positive ACTH test were more likely to develop fever during treatment (P=0.04), but did not develop overt electrolyte disturbances, reduced mean arterial pressure or elevated eosinophil levels.
Summary/Conclusion: Our study demonstrates that patients who received DA- EPOCH- R were more likely to develop AI. We believe that clinicians should be aware of this limited adrenal response to stress and that the option of AI as an alternative cause of fever in these patients should be considered.

PB1773

PRIMARY PANCREATIC LYMPHOMA: REPORT ON 10 CONSECUTIVE PATIENTS

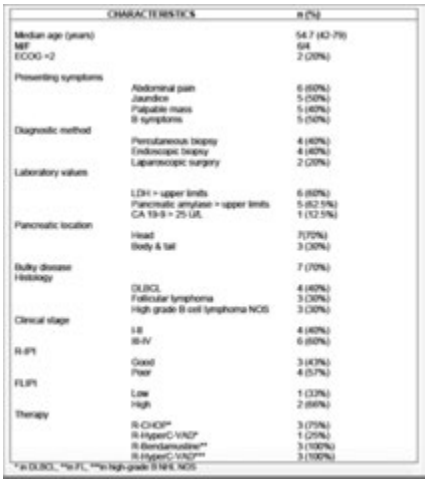
D. Facchinelli[1,*], A. Polino[1], A. Borin[2], E. Boninsegna[3], M.C. Tisi[4], C. Visco[4], M. Krampera[1], C. Tecchio[1]

[1]Department of Medicine, Section of Hematology, [2]Department of General and Pancreatic Surgery, [3]Department of Radiology, University of Verona, Verona, [4]Department of Medicine, Section of Hematology, San Bortolo Hospital, Vicenza, Italy

Background: Primary Pancreatic Lymphoma (PPL) is a very rare disease, representing only 0.1% of lymphomas and 0.2% of pancreatic tumors. According to the WHO classification the diagnostic criteria for PPL are: i) the bulk of the disease has to localize in the pancreas; ii) although adjacent and distant spread may be exist the primary clinical presentation has to involve the pancreatic gland. PPL presentation is variable and may overlap the onset of other neoplastic or inflammatory pancreatic diseases, often resulting in diagnostic difficulties. Histopathological examination usually reveals a diffuse large B cell lymphoma (DLBCL), nonetheless other types of lymphomas have been reported including the indolent ones. Diagnosis can be obtained through a percutaneous/endoscopic ultrasound-guided biopsy or surgery. An accurate histological characterization is essential because, unlike epithelial tumors, PPL does not benefit from surgery.

Aims: to evaluate retrospectively clinical, laboratory and histological characteristics, diagnosis, treatment, and outcome of a series of PPL patients diagnosed in 2 hematology units.
Methods: patients diagnosed with PPL between 2008 and 2017 were retrieved from the medical record databases of the Verona and Vicenza Hematology Units. Inclusion criteria were the availability of information related to clinical onset, histopathological diagnosis, laboratory tests, radiology imaging, treatment and follow-up. Patients with secondary pancreatic involvement of lymphoma were excluded. All cases herein presented were revised to ensure that they fit the latest WHO criteria for PPL.
Results: 10 patients with PPL were identified: median age was 54 years, 6 patients were males. Clinical manifestations included abdominal pain, jaundice, a palpable epigastric mass and systemic symptoms. None of these patients had a history of previous pancreatitis, one had a positive serology for hepatitis B. Laboratory tests evidenced a normal hemogram in all cases, while LDH and pancreatic amylase were augmented in 6 and 5 patients, respectively. CA 19-9 was slightly increased in 1 patient only. The histopathological diagnosis was obtained by percutaneous or endoscopic biopsy or surgery and revealed: DLBCL (n=4), follicular lymphoma (FL) (n=3) and high-grade B-cell lymphomas not otherwise specified (n=3). In 7 cases PPL was bulky and occupied the pancreatic head. All patients underwent a first line immune-chemotherapy: R-Bendamustine (n=3), R-CHOP (n=3) or R-HyperC-VAD (n=4). Only 2 patients with a histopathologica diagnosis of FL achieved a complete remission (20%). The 7 patients with aggressive lymphoma had a median progression free survival of 4 months and an overall survival of 24 months.

Table 1.



CHARACTERISTICS		n (%)
Median age (years)		54.7 (42-79)
M/F		6/4
ECOG >2		2 (20%)
Presenting symptoms		
	Abdominal pain	6 (60%)
	Jaundice	5 (50%)
	Palpable mass	5 (40%)
	B symptoms	5 (50%)
Diagnostic method		
	Percutaneous biopsy	4 (40%)
	Endoscopic biopsy	4 (40%)
	Laparoscopic surgery	2 (20%)
Laboratory values		
	LDH > upper limits	6 (60%)
	Pancreatic amylase > upper limits	5 (62.5%)
	CA 19-9 > 25 UI/L	1 (12.5%)
Pancreatic location		
	Head	7(70%)
	Body & tail	3 (30%)
Bulky disease		7 (70%)
Histology		
	DLBCL	4 (40%)
	Follicular lymphoma	3 (30%)
	High grade B cell lymphoma NOS	3 (30%)
Clinical stage		
	I-II	4 (40%)
	III-IV	6 (60%)
R-IPI		
	Good	3 (43%)
	Poor	4 (57%)
FLIPI		
	Low	1 (33%)
	High	2 (66%)
Therapy		
	R-CHOP*	3 (75%)
	R-HyperC-VAD*	1 (25%)
	R-Bendamustine**	3 (100%)
	R-HyperC-VAD**	3 (100%)

*in DLBCL, **in FL, ***in high-grade B NHL NOS

Summary/Conclusion: PPL represents a rare and difficult-to-recognize disease. Most patients in our series complained symptoms characteristics of pancreatic adenocarcinoma such as abdominal pain, jaundice, and weight loss. However increased LDH serum levels and normal CA19-9 were associated to a diagnosis of NHL, as confirmed by histopathological analysis. The poor outcome of patients with high-grade PPL suggests the need of aggressive first-line immune-chemotherapy regimens, possibly followed by autologous stem cell transplantation. Obviously larger series of patients are awaited in order to drawn definitive conclusions on this rare extranodal NHL.

PB1774

PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA-THE ROLE OF MEDIASTINAL RADIATION

S. Afonso*, I. Carvalhais, A. Carneiro, F. Príncipe, J.E. Guimarães

Department of Clinical Hematology, Centro Hospitalar de São João, Porto, Portugal

Background: Primary mediastinal large B-cell lymphoma (PMLBCL) is a rare subtype of diffuse large B-cell lymphoma. The most standard approaches include a combination of immunochemotherapy and mediastinal radiotherapy (RT).
Aims: Because mediastinal RT is associated with significant long-term toxicities, it was necessary the development of effective therapeutic strategies (rituximab with increased dose intensity regimens) that changed the need for routine RT.
Methods: Fifteen patients with diagnosis of PMLBCL between 2005-2015 were treated according to protocol R-DA- EPOCH (infusional dose-adjusted etoposide, doxorubicin and cyclophosphamide with vincristine, prednisone and rituximab). Patients were classified into 3 risk groups (according to presence of pleural/pericardial effusion and IPI high/intermediate-risk or high-risk). Residual disease (RD) was evaluated by FDG-PET scan and defined as score 2 according to Deauville Criteria and partial response (PR) defined as score 4-5 with a reduction >50% in size of mass.
Results: Median age at diagnosis was 29 (21-43) years, 86.7% of patients were aged<40 years (n=13). Eleven patients (78.6%, n=14) had bulky disease (tumor mass ≥10 cm) and 6 (42.9%, n=14) had superior vena cava syndrome (SVCS) at presentation. Presence of pleural/pericardial effusion in 7 patients (53.8%, n=13) and pulmonary involvement in 5 (38.5%, n=13). According to the prognostic score, two patients (15.4%) were classified as high risk (2 adverse factors), five (38.5%) in intermediate risk (1 factor) and six (42.2%) in low risk (0 factors). All patients were treated with 6 cycles of immunochemotherapy. Seven patients (46.7%) achieved complete response (CR), confirmed by FDG-PET in five. Another 3 (20%) had RD. All patients who achieved CR/RD did not perform RT with an event-free survival (EFS) of 100%. PR was attained in 3 patients (20%), two had high-risk disease. All patients in PR were submitted to RT, reaching CR (confirmed by FDG-PET) without relapse during the follow-up time. Progressive/stable disease was observed in 2 patients (13.3%). They were submitted to autologous stem cell transplant reaching CR. With a median follow-up of 64 (26-128) months, an overall survival/EFS of 100% was reached without evidence of disease. No cardiac events or secondary tumors were observed, so far.
Summary/Conclusion: The use of therapeutic approaches with rituximab and increased dose intensity regimens (like R-DA-EPOCH) has shown excellent efficacy and challenge the need for mediastinal radiation (5-years OS and EFS >90%, according to data from several studies). In conclusion, our results indicate that patients who had CR/RD evaluated by FDG-PET after treatment with R-DA-EPOCH, attained an EFS was 100% with a median follow up of 64 months. In these patients, the use of R-DA-EPOCH obviated the need for routine mediastinal RT. In patients with persistent disease after treatment, RT is a necessary approach though.

PB1775

R-DA-EPOCH AS OUTPATIENT REGIMEN FOR AGGRESSIVE NON HODGKIN B CELL LYMPHOMA: A SINGLE CENTER EXPERIENCE

F. Cocito[1,*], I. Casaroli[1], E. Doni[1], F. Rossini[1], L. Verga[1], A. Aroldi[1], F. Brioschi[1], C. Borella[1], C. Gambacorti Passerini[1,2], B. Silvia[1]

[1]Hematology, Ospedale San Gerardo-ASST Monza, [2]Department of Internal Medicine, University of Milano-Bicocca, Monza, Italy

Background: R -CHOP still remains the standard of care for large B cell Lymphoma(LBCL) patients(pts) but it's clearly unsatisfactory for about 30-40% of them, especially for pts characterized by high-risk molecular profile,carrying expression of MYC and BCL2 proteins (Double Expressor Lymphoma[DEL]) associated or not to the underlying translocation(Double Hit Lymphoma[DHL]).More intensive programs and in particular R-DA-EPOCH scheme obtained good results in retrospective studies. We report our prospective experience with this program.
Aims: to analyze the outcome in terms of response, Progression free survival (PFS), Overall Survival (OS), feasibility and toxicity of consecutive pts with Diffuse Large B cell Lymphoma(DLBCL),High grade Lymphoma(HGL) or Primary mediastinal B cell Lymphoma(PMBCL) diagnosed in a single Italian hematological center and all treated with the R-DA-EPOCH, which was delivered either as an inpatient regimen or administered on an outpatient basis using ambulatory infusion pumps.
Methods: We reviewed chart data of 36 LBCL pts consecutively diagnosed from December 2014 to February 2018 at our center. For pts diagnosed since 2016 we introduced the new category of HGL according to WHO 2016.
Results: Median follow up of our pts is 533 days (42 -1154). Regarding pts characteristics 23/36 (63.9%) are male, 13/36 (36.1%) female. Response is available for 33/36 pts, 1 is waiting for restaging and 2 pts are still receiving therapy at time of analysis. Median age at diagnosis was 50 years(range 22-69). Considering histology 23/36 (63.9%) were DLBCL, 6/36(16.7%) HGL and 7/36 (19.4%) PMBCL. Median ki67% was 80% (range 50- >90). Regarding molecular profiles a total of 18/36 (50%) pts show bcl2 expression,28/36 (77,8%) are bcl6 and 7/36 (19,4%) myc positive. In our series 4/36(11,1%) pts were DEL (bcl2 and myc positive) but FISH data were available only for 2 pts and only 1 resulted DHL. Regarding the disease stage 17/36(47,2%) have IPI score 0-2 and 19/36 (52,8%) IPI score 3-5. Twenty out of 36 pts (55,6%) have an extra-nodal (EN) localization at diagnosis; 15/36 (41,7%) >1 EN localization. Of note 4/36 (11,1%) were HIV positive pts. Data regarding the feasibility of this approach are available for all pts: 28/36(77,8%) pts completed six planned cycles and 23/36(63,9%) received therapy on an outpatient basis (123 outpatient cycles administrat-

ed).Four pts didn't complete 6 cycles because of disease progression. Dose escalation was possible in 23/36(63.9% [95% C.I. 57%-85%]) pts, but rarely in pts >65 yrs (only in 2/36 [5,6%, 95% C.I. 1.7%-17%]). The median dose level reached was 2 (range level -1-5). Grade4 neutropenia occurred in 36/36 (100%)pts, thrombocytopenia grade 4 in 8/36 pts (22,2%). A total of 15/36 (41,7%) pts had an infectious event and 10/36(27.8%) had therapy delay due to toxicities. Grade 3/4 non-hematological toxicities, especially neurotoxicity, were infrequent and manageable. We observed cardiotoxicity in 2/36 pts, which was related to tumor lysis syndrome and HIV infection. Among pts evaluable for response, 27/33(81,8%) obtained a complete remission (CR). A total of 6/36 pts died, all due to disease progression. PFS at 2 years was 74,7% (Figure 1). OS at 2 years 77%.

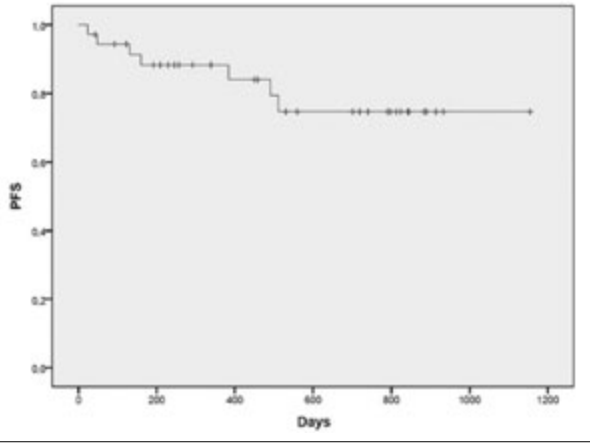


Figure 1.

Summary/Conclusion: Our results confirm R-DA-EPOCH as a feasible program with promising response rate.This scheme allows to obtain CR in 81,8% of pts with an acceptable toxicity profile. Especially in patients with high risk DLBCL (high IPI score, DEL and DHL) R-DA-EPOCH is an alternative to standard induction therapy which can be administered as an outpatient regimen in the majority of pts.

PB1776

R-HCVAD/MTX-ARA-C AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN MANTLE CELL LYMPHOMA-RETROSPECTIVE ANALYSIS OF A SINGLE INSTITUTION EXPERIENCE IN THE RITUXIMAB ERA

P. Chorão[1,*], M. Badior[2], I. Carvalhais[1], A. Carneiro[1], F. Príncipe[1], J.E. Guimarães[1,3]

[1]Clinical Hematology Department, Centro Hospitalar São João, Porto, [2]Clinical Hematology Department, Centro Hospitalar Vila Nova de Gaia/Espinho, Gaia, [3]Faculty of Medicine of Porto University, Porto, Portugal

Background: In fit patients with mantle cell lymphoma (MCL), intensive chemotherapy containing rituximab (R) and cytarabine (Ara-C) followed by autologous stem cell transplantation (ASCT) is the backbone for treatment. Despite new data on novel induction schemes, reports on R-HCVAD/MTX-Ara-C (R-hyperfractionated-cyclophosphamide-doxorubicin-vincristine-dexamethasone and R-methotrexate-Ara-C) are lacking.
Aims: To analyze the outcome of MCL patients treated with R-HCVAD/MTX-Ara-C followed by ASCT.
Methods: From 2005 to 2017, data on MCL patients treated with R-HCVAD/MTX-Ara-C (3+3 cycles) followed by BEAM (BCNU, Etoposide, Ara-C, Melphalan) and ASCT was retrospectively analyzed. Five-year survival (5y-OS) and progression free survival (5y-PFS) from time of ASCT was calculated.
Results: A total of 28 patients (6 were external referrals) received induction with R-HCVAD/MTX-Ara-C. Median age was 60 years (interval 42-68) and 71% were male. At diagnosis, 96% had Ann-Arbor stage IV, 50% had B symptoms, 93% had ECOG performance status<2, 39% had increased lactic dehydrogenase, 57% had leukemic expression and 32% gastrointestinal invasion. MIPI was low-risk (LR) in 39%, intermediate-risk (IR) in 25% and high-risk (HR) in 36%. For the 22 patients treated in our institution, overall response rate was 86% with complete response (CR) rate of 64%. Three patients died due to disease progression (2 during treatment) and 1 patient was ineligible for ASCT because of cardiovascular toxicity. The 6 patients externally referred to ASCT were all in CR. Of the 24 patients who were submitted to ASCT the median age at transplantation was 60 years (17% had >65 years) and MIPI was LR in 46%, IR in 25% and HR in 29%. All patients made successful peripheral blood stem cell progenitors (PBSCP) harvest, 71% at the secondo R-MTX-Ara-C and the remaining in following cycles. Only 2 patients required plerixafor in addition to G-CSF. Median harvest for infusion was $2.90x10^9$ CD34+ cells per kilogram (interval 2.08-7.70), with no differences between harvesting at the second R-MTX-Ara-C or after (p=0.260). There was no mortality associated with BEAM conditioning and ASCT. All engraftments were successful, with median time to neutrophil recovery of 11 days (interval 9-14) and to platelet recovery of 17 days (interval 12-42). At the time of analysis, data was available on 22 patients (2 have yet to reach 100 days after ASCT). CR rate at +100 day was 100%, with 63% 5y-PFS and 77% 5y-OS after ASCT. Five patients relapsed and 4 deaths occurred (2 from lymphoma relapse and 2 due to secondary solid malignancies). Patients with HR MIPI had worst outcomes (median OS HR 58 months *versus* LR/IR not reached, p=0.007). Patients older than 60 years had inferior 5y-PFS (35% [median 59 months] for >60 years *versus* 82% for ≤60 years, p=0.022) but similar 5y-OS (57% *vs* 90%, p=0.060).
Summary/Conclusion: Even in an older and higher risk patient cohort, our results show similar outcomes to other induction schemes. R-HCVAD/MTX-Ara-C followed by ASCT remains a practical option for fit MCL patients.

PB1777

SERUM ALBUMIN IS A STRONG PREDICTOR OF SURVIVAL IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA TREATED WITH R-CHOP

L. Villela[1,*], E. Montaño[2], G. Ruiz-Argüelles[3], S. Baltazar[4], A. Isassi-Chapa[5]

[1]Hematology and blood bank of Centro Medico Dr. Ignacio Chavez, ISSSTESON, ISSSTESON, HERMOSILLO, [2]Hematology Service, Hospital General de México, Hospital Dr. Eduardo Liceaga, Ciudad de México, México, Secretaria de Salud, Mexico City, [3]Clinica Ruiz de Puebla, Clinica Ruiz de Puebla, Puebla, [4]Hematology Department, UMAE [25] IMSS, [5]Hematology and oncology department, ITESM, Monterrey, Mexico

Background: Traditional International Prognostic Index before (tIPI) and post-rituximab era[Revised IPI (R-IPI) and enhance National Cancer Comprehensive Network-IPI (NCCN-IPI)] has been used to obtained risk groups for overall survival (OS) and progression free survival (PFS). Serum albumin (SA) has proven to be a prognostic marker in many hematological malignancies, including diffuse large B-cell lymphoma (DLBCL), but it was not analized in any IPI. SA could be a surrogate for age, poor nutritional status, comorbid status, and aggressive disease.
Aims: Analyze in DLBCL patients the predictive factor of albumin in relation to OS.
Methods: From 2010 to 2016, 5 databases of public and private institutions from 4 different states were analyzed retrospectively. Where *de novo* DLBCL patients were identified and obtained 113 from 160 patients with next inclusion criteria: 18 years old and older, and R-CHOP treatment. Minimum the following clinical data for calculating tIPI,R-IPI and NCCN-IPI : Age, lactic dehydrogenase (LDH), performance status (PS, ECOG scale), number of extranodal disease (EN), Ann Arbor stage (localized *vs* Advance), and others like SA. Diagnosis of DLBCL was confirmed through review the initial pathology report. 47 patients were excluded because they did not have some variable before mentioned. Primary clinical outcome was defined as date of diagnosis to date of death or date of last contact for those censored. For the descriptive statistics the square Chi was used. Time-to event data (OS) were estimated using the KaplanMeier method. Hazard Ratios (HR) were used to identify potential risk factors in different risk groups conformed. To obtain a correct cut-off point for albumin, we used the ROC curve and later we evaluated the best positive and negative likehood ratio (LHR+ and LHR-, respectively).
Results: From 113 DLBCL-patients, median age was 56.5 (range: 19-89); 52% were women; 58% were advanced stage (III/IV Ann Arbor); age >60 y/o,47%, but 15% >75 y/o; ECOG 2, 28.3%; EN disease (≥1), 35.5%; LDH elevated, 57.2%; Albumin<4g/dL, 46%. The median follow-up of whole series was 19 months; the median follow-up depending on the response after treatment was for complete response (CR): 32 months, partial response (PR): 13.6 months and Progression/failure (P): 8 months. The OS curves creation for tIPI, R-IPI and NCCN IPI was in accordance with already reported in other series. The cut-off point for albumin using the ROC curve was ≤3.7g/dL (Sensitivity 63.4%, Specificity 80.6, AUC 0.77; p<0.001). Interestingly, the LHR+ in 19.32 and LHR- in 0.74 were found in accordance to 2.7g/dL cut-off point of albumin, thus identifying a value lower than the original point of 3.7 of albumin that could be associated with higher mortality. Then, we

can build two risk groups: one called low (3.7 to 2.8g/dL) and another ultra low-albumin (≤2.7g/dL). When we capture these groups in the OS curves, 3 groups can be identified perfectly (Figure 1).

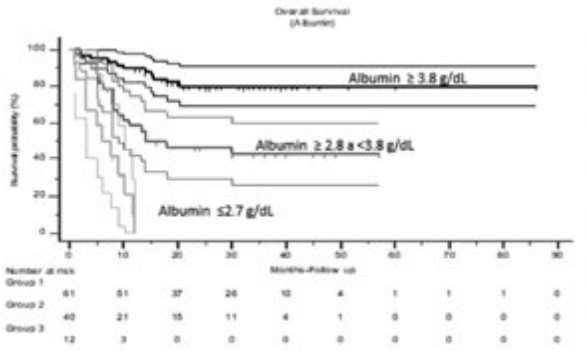


Figure 1.

Summary/Conclusion: The low and ultra low levels of serum albumin are a strong predictive factor for the early mortality of patients with DLBCL. These findings guarantee to evaluate this biomarker more closely

PB1778

FEASIBILITY OF PIXANTRONE CONTAINING R-CPOP AS FIRST LINE TREATMENT FOR PATIENTS WITH AGGRESSIVE B CELL LYMPHOMA WITH CONGESTIVE HEART FAILURE OR AT RISK OF ANTHRACYCLINE INDUCED CARDIOTOXICITY

R. Marks*, T. Strüßmann, U. Krohn, J. Duyster, J. Finke, K. Fritsch

University Hospital Freiburg, Freiburg, Germany

Background: Anthracyclines are essential in the treatment of aggressive B cell Non Hodgkin Lymphoma (NHL). Nevertheless, in clinical practice the substantial risk of developing drug induced cardiotoxicity, in particular congestive heart failure (CHF), results often in ommission of anthracyclines in first line treatment of this otherwise curable malignant lymphomas in patients at risk. Known cofactors for developing anthracycline induced CHF are cumulative anthracycline dose, female sex, age >65, diabetes, impaired cardiac function, and hypertension. No standard treatment for patients with aggressive B cell NHL having one of this cofactors exist. Pixantrone is an aza-anthracenedione showing reduced cardiotoxicity *in vitro* compared to standard anthracyclines. Recent data by Herbrecht *et al.* (Ann Oncol, 2013, 24:2618) showed that first line combined rituximab based immunochemotherapy treatment for DLBCL with the R-CPOP protocol (derived from R-CHOP in which doxorubicin is substituted with pixantrone 88mg/m^2) resulted in similar PFS when compared with R-CHOP, while reduced grade 3 CHF rates could be observed.
Aims: We wanted to explore the feasibility of R-CPOP treatment in aggressive B cell NHL patients with cofactors for developing anthracycline induced CHF.
Methods: In an unicenter setting we started in 2015 to use this protocol (6 cycles CPOP, 8x rituximab, every three weeks) in ten patients. Diagnoses included DLBCL (8 pat.), Richter transformation and high grade B cell lymphoma (1 pat. each). Median age was 72,4 years (range: 61-84) with an IPI 3-5 in 70% of patients. The main cardiac risk factor in all patients was known clinical CHF with a median left ventricular ejection fraction (LVEF) of 37% (range: 25-55). Known causes were ischemic heart disease in 5 patients. Three patients had exposure to anthracyclines in prior cancer treatment.
Results: Eight patients received four to six cycles of R-CPOP. Among those, treatment was well tolerated with hematological toxocities as expected in this patient cohort and no deaths due to neutropenic complications. No one experienced higher grade acute cardiac toxicity during treatment. Two patients did not complete the treatment, one with an initial LVEF of 25% experienced a deterioration of the CHF after the first cycle, the other with Richter transformation showed an early disease progress. While treatment is still ongoing, all of the remaining patients responded to R-CPOP with confirmed complete remission in 5 (62%) patients. This results in a median overall survival of 10 months (range: 2-31 months) so far.
Summary/Conclusion: In conclusion, using pixantrone in a first line combined R-CPOP treatment protocol for aggressive B cell NHL is feasible. An ongoing study (DRKS-ID: DRKS00000718) is active in recruiting patients and will further explore duration of response and incidence of longterm toxicity of this approach.

PB1779

PLEURAL EFFUSION IN PATIENTS WITH NON-HODGKIN LYMPHOMA

A. Roque[1,2,*], M.C. Afonso[1], M. Gomes[1], M.L. Ribeiro[1]

[1]Clinical Hematology Department, Centro Hospitalar e Universitário de Coimbra, [2]Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Background: Pleural effusion (PE) is reported in up to 20% of patients (pts) with non-Hodgkin lymphoma (NHL), and it is mainly due to pleural infiltration or lymphatic and thoracic duct obstruction. However, the risk factors for its development, as well as the evaluation of the diagnostic tools for lymphoma cells identification are not yet well established.
Aims: We aimed to evaluate the risk factors and diagnostic techniques for PE study in pts with NHL.
Methods: We analyzed all pts that performed a diagnostic thoracentesis and had a previous or simultaneous diagnosis of NHL, between January/2007 and December/2017. Inter-technique reliability was evaluated using the Cohen's kappa test. The overall survival (OS) between the groups was compared using a log-rank test, followed by subset analysis for estimated survival among patients using univariate Cox proportional hazard regression.
Results: During this period, we identified 55 pts with a median age of 76 years (26-89), 49.1% were males. The main NHL subtypes were diffuse large cell lymphoma (DLCL) (39.6%; n=21), follicular lymphoma (28.3%; n=15), chronic lymphocytic leukemia/small lymphocytic lymphoma (9.4%; n=5), marginal zone lymphoma (7.6%; n=4) and T-cell NHLs (5.7%; n=3). All the pts had stages III-IV. At the time of PE identification, 79.3% of pts (n=42) had mediastinal lymphadenopathies (28.6% bulky mediastinal disease) and 11.3% (n=6) were in leukemic form. 51.5% (n=31) pts had PE at presentation of NHL. Among those that developed PE later, during the course of the disease, the median time to diagnosis was 18.4 months; 20 had been treated with chemotherapy and 3 pts received mediastinal radiotherapy. In 17.0% of the pts the PE was bilateral; 37.7% had a chest tube drainage and only 13.2% had performed a pleurodesis. Considering PE biochemical characteristics, 84.9% pts (n=45) presented an exudate (79.2% with ≥2 Light's Criteria), 9.4% (n=5) a chylothorax and 5.7% (n=3) a transudate. Curiously, the presence of bulky mediastinal disease was not associated with the risk to develop chylothorax (OR 1.9; p=NS). The flow cytometry study was performed in 75.5% (n=40) pts and was positive in 80.5% (median effusion cellularity 3200 cells/uL; median infiltration by NHL cells 33%). NHL subtype was identified in PE in 75% of the cases. Cytology recognized abnormal cells in 42.6% pts. The consistency between flow cytometry and cytology to evaluate the presence of lymphoma cells in PE was moderate (κ=0.42). Effusion relapse occurred in 20.8% (n=11) pts, with a median time of 3.7 months. The identification of lymphoma cells in immunophenotyping (OR 2.6; p=NS) or morphological (OR 4.8; p=NS) studies did not modify the risk for relapse. Only 2 pts had bacterial growth in effusion cultures. However, although no pt had positive cultures for *Mycobacterium spp.*, 49.1% had increased levels of pleural adenosine deaminase (ADA) (>40 IU/L). The median OS for the cohort was 3.7 months. There was no difference in OS between pts with positive effusion flow cytometry (HR 1.5; p=NS) or cytology (HR 1.5; p=NS).
Summary/Conclusion: Our study shows a higher sensitivity in the identification of lymphoma cells in PEs by flow cytometry compared with cytology, with a moderate concordance between the two techniques. Pleural ADA levels had shown low specificity for tuberculosis pleuritic infection in NHL pts, suggesting that cultures for *Mycobacterium spp.* should always be performed. The presence of lymphoma cells in PE of NHL pts does not seem to have a prognostic relevance in OS.

PB1780

CIRCULATING EXSOMAL MIR-451A FOR THERAPY RESPONSE MONITORING IN DIFFUSE LARGE B CELL LYMPHOMA

C. Xu*, D. Cao, Y. Jiang, J. Xu, Y. Feng, C. Jing

West China Hospital of Sichuan University, CHENGDU, China

Background: Emerging evidence have indicated that Exosomal microRNAs(miRNAs) in serum could be used as noninvasive biomarkers for tumor early diagnosis and/or prognosis. Here we hypothesized circulating exosomal miRNA can work as a candidate for diffuse large B cell lymphoma (DLBCL) diagnosis.
Aims: To investigate the expression level and significances of therapy monitoring of circulating exsomal miR-451a in diffuse large B cell patients.
Methods: We isolated exsomal RNAs fractions from serum of DLBCL patients before treatment during treatment and after treatment, the serum of healthy controls was collected at the same time. Real time polymerase

chain reaction RT-PCR were performed to detected the expression level of circulating exsomal miR-451a.
Results: A total of 112 participates, including 56 DLBCL patients and 56 healthy controls were enrolled. Circulating exsomal miR-451a were down-expression in DLBCL compared with healthy controls(P<0.0001) and the area under the curve was 0.737 95%CI 0.645~0.816 . In patients who obtained remission including complete remission CR and partial remission PR , the levels of circulating exsomal miR-451a were gradually increased to healthy control. While in patients who did't get remission including stable disease SD and progression disease PD , the changes of the level without significant difference and still lower than healthy controls. The AUC was 0.867 95%CI 0.728~0.951 when compared remission group and non-remission group.
Summary/Conclusion: Circulating exsomal miR-451a are suitable for therapy response monitoring in DLBCL.

PB1781

LONG-LASTING RESPONSES WITH EXTRACORPOREAL PHOTOPHERESIS FOR SÉZARY SYNDROME. SINGLE CENTER EXPERIENCE

M.M. Andrade Campos[1,2,*], S. Sanchez Ortega[3], C. Muniesa[4], N. García-Muñoz[3], L. Martinez[4], E. Domingo Domenech[5], E. De la Banda[6], F. Climent[6], A. Sureda[5], E. Gonzalez Barca[7], O. Servitje[8]

[1]Department of Hematology, Duran I Reynals Hospital, Catalan Institute of Oncology, IDIBELL, Barcelona, [2]U-752 Research gruop on Hematology and Lysosomal disorders., CIBER Enfermedades Raras, CIBERER, IISCIII, Madrid, [3]Catalan Blood and Tissue Bank, Barcelona, [4]Dermatology, Bellvitge Universitary Hospital, [5]Department of Hematology, Duran I Reynals Hospital, Catalan Institute of Oncology, IDIBELL, [6]Department of Pathology, Bellvitge Universitary Hospital, [7]Department of Hematology, Duran I Reynals Hospital, Catalan Institute of Oncology, IDIBELL. University of Barcelona, [8]Department of Dermatology, Bellvitge Universitary Hospital, Hospitalet de Llobregat, Barcelona, Spain

Background: Sézary syndrome (SS) is a rare (7.7 cases per million per year) and incurable disease, it is one of the major variants of cutaneous T-cell lymphomas (CTCL). Extracorporeal photopheresis (ECP) is a process by which peripheral blood mononuclear cells are isolated from circulation by discontinuous leukapheresis, exposed ex-vivo to pro-apoptotic doses of 8-methoxypsoralen and ultraviolet A radiation and reinfused. Nowadays is a technique used for different lymphocyte-mediated diseases, including CTCL, graft-versus-host disease and solid organ transplant rejection.
Aims: To analyze response rate and duration of response of ECP for advanced stage MF/SS.
Methods: Patients >18y.o, diagnosed with SS not previously transplanted treated and treated with ECP were consecutively included since August 2015. The ECP protocol included 2 procedures administered on 2 consecutive days at 2-weeks intervals until the procedure number 20, when an evaluation was performed (ECP20) assessing the skin (dermatological) and hematological responses according to Olsen criteria (Olsen *et al*., JCO 2011;29(18):2598-607). For complete dermatological response (CDR): 100% skin clearance, partial DR (PDR): 50-99% clearance of lesions without new tumors, stable D disease (SDD):<25% increase or<50% clearance of skin lesions, progressive D disease (PrDD) >25% increase or new tumors, for blood involvement: complete hematological response (CHR): B0, partial HR (PHR): >50% decrease in Sézary cells, progressive H disease (PrHD): move form B0 to B2 or >50% increase in blood burden with at elast 5,000 Sézary cells/microL, stable HD (SHD): fails to CHR, PHR or PrHD. In cases without response or progressive disease (PD), ECP was stopped; in cases with partial response (PR), bi-weekly ECP is continued until procedure number 30, in cases with complete response (CR), ECP continued 2 consecutive days every 4-week with progressive withdrawal in 10 procedures.
Results: 9 patients were eligible for ECP, 7 completed at least 20 procedures and were included in the analysis, 1 patient discontinued therapy due to PD after 18 ECP, and 1 patient due to a new diagnosis of a degenerative disease (lateral amyotrophic sclerosis). Among the 7 patients that could be evaluated at ECP20, 4 were males, mean age was 62 y.o (range: 36-72), and stage was IVA1in 5 and IVA2 in 2. In 4 patients, ECP was started in progression at a median time from diagnosis of 10 months (limits 1-101) (median previous lines 3), and in 3 cases was used as front-line therapy combined with prednisone, retinoids or interferon. ECP20 evaluation showed CR in 1 patient and PR in 6. Specific 20ECP responses were: PHR+SDD: 2, PHR+PDR: 2, CHR+PDR: 2, CHR+CDR: 1. After ECP20, 3 patients (1CHR+CDR, 2 CHR+PDR) continued bi-weekly ECP until procedure 30 and then were switched to the 4-week schedule, the other 4 patients continued in the 2-weeks schedule. With a median follow-up of 19 months (limits 8-25), none of the 7 patients lost their response. Median duration of response (PFS since ECP20) was 14 (limits 23-21) months. Currently 1 patient continues in CHR+CDR, 4 are in CHR+PDR and 2 in PHR+PDR. The patient in CHR+CDR stopped ECP after 34 procedures and continued free of relapse 21 months later. No mortalities neither hospitalization were registered during therapy. Fig1 shows two illustrative cases.

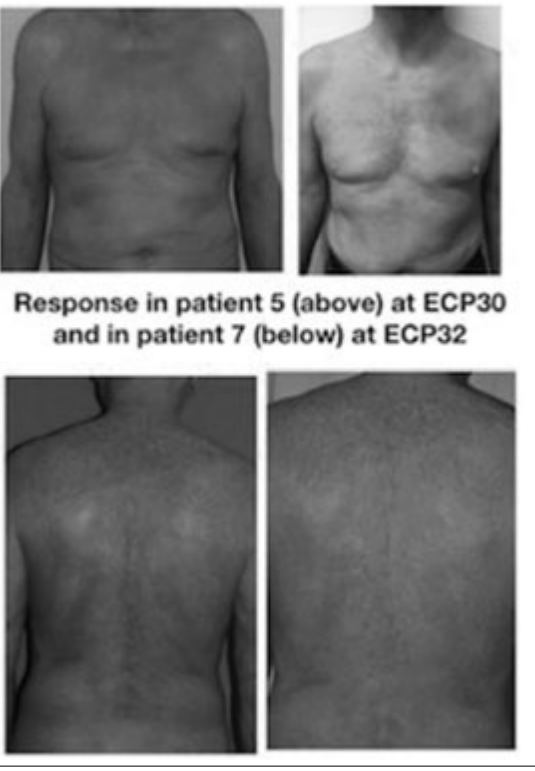


Figure 1.

Summary/Conclusion: Patients showed long-lasting responses with ECP without toxicity. Moreover, none of the responder patients at ECP20 lost their response during the follow-up.

PB1782

RISK FACTORS FOR HIGH DOSE METHOTREXATE ASSOCIATED ACUTE KIDNEY INJURY

I. Amitai[1,*], U. Rozovski[1,2], R. El-Saleh[2], P. Raanani[1,2], A. Gafter-Gvili[1,2,3], R. Gurion[1,2]

[1]institute of hematology, Davidoff Cancer Center, Rabin Medical Center, Petah-Tikva, [2]Sackler Faculty of Medicine, Tel Aviv University, Tel-Aviv, [3]Internal Medicine A, Rabin medical center, israel, Petah-Tikva, Israel

Background: High-dose methotrexate (HDMTX) is an important component in the treatment protocols of certain hematological malignancies. A significant complication of this treatment is renal failure. There is paucity of data regarding the occurrence of acute kidney injury (AKI) following HDMTX in hemato-oncological patients. Herein we aimed to explore the incidence and risk factors of AKI after HDMTX therapy.
Aims: 1. explore the incidence of AKI after HDMTX administration. 2. define the putative patients' baseline characteristics that predict the occurrence of AKI.
Methods: This is a single center, retrospective study. We reviewed the medical records of all consecutive patients who received a total MTX dose of 1500 mg or more. For these patients we collected demographic, clinical and outcome data. Acute kidney injury (AKI) was defined according to Acute Kidney Injury Network (AKIN) criteria (an increase in serum creatinine of at least 0.3 mg/dL). We compared patients with or without renal toxicity. For categorical variables, we used the χ^2 test. We used a logistic regression model with the exp(β) as an estimator of the odds ratio and the confidence interval around it in order to define which baseline variables, predict renal toxicity. We used receiver operator characteristics (ROC) curves and the area under the curve to define the best cutoff for continuous variables. The probability of overall survival (OS) was estimated by the Kaplan-Meier method. The log-rank test was used to compare survival distributions.
Results: Between January 2012 and December 2016, 136 patients received

at least one course of HDMTX in our Institute. Indications for HDMTX included: primary CNS lymphoma (n=12, 9%), other types of lymphoma (including as primary prophylaxis) (n= 96, 70%), acute lymphatic leukemia (n=24, 18%) or for various other diagnoses (n=4, 3%). The median age at diagnosis was 58 years (range 22 to 84), and 68 were males (50%). The vast majority were of good functional status, and 46 (34%) had at least one major comorbidity: vascular (n=18, 13%), diabetes (n=23, 17%) or prior malignancy (n=9, 7%). The median number of cycles per patient was 2 (range 1 to 10) and the median dose of MTX adjusted to body surface area (BSA) was 1670 mg/m^2 (range: 743 to 3865). The median time until MTX clearance was 5 days (range 3 to 20). During this time AKI developed in 12% of patients (n=16). In a univariate analysis for prediction of acute nephrotoxicity: age above 58 years (p=0.03), LDH above 470 units/L (p=0.02), MTX dose adjusted for BSA above 1640 mg/m^2 (p=0.015) and low levels of albumin at baseline (p=0.04) predicted acute MTX associated renal toxicity. In a multivariable analysis only age above 58 years remained predictive (Odds Ratio 4.6, 95% confidence interval 1.16-18.4). In 80% of cases, the creatinine levels returned to normal within 1 month. Yet, the median survival of patients who developed acute toxicity was only 38 months, while it was not reached in patients who maintained normal kidney function (Log rank= 0.06).

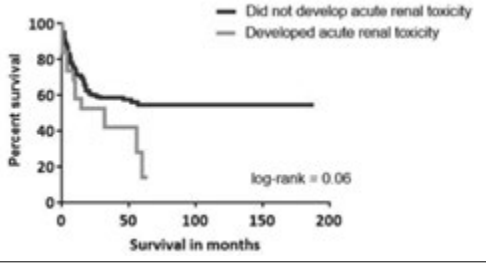


Figure 1.

Summary/Conclusion: Older age is the strongest risk factor which predicts AKI in patients receiving HDMTX. While the rise in creatinine levels is usually reversible, AKI was associated with increased mortality rates. Whether decreasing the MTX doses in the elderly population will improve outcome in these patients warrants further investigation.

PB1783

CLINICAL IMPACT OF PROGNOSTIC NUTRITIONAL INDEX IN DIFFUSE LARGE B-CELL LYMPHOMA

G.-W. Lee[1,*], S.-I. Go[1]

[1]Hematology and Oncology, Gyeongsang National University Hospital Gyeongsang National University College of Medicine, Jinju, Korea, Republic Of

Background: Cachexia is an indicator of tumor progression in patients with malignancy. Cachectic patients are intolerant to cytotoxic chemotherapy, exhibit a reduced response to antitumor therapy, and have an unfavorable prognosis. An international consensus proposed diagnostic criteria for cancer cachexia including weight loss, low body mass index (BMI), and/or presence of sarcopenia. Other potential biomarkers (e.g., albumin, C-reactive protein, pro-inflammatory cytokines, and microRNAs) and scoring systems (e.g., cachexia score [CASCO], Glasgow prognostic score [GPS], prognostic nutritional index [PNI]) have also been studied to diagnose cancer cachexia and assess its severity.
Aims: We evaluated the association between the prognostic nutritional index (PNI) and the clinical features of diffuse large B-cell lymphoma (DLBCL) and developed a novel prognostic model using a nomogram including the PNI and other biomarkers for cancer cachexia.
Methods: PNI was positively correlated with skeletal muscle index, body mass index, and serum levels of albumin. The low PNI group had a lower complete response rate (60.3% *vs* 87.6%), increased treatment-related toxicity, and more frequent treatment discontinuation (43.5% *vs* 8.8%) than the high PNI group. The median OS was shorter in the low PNI group than the high PNI group (15.6 months *vs* not reached; p<0.001). Multivariate Cox regression analyses showed that PNI, sarcopenia, and the international prognostic index (IPI) were independent prognostic factors for OS. The nomogram developed using this regression model showed excellent discriminatory ability for predicting OS (c-index, 0.80) compared to the IPI alone (c-index, 0.75).
Results: PNI was positively correlated with skeletal muscle index, body mass index, and serum levels of albumin. The low PNI group had a lower complete response rate (60.3% *vs* 87.6%), increased treatment-related toxicity, and more frequent treatment discontinuation (43.5% *vs* 8.8%) than the high PNI group. The median OS was shorter in the low PNI group than the high PNI group (15.6 months *vs* not reached; p<0.001). Multivariate Cox regression analyses showed that PNI, sarcopenia, and the international prognostic index (IPI) were independent prognostic factors for OS. The nomogram developed using this regression model showed excellent discriminatory ability for predicting OS (c-index, 0.80) compared to the IPI alone (c-index, 0.75).
Summary/Conclusion: Low PNI was associated with adverse clinical features in patients with DLBCL. The proposed nomogram supports the clinical impact of cachexia on survival and may contribute to individualized therapy in patients with DLBCL.

PB1784

RESULT OF TREATMENT OF PRIMARY LYMPHOMAS OF THE BRAIN

A. Jazyltayeva*, R. Karakulov, S. Gabbasova, E. Ishkinin, G. Sagindykov, B. Nassipov, N. Kemelbekov, A. Akanov

Hemoblostosis, Kazakh Scientific Research Institute of Oncology and Radiology, Almaty, Kazakhstan

Background: Primary non-Hodgkin's lymphomas occur in 3% of all brain tumors. This is a rare aggressive form of non-Hodgkin's lymphoma, localized in the brain, in the cerebral membranes, the spinal cord, in the orbit of the eyes.
Aims: The aim of the study is to evaluate five year overall and disease-free survival of patients with primary CNS lymphomas depending on the risk factors.
Methods: It is retrospective study of the 45 patients with primary lymphoma of the central nervous system (CNS) treated at the Kazakh Institute of Oncology and Radiology, from June 2006 to September 2016. The histological variants of the disease are presented in the form of diffuse large B-cell lymphoma in 28 patients, in the B-cell unspecified lymphoma in 16 patients, in the marginal zone-one patient. Mean age 48.4 (21-66). Women-24 (53.3%), men-21 (46.7%). Local forms-15 (33,3%), advance forms-30 (66,7%) of CNS lesion. The increase in LDH was registered in 32 patients. All patients received 3 cycles of chemotherapy (methotrexate 3.5 mg / m^2 1d, cytarabine 2 g / m^2 twice a day 2+3 d) with an interval of 21 days, following whole brain radiotherapy and for patients with PR adding boost for residual tumor.
Results: Complete response (CR) was achieved-28 (62.2%), partial response (PR) -16 (35.5%), progressive disease (PD) -one (2.2%). The OS for all patients 48.9%, median was 21.6 months, SE 17.4, CI 95% (0-55.6). Patient performance status assessed by Karnovskiy scale before treatment 63.3, in the end of treatment 72.7. The recurrences of the disease were registered 21 patients (46.7%), the majority of patients were included in the unfavorable group on the Extranodal Lymphoma Study Group scale. Seventeen patients had a local recurrence, two-spinal cord recurrence. Recurrence-free median 24.6, SE 5.5, CI 95% (13.8-35.3). According to the Extranodal Lymphoma Study Group scale we have analyzed risks factors such as age, tumor lesion. It was presented that in the group of age >50, OS 46.4%, median was 31.5 months, SE 4.2, CI 95% (23.4-39.7). Age<50 OS 52.9%, median was 37.5 months, SE 10.8, CI 95% (16.3-58.7). For age risk factor differences not significant (χ2=0,2 p=0,65). Recurrence for older than 50 years, and younger was 42.9% and 52.9% respectively, differences not significant (χ2=0,16 p=0,69). In the local forms, OS 66.7%, median was not achieved yet. In that time advance forms OS 40.0%, median was 15.7 months, SE 5.3, CI 95% (5.3-26.0). For dissemination risk factor differences significant (χ2=5,2 p=0,02). Recurrence for local and advance forms was 40% and 50% respectively, differences not significant (χ2=0,9 p=0,34). Infectious complications were diagnosed in seven patients. Neurological toxicity was observed in 12 patients, in the form of intense headaches, aggressive behavior and sleep disturbances after radiation therapy.
Summary/Conclusion: The analysis of the results obtained during chemotherapy for patients with primary CNS lymphoma, according to the prognostic factors (prevalence of the process, age), showed that relapses of the disease were more frequent in the group with unfavorable prognostic risk factors, the overall survival of patients in this group was lower than in a group with a favorable prognosis.

PB1785

VEINO-OCCLUSIVE DISEASE (VOD) DURING AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT): WARNING ABOUT SUSPECTED INCREASED INCIDENCE AFTER OXALIPLATIN-CONTAINING SALVAGE REGIMEN

P.-E. Debureaux[1,*], D. Le Febvre de Nailly[2], K. Belhadj[1], F. Le Bras[1], L. Roulin[1], C. Salvado[1], T. El Gnaoui[1], M. Hammoud[1], F. Lemonnier[1,3], D. Azoulay[3,4], J.P. Richardet[5], A. Luciani[3,6], S. Mulé[6], J. Calderaro[7], M. Carvalho[2], C. Haioun[1,3], J. Dupuis[1]

[1]Lymphoid Malignancies Unit, [2]Pharmacy Unit, Henri Mondor University Hospital,

[3]Paris Est-Créteil University (UPEC), [4]Department of Hepatobiliary and Pancreatic Surgery and Liver Transplantation, [5]Departement of Hepatology and Gastro-Enterology, [6]Department of Radiology and Medical Imaging, [7]Department of Pathology, Henri Mondor University Hospital, Créteil, France

Background: ASCT is a cornerstone in lymphoma therapeutics, especially in the relapse setting. BEAM (Carmustine, Etoposide, Cytarabine and Melphalan) conditioning regimen is the most widely used with a low toxic mortality rate. Bendamustine has been occasionally used as a substitute for Carmustine ("BendaEAM") during shortage periods. VOD is a potentially life-threatening complication, rarely observed after ASCT.
Aims: During the last 2 years, we observed 5 cases in patients treated with BEAM or BendaEAM in our department. Two patients required hepatic transplantation (HT).
Methods: We recorded cases of VOD observed in our department after ASCT. The new EBMT classification for VOD was used [1].

Table 1.

Age at ASCT/Sex	Type of lymphoma	First line	Second line / Third line	Risk factor of VOD before ASCT	Conditionning	VOD Grade	Delay ASCT-VOD	Treatment	Post ASCT Outcomes (months)
Five cases in Mondor between 2015 and 2018: DHAOX followed by ASCT									
44/F	MCL	R-DHAOx	None	Hydroxyurea, SGOT at 1.5 ULN	BEAM	Moderate	5	Support	Alive at 19m
58/M	FL	Mini-CVP+Rtx	BR+Rtx / R-DHAOx	None	Benda-EAM	Moderate	5	Defibrotide	Alive at 18m
62/M	DLBCL	R-CHOP21	R-DHAOx+Ibrutinib	Metabolic syndrom, Carmustin 80%	BEAM	Very severe	8	Defibrotide+HT	Dead at 1.5m
58/F	FL	R-CHOP21+ Rtx	R-DHAOx	None	Benda-EAM	Very severe	5	Defibrotide+HT	Alive at 29m
63/M	MCL	CALGB+DHAOx	None	Carmustin 80%	CALGB-BEAM	Severe	15	Defibrotide	Alive at 1m
Last case in Mondor in 2009									
41/F	HL	ABVD	BEACOPP	None	BEAM	Mild	12	Support	Alive at 90m

MCL : mantle cell lymphoma / FL : follicular lymphoma / DLBCL : diffuse large B-cell lymphoma / HL : Hodgkin Lymphoma
CVP : Cyclophosphamide Vincristine Prednisone / Rtx : Rituximab in abbreviation / BR : Bendamustine Rituximab / HT : Hepatic transplantation
CALGB : Chemotherapeutic / IFN : Interferon / ULN : upper limit normal

Results: Case 1: A 54 y.o. woman was diagnosed in 2010 with grade I-II follicular lymphoma. Complete remission (CR) was obtained after 6 cycles of R-CHOP21 and 2 years of Rituximab maintenance. Upon relapse in March 2015, a second CR was obtained after 4 cycles of R-DHAOX (Rituximab, Dexamethasone, High-dose Cytarabine, Oxaliplatin) delivered every 3 weeks [2]. ASCT was performed in the setting of the "BENEFIT" study (NCT02008006) using BendaEAM with Bendamustine 200 mg/m². VOD started at day 5 with ascites, painful hepatomegaly, weight gain (11%) and jaundice. Factor V dropped and hepatic encephalopathy occurred. At day 15, HT permitted recovery. At 29 months of follow-up, she is in persisting CR and didn't present any major complication of HT.Case 2: A 60 y.o. man was diagnosed in 2015 of diffuse large B-cell lymphoma treated by 8 cycles of R-CHOP. In May 2017, relapse occurred. Second CR was obtained with 3 cycles of R-DHAOX + Ibrutinib in the setting of the "BIBLOS" study (NCT02055924) followed by BEAM/ASCT. VOD started at day 8 with ascites, tender hepatomegaly, weight gain (8%) and jaundice. Low factor V and encephalopathy led the patient to receive an HT at day 12. After transient improvement, he died of septic choc. The same pattern was observed in 5 cases of VOD in last 3 years : R-DHAOx inductive salvage regimen followed by consolidative ASCT (conditioning by BEAM or BendaEAM). The last case before this period was a woman in 2009 who presented mild VOD after BEACOPP and BEAM/ASCT. Characteristics of these patients are described in table 1. None had major comorbidities. Between 2008 and 2017, incidence of VOD in our department following DHAOX/ASCT was 6.4% (5/78) *versus* 1.3% (1/75) after other salvage regimen/ASCT for lymphoma.
Summary/Conclusion: In the last decade, Cisplatin was often replaced by Oxaliplatin in the DHAP protocol in order to avoid renal impairment. Preoperative Oxaliplatin in hepatic metastatic of colorectal cancer leads to asymptomatic sinusoidal damage in half of patients and could explain the increase of VOD in ASCT [3]. We need to be more aware of risk in this setting and national report is ongoing. Management of VOD could include HT with limits about status of disease and multi-organ failure.

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PB1786

PROGNOSTIC FACTORS AND TREATMENT OUTCOMES IN PATIENTS WITH PERIPHERAL T-CELL LYMPHOMAS-A SINGLE CENTRE EXPERIENCE

T. Skrypets[1,*], O. Novosad[1], I. Pastushenko[1], T. Kadnikova[1], I. Tytorenko[1], O. Aleksik[1], Y. Stepanishina[1], K. Filonenko[1], A. Martynchyk[1], K. Ulianchenko[1], E. Kushchevyy[1], O. Gorbach[2], I. Kriachok[1]

[1]Oncohematology, [2]Experimental Oncology, National Cancer Institute, Kiev, Ukraine

Background: Peripheral T-cell lymphomas (PTCLs) are a rare heterogeneous group of lymphoid malignancies (5-15% of all non-Hodgkin lymphomas) with aggressive clinical behavior. There are three most common subtypes of PTCLs: Peripheral T-Cell Lymphoma (NOS), ALCL and AICL. Compared to B-cell lymphomas, which incidence remains stable, level of T-cell lymphomas have been rising continuously. Despite worldwide standard of first-line treatment, using anthracycline-based regimens, there is a need for better management of this patients. Unfortunately, due to lack of cases and randomized clinical trials, there are still inconsistency of specific prognostic factors in PTCLs.
Aims: The primary endpoint was event-free survival (EFS) and overall survival (OS), depending on PTCL type. We evaluated the influence on patients outcomes of different prognostic factors, which are included in PTCLs prognostic scores (IPI, IPTCL, PIT, mPIT).
Methods: We analyzed 50 patients (median age: 57, range: 22-80 years; males: 34, females: 16) with PTCLs treated from Sep 2006 to Feb 2018 in NCI (Kiev, Ukraine). Twenty two patients (44%) have PTCL (NOS), ALK –ve were 11 (22%) pts, ALK +ve-11 (22%) pts and 6 (12%) have unknown ALK status. 82% and 18% pts were younger and older than 65 y.o, respectively. They were assessed with IPI, IPTCLP, PIT and mPIT prognostic scores. Patients received CHOP-like chemotherapy regimens (CHOP, CHOEP, EPOCH).
Results: ORR was observed in 66% cases, progression on therapy had 34% pts. We registered 48% of relapses after the 1-st line therapy during the follow-up period (median-11 months; range 1–85 months). EFS was associated with LDH level (>620 U/l) and stage (III-IV) by ROC analysis [Sp=64.71%, Se=82.35%, AUC= 0.74, p=0,0011] and [Sp=66.67%, Se=65.38%, AUC= 0.66, p=0,03], respectively. 3-year EFS showed worse outcomes in pts with high LDH level 25% *vs* 75% in pts with normal LDH (p<0.05). Patients with stage I-II had better 3-years EFS compared to stage III-IV, 62% *vs* 15%, respectively (p<0.05). Also, multivariate analysis revealed that ECOG influence on EFS with HRs of 3.3 [95% CI 1.16–9.5, p=0.001]. The 3-year EFS in patients with ECOG<1 was 50% *vs* 15% with ECOG >1. Any significant difference was found between bone marrow involvement, age, gender, B-symptoms, Ki-67, albumin, ANC, platelets level and EFS rate. Also, there was no superiority between conventional CHOP *vs* more intensive CHOEP ana EPOCH (p=0.16). 5-year OS was 33%, 45% and 90% in PTCL (NOS), ALK –ve and ALK +ve, respectively (p<0.05).
Summary/Conclusion: PTCLs are still remaining a rare group of lymphoid malignancies, which are difficult to cure. Over the last decades, several studies showed prognostic evaluation in clinical and pathologic features of PTCLs. In our retrospective analyses we identified LDH level (>620 U/l), stage (III-IV) and ECOG >1, as the most valuable factors that predict a lower level of EFS in patients with PTCL (NOS), ALK –ve, ALK +ve lymphomas.

PB1787

PROGNOSTIC IMPACT OF PRETREATMENT ALBUMIN TO GLOBULIN RATIO IN ELDERLY PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH R-CHOP

S.-H. Kim[1,*], G.-W. Lee[2]

[1]Division of Hematology and Medical Oncology, Department of Internal Medicine, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, [2]Division of Hematology-Oncology, Department of Internal Medicine, Gyeongsang National University School of Medicine, Jinju, Korea, Republic Of

Background: Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma, representing about 30% of cases. The introduction of rituximab (R) in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone) chemotherapy, usually also known as 'R-CHOP,' improved survival outcomes, and has affected the importance of formerly recognized prognostic markers. In the pre-R era, the International Prognostic Index (IPI) was usually used to predict responses and prognoses in patients with high-risk non-Hodgkin's lymphoma, but in the post-R era, the original IPI could not well identify a poor prognostic group with under half risk for survival.
Aims: We evaluated the clinical implication of the albumin to globulin ratio (AGR) in patients with diffuse large B-cell lymphoma (DLBCL) treated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP).
Methods: Data for 232 DLBCL patients treated with first-line R-CHOP from 2004 to 2017 were reviewed retrospectively. Patients with AGR ≥1.22

and<1.22 were assigned to the high and low AGR groups, respectively. Treatment response and survival were compared according to AGR.
Results: The low AGR group (median overall survival [OS]=26.87months; 95% confidence interval[CI]=4.19-49.55) showed a significant decrease in OS compared to the high AGR group (median OS=148.83months;95% CI=76.26-221.41;*p*<0.001). Progression-free survival (PFS) was also significantly decreased in the low AGR group (median PFS=14.29months; 95% CI=2.58-26.01) compared to the high AGR group (median PFS=148.83months; 95% CI=76.21-221.45; *p*<0.001). However, in a multivariate analysis, low AGR was an independent poor prognostic factor for OS (hazard ratio [HR]=0.55; 95% CI=0.35-0.86; *p*=0.008) and PFS(HR=0.54;95% CI=0.35-0.83; *p*=0.005) only in elderly.
Summary/Conclusion: Pretreatment AGR can be useful for predicting clinical outcomes and prognosis in elderly patients with DLBCL treated with R-CHOP. Further large prospective studies will be necessary to validate our findings.

PB1788

THE FREQUENCY AND CLINICAL FEATURES OF DOUBLE-HIT DIFFUSE LARGE B-CELL LYMPHOMA-THE EXPERIENCE OF A SINGLE INSTITUTE IN TAIWAN

Y.-C. Su[1,2,*], C.-H. Lee[3], Y.-K. Lin[4], S.-M. Hsieh[5], M.-L. Liu[5], T.-Y. Chao[2], J. Whang-peng[6], Y.-Y. Hsieh[2], W.-H. Cheng[2]

[1]The PhD Program for Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taipei, [2]Hematology-oncology, Taipei Medical University-Shuang Ho Hospital, New Taipei, [3]Pathology, Taipei City Hospital Heping Fuyou Branch, [4]Research Center of Biostatistics, College of Management, Taipei Medical University, Taipei, [5]Pathology, Taipei Medical University-Shuang Ho Hospital, New Taipei, [6]Cancer Center, Wan Fang Hospital, Taipei, Taiwan, Republic of China

Background: Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin's lymphoma and it accounts for about half of all lymphomas in Taiwan. The gene expression profiles (GEP) may confer some clues for clinicians to predict the prognosis. According to the GEP, DLBCL can be divided into 3 subtypes: germinal center type, activated B-cell type and type III. The germinal center B-cell (GCB) phenotype of DLBCL usually results in a good prognosis with a 5-year overall survival (OS) of 59%, while the 5-year OS of activated B-cell (ABC) phenotype is poor (about 31%). Recent studies showed DLBCL with concurrent translocation of Myc and BCL2/BCL6 (double hits), the so-called double-hit lymphoma (DHL) may have a dismal prognosis in spite of the GCB phenotype.The previous studies showed concurrent BCL2 and Myc translocations account for about 75% of DHL while BCL6 translocated DHL is less 25%. However,we did not have any data about the frequency and clinical features of DHL in Taiwan.
Aims: We would like to understand the frequency of double hits in patients with DLBCL in Taiwan and figure out their clinical features.
Methods: We retrieved 88 patients with newly-diagnosed DLBCL in the Shuang-Ho Hospital from 2009 to 2016. The translocations of Myc, BCL2 and BCL6 were detected by the fluorescence in-situ hybridization (FISH), using the break-apart probes of target genes. We tested all samples with the Myc probe first and both BCL2 and BCL6 translocations would be screened for samples with positive Myc translocation.
Results: Among 88 DLBCL patients, we found 15 patients with Myc translocation, accounting for 17% of all DLBCL patients. Meanwhile, there were 9 patients with concurrent Myc and BCL2/BCL6 translocations, account for 10.2% (Figure 1A). Among them, two-thirds (6 patients) were BCL6-translocated while the others (3 patients) had BCL2 translocation(Figure 1B). This finding was quite different from the results of prior reports in which BCL2 translocation accounted for the majority of the double-hit lymphoma. The median age of patients with DHL was 62 years(Table 1). In addition, the DHL group had a high ratio of advanced disease (88.9%), indicating a more extensive involvement at presentation. Meanwhile, the male gender was predominant in the DHL group (77.8%). Phenotype could not be identified in 1 BCL2-translocated patient because of lack of the residual sample. The others in this group were exclusively GCB phenotype. In contrast, two-thirds of BCL6-translocated DHL patients had non-GCB DLBCL. This result caused our attention because the majority of DHL patients were GCB phenotype in the prior reports. In our cohort, the median OS of DHL patients was 344 days, compatible with results of previous studies, while that of the non-DH DLBCL patients were 574 days (Figure 2). It seemed that the DHL patients survived worse than its non-DH counterpart. In spite of this, there was no significant difference between these 2 groups in OS and the major factor was probably the small sample size of the DHL group.

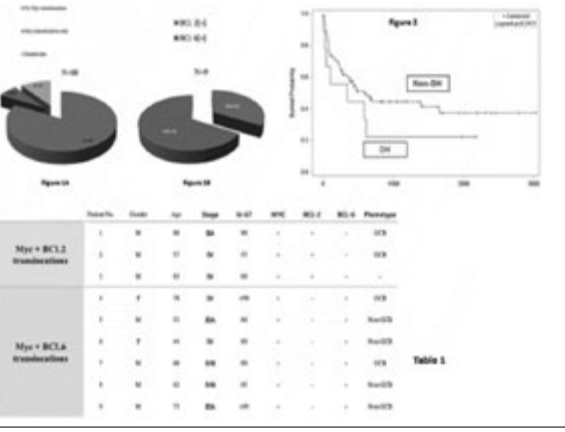

Figure 1.

Summary/Conclusion: The frequency of DHL in our institute was 10.2% and the majority of DHL were BCL6-translocated with an advanced disease(88.9%). The DHL patients seemed to have a worse prognosis than non-translocated ones without a clinical significance.

PB1789

OXIDATIVE STRESS EVALUATION IN DIFFUSE LARGE B-CELL LYMPHOMA

M.-A. Gaman[1,*], A.M. Gaman[2,3]

[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Bucharest, [2]Pathophysiology, University of Medicine and Pharmacy of Craiova, [3]Hematology, Filantropia City Hospital of Craiova, Craiova, Romania

Background: Diffuse large B-cell lymphoma (DLBCL) is an aggressive form of Non-Hodgkin's lymphoma (NHL) which can occur *de novo* or by transformation of a less aggressive NHL of B-cell origin such as follicular lymphoma (FL). The genesis of lymphoma has yet to be explained. Several studies have suggested that oxidative stress, via chronic inflammation, might play a role in DLBCL, since its involvement has been shown in aging and in many hematological disorders[1-8].
Aims: The major aim of this study is to evaluate the levels of reactive oxygen species and the total antioxidant capacity in patients with DLBCL compared to healthy controls before and after R-CHOP therapy. The minor aim is to observe if the level of HDL-cholesterol was related to the oxidative status of the study group.
Methods: We enrolled 32 Romanian patients with DLBCL and 20 healthy controls. Informed consent was obtained from all subjects involved. The positive diagnosis was based on lymph node biopsy with histopathological and immunohistochemical examination. The Ann-Arbor staging system was used for staging. The standard treatment consisted of 6-8 cycles of R-CHOP. Oxidative stress was evaluated at diagnosis and at the end of therapy using a CR3000 analyzer from a single drop of capillary blood. Reactive oxygen species were evaluated by FORT (Free Oxygen Radicals Testing) and the total antioxidant capacity by the FORD (Free Oxygen Radicals Defense) assays. The normal ranges for the assays are: FORT<2.3 mmol/L H_2O_2 and FORD=1.07-1.53 mmol/L. HDL-cholesterol was also evaluated. Statistical data analysis was performed using the student T-test and a p-value ≤0.05 was considered significant.

Table 1.

Stage	HDL [mg/dL]	FORT [mmol/L H_2O_2]	FORD [mmol/L]	Evaluation
IIB	67	2.5	1	At diagnosis
IIB	78	1.1	1.5	After treatment
IIIB	47.5	3.1	0.6	At diagnosis
IIIB	66	1.7	1.4	After treatment
IVB	32.5	3.6	0.3	At diagnosis
IVB	53.8	2.4	1	After treatment
Study group	47	3.1	0.6	At diagnosis
Study group	65	1.8	1.3	After treatment

Results: The study group had the following characteristics: median age=52.0 years, 62.5% males, 37.5% females, 75% patients from urban areas, 25% patients from rural areas, 93.75% *de novo* DLBCL cases, 6.25% cases transformed from FL. The control group had a median age=64.1±2.26 years. DLBCL staging revealed: stage IIB=5 cases, stage IIIB=19 cases and stage IVB=8 cases. The evolution was favorable with complete remission in 24 cases, 6 patients had partial remission after conventional treatment, and 2 patients had refractory disease. Before treatment, HDL-cholesterol value was normal (>60 mg/dL) in 9 patients (5 in stage IIB and 4 stage IIIB) and low in 23 patients. The results of oxidative status evaluation and HDL-cholesterol are depicted as mean values in the attached table. After treatment, FORD and HDL-cholesterol values significantly increased, whereas FORT values significantly decreased ($p \leq 0.05$), with the exception of the 2 cases of refractory DLBCL.
Summary/Conclusion: We found an unbalanced oxidative status in DLBCL patients compared to controls: FORT levels were increased, whereas FORD and HDL-cholesterol levels were decreased. FORT levels decreased, whereas FORD and HDL-cholesterol values increased after chemotherapy regimens. Further comprehensive studies must be performed to highlight the involvement of oxidative stress in DLBCL.

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PB1790

IMMUNOGLOBULINOPATHIES IN PATIENTS WITH ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA

N. Chernova[1,*], N. Soboleva[2], S. Maryna[2], Y. Sidorova[3], M. Sinitcina[4], V. Dvirnyk[2], E. Zvonkov[1]

[1]Department Chemotherapy of Lymphomas, [2]Clinical Diagnostic Laboratory, [3]Department of Molecular Hematology, [4]Department of Pathology, National Research Center for Hematology, Moscow, Russian Federation

Background: Angioimmunoblastic T-cell lymphoma (AITL) is a rare form of non-Hodgkins lymphoma, characterized by generalized lymphadenopathy, hepatosplenomegaly and dysproteinemia. Hypergammaglobulinaemia is revealed in 50–83% pts with AITL. However, the characteristics of immunoglobulinopathies observed in AITL are scarce.
Aims: The aim of the study was to characterize quantitative and qualitative immunoglobulinopathies in patients with AITL at the onset of the disease.
Methods: 55 patients with newly diagnosed AITL were enrolled in the study, the male/female ratio was 30/25; median age was 61 (29-81) years. Diagnosis was based on standard WHO criteria. Immunochemical studies of blood serum included serum protein electrophoresis/immunofixation, nephelometric quantification of total immunoglobulins, serum free light chain assay.
Results: Quantitative and qualitative immunoglobulinopathies were determined in 49 (89,1%) of 55 pts. Quantitative immunoglobulinopathies were revealed in 47 (85.5%) of 55 cases, qualitative-in 14 (25,5%). Combination quantitative and qualitative immunoglobulinopathies was observed in 12 (21,8%) of 55 pts. The detected immunoglobulinopathies were divided into 4 groups: polyclonal hypergammaglobulinaemia, hypogammaglobulinaemia, oligoclonal gammapathy, and monoclonal gammapathy. Polyclonal hypergammaglobulinaemia was marked in 41 (74.5%) of 55 pts, elevated level of IgG was determined in 27 (49,15%) of 55 cases, IgM-in 18 (32,7%) and IgA-in 21 (38.2%). Interestingly, polyclonal IgE hypergammaglobulinaemia was detected in 12 (48,0%) of 25 cases of performed studies. Simultaneous increase of serum level of *3 immunoglobulin classes* was observed in 8 (24.2%) of 40 pts, 2 classes-in 13 (39.4%), 1-in 12 (36.4%). Hypogammaglobulinaemia was detected in 8 (14,5%) of 55 cases. Oligoclonal gammapathy was determined in 4 (7.3%) of 55 pts. Monoclonal gammapathy was revealed in 11 (20,0%) of 55 cases. The amount of monoclonal immunoglobulin varied from 2.6 to 14.1 g/l. Monoclonal immunoglobulin Gk was detected in 5 of 11 pts, Gλ-in 2, Mλ-in 2, Mk-in 2. Monoclonal gammapathy was accompanied by polyclonal hypergammaglobulinaemia in 9 of 11 cases, hypogammaglobulinaemia-in 2.
Summary/Conclusion: Quantitative and qualitative immunoglobulinopathies are observed in most patients at the onset of AITL. Quantitative abnormalities were determined more often than qualitative. Monoclonal gammapathy can be a manifestation of lymphoproliferation and other concomitant disorders. The prognostic value of immunochemical parameters is still unclear and requires dynamic observation and study.

PB1791

PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA AND EXTRAMEDIASTINAL LESION

Y. Mangasarova*, A. Magomedova, A. Misyurina, E. Nesterova, S. Kravchenko

National Research Center for Hematology, Moscow, Russian Federation

Background: Primary mediastinal large B-cell lymphoma (PMBCL) is derived from a thymic B-cell and commonly presents as a bulky lesion in anterior-superior mediastinum. Extramediastinal lesions is an extremely rare situation and require differential diagnostics with diffuse large B-cell lymphoma (DLBCL).
Aims: To evaluate clinical features and treatment efficacy in patients with PMBCL with extramediastinal lesion.
Methods: From 2007 to 2018 years, 157 patients were diagnosed with PMBCL (according to WHO criteria) in National Research Center for Hematology Ministry of Health, Moscow, Russian Federation. Extramediastinal involvement was detected in 16 patients, 3 of them were at different stages of pregnancy. The median age of patients was 27 (23-69) years. 8/16 patients underwent molecular analysis for determination of gene overexpression JAK2, TRAF1, MAL, PDL1, PDL2. In all 8/8 of cases, overexpression of 2 or more genes was determined, which allowed confirming and in some cases to revise diagnosis in favour of PMBCL.
Results: In 69% of cases, nonlymphoid extramediastinal lesions below diaphragm were verified, which involved internal organs. In 31% of cases, there were multiple involvements of organs. The most frequently were noted involvement of kidney-4/16 of cases, ovarian-3/16 of cases, pancreas-3/16 of cases and bone marrow-3/16 of cases (confirmed by the molecular study). Gastric involvement was revealed very rarely-1/16 of cases, an involvement of bones-1/16 of cases, soft tissues involvement-1/16 of cases. In 15 from 16 cases, an isolated extramediastinal lesion was combined with involvement of antero-superior mediastinum and only in one from 16 patients was revealed isolated thoracic soft tissues involvement (without involvement of mediastinal structures).
Summary/Conclusion: in 15/16 of PMBCL cases extramediastinal lesions were revealed in addition to anterior-superior mediastinum involvement. In 1 of 16 cases, thoracic soft tissue involvement was not accompanied by presence of a tumour in mediastinum. This clinical feature required an additional molecular study that allowed to diagnose primary mediastinal large B-cell lymphoma. Thus, in case of mediastinal tumour and presence of extramediastinal lesions in young patients differential diagnosis between primary mediastinal large B-cell lymphoma and advanced stage diffuse large B-cell lymphoma with extramediastinal lesion should be performed.

PB1792

PRIMARY CNS LYMPHOMA: SINGLE INSTITUTION EXPERIENCE WITH THE 2015 GELTAMO PROTOCOL

A. Lancharro, C. Cañigral*, J. Clavel, A. Gascon, D. Linares, R. Garcia, M. Fernandez-Delgado, L. Serrano, I. Garcia, M. Mas, I. Cuadrado, P. Carrascosa, M.B. Contreras, G. Cañigral

Hematology, Hospital General De Castellon, Castellon, Spain

Background: Primary central nervous system lymphoma (PCNSL) is an uncommon variant of non-Hodgkin lymphoma, being immunodeficiency its main predisposing factor. Without treatment, the evolution of PCNSL is rapidly fatal. Published data support the use of penetrating chemotherapy in CNS to avoid the side effects of radiotherapy. In this sense, the GELTAMO group developed in February 2015 a protocol with carmustine, rituximab, cytarabine and high dose methotrexate (BRAM) followed by high-dose chemotherapy with carmustine and thiotepa and autologous stem cell transplant rescue (ASCT).
Aims: This retrospective study aimed to analyse the characteristics and outcomes of patients diagnosed of PCNSL in our institution since the implementation of the *2015 GELTAMO* protocol.
Methods: All adult (>16 years) patients with PCNSL diagnosed in our centre from February 2015 to February 2018 were identified. Clinical, histopatho-

logical and cytogenetic data at diagnosis were collected, as well as information of treatment outcomes and toxicities.

Figure 1.

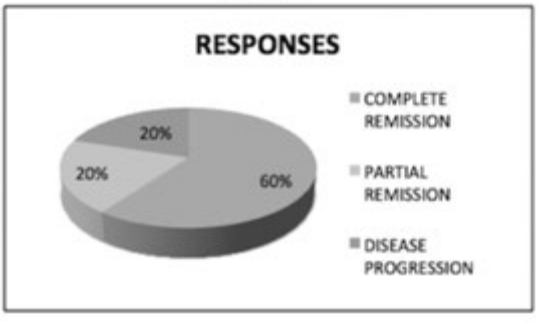

Figure 2.

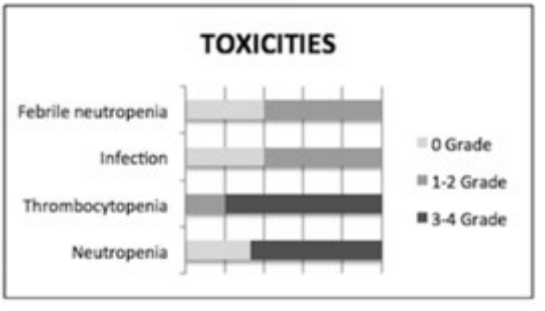

Figure 1.

Results: Between February 2015 and February 2017, 7 patients were diagnosed of PCNSL. Their clinic and histologic characteristics are shown in *table 1.* Two patients were managed by palliative care because of their age (above 74 years) and general condition (ECOG >3); they died within 6 months from diagnosis.The remaining 5 patients received 2 cycles of BRAM according to the *2015 GELTAMO* protocol.Three patients (60%) received BRAM full doses and all of them achieved complete remission.Two patients (40%) required dose reduction of methotrexate and cytarabine due to treatment related toxicities and advanced age respectively. Of these, one reached stable disease and required radiotherapy prior to ASCT and one reached complete remission and did not receive ASCT but relapsed after 4 months.Two patients (40%) did not undergo ASCT because of comorbidities, being now one in relapse and one in complete remission.Of the three patients who received ASCT (60%), two achieved complete remission and one is in now in the ASCT procedure.The responses and mayor toxicities are shown in figures 1 and 2 respectively.Toxicities were mainly hematologic, infectious and renal. They were manageable, but forced to reduce doses in one patient.Their progression free survivals are in the range of 9 to 20 months.

Table 1. Patient Characteristics (n=7).

Gender-no. (%)	
Female	2(29)
Male	5(71)
Median age- yr (range)	66 (52-76)
Diagnosis-no. (%)	
- DLBCL	5(71)
- High grade B cell lymphoma, NOS	2(29)
ECOG median (range)	1(1-4)
Immunodeficiency-no. (%)	0 (0)
Intracranial lesion-no. (%)	
- solitary	4(57)
- multiple	3(43)
- periventricular	3(43)
- non periventricular	4(57)
Immunohistochemistry- no.	
- Bcl6	7
- Bcl2	7
- MUM1	6
- c-myc	2
- ki-67>80%	7
Treatment- no. (%)	
Palliative	2 (29)
BRAMx2 cycles	2 (29)
BRAMx2 cycles + autologous HCT	3(43)
Radiotherapy prior to autologous HCT	1 (14)

Summary/Conclusion: PCNSL continues to be a challenge for haematologists. Our results show that the *2015 GELTAMO* protocol is an effective and feasible treatment scheme. Toxicities were manageable, being grade 2-3 haematological toxicity the most frequent complication.The ASCT role in the duration of remission and survival should be evaluated with longer follow-up.

PB1793

AUTOLOGOUS STEM CELL TRANSPLANTATION AS FIRST-LINE TREATMENT OR SALVAGE REGIMEN FOR LARGE B-CELL LYMPHOMA IN THE RITUXIMAB ERA: A SINGLE CENTRE EXPERIENCE

M. Riva*, A. Visentin, G. Scapinello, L. Ammirati, L. Frison, A. Branca, T. Berno, C. Gurrieri, R. Zambello, L. Trentin, F. Piazza, G. Semenzato

Department of Medicine, Hematology and Clinical Immunology Unit, Padova, Padova, Italy

Background: Diffuse large B-cell lymphoma (DLBCL) is an aggressive but potentially curable B cell tumor, characterized by an extreme biological complexity and different clinical outcomes. Recent studies suggested that the use of front-line high-dose therapy (HDT) followed by autologous stem cell transplantation (ASCT) in untreated high-risk DLBCL does not improve the outcome, while this approach remains the standard of care for chemosensitive relapse/refractory (R/R) DLBCL. However, the outcome of many R/R DLBCL still remains unsatisfactory.
Aims: We analyzed the outcome and toxicities in a cohort of DLBCL patients treated with HDT and ASCT as first-line or salvage approach at our centre.
Methods: We retrospectively evaluated a group of 34 high-risk DLBCL patients, who underwent ASCT (12 as first-line and 22 as salvage regimens). Median age at diagnosis was 50 years (range 21-70 years). Adverse clinical features in the upfront HDT and R/R groups, were: Ann Arbor stage III to IV (77% *vs* 86%), ECOG ≥2 (25% *vs* 10%), bulky disease (58% *vs* 45%), ≥2 extranodal sites (25% *vs* 32%), B symptoms (67% *vs* 41%), R-IPI poor (58% *vs* 68%), CNS-IPI intermediate to high-risk (83% *vs* 86%), primary refractory/early relapse (42% *vs* 77%), cell of origin (COO as per Hans' immunohistochemistry (IHC) algorythm: GCB 33% *vs* 36% and non-GCB 42% *vs* 50%), BCL2 positivity in IHC (50% *vs* 73%), high proliferation index (Ki67 ≥70: 42% *vs* 45%). c-Myc was evaluated only in 8/34 patients (23%). Cytogenetics and FISH analysis were performed at diagnosis in 30/34 patients (88%). Results were: poor/complex karyotype (17% *vs* 23%) and double hit lymphoma (2 patients in the upfront HDT group *vs* 1 patients in the R/R group). Salvage regimens were R-DHAP, R-ICE or R-CODOX/IVAC; ASCT conditioning was BEAM (10 patients), BEAM-like (15 patients) or Mitox/L-PAM (7 patients). Response was assessed using the revised Lugano criteria.
Results: There were not significant differences in CR rate, OS and PFS between the upfront HDT and R/R group (CR: 58% *vs* 55%, p=0.9; 3-year OS: 54.5% *vs* 58.4%, p=0.9; 3-year PFS: 56.3% *vs* 59.1%, p=0.9). Grade ³3 hematological and non-hematological adverse events were reported in 58% patients in the upfront HDT group *versus* 55% patients in the R/R group. Conditioning regimens provided comparable results, both in term of toxicity and efficacy against lymphoma. Five patients (42%) in the upfront HDT group and nine patients (41%) in the R/R group died. No differences in OS and PFS were observed according to the COO, BCL2 positivity, lymphocyte/monocyte ratio at diagnosis and subdiaphragmatic disease. The presence of R-IPI poor and Ki67≥70% at diagnosis significantly impacted on OS and PFS (for R-IPI poor, 3-year OS 45%, p=0.07, 3-year PFS 46%, p=0.05; for Ki67≥70%, 3-year OS 30%, p=0.03, 3-year PFS 33%, p=0.03: data confirmed also in multivariate analysis).
Summary/Conclusion: Our analysis is a "real life" experience of treatment choices for high risk DLBCL; we confirmed that front-line HDT followed by ASCT in untreated high-risk DLBCL does not improve CR, PFS and OS, compared to ASCT in the R/R setting. Hematologic and non-hematologic toxicities were similar in the upfront HDT and R/R groups. Importantly, in our cohort an high R-IPI and elevated Ki67 positivity at diagnosis significantly impacted on OS and PFS. These markers together with other recognized prognostic factors validated in the R/R setting (as described in the CORAL study and in REFINE analysis), should be assessed to help discriminate patients in which conventional salvage therapies is not appropriate.

PB1794

DIFFUSE B CELL LYMPHOMA: AN AUDIT ON DEPARTMENTAL ADHERENCE TO BRITISH HAEMATOLOGICAL SOCIETY (BSH) GUIDELINES FOCUSING ON APPROPRIATE PROPHYLAXIS AND CHARACTERISTICS OF PATIENTS WITH HIGH MORTALITY

H. Vijayenthiran*, C. Gregory

Haematology, Wigan Royal Infirmary, Wigan, United Kingdom

Background: Diffuse B cell lymphoma, the most common type of Non-Hodgkin's lymphoma, typically presents as a clinically aggressive and heterogeneous group of disorders with a variable response rate to standardised rituximab; cyclophosphamide, doxorubicin, vincristine, prednisone(R-CHOP) chemotherapy. Recent BSH guidelines have addressed diagnostic work up and prognostic dependent management steps with emphasis on high risk and frail individuals. Patients with a poor performance ability are recommended for G-CSF prophylaxis in patients above the age of 65 years. Those with high numbers of comorbidities and impaired performance should also be considered for a steroid course prior to chemotherapy or modified treatment. The International Prognostic index (IPI) takes into account age at diagnosis, raised serum Lactate dehydrogenase, stage, bulky disease and performance status and gives an indication for low risk and high risk mortality.
Aims: To evaluate departmental adherence to national guidelines specifically focusing on the management of high risk and frail individuals. Also to study the trends between the prognostic scores and characteristics of patients who developed complications and mortality.
Methods: Data was collected from electronic records of 48 patients at Wigan Royal Infirmary. 32 Patients before Guidelines and 17 patients post guidelines.
Results: 93%(14 of 15) of WHO performance scores were not calculated post guidelines as compared to 66%(21 of 32) pre guidelines. 86% of patients above 65 years, did not have scores but incidentally had above stage three disease and more than two comorbidities, classing them frail. 33% of these did not receive GCSF or steroid prophylaxis. 29% of patients without a score died and 14% relapsed. 67% (10 of 15) of patients developed complications such as Neutropenic sepsis, oesophageal ulcers, recurrent infections, pulmonary embolisms and superior vena cava thromboses. No prognostic scores were calculated in this group hence the difficulty in understanding whether certain individuals would have benefited from appropriate prophylaxis. However 60% (6 of 10) of patients with complications had high co-morbidities numbers and were complicated by 3 deaths. There was a 19% mortality (6 of 32) pre guidelines of which 34% had an IPI score of Intermediate risk and 33% were under the age of 51. Mortality post guidelines were 46%(7 of 15) of which 57% (4) were under the age of 51years. Out of these young mortalities, one case was given steroid prophylaxis and 1 case had a low risk IPI.
Summary/Conclusion: This small capture of data may give an indication of how the current IPI score may not encompass all the key characteristics that predict high mortality. With surplus deaths in ages below 65; this may also indicate the need to expand the criteria for individuals who might benefit from prophylaxis treatment. Further study into characteristics of young mortality, associated family history, autoimmune disease, weight and occupational exposures can guide better management of potential high risk individuals. The numbers of non-adherence to the guidelines in this data set may also indicate the need to evaluate how long it takes for guidelines to be practiced and whether alternate means of education and delivery is needed apart from publishing.

PB1795

FOLLICULAR LYMPHOMA WITH C-MYC REARRANGEMENT PLACE OF THERAPY INTENSIFICATION

A. Misyurina[1,*], S. Kravchenko[1], A. Kovrigina[1], L. Plastinina[1], E. Nesterova[1], T. Obukhova[1], V. Misyurin[2], A. Misyurin[2], A. Magomedova[1], F. Babaeva[1], A. Vorobiev[1]

[1]National Research Center for Hematology, [2]N.N. Blokhin National Research Center for Oncology, Moscow, Russian Federation

Background: Presence of secondary *c-MYC* rearrangement (c-MYC-R) in patients with follicular lymphoma (FL) can predispose to a histologic transformation of FL and aggressive clinical course of the disease. That could be explained by the fact that finding of a secondary chromosomal break of *c-MYC* along with inactivation of tumour suppressor genes (*TP53, CDKN2A/B, B2M*) is one of predisposing factors to FL transformation Therapeutic approaches to treatment of FL with -*MYC*-R have not yet been clearly defined.
Aims: To describe clinical characteristics and treatment efficacy of FL patients presenting with *c-MYC*-R.
Methods: From 2015 to 2018 in National Scientific Center, were diagnosed 8 cases of FL with *c-MYC*-R (5 males and 3 females). Median age was 34,5 years (from 30 to 57). Median of follow-up was 8,8 months (from 5 to 25,6). Taking into account that in the majority of cases, except of *c-MYC* locus translocation, a tumour was represented by FL 3B, transformed FL or blastoid variant of FL, we had an intention to treat with R-(DA)-EPOCH, what was more intense than commonly used treatment approaches of FL. In case of incomplete antitumor response, autologous stem cell transplantation (auto-SCT) was planned.
Results: 7 out of 8 pts had a widespread tumour, ECOG≥2, 6-increased LDH activity, multiple extranodal involvements. In 6 cases tumour manifested with FL grade 3B; in 3 of them were revealed signs of large-cell transformation; in 2-with FL grade 3A with blastoid morphological features. In 4 out of 4 cases which had been stained with c-MYC, c-MYC expression was≥40%. 3 cases (FL grade 3B with foci of DLBCL) were BCL2-negative (<50%). Median of Ki-67 was 80% (40-85%). All 8 cases were positive for *c-MYC*-R. Pts, who manifested with FL grade 3A, had *c-MYC* and *BCL2* rearrangements (*c-MYC/BCL2*-R), with FL grade 3B-*c-MYC/BCL6*-R in 3 cases, in case of FL transformation into DLBCL were revealed *c-MYC/BCL2/BCL6-R*. This distribution highlights common pathogenesis between FL3B and DLBCL. G-banding was performed in 7 pts and revealed complex karyotype abnormalities. Five out of 8 of pts with FL underwent R-(DA)-EPOCH, 3-R-CHOP-21. In a group of 5 pts after R-DA-EPOCH, 2 pts had complete remission (CR) (one case of FL 3A with *c-MYC/BCL2*-R, another case of FL 3B with solely *c-MYC*-R), 3 pts needed second-line treatment (R-DHAP) due to progressive disease (PD) (2 cases of FL 3B with *c-MYC/BCL6-R*) or partial remission (PR) (1 case of FL 3A with *c-MYC/BCL2*-R). The last one achieved CR after 2 R-DHAP, later auto-SCT was performed. Another pt had stable disease (SD), auto-SCT was planned. Third one died due to progressive disease (PD). Three pts underwent R-CHOP: one case of FL 3B with *c-MYC/BCL6*-R had CR after 5 R-CHOP, but soon died due to bleeding of unknown source. Two another pts had PD after R-CHOP (one pt with triple-hit lymphoma died due to PD, another pt with FL 3B and solely *c-MYC*-R is alive with signs of disease). Thus, from 8 pts only 5 are alive, 2 of them with disease. Auto-SCT wasn't performed in spite of intention in two cases due to fast lymphoma progression.
Summary/Conclusion: FL with *c-MYC* rearrangement especially in combination with translocations involving *BCL2* and/or *BCL6* represents an aggressive tumour which requires more intensive approach than R-CHOP. Patients with FL who didn't achieve CR at the first line of treatment might have benefited from high dose therapy following auto-SCT. Otherwise, new treatment approaches are needed (CAR T-cells, clinical trials).

PB1796

A CNS INVOLVEMENT IN PATIENT WITH HAIRY CELL LEUKEMIA-PRIOR CNS INFECTION WITH SUBSEQUENT LEUKEMIA BREAKTHROUGH OR DISGUISED LEUKEMIA IN CNS FROM ONSET?

M. Smiljanic[1,*], M. Todorovic Balint[1,2], D. Antic[1,2], B. Mihaljevic[1,2]

[1]Clinic of Hematology Clinical Centre of Serbia, [2]Faculty of Medicine, Belgrade University, Belgrade, Serbia

Background: Hairy cell leukemia (HCL) is a rare chronic lymphoproliferative disease, comprising 2% of all adult leukemia cases. With current treatment, including purine nucleoside analogs, the prognosis of HCL is favorable, with some reports of 12-y overall survival rate of 87%. Usually HCL presents with cytopenias, enlarged spleen, massive lymphadenopathy and secondary infections, as tumor cells predominantly affects bone marrow and spleen, but rarely it can be presented with atypical involvement of bone, liver, skin, neurologic, or vascular tissue. CNS involvement, reported in only few cases, is extremely rare, most neurological signs and symptoms are usually related with infections.
Aims: To present diagnostics, clinical course and treatment outcome of our patient diagnosed with variant HCL (vHCL) and very rare leukemia CNS involvement.
Methods: Case report of a rare vHCL with CNS involvement.
Results: Our female patient, aged 68, was initially admitted as a high suspicion for subacute meningoencephalitis, in the Clinic for Infectious Disease, Clinical Centre of Serbia. She presented with persistent high fever and neurological/ neurological related symptoms (generalized convulsions, aphasia, confusion, nausea and vomiting) of which some (confusion, nausea) appeared 3 months earlier. Magnetic resonance (MR) of the brain was highly suspicious for bilateral frontal lobes empyema. Serological and cerebrospinal fluid (CFS) analyses for WNV, Wright, HSV1 and 2, Toxoplasma Gondii, Rubeolla, CMV and B. Burdgoferii, as well as for TBC and Cryptococcus were all negative. Lumbal puncture test for malignant cells was negative, but revealed a large number of small and active lymphocytes. Blood count showed bicytopenia (hemoglobin 9.7g/dl, WBC $1.7x10^9$/l). Laboratory analyses showed high C-reactive protein and ESR. Radiography findings (ultrasound and CT) revealed no enlarged spleen or lymph nodes. However, CSF Flow cytometric immunophenotyping (FCI) analysis revealed signifi-

cant (56%) presence of B-Non Hodgkin Lymphoma (B-NHL) cell population, $CD19^{+high}CD20^{+medium}CD103^{+low}CD45^{+high}/SSC^{low}$, confirming malignant CNS involvement. Patient was initially treated with antiepileptics, broad spectrum antibiotics, antituberculosis and antifungal therapy. After subsequent admission in our Clinic, hematological diagnostics was performed. Peripheral blood smear was indicative for vHCL, showing atypical TRAP+. FCI analyses of the peripheral blood lymphocytes and of bone marrow revealed presence of atypical monoclonal mature B cells in peripheral blood (170cells/µl, 10% of WBC) and in bone marrow respectively (24% of nuclei cells), implying presence of B-NHL, CD5-, CD10-, CD103+ in leukemic phase. CLL score was 3. Aspiration biopsy, as well as bone marrow biopsy, showed significant (up to 90%) presence of small cell lymphoid infiltrates. Following these findings and regarding absence of lymphoid tissue tumor burden, lack of enlarged spleen, and aggressive clinical presentation, we diagnosed our patient with vHCL and extremly rare leukemia involvement of CNS. Patient was treated accordingly with Cladribine, one cycle, plus intrathecal terapy with Metotrexate. Post treatment analyses showed good partial responce with complete regresssion of neurological signs and symptoms and normal posttherapeutical brain MR finding.
Summary/Conclusion: We presented a case of an extremely rare leukemia involvement of CNS with vHCL cells. Leukemia cells were found in CSF and final regression of neurological signs and symptoms was registered after Cladribine treatment.

PB1797

MODIFIED R-IDARAM TREATMENT IN CENTRAL NERVOUS SYSTEM LYMPHOMAS: A SINGLE CENTRE EXPERIENCE

S. Maral[1,*], M. Albayrak[1], C. Pala[1], H.B. Afacan Ozturk[1], A. Yıldız[1], O. Sahin[1], H. Okutan[2]

[1]Hematology, Dıskapı Research and Training Hospital, [2]Hematology, Losante Hospital, Ankara, Turkey

Background: Central nervous system (CNS) lymphomas are aggressive entities which have a poor prognosis. Treatment has improved significantly after the high-dose methotrexate(HD-MTX)-based regimen used in recent years. The optimal treatment approach for patients with CNSL is still unclear, and there is no standard therapeutic approach for these patients.
Aims: The aim of this paper was to present the cases of 9 CNS lymphoma patients treated with the modified R-IDARAM protocol to shed some light on the effectiveness, tolerability and toxicity of this protocol.
Methods: A total of 9 patients, 7 with PCNL and 2 with SCNL, were treated with the modified R-IDARAM regimen,comprising rituximab 375 mg/m^2 (day 1), idarubicin 10 mg/m^2 (days 2 and 3), dexamethasone 100 mg/m^2 (12-hr infusion on days 2, 3 and 4), cytosine arabinoside (ARA-C) 1 g/m^2 (1-hr infusion on days 2 and 3), methotrexate (MTX) (3 g/m^2, 6-hr infusion on day 4) with folinic acid rescue, ARA-C 40 mg plus 15 mg MTX and dexamethasone 8 mg (via intrathecal route on days 1 and 8)The PCNSL patients were diagnosed from the examination of mass biopsy materials or surgically resected specimens. The Karnofsky Performance Status (KPS) scale was used to evaluate the performance status of patients. The risk profile and prognosis of patients were determined according to Memorial Sloan-Kettering Cancer Center (MSKCC) Prognostic Scoring system.Two additional courses of R-IDARAM (total four courses) were applied and all courses were given every 28 days.Complete remission (CR) referred to resolution of all apparent tumors. Partial Resolution (PR) of >50% of assessable disease, determined as the product of two diameters of measurable lesions, minor response/no change (MR/NC) was defined as a reduction of<50% of measurable disease but with no disease progression, and progressive disease (PD). The response was evaluated with cranial magnetic resonance imaging (MRI) for the PCNSL patients and additional thoraco-abdominal CT was performed for SCNSL.In patients where CR was achieved, follow-up was made every 3 months for 2 years and then every 6 months. The toxicity of the regimen was evaluated after all courses and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE v4.0).
Results: CR was achieved in 4/7 PNCLS and 1 / 2 SCNCL patients after 2 courses. These PNCLS patients and 1 SCNCL patient are still being followed up without disease progression to date (66, 75 and 4 months, respectively).During the first course, 2 PCSNL patients died because of severe infection and 1 SCNSL patient died because of progressive CNS lymphoma with 1-month survival.All patients had grade 3-4 hematological side-effects including thrombocytopenia and neutropenia, and intravenous antibiotherapy was required during febrile episodes. Severe mucosal problems were experienced in 2 patients and grade 3 peripheral neuropathy in 1 patient. No cranial or neurological complications attributed to radiotherapy were detected.No cardiac or renal side-effects were seen in any patient.
Summary/Conclusion: The data of a small number of patients are presented here. Most patients achieved CR after induction therapy but this was not maintained for a long time. Few patients were able to complete the therapy completely due to poor performance status and social problems. Therefore, the long-term results are not satisfactory. More clinical trials are still needed to develop new therapeutic methods for CNSL. Nevertheless, it seems to be a good option with response rates, manageable toxicity and a well-tolerated regimen.

PB1798

MEDIASTINAL GREY ZONE LYMPHOMA

Y. Mangasarova*, A. Magomedova, A. Misyurina, E. Nesterova, S. Kravchenko

National Research Center for Hematology, Moscow, Russian Federation

Background: Mediastinal B-cell lymphomas present in the mediastinum and are most frequent in young patients. Nodular sclerosis Hodgkin lymphoma (NS HL) and primary mediastinal B-cell lymphoma (PMBL) are common types, whereas mediastinal grey-zone lymphoma (MGZL) is extremely rare and has pathological features intermediate between NS HL and PMBL. The indeterminate pathobiology of MGZL led to uncertainty regarding therapeutic strategy. Clinical characteristics of MGZL and treatment strategy have not been characterized.
Aims: To describe clinical features of patients with MGZL.
Methods: From 2007 to 2018 years, 20 patients were diagnosed with MGZL (according to WHO criteria) in National Research Center for Hematology scow Russian Federation. The median age of patients was 32 years (from 19 to 54). There were 7 males, 13 females. Increased LDH activity was revealed in 69% of pts. Bulky mediastinal disease manifested in 45% of pts. A metachronous development of NS HL and PMBCL was observed in 2 out of 20 pts. Synchronous onset of classical HL and PMBL occurred in 18 out of 20 pts. reatment was completed in 16 out of 20 pts. Therapeutically, the Hodgkin-like pathological features of MGZL was treated like HL. Cases with strong expression of the CD20 were treated with rituximab. Cases with features of PMBL, with rituximab-based regimens as with PMBL. R-DA-EPOCH/m-NHL-BFM-90 were used in 12 out of 16 pts, R-BEACOPP-14 in 4 out of 16 patients. Autologous peripheral blood stem cell transplantation (auto-SCT) with BEAM conditioning regimen after first-line treatment was performed in 6 out of 16 pts. Therapeutically, the Hodgkin-like pathological features of MGZL was treated like HL. Cases with strong expression of the CD20 were treated with rituximab. Cases with features of PMBL, with rituximab-based regimens as with PMBL. R-DA-EPOCH/m-NHL-BFM-90 were used in 12 out of 16 pts, R-BEACOPP-14 in 4 out of 16 patients. Autologous peripheral blood stem cell transplantation (auto-SCT) with BEAM conditioning regimen after first-line treatment was performed in 6 out of 16 pts.
Results: After R-DA-EPOCH/m-NHL-BFM-90 therapy CR was achieved in 6 out of 16 pts. Four out of 16 pts had PR and underwent 2 R-DHAP courses followed by auto-SCT. Early relapse occurred in 1 out of 16 patient. Two out of 16 were primary resistant and underwent the PD-1 blockade.
After R-BEACOPP-14 therapy CR was achieved in 3 out of 16 pts, 1 out of 16 pts had PR and underwent 2 R-DHAP courses followed by auto-SCT, but early disease progression led to lethal outcome. Median follow up was 32 months.
Summary/Conclusion: Our data suggest that intensive treatment could improve outcome of this rare and aggressive disease.

PB1799

CLINICAL AND HEMATOLOGICAL MARKERS OF THE RESPONSE FORECAST TO FIRST-LINE THERAPY IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

S. Samarina[1,*], E. Nazarova[1], N. Minaeva[1], E. Osipova[1], S. Gricaev[2]

[1]Federal State Institute of Science "Kirov Research Institute of Hematology and Blood Transfusion of the Federal Medical and Biological Agency of Russia", Kirov, [2]Federal State Institute "Russian Research Institute of Hematology and Transfusion of the Federal Medical and Biological Agency of Russia", St. Petersburg, Russian Federation

Background: Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive type of non-Hodgkin's lymphoma, which is characterized by pronounced biological heterogeneity and manifested by various response to induction therapy and the timing of the relapse onset. The risk factors used in clinical practice is not sufficient for correct stratification of patients. This

makes it difficult to plan the duration and intensity of therapy. Thus, the current direction is the allocation of new highly informative markers, which help to individualize the choice of the treatment method of DLBCL.
Aims: To evaluate the prognostic potential of individual clinical and hematological indicators as predictors of the first-line of therapy effectiveness in DLBCL patients.
Methods: A retrospective analysis of the 99 patient's cases was conducted, the age median was 57 (37-80) years. The criteria for study inclusion were: patient's age, DLBCL type, DLBCL morphological subtype, IPI index, disease stage, B-symptoms, LDH level, the method and effectiveness of induction therapy, the status of the patient and the disease in post-induction monitoring. All patients receiving R-CHOP treatment were divided into 4 groups: 54 patients with complete response (CR), 14 with partial response (PR), 16 with resistance to first-line therapy and 9 with early relapse (ER). The median of follow-up duration was 51 (1-60) months.
Results: Among the examined, patients younger than 60 years with a low IPI index were significantly more likely observed (37.7 *vs* 13.3%, $p<0.0076$). Low IPI was reported more often in patients with CR than in resistant form (37 *vs* 0%, $p<0.001$). The frequency of high IPI in groups with CR, PR and resistance to therapy was also significant differed: 7.4, 50 and 68.8%, respectively ($p=0.002$). B-symptoms in the beginning of the disease in 1, 2 and 4 groups were observed in 42.6, 71.4 and 88.9% of patients, respectively. At the same time, in the group with PR, the frequency of B-symptoms was significantly higher than in patients with CR and ER ($p=0.05$ *vs* $p=0.013$, respectively). At the first stage of the disease, the CR frequency was significantly higher than the PR (25 *vs* 0%, respectively, $p=0.016$). It was found that the depth of response correlated with the serum LDH level. LDH value within the references were more often observed in patients with CR than with PR (44.4 *vs* 14.3%, $p=0.039$) or resistant variant (44.4 *vs* 6.3%, $p=0.005$). There was a tendency to decrease the effectiveness of R-CHOP in patients with non-GCB subtype of DLBCL in comparison with GCB type: 26.9 *vs* 73.1% in group 1 ($p=0.174$). Four-year disease-free survival (DFS) in patients with CR was not achieved and was 6 months in the group with PR ($p=0.001$). Four-year overall survival (OS) in the patients with CR, PR and ER was not achieved, and in patients with resistant variant it was 12 months ($p<0.001$).
Summary/Conclusion: A correlation was found between the IPI index, the presence of B-symptoms and the LDH level with the depth of response to induction therapy R-CHOP in DLBCL patients, which indicates a correlation of tumor burden with the effectiveness of treatment. The variability of individual indicators does not allow to correctly predict the probability of reaching a response to treatment. It was found that CR is associated with an increase of the DFS and OS duration. It seems reasonable to search for new risk markers for the early initiation of aggressive treatment in the case of the predicted low efficacy of the standard R-CHOP induction regimen.

PB1800

ACQUIRED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS AMONG HEMATOLOGIC PATIENTS: A SINGLE CENTRE EXPERIENCE

N.S. Fracchiolla*, L. Pettine, V. Mattiello, A. Freyrie, M.C. Goldaniga, M. Sciumè, A. Cortelezzi

Oncohematology Division, IRCCS Ca' Granda-Maggiore Policlinico Hospital Foundation and University of Milan, Milan, Italy

Background: Hemophagocytic Lymphohistiocytosis (HLH) is characterized by activation of cytotoxic T-cells and overwhelming cytokine production and release, resulting in an extreme inflammatory response. Primary HLH is most common in children and is genetically driven. Secondary HLH recognises multiple causes, as drugs, autoimmune disease, infection or cancer. Secondary HLH may occur during hematologic neoplasia, as first manifestation of them, before or during the treatment of a recognized malignancy, or associated with relapsing/progressive disease. Hematological malignancy-associated HLH may present an even more severe outcome than primary HLH. Despite the availability of diagnostic criteria, the diagnosis of HLH remains challenging, due to the multiple variables involved and its potential rapidly catastrophic presentation.
Aims: To describe the treatment and outcome of a monocentric, real-life, small cohort of secondary HLH.
Methods: We describe the diagnosis, clinical features, treatment and outcome of five consecutive patients (pts) with secondary HLH presenting at our Centre between March 2016 and December 2017.
Results: All pts received a diagnosis of HLH based on the HLH-2004 criteria (Table 1). Median age was 54 years (range 23-70), and 3 were male. Associated conditions included hematologic malignancies (80%) and infections (40%). Four pts had hematologic malignancy in relapse (80%): 3 non-Hodgkin lymphoma (B-cell, n=1; T-cell, n=2), 1 CLL. Two pts had also an underlying CMV and EBV viral infection. One patient developed HLH in the setting of Kaposi's sarcoma. All cases presented with fever, elevated ferritin (≥500 µg/l) (median 34.126 µg/l, range 9.130-100.000 µg/l) and high triglycerides (≥265mg/dL)(median 371 mg/dl, range 331-428 mg/dl). Other features at HLH diagnosis were: 4 pts (80%) had splenomegaly, 4 pts (80%) had new onset of ≥2 cytopenias, and 1 pt (20%) had low fibrinogen (≤150 mg/dl). Bone marrow examination was performed in 3 pts, 2 had morphological evidence of hemophagocytosis (40%). NK-cell activity and sIL2R testing were not performed. The median lactate dehydrogenase (LDH) was 1.784 U/L (range, 458-4.060 U/L). Increase in bilirubin and transaminases or creatinine were seen in 3 and 2 patients, respectively. All patients received etoposide and dexamethasone treatment, one patient started CHOEP, and one started ibrutinib to control underlying malignancy. One pt received rituximab for EBV infection and one patient received valganciclovir for CMV infection. Two pts died within 30 days from diagnosis, while 3 pts are in HLH CR.

Table 1.

Patients characteristics	
Number	5
Median age, years (range)	54 (23-70)
Male gender, number (%)	3 (60)
Underlying disease, number (%):	
B-cell lymphoma	1 (20)
T-cell lymphoma	2 (40)
CLL	1 (20)
Kaposi sarcoma	1 (20)
HLH-2014 diagnostic criteria, number (%):	
Fever	5 (100)
Splenomegaly	4 (80)
Cytopenias affecting ≥2 lineages	4 (80)
Hypertriglyceridemia (≥ 265 mg/dL)	5 (100)
Hypofibrinogenemia (≤ 150 mg/dl)	1 (20)
Hemophagocytosis in tissue	2 (40)
Ferritin > 500 mg/L	5 (100)
Outcome, number (%):	
< 30 day mortality	2 (40)

Summary/Conclusion: Diagnosis of malignancy-associated HLH in adults is particularly challenging due to the multiplicity of confounding variables and evolution rapidity, possibly leading to a delayed recognition. HLH should be suspected in patients with hematologic malignancies, in particular in T-cell lymphomas, or in the presence of EBV and CMV infections, presenting fever, cytopenias, splenomegaly and ferritin elevated to more than 500 mg/L, that indicate an underlying uncontrolled inflammatory state. A high degree of clinical suspicion allows a prompt diagnosis and treatment, that remain the key factors in the prevention of a HLH catastrophic clinical course.

PB1801

IBRUTINIB AS SALVAGE TREATMENT IN TWO MANTLE CELL LYMPHOMA (MCL) PATIENTS WITH CENTRAL NERVOUS SYSTEM INVOLVEMENT (CNSI)

N. Giannakoulas*, F. Panitsas, M. Palassopoulou, G. Stefani, A. Markou, L. Mavroeidis, T. Testa, E. Bouronikou, G. Vassilopoulos

Hematology Department, School of Health Sciences University of Thessalia, Larissa, Greece

Background: CNSi is extremely rare at MCL diagnosis but more frequent at relapse (4-8%) with poor prognosis (median survival<6 months). Leptomeningeal infiltration is more frequent than parenchymal disease. Treatment includes CNS directed chemotherapy crossing the blood-brain barrier (BBB). Consolidation with HDT/ASCT can be considered for fit patients. Irradiation and palliative care are also options in relapsed/refractory (R/R) or unfit cases. Ibrutinib has shown significant single agent activity in R/R MCL.
Aims: We present two cases of MCL patients with secondary CNSi, successfully treated with Ibrutinib.
Methods: Case 1: A 58-year-old male was diagnosed with MCL stage IV, MIPI score high and ki-67 high (40%) in November 2011. He achieved CR with first line treatment (R-CHOP, 8 cycles followed by R maintenance). Three years after initial diagnosis (November 2014) he developed eye proptosis with pain and periorbital oedema. MRI scan showed intraorbital mass infiltrating the rectus muscles and extending into the maxillary sinuses. Further imaging only showed cervical lymphadenopathy. CSF examination was

negative. He was treated with high dose Methotrexate and Cytarabine and achieved partial response. Additional treatment with Bendamustine-Rituximab (6 cycles, April 2015-September 2015) led to further radiological improvement.He suffered second disease recurrence in the same area 12 months later (September 2016). Whole body imaging with PET/CT scan revealed hypermetabolic foci inside the right frontal lobe with high uptake compared to brain parenchyma (SUV 16.7). CSF examination was negative. MRI confirmed the presence of intraparenchymal solid lesion. He was commenced on Ibrutinib 540 mg/d in November 2016. Follow-up imaging in March 2017 showed significant shrinkage of the intracranial tumor and complete response was seen in July 2017. Latest MRI imaging in January 2018 showed that he remains in continuous CR on ibrutinib treatment.
Results: Case 2: A 58 year-old male was diagnosed with stage IV, MIPI high MCL in November 2013. He achieved good PR after 6 cycles of R-CHOP. He was not considered eligible for HDT/autologous transplant consolidation. Six months later (October 2014) disease relapsed with Waldeyer ring involvement (confirmed by biopsy, Ki-67 75%) and CNSi (leptomeningeal disease only). He was treated with three cycles of HyperCVAD/MA and IT chemo to CR. In June 2015, there was systemic relapse with CNSi (leptomeningeal and parenchymal disease). He received salvage R-DHAP with responding systemic disease but only partial CNS response. There was CNS progression in October 2015, refractory to high dose Methotrexate. He was started on Ibrutininb 560 mg/day in November 2015. There was immediate response of CNS disease with CR on imaging 10 months later. Systemic disease remained in remission throughout, however he developed isolated CNS relapse in March 2017 (leptomeningeal and parenchymal). Disease was refractory to high dose Methotrexate but responded (PR) to Bendamustine, Cytarabine, Dexamethasone and Rituximab plus IT chemo (4 cycles). There was progression of CNS disease (parenchymal only) again in February 2018. He was started on Rituximab- Ifosfamide-Etoposide. Formal response assessment is pending.
Summary/Conclusion: Ibrutinib has been shown to rapidly cross the BBB and has been reported to have impressive clinical results in cases with CNS MCL relapse. Our experience described herein has also been positive. Salvage of MCL patients progressing on Ibrutinib treatment is difficult and outcomes are generally poor.

PB1802

PRIMARY TONSIL DIFFUSE LARGE B CELL LYMPHOMA IS CLINICOPATHOLOGICALLY DIFFERENT FROM NODAL DIFFUSE LARGE B CELL LYMPHOMA IN TAIWAN

C.-F. Yang*

Pathology and Laboratory Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, Republic of China

Background: Diffuse large B cell lymphoma (DLBCL) is the most common lymphoma. It is a highly heterogeneous group of neoplasms and patients have a variable clinical course and prognosis.
Aims: In this work, we try to study the difference of primary tonsil DLBCLs and nodal DLBCLs.
Methods: We analyzed a cohort of 103 primary tonsil diffuse large B cell lymphomas (DLBCLs) retrieved from our pathological file from 1998 to 2015, and compared 74 nodal DLBCLs from 2012 to 2013.
Results: In those cases with available data, the primary tonsil DLBCLs were more common confined to stage I–II disease (71%, 65/92 *vs* 33%, 24/73, P<0.001), and more often revealed a germinal center B-cell-like (GCB) immunophenotype (56%, 15/27 *vs* 29%, 17/59, P=0.029). The 5-year overall survival (OS) of primary tonsil and nodal DLBCL patients were 72% *vs* 58.9%(P=0.008).
Summary/Conclusion: Primary tonsil DLBCLs display distinct clinicopathologic features compared with nodal DLBCLs in Taiwan, with usual localized-stage disease, common GCB immunophenotype, and a better outcome.

PB1803

CLADRIBINE IN TYPE II REFRACTORY CELIAC DISEASE (RCD): A SINGLE INSTITUTION EXPERIENCE

A.I. Gregorini[1,*], V. Ferla[1], F.G. Rossi[1], A. Pompa[1], F. Guidotti[2], M.C. Goldaniga[1], V.B. Valli[2], A. Cortelezzi[1,2], L. Baldini[1,2]

[1]Hematology Unit, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, [2]Oncology and Hemato-oncology, University of Milan, Milan, Italy

Background: RCD is defined by no response to a year of gluten free diet (GFD) with persistence of malabsorption and intestinal villous atrophy. A biopsy of upper gastrointestinal tract is necessary for diagnosis of the two RCD subtypes: type I characterized by a normal phenotype of intraepithelial lymphocytes (IEL) with surface CD3 and CD8 expression; type II characterized by clonal expansion of abnormal IEL lacking surface markers CD3, CD8 and T-cell receptor and preserved intracellular CD3 expression. RCD II has a severe clinical presentation due to ulcerative jejunitis responsible for severe protein loss enteropathy and malnutrition. The risk of transformation in enteropathy associated T cell lymphoma (EATL) is about 33-52% within 5 years after diagnosis. Only 30-40% of patients (pts) achieve histological response and in most cases the clinical improvement is transient with steroids. Immunosuppressive therapy has failed to show a histological response, even increasing the risk of evolution in EATL. Cladribine, a purine analogue, at the dosage of 0.14mg/kg per day for 5 days has induced clinical and histological response.
Aims: To achieve an early and accurate diagnosis of RCD type II pts and to evaluate clinical and histological response to purine analogues treatment.
Methods: We retrospectively collected clinical, laboratory, endoscopic end histological data of two RCD type II pts treated with subcutaneous (SC) cladribine from 2015 in our haematology unit.
Results: The first pt was a 63 years old man diagnosed with CD since 2007. In 2012 he underwent esophagogastroduodenoscopy (EGD) for diarrhea and weight loss with diagnosis of RCD I. A steroid therapy was started with transient clinical benefit. In July 2015, a new EGD was performed for recurrence of symptoms and showed a 60% T-IEL infiltrate with aberrant phenotype and a monoclonal TCR on a polyclonal basis. Video capsule endoscopy (VCE) demonstrated widespread atrophy in more than half of the small intestine, while CT scan and PET resulted negative. Because of steroid refractoriness, a treatment with SC cladribine was started. He underwent three cycles every six months, with a 30% dose reduction for the first two cycles. Treatment was well tolerated, without hematological toxicity. After III cycles he had weight gain and resolution of diarrhea and a macroscopic improvement of ileal athrophy and ulcers, without significant reduction of T cell infiltration. The second pt was a 62 years old woman diagnosed with CD in January 2016. A EGD revealed a 60% T-IEL infiltrate with aberrant phenotype and monoclonal TCR on a polyclonal basis. VCE demonstrated widespread signs of jejunal atrophy and erosions, while CT scan and PET were negative. Since steroids were ineffective, she underwent two cycles of SC cladribine. Treatment was well tolerated. There was an improvement of clinical symptoms (weight gain and resolution of diarrhea) and of ileal atrophy and ulcers, with a 50% reduction T IEL infiltrate.
Summary/Conclusion: In conclusion both pts showed a clinical and histological response to SC cladribine without hematologic toxicity or infectious complications: this indicate that this treatment could be a safe and effective option in elderly pts affected by type II RCD. Furthermore the outpatient administration of the treatment also lead to a reduction of pt discomfort and costs related to hospitalization.

PB1804

PLASMABLASTIC LYMPHOMA IN HIV-NEGATIVE PATIENTS: EXPERIENCE OF SINGLE CENTER IN SPAIN

G. Pinto*, M. Calbacho, J. López

Hematology, Hospital Ramon Y Cajal, Madrid, Spain

Background: Plasmablastic lymphoma (PB) is a low incidence subtype of lymphoma with special clinical features, which is mainly seen in patients with HIV, but not exclusively. Currently, there is no standard of care and prognosis remains poor. Besides, little is known about its clinical behavior in HIV-negative patients.
Aims: Describe the cases of PB in a single center in order to find diferences between HIV-negative and HIV-positive patients.
Methods: 9 patients were diagnosed between 2011-2016. Clinical features, treatment and overall survival (OS) were analized both in HIV-positive and HIV-negative patients.
Results: The main clinical features in the group HIV-positive *vs* HIV-negative are described in table 1. Median follow-up was 7.23 months. In the HIV-negative group, 3 patients (50%) had a post-transplantation PB (2 patients after bone marrow transplant, and 1 after liver transplant). Most of patients received treatments based on bortezomib added to standard doses of chemotherapy (VR-CAP scheme) or an intensified protocol (DA-EPOCH scheme). Other protocol (Rituximab monotherapy) was used in only one HIV-negative patient with stage II PB. The median OS in HIV-positive and HIV-negative patients was 3.5 and 25.3 months respectively. All HIV-positive patients died during the first year, whilst HIV-negative patients had a survival rate of 66% at 1 year and 50% at 2 years.
Summary/Conclusion: Among the patients with PB, HIV-negative group tends to share the same clinical features than HIV-positive group, but has a

better prognosis in terms of OS even in the post-transplant setting. Both treatments (standard *vs* intensified) show to be effective, but it is necessary further studies to determine an optimal treatment protocol. On the other hand, outcome in HIV patients with PB is dismal despite bortezomib based or intensified protocols.

Table 1.

CLINICAL FEATURES	HIV-positive (N=3)	HIV-negative (N=6)
Mean age	48.6	56.8
Male sex	66%	85%
Extranodal disease	66%	85%
Stage III/IV	100%	71%
MYC mutation	33%	42%
Histological detection of EBV	66%	42%
Human herpes virus-8	33%	0%
CNS involvement	0%	0%
VR-CAP scheme	66%	50%
DA-EPOCH scheme	33%	33%

Bleeding disorders (congenital and acquired)

PB1805

INCORPORATION OF EVIDENCE BASED GUIDELINES ON BLEEDING RISK ASSESSMENT PRIOR TO ENT SURGERY INTO PRACTICE: REAL TIME EXPERIENCE

N. Al-Marhoobi[1], M. Maktoom[2,*], M. Elshinawy[3,4], H. Nazir[4], K. Al hashmi[5], R. Al-abri[6], K. Macki[7], A. Al-Rawas[4], Y. Wali[4]

[1]ENT surgery, [2]Pediatrics, Oman Medical Specialty Board, Muscat, Oman, [3]Pediatrics, Faculty of Medicine, Alexandria University, Alexandria, Egypt, [4]Pediatrics, Sultan Qaboos University Hospital, [5]Hematology, Armed Forces Hospital, [6]ENT surgery, Sultan Qaboos University Hospital, [7]ENT surgery, Armed Forces Hospital, Muscat, Oman

Background: In spite of guidelines recommending the no need for coagulation profile prior to ENT surgeries when challenging history of bleeding is negative, yet surgeons still practice it. Cost and delaying surgeries are major issues faced when insignificant abnormalities are found in the coagulation profile results. In 2008, British Committee for Standards of Hematology has published guidelines (1) on assessing the bleeding risk prior to surgeries or invasive procedures, which stated that the indication for sending a coagulation profile is based on the bleeding history of the patient.
Aims: This study aims to identify the reasons of ENT surgeons for requesting pre-operative coagulation screening; prothrombin time (PT) and activated partial thromboplastin time (APTT) in spite of the available evidence and guidelines of their poor correlation with bleeding risk.
Methods: The current work was based on a survey conducted at 3 tertiary care facilities in the Sultanate of Oman for surgeons who performed adenoidectomy/adeno-tonsillectomy and other ENT surgical procedures during the period from 1st Jan 2017 to 1st September 2017. The Survey was conducted for identifying the practice of the ENT surgeons prior to surgeries, either getting a challenging bleeding history from the patients/guardians or requesting a coagulation profile (PT, APTT) as well. Surgeons who decide to do coagulation profile were requested to identify their reason. Patients with proven or suspected bleeding disorder (based on the past and family history) were excluded.
Results: The study included data from 730 patients who underwent ENT surgical procedures. They were 432 males and 298 females. Their mean age was 19.6 ± 16.92 year. Out the 730 patients, 372 patients were interviewed for a challenging bleeding history alone (group 1) and 358 were interviewed plus a pre-operative coagulation profile check (Group 2). Two patients had an intraoperative minor bleed that requires stitching (one in each group). Three patients in group 2 had post-operative secondary bleeding after 1 week that responded to local hemostatic measures. Twenty eight surgeons who preferred to do the coagulation profile for their patients answered the survey. Twenty two (78.5%) gave the reason of habitual practice and overprotection, 4 were not confident with the current evidence and 2 had previous bleeding experience with their patients.
Summary/Conclusion: Despite the current evidence of meta-analysis and the hospital guidelines, still many surgeons prefer to do coagulation check before ENT surgeries for different reasons. Surgeons need to be educated more on the current evidence of the superiority of the challenging bleeding history over the routine PT and APTT tests. This might reduce the waiting time for performing these procedures.

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PB1806

GENETIC FACTORS POSSIBLY CONTRIBUTING TO COMPLICATIONS AFTER ORTHOPEDIC OPERATIONS IN HEMOPHILIA PATIENTS

T. Polianskaia*, I. Fevraleva, E. Karpov, N. Sadykova, M. Sampiev, G. Mishin, A. Golobokov, D. Petrovskiy, A. Koroleva, T. Tupoleva, A. Kovrigina, A. Sudarikov, V. Zorenko

National Research Center for Hematology, Moscow, Russian Federation

Background: The intensity and frequency of hemorrhagic manifestations in patients with certain form of hemophilia may vary. There are still no convincing data explaining the different clinical course of hemophilia. Despite comprehensive substitutive hemostatic therapy, some patients devel-

op severe arthropathy requiring surgical support. Frequency of purulent complications after arthroplasty in patients with hemophilia is higher than in the general population.
Aims: To study possible genetic factors that aggravate the course of hemophilia and increase the risk of complications after orthopedic operations.
Methods: 96 patients with severe hemophilia and arthropathy who underwent endoprosthesis replacement of the affected joint were included in the study. The mean age was 39.6 years (min 23, max 68): 75 (78.1%) with hemophilia A, 16 (16.7%) with hemophilia B and 5 (5.2%) with an inhibitory form of hemophilia. Histological studies of bone tissue in the area of the affected joint, taken during arthroplasty of the joint, were performed. Patients were examined for antibodies to hepatitis C and hepatitis C RNA. DNA samples were examined for genetic markers of hereditary hemochromatosis (mutations H63D and C282Y of HFE gene), thrombophilia (F5, MT677, MT1298, F2), polymorphisms of the vitamin D receptor gene (Taq1, Apa1 and Fok1) by means of real-time allele-specific polymerase chain reaction.
Results: Homozygous Taql and Apal polymorphisms of the vitamin D receptor gene in hemophilia patients was found in 7.3% and 18.8%, respectively, which corresponds to the literature data for the Russian population. Homozygous Fokl marker was detected in 27 patients (28%), which is 2.8 times more frequent than in general population (10%). Nine heterozygous cases (9.4%) of C282Y HFE gene mutations (none homozygous) and 38 heterozygous (39.5%) cases of H63D mutation (4 homozygous-4.2%) were found in hemophilia patients which is 1.5-2 times higher than the average incidence of these alleles in the Russian population. For thrombophilia markers frequencies were: F5-7 heterozygotes and no homozygotes; MT677-36 for the heterozygotes and 9 homozygotes; MT1298-35 heterozygotes and 8 homozygotes, and. Twelve patients had heterozygous mutations at the same time for MT677 and MT1298. In patients with hemophilia B, the frequency of homozygous thrombophilia mutations was higher (31.25%) than in hemophilia A (13.3%).
Summary/Conclusion: HFE gene mutations may possibly increase the deposition of hemosiderin in the joint tissues subsequently accelerating arthropathy progression. Since 91.4% patients in our sample are infected with viral hepatitis C, hemochromatosis markers may also complicate the course of hepatitis and increase the risk of complications. Homozygous Fokl marker of the vitamin D receptor gene is linked to increased risk of developing infectious complications, therefore in our cohort may increase the risk of purulent postoperative complications. Several alleles of the vitamin D receptor gene are correlated with increased rate of bone resorption and may also increase the risk of aseptic instability in the components of endoprostheses. The presence of thrombophilia markers in patients with hemophilia also may change the course of hemophilia in patients with the same initial level of the deficient factor requiring more individualized approach for hemostatic replacement therapy.

PB1807

UNDERLYING DISEASE OF THE ACQUIRED HAEMOPHILIA AFFECTS THE TREATMENT OUTCOME AND PROGNOSIS-CROATIAN EXPERIENCE

M. Vodanović[1,*], D. Pulanić[1,2], A. Boban[1,2], S. Zupančić Šalek[1,2,3]

[1]Dpt of internal Medicine, Division of Haematology, University Hospital Centre Zagreb, [2]School of Medicine, University of Zagreb, Zagreb, [3]School of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

Background: Acquired haemophilia A (AHA) is a rare autoimmune disease, caused by antibodies (inhibitors) against coagulation FVIII and characterized by spontaneous hemorrhage in patients with no previous history of bleeding. Risk factors for the occurrence of AHA include advanced age and underlying diseases (malignancy, autoimmune disorders, pregnancy, and the postpartum period).
Aims: In this work we showed how the underlying diseases of acquired haemophilia A affect the treatment outcome and prognosis (deaths in patients).
Methods: We analyzed the results of fifteen patients (10 (66,7%) male,5 (33,3%) female), median age 71 (51-81) with AHA from July 2010 until December 2017, treated at the UHC Zagreb. Severe bleeding had 66,7% patients. Almost half of patients (46,7%) had FVIII activity<5 U/ dL. The median inhibitor titre at diagnosis was 5,8 (2,3–2000) BU/mL. In 7 patients (46,7%) were not identified underlying diseases associated with AHA. In 8 patients (53,7%) were identified underlying diseases; malignancies 3 (oligodendroglioma, B-CLL, IgM monoclonal gamapathy), autoimmune diseases 4 (autoimmune haemolytic anaemia in 2 patients, rheumatoid arthritis, polymyalgia rheumatica), 1 postinfective.
Results: Haemostatic therapy was initiated in 86,7% of patients (100% with severe bleeding) with AHA. First-line therapy consisted of bypassing agents (recombinant FVIIa in 13,3%, activated prothrombin complex concentrates in 73,3%), and all patients were treated with immunosupressive treatment (combination of steroids and cyclophosphamide in 13 patients, steroids alone in 2 patients). Rituximab alone was used in one patient as the second line treatment. Both group with underlying and without underlying disease were similar according to the median age 71, FVIII activity 5 *vs* 6 U/dL, time to achieve remission was the same 28,2 days. The titre inhibitor was significantly higher in the group with underlying disease (22 *vs* 3,5 BU/ml), and the rate of major bleeding was higher (86,7 *vs* 42,6%). At follow up of 31 months (1-71) 11 patients (73,3%) are alive, 4 (26,6%) dead. No death was due to bledding. Three (75%) of deaths was reported in a group of patients with underlying disease (two deaths associated with sepsis, one with progressive malignant disease) with mortality rate of 37,5 *vs* 14,2% on behalf of group with known cause of AHA. One death was associated with cardiogenic shock in patient without underlying disease of AHA. One patient (7%) had thrombotic complication (myocardial infarction and venous thromboembolism).
Summary/Conclusion: Mortality in patients with acquired haemophilia was associated with the underlying disease, cause of AHA (malignant disease), as well as with infective complications of immunosupresive treatment in elderly with comorbidites, according to recent literature data.

PB1808

COMPARISON BETWEEN ELISA BASED TEST FOR DETECTION OF FACTOR VIII ANTIBODIES SCREEN AND STANDARD BETHESDA

S. Bakr[1,2,*], R. Nasr[3], A.-K. Al-Momen[4], R. Al-Nounou[3], T. Owaidah[3]

[1]Department of Clinical Pathology, Faculty of Medicine, Fayoum University, Fayoum, Egypt, [2]College of Medicine, Princess Nourah bint Abdulrahman University, [3]Department of Pathology and Laboratory Medicine, King Faisal Specialist hospital and RC, [4]Center of excellence in Thrombosis and Hemostasis, College of Medicine, King Saud University, Riyadh, Saudi Arabia

Background: Inhibitors of Factor VIII (FVIII) which arise in up to 35% of patients with hemophilia A causing the most detrimental complications, are usually IgG polyclonal antibodies. Unluckily, the unavailability of the gold standard Bethesda assay that requires expertise in every hemophilia treating centers makes detection of anti FVIII antibodies seems difficult especially for low titer inhibitors.
Aims: To assess the efficacy of ELISA assay for detection of FVIII inhibitors in comparison to the Bethesda test.
Methods: A total of 102 patients with hemophilia A who were screened for inhibitors by APTT then confirmed by Bethesda assay, were tested using enzyme linked immune-sorbent assay (ELISA) for the presence of FVIII specific IgG antibody. Thirty control samples as well as negative and positive samples were included with each run.
Results: Out of all tested samples, 17 (16.7%) were positive by Bethesda assay. Nine samples had high titer (>10 BU), while 8 samples had low titer (<10 BU). In comparison between Bethesda test and ELISA, all the samples that were positive by Bethesda at both high and low titer were detected by ELISA test and all negative samples by Bethesda were also negative by ELISA. Only 6 samples that were positive by Bethesda but at very low titer (<1BU), were missed by ELISA.
Summary/Conclusion: This study results demonstrates strong correlation between the ELISA and Bethesda assay in detecting immune responses to FVIII, as what had been reported in previous literature. ELISA for FVIII antibody detection provides rapid screening test that could be available in small coagulation laboratory.

PB1809

CONTINUOUS INFUSION OF TUROCTOCOG ALFA DURING SURGERY: AN EFFECTIVE AND SAFE WAY OF TREATING PATIENTS WITH HAEMOPHILIA A

L. Koers[1,*], N. Uitslager[1], E. Mauser-Bunschoten[1], A. Huisman[2], I. Kremer Hovinga[1], P. van der Valk[1], R. Schutgens[1]

[1]Van Creveldkliniek, University Medical Centre Utrecht, Utrecht University, [2]Clinical Chemistry and Hematology, University Medical Centre Utrecht, Utrecht, Netherlands

Background: Turoctocog alfa (NovoEight®, N8) is a third generation recombinant factor VIII, developed for prevention and treatment of bleeding episodes in patients with haemophilia A.
Aims: To investigate the efficacy and safety of turoctocog alfa as continuous infusion (CI) peri-operatively in patients with haemophilia A.

Methods: Ten adult patients undergoing eleven surgical procedures were included in this retrospective, observational cohort study. Primary objective was the efficacy of turoctocog alfa via CI based on the stability of FVIII levels, blood loss during surgery and bleeding complications. Secondary objective was the safety of turoctocog alfa as CI, also in terms of blood loss and bleeding complications and in terms of *de novo* FVIII-inhibitor development. Dose was started at 4 U/kg/hr and adjusted daily based on FVIII levels. Target levels were predefined.
Results: Ninety-three percent of the achieved FVIII levels were equivalent to or higher than the target levels, with a mean deviation of 37% above target. All patients showed a higher *in vivo* recovery than expected, with a mean recovery of 0,029 IU/kg. All patients had an adequate perioperative haemostatic response. A change in treatment because of a bleeding complication was not required in any case. There were no thromboembolic complications. No new cases of FVIII-inhibitors were detected.

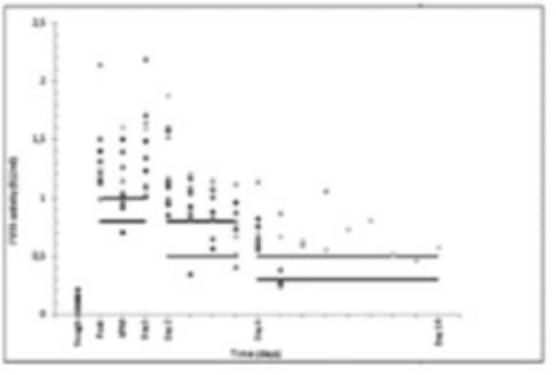

Figure 1.

Summary/Conclusion: Continuous infusion of turoctocog alfa is a feasible, effective and safe way of treating patients with haemophilia A during surgery.

PB1810

RETROSPECTIVE EVALUATION OF ACQUIRED HEMOPHILIA IN A SPANISH CENTER

F. Martín Moro*, E. Arrontes Caballero, C. Guillén Rienda, I. García García, B. Michael Fernández, C. Martínez-Geijo Román, K. Velázquez Kennedy, A. Sanz Rupérez, J. Marquet Palomanes, F.J. López Jiménez

Hematology and Hemotherapy, Hospital Universitario Ramón y Cajal, Madrid, Spain

Background: Acquired hemophilia (AH) is a rare autoimmune bleeding disorder caused by the development of an autoantibody directed against a coagulation factor, most frequently factor VIII (FVIII).
Aims: To review the characteristics and management of AH in our center during the last five years (2013-2017).
Methods: Retrospective single-center study. There is no Department of Obstetrics in our hospital. Data was collected from clinical records.

Table 1. Summary of the 7 patients who received treatment to eradicate FVIII inhibitor.

Underlying disease (age/sex)	FVIII (%)	Inhibitor (BU)	1st line treatment	2nd line treatment	Time to response*** (days)		Complications	Relapse ***
					Partial	Complete		
Neoplasia (85/M)	5	NA	CS + CTX	-	49	-	-	Yes
Idiopathic (81/F)	<5	NA	CS + CTX	-	51	61	-	-
Autoimmune (88/F)*	<5	32	CS + CTX	-	30	60	-	Yes
Autoimmune (85/M)	7	1.4	CS + Rituximab	-	17	48	Sepsis	-
Neoplasia (77/M)	<5	95	MBMP**	-	24	-	IFI, CMV	-
Autoimmune (76/F)	<5	64	MBMP	Rituximab	20	55	-	-
Neoplasia (79/F)	<5	64	MBMP	Rituximab	27	51	Neutropenia	-

**Relapse; **no plasmapheresis; ***GTH-AH 01/2010. CMV: Cytomegalovirus; CS: Corticosteroids; CTX: Cyclophosphamide; IFI: Invasive Fungal Infection; MBMP: Modified Bonn-Malmö Protocol; NA: Not Available.*

Results: Eleven patients were found: 10 with FVIII inhibitor and 1 with FXI inhibitor. Of the 10 patients diagnosed with AH A (4M, 6F), 1 was a relapse. The median age at diagnosis was 85 years (76-93). Median time to diagnosis from symptom onset was 1 month (0.4-18). Underlying disease: autoimmune (4), solid neoplasia (3), hematologic neoplasia (2) and idiopathic (1). All cases had bleeding as initial presentation (one life-threatening): subcutaneous (6), genitourinary (3), muscle (1), gastrointestinal (1) and/or after surgery (1). All patients had anaemia at diagnosis. FVIII level at diagnosis was<5% in most cases (8/10), the median inhibitor level was 64 BU (1.4-134). Regarding treatment, 3 patients received Corticosteroids alone due to comorbidities, without follow-up; 3/3 died due to underlying disease/unknown cause. The remaining 7 patients were treated to eradicate FVIII inhibitor (Table 1). Recombinant FVII activated was chosen as first-line haemostatic agent. Bleeding stopped in all cases and none presented thrombosis. Three of these 7 patients passed away, 2 due to underlying disease/unknown cause and 1 due to immunosuppression. The patient with acquired FXI inhibitor was a 73 year-old female with no underlying disease. No bleeding at diagnosis. The first-line of eradicator treatment was CS + CTX, then second-line Rituximab, without response. She passed away due to immunosuppression-related complications.
Summary/Conclusion: All patients were elderly at diagnosis, influenced by the lack of pregnancy-associated cases. An underlying disease was found in almost all cases. In our experience all patients with FVIII inhibitor who received eradicator treatment achieved a response (partial +/- complete). Treatment with higher immunosuppression involved more complications. Most deaths were due to underlying disease/unknown cause.

PB1811

THE ASSOCIATION BETWEEN HLA CLASS I,II ALLELES AND THE OCCURRENCE OF INHIBITOR IN TURKISH PATİENTS WITH HEMOPHILIA A: A PILOT STUDY

T. Patiroglu[1,*], M. Cansever[2], S. Akbayram[3], H. Gulen[4], K. Oncel[5], O. Bor[6], Y. Oymak[7], Y.Z. Aral[8], Y. Ay[9], R.K. Kavaklı[10], H. Oren[11], Y. Kılınç[12]

[1]Pediatric Hematology, [2]Pediatric Immunology, Erciyes University Medical Faculty, Kayseri, [3]Pediatric Hematology, Gaziantep University Medical Faculty, Gaziantep, [4]Pediatric Hematology, Celal Bayar University, Manisa, [5]Pediatric Hematology, Ankara Unıversty, Ankara, [6]Pediatric Hematology, Osmangazi Unıversty, Tokat, [7]Pediatric Hematology, Gazi Unıversty, Ankara, [8]Pediatric Hematology, Adnan Menderes Unıversty, Aydın, [9]Pediatric Hematology, Behçet Uz Research Hospıtal, [10]Pediatric Hematology, Ege Unıveresty, [11]Pediatric Hematology, Dokuz Eylül Unıversty, [12]Pediatric Hematology, Dokuz Eylul Universty, İzmir, Turkey

Background: Inhibitor is the most severe complication of hemophilia A treatment. Approximately, antibodies against Factor VIII develop in 25% of severe hemophilia A patients. There are both genetic and enviromental factors contributing the development of inhibitor. Genetic factors include Factor VIII gene defects (deletion of intron 22),and polymorphisms of immune response genes including genes encoding the human leukocyte antigens, tumor necrosis factor-a (TNF-a), interleukin-10 (IL-10) and cytotoxic T-lymphocyte antigen-4 (CTLA-4). It has been shown the association with some HLA alleles and inhibitor against Factor VIII.
Aims: The purpose of the pilot study is to evaluate the frequencies of HLA class I (A, B, C) and class II (DRB1, DQB1) alleles in patients with Turkish hemophilia A and compare to the results of unrelated healthy controls.
Methods: The distribution of HLA class I and II alleles in 30 hemophilia A patients with inhibitor and 30 unrelated healthy subjects as controls was determined using the PCR- Sequence Based Typing (PCR-SBT) method, and the association between the occurrence of factor VIII (FVIII) inhibitor and the presence of certain HLA class I and II alleles was investigated.
Results: The frequency of HLA-DQB1*02:02 was significantly higher in the hemophilia A patients with FVIII inhibitor as compared to controls (p=0.029). On the contrary, HLA-C*08:02, HLA-DRB1*03:01, and HLA-DRB1*04:02 alleles frequencies lower than controls (p=0.014, p=0.029, and p=0.014, respectively).
Summary/Conclusion: The study's findings show that the DQB1*02:02 allele might have contributed to the occurrence of inhibitor in hemophilia A patients; however, additional research using larger samples is warranted.

PB1812

CYTOKINE ADSORPTION IN A PATIENT WITH SEVERE COAGULATION ABNORMALITIES DUE TO HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS (HLH)

S. Birndt[1,*], S. Fetscher[2], M. Frimmel[3], A. Hochhaus[1], P. La Rosée[4]

[1]Klinik für Innere Medizin II, Universitätsklinikum Jena, Jena, [2]Medizinische Klinik III, [3]Interdisziplinäre Intensivmedizin, Sana Kliniken, Lübeck, [4]Medizinische Klinik II, Schwarzwald-Baar-Klinikum, Villingen-Schwenningen, Germany

Background: Hemophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory syndrome evolving from a pathologic immune response, leading to

uncontrolled cytokine release and organ damage. In adults, HLH can be triggered by various conditions like infections, malignancies, or autoimmune diseases. HLH treatment is based on immunosuppression and trigger directed therapies. However, despite improved treatment protocols, patients with severe HLH still have a poor prognosis. Cytokine adsorbing devices are increasingly used in treating critically ill patients with inflammation and hypercytokinemia to prevent and reduce the effects of pro-inflammatory cytokines[1]. Thus, cytokine adsorption may be a suitable therapeutic option in severe, life-threatening HLH.
Aims: We report the case of a 49-year old male patient presenting with severe coagulation abnormalities due to hemophagocytic lymphohistiocytosis.
Methods: Case report. A 49-year old male patient was admitted to intensive care unit (ICU) because of suspected sepsis. A prodromal two week episode had led to previous emergency room admissions due to fever of unknown origin (FUO). We began fluid replacement therapy and started empiric antibiotic treatment with piperacillin/tazobactam, leading to initial improvement of the patients clinical condition. However, pancytopenia progressed and coagulation parameters deteriorated to unmeasurable levels (fibrinogen 0.31 g/l [reference range 1.8-3.5], INR >8) making large scale substitution treatment necessary. Despite extensive diagnostics, no infectious focus was found. Indicated by markedly elevated ferritin values (81,393 µg/l), HLH was diagnosed with 7 of 8 HLH-2004 criteria. A combination therapy using dexamethasone, polyvalent immunoglobulins and etoposide was administered without improvement of coagulation parameters. Due to progressive multiple organ failure (respiratory/renal/hepatic/hematopoietic) we initiated cytokine adsorption using Cytosorb columns in serial application with continuous hemofiltration. In marked association with cytokine adsorption, coagulation features significantly improved and inflammatory markers decreased. Multiple organ failure reversed and the patient was moved to the general ward.
Results: Overall, hemoadsorptive treatment was performed over 12 days. Cytokine adsorption resulted in a substantial decline of C-reactive protein and interleukin-6. Using immunosuppression and hemoadsorption, coagulation slowly improved. Moreover, ferritin and lactate dehydrogenase levels decreased, whereas no effect on soluble interleukin-2 levels was observed. Utilization of the cytokine adsorber was safe and well-tolerated, and no relevant side effects were noticed. No HLH-triggering disease was identified. Functional tests including degranulation assays and perforin expression were within normal range. Currently, the patient is in follow-up one year after initial HLH diagnosis with tapered immunosuppressive treatment.
Summary/Conclusion: HLH treatment remains challenging as multiple organ systems can be affected. Besides immunosuppression and trigger specific therapies, cytokine adsorption is a useful tool in imminent or present multiple organ failure, when drug treatment intensification is limited by organ function.

Reference

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PB1813

UNIQUE CASE OF TYPE 3 VON WILLEBRAND DISEASE

M. Mandava[1,*], S. Bergmann[1], E. Curl[2], J. Lazarchick[3]

[1]Pediatric hematology oncology, [2]Departmen of pathology, [3]Department of Pathology, medical university of South carolina, charleston, United States

Background: VonWillebrand disease (vWD) is the most common inherited bleeding disorder worldwide. Genetic mutations in the von Willebrand gene may result in either quantitative (Types 1 and 3 vWD) or qualitative defects (Type 2 vWD) of von Willebrand Factor (vWF), which plays a major role in hemostasis. Type 3 is the rarest and most severe form of vWD, resulting in a virtual absence of vWF. Type 3 vWD follows autosomal recessive inheritance and is most often reported in patients who are homozygous for the same gene mutation.
Aims: We report a case with type 3 vWD inheriting two different mutations, one from each parent, resulting in compound heterozygosity.
Methods: Array Comparative Genomic Hybridization (aCGH)/Duplication analysis was performed on our patient sample. Family was tested by PCR amplification and bi-directional DNA sequence analysis of requested exons.
Results: Our patient presented with a normal CBC and normal levels of Factor IX, XI, and XII activity. Her PTT was prolonged at 59 (reference range 23.3-35.7) with a normal INR. A von Willebrand panel showed markedly decreased Factor VIII (2%), vWF antigen (6%), and vWF activity (8%). VWF multimers were absent, consistent with a diagnosis of type 3 vWD. VWF gene sequence analysis was sent to the Blood Center of Wisconsin and demonstrated two pathologic variants, one on each allele: c2345delC in exon 18 and a deletion within exon 6. Subsequent analysis revealed that the patient's mother is heterozygous for the c2345delC variant and the patient's father is heterozygous for the deletion within exon 6 of the VWF gene. The patient's older sibling inherited only the maternal mutation, resulting in a diagnosis of Type 1 vWD, and a younger brother was negative for both mutations.
Summary/Conclusion: Type 3 vWD is quite rare, with a prevalence ranging from 0.1-5.3 per million. Our case is especially interesting due to the unique inheritance pattern resulting in our patient's type 3 vWD phenotype. Type 3 vWD cases are most often described in patients homozygous for a mutation in the VWF gene, frequently as a result of consanguinity. Our patient inherited a unique variant from each parent, resulting in heterozygous expression of two defective VWF alleles (compound heterozygosity). In our patient's family, because each parent is heterozygous for a mutation in the VWF gene, future children have a 75% chance of inheriting at least one mutation, and a 25% chance of inheriting both mutations. Type 3 vWD patients have impaired endogenous synthesis of functional vWF, thus therapies such as desmopressin, used in other types of vWD to stimulate secretion of endogenous vWF, are ineffective. Instead, first-line treatment in Type 3 is replacement therapy with Humate-P as needed during bleeding episodes and/or as prophylaxis. Humate P is VWF/FVIII concentrate is obtained from pooled human plasma from many carefully screened plasma donors and contains the clotting proteins VWF and FVIII. Humate-P has a VWF:FVIII ratio of approximately 2.4:1. Adjuvant treatment with fibrinolytic agents has been attempted in some patients, and platelet transfusions have been administered in patients with excessive bleeding despite first-line treatment, with some success. Complications of therapy include the rare development of anti-vWF alloantibodies, which most often occurs in patients with partial or complete VWF gene deletions. Our patient has received aminocaproic acid for minimal bleeding episodes and plasma derived vWF/FVIII concentrates for multiple episodes of moderate bleeding. She has not developed antibodies, but is at high risk.

PB1814

CLINICAL FEATURES AND TYPES OF VON WILLEBRAND DISEASE IN WOMEN WITH MENORRHAGIA REFERRED TO HEMATOLOGY CLINIC OF KERMANSHAH

M. Aeinfar*, M. Payandeh

KUMS, KUMS, Kermanshah, Iran, Islamic Republic Of

Background: Menorrhagia is the most common symptom that is experienced by women with bleeding disorders. Von Willebrand disease (VWD) is the most common congenital human bleeding disorder that is manifested as a quantitative deficiency in Von Willebrand factor (VWF) or dysfunction of this factor.
Aims: The present study was carried out to find the frequency of VWD, its types, and clinical features of the disease among women with menorrhagia who referred to the Hematology Clinic of the Kermanshah University of Medical Sciences.
Methods: Case study
Results: The study comprised 482 women with menorrhagia. After excluding patients with confounding factors, 56 (11.6%) patients were evaluated for inherited bleeding disorders. We detected 31 (55.3%) patients with VWD. Type 3 of VWD was the most frequent subtype (45.1%) followed in frequency by type 2, (32.2%), and type 1 (22.5%).
Summary/Conclusion: In conclusion, our study indicated that menorrhagia can be the first symptom of VWD. Therefore, rare coagulation disorders should be considered in women with idiopathic menorrhagia, particularly in regions with high rates of consanguinity.

PB1815

KOREAN VON WILLEBRAND DISEASE REALITIES

S.K. Park[1,*], S.K. Kim[2], J.H. Seol[1]

[1]Pediatrics, Ulsan University Hospital, Ulsan, [2]pediatrics, inha univ hospital, Incheon, Korea, Republic Of

Background: von Willebrand disease (VWD) is the most common inherited bleeding disorder with a prevalence of up to 1%. However in Korea, only 126 VWD patients were registered in Korea Hemophilia Foundation (KHF).
Aims: The aim of this study was to determine the status of VWD patients in Korea. We analyzed VWD patients by age, gender, blood group, family history and bleeding history.

Methods: One hundred twenty-six VWD patients registered in the KHF by December 2016, and 74 patients diagnosed at six university hospitals were enrolled in this study. We evaluated the medical records from the KHF and the questionnaires from six university hospitals retrospectively.
Results: Seventeen patients misdiagnosed and ten patients duplicated were excluded. One hundred nine patients registered in the KHF and 64 patients diagnosed at six university hospitals met the criteria for VWD. The blood type O accounts for 72 (51.8%). VWF mutation was detected in 30 patients (17.3%). Median age at diagnosis was 10.5yr. The bleeding score of adults was higher than that of children (P 0.001). The most common bleeding symptom was epistaxis (48.5%). The distribution of VWD types was: 67% of type 1, 30.1% of type 2, and 2.9% of type 3.
Summary/Conclusion: Even though only six hospitals responded to the survey, 64 patients not registered in the KHF were diagnosed with VWD. Our results suggest the prevalence of Korean VWD might be higher than previously reported. A nationwide registration system is warranted in order to accurately identify the national prevalence of VWD.

PB1816

TRAINING THE PATENT AND PARENTS IN SELF-ADMINISTERING THE MISSING BLOOD FACTOR AT HOME

P. Laguna*, M. Matysiak

Ped Hem/Onc, Medical University, Warsaw, Poland

Background: Since 2008 all patients with severe forms of haemophilia A and B aged 0-18 have been included in the programme of prophylactic administration of the missing factor.
Aims: The programme consists of: ensuring the availability of the factor, delivering the factor to patients homes and collecting the waste after the procedure. In order to improve the independence of patients and their families, since 2014 qualified nurses have been educating and instructing them how to self-administer the factor intravenously and to central venous catheters. Such training take place throughout Poland.
Methods: Materials and Methods Two nurses, who were employed by the Shire company, at the request of a physician and after receiving parental consent, performed the training at patients houses where they showed how to apply the missing factor using. There were 560 dummies educational visits. Another role of these nurses was to train medical staff and staff in schools close to the patients' places of residence.150 training sessions took place (45 of them took place in schools and 105 in hospitals). In addition, those nurses also took part in conferences about haemophilia intended for patients and medical staff.
Results: Results The quality of life of our patients has significantly improved since introducing such training. Self-administering the missing factor by parents or even patients themselves is not a problem anymore. As a result of participating in the training, our patients became more independent and level-headed.
Summary/Conclusion: The training of self-administering the missing factor by patients and parents, as well as by teachers and medical staff close to the patients' places of residence is an important part of improving the quality of their lives

PB1817

HEMOPHILIA CARE IN PAKISTAN

M. Borhany*, N. Fatima, M. Abid, T. Shamsi

National Institute of Blood Disease and Bone Marrow Transplantation, Karachi, Pakistan

Background: Hemophilia is a rare congenital disorder characterized by prolonged bleeding, either spontaneously, or after injury. In the developing world, where majority of the hemophiliacs live, awareness of this disease and its management is poorly done. It is a significant cause of morbidity and mortality and is responsible for psychological, social and economical stress to patients and their families.
Aims: The objective of this study was to evalute the frequency of Hemophilia patients diagnosed among bleeding disorders, their demographics and treatment options available for patients with hemophilia in countries with limited resources.
Methods: This is a cross sectional, observational study was carried out at the National Institute of Blood Disease Karachi, Pakistan after getting approval from the Institutional ethics committee. Diagnosed adult and pediatric hemophilia A (HA) and hemophilia B (HB) patients of various ages and severity were included from 2012-2017. Demographic and management history of hemophila patients were recorded and analyzed.
Results: A total of 149 male patients diagnosed as HA (n=107) and HB (n=42) were evaluated. Mean age of HA 13.1±10.6 and for HB is 15.1±11.1 years. Age at diagnosis ranged from birth to 3 years. History of consanguinity was present in 85% of cases and significant family history of bleeding in 69% of patients. Hemarthrosis and hematoma were the more frequent symptoms in these patients. Surgical history including circumcision was done in 55%patients while 4 had major surgeries (hip& femur bones fracture, extensive nasal septum, and head surgery). 29% of patients had transfusion-transmitted infections in which HCV (68%) was most prevalent followed by HBV (11%) and HIV (4%). 14 HA patients (13%) were found to have positive results for inhibitors and none in HB. Treatment included tranexamic acid, fresh frozen plasma, cryoprecipitate, cryosupernatant and factor concentrates on demand basis. Recently we have started low dose prophylaxis regimen for 15 children<10 years old with the help of World Federation of Hemophilia. We administer 250 IU vial once a week and monitor for bleeds. If no bleed, continue same dose. If 2-3 joint bleeds occur in 3 months, increase the dose to twice weekly and treat the bleed (20-30 IU/kg).
Summary/Conclusion: Hemophilia A and B are common among congenital bleeding disorders. Rate of transfusion-transmitted diseases, particularly hepatitis C infection, has gained a huge proportion. Comprehensive haemophilia care center with multidisciplinary approach needs to be established.

PB1818

A RARE CASE OF ACQUIRED HEMOPHILIA A INDUCED BY THE USE OF RIVAROXABAN

C. Freitas De Andrade Rodrigues[1,*], M.T. Hercos Juliano[2], I. Marinho de Azevedo[2]

[1]Hematology, [2]Cardiology, Hospital Universitário-Ufma, Sao Luis, Brazil

Background: Rivaroxaban is an anticoagulant and the first orally active direct factor Xa inhibitor. It is indicated for the prevention of venous thromboembolic events, especially stroke and systemic embolism in patients with nonvalvular atrial fibrillation. Routine lab monitoring is not necessary. At advanced age, hemorrhage may become a serious side effect of anticoagulants and it must be investigated to exclude other causes of acquired coagulopathies. One of these is Acquired Hemophilia A (AHA), a rare bleeding disorder caused by an autoantibody to coagulation factor VIII. It is characterized by soft tissue bleeding in patients without a personal or family history of bleeding. Bleeding is variable, ranging from acute with up to 22% mortality to mild bleeding that requires no treatments. Initial treatment involves the control of acute bleeding with bypassing agents. Immunosuppressive treatment to eradicate the FVIII inhibitor should be started as soon as the diagnosis is confirmed to reduce the time the patient is at risk of bleeding.
Aims: Report a rare case of a patient taking rivaroxaban with bleeding associated with acquired hemophilia A.
Methods: We report a case of a 72-year-old man suffering from atrial fibrillation/coronary, artery disease/ hypertension for several years and taking Rivaroxaban 20mg/24h since 2014. He presented at an emergency with genital urinary and muscle bleedings specially in hands and abdomen.
Results: Hemostatic tests indicated prolonged activated partial thromboplastin time (APTT) to 107 sec (norm 26-36 sec), normal value of the prothrombin index which was 85% (norm 70-130%), fibrinogen concentration to 380 (normal value 200-400 mg/dl), the bleeding time was 5 min (norm<10 min) and the platelet count was 325 x 10^9/l (norm 130-400 x 10^9/l). Therapy with Rivaroxaban was discontinued. 48 hours later the patient presented the same alterations in exams. A coagulation disorder was therefore suspected. The autoantibody against factor VIII in a titer 205 Bethesda Units/ml (BU/ml) and decreased factor VIII activity to 0,1% (norm 50 -150%) with normal plasma concentration of factor IX. Activated (FEIBA, Baxter) had been used in the treatment of bleeding episodes for five times. immunosuppressive treatment with oral prednisone 80mg/24h was administered for three weeks without improvements. Then, cyclophosphamide 100mg/24h was added in order to remove the factor VIII inhibitor. One month later, APTT decreased to 57 sec but little bleedings continued. We decided to use Rituximab 375 mg/m^2 weekly for four doses. After this, reduction of the factor VIII inhibitor titer to 2,6% and increased of factor VIII activity to 128% led to normalization of hemostatic parameters. We investigated solid tumors and lymphoproliferative diseases by using computerized tomography, PET scan and tumor markers which were negative. Autoantibodies to rheumatologic diseases were negative.
Summary/Conclusion: Although the new anticoagulants are safe, hemorrhages may occur and clinicians should conduct these alterations carefully. Delay or undertreatment may be life-threatening. If atypical hemorrhage occurs during anticoagulant therapy, *e.g.* severe nontraumatic skin hemor-

rhage, an additional coagulation disorder should be considered. In order to confirm diagnosis, clotting testing can be performed again 48 hours after anticoagulant treatment is interrupted. If APTT, for example, remains abnormally prolonged, clotting disorders such as deficiency of clotting factors VIII, IX, or XI should be ruled out.

PB1819

5Q35.2Q35.3 MICRODUPLICATION ENCOMPASSING NSD1 WITH UNEXPLAINED PANCYTOPENIA: CASE REPORT

S. Park[1,*], G.-W. Lee[2], H.-N. Song[1]

[1]Gyeongsang National University Hospital, Jinju, Korea, Republic Of, [2]hemato-oncology, Gyeongsang National University Hospital, Jinju, Korea, Republic Of

Background: Variety of diseases, haematological and non-haematological can affect the bone marrow primarily or secondarily, resulting in pancytopenia. But the incidence of pancytopenia with normal bone marrow was 3.38% to 10.5% in various international studies. Meanwhile, Sotos syndrome is an autosomal dominant childhood overgrowth syndrome with additional features of characteristic dysmorphisms, mild-to-severe learning disabilities (LD) and advanced bone age. As, in contrast, the duplication 5q35.2.q35.3 phenotype is characterized by growth delay, microcephaly and delayed bone age in some patients, it has been referred to as a reversed Sotos phenotype. However, there has been no report on the occurrence of pancytopenia as duplication 5q35.2.q35.3 phenotype.

Table 1.

Table 1. Haematological parameters of the patient during the stay in hospital

Parameters	3rd day	9th day	24th day	40th day	51th day	65th day
Hb(gm/dl)	8.6	6.9	10.2	7.3	10.7	12.7
TLC(cells/cumm)	1950	750	1170	1580	1950	2840
ANC(cells/cumm)	(-)	190	570	420	1150	510
platelet(cells/cumm)	71,000	2,000	51,000	1,000	70,000	2,000

Abbreviation: Hb, hemoglobin; TLC, total leucocyte count; ANC, absolute neutrophil count

Table 2. Evaluation of the pancytopenia

Infectious disease	
Parvo B19 virus	negative
HIV	negative
HBV	negative
HCV	negative
CMV	negative
EBV	negative
Immunology	
ANA	negative
ANCA	negative
RF	negative
Cold agglutinin	negative
Direct/indirect coombs	negative
Other	
Vitamin B12 (pg/mL)	>2000.0
Folate (ng/mL)	>20
PNH screen	negative
Fanconi test	negative
Gaucher test	negative
Chromosomal Karyotype	46, XY, t(6;9) (q21;q21)

Abbreviation: HIV, human immunodeficiency virus; HBV, Hepatitis B virus; HCV, Hepatitis C virus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; ANA, antinuclear antibody; ANCA, Anti-neutrophil cytoplasmic antibody; RF, Rheumatoid Factor; PNH, paroxysmal nocturnal hemoglobinuria

Aims: We report the a 42-year-old male patient who has the microduplications of 5q35.2-q35.3 including NSD1 with unexplained pancytopenia was not improved by any treatment, but experienced no serious complications.
Methods: A 42-year-old male visited emergency room due to multiple trauma. He was diagnosed with mental retardation in the past. Physical examination was unremarkable except tenderness from bone fracture. Peripheral blood test showed leukocyte 3,510/mm^3, neutrophil 190/mm^3, hemoglobin 8.3 g/dL, hematocrit 25.0% and platelet 4,000/mm^3. There was no relevant history of intake of any medications and exposure to radiation. And there were no other haematological parameters to lead pancytopenia. The finding of bone marrow biopsy was hypercellular marrow with trilineage hematopoiesis. The fluorodeoxyglucose (FDG) uptake increased in multiple lymph node, bone and spleen in positron emission tomography–computed tomography (PET-CT) and lymph node biopsy was undergone in right axilla, but histologic finding was unremarkable. The chromosomal study in bone marrow showed 46 XY, t (6:9) (q21;q21) and the microarray revealed a gain of about 3.5 Mb at the 5q35.2q35.3 site including NSD1.
Results: Any treatments including vitamin and folic acid supplement, platelet transfusion, granulocyte colony-stimulating factor, steroid and intravenous immunoglobulin had no effects in the patient. But there were no severe complications associated with pancytopenia during a follow-up of 3 months. And periodic pattern of deterioration and improvement appeared in pancytopenia, spontaneously.
Summary/Conclusion: Since it is rare for these distinctive feature of pancytopenia and chromosomal abnormality to coexist together, it is necessary to investigate the association. We describe the first case of 5q35.2q35.3 microduplication encompassing NSD1 with unexplained pancytopenia. Molecular cytogenetic testing in individuals with unexplained pancytopenia could led to the discovery of new phenotype of 5q35.2q35.3 microduplication syndromes.

PB2545

ANALYSIS OF ANTI-THROMBOPOIETIN RECEPTOR ANTIBODYIES IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA

T. Satoh[1,2,*], K. Miyazaki[3], R. Horie[1,2]

[1]Division of Hematology, Kitasato University School of Allied Health Sciences, [2]Division of Molecular Hematology, Kitasato University Graduate School of Medical Sciences, [3]Department of Transfusion and Cell Transplantation, Kitasato University School of Medicine, Sagamihara, Japan

Background: Immune thrombocytopenia (ITP) is an autoimmune disease characterized by the presence of autoantibodies against platelet membrane glycoproteins, such as GPIIb/IIIa. An *in vitro* study showed that plasma containing anti-GPIIb/IIIa antibodies from adults with severe ITP inhibited the maturation of hematopoietic stem cells into megakaryocytes. In addition, some patients with ITP had anti-thrombopoietin receptor (TPOR) antibodies that suppressed megakaryocyte differentiation. Eltrombopag is a TPOR agonist that increases platelet counts by stimulating the differentiation and proliferation of megakaryocytes in ITP.
Aims: To determine the prevalence and pathogenic role of anti-TPOR antibodies in Japanese patients with ITP.
Methods: Anti-TPOR antibodies from 132 patients with ITP and 70 healthy controls were measured by enzyme-linked immunosorbent assay (ELISA). TPO levels in platelet-poor plasma were measured using an ELISA kit. To investigate whether anti-TPOR antibodies inhibited functional interactions between TPO and TPO receptors, we examined extracellular signal-regulated kinases (ERKs), downstream signals induced by recombinant human TPO (rhTPO). The binding of rhTPO to TPO receptors induced the phosphorylation of ERK in TPOR-expressing UT-7/TPO cells. Furthermore, for functional analyses, eltrombopag was used instead of rhTPO.
Results: Anti-TPOR antibodies were detected in fifteen ITP patients (11.4%) and none of the healthy controls. There was no difference in plasma TPO levels between ITP patients with anti-TPOR antibodies and patients without anti-TPOR antibodies (77.5±78.7 pg/ml versus 52.7±57.4pg/ml). Six of 10 anti-TPOR antibody-positive samples inhibited the phosphorylation of ERK in UT-7/TPO cells, which might be related to the blocking of rhTPO by anti-TPOR antibodies. In contrast, healthy control and anti-TPOR antibody-negative samples had no inhibitory effect. Eltrombopag improved the phosphorylation of ERK in UT-7/TPO cells in the presence of functional anti-TPOR antibodies.
Summary/Conclusion: Our findings suggest that functional anti-TPOR antibodies impair megakaryocyte differentiation and proliferation in patients with ITP. Eltrombopag might be a therapeutic option for ITP patients with anti-TPOR antibodies.

Bone marrow failure syndromes incl. PNH – Biology & Translational Research

PB1820

EPIDEMIOLOGICAL, CLINICAL AND GENETIC CHARACTERIZATION OF APLASTIC ANEMIA PATIENTS IN PAKISTAN

Z. Akram[1,2,3,*]

[1]Department of Hematology, National Heart, Lung and Blood Institute, NIH, Bethesda, United States, [2]Biochemistry, Quaid i Azam University,, Islamabad, [3]Stem Cell Research Lab, Armed Forces Bone Marrow Transplant Centre, rawalpindi, Pakistan

Background: Aplastic anemia (AA) is a rare and life threatening hematopoietic disease characterized by peripheral blood pancytopenia accompanied with trilineage bone marrow (BM) aplasia. AA may develop at any stage of lifeAccording to epidemiological studies, the estimated annual incidence of AA is two per million in Western countries. The incidence rate is 2-3 times higher in Asian population but the cause for this is still not clear. In Pakistan, AA is the second most common serious non-malignant blood disorder after thalassemia. AA has been reported predominantly in young adult male population.
Aims: To gain insight into the genetics and pathophysiology of AA in the Pakistani population, we investigated samples from AA patients reporting at the Armed Forces Bone Marrow Transplant Centre, Rawalpindi, Pakistan. This is the first study where examination of socio-etiological data, cytokine profiling, HLA allelic association and mutations in *TERT* and *TERC* genes were simultaneously studied to dissect a high incidence of AA cases in Pakistani population.
Methods: Genomic DNA (gDNA) was extracted from whole blood and peripheral blood mononuclear cells (PBMCs) of AA patients using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA).For mutation analysis, polymerase-chain-reaction (PCR) amplification of *TERT* and *TERC* genes was performed.Purified PCR products were subjected to direct sequencing with the BigDye Terminator v3.1 Cycle Sequencing kit (ThermoFisher Scientific, Waltham, MA, USA) and the 3130*xl* Genetic Analyzer (ThermoFisher Scientific). Serum cytokine levels were measured by Magnetic Luminex Screening Assay using the Human Premixed Multi-Analyte kit. HLA typing was carried out in 74 of our patients by PCR with sets of sequence specific primers (SSP) or serological technique.The descriptive statistics were performed using SPSS version 17.0. GraphPad PRISM software version 6 was used for analysis of variance using Krusk-Wallis test to compare serum levels of cytokines, growth factors and hormones between different patient groups and control subjects. A P value<0.05 was considered statistically significant.
Results: Epidemiological data revealed 2.75-fold higher frequency of AA among males. A single peak of disease onset was observed between age 10-29 years followed by a steady decline. AA was strongly associated with lower socioeconomic profile, rural residence and high rate of consanguineous marriages. Serum G-CSF and TPO levels were significantly elevated in AA patients, compared to healthy controls (P<0.001), while there was no statistical significance in other nine cytokine levels screened. Allele frequencies of DRB1*15 (56.8%) and DQB1*06 (70.3%) were predominantly high in AA patients. Ten mutations were found in TERT and TERC genes, including two novel mutations (Val526Ala and Val777Met) in exon 3 and 7 of TERT gene.
Summary/Conclusion: Despite specific features of the AA cohort, this study suggests that epidemiologic and etiologic factors as well as host genetic predisposition exclusively or cooperatively trigger AA in Pakistan.

PB1821

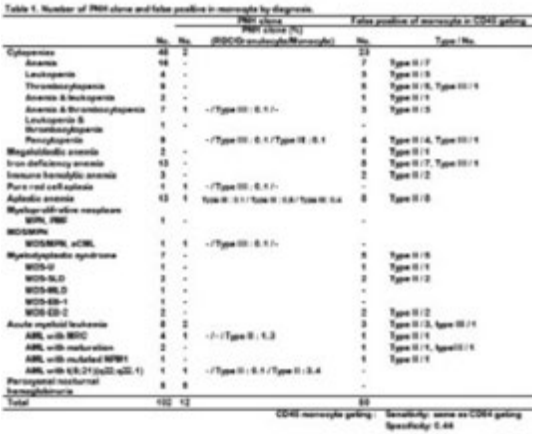
HOW USEFUL IS CD14 AND CD64 FOR GATING MONOCYTES IN FLOW CYTOMETRIC STUDY FOR PROXIMAL NOCTURNAL HEMOGLOBINURIA?

W.-J. Kwoun*, J.-Y. Ahn, P.-W. Park, Y.-H. Seo, K.-H. Kim, J.Y. Seo, J.-H. Jeong, H.T. Lee

Laboratory Medicine, Gachon University Gil Medical Center, Incheon, Republic of Korea, Incheon, Korea, Republic Of

Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare hematopoietic stem cell disorder acquired by somatic mutation of phosphatidylinositol glycan–class A (PIG-A) gene. PIG-A mutation leads partial or absolute defect in expression of GPI-anchored cell surface structure, and this result in episodes of intravascular hemolysis that are typical of the disease. Flow cytometric techniques with utilizing the FLAER enable to sensitive identify very small numbers of GPI-deficient cells. Flow cytometric PNH testing uses CD15 to gate on neutrophils, and recently uses CD64 to gate on monocytes.
Aims: In this study, we investigate utility of CD14 and CD64 in PNH study of monocyte by compare with CD45 gating in PNH study of all cytopenia cases including underlying hematologic malignancy.
Methods: Total 102 PNH study cases were recruited from July 2017 to February 2018 at Gachon University Gil Medical Center in Korea. The PNH study was done on EDTA blood specimens. Two tubes of whole blood were stained for white blood cells with two combinations consisting of FLAER and CD45 with CD15, CD24 and CD64, CD14 to gate on granulocyte and monocyte, respectively. The sample was analyzed on Cytomics FC500 cytometer. Monocyte proportions were estimated in two ways, one is gating monocyte region in CD45 plus light scatter plot and the other is gating monocyte region in CD64 plus CD14 scatter plot. Reference monocyte proportions were measured by ADVIA 2120i using samples collected together with PNH study sample. Statistical analysis of result with Bland-Altman plot was done with medcalc 15.2.
Results: Prominent PNH clone of RBCs, granulocytes and monocytes were identified in 5 cases and minor PNH clones population were detected in 7 cases. 36 cases were undergoing bone marrow biopsy and 17 cases were diagnosed as hematologic malignancy including myelodysplastic syndrome and acute leukemia. Minor PNH clones are identified in 3 hematologic malignancy cases. Aplastic anemia diagnosed in 13 cases, minor PNH clone was detected in one case. CD45 monocyte gating method showed 50 false positive cases. False negative case was not found in this study (Table 1). Compare with CD64 monocyte gating result, specificity of CD45 gating is 0.44 and sensitivity is same as CD64 gating. Difference between reference and CD45 gated monocyte proportion was 105.57(%) with 35.48 standard deviation(SD). Difference between reference and CD14 plus CD64 gated monocyte proportion was 15.87(%) with 36.11 SD.

Table 1.



Summary/Conclusion: Flow cytometry allow to detect GPI-deficient PNH phenotype highly sensitive. Adding appropriate monocyte specific lineage markers, such as CD14 and CD64, for gating monocyte result in reduce difference of monocyte proportion between reference method and increases accuracy of monocyte gating. Specificity of detecting PNH clones also increased compare. All 50 false positive samples show single normal monocyte population when tested with CD14 plus CD64 monocyte gating method. Minor PNH population was also detected in CD45 gating method when detected by CD14 plus CD64 gating method. Percentage of type in each minor PNH clone was not much different by gating method. However, Case with prominent PNH clone, proportion of Type III monocytes were less in CD14 plus CD64 gating than CD45 gating, and show more clear population. CD14 and CD64 are useful in PNH study for monocytes detect and identify a small amount of PNH clone, thus these two markers are worthy to include in routine PNH study.

Bone marrow failure syndromes incl. PNH – Clinical

PB1822

A COMPARISON OF THE ACCURACY IN DETECTING MINOR PNH CLONES IN PATIENTS WITH BONE MARROW FAILURE BETWEEN TWO HIGH-SENSITIVITY FLOW CYTOMETRY ASSAYS: CLSI AND OPTIMA

K. Hosokawa[1,*], K. Ishiyama[1], T. Ikemoto[2], C. Sugimori[3], H. Noji[4], T. Shichishima[4], N. Obara[5], S. Chiba[5], H. Ninomiya[5], Y. Shirasugi[6], Y. Nakamura[6], K. Ando[6], Y. Ueda[7], Y. Yonemura[8], T. Kawaguchi[8], J.-I. Nishimura[7], Y. Kanakura[7], S. Nakao[1]

[1]Kanazawa University, Kanazawa, [2]Osaka Medical College, Takatsuki, [3]Ishikawa Prefectural Central Hospital, Kanazawa, [4]Fukushima Medical University, Fukushima, [5]University of Tsukuba, Tsukuba, [6]Tokai University, Tokai, [7]Osaka University, Osaka, [8]Kumamoto University, Kumamoto, Japan

Background: Minor populations of glycosylphosphatidylinositol-anchored protein (GPI-AP)-deficient (GPI[-]) cells are often detected in patients with acquired aplastic anemia (AA) or low-risk myelodysplastic syndromes (MDS) and are known to predict a good response to immunosuppressive therapy (IST) and a favorable prognosis. Recently, the Clinical and Laboratory Standards Institute (*CLSI*) established a high-sensitivity flow cytometry (FCM) assay using fluorescence-labeled aerolysin (FLAER)/CD24/CD15/CD45 (CLSI method) that can detect GPI(-) cells at a sensitivity of 0.01%. We have been using a different high-resolution FCM method that defines ≥0.003% GPI(-) granulocytes and ≥0.005% GPI(-) erythrocytes as an abnormal increase. The principle of this method (OPTIMA method) is based on the elimination of as many false GPI(-) cells that can be detected in healthy individuals as possible. However, whether GPI(-) cells detected by the two different FCM assays identify the same GPI(-) cell population is unclear.
Aims: To compare these two high-sensitivity FCM assays, we performed the clinical study "Comparison of Methods in PNH clone size Assessment by CLSI Recognized or Enhanced high-sensitivity flow cytometry (COMPARE)", which aimed to determine the percentage of GPI(-) cells in peripheral blood of BM failure (BMF) patients possessing minor populations (0.003%-0.01%) of GPI(-) cells, and studied the clinical characteristics of these patients.

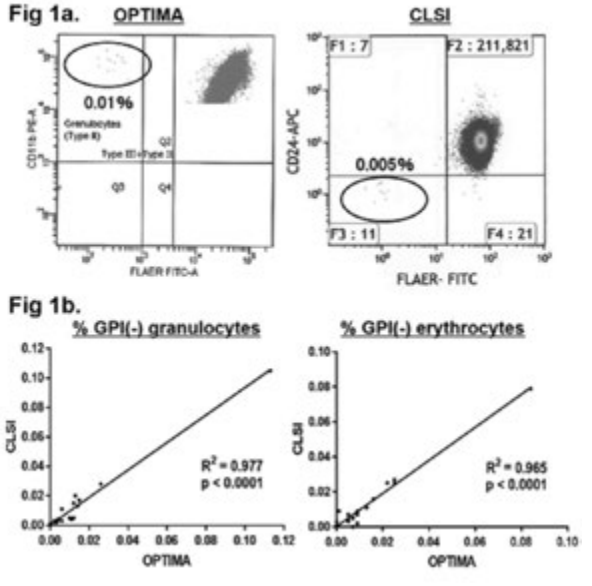


Figure 1.

Methods: We analyzed the peripheral blood of 18 BMF patients (13 AA, 3 MDS, and 2 undifferentiated BMF) who had been judged positive for GPI(-) cells (around 0.01%) by previous analyses using the OPTIMA method. New blood samples were subjected to CLSI and OPTIMA assays on the same day at two different institutions. For 8 of the 18 patients, chronological changes in the GPI(-) clone size were studied over a 3-year period.
Results: GPI(-) granulocytes≥0.003% were detected in 14 patients (78%) by the OPTIMA method, while GPI(-) granulocytes≥0.01% were detected in 7 patients (39%) by the CLSI method. The OPTIMA method revealed 0.003%-0.012% GPI(-) granulocytes in 7 patients who were judged negative by the CLSI method because their GPI(-) granulocyte percentages were less than 0.01% (0.002%-0.005%) (Fig 1a). Four BMF patients who had 0.003%-0.012% GPI(-) granulocytes in previous OPTIMA analyses were judged GPI(-) cells negative by both methods, due to spontaneous regression of their PNH clones. The median GPI(-) granulocyte clone size of the PNH-positive patients was 0.011% (0.003%-0.113%) with the OPTIMA method and 0.017% (0.011%-0.105%) with the CLSI assay. The clone sizes of GPI(-) cells revealed by each assay were positively correlated (Fig. 1b). Of the 14 BMF patients with GPI(-) cells revealed by the OPTIMA, 9 patients (8 AA and 1 MDS-RCMD) received IST (4 with ATG+ CsA and 5 with CsA monotherapy), and all of them achieved partial or complete remission. The GPI(-) clone size of 8 BMF patients (5 AA and 3 MDS) was monitored over a 3-year period, and 0.003%-0.048% GPI(-) clones were consistently revealed by the OPTIMA method in all cases.
Summary/Conclusion: Both high-sensitivity FCM methods produced comparable results in terms of detecting minor GPI(-) cell populations. However, when the CLSI method is used to detect GPI(-) cells in BMF patients, some patients who were actually positive for minor GPI(-) cell populations may be judged negative due to its high cut-off level (0.01%). Thus, caution is needed when<0.01% GPI(-) cells were revealed by the CLSI method; the patients may be positive for increased PNH-type cells and likely to respond to IST.

PB1823

FEASIBILITY AND EFFICACY OF COMBINED TREATMENT WITH ELTROMBOPAG AND DEFERASIROX IN PATIENTS AFFECTED BY APLASTIC ANEMIA

D. Cilloni[1,*], L. Biale[2], D. Capelli[3], C. Frairia[4], S. Galimberti[5], L. Giaccone[6], S. Macchi[7], M. Ruggeri[8], F. Russo[9], M. De Gobbi[1], A. Tendas[10], C. Finelli[11]

[1]Dept of Clinical and Biological Sciences, University of Turin, Orbassano, [2]banca del Sangue, Città della Salute e Della Scienza, Turin, [3]Division of Hematology, University of Ancona, Ancona, [4]Division of Hematology, Città della Salute e Della Scienza, Turin, [5]Division of Hematology, University of Pisa, Pisa, [6]Division of hematology, University of Turin, Turin, [7]Banca del Sangue, Ospedale di Borgomanero, Borgomanero, [8]Division of Hematology, ULSS [8] Berica, Vicenza, [9]Division of Hematology, University of Parma, Parma, [10]Division of Hematology, San Eugenio Hospital, Rome, [11]Dept of Hematology, Seragnoli Institute, University of Bologna, Bologna, Italy

Background: Aplastic anemia (AA) is characterized by immune-mediated bone marrow hypoplasia and pancytopenia, and can be effectively treated with immunosuppressive therapy or allogeneic transplantation. Nevertheless, one third of patients are refractory to immunosuppression, with persistent, severe cytopenia and transfusion requirement. Eltrombopag (ELT) is an orally bioavailable thrombopoietin receptor (TPO-R) agonist approved for the treatment of refractory idiopathic thrombocytopenia and aplastic anemia. Additionally It was shown that ELT is also able to decrease labile iron within the cells. Since in many studies hematological response was reported during iron chelation therapy (ICT) in patients with AA, similarly, in principle, some beneficial effects of ELT on hematopoiesis could also derive from its property to remove iron".
Aims: The study was a retrospective collection of clinical data of patients with AA treated concomitantly with eltrombopag and deferasirox (DFX) with the aim of investigating the tolerability of the association and the response rate of AA patients treated in 11 Italian hematological centers.
Methods: We collected 14 AA, 2 cases of MDS and 1 case of B-CLL. Four patients with AA were excluded because the follow-up was less than 2 months or because the ICT and ELT were not concomitant. The median time of ELT therapy in the 10 AA patients was 15 months. In 8 out of 10 patients DFX therapy was started before ELT. The median time from diagnosis to ELT therapy was 9 months. All the patients were heavily transfused before ELT therapy. The median number of transfusions before ELT therapy was 33.5 U of RBC and 31 U of platelets. The median serum ferritin level was 2600 ng/mL at ELT starting and 1480 ng/mL at the end of ELT and DFX treatment. All the patients gradually reached the dose of 150 mg/d of ELT and were treated with a median dose of 10 mg/kg of DFX.
Results: Seven out of 10 AA patients (70%) obtained complete hematological response with transfusion independence, one a partial erythroid response and one has a significant reduction of platelets transfusion. One patient did not obtain any benefit. The two MDS cases and the B-CLL case obtained transfusion independence. The combination was well tolerated and no unexpected side effects or significant alterations of blood chemistry occurred.
Summary/Conclusion: Although the retrospective nature of our observation and the limited number of patients included, our multicenter experience with the association of ELT and DFX results in a good rate of tolerability

and a surprising rate of responses. If these data will be conformed in a larger cohort of patients, these data will pave the way to prospective trials and biological studies to investigate the eventual role of iron removal in favoring eltrombopag response.

PB1824

DISTINCT DYNAMICS OF PNH CLONE AND HEMOLYTIC MANIFESTATIONS ACCORDING TO BASELINE CLONE SIZE IN PATIENTS WITH APLASTIC ANEMIA/PNH AFTER IMMUNOSUPPRESSIVE TREATMENT: PROSPECTIVE OBSERVATIONAL STUDY

S.-S. Park*, G.J. Min, Y.-W. Jeon, J.-H. Yoon, S.-E. Lee, B.-S. Cho, K.-S. Eom, Y.-J. Kim, S. Lee, H.-J. Kim, C.-K. Min, S.-G. Cho, D.-W. Kim, J.W. Lee

Division of Hematology, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic Of

Background: Clonal expansion or its clinical impact after immunosuppressive therapy (IST) was not fully elucidated in patients with paroxysmal nocturnal hemoglobinuria (PNH) in the context of aplastic anemia (AA) (AA/PNH).
Aims: This study aimed to identify the dynamics of PNH clone and proportion of hemolytic manifestations according to baseline clone size during the course of AA/PNH after IST.
Methods: A cohort of 63 patients who received IST at the time of diagnosis of AA/PNH was enrolled in this study. Hemolytic AA/PNH was defined as AA/PNH with elevated lactate dehydrogenase (LDH) ≥1.5 times the upper limit of normal (ULN). Data of each PNH clone were collected at diagnosis, 6, 12, 24 months after diagnosis of AA/PNH. PNH clone of granulocyte was measured by high-sensitivity flow cytometry (FC). The sensitivity limit of FC was 0.01% of granulocyte population. We divided patients into two groups according to granulocyte clone size at the time of diagnosis for AA/PNH: group-A (n=17) consisted of patients who showed PNH clone size ≤1%, and group-B (n=46) patients who had PNH clone size >1%.

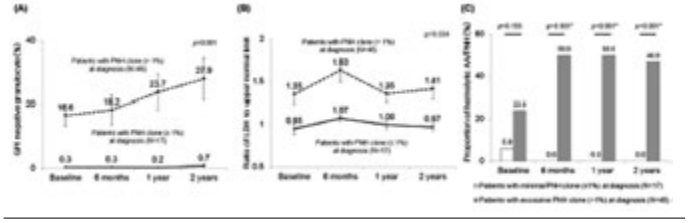


Figure 1.

Results: The median age of patients was 44 years (range, 18-79) and 12 patients (19.0%) had hemolytic AA/PNH at diagnosis of AA/PNH. During median follow-up periods of 36.0 months (5.8-90.6) from diagnosis of AA/PNH, overall response rate (ORR) of IST was 82.5% without significant intergroup difference between group-A and B (88.2% *vs* 80.4%, *p*=0.712). The median clone size and LDH value were 2.5% (range, 0.1-85.7) and 1.07 (range, 0.50-4.36) x ULN, respectively. Twenty-five patients (39.7%) developed hemolytic AA/PNH during the clinical course. Compared to dynamics of group-A, those of group-B showed significant upward trend of clone size over time (p<0.001) (Fig A). After 2-years from diagnosis, average PNH close size of group B was significantly increased from the baseline (16.6% at diagnosis to 27.9% at 2 years, *p*=0.024), whereas that of group A was not changed (0.3% at diagnosis to 0.7% at 2-years, *p*=0.932). There were no significant difference of median LDH value between baseline and 2-year in both groups (0.95x ULN to 0.97x ULN in group A, *p*=0.600, whereas 1.35x ULN to 1.41x ULN in group B, *p*=0.063) (Fig B). However, despite similar frequencies of hemolytic AA/PNH at baseline between group-A and B (5.9% in group-A *vs* 23.9% in group-B, *p*=0.155), the proportions of hemolytic AA/PNH in group-B were significantly higher at 2-year compared to those of group-A (46.9% *vs* 0.0%, *p*<0.001) (Fig C).
Summary/Conclusion: Current study demonstrate that there were significant differences in the dynamics of the PNH clone and its hemolytic manifestations according to 1% of baseline PNH clone size as cut-off in patients with AA/PNH after IST. Although ORR of IST was similar between two groups, it is suggested that the regular monitoring of PNH clone and LDH value in AA/PNH should be performed in patients with clone size >1% at baseline to identify the clonal expansion and hemolytic manifestations earlier after IST.

PB1825

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN ADULTS-A RETROSPECTIVE ANALYSIS FROM A SINGLE INSTITUTION

B. Batko[1], J. Biernat[1], M. Pukal[1], P. Krawiec[1], M. Machaczka[2,3,*]

[1]Department of Rheumatology, J. Dietl Specialist Hospital, Krakow, [2]Medical Faculty, University of Rzeszow, Rzeszow, Poland, [3]Hematology Center Karolinska, Karolinska University Hospital Huddinge, Stockholm, Sweden

Background: Hemophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory condition caused by highly stimulated but dysregulated and ineffective immune responses. Cardinal features of HLH are fever, hepatosplenomegaly, pancytopenia, and lymphohistiocytic proliferation. HLH is an underdiagnosed but potentially life-threatening clinicopathological syndrome often occurring in adults with hematological malignancies and autoimmune disorders.
Aims: To describe clinical characteristics of adults with reactive HLH/macrophage activation syndrome (MAS).
Methods: From Jan 2009 onwards, data on adults referred to the Rheumatology Department (2000 hospitalized patients per year) of the J. Dietl Specialist Hospital in Krakow (inhabited by approx. 1 million people) with suspected HLH/MAS were collected. The diagnosis of HLH was based on the HLH-2004 guidelines and HScore. In all patients, aspiration biopsy of bone marrow (BM) and/or lymph node was performed to evaluate a presence of hemophagocytosis. Connective tissue diseases were diagnosed according to international criterias. Infection as a possible additional trigger of HLH was carefully studied in all patients. EBV and CMV were routinely examined in whole blood; blood and urine cultures were performed in order to reveal any bacterial infections. The patient's medical records were reviewed to collect relevant clinical data. The patients provided their informed consent. The study was performed according to the ethical guidelines of the Declaration of Helsinki.
Results: Seven adults (4 female, 3 male), aged 20–59 years (median age 31 years), were diagnosed with aggressive HLH during the 8.5-year period between Jan 2009 and Jul 2017. Of these, two patients suffered from adult onset Still disease, one from childhood onset ankylosing spondylitis, one from systemic lupus erythematosus, one from systemic onset juvenile idiopathic arthritis, one from connective tissue disease UNS, and one from Erdheim-Chester disease. All patients presented with unremitting fever, splenomegaly, and hyperferritinemia ≥500 μg/L (median 5731, range 1469-27760). Hemophagocytosis was present in all but one patient, though only in 3 patients on the first examination. A newly developed cytopenia was present in 3/7 patients according to HLH-2004 and in all patients according to HScore. HLH-2004 criterion of hypertriglyceridemia ≥3 mmol/L and/or hypofibrinogenemia ≤1.5 g/L was fulfilled in 5/7 patients. An inflammatory state was observed both from elevated CRP (median 49,4 mg/L, range 14-277) in all patients, and low ESR (median 17 mm, range 8-45) in all but one patient. In 3 patients, the HLH trigger was identified as an active EBV infection (IgG EBNA, VCA), and for one patient HSV was suspected. One patient presented with neuropsychiatric symptoms that were not accompanied at first by any MRI abnormalities, though CSF revealed hemophagocytosis.
Summary/Conclusion: Clinical and biological features of HLH (*e.g.*, fever, splenomegaly, cytopenia, hyperferritinemia) are not specific and can also occur in other disorders. It may be difficult to distinguish HLH from other diseases such as severe sepsis, hematologic malignancies or exacerbation of rheumatic disease. Thus, the HLH/MAS diagnosis may be overlooked or delayed. Timely diagnosis is crucial since early administration of effective treatment (*e.g.*, HLH-94 therapy) may improve survival. The knowledge of HLH-2004 and HScore diagnostic criteria among physicians is essential to increase a recognition of this syndrom.

PB1826

IMMUNOLOGICAL STATUS OF PATIENTS WITH DIAMOND BLACKFAN ANEMIA

F. Gumruk[1,*], G. Soltanova[2], N. Akarsu[1], M. Cetin[1], S. Unal[1]

[1]Center for Fanconi Anemia and Inherited Bone Marrow Failure Syndromes, [2]Department of Pediatrics, Hacettepe University, Ankara, Turkey

Background: Diamond Blackfan anemia (DBA) is a rare genetic disease characterized with anemia, reticulocytopenia, paucity of erythroid precursors in the bone marrow, dysmorphic features and propensity for malignancy.
Aims: DBA is a ribosomopathy that affects mainly erythroid lineage, however as being a ribosomopathy it may have impact in other cell lines. Besides, there is limited data on the immunological status of DBA patients in the reported literature.

Methods: Out of 45 DBA patients, 32 (71%) were evaluated with serum immunoglobulin (Ig)A, IgG and IgM levels and 28 (62%) were evaluated with lymphocyte subset analyses. The values of the patients were compared to age appropriates.
Results: Of the 32 patients analyzed for Ig levels, 14 (43.7%) were found to have low levels of at least one type Ig. Two patients were found to have low IgA (6.2%), three (9.4%) had low IgM, four (12.5%) had low IgG, one (3.1%) had combined low levels of IgA and IgM, one (3.1%) had combined low levels of IgA and IgG, three (9.4%) had low levels of IgA, IgM and IgG at the same time compared to age appropriates. Six (21.4%) out of 28 patients analyzed for lymphocyte subsets were found to have low CD19+ B cells and four (14.3%) had low NK cells. Five of the patients analyzed for immunological status were found to have *CECR1* gene mutation, causing ADA2 enzyme deficiency associated DBA-like phenotype. Of these five ADA2 deficient patients four (80%) were found to have low Ig levels and three (60%) had lymphocye subset abnormalities.
Summary/Conclusion: There is limited data on the immune status of patients with DBA. Our results indicate a high rate of immune function abnormalities in patients with DBA. ADA2 deficiency is autosomal recessively inherited and could be higher in rate in our study compared to other registries, related to higher rates of consanguineous marriages in our country. ADA2 deficiency has been reported to cause subtle immunological defects and mild immunodeficiency symptoms, in addition to DBA. hematological findings. This might also have contributed to higher rate of immune function abnormalities in our registry.

PB1827

ELTROMBOPAG'S ADDITIVE VALUE IN THE THERAPY OF PEDIATRIC ACQUIRED APLASTIC ANEMIA: A SINGLE INSTITUTION EXPERIENCE

M. Filippidou[1,*], G. Avgerinou[1], K. Katsibardi[1], N. Tourkantoni[1], H. Tsipou[1], K. Roka[1], E. Rigatou[1], N. Selenti[2], A. Kattamis[1]

[1]Division of Pediatric Hematology-Oncology, First Department of Pediatrics, [2]Department of Medical Genetics, University of Athens, Aghia Sophia Children's Hospital, Athens, Greece

Background: Acquired Aplastic Anemia(AAA) is a rare bone marrow failure syndrome characterized by peripheral blood pancytopenia and marrow aplasia, probably caused by an immune-mediated destruction of hematopoietic stem and progenitor cells. Treatment includes hematopoietic stem-cell transplantation (HSCT), particularly by a matched sibling donor or immunosuppressive therapy(IST) with anti-thymocyte globulin(ATG) and cyclosporine A(CSA). Eltrombopag(ELT), a small, oral, non-peptide thrombopoietin receptor agonist has been shown to improve trilineage hematopoiesis and was recently approved as front-line treatment for AA in adults.
Aims: We report the therapeutic approach of children with AAA, treated in our Department in the last 5years.
Methods: Sixteen children (10males/6females) of mean age 8.7 (range:2-16years) were diagnosed with severe AAA since 2012. Causative agents or concurrent events included treatment with NSAIDs(1/16), infuenza virus infection(1/16), autoimmune hepatitis(1/16), transaminasemia of unknown etiology(2/16). Diagnosis was established by bone marrow aspirate and biopsy. Other bone marrow failure syndromes, either inherited (Fanconi anemia, Shwachman-Diamond syndrome) or acquired (Paroxysmal Nocturnal Hemoglobinuria) and hypoplastic myelodysplastic syndromes were excluded.
Results: Should a fully-matched sibling donor be available, patient underwent HSCT (3 patients). One patient received autologous cord blood, after standard HSCT preparatory regimen. The remaining 12 patients were treated with standard IST. ELT was added to IST as an off-label treatment, after approval from regulatory authorities in 11 patients. It was initiated on day 0, ≤4 weeks or >4 weeks post IST in 3,6 and 2patients respectively. ELT was given at a starting dose between 25-150mg/d depending on age/weight of the patient and it was further adjusted for platelet count >100,000 and <150,000/mm^3 (max dose 150mg/d). All 4 patients having received HSCT as first line treatment achieved complete remission. Of the patients treated with IST, 1 patient is still under ELT treatment showing progressive improvement of peripheral blood values 4 months post IST, 8 patients are in remission after receiving ELT treatment for a mean time of 10.7 months. Treatment with ELT was well tolerated and there was no evidence of clonal evolution or marrow fibrosis. The remaining 3patients (including the one that did not receive ELT) failed IST, and subsequently underwent MUD-HSCT (3, 4 and 18 months post IST, respectively), with fatal outcome in 2 and severe morbidity in 1 (renal failure).
Summary/Conclusion: Survival in severe AAA has remarkably improved due to early therapeutic interventions (allo-HSCT or immunosuppressive therapy) and improved supportive care. This small series of children with AAA underlines the option of new effective, well-tolerated modalities including autologous cord blood transplantation and adding ELT in IST, and the possible significant mortality/morbidity of MUD when used as a 2nd line therapy. ELT seems to enhance tri-linear recovery in children, in a manner similar to the one observed in adults with AA. Long-term-follow-up remains important to identify possible late complications (clonal evolution, leukemia or autoimmunity).

PB1828

BEST PRACTICE GUIDANCE FOR NURSES MANAGING PATIENTS WITH PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA ACROSS THE LIFE SPECTRUM

L. Arnold[1,*], M. Brown[2]

[1]Haematology, Leeds Teaching Hospitals Trust, Leeds, United Kingdom, [2]Royal Melbourne Hospital, Melbourne, Australia

Background: Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired disease characterized by chronic, complement mediated intravascular haemolysis. Nurses play a critical role in the holistic management of PNH. Due to the rarity of the disease, experience and knowledge in managing patients with PNH is lacking for many nurses, and there is currently no literature specific to the nurse care setting published to date.
Aims: To address different issues and aspects of PNH that commonly arise across the different stages of a patient's life, and provide specialised guidance for nurses.
Methods: This is a collaboration of real-world experience and expert opinion by two world-leading PNH nurse specialists, and is supported by evidence based on current literature. Common issues are discussed in relation to different life stages; 1) teenagers and adolescents 12-20 years, 2) early adults 20-40 years, 3) middle age adults 40-60 years, 4) retirees >60 years, and 5) all life stages, with the intent to prompt and raise discussions that are relevant to each patient.
Results: The most commonly identified issues for each life stage discussed are 1) Behavioural and psychological issues, acceptance of their condition, and adherence to prophylactic antibiotics for teenage and adolescent patients. 2) Contraceptive methods, reproductive concerns, and pregnancy planning and management for early adults. 3) Alternative/holistic therapies and age related comorbidities for middle aged adults. 4) Home care services, cannulation and central device issues and when to stop eculizumab/start palliative care for the retiree age group. 5) Managing travel and preserving vein integrity for all age groups.
Summary/Conclusion: Nurses play a vital role in managing patients with PNH across their life course. By identifying potential issues and providing best practice guidance on matters that may arise during a patient's life journey, nurses can be better educated and equipped to provide the best care for their patients.

PB1829

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA IN CHILDHOOD AND ADOLESCENCE- A 5-YEAR RETROSPECTIVE ANALYSIS FROM A SINGLE TERTIARY CARE CENTER FROM NORTH INDIA

R. Halder*, M. Aggarwal, M. Mahapatra, R. Dhawan, T. Seth, S. Tyagi, H.P. Pati, R. Saxena, S. Mirgh, S. Kothari, U. Kumar, R. Chauhan

Department of Hematology, All India Institute of Medical Sciences, New Delhi, India

Background: Paroxysmal nocturnal hemoglobinuria is not well recognized in pediatric age group.
Aims: To Study the clinical profile and laboratory data of children with PNH positive clone.
Methods: We analyzed the hospital records for patients of classical paroxysmal nocturnal hemoglobinuria and aplastic anemia/hypocellular bone marrow patients in the pediatric age group (<18years age) from 2013 to 2017. The clinical and laboratory data of patients with PNH clones were collected from the hospital records. PNH clone was identified by flowcytometry. Patients with classical PNH were treated with a combination of steroids with or without androgens and those with PNH/AA were treated with cyclosporine+/-androgens.

Results:

Table 1.

Symptoms at diagnosis	Classical PNH n=10(%)	PNH/AA n=29(%)
Palloe/Fatigue	10(100.00)	29(100.00)
Jaundice	5(50.00)	2(6.89)
Hemoglobinuria	5(50.00)	3(10.34)
Bleeding (Petechiae/Purpura/wet bleeds)	0	11(37.93)
Seizures	1(10.00)	0
Fever	5(50.00)	14(48.27)
Thrombosis	1(10.00)	0
Dysphagia/Chest pain/Abdominal Pain	2(20.00)	1(3.44)

Table 2.

	Classical PNH	PNH/AA	Total
Median age to presentation, years (range)	16.0 (13.7-16.7)	16.0(14.0-18.0)	16.0(14.0-18.0)
Median age to diagnosis, years (range)	18.0(16.0-19.0)	16.0(14.0-18.0)	16.0(15.0-18.0)
Duration of symptoms, months (range)	30(24-36)	3(2-6)	5(2-12)
Laboratory results			
Hb, g/dL	5.8(4.6-8.2)	5.4(4.5-7.3)	5.8(4.5-7.5)
WBC x 10⁹/L	4.1(3.2-5.6)	3.1(2.5-3.6)	3.2(2.6-3.7)
ANC x 10⁹/L	1.6(0.8-3.0)	0.5(0.3-0.7)	0.6(0.4-1.1)
Platelet x 10⁹/L	74.0(52.0-150.0)	20.0(10.0-40.0)	30.0(15.0-59.5)
Reticulocyte x 10⁹/L	57.0(50.0-67.0)	1.0(0.06-2.0)	20.0(0.9-40.0)
Total Bilirubin, mg/dl	1.4(0.8-2.0)	0.7(0.5-0.8)	0.8(0.5-1.1)
PNH clone size			
Granulocyte clone size, %, median (range)	79.0(40.6-86.0)	3.0(1.0-8.0)	5.2(2.0-28.2)
Monocyte clone size, %, median (range)	91.5(86.3-97.5)	6.0(3.2-14.4)	10.8(3.8-64.6)

14 patients were in CR, 11 patients were in PR and rest did not have any response. Only one patient was able to undergo allogenic stem cell transplantation.

Summary/Conclusion: There is significant delay in the diagnosis of the classic form of PNH in children and more awareness is to be created regarding this disorder in children. Thrombotic events were also less common in our patient population.

PB1830

CHROMOSOME FRAGILE SITE 16B AND BENIGN NEUTROPENIA: A FAMILIAR CASE STUDY

N. S. Fracchiolla[1,*], V. Ferla[1], E. Fermo[1], D. Morotti[2], W. Barcellini[1], A. Cortelezzi[1]

[1]Hematology Unit, [2]Cytogenetics Laboratory, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy

Background: Neutropenia is defined as an absolute neutrophil count (ANC)<1500 cells/mmc, can be acquired or inherited (autosomal dominant). The latter is rare and may be mild or severe, some forms may be associated with a malignant transformation in Acute Myeloid Leukemia and Myelodisplastic Syndrome. A fragile site on chromosome 16 associated to severe familiar neutropenia with variable clinical severity has been described in two cases, one of which with recurrent infections.
Aims: We have identified an additional case of a familial benign chronic neutropenia with karyotype abnormalities represented by deletion and fragile site (FRA16B) on chromosome 16 at band q22.
Methods: Chromosome studies: for evidencing FRA16B, 16q22.1, DAPI colture was used. Molecular studies: Array comparative genomic hybridization (a-CGH) was performed using Agilent SurePrint G3 Human 4x180K kit. Molecular analysis of ELANE gene (19p13.3) was performed
Results: A 35 years old Caucasian female presented with neutropenia known since the age of 15 years old with average values of neutrophils of 500/mmc. Bone marrow (BM) was normal 25 yrs before. No history of recurrent infections was reported. Hepatosplenomegaly or lymphadenopathy were absent. Blood analysis showed hemoglobin 12 g/dl, white blood cells 2000/mmc, neutrophils 640/mmc, lymphocytes 1190/mmc, monocytes 120/mmc and platelets 334.000/mmc. Screening tests for autoimmunity and anti-neutrophil antibodies (direct and indirect granulocyte immunofluorescence test; GIFT method), antiphospholipid antibodies, HIV, HCV and HBV were negative. Coagulation tests were normal. BM aspirate and biopsy resulted normal. At the age of 37 and 38 years old, the patient experienced severe pneumonia responding to antibiotic intravenous therapy with normal neutrophils and increased inflammatory markers. Karyotype on bone marrow sample evidenced the presence of a del(16)(q22) in 12 out of 20 examined metaphases. Unstimulated peripheral blood karyotype evidenced the presence of a cells population (~ 35%) with the same fragile site FRA16B (16q22.1). ArrayCGH analysis of patient was normal. No mutations in ELANE gene were detected. Due the heritability of fragile site a constitutional study of her family was performed. The patient's mother, 74 years old, had a severe neutropenia with neutrophils 500/mmc, without recurrent infections. Cytogenetic analysis on peripheral blood uncultured and coltured with fragile site inducting factors showed fra(16)(q22). The 52 years old brother had a mild neutropenia with neutrophils 1500/mmc. And the fra(16) was detected in PB only after culture with fragile site inducting factors. The patient's father and sister showed normal blood count, with a normal karyotype
Summary/Conclusion: We describe a family that presents the simultaneous occurrence of FRA16B (16q22.1) and neutropenia in three of its members (the patient, the mother and one of two siblings). In this family, the presence of FRA16B (16q22.1) correlated specifically with neutropenia, that, interestingly, was deeper in those members (the patient and the mother) in which the fragile site was evident without the need of fragile site inducing factors culture. Even it is not possible to substantiate a causative role of FRA16B (16q22.1) in familiar neutropenia, the family described here prompts further studies to explore the possibility that this association may represent a subset of familiar neutropenias with specific, albeit not yet unraveled, genetic lesions.

PB1831

ELTROMBOPAG FOR THE TREATMENT OF APLASTIC ANEMIA-CROATIAN EXPERIENCE

D. Pulanic[1,*], A. Boban[1], M. Vodanovic[1], I. Krecak[2], V. Blaslov[3], V. Gveric-Krecak[2], M. Jakic-Bubalo[3], S. Zupancic Salek[1]

[1]Department of Internal Medicine, Division of Hematology, University Hospital Center Zagreb and Medical School University of Zagreb, Zagreb, [2]Department of Internal Medicine, General Hospital of Sibenik-Knin County, Sibenik, [3]Department of Internal Medicine, Division of Hematology, University Hospital Center Split, Split, Croatia

Background: Eltrombopag, a peroral thrombopoietin receptor agonist, has been introduced recently as a possible novel therapeutic option for adult patients with acquired severe aplastic anemia (SAA) who were either refractory to prior immunosuppressive therapy or heavily pretreated and were unsuitable for hematopoietic stem cell transplantation (HSCT). However, it is still not known how eltrombopag is used for that indication in the real-world setting.
Aims: In this work we analyzed results of eltrombopag usage in Croatia for the treatment of patients with SAA or very severe aplastic anemia (vSAA) outside of clinical trials.
Methods: We conducted a retrospective analysis on the use of eltrombopag for SAA or vSAA patients among Croatian hematology centers.
Results: From March 2015 until February 2018 seven adult patients (4 (57%) female, median age 63 (38-79) years) with acquired aplastic anemia were treated with eltrombopag in Croatian hematology centers: 3 among them with SAA, 3 with vSAA, and 1 with PNH with SAA fenotype. All patients received eltrombopag after at least one previous line of immunosuppressive treatment, either as a monotherapy (2/7) or in combination with CyA +/- low dose methylprednisolone. Complete remission (CR) (defined as Plt >100/mcL and Hb >100 g/L and Neutrophils >1.5/mcL) achieved 2 patients, partial remission (PR) (defined as transfusion independence) 1 patient, minimal response (defined as some improvement in 1 or more lineage but not fulfilling the criteria of PR) 1 patient, and 3 patients were refractory to eltrombopag. Median time of eltrombopag administration was 5 (3-25) months. Median eltrombopag dose was 150 mg per day and adverse events were consistent with the known safety data of the drug. Two patients in CR are receiving eltrombopag for 25 and 14 months, respectively. One patient who achieved minimal response relapsed after 8 months of treatment and developed new cytogenetic change (trisomy 8). 85.7% of patients are alive at a median follow up of 12 (5-32) months. Only one patient died, 8 months after discontinuation of eltrombopag therapy, with refractory vSAA, comorbidities and infections.
Summary/Conclusion: Presented data from Croatian hematology centers in the real-world setting confirm that eltrombopag is interesting new modality to treat patients with acquired SAA, especially for those who are refractory to prior immunosuppressive therapy and/or unsuitable for HSCT.

PB1832

PIGA MUTATIONS AS A PREDICTORS OF TREATMENT RESPONSE IN PNH

H.S. Park[1,*], S. Choi[1], H. Seo[2], S.-M. Kim[3], K. Im[4], J. Yun[1], D. Jeong[1], J.-A. Kim[5], S.M. Hwang[6], K. Kim[7], J.H. Kim[2], D.S. Lee[1]

[1]Department of Laboratory Medicine, [2]Division of Biomedical Informatics, [3]Cancer Research Institute, [4]Institute of Reproductive Medicine and Population Medical Research Center, Seoul National University, [5]Department of Laboratory Medicine,

Chung-Ang University Hospital, Seoul, [6]Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seongnam, [7]Division of Clinical Bioinformatics Research Institute, Seoul National University Hospital, Seoul, Korea, Republic Of

Background: The detection of extremely small clone (<0.01%) became possible through high-sensitive flow cytometry (FCM), but the clinical significance of small PNH clone has not been elucidated.
Aims: To investigate a correlation of *PIGA* mutation and small PNH FCM clone, we measured PNH FCM clone size and mutant burden of *PIG* gene, with their correlation to treatment response.
Methods: A total of 89 specimens from 63 patients whose PNH clone size was ≥0.1% by FCM was enrolled (classic PNH 9, PNH related with bone marrow disorder 47, subclinical PNH 10). To detect minor cell population with *PIG* mutation, we adopted ultra-deep sequencing for *PIGA*, *PIGM*, *PIGX and PIGT* mutation.
Results: Twenty two% of 63 patients with PNH FCM clone harbored *PIG* gene mutation and 92.8% of patients with *PIG* mutation had >10% PNH FCM clone in RBC and granulocyte. In classic PNH patients (n=6), the average of PNH FCM clone size was 56.8% in RBC and 89.6% in granulocyte, and all patients had *PIG* gene mutation. In patients with subclinical PNH clone, the average of PNH FCM clone was 1.8% in RBC and 3.3% in granulocyte, while *PIG* gene mutation was not detected. In the patients with coexisting bone marrow disorder (BMD), the average of PNH FCM clone size was 8.0% in RBC and 14.9% in granulocyte. Among 6 patients with Eculizumab treatment, hemoglobin increment and decrease of FCM RBC clone size correlated, while LDH decreased in all patients, irrespective of treatment response. Decrease of the ratio over 0.15 (type III/type II+III PNH clone in RBC) was a predictive factor for complete response at 6 months from treatment initiation. Of the 11 patients with consecutive results of *PIG* mutation, 88% of patients with *PIG* mutations was non-responsive to supportive treatment, while 33% of patients without *PIG* mutations was non-response (p=0.072). Mutant burden of *PIG* gene mutation were not changed during treatment irrespective of types of treatment.
Summary/Conclusion: The *PIG* gene mutation was detected only in patients with >10% FCM PNH clone and The mutation burden of *PIG* gene was related to the granulocyte FCM PNH clone size. The presence of *PIG* gene mutations was correlated with adverse treatment response. We suggest monitoring of PNH clone in RBC can be a potential predictor of treatment response as well as Hb during treatment with Eculizumab.

PB1833

CLINICOHEMATOLOGICAL AND CYTOGENETIC PROFILE OF APLASTIC ANEMIA IN PAKISTAN

N. Anwar[1,*], A. Arshad[1], Z. Sharif[1], S. Sharif[2], N. Fatima[2], T. Shamsi[1]

[1]Hematology, [2]Research and development, National Institute of blood diseases and bone marrow transplant karachi Pakistan, Karachi, Pakistan

Background: Aplastic anemia are acquired or congenital anemias associated with hypocellular bone marrow. The exact etiology is unknown but several factors are considered to be causative for suppression of hematopoitic cell production resulting in aplastic anemia.
Aims: This entity considered to be uncommon is frequently prevalent with rising trend seen especially in Pakistan. This is quite contrast to what is observed in west. Moreover, there is scarce local data. Hence the study was done to assess baseline clinical and cytogenetics features of patients presenting with aplastic anemia.
Methods: This study was approved by the Institutional Ethics Committee of National Institute of Blood Diseases and Bone Marrow Transplantation. In this cross sectional study,122 patients with aplastic anemia were enrolled during the period of June 2016 to January 2018. Informed consent was obtained prior to the study. Data collected included demographic information, laboratory information, includinggender, symptoms, treatments, blood counts and chemistry parameters including urea, creatinine and liver function tests.Viral profile included Anti-HCV, Anti-HBsAg, HIV I/II and Cytomegalovirus (CMV) performed. Cytogenetic analysis was performed on bone marrow samples and karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN) 2013, karyogram were made using Meta system. Variables were evaluated using SPSS version 23.
Results: A total of 122 patients were included in the study. The median age of the patients was 14±12.59. A slight predominance of males were observed which were (n=76, 62%). Fever and weakness were the most complain found in (n=56, 46%) followed by colitis (n=24, 20%), gum bleeding (n=20, 16%) bruises (n=12, 10%) and shortness of breath in (n=10, 8%) patients. All patients were grouped according to camitta classification. Non severe aplastic anemia were observed in (n=100, 82%) followed by very severe aplastic anemia (n=12, 10%) and severe aplastic anemia (n=10, 8%). The mean hemoglobin was 7.61±2.11g/dl, red blood cells 3.17±3.4x10^{12}/l, MCV 89.2±11.56fl, total leucocytes counts (tlc) 3.09±2.03x10^{9}/l, absolute neutrophils count 0.7±0.99×10^{9}/l and platelets counts were 27±52.29x10^{9}/l. The mean total bilirubin was 0.76±0.38mg/dl, direct bilirubin 0.37±0.27mg/dl, alanine aminotransferase (SGPT) 61±89.5u/l and alkaline phosphatase was 201±116.5u/l. Out of 122 patients, chromosomal breakage was observed in (n=10, 8%) patients. Anti HbsAg was positive in (n=04, 3%) and anti HCV in (n=02, 1.6%).CMV was positive in (n=02, 1.6%) patients.
Summary/Conclusion: In our study we have observed lower median age andmale predominance.Non severe aplastic anemia was most common. Raised SGPT was seen in many indicating liver damage. To overcome adverse prognostic implications, early identification of such patients with close clinical follow up and upfront stem cell transplant must be considered. This study was done retrospectively yet represents a large cohort of aplastic anemia in the country. In future, prospective studies are needed to be done to elaborate disease biology and clinical outcome of the baseline adverse disease characteristics observed in our study.

PB1834

ADA 2 ENZYME DEFICIENCY MANIFESTING AS PURE RED CELL APLASIA

G. Ersoy[1], C. Bayram[1], M. Gökçe[1], S. Ünal[2], N.N. Ozdemir[1,*]

[1]Pediatric Hematology Oncology, Kanuni Sultan Suleyman Education and Research Hospital, Istanbul, [2]Pediatric Hematology Oncology, Hacettepe University, Ankara, Turkey

Background: Pure red cell anemia is characterized by normochrome normocytic anemia with severe reticulocytopenia and marked decrease or absence in red cell lineage in bone marrow. We herein present a child with congenital pure red cell anemia diagnosed as ADA2 enzyme deficiency to emphasize this rare etiology of pure red cell anemia.
Aims: A 40 day old boy was admitted to our hospital with pallor. On physical examination, there was no hepatosplenomegaly or any stigmates. His laboratory work up showed severe anemia, hgb:2 gr/dl; htc:%5.8; wbc: 9750/mm^3; neu: 1800/mm^3; plt: 690 000/mm^3; MCV:89 fL; LDH:360 U/L. Electrolytes, kidney function tests, and liver function tests were normal. Direct coombs test was negative, reticulocyte count was low (0.2%). On blood smear, erythrocytes were normal in shape and size with no signs of hemolysis. On follow up he was transfusion dependant monthly. Bone marrow aspiration showed decreased erythrocyte progenitor cells. HbF was 5.8%. Viral serology including Parvovirus was negative. Vitamin B12 and folate levels were normal, erythropoetin was 39.9 mU/ml (increased). The diagnosis of pure red cell aplasia was established. He also had a history of recurrent infections and his immunoglobulins were low. The genetic analysis for DBA was negative.
Methods: Whole exome sequencing, showed CECR1 mutation causing ADA-2 enzyme deficiency. His parents were silent carriers. The patient's follow up continues in our outpatient clinic, he receives erythrocte tranfusion and intravenous human immunglobulin monthly.
Results: CECR1 gene is responsible for the synthesis of ADA 2 enzyme which is the major extracellular adenosine deaminase and functions as a growth factor. It was first identified in 2014 in patients with poliarteritis nodosa by exome sequencing and is responsible for a spectrum of autoinflammatory symptoms from vasculitis to thromboembolic events. ADA- 2 enzyme deficiency has been described in around 100 patients until now. Most of the patients had inflammatory symptoms like vasculopathy, lacunar strokes, hepatosplenomegaly or livedo reticularis. Hematologic manifestations were poorly described and unexpected without the inflammatory symptoms or immunodeficiency. In 2016, 5 patients from Israel were reported with hematological manifestations, 2 siblings had congenital pure red cell anemia without any vasculopathy.
Summary/Conclusion: Together with other case reports, our patient represents a new phenotype of this mutation and causes the need for evaluating the functions of ADA 2 in bone marrow.

PB1835

BREAKTHROUGH HEMOLYSIS AND THROMBOEMBOLISM CONTROLLED BY ECULIZUMAB DURING PREGNANCY IN PAROXYSMAL NOCTURAL HEMOGLOBINURIA (PNH): A SINGLE INSTITUTION EXPERIENCE

C. Frairia[1,*], S. Aydin[1], E. Audisio[1], S. D'Ardia[1], G. Iovino[1], B. Nicolino[1],

C. Macchi[2], F. Zizzo[2], F.M. Comoglio[2], T. Todros[2], U. Vitolo[1]

[1]Hematology, AOU Città della Salute e della Scienza di Torino, [2]Obstetric and Gynecology, Department of Surgery, University of Turin, Turin, Italy

Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired clonal stem cell disorder characterized by intravascular hemolysis, cytopenia and thrombophilia. Thromboembolism, infection and premature birth are main reasons for significantly increased maternal and fetal morbidity and mortality during pregnancy and the following post-partum period in PNH patients. Therefore, PNH has been considered a relative contraindication for pregnancy. The terminal complement cascade inhibitor Eculizumab prevents fatal complications and nearly normalizes overall survival in PNH. Consequently, it has become the standard treatment in patients with symptomatic PNH. However, there are limited published data regarding the use of Eculizumab during pregnancy, the postpartum period or, more less, during lactation.
Aims: To evaluate the management of pregnancy in PNH in our institution.
Methods: We report three cases of pregnancy in PNH.
Results: A 28 year old PNH patient became pregnant while on Eculizumab and the therapy was continued throughout the whole pregnancy. At diagnosis of her pregnancy (6th week of gestation) anticoagulation therapy with low molecular weight heparin was initiated and continued although no clinical sign of thrombosis was present. She was immediately introduced in an interdisciplinary team for high risk pregnancies consisting of specialists for gynecology, internal medicine, anesthesia and hematology. During the third trimester she developed a breakthrough hemolysis in terms of symptomatic anemia requiring repeated blood transfusions. Hemolysis was successfully controlled by dose escalation of Eculizumab first from 900mg to 1200mg biweekly and consequently shortening the administration interval from a biweekly to a weekly scheme until the birth of the baby. She successfully delivered a healthy baby at term by natural birth without complications. One month after delivery the patient has returned to her usual therapeutic Eculizumab regimen (900mg biweekly). She breastfeed her baby for six months without complications. The anticoagulation treatment was continued, as recommended, for the first three months. The baby girl is developing well according to her age and she is now one years old. Currently, we are managing other two young pregnant PNH patients. Our second patients is a 27 years old girl who became pregnant while she was not on Eculizumab but only in follow up due to indolent disease without hemolysis. She was started with anticoagulation prophylaxis with low molecular weight heparin as soon as the pregnancy was noted, and she started the standard therapeutic Eculizumab regimen (900mg biweekly) from the beginning of second trimester. She is now at the end of the third trimester, fetal growth is regular, no signs of hemolysis on the mother and no thromboembolic complications were noted. The estimated date of delivery is scheduled for the end of March, 2018. The third pregnancy is on a 27 years old PNH patient currently at the 15th week of gestation; she was on Eculizumab standard regimen treatment from 2009 due to breakthrough hemolysis and we are managing her pregnancy according to the policies followed for the first described case.
Summary/Conclusion: Our single center experience reports a favorable outcome of a PNH patient who became pregnant while under Eculizumab, supporting the scarce published experience that this drug can be given safely and can even be escalated during pregnancy in PNH patients, with a good disease control. We are currently managing other two pregnant PNH patients, at the moment without complications.

PB1836

A CASE OF SEVERE CONGENITAL NEUTROPENIA CARRYING A NOVEL HOMOZYGOUS MUTATION IN CSF3R GENE

H. Tokgoz[1], U. Caliskan[1,*], F. Ozkınay[2], H. Onay[2]

[1]Pediatric Hematology and Oncology, Necmettin Erbakan University Meram Medical Faculty, Konya, [2]Medical Genetics, Ege University Medical Faculty, İzmir, Turkey

Background: Severe congenital neutropenia (SCN) is characterized by profound neutropenia and a predisposition to life-threatining bacterial infections. Autosomal dominant, autosomal recessive, X-linked and sporadic SCN forms have all been described. Mutations in several genes including ELANE, GFI1, HAX-1, G6PC3 and WAS are responsible for SCN. CSF3R mutations are extremely rare and usually somatic.
Aims: Here we describe an SCN case having a novel homozygous CSF3R mutation.
Methods: A-5-month-old boy was referred to our department because of neutropenia. There was a history of hospitalization because of cervical lymphadenitis and neck abscess at the age of 3 months. The parents were consanguineous. Physical examination was normal (no dysmorphic findings such as leukoplaki, nail dystrophy, brown ridging on the neck, thumb abnormalities, etc.were present). Laboratory tests revealed a severe neutropenia (300/mm^3). Lymphocyte count, serum immunoglobulin levels and peripheral lymhocyte subset analysis were all normal. Viral infection markers were negative. Neutropenia persistedat the level of 200-400/mm^3. In the following8 weekperiod, the neutrophile count was monitored twice weekly. All neutrophile counts were below 500/mm^3. A bone marrow examination revealed normocellularity and maturation arrest of granulocytic series at the level of band form. Cytogenetic studies were normal. The patient did not respond to G-CSF therapy and the neutropenia persisted. The patient is now 21-month-old and has experienced several febrile neutropenia episodes. Bone marrow transplantation has been planned. Unfortunately to date no HLA matched donor within the family has been found.
Results: Molecular genetic analysis for ELA-2, HAX-1, G6PC3, SBDS were normal. However, in the CSF3R genea novel homozygous p.V224D (c.671 (T>A)) mutation was detected. Using in silico analysis this novel mutation was found to be pathogenic. A functional analysis has been planned.
Summary/Conclusion: CSF3R mutations in SCN cases are usually somatic or *de novo* and inherited form is extremely rare (Triot A, *et al.* 2014). It has been considered that the SCN case described here will expand the mutation spectrum of GCSF3R gene and help to make phenotype-genotype correlations.

Rerefence

1. Triot A, Jarvinen PM, Arostegui JI, Murugan D, Kohistani N, Dapena Diaz JL, *et al.* Inherited biallelic CSF3R mutations in severe congenital neutropenia. Blood. 2014 Jun 12;123(24):3811-7.

PB1837

BONE MARROW FINDINGS IN COLLAGEN VASCULAR DISEASES ASSOCIATED WITH CYTOPENIAS

D. Elshamy*

Hematology Department, Menoufia Faculty of Medicine, Shebin Elkom, Egypt

Background: the collagen vascular diseases area diverse group of inflammatory systemic disorders thought to be immunologically mediatedincluding systemic lupus erythematosus (SLE), scleroderma, polymyositis and polyarteritisnodosa, they are frequently included in the differential diagnosis for unexplained cytopenias and often prompt a bone marrow evaluation in this population is important.
Aims: to study morphologic and immunohistochemical characteristics of bone marrow involvement in patients with collagen vascular diseases associated with cytopenias
Methods: In the current study, we examined 100 patients. The diagnosis of collagen vascular diseases was made according to diagnostic criteria for each as SLICC for SLE.All patients were subjected to thorough history taking, physical examination, and many investigations were done for them including serology (ANA, Anti dsDNA, AntiCCP, RF), and bone marrow examination was done for all patients.
Results: collagen vascular diseases show female predominance regarding SLE and RA with male: female ratio equal 3:14 and 1:4 respectively. Nephritis and arthritis were the most common presentation in SLE and RA respectively and almost present in all patients followed by hematological manifestations as fatigue and ecchymosis. Normocellular bone marrow was the commonest finding in studied patients followed by hypercellular marrow then hypocellular marrow while dysplastic changes affected erythroid element mostly followed by myloid and megakaryocytic elements regarding eosinipgilic, plasma cells and lymphocytic infiltrations; none of them shows statistically significance
Summary/Conclusion: There were no significant statistical differences between CVDs patients as regard bone marrow findingalso there were no specific bone marrow finding detected in SLE and RA patients.

PB1838

A RARE CAUSE OF ANEMIA: GHOSAL TYPE HEMATO-DIAPHYSEAL DYSPLASIA

K. Yılmaz[1], B. Koc[2,*], G. Dikme[2], H. Kızılocak[2], S. Kurugoglu[3], T. Celkan[2]

[1]Pediatric, Istanbul University, [2]Pediatric Hematology and Oncology, [3]Radyology, Istanbul University, Cerrahpasa Medical Faculty, Istanbul, Turkey

Background: Ghosal type hemato-diaphyseal dysplasia (GHDD) is a rare, autosomal recessive disorder characterized by increased bone density with both diaphyseal and metaphyseal involvement and bone marrow dysfunction marked by a corticosteroid responsive, myelopthisic anemia. The gene

responsible for this disease was *TBXAS1* which encodes the thromboxane synthase (TXAS) enzyme.
Aims: We aimed to emphasize the importance of rare genetic disordes in differential diagnosis of anemia.
Methods: Here is reported such a rare case of Ghosal type hemato-diaphyseal dysplasia.
Results: Three year-old-boy was admitted with history of pallor, progressive weakness, pain at lower extremities and abdominal distension for last few months. He was suffering from progressive pallor for the last three months requiring multiple blood transfusions without any bleeding diathesis. His birth, development, immunization, dietary histories were normal. There was history of consanguinity. He had height of 98 cm (25-50th percentile of NCHS), weight of 16 Kg (50-75th percentile of NCHS) head circumference of 50 cm (25-50th percentile of NCHS). Physical examination revealed hepatosplenomegaly and generalize lymphadenopathy (<1cm). Laboratory investigation revealed hemoglobin 6.7 g/dL, MCV: 82.7fl, wbc: 6800/mm^3, plt: 134.000/mm^3, neut: 1600/mm^3 and reticulocyte count 2%. Peripheral smear was hypochromic normocytic with normoblasts. Serum electrolytes, calcium and renal function were within normal limit. Multiple bone marrow aspirations were dry tap. However, bone marrow biopsy revealed myelofibrosis. After biopsy, steroid treatment was initiated and never required blood transfusion. Ultrasonography of abdomen revealed hepatosplenomegaly. Chest X-ray was within normal limit. X-rays of long bones and skull base showed thickening of diaphysis and moderately thickening of skull base. Distal part of femur revealed Erlen-Mayer deformity. The case had myelofibrosis, bone deformities and steroid responsive anemia showing both hematologic and diaphyseal dysplasia of Ghosal hemato-diaphyseal dysplasia. The genetic counseling of the case revealed TBXAS1 homozygous mutation.
Summary/Conclusion: Our report illustrates the need to consider GHDD in children with anemia, myelofibrosis, bony abnormalities, and consanguineous parents. Moreover, this case demonstrates the efficacy of chronic, low-dose steroid therapy.

PB1839

CHRONIC PRIMARY NEUTROPENIA IN ADULTS. A MONOCENTRIC UNIVERSITY HOSPITAL COHORT STUDY

L. Njue*, N. Bonadies, A. Rovo

Hematology, University hospital Bern, Bern, Switzerland

Background: Chronic primary neutropenia (CPN) is a rare benign entity with increased susceptibility to bacterial infections. So far there is no epidemiological data from Swiss CPN patients available.
Aims: We aimed to evaluate patients characteristics, rate of infections and long-term outcomes of adult CPN patients at our policlinic.
Methods: This is a retrospective study, in which we reviewed all patients recorded as chronic neutropenia in our policlinic. Patients with neutropenia secondary to infection, drugs, tumor, malnutrition, vitamin B12 deficiency, benign ethnic neutropenia, T-cell large granular leukemia or associated to pancytopenia were excluded. Repetitive infections were defined as ≥3 episodes per year. Infections requiring hospitalization were defined as severe.
Results: From 1998 to February 2017, we identified 20 adults with CPN. CPN patients were more often female 16/20 (80%). The median age at diagnosis was 27 (range 1-66) years. Five patients were<18 years at diagnosis. The median absolute neutrophil count (ANC) at diagnosis was 0.41 G/L (range 0.0-0.90), and the median ANC at last follow-up was 0.8 G/L (range 0.03-7.36). All patients underwent bone marrow evaluation and there was no clear bone marrow pathology pattern. Two of twelve (16%) patients evaluated for anti-granulocyte autoantibodies were positive. Regarding management, therapy was aimed to provide a normal lifestyle. Thus 12/20 patients (60%) received G-CSF either sporadically (6/12) or continuously (6/12) and all responded with increase in the number of neutrophils. Repetitive infections were recorded in 7/20 (35%) patients. 5 patients (25%) presented a total of 23 severe bacterial infections; of these, 3 were diagnosed during childhood. 13 patients (65%) neither had repetitive nor severe infections, 6 of them were treated with different G-CSF modalities. Three infection episodes occurred in 3 patients who were under treatment with G-CSF and had normal neutrophil count. 19 pregnancies were documented (12 before initial diagnosis), 1 patient received G-CSF during 2 of her 6 pregnancies with good outcome and one patient had an uneventful pregnancy without G-CSF. The median follow-up of this cohort was 122 months. 3 patients were lost to follow-up. No death due to bacterial infections or hematologic malignancies occurred. One patient died due to glioblastoma multiforme 10 years after diagnosis of CPN.
Summary/Conclusion: Our adult CPN patient cohort is characterized by a female predominance and a benign outcome. Patients diagnosed during childhood seem to have a higher risk of infections. No secondary hematological malignancies were observed. G-CSF therapy was based on individual schedules and was well tolerated. The reasons why some patients are susceptible to repetitive infections and others are not, remain to be elucidated.

PB1840

CLINICAL OUTCOMES IN ADULT PATIENTS WITH APLASTIC ANEMIA: A SINGLE INSTITUTION EXPERIENCE

Y. Petrov[1,*], G. Arnaudov[1], P. Ganeva[1], Z. Popova[2], V. Stoeva[2], S. Mishkarova[2], S. Lozenov[2], M. Donchev[2], G. Balatzenko[3], M. Jagurinoski[4], M. Guenova[4], B. Spassov[2]

[1]Stem cell transplantation, [2]Clinical Hematology, [3]Laboratory of Cytogenetics and Molecular biology, [4]Laboratory of Hematopathology & Immunology, National Hospital for Active Treatment of Hematological Diseases, Sofia, Bulgaria

Background: Severe aplastic anemia (SAA) is a life-threatening disorder characterized by pancytopenia in the peripheral blood and hypocellular marrow. Although both allogeneic hematopoietic stem cell transplantation (HSCT) and immunosuppressive treatment (IST) are available treatments, allogeneic HSCT from a HLA-matched familial donor is preferred by SAA patients. However, approximately two-thirds of patients do not have a suitable HLA-matched sibling donor; therefore, either IST or alternative donor HSCT is employed in such cases. However, previous reports have shown that IST is related with a substantial risk of relapse and clonal evolution. Furthermore, IST often leads to the amelioration of cytopenia rather than cures. Advances in HSCT, such as modification of the conditioning regimen, a better selection of donors by high degree HLA allele level matching and the introduction of low-dose TBI, have improved the outcomes of HSCT in patients with SAA.
Aims: The aim of our study was to compare the outcomes of immunosuppressive treatment (IST) and hematopoietic stem cell transplantation (HSCT) as frontline therapy in young adults with severe aplastic anemia (SAA).
Methods: We retrospectively reviewed the medical records of 24 patients with acquired severe aplastic anemia (SAA). Eleven patients received immunosuppressive treatment (IST) and 13 patients received hematopoetic stem cell transplantation (HSCT) from matched related or unrelated, or alternative donor as a frontline therapy. SAA was defined according to the standard criteria; if patients met these criteria and had a neutrophil count<0.2×109/L, their disease was considered to be very severe aplastic anemia. To assess the effect of treatment modalities on the outcomes, the probable overall survival (OS), failure-free survival (FFS) or event-free survival (EFS) rates were estimated. Complete response was defined as a normal hemoglobin level for age, neutrophil count >1.5×109/L and platelet count >150×109/L. Partial response was defined as transfusion independent and no longer meets the criteria for severe disease. Treatment failure or an event after HSCT was defined as death, primary graft failure, late rejection, relapse and secondary malignancy, whichever occurred first.
Results: Sex, age and the disease status at treatment were comparable between the 2 groups; however, the median time from diagnosis to treatment was longer in the frontline HSCT group than in the frontline IST group (10.1 months *vs* 4.0 months respectively, P=0.025). The overall response rate of IST was 42.1%. Among the 13 patients who underwent frontline HSCT, 11 experienced FFS. Although the 5-year OS was comparable between the frontline HSCT and frontline IST groups (91.3% *vs* 71.2% respectively, P=0.187), the 5-year FFS was significantly higher in the former than the latter (91.3% *vs* 30.7% respectively, P<0.001).
Summary/Conclusion: Both IST and alternative donor HSCT have improved the survival rate of patients with SAA. Our data suggests that frontline HSCT may be a better treatment option than IST for young adults especially in situations where ATG is not available.

PB1841

AN UNUSUAL CASE OF PURE RED CELL APLASIA

M.L. Tung*

Haematology-Oncology, National University Cancer Institute, National University Health System, Singapore, Singapore, Singapore

Background: Acquired pure red cell aplasia (PRCA) is a rare condition and is known to be associated with thymomas. Other associations have been described such as drugs, autoimmune conditions, malignancies and ABO incompatible stem cell transplants.
Aims: Here, we describe an unusual case of PRCA which was associated with both a thymoma and parvovirus infection. As part of the work-up for her thymoma, she was also found to have hypogammaglobulinaemia (Good's syndrome) and positive anti-acetylcholine receptor antibodies without clinical evidence of myasthenia gravis.

Methods: A 45-year-old lady presented with symptomatic anaemia to the haematology clinic. She had no past medical history of note prior to this consultation. She presented with symptoms of a viral illness at another hospital a few weeks prior and was found to have anaemia requiring transfusion. On examination, she was well with mild pallor. The rest of her physical examination was unremarkable with no lymphadenopathy or hepatosplenomegaly. Her heart rate was 87 beats per minute (bpm), blood pressure was 100/53mmHg and her oxygen saturation was 100% on room air. Initial investigations revealed a normochromic, normocytic anaemia with a haemoglobin level of 8.3g/dL. Her white blood cell count (WBC) was $4.2x10^9$/L and absolute neutrophil count (ANC) of $2.25x10^9$/L. Her platelet count was normal. There was also reticulocytopenia with an absolute reticulocyte count of $5.80x10^9$/L. The peripheral blood film showed a normochromic, normocytic anaemia with no other abnormalities seen. A bone marrow examination was performed and revealed an absence of erythropoiesis with preserved granulopoetic and megakaryopoietic activity. Parvovirus PCR was positive as well from the bone marrow sample. A chest computed tomography (CT) demonstrated an anterior mediastinal mass (Figure 1). Given the CT findings, conditions associated with a thymoma was screened for and she was found to have a positive anti-acetylcholine receptor antibody titre of 1.76 nmol/L. Flow cytometry of the bone marrow showed B cell aplasia and the possibility of Good's Syndrome was raised. She had evidence of hypogammaglobulinaemia, with an IgG level of 4.83g/L, IgA of 0.27 g/L and IgM of<0.2g/L, consistent with Good's syndrome.
Results: She continued to require red cell transfusions to maintain an adequate haemoglobin level. She eventually agreed for a thymectomy 9 months after the initial diagnosis. She received red cell transfusions and intravenous immunoglobulin prior to surgery. The histology of the anterior mediastinal mass was consistent with a WHO type AB thymoma. She was commenced on immunosuppressive therapy with cyclosporine as she remained transfusion dependent despite the thymectomy. After 8 months of immunosuppressive treatment post thymectomy, she is currently transfusion dependent for 9 months.

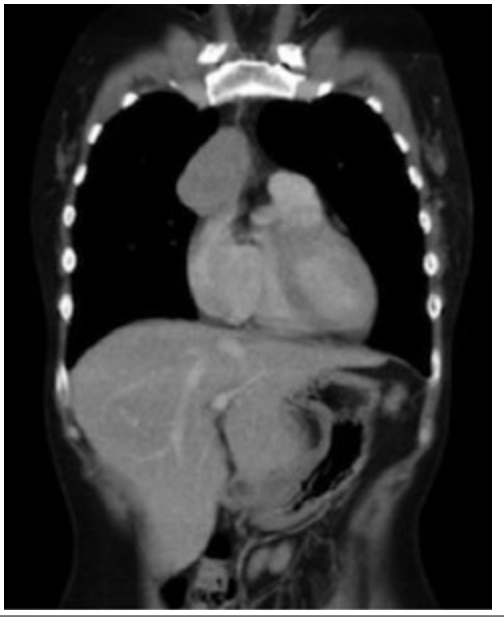


Figure 1.

Summary/Conclusion: Good's syndrome which was associated with the thymoma was important to exclude in this patient prior to her thymectomy to reduce the risk of post surgical infective complications. Given that infective complications were the major cause of death in a case series, I believe that thymoma-associated PRCA should be screened for Good's syndrome prior to their surgery to improve patients clinical outcome.

PB1842

FOUR CASES OF FANCONI ANEMIA WITH VARIABLE ABNORMALITIES

E. Turkkan*

Pediatric Hematology, Okmeydanı Research and Training Hospital, Istanbul, Turkey

Background: Fanconi anemia (FA) is an autosomal recessive genetic chromosomal instability syndrome characterized by congenital malformations, hematological problems and predisposition to malignancies. FA can present a variety of congenital defects but invariably results in defective haemopoiesis, which is the major cause of morbidity and mortality. Numerous congenital abnormalities have been reported in approximately 75% of patients with FA, and the most common of these abnormalities are skeletal (radial ray, hip, vertebral bones, rib), skin dyspigmentation, short stature, abnormalities of the eyes (microphthalmi and strabismus), renal and genito-urinary tract abnormalities, microcephaly.
Aims: In this paper, four cases of FA patients between 7-18 ages (3 female and one male) with different skeletal, skin and hematologic abnormalities are presented.
Methods: All 4 cases had microcephaly, skin dyspigmentation, dysmorphic face appearance, There was 1 case with an unusual thumb polydactyly, and the remaining 3 patients had normal fingers. The extra thumb had nail, but no active motion. Thumb polydactyly is a common hand anomaly but its concurrence with FA is a very rare.
Results: Repeated complete blood cell counts indicated persistent mild thrombocytopenia; diepoxibutane and nitrogen mustard induced chromosome breakage testing on lymphocyte cultures were compatible with the FA and levels of fetal hemoglobin were increased in all of the patients.
Summary/Conclusion: The disease is a heterogeneous condition that can present with a variety of congenital defects. There have been conflicting reports about the severity and type of congenital malformations of the disease. Although diagnosis of FA should be verified by identification of chromosomal instability. Recognition of the numerous pathologies associated with FA is important in order to diagnose of the syndrome.

Chronic lymphocytic leukemia and related disorders – Biology & Translational Research

PB1843

MONOCLONAL GAMMOPATHY AND HYPOGAMMA GLOBULINEMIA AS INDEPENDENT PROGNOSTIC FACTORS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA: A RETROSPECTIVE MONOCENTRIC EXPERIENCE

A. Corbingi, I. Innocenti, F. Autore, F. Morelli, D. Soldati, S. Sica, L. Laurenti*

Policlinco Agostino Gemelli Roma, Roma, Italy

Background: Chronic Lymphocytic Leukemia (CLL) is an indolent B-cell lymphoproliferative disorder. Several prognostic factors such as IGHV mutation status and chromosomal aberrations as trisomy 12, del11q, del13q or del17p have been detected so far. More recent genetic mutations such as BIRC3, SF3B1, NOTCH1 and TP53 stratifies the prognosis and outcome in CLL patients (pts). However all data above reported, because of expensive techniques and experience typical of big laboratories, are not available to all medical centers. Data concerning the presence of IgM, IgG paraprotein and their impact on natural history of CLL pts are controversial and contradictory while more certain seem to be the ones concerning hypogamma/CLL pts.
Aims: The aim of the study is to evaluate the prevalence and the outcome of monoclonal IgM/CLL, IgG/CLL and hypogammaglobulinemia compared with CLL pts with normal immunoglobulin (Ig) levels.
Methods: Our center collected from our CLL database 404 pts diagnosed from 1999 to 2017 with a baseline assessment of serum Ig, immunofixation, immunophenotype, chromosomal aberrations and clinical features evaluating time to progression (TTP), time to treatment (TTT) and overall survival (OS).
Results: Among 404 pts who met eligibility criteria, 26 pts had IgM/CLL, 33 pts had IgG/CLL, 46 pts had hypogamma/CLL and 299 showed no evidence of paraprotein. Median IgM level was 314 mg/dL in IgM/CLL, median IgG level was 1033 mg/dL in IgG/CLL, median gamma globulin was 7.8% in hypogamma/CLL. Median age was similar in all the groups. The worst time-dependent parameters such as TTP, TTT and OS were identified in the IgM/CLL group. These data probably reflect a more aggressive disease with more than 68% of pts in an advanced stage at diagnosis (Rai B/C).

Table 1.

	IgM/CLL	IgG/ CLL	Hypogamma	Absence of gammopathy
Patients	26	33	46	299
Age (y.o.)	69	63	68	66
Range	(48-82)	(45-86)	(39-89)	(26-85)
Gamma protein (%)	14.4	14.3	7.8	13.1
Range	(7.9-21.5)	(9.1-32)	(5.3-9)	(9-49.5)
IgM level (mg/dL)	314	52	19	54
Range	(31-3300)	(19-330)	(3-300)	(8-1443)
IgG level (mg/dL)	921	1033	535	954
Range	(464-2700)	(471-3939)	(320-1151)	(447-2382)
IGHV				
Mutated	11 (61%)	13 (62%)	17 (52%)	147(63%)
Unmutated	7 (39%)	8(38%)	16 (48%)	88 (37%)
CD38				
Positive	11 (58%)	15(52%)	15 (39%)	43 (16%)
Negative	8 (42%)	14(48%)	23(61%)	231 (84%)
Rai Stage				
A	8 (32%)	18(55%)	21 (45%)	173 (58%)
B	14 (56%)	12(36%)	17 (37%)	111 (37%)
C	3 (12%)	3(9%)	8 (18%)	15 (5%)
TTP (months)	3	19	16	28
TTT (months)	3	22	16	30
OS (months)	49.5	75	72	65

Summary/Conclusion: This study highlights the frequency of clonal IgM, IgG and hypogammaglobulinemia in CLL patients. Among the 4 groups, IgM/CLL group seems to have the worst outcome which proved to be a negative prognostic marker in newly diagnosed CLL patients.

PB1844

THE POSSIBLE ROLE OF TLR2 AND TLR9 RECEPTOR DYSFUNCTION IN THE PATHOGENESIS AND PROGRESSION OF CHRONIC LYMPHOCYTIC LEUKEMIA

A. Drobiecki[1], A. Szymczyk[2,*], A. Bojarska-Junak[3], A. Szymańska[2], W. Tomczak[4], J. Roliński[3], I. Hus[2]

[1]Department of Hematology and Bone Marrow Transplantation, Cancer Centre in Kielce, Kielce, [2]Independent Clinical Transplantology Unit, [3]Chair and Department of Clinical Immunology, [4]Chair and Department of Haematooncology and Bone Marrow Transplantation, Medical University of Lublin, Lublin, Poland

Background: Recent data indicate a significant role of antigenic stimulation in chronic lymphocytic leukemia (CLL) development. It has been suggested that severe, symptomatic infections of bacterial and viral etiology can initiate immune disorders leading to the development of CLL. A special role is attributed to infections of the respiratory tract of bacterial etiology, especially those with *Streptococcus pneumoniae*. The induction of a humoral response against the pneumococcal sheath of antigens depends on the B-cell receptor (B-cell receptor) and receptors recognizing pathogen-associated molecular patterns (PAMPs), which also include Toll-like receptors (TLRs), especially TLR4 signaling pathway with the synergistic effect of ligands for TLR2 derived from the cell wall of the pathogens and TLR9, which recognizes fragments of nucleic acids.
Aims: The aim of the study was to evaluate the relationship between the expression of TLR2 and TLR9 responsible for the recognition of *Streptococcus pneumoniae* antigens and prognostic factors, treatment free survival (TFS) and the incidence of infectious complications in patients with CLL.
Methods: The study group consisted of 119 patients aged 49-87 with newly diagnosed CLL. Expression of the studied receptors was assessed by flow cytometry on CD19+ lymphocytes B and CD14+ monocytes with the use of monoclonal antibodies: FITC antihuman CD282 (TLR2) and PE Rat anti-Human CD289 (TLR9).
Results: In patients with CLL percentages of B lymphocytes expressing TLR2 and TLR9 were significantly lower compared to the control group (Md 0.38% *vs* 1.58%, p<0.05) and Md 0.6% *vs* 16.84%; p<0.01, respectively). The lower percentage of CD19+/TLR2+ and CD19+/ TLR9+ cells was associated with unfavorable prognostic factors (deletion of 17p and/or deletion of 11q, ZAP-70 and CD38 expression). Percentages of CD14+/TLR9+ monocytes were lower in the group of patients with advanced disease stages according to the Rai classification, CD38 and ZAP-70 expression and unfavorable cytogenetic aberrations. Percentages of CD14+/TLR2+ cells were lower in CD38-positive and ZAP-70-positive patients. There was also a significantly lower percentage of CD19+/TLR9+ B cells and CD14+/TLR9+ monocytes in patients requiring treatment during observation in comparison with the group of patients with no need to start the therapy. TFS was significantly longer in patients with a higher percentage of monocytes expressing TLR9 (25 *vs* 20 months, p<0.05). The percentage of B lymphocytes expressing TLR9 was significantly lower in patients with recurrent infections. In addition, the reverse correlation between the percentage of monocytes expressing TLR2 and TLR9 and the overall incidence of infections and the incidence of the bacterial infections was observed. Moreover, in patients who required IgG supplementation, the percentages of monocytes CD14+/TLR9+ and lymphocytes CD19+/TLR9+ were significantly lower compared to the patients without the need for supplementation (p<0.05).
Summary/Conclusion: The presented data suggest that the reduced expression of TLR2 and TLR9 might be involved in CLL pathogenesis and progression. One of the potential mechanisms in which the aberrant expression of TLR2 and TLR9 may affect the development and progression of CLL is the increasing susceptibility to infections that from one side are one of the most important causes of morbidity and mortality in patients with CLL, and on the other side may contribute to the progression of leukemia.

PB1845

DETECTION OF MEASURABLE RESIDUAL DISEASE BY NEXT-GENERATION SEQUENCING IN PAIRED BLOOD AND BONE MARROW SAMPLES FROM PATIENTS WITH LYMPHOID MALIGNANCIES

D. Miklos[1], L.W. Lee[2], B. Crossley[2], L. Muffly[1,*]

[1]Stanford Hospitals and Clinics, Stanford Medicine, Stanford, [2]Translational Medicine, Adaptive Biotechnologies, Seattle, United States

Background: Cytomorphologic examination of bone marrow samples from patients with leukemia has been a hallmark of clinical management for decades. More recently, measurable residual disease detection (MRD) has become an important tool for the monitoring of patients with lymphoid

malignancies during their course of therapy and for early detection of impending relapse. Conventionally in the USA, bone marrow is collected and analyzed by multiparametric flow cytometry (mpFC) to evaluate MRD status. The sensitivity of mpFC varies but generally is in the range of 10^{-3} to 10^{-5} if one million cells are subjected to analysis. More recent molecular methods such as next-generation sequencing (NGS) allow for more sensitive detection of MRD at 1 in 10^{-6} if one million cells are analyzed. Thus, for the same number of cells analyzed NGS is approximately 10-100 times more sensitive than flow cytometry. The higher sensitivity of NGS raises the possibility that peripheral blood (PB) samples with an overall lower total tumor burden than bone marrow may nevertheless be suitable for MRD tracking by NGS in certain lymphoid malignancies.
Aims: We conducted a comparative analysis of the levels of MRD by NGS of paired PB and bone marrow samples among patients with lymphoid malignancies in order to determine concordance of MRD between marrow and PB, as well as the sensitivity and specificity of tracking MRD in the PB by NGS.
Methods: Forty-seven paired marrow and PB samples were obtained from 33 patients with lymphoid malignancies: 20 with acute lymphoblastic leukemia (ALL), 11 with chronic lymphocytic leukemia (CLL), and 2 mantle cell lymphoma patients. Genomic DNA was extracted from pre-treatment bone marrow samples and Adaptive Biotechnologies' NGS MRD assay was used to evaluate the complete B and/or T cell repertoires in order to identify dominant sequences appropriate for MRD tracking. Subsequently, paired PB and bone marrow samples were obtained from this patient cohort and evaluated for MRD via NGS.

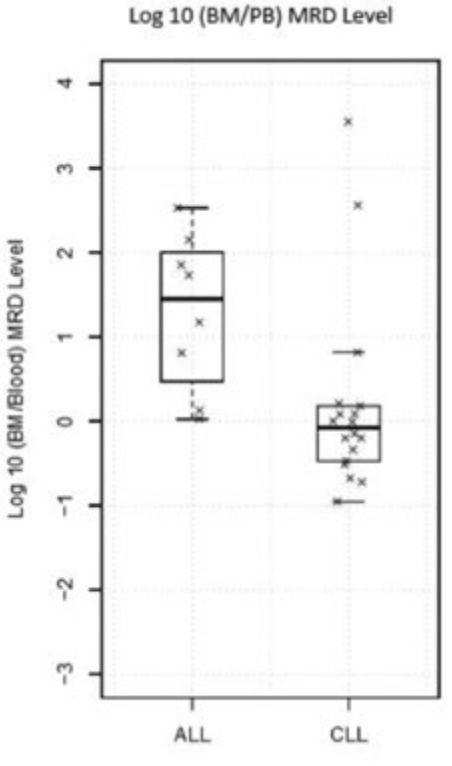


Figure 1.

Results: Twenty of 47 paired samples were negative for MRD in both bone marrow and PB. 27 of 47 paired samples were positive for MRD in the bone marrow; 23 of these were also positive for MRD in the corresponding blood sample. When compared to bone marrow, the sensitivity of detection in PB for ALL is 75% and for CLL is 88.9% with a specificity of 100% for both. Comparison of the MRD levels from PB and bone marrow for the 27 MRD positive cases generated a Lin's concordance coefficient of 0.92. Overall, MRD levels in the marrow were a median of 1.2 times higher than MRD in levels in the PB. Using the criterion of a one hundred fold difference between bone marrow and PB (roughly corresponding to an average difference in sensitivity of mpFC and NGS), 91.5% of the paired samples demonstrated concordance between PB and marrow MRD NGS results. The median ratio of marrow to PB MRD differed significantly between diseases (Marrow: PB MRD of 0.8 in CLL and 28.3 in ALL), Wilcoxon p value=0.007, see figure. Individual cases with discordant MRD results will be described in detail in the formal presentation.

Summary/Conclusion: Monitoring of MRD levels via NGS of the peripheral blood over time may provide an alternative to more invasive bone marrow aspiration and mpFC analysis in patients with lymphoid malignancies. Confirmatory prospective studies of PB MRD monitoring by NGS in patients with lymphoid malignancies are warranted.

PB1846

AGE-RELATED BACH2/PRDM1 GENE EXPRESSION ALTERATIONS IN T AND B LYMPHOCYTES FROM CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS AND AGE-MATCHED HEALTHY DONORS

P. De Silva[1,2,*], V.L. Dang Chi[1,2], S. Garaud[1], B. Stamatopoulos[3], C. Gu-Trantien[1,4], H. Duvillier[1], J.-N. Lodewyckx[1], K. Willard-Gallo[1], C. Sibille[5], D. Bron[2]

[1]Molecular Immunology Unit, [2]Hematology, [3]Laboratory of Clinical Cell Therapy, [4]Institute of Medical Immunology, [5]Department of Pathology, Institut Jules Bordet, ULB, Brussels, Belgium

Background: With advancing age, especially after the six decade of human life, the probability of developing cancer, including malignant hemopathies such as chronic lymphocytic leukemia (CLL), increases. This happens due to progressive decline in immunosurveillance that favors tumor escape and progression in elderly. With increasing age, the human body is more prone to genetic and epigenetic changes that are important contributing factors in the pathogenesis of cancer by negatively impacting on immune cell function. Moreover, these genetic or epigenetic modifications associated with aging could also impact on tumor suppressor genes (TSGs).
Aims: We previously reported a correlation between 6q deletion and progression into a T cell lymphoproliferative disease, identifying the *BACH2* gene as a candidate TSG. We thus examined the expression of specific transcription factors; BACH2/PRDM1 in T and B cells for their potential role in immunosenescence.
Methods: Peripheral blood mononuclear cells were isolated from whole blood obtained from untreated B-CLL patients (n=41) and age-matched healthy donors (HD; n=60) using Lymphoprep (Stemcell Technologies) density gradient centrifugation. T cells ($CD3^+CD4^+$, $CD3^+CD8^+$) and B cells ($CD19^+$) were purified (purity between 95%>99%) using magnetic isolation for subsequent molecular analyses. *BACH2*, *PRDM1 and CDKN2A (p16INK4A)* transcripts were quantified using RT-qPCR. *BACH2* and *BLIMP1 (PRDM1)* protein expression were examined by Western blotting. To measure ß-galactosidase activity, we used flow cytometry with Fluorescein-di-beta-D-galactopyranoside (FDG) as a substrate for beta-galactosidase. T and B cell apoptosis was analyzed after intracellular oxidative stress-inducing etoposide treatment in both populations.
Results: *BACH2* gene expression in the HD groups is significantly down-regulated in $CD4^+$, $CD8^+$ T cells and $CD19^+$ B cells from the older HD group (p=0.0012; 0.0045 and 0.0367, respectively). *BACH2* expression further reduced in $CD4^+$, $CD8^+$ T cells and $CD19^+$ B cells from CLL patients compared to age matched-HD (p=0.001;<0.0001 and 0.0043). *PRDM1* gene expression inversely correlated with *BACH2* in $CD4^+$, $CD8^+$ T cells and $CD19^+$ B cells (r=0.61; 0.71 and 0.65, respectively). Moreover, *PRDM1* was as expected significantly upregulated in $CD4^+$ and $CD8^+$ T cells (p=0.0034; p=0.0017) from B-CLL patients but not in their leukemic-B cells. Western blotting analysis demonstrated that *BACH2* and *BLIMP1* (*PRDM1*) protein expressions in the T and B cell subpopulations significantly correlated with transcript expression. We further studied correlation between BACH2 expression and other senescence markers, such as *p16INK4A* (encoded by *CDKN2A* gene) and ß-galactosidase in both T cells and B cells. *CDKN2A* gene expression inversely correlated with BACH2 in $CD4^+$, $CD8^+$ T cells and $CD19^+$ B cells (p=0.036; 0.025 and 0.043, respectively). ß-galactosidase activity showed a twofold increase compared to the BACH2 deficient lymphocytes ($CD4^+$, $CD8^+$ and $CD19^+$) in older HD compared to young. We also observed a strong correlation between age-related BACH2 down-regulation and a decrease in $CD4^+$ T cell and $CD19^+$ B cell apoptosis (p=0.0127 and 0.0218 respectively).
Summary/Conclusion: Our data suggest that downregulation of *BACH2* and upregulation of *PRDM1* expression in major lymphocyte subsets from CLL patients and older healthy controls are significantly correlated with the aging immune cells and could be part of the resistance to apoptosis in B-CLL cells.

PB1847

CORRELATION BETWEEN IGHV MUTATIONAL STATUS AND AID "ON-TARGET" MOTIFS (WGCW) COUNT IN A SPANISH CLL COHORT

A. Fuentes[1,*], A. Serrano[2], B. Ferrer[3], B. Navarro[3], M. Ortiz[4],

A. Rodriguez[5], A. Medina[6], M. Fernández[7], V. Martín[7], E. Rios[8], C. Chic[9], M.J. Berruezo[10], C. Benet[11], M.A. Ruíz[12], M. Montagud[13], I. Navarro[14], A.I. Teruel[3], M.J. Terol[3], J.F. Chaves[1]

[1]Genotyping and Genetic Diagnosis Unit, [2]Hematology, INCLIVA Institute, [3]Hematology, Clinic University Hospital, Valencia, [4]Hematology, University Hospital Carlos Haya, Malaga, [5]Hematology, University Hospital Virgen de la Macarena, Sevilla, [6]Hematology, Hospital Costa del Sol, Malaga, [7]Hematology, University Hospital Reina Sofía, Córdoba, [8]Hematology, University Hospital Virgen de Valme, Sevilla, [9]Hematology, Hospital Infanta Margarita, Córdoba, [10]Hematology, University Hospital de Jerez, Jerez de la Frontera, [11]Hematology, University Hospital Arnau de Vilanova, [12]Hematology, University Hospital San Francisco de Borja, Valencia, [13]Hematology, Hospital de Vinaroz, Castellón, [14]Hematology, Hospital de Sagunto, Valencia, Spain

Background: The mutational status of *immunoglobulin heavy variable* (*IGHV*) genes has been established as one of the most reliable prognostic markers in CLL. Unmutated CLL (U-CLL), is associated with an aggressive phenotype and shorter survival than patients with less than 98% identity to germ line ("mutated CLL"). The molecular mechanisms underlying these subtypes are incompletely understood. Human immunoglobulin (Ig) genes are diversified in mature B cells by distinct processes known as Ig heavy chain class switch recombination and Ig variable region exon somatic hypermutation. These DNA modification processes are initiated by activation-induced cytidine deaminase (AID), a DNA cytidine deaminase predominantly expressed in activated B cells. In malignant lymphoid tumors of germinal origin, activation-induced deaminase appears to be pathogenic. More recent work has suggested that WGCW sites, and in particular certain AGCT sites, may play a special role as AID "entry sites" that not only mutate at high frequency, but also facilitate further mutations close to the site of the original mutation and throughout the V region.
Aims: In this study, we investigated the frequency and mutation status of *IGHV* in a cohort of 489 CLL patients from Mediaterranean region of Spain. We also analyzed the relationship between the number of WGCW hotspots in the germline V-region and the observed mutation frequency in this cohort.
Methods: Initial FASTA files with IGH (BIOMED-2) sequences from 489 patients sequenced by Sanger were converted to FASTQ with simulated qualities to test our IGH NGS-designed analysis pipeline. Reads are aligned against IGHV, IGHD and IGHJ genes from IMGT References Database using BWA mem, and then mapping statistics are generated to select the 10 more represented alleles for each sample. IGHV and IGHJ genes are determined and compared with IMGT-VQuest results. Homology and mutational status in IGHV is achieved by performing local alignment (EMBOSS Water) against the candidate alleles, and those with a homology percent less than 85 are discarded (30 out of 489 samples). All the sequences were productive. IGHD allele is determined extracting CDR3 according to IMGT Unique numbering recommendations and then the subset is aligned against IGHD alleles using BLAST, choosing the hit with higher score. WGCW motif count was performed on each sequence using custom R scripts. Secondary AID hotspots (WRC, GYW, SYC, GRS) were quantified separately not involving possible overlappings with WGCW motif.
Results: The mean number of AID hotspots (WGCW) in the germline V region of U-CLL cases (11 motifs), is significantly higher than for M-CLL patients (6,8 motifs) whereas in the secondary hotspots we could not find any significative differences (Fig1.a). We compared the mutational status of each V gene, to the mean number of WGCW motifs (vertical axis) in the corresponding germline V region, and contradictory, V regions with more WGCW motifs are more likely to be U-CLL (Fig1.b). All of patients with IGHV1-69 rearrangement have a mean mutation frequency of 0.35%, and paradoxical, this germline sequence contains a mean of 13 WGCW sites; whereas cases expressing IGHV3-15 or IGHV3-72 clones, are commonly mutated, with a mean mutation frequency of 6% and 5.2%, respectively, and contains less hotspots (5-7 WGCW sites) (Fig1.c).
Summary/Conclusion: We confirm a geographical-dependent leukaemic repertoire and reproduced previous studies which observed both V-regions with more WGCW hotspots in the germline sequences are more likely to be unmutated and this relationship is restricted to WGCW hotspots.

PB1848

QUADRANT ANALYSIS OF DRUG APPROVAL AND EMERGING TREATMENT TRENDS IN CHRONIC LYMPHOCYTIC LEUKEMIA-A RETROSPECTIVE STUDY ON PIVOTAL CLINICAL TRIALS

M. Chhikara[1,*], D. Mazumder[2], R. Tyagi[2]

[1]Oncology, SmartAnalyst Inc., New York, United States, [2]Real World Evidence, SmartAnalyst India Pvt Ltd, Gurgaon, India

Background: CLL is an incurable lymphoproliferative disease where treatment landscape has rapidly evolved with the approval of several novel oral targeted therapies across various settings. Most settings are unique based on baseline risk profiles of the patients (pts). In absence of head-to-head trials, it is challenging to compare treatments for researchers and physicians as different treatments are approved in different patient segments. There are important prognostic and predictive parameters that guide the therapy choices and outcomes. Using appropriate methods, CLL can be classified into three risk groups differing in efficacy and safety outcomes that can guide patient selection for specific therapies.
Aims: The primary aim of the study is to evaluate risk adjusted relative scores of efficacy and safety for approved drugs in CLL.
Methods: The study used data from SMARTOncology Database (SmartAnalyst Inc., USA) developed for internal research purposes using public sources such as ClinicalTrials.gov, publications, company websites and FDA labels. The study derived patient risk scores based on prognostic variables such as median prior lines of therapy, ECOG, staging, co-morbidities to categorize pts into low, medium and high-risk groups. This study also considered pts with 17p deletion (del)/TP53 mutations (mut) as high-risk pts. For the trials where 17p del/TP53 mut information was missing, the risk categorization was done based on above prognostic factors. The risk-adjusted relative scores of efficacy and safety endpoints (ORR, mPFS and grade ≥3AEs) were derived based on patient risk scores to design the quadrants. The individual quadrants were designed for ORR and safety endpoints and for mPFS and safety endpoints. The X-axis represented efficacy from 'Low-High' and the Y-axis represented safety from 'Low-High'. The adjusted relative score for each drug was plotted and collectively rated based on the position of the drug in the respective quadrant. The drugs represented in the quadrant of 'High efficacy' and 'High safety' can also be called as "quadrant of effectiveness". The analysis was carried out separately for the drugs approved in frontline and relapsed/refractory (RR) settings.
Results: The study revealed that Ibrutinib and Ofatumumab+chlorambucil were observed in the quadrant of effectiveness based on ORR and safety endpoints, while Ibrutinib and Obinutuzumab +chlorambucil were observed in the quadrant of effectiveness based on mPFS and safety endpoints in frontline. The study revealed that Ibrutinib has the most promising efficacy and safety as compared to other novel drugs. In RR setting, Ibrutinib and Venetoclax were observed in the quadrant of effectiveness based on both ORR and safety endpoints as well as mPFS and safety endpoints. While Idelalisib+Rituximab represented relatively lower effectiveness compared to the other drugs. The study also revealed that Ibrutinib and Venetoclax were the most promising drugs in RR setting compared to others.

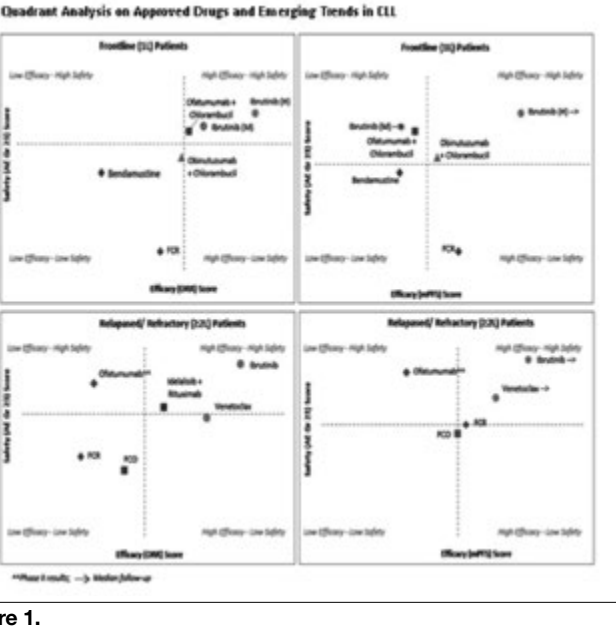


Figure 1.

Summary/Conclusion: Despite multiple approvals of novel therapies in CLL, unmet need still persists. These approved therapies may be tested in wide range of patient settings. The current analysis suggests that if the therapies were observed in the quadrant of effectiveness the probability of approval may increase and so does the broader applicability across multiple settings. Further analysis will help in understanding the correlation of differences in patient characteristics and cytogenetic testing practices on treatment decisions and outcomes.

PB1849

UNCLASSIFIABLE ISOLATED MONOCLONAL HYPERLYMPHO CYTOSIS: COMPREHENSIVE DESCRIPTION OF A RETROSPECTIVE COHORT

M. Degaud[1,*], L. Baseggio[1,2], B. Grange[1], D. Manzoni[1], S. Huet[1,2], E. Callet-Bauchu[1,2], A. Traverse-Glehen[2,3], H. Ghesquières[2,4], G. Salles[2,4], P. Sujobert[1,2]

[1]Laboratoire d'hématologie, Hospices Civils de Lyon-Centre Hospitalier Lyon Sud, [2]INSERM 1052, CNRS 5286, Université Claude Bernard-Faculté de Médecine Lyon Sud Charles Mérieux, [3]Laboratoire d'anatomopathologie, [4]Service d'hématologie clinique, Hospices Civils de Lyon-Centre Hospitalier Lyon Sud, Pierre Bénite, France

Background: With the emergence of targeted therapy, the precise classification of B-cell disorders has become critical. Indeed, a B-cell disorder presenting an altered TP53 gene should be treated by BCR inhibitors if it is a chronic lymphocytic leukemia (CLL), but by immunochemotherapy if it is a marginal zone lymphoma (MZL). For B-cell disorders without tumoral syndrome, the diagnosis relies only on the analysis of circulating lymphocytes. Most of the time, cytologic, phenotypic, cytogenetic and molecular data allow to correctly classify those patients according to the WHO classification. However, for a few patients, the comprehensive analysis of tumoral cells doesn't allow to retain a precise diagnosis.
Aims: The aim of this study was to better characterize the clinical presentation and outcome of patients with absolute lymphocytosis that cannot be precisely classify in the WHO classification.
Methods: We describe a monocentric retrospective cohort of patients presenting a lymphocyte count >5 G/L, but without clinically detectable tumoral syndrome. We excluded patients with evident diagnosis according to the WHO classification, such as CLL, mantle cell lymphoma, or splenic MZL. Overall, 18 patients fulfilling these criteria were identified between 1999 and 2015 in our tertiary care center. We have reviewed cytological, phenotypical, cytogenetical and molecular features of these patients. For 8 patients, a panel of 94 genes was sequenced.
Results: Most patients were old (median age: 79 year-old) and without clinical signs or cytopenia. Cytological and phenotypical features were intermediate between CLL and MZL (notably with the RMH score<3 and positive CD5 staining for 16/18 cases). Cytogenetic analysis highlighted a chromosome 12 trisomy for 13/18 cases, and other alterations found in CLL (17p deletion, 13q deletion). Sequencing of the gene panel highlighted molecular anomalies found in both CLL and MZL, with frequent mutations of TP53 (3/8 patients). The disease seems relatively indolent as 5/18 patients were treated after a median follow-up of 4 years.
Summary/Conclusion: We have identified a group of patients presenting biological features intermediate between CLL and MZL. These B-cell disorders are characterized by a high frequency of chromosome 12 trisomy and a relatively indolent evolution. As these cases cannot be definitively classified in a category of the WHO classification, the choice of the first line treatment might be problematic: for patients presenting a TP53 alteration, if they are classified as CLL, ibrutinib is indicated, but this is not the case if they are classified as MZL. Other case series are mandatory to precise the optimal treatment of this B-cell disorder with intermediate features between CLL and MZL.

PB1850

TARGETING THE INTERPLAY BETWEEN HSP70 (HEAT SHOCK PROTEIN OF 70KDA) AND HSF1 (HEAT SHOCK FACTOR 1) IN CHRONIC LYMPHOCYTIC LEUKEMIA

F. Raggi[1,2,*], F. Frezzato[1,2], V. Martini[1,2], F. Severin[1,2], V. Trimarco[1,2], A. Visentin[1,2], E. Scomazzon[1], M. Facco[1,2], G. Semenzato[1,2], L. Trentin[1,2]

[1]Department of Medicine Hematology and Clinical Immunology Branch, Padua University school of Medicine, [2]Venetian Institute of Molecular Medicine VIMM, Padua, Italy

Background: Chronic Lymphocytic Leukemia (CLL) is a heterogeneous disease characterized by the accumulation of clonal B cells in peripheral blood, bone marrow and lymphoid tissues[1]. Recently, we demonstrated that two proteins endowed with cytoprotective activity, the Heat Shock Protein of 70kDa (HSP70) and the Heat Shock Factor 1 (HSF1), are overexpressed in CLL B cells and correlated to poor prognosis. HSF1 is regulated by a fine balance of activatory/inhibitory phosphorylations mediated by kinases belonging to RAS-triggered pathways[2]. Thanks to our previous study[3], we hypothesize a model by which HSF1 is regulated through different RAS pathways, thus helping to gather information and dissect these networks in CLL. Particularly, the activation of RAS/PI3K/AKT is able to up-regulate HSP70 production, while the activation of the RAS/RAF/MEK/ERK pathway leads to the down-modulation of HSP70 expression.
Aims: Since we found that HSP70 and HSF1 were overexpressed in CLL and considering the pro-survival role of HSP70/HSF1 axis in cancer[4], we are herein aimed at testing their druggability in CLL neoplastic B cells. In this context, we used molecules whose activity simultaneously affected the two RAS-mediated pathways, inhibiting AKT and activating ERK to the final purpose of down-modulating HSP70.
Methods: Freshly isolated leukemic B cells from therapy-free CLL patients were cultured in RPMI 1640 supplemented with antibiotics and 2% FBS and treated separately with: 40μM Resveratrol (a phenol); 10, 20 and 30μM Pterostilbene (a natural analogue of Resveratrol); 10, 50 and 100μM Triacetyl Resveratrol (a Resveratrol prodrug displaying superior bioavailability to Resveratrol); 5, 10 and 20μM Honokiol (a poly-phenolic compound whose action resemble that of Resveratrol). Apoptosis was evaluated after 24 hours by Annexin V/Propidium iodide flow cytometry test and by the presence of cleaved PARP in Western blotting.
Results: We found that in CLL, Resveratrol, or molecules with the same mechanism of action (*i.e.* inhibiting AKT activity and enhancing that of ERK) are able to induce apoptosis of neoplastic B cells in a dose-dependent manner. Particularly, we observed apoptosis after treatment with: 40μM Resveratrol (54±20% of living cells *vs* untreated cells, 70±18%; p<0.01, paired Student's *t* Test); 10μM Triacetyl Resveratrol (65±8% of living cells *vs* untreated cells, 80±5%; p<0.01, paired Student's *t* Test); 20μM Honokiol (26±29% of living cells *vs* untreated cells, 77±8%; p<0.05, paired Student's *t* Test). Preliminary data on Pterostilbene show similar results.
Summary/Conclusion: HSP70 and HSF1 overexpression and correlation with poor prognosis in CLL patients underline their pivotal role in the regulation of leukemic B cell survival. For this reason, they represent interesting targets for anti-leukemic therapies. Of note, the use of molecules that simultaneously act at two different levels in the regulation of HSF1, and consequently of HSP70, should be considered in this field.

PB1851

ANALYSIS OF THE 3′UTR REGION OF THE NOTCH1 GENE IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

N. Bilous[1,*], I. Abramenko[1], A. Chumak[1], Z. Martina[2], I. Dyagil[2]

[1]Clinical Immunology, [2]Hematology, Research Center for Radiation Medicine, Kyiv, Ukraine

Background: *NOTCH1* mutations are associated with poor prognosis for chronic lymphocytic leukemia (CLL) patients. They are represented mostly by c.7541_754delCT deletion and, in the minority of cases, point mutations in the 3 UTR region of *NOTCH1. Both types of mutations* resulted in the removal of C-terminal PEST domain and deregulation of *NOTCH1*-dependent signaling pathways.
Aims: To evaluate the frequencies of mutations in the 3 UTR region of the *NOTCH1 in CLL patients in Ukraine and the distribution of* rs3124591 genotypes located in the amplified area.
Methods: Detection of mutations in the 3 UTR region of the *NOTCH1 was performed by* direct sequencing in *109 CLL patients in whom typical* c.7544_7545delCT mutation was not found. *IGHV* rearrangement were analyzed by IMGT/V-QUEST (Brochet *et al.*, 2008). Analysis of rs3124591 genotypes was performed using SNPstats tool (http://bioinfo.iconcologia.net/ snpstats/start.htm).
Results: Mutations in the 3 UTR region of the *NOTCH1 were revealed in 3 of 109 CLL patient (2.75%), all three among 92 cases with in-frame unmutated IGHV genes (5.4%).* Non-coding mutations were represented by 139390152A>G (*two cases*) and 139390145A>G (one case). *Two cases with* mutations were related to subset #1, and one case belonged to stereotyped subset #28a. In addition to previously revealed c.7544_7545delCT mutations in 34 CLL patients, four of eight cases of subset #1 and both cases belonging to subset #28a in our CLL cohort had *NOTCH1* mutations. The distribution of rs3124591 genotypes was as follows: CC genotype-22 cases (20.2%), CT genotype-47 cases (43.1%), and TT genotype-40 cases (36.7%). The spectrum of used *IGHV* genes was significantly larger in TT homozygotes than in carriers of CT and CC genotypes (; p=0.046). When in-frame unmutated *IGHV* rearrangements were analyzed, we found a reduced *IGHV*1-69 gene usage in carriers of TT genotype compared to carriers of CT and CC genotypes (14.7%, 38.5%, and 47.4%; p=0.023) and, on the contrary, more frequent *IGHJ4* gene usage (50.0%, 25.6%, and 21.1%; p=0.046). In addition, the frequency of "major" stereotyped subsets (according to Agathangelidis *et al.*, 2012) was higher in TT carriers compared to carriers of CT and CC genotypes (26.5%, 10.2%, and 5.5%; p=0.021). The number of N nucleotides inserted in the V_HD junctions in

unmutated *IGHV* rearrangements was significantly less in carriers of TT genotype than in carriers of CT and CC genotypes (5.28±0.74, 9.21±0.94, and 9.21±1.03, correspondingly; p=0.003). The HCDR3 length in carriers of TT genotype was significantly shorter than in carriers of others genotypes (18.88±0.71 a.a. *vs* 21.15±0.68 a.a. in CT genotype, and 21.36±1.13 in CC genotype; p=0.05). The comparison of CLL sequences with non-CLL sequences available from public databases showed that most cases that had HCDR3 homology with antibacterial or antiviral Ig clones were present in TT homozygotes (18.7% *vs* 6.5% in carriers of CT genotype and 0% in carriers of CC genotype; p=0.042). All CLL sequences homologous with autoreactive clones were revealed in carriers of TT (10.6%) and CT (8.7%) genotypes.

Summary/Conclusion: Our data confirmed current data on the association between the structure of the B-cell receptor and appearance of *NOTCH1* mutations. Some features of HCDR3 structure were identified in carriers of TT and CC genotypes. Taking into account data of Cao *et al.* (2014) on the functional significance of this polymorphism, it can be suggested that rs3124591 can influence on the selection of B-cell clones during early stages of CLL development.

PB1852

MUTATIONAL STATUS, IGHV GENE REARRANGEMENTS AND STEREOTYPED RECEPTORS IN CHRONIC LYMPHOCYTIC LEUKEMIA: RESULTS FROM A SOUTH AMERICAN MULTICENTER STUDY

C. Stanganelli[1,*], N. Sotelo[2], M.E. Márquez[3], D. Torres[4], P. Segges[4], M.T. Muniz[5], R. Bigni[6], P. Campregher[7], E. Abdelhay[8], J. Cabrera[9], P.-A. Deglesne[3], J.L. López[10], R. Bezares[11], H. Fernández Grecco[12], M. Cabrejo[12], R. Gabús[13], G. Dighiero[14], P. Oppezzo[14], R. Hassan[8], I. Slavutsky[15]

[1]Patología Molecular, Academia Nacional de Medicina de Buenos Aires, Buenos Aires, [2]Instituto de Biotecnología de Misiones, Universidad Nacional de Misiones, Misiones, Argentina, [3]Centro de Medicina Experimental, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela, Bolivarian Republic Of, [4]Centro de Transplante de Medula Óssea, Instituto Nacional de Câncer, Río de Janeiro, [5]Centro de Oncohematologia Pediátrica, Hospital Universitário Oswaldo Cruz, Instituto de Ciências Biológicas, Universidade de Pernambuco, Recife, [6]Serviço de Hematologia, Instituto Nacional de Câncer, Rio de Janeiro, [7]Departamentos de Hematologia e Patologia Clínica, e Centro de Pesquisa Experimental, Hospital Israelita Albert Einstein, São Paulo, [8]Centro de Transplante de Medula Óssea, Instituto Nacional de Câncer, Rio de Janeiro, Brazil, [9]Patologia Molecular, Academia Nacional de Medicina de Buenos Aires, Buenos Aires, Argentina, [10]Banco Municipal de Sangre, Caracas, Venezuela, Bolivarian Republic Of, [11]Hospital Álvarez, [12]Sanatorio Méndez, Buenos Aires, Argentina, [13]Hospital Maciel, [14]Instituto Pasteur de Montevideo, Montevideo, Uruguay, [15]Laboratorio de Genética de Neoplasias Linfoides, Instituto de Medicina Experimental, CONICET- Academia Nacional de Medicina, Buenos Aires, Argentina

Background: The *IGHV* (immunoglobulin heavy-chain variable region) gene mutational status is considered as an important prognostic factor in chronic lymphocytic leukemia (CLL), readily identifiable at diagnosis and independent of clinical stage or other prognostic factors. In addition, about 30% of CLL patients carry quasi-identical B-cell receptor (BCR) immunoglobulins that can be assigned to distinct stereotyped subsets. At present, information about *IGHV* repertoire and stereotyped BCRs (BCRSs) in CLL patients from South America is scarce.

Aims: In the current study, we have evaluated *IGHV* mutational status, gene usage and BCRSs in CLL patients from a multicenter study developed in the context of the Latin American Group of CLL.

Methods: Our cohort included 707 unselected CLL patients from Argentina (253), Brazil (276), Uruguay (99) and Venezuela (79) (413 males; mean age: 66.6 years, range: 27-105 years; Rai clinical stages: 0: 35.8%, I-II: 41.9%, III-IV: 22.3%). The study was approved by the local Ethics Committees of each Institution. All individuals provided their informed consent. PCR was performed on cDNA or gDNA using VH framework region 1 consensus family specific (VH1-VH6) or leader primers and antisense primers JH or Cμ. PCR products were analyzed on an automated DNA sequence analyzer. Sequence data were evaluated using IgBLAST and IMGT/V-Quest databases. *IGHV* sequences with ≥98% homology with respect to the germline counterpart were considered as unmutated. Stereotyped rearrangements and clusters were assigned by means of pair-wise alignment with known stereotyped sequences available from different public databases and using the new bioinformatics approach to identify major clusters.

Results: Our cohort showed over-representation of mutated cases, except for Brazilian patients. Concerning IGHV family usage, VH3>VH4>VH1 distribution was found in Argentina, while VH3>VH1>VH4 was present in the other countries. The most frequently used *IGHV* genes were *IGHV1-69* (12.3%), *IGHV3-23* (8.2%) and *IGHV4-34* (9.5%). *IGHV3-21* was present in 10% of Venezuelan patients but showed lower frequencies in Uruguayan (2%) and Brazilian (2.1%) cohorts and intermediate frequency in the Argentinean series (6.5%). BCRSs were present in 15.2% of total cases, 73.9% belonged to major clusters; three novel potential subsets were detected. As a whole, our cohort showed the following frequencies: cluster #1 (11; 1.53%), cluster #2 (9; 1.26%), cluster #4 (16; 2.23%) and cluster #8 (5; 0.70%). Argentinean series exhibited similar frequencies of clusters #1 (2.00%) and #2 (3.13%) than series previously reported. These clusters were under-represented or absent in the other studied countries, meanwhile cluster #4 was over-represented in South American cohort, particularly in Uruguay, followed by Argentina and Venezuela On the contrary, Venezuelan CLL patients showed very low percentages of major clusters (5.1%). A graphic showing cluster frequencies among different countries is shown in Figure 1. Interestingly, the analysis of the distribution between stereotyped and heterogeneous (not stereotyped) receptors showed that *IGHV3-21* gene was always included in the heterogeneous group in Brazilian and Venezuelan cohorts.

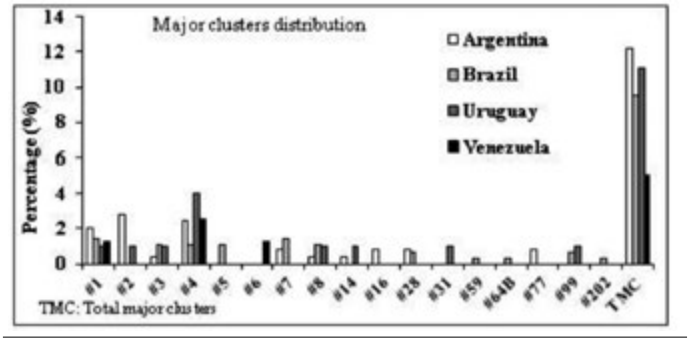


Figure 1.

Summary/Conclusion: Our results showed interesting differences compared to those from other geographical regions, highlighting the need for further research to understand the role of genetic background and environmental factors operating in CLL pathogenesis in the different geographical regions.

PB1853

A METHYL-CPG BINDING DOMAIN SEQUENCING STUDY SHOWED DYSREGULATED METHYLATION IN GENES INVOLVED IN CANCER, IMMUNITY, LYMPHOID DIFFERENTIATION AND MAJOR SIGNALING PATHWAYS IN ASIAN CLL

E. Lee[1,*], K. Jeon[1], M. Kim[1], J. Lee[1], H.-S. Kim[1], H.J. Kang[1], Y.K. Lee[1], S. Hwang[2]

[1]Laboratory medicine, Hallym Sacred Heart Hospital, Anyang, [2]Korea Research Institute of Bioscience and Biotechnology, Korean BioInformation Center, Daejeon, Korea, Republic Of

Background: CLL is the most common leukemia in Western countries, but rare in Asia including Korea (5/100,000 *vs* 0.5/100,000 per year), even though the incidence is increasing in Korea (0.5/100,000 in 1999-2001 to more than 1.2/100,000 in 2005-2010). Epigenetic research in Asian CLL is very rare and restricted to a few genes.

Aims: We hypothesized that CLL in Korea shows unique features compared to CLL in Western countries in terms of epigenetics. We performed Methyl-CpG Binding Domain Sequencing on CLL patients for the second time in the world and for the first time in Asia.

Methods: Nine Korean CLL patients and 5 age-matched Korean healthy individuals were included in the study. All the 9 patients showed TP53 mutation, and 1 patient showed IGHV hypermutation. One patient with IGHV hypermutation was excluded from the further analysis. B lymphoid cells were extracted using magnetic bead sorting technology. MBD-seq was performed using Invitrogen MethylMiner Methylated DNA Enrichment Kit and Illumina HiSeq 2000 platforms. The windows with FDR<0.01 were deemed as differentially methylated. The list of differentially methylated genes (DMGs) was then finally obtained. Functional enrichment analysis was performed by the clusterProfiler program with respect to Gene Ontology (GO) Biological Process and KEGG pathways, with significance criteria of p-value<0.05. For GO analysis, the options *minGSSize* of 20 and *maxGSSize* of 1,000 were applied.

Results: Our approach with MBD-seq enables a comprehensive genome-wide interrogation of differentially methylated regions (DMRs) in CLL.

There were more hypomethylated regions (2,062 windows) than hypermethylated regions (777 windows). Distal intergenic and intron regions contain the largest number of DMRs followed by promoter, 3'UTR and exon. As to the proportion of the DMRs, promoters contained the highest proportion of DMRs (around 0.08%), followed by 3'UTR, 5'UTR, downstream, distal intergenic region, exon and intron. A supervised hierarchical clustering clearly distinguished CLL patients from healthy individuals. The top forty differentially methylated genes (DMGs) include ERBIN, LINC00273, AGBL5, MIR4537, HCN1, LHPP, KIF16B, MYH3, LMNTD1 and CWH43. Immune process and cancer-related GO terms were dysregulated in CLL by DNA methylation either by hypo- or hyper-methylation. The dysregulated GO terms include Ras protein signal transduction, lymphocyte differentiation, immune system development, hematopoietic or lymphoid organ develoment, lymphocyte activation and positive regulation of apoptotic process. Dysregulated KEGG pathways included those that are directly related to immune process and cancer, mainly by hypomethylation. Most KEGG pathways were dysregulated by differential methylation in introns. The dysregulated KEGG pathway includes gastric cancer pathway, hepatocellular carcinoma pathway, CML pathway, breast cancer pathway Wnt signaling pathway, Ras signaling pathway, AML patway and transcriptional misregulation in cancer pathway. The reason why CLL pathway was not listed on top dysregulated KEGG pathways is simply because it is missing from KEGG pathway database content.

Summary/Conclusion: MBD-sequencing revealed that CLL patients have a distinct methylation profile from that of healthy individuals. Genes involved in cancer, immunity, lymphoid differentiation and major signaling pathways were dysregulated suggesting the role of dysregulated methylation in the pathogenesis of CLL. A further analysis will provide an important insight into the difference in methylation between Asian CLL and Western CLL.

PB1854

DRUG SENSITIVITY SCREENING IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) FOR PRECISION CANCER THERAPY

D.B. Thimiri Govinda Raj[1,*], A. Cremaschi[2], S.S. Skånland[2], A. Gade[2], D. Wang[3], F. Schjesvold[4], G.E. Tjønnfjord[4], L.A. Munthe[4], K. Tasken[1]

[1]NCMM UIO and Oslo Univeristy hospital, [2]NCMM UIO, [32]Centre for Immune Regulation, Institute of Clinical Medicine, UiO, [4]Department of Haematology, Oslo University Hospital, Oslo, Norway

Background: Personalized cancer therapy is a rapidly developing field that includes predictive medicine, preventive medicine and various personalized or individualized therapies, *e.g.* labeled "precision medicine". One particular challenge with cancer is tumor heterogeneity evolving after the original clonal event. We focus on Chronic Lymphocytic Leukemia (CLL), Multiple Myeloma and Follicular lymphoma (FL), diseases currently considered incurable. Although current treatment regimens prolong life for patients, CLL eventually relapse. Current potential in using therapeutics against CLL include design of optimal treatment for individual patients based on characterization of the tumor and its intratumor heterogeneity as observed by whole genome sequencing. Efficient therapies require a personalized approach that combines targeting cancer cells and the tumor microenvironment by restoring the patient's own anti-tumor immunity. Another major limitation is that there exists no efficient approach to identify the most efficient drugs for each patient and also for different cancer stage. Using our drug sensitivity screening platform, we aim to address the limitation in identifying the efficient drugs for individual patients.

Aims: To introduce individualized treatment for patients, we aim to establish cell-based assays and a drug sensitivity platform. We aim to establish a pipeline for drug sensitivity screening in CLL. To complement the results from the drug sensitivity screening, we aim to perform phosphoflow analysis. We aim to complement our approach using xenografted mice. We propose to use the drug sensitivity screening platform for CLL individualized treatment with an effective combination of targeted therapies.

Methods: To define drugs that inhibit malignant B cell growth, we use the cell-based assays CellTiter-Glo® luminescent cell viability assay and CellTox™ green cytotoxicity assay. We have established culture settings that mimic the tumor microenvironment for CLL. We perform drug sensitivity screening with 517 drugs at 5 concentrations to select drug candidates and pathway inhibitors. Selected drug candidates will be further analyzed by bioassays and flow cytometry to assess effects on intracellular signaling (phosphoflow-based approach). We propose to use the drug sensitivity screening platform to identify and validate drug candidates for xenografting and precision medicine clinical trials.

Results: We established an experimental setting that mimics the tumor microenvironment for CLL (Thimiri Govinda Raj *et al.* EHA meeting abstract 2017 Haematologica 102, 711-711). We have performed drug screening on 20 patient samples with 517 drugs at 5 concentrations and are currently aggregating data and expanding the data set. We have also performed drug screening on healthy B cells as a control.

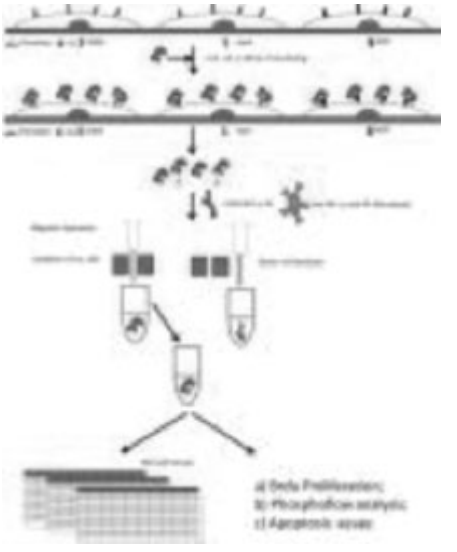


Figure 1.

Summary/Conclusion: Using our established CLL drug sensitivity screening at NCMM, we have performed drug screening on a number of patient samples. We aim to use a statistical approach to identify synergistic drug effects and validate the drug combinations experimentally. As a future perspective, we would combine machine learning strategies with the experimental drug screening strategies for the precision medicine clinical trials in other cancer settings.

PB1855

MONOCLONAL B-CELL LYMPHOCYTOSIS IN GREEK POPULATION

Z. Saouli, C. Savopoulos, Z. Kontoninas, E. Ztriva*, S. Mpountola, G. Polychronopoulos, D. Milonas, A. Papadopoulos, A. Hatzitolios, G. Kaiafa

[1]st Propedeutic Department of Internal Medicine, AXEPA General University Hospital of Thessaloniki, Thessaloniki, Greece

Background: The diagnostic term monoclonal B-cell lymphocytosis (MBL) is used to characterise individuals with a circulating population of clonal B-cells, a total count of<5 x 10(9)/L, with no other features of a B-cell lymphoproliferative disorder. In the recent literature, it has been reported that circulating chronic lymphocytic leukemia (CLL)-like B cells can be detected using multicolor flow cytometry in 0.57%>12% of adults with normal lymphocyte counts.

Aims: As far as we know, this is the first study in Greek population, investigating the frequency of B-cell lymphocytosis in healthy individuals, trying to understand the prognostic factors that lead the occurence of MBL to evove into B-CLL.

Methods: We investigate the frequency of circulating monoclonal B cells in 815 healthy blood donors aged 30-70 years with no evident history of malignant disease and normal blood counts, who provided signed informed consent. We used flow cytometric analysis of CD19/CD5/CD79b/CD20/CD23/CD38 expression, while other parameters such as LDH, beta-2 microglobulin and C reactive protein were measured. Monoclonality was demonstrated by immunoglobulin light-chain restriction in all cases with CLL phenotype cells.

Results: The monoclonal CLL phenotype cells were detected in 1.6% of individuals, with a higher frequency in men (male-to-female ratio, 1.2:1) and elderly individuals (1.9% of 40- to 59-year-olds *versus* 4.0% of 60- to 89-year olds, P=0.01) which is within the framework of international studies.

Summary/Conclusion: Our study shows that a healthy percentage of the population in Greece, equal to 1.6% is bearing cells with CLL immunophenotype. The use of more extensive immunophenotype-order control or control of clonality seems to be a valuable prognostic factor for the occurence of monoclonal lymphocytosis, which may potentially evolve into B-CLL over time. Furthermore it is of great importance monitoring of these patients over time.

PB1856

THE EXPRESSION LEVEL OF CD 177 IS INCREASED IN PATIENTS WITH CHRONIC LYMPHOPROLIFERATIVE DISEASES AND MYELOID NEOPLASIAS

I. Krobinets*, N. Mineeva, A. Chechetkin, I. Bogdanova

FSBI "Russian research Institute of Hematology and Transfusiology of FMBA", St.Petersburg, Russian Federation

Background: CD177 is a glycosyl-phosphatidylinositol (GPI)-linked cell surface glycoprotein that has been shown to be a counter-receptor for platelet endothelial cell adhesion molecule-1 (PECAM-1). The interaction of CD177 with endothelial PECAM-1 supports neutrophil transendothelial migration. The phenotype of neutrophils can change in patients with malignant diseases of the hematopoiesis system. Several studies have confirmed the overexpression of neutrophil CD177 mRNA in polycythemia rubra vera, and two other myleoproliferative disorders, essential thrombocythemia and idiopathic myelofibrosis. It was also shown that CD177 is absent from neutrophils from paroxysmal nocturnal hemoglobinurea patients who are deficient in the ability to synthesize GPI linkages. Neutrophils from patients with chronic lymphoproliferative diseases (CLD) are characterized by increased CD54 expression, which is associated with cell activation. Little is known about the level of CD177 expression in patients with CLD.
Aims: The aim of the study was to evaluate and compare the expression level of CD 177 in donors and patients with CLD and myeloid neoplasias (MNP).
Methods: Blood samples of 144 healthy donors (group 1), 94 patients with CLD: chronic lymphocytic leukemia, Hodgkin's lymphomas, non-Hodgkin's lymphomas, multiple myeloma patients (group 2), and 61 patients with MNP, including chronic idiopathic myelofibrosis, chronic myelogenous leukemia, essential thrombocytosis, polycythemia vera patients (group 3) were included in the study. The mean age of patients in groups 2 and 3 was 64 years (range, 22 to 84) and 65 years (range, 21 to 81), respectively. Flow cytometry immunophenotyping analysis of CD 177 was performed using anti-human CD 177-PE, clone MEM-166. The level of CD177 expression was evaluated using mean fluorescence intensity (MFI) rate. MFI for CD 177-PE in group 1 was taken as the reference value.
Results: MFI for anti-CD 177-PE in group 1 was 31.8 to 149 (median 79.2). MFI in groups 2 and 3 was significantly higher (p=0.000001) than in group 1 (median 114, CI: 38.58-706.7 and median 131.1, CI: 40.39-360.2 *vs* median 79.2, CI: 41.25-136.37, respectively). No significant differences were revealed when MFI between groups 2 and 3 were compared (p=0.19).
Summary/Conclusion: The expression level of CD 177 is increased in patients with CLD and MNP compared to healthy donors. Neutrophils in patients with CLD have an activated phenotype, probably associated with a systemic inflammation. The presence of a chronic systemic inflammatory response may also explain the increase in CD 177 expression in patients with MNP.

PB1857

DEFINING A TECHNICAL WORKFLOW FOR GENE EXPRESSION PROFILING IN MRD+ CHRONIC LYMPHOCYTIC LEUKEMIA

A. Mora[1,2,*], R. Bosch[1,2], C. Cuéllar[1,2], R. Martino[2], J. Sierra[2], J. Nomdedeu[3], C. Moreno[1,2]

[1]Laboratory of Oncology/Hematology and Transplantation, IIB Sant Pau, [2]Department of Hematology, [3]Laboratory of Hematology, Hospital de la Santa Creu i Sant Pau, Autonomous University of Barcelona, Barcelona, Spain

Background: In CLL, the persistence of residual leukemic cells (MRD) after therapy is associated with poor outcome. Therefore, a better understanding of biological MRD characteristics would help to design newer and specific therapeutic approaches aimed at preventing clinical relapse. Nevertheless, working with a small amount of leukemic cells entails intensive labor to obtain enough material for gene expression profile studies. Here, we set forth an effective gene expression approach to study residual CLL cells.
Aims: The aim of this study was to set forth an effective gene expression approach to study residual CLL cells.
Methods: Leukocytes from fresh peripheral blood samples from 3 CLL patients were sorted based on MRD phenotype by multiparametric flow cytometry using a panel of 6 markers (CD43/CD5/CD19/CD81/ CD79b/ CD20). The quality and quantity of RNA isolated from two different inputs of cells $1x10^4$ and $5x10^5$ were compared by using two silica columns protocols (RNeasy Micro and RNeasy Mini, Qiagen). Furthermore, RNA amplifications were carried out according to two manufacture protocols (Ovation Pico SL and Ovation Pico WTA system, Nugen) to further compare the quality and quantity of cDNA obtained. A total of 3.5µg of cDNA obtained with Pico WTA System was labeled with Encore Biotin Module (Nugen) and hybridized with GeneChip Hybridization Wash and Stain Kit (Thermo Fisher) to GeneChip Human Gene 2.0 ST arrays (Thermo Fisher Scientific). The reproducibility of arrays resulting from the different amount of input cells was compared.
Results: RNA extracted from $1x10^4$ cells by RNeasy Micro and Mini Kit showed similar RNA Integrity Number (RIN), however, the amount of RNA obtained was higher using the RNeasy Micro Kit (mean: 116.0ng *vs* 80.0ng). Of note, the quantity of RNA obtained from $1x10^4$ cells was not significantly different from that isolated when the experiment was performed with a total of $5x10^5$ cells.
Furthermore, RNA samples obtained after using RNeasy Micro Kit were amplified with two different kits. The amount of cDNA as a result of RNA amplification was enough to proceed for an array experiment by using any of the two different protocols (see table 1). In terms of cDNA quality, the mean 260/280 ratios were in all cases above 1.8. Nevertheless, samples processed by Ovation Pico SL WTA showed smaller amplified fragments that were not observed in samples processed by Ovation Pico WTA system. Microarray data analysis of RNA extracted from $1x10^4$ and 5 $x10^5$ cells with Micro RNeasy and amplified with Ovation Pico WTA System, displayed a Pearson s correlation average of 0.964.

Table 1.

No. input cells	RNEasy Protocol	Mean RNA (ng, range)	Mean 260/280 ratio (range)	RIN range	Ovation WTA System	Mean cDNA (µg, range)	Mean 260/280 ratio (range)
10.000	Micro	116.6 (81.6-152.4)	1.4 (1.4-1.5)	2.4-3.1	Pico	8.6 (8.0-8.9)	1.9 (1.9-2.0)
					Pico SL	4.8 (4.6-5.1)	2.0 (2.0-2.0)
	Mini	80.0 (69.0-90.0)	2.7 (2.0-3.6)	2.6-3.2	-	-	-
500.000	Micro	117.6 (94.8-153.6)	1.8 (1.5-1.8)	4.9-7.4	Pico	8.8 (8.0-9.6)	2.0 (1.9-2.0)
					Pico SL	5.4 (5.2-5.6)	2.0 (2.0-2.0)
	Mini	411.0 (294.0-534.0)	1.9 (1.9-2.0)	2.7-3.3	-	-	-

Summary/Conclusion: By using the methodology herein presented, a total of $1x10^4$ leukemic cells were enough to obtain quantity and quality samples to perform array studies. Thus, this methodology can be useful to carry out gene expression profiling experiments involving MRD in CLL.

PB1858

EXPRESSION OF THE GENES OF THE MAIN RECEPTORS AND LIGANDS OF THE EXTERNAL PATHWAY OF APOPTOSIS IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC LYMPHOCYTIC LEUKEMIA DURING RFC THERAPY

S. Zakharov[1,*], A. Golenkov[1], A. Misyurin[2], E. Kataeva[1], M. Baryshnikova[1], T. Mitina[1], E. Trifonova[1], L. Vysotskaya[1], Y. Chernykh[1], E. Klinushkina[1], K. Belousov[1], Y. Finashutina[2], A. Misyurina[3], V. Misyurin[2]

[1]Moscow Regional Research and Clinical Institute, [2]"N.N.Blokhin Russian Cancer Research Center" Ministry of Health, [3]National Research Center for Hematology, Moscow, Russian Federation

Background: The expression of *FAS*, *TRAIL*, *TNFR2*, *DR4/5* and *DR3* are dysregulated in set of hematological malignancies. As result, apoptotic process in malignant cell is blocked due to lack of membrane apoptotic receptors. It may have negative clinical significance. We wanted to investigate the most important gene among listed genes.
Aims: To determinate clinical significance of *FAS*, *TRAIL*, *TNFR2*, *DR4/5* and *DR3* genes in primary B-CLL.
Methods: The expression level of *FAS*, *TRAIL*, *TNFR2*, *DR4/5* and *DR3* genes was studied in 23 patients with newly diagnosed chronic lymphocytic leukemia (B-CLL) before and after RFC-therapy. Multivariate regression analysis to perform clinical significance of each gene.
Results: According to multivariate regression analysis results, among these genes the *FAS* expression level has most value in clinical outcome. There are two conditions of *FAS* activity was observed-high expression level (median 7717%), and low (373%). Patients with high expression level of *FAS* has I-II stage of diesis (p=0.0205), lowest level of lymphocytes (p=0.0016) and highest level of erythrocytes (p=0.0159), and highest *TNFR2* and *TRAIL* expression level (p=0.0015 and p=0.0053, respectively). Patients

with low expression level of *FAS* has poorer clinical outcome (p=0.026).
Summary/Conclusion: Highest level of *FAS*, *TRAIL TNFR2* is associated with sensitivity oh B-CLL cells to induction of apoptosis. Moreover, it associated with better effectiveness RFC-therapy.

PB1859

THE IMPORTANCE OF MOLECULAR AND CYTOGENETIC ABERRATIONS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

E. Nazarova*, M. Sarpova, N. Minaeva, J. Suhorukova, I. Paramonov, E. Zotina, I. Dokshina, V. Shardakov

Kirov Research Institute of Hematology and Blood Transfusion, Kirov, Russian Federation

Background: Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults and is characterized by clinical and biological heterogeneity. This heterogeneity is caused in some cases by structural aberrations in malignant cells, as evidenced by the presence of an abnormal karyotype. It is known that, in addition to cytogenetic aberrations, mutations of several genes involved in the pathogenesis of the disease and determining its differential prognosis are considered as important prognostic factors of CLL.
Aims: To identify the relationship of a number of immune response genes mutational status to the existing of numerical and structural changes in chromosomes in patients with CLL.
Methods: Sixty patients with CLL in the onset of the disease were examined, aged of 39 to 79 years (median-62 years). Men-37 (61.7%), women-23 (38.3%). All patients underwent FISH-diagnostics of nuclei of interphase bone marrow cells with DNA probes Kreatech FISH Probe. Genotyping of 20 polymorphic locus of the 13 immune response genes of peripheral blood leukocytes genomic DNA was carried out by polymerase chain reaction with allele-specific primers (single nucleotide polymorphism-SNP).
Results: The patients were divided into two groups. The first group included 26 (43.3%) patients with CLL who had no chromosomal abnormalities in the FISH analysis. The second group consisted of 34 (56.7%) patients with various cytogenetic aberrations in the form of monosomy, trisomy 12 and 13 chromosomes, del13q14, del11q22, del17p13. Comparing the obtained cytogenetic data with the results of detection of the immune response genes SNP, it was found that the presence of mutant alleles in the haplotypes of the Toll-like receptor (TLR) gene 9 (*TLR9*, T-1237C) and interleukin (*IL*) 2 (T-330G) is almost 14 (p=0.01) and 5 times (p=0.03), respectively, reduces the frequency of cytogenetic aberrations detection in CLL. Survival and proliferation of CLL cells are directly dependent on external factors-pathogen-associated (PAMPs) and damage-associated molecular patters (DAMPs). PAMPs and DAMPs initiate the development of the malignant process and its progression by stimulating the B-cell receptor and others, including TLRs. The TLR9 agonist-CpG-ODN upon binding to the receptor lead to activation of malignant CLL cells. The response of transformed cells to CpG-ODN is significantly different with IgVH-non-mutated and mutated CLL. In the first case, CpG-ODN protect CLL cells from spontaneous apoptosis and trigger their proliferation, whereas in case of mutated CLL, induction of programmed cell death is observed, which is reflected in a different clinical course of the disease. The study of this mechanism can provide additional information on the pathogenesis of CLL and, thus, leads to the development of new therapeutic strategies. Experimental dates have shown that CpG-ODNs induce proliferation, cytokine production and expression of the high-affinity receptor for IL-2 on malignant cells in CLL. IL-2 weakens the apoptosis of proliferating B cells and increases the frequency of their detection, which is used in the FISH analysis to increase their ability to stimulate the proliferation of T lymphocytes and their production of IL-4 and IL-5, which in the future can be used in immunotherapy for CLL.
Summary/Conclusion: Cytogenetic and molecular genetic studies in CLL are used to predict the course of the disease, and are one of the bases for the development of new targeted therapy drugs.

PB1860

BRAF MUTATION IN BOTH HAIRY CELL LEUKEMIA AND PAPILLARY THYROID CANCER IN THE SAME PATIENT

S. Mohamed*, M. Yassin, H. El Omri, F.I. Brahim, A. Nashwan, S. El Kiki

Hematology, HGH, DOHA, Qatar

Background: Hairy cell leukemia (HCL) is an uncommon form of mature B-cell neoplasm that originates from activated late B-cells. BRAF mutation associated with all cases of HCL, and 40% papillary thyroid (PTC) cancer.
Aims: BRAF mutation is well known finding in both HCL and PTC. Although the association of both cancers (HCL &PTC) with BRAF mutation is well established in the literature, up to our knowledge, this specific combination has not been previously reported in one patient.
Methods: 48-year old male, presented with bilateral hip pain found to have lytic bone lesions on both x-ray and MRI. His CBC were normal and abdominal US didn't show any splenomegaly.Bone marrow examination and flow cytometry results confirmed the diagnosis of HCL. The patient treated with cladrabine.Responded but continue to have fever, PET CT showed abnormal uptake in thyroid. PTC diagnosis confirmed underwent total thyroidectomy followed up with RAI 30 micori BRAF from both bone marrow biopsy and thyroid tissue which turn out positive in both.
Results: The case is unique for several reasons: 1- Patient did not present with pancytopenia, which is common at presentation and reported in 50% to 70% of patients with HCL; 2- He did not have splenomegaly. Splenomegaly is the most common physical finding in HCL and is reported in 70%>100% of cases; 3- HCL presenting as a lytic lesion is very uncommon. Most important the association of BRAF-positive papillary thyroid cancer. Finding of the activating point mutation in the kinase-encoding *BRAF* gene in all patients with classical HCL The mutation results in substitution of adenine for thymine at position 1799 in exon 15 of the BRAF that replaces Valine (V) by glutamate (E) at amino acid 600(*BRAF* V600E). Although the BRAF V600E mutation is frequently present in different neoplasms, including PTC. The *BRAF*V600E mutation is found to be highly specific for HCL and testing for this mutation is particularly useful in differentiating classic HCL from other B- cell neoplasm with overlapping features, such as HCL variant.
Summary/Conclusion: The presence of BRAF mutation in both papillary thyroid cancer and HCL may raise the possibility of common pathogenesis or BRAF maybe driving mutation

PB1861

CHARACTERIZATION OF IGHVDJ REARRANGEMENTS AND CHROMOSOMAL ALTERATIONS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

M.A. Perera Alvarez[1,2,*], C. Bilbao Sieyro[1], A. Suarez Cabrera[1], Y. Florido Ortega[1], R. Stuckey[1], A. Saez Salinas[1], M.D.L.N. Saez Perdomo[1], S. Sanchez Sosa[2], J.M. Gonzalez Martin[3], D. V. Fiallo Suarez[1], M.T. Gomez Casares[1], T. Molero Labarta[1]

[1]Hematologia, Hospital Universitario de Gran Canaria Dr. Negrín, [2]Universidad de Las Palmas de Gran Canaria, [3]Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Spain

Background: The mutational status of *IGHV* genes is a well-established prognostic biomarker in chronic lymphocytic leukemia (CLL). Patients with unmutated *IGHV* genes experience a more progressive course of the condition and their survival is significantly reduced with respect to patients with mutated *IGHV* genes. Increasingly, studies are reporting the type and composition of IGH-VDJ rearrangements and defining specific stereotyped CLL subsets.
Aims: Analyze the IGH-VDJ rearrangements in a series of patients with CLL, determine their distribution and compare to results from similar studies. Group the rearrangements according to stereotype subsets and evaluate if a relation exists between subsets and other common genetic alterations in CLL.
Methods: The cohort included 49 patients diagnosed with CLL at the Hospital Universitario de Gran Canaria Doctor Negrín between 2014 and 2017. The characterization of IGH-VDJ rearrangements was determined from cDNA obtained from peripheral blood samples by Sanger sequencing using a 3130 Genetic Analyzer (Life Technologies, Carlsbad, CA). Chromosomal alterations were determined using FISH as part of routine diagnosis procedures. The statistical analyses were performed using R software.
Results: Of the 49 patients, 30 (61%) presented mutated *IGHV* genes *vs* 19 (39%) that were ummutated. With respect to the genetic alterations detected by FISH, 8% harbored del(11q), 10% del(17p), 18% +12, and 60% del(13q). There was a statistically significant association between del(11q) and unmutated *IGHV* genes (p=0.018, Fisher) and a marginally significant association between del(13q) and mutated *IGHV* genes ((p=0.075, Fisher), both in agreement with previously published results. The distribution of VH family usage and its association with the mutational status of IGHV was studied (Figure 1). The distribution of used VH families from highest to lowest incidence was IGHV3 (46.9%), IGHV4 (24.5%), IGHV1 (22.4%), IGHV5 (4.1%) and IGHV2 (2%), similar to that previously described in a series of Spanish CLL patients (González-Gascón *et al.*, 2013). The frequency of mutated *vs* unmutated for VH3 was 56.54% *vs* 43.5%, 45.5% *vs* 54.5% for VH1, and 75% *vs* 25% for VH4, respectively. These

frequencies were similar to those previously published with the exception of IGHV1 and VH3, for which the number of unmutated cases in IGHV1 was reported to be twice the number of mutated cases, with the opposite reported in VH3. With respect to the subfamily distribution (Figure 2), the highest incidence was VH3-30 (16%) followed by VH3-2, VH1-69 and VH4-59 (8% each). However, according to González-Gascón *et al*., the predominant subfamilies were VH1-69 and VH3-23 (18%) followed by VH4-34 (16%) and VH3-30 (12%). Table 1 shows the characteristics of the amino acids that constitute the HCDR3 region (quantity and proportion of hydrophilic/hydrophobic residues) and their relation with *IGHV* mutational status. We identified three cases that belonged to described stereotypes defined by Agathangelidis *et al.* (Blood, 2012): two were # 2 and one #202.

Table 1.

	MUTATED	UNMUTATED
MEAN AA NUMBER	15	18
HYDROPHILIC AA	24 (80%)	11 (57,9%)
HYDROPHOBIC AA	6 (20%)	8 (42,1%)
TOTAL	30	19

Table 1:Aminoacid distribution and its assotiation with the IgHV mutational status.

Summary/Conclusion: In general, our results are similar to those published on other Spanish CLL patients in terms of the IGHV family distributions, subfamilies and associations with other chromosomal alterations. The differences observed could be a result of the limited size of the patient series analyzed in this study. The different distribution of hydrophilic/hydrophobic amino acids among mutated and unmutated cases of CLL is a novel result and could possibly be associated with prognosis.

PB1862

EXPRESSION OF THE APOPTOSIS-RELEATED GENES IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC LYMPHOCYTIC LEUKEMIA IN CLINICAL DATA CONTEXT

S. Zakharov[1,*], A. Golenkov[1], V. Misyurin[2], E. Kataeva[1], M. Baryshnikova[2], T. Mitina[1], A. Misyurin[2], E. Trifonova[1], L. Vysotskaya[1], Y. Chernykh[1], E. Klinushkina[1], K. Belousov[1], Y. Finashutina[2], A. Misyurina[3]

[1]Moscow Regional Research and Clinical Institute, [2]N.N.Blokhin Russian Cancer Research Center, [3]National Research Center for Hematology, Moscow, Russian Federation

Background: The given data of fundamental studies of apoptosis processes in B-CLL testifies about the complexity and variety of mechanisms affecting the kinetics of normal cells and tumor lymphocytes in this disease. It is important to study the severity of clinical manifestations of the disease depending on the expression of the genes that modulate apoptosis.
Aims: The aim of the study is to compare the activity of genes encoding apoptosis modulators, the cell cycle and cancer-testicular PRAME protein with clinical manifestations of the disease in primary patients with B-CLL.
Methods: The level of expression of the proapoptotic genes *FAS*, *TRAIL*, *TNFR2*, *DR4/5* and *DR3*, as well as the *HSP27*, *XIAP* genes, blocking apoptosis was determined in 23 patients with newly diagnosed chronic B-cell lymphocytic leukemia (B-CLL). In addition, expression of genes *TP53* and *P21* and cancer-testis gene *PRAME* are tested.
Results: According to the multivariate regression analysis, the *FAS* gene expression in the onset of the disease had the greatest impact on the clinical characteristics of the disease. In this connection, the patients were divided into groups with normal (group) and low gene level (group II). A low level of *FAS* expression (Me 387%) was associated with stage II disease (p=0,03), a large number of lymphocytes (p=0,001), fewer erythrocytes (p=0,08), and a lower level of *TNFR2* gene expression (p=0,08), high level of expression of *XIAP*, *HSP27*, *P21*. Overall, the anti-apoptotic potential in Group II patients was higher, which was accompanied by more pronounced clinical manifestations of the disease.
Summary/Conclusion: The increased anti-apoptotic potential of tumor lymphocytes in newly diagnosed B-CLL is accompanied by a larger tumor mass and greater clinical and hematological manifestation of the disease.

Chronic lymphocytic leukemia and related disorders – Clinical

PB1863

DISCORDANCE IN THE THRESHOLDS OF EARLY RELAPSE IN CLL GUIDELINES

V. Strugov*

Hematology Institute, National Almazov Medical Research Centre, Saint Petersburg, Russian Federation

Background: It is generally accepted that CLL patients with late disease progression after chemoimmunotherapy (CIT) remain chemosensitive and can be successfully retreated with the same regimen if they are still "fit" and no del17p/TP53mut is acquired. Accordingly, newer treatment options, such as Btk and PI3K inhibitors, are reserved to patients with so called "early relapse". As switch to newer drugs is often a difficult medical decision with consequences both to patients (different toxicity profile) and economy (higher treatment costs), a stringent evidence-based definition of "early relapse" is desirable.
Aims: We tried to analyze consistency and evidence base in the proposed thresholds of "early relapse" in different publicly available CLL guidelines.
Methods: PubMed and Google were searched for terms "CLL guideline" and "CLL recommendation". Relevant documents were collected from search results and analyzed for clinical guidance and references.
Results: Results are summarized in Table 1. All documents specified different timings of "early relapse" (namely, within 24, 24-36 or 36 mos after CIT). In two documents relevant clinical guidance was marked with II-III evidence level, however no prospective trials were cited with time-of-relapse based interventions or prespecified outcome analysis. Some guidelines didn't provide rational for a particular choice of threshold at all. Those documents, that gave references, cited MDACC (Keating *et al*., 1998; Keating *et al.* 2005; Tam *et al*., 2008; Keating *et al*., 2009; Tam *et al*., 2014), GCLLSG (Stilgenbauer *et al*., 2010; Cramer *et al*., 2015) or French Group (Fornecker *et al*., 2015). However, these sources appear to have serious limitations. For example, in publications from MDACC overall survival difference was calculated for a threshold of 36 mos *from the start* of FCR (that is, approx. 30 mos *post* CIT). Same issue applies to CLL8 data provided in Stilgenbauer *et al*., 2010 (PFS of 24 mos, approx. 18 mos *post* CIT). Hence, thresholds of 24 or 36 mos *after* CIT appear to be not properly validated. Also, large meta-analysis of 1558 pts by Cramer *et al.* 2015 didn't find statistically significant difference in OS at 1, 2 or 3 year thresholds and French Group results are based on relatively small (n =132) retrospective cohort. Additionally, although all cited studies analyzed prognostic role of early relapses solely after 1st line CIT, none of the guidelines mentioned the fact that suggested thresholds can't be extrapolated to relapses after further lines of therapy.

Table 1.

Table 1. Thresholds of "early relapse" after CIT in different recommendations.

Source	Cited studies*	Implied "early relapse" definition	Country	Year
IWCLL (Hallek et al., 2008)	-	No definition	Global	2008
NCCN guidelines 2.2018	-	No definition	USA	2018
EBMT CLL transplant consensus	MDACC, GCLLSG	≤ 24 mos post CIT	Europe	2007
SIE, SIES, GITMO	MDACC, GCLLSG	≤ 24 mos post CIT	Italy	2012
BCSH Guidelines	MDACC, GCLLSG	< 2 years post FCR/FC	UK	2012
East Midlands Cancer Network	-	< 2 years post FCR/FC	UK	2013
London Cancer CLL Guidelines	-	≤ 24 mos post FC	UK	2015
CancerCare Manitoba CLL Guidelines	MDACC	< 2 years post FCR	Canada	2015
KroHem CLL Guidelines	-	< 2 years post FCR/FC	Croatia	2017
Brazilian CLL Group	-	≤ 24 mos post CIT	Brazil	2016
AWMF, DKG and DKH guideline	French, GCLLSG	≤ 24 mos post CIT	Germany	2017
"Ultra high-risk CLL" (Stilgenbauer et al., 2016)	MDACC, GCLLSG	≤ 24-36 mos post CIT	-	2016
Revised Dutch CLL Guidelines	MDACC	≤ 24-36 mos post FCR/BR	Netherlands	2018
Czech CLL Group	-	< 2-3 years post CIT	Czech Republic	2016
Swedish National Care Program	MDACC	< 2-3 years post CIT	Sweden	2016
BHS Guidelines	French, MDACC, GCLLSG	< 2-3 years post CIT	Belgium	2016
ESMO Guidelines	-	≤ 24-36 mos post CIT	Europe	2017
Danish Lymphoma Group	-	< 3 years post CIT	Denmark	2017
ARS Clinical Practice Guideline LYHE-007 v.4	MDACC	< 3 years post FCR, < 2-3 years post BR	Canada	2017

* MDACC – MD Anderson Cancer Center, GCLLSG – German CLL Study Group, French CLL Group

Summary/Conclusion: There is a considerable variability and paucity of evidence base in the thresholds for "early relapse" proposed by current CLL guidelines. This can potentially impede clinical practice, leading to untimely or/and unjustified therapeutic decisions when switch to newer inhibitors

and allogeneic stem cell transplantation is considered. Also, a question remains on which duration of remission can sufficiently predict sensitivity to CIT in the context of subsequent relapses. Analysis of large prospective datasets is needed.

PB1864

IL-2, IL-6, IL-17 AND IL-33 IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA COMPLICATED BY AUTOIMMUNE CYTOPENIAS

O. Vyhovska*, O. Danysh, K. Susida, K. Kotlyarchuk, O. Tsyapka, L. Lukavetskyy, Y. Vyhovska, Z. Maslyak

SI "Institute of Blood Pathology and Transfusion Medicine, NAMS of Ukraine", Lviv, Ukraine

Background: One of the important issues that should be considered within the studies concerning pathogenesis of the immune cytopenia in chronic lymphocytic leukemia (CLL) is impairment of the cytokine balance resulting from the disorders of the intercellular interactions between cytokine producing blood cells.
Aims: of the study were to investigate the level of certain regulatory cytokines in patients with CLL, including those with development of autoimmune hemolytic anemia (AIHA) and immune thrombocytopenia (ITP), which frequently may complicate the course of leukemia.
Methods: 68 CLL patients aged 45-77 years, including 39 without immune complications and 29 with presence of immune cytopenia not related to previous therapy were investigated. The male to female ratio was 1.3:1, the distribution by Rai stage was as follows: stage II-15 patients, stage III-31 patients, stage IV-13 patients. The control group consisted of 12 healthy subjects aged 50-80 years. The concentration of IL-2, IL-6, IL-17A and IL-33 was measured using the validated commercial ELISA kits.
Results: 39 patients with CLL without autoimmune complications appeared to have significantly increased IL-2 and IL-6 levels compared to healthy individuals, while IL-17A and IL-33 concentrations did not significantly differ from the control group (Tab.1). Positive DAT, elevated serum indirect bilirubin (55,27±18,6 umol/l), reticulocytosis (4,7±1,0%), and erythroid hyperplasia in bone marrow have been confirmed in 15 patients with CLL AIHA. There is a significant increase of IL-2, IL-6 and IL-17A levels compared to the healthy individuals in this group of patients (Tab.1). The concentration of IL-33 was 3 times higher than in the healthy individuals, but the difference was unreliable due to significant fluctuations of the results in some patients (Tab.1). In 14 patients with CLL mean platelet count was $37,6\pm10,7*10^9$/l, ITP was established on the basis of rapid blood thrombocytopenia and hypermegakaryocytosis in the bone marrow. Just like in patients from the previous two groups, their IL-2 and IL-6 levels were also significantly higher than those in healthy subjects. The concentration of IL-17A did not differ significantly from the control group and patients without immune complications, but was significantly lower than in subjects with AIHA. The level of IL-33 in this group was not significantly different from healthy individuals and patients from other groups (Tab.1).

Table 1.

Table 1. IL-2, IL-6, IL-17A, IL-33 in CLL patients without immune cytopenia, with AIHA and with ITP

Patients	IL-2 (pg/ml)	IL-6 (pg/ml)	IL-17A (pg/ml)	IL-33 (pg/ml)
Healthy individuals	8,12 ± 2,59	0,30 ± 0,17	10,02 ± 1,80	2,20 ± 0,50
CLL without AIHA or ITP	18,07 ±1,55*	2,89 ± 0,46*	14,28 ±1,86	1,77 ± 0,26
CLL with AIHA	16,35 ± 2,20*	6,86 ± 0,20*	16,88 ± 0,65*	3,71 ± 1,90
CLL with ITP	15,76 ± 2,04*	2,80 ± 0,59*	10,80 ± 1,87**	1,11 ± 0,29

*significant as compared to the healthy individuals
**significant as compared to the CLL with AIHA group

Summary/Conclusion: The obtained results reflect activation of the IL-17 production in patients with CLL-associated AIHA. Similar but not statistically significant changes were found also for IL-33, elevation of which was also reported in certain publications for cases of AIHA without leukemic background. However, in patients with CLL ITP levels of these cytokines were lower as compared to CLL AIHA, and did not significantly differ from the healthy individuals and CLL patients without immune cytopenia. This could possibly be a result of difficulties in differentiation between metaplastic and immune thrombocytopenia in CLL. At the same time significantly increased levels of IL-2 and IL-6 were observed in all three groups of patients which apparently reflects major impairments of the spectrum of regulatory cytokines in patients with advanced stages of CLL. Investigating the dynamic changes in concentration of these cytokines may be important for prognosis of the leukemia progression as well as development of CLL-associated AIHA.

PB1865

MITIGATION OF TUMOR LYSIS SYNDROME (TLS) COMPLICATIONS WITH VENETOCLAX (VEN) IN CLL

J.F. Seymour[1,2,*], M.S. Davids[3], A.W. Roberts[2], B. Eichhorst[4], T. Kipps[5], A. Mato[6], S. Stilgenbauer[7], M. Mobasher[8], M. Desai[9], G. Pena[9], J. Potluri[9], M. Verdugo[9], J. Nielsen[9], A. Cyr[9], L.L. Lash-Fleming[9], M. Hallek[4]

[1]Peter MacCallum Cancer Centre, and University of Melbourne, [2]Royal Melbourne Hospital, Melbourne, Australia, [3]Dana-Farber Cancer Institute, Harvard Medical School, Boston, United States, [4]Department of Internal Medicine, Center of Integrated Oncology Köln Bonn, and Cluster of Excellence on Cellular Stress Responses in Aging (CECAD), University of Cologne, Cologne, Germany, [5]University of San Diego, San Diego, [6]CLL Program, Leukemia Service, Division of Hematologic Oncology, Memorial Sloan Kettering Cancer Center, New York, United States, [7]Internal Medicine III, Ulm University, Ulm, Germany, [8]Genentech Inc., South San Francisco, [9]AbbVie, Inc., North Chicago, United States

Background: VEN can cause rapid tumor debulking in patients (pts) with relapsed/refractory CLL. Current VEN dosing schedule with TLS prophylaxis/monitoring averts overt TLS, but further data on emergent biochemical changes during VEN initiation are needed to guide practice.
Aims: To report on laboratory abnormalities observed during VEN initiation with 5-wk dose ramp up and current TLS prophylaxis/monitoring recommendations.
Methods: Pts were included from phase 2 trials of VEN for CLL with del(17p) (n=51) or prior BCRi therapy (n=117). VEN started at 20 mg QD with ramp up to 400 mg over 5 wk. Pts were categorized as low (L) TLS risk (nodes<5 cm and ALC<25), medium (M; node ≥5-<10 cm or ALC ≥25), or high (H; node ≥10 cm or ≥5 cm and ALC ≥25). Laboratory values, AEs, and relevant concomitant medications during ramp up were analyzed.
Results: TLS risk was categorized as 36% L, 36% M, and 27% H. 96% of pts completed ramp up; most in 5 wk. TLS AEs were reported in 4 pts (2 M; 2 H), with no clinical TLS and 1 laboratory TLS meeting Howard criteria (HC*). During VEN ramp up, 114 pts had potassium (K) >upper limit of normal (ULN) (1 K>HC); 21 were treated for rising or sustained K >ULN. 16 of these 21 had pre-VEN initiation K>ULN. 5/21 pts interrupted VEN for 1 day and all restarted VEN. 119 pts had phosphate (P) >ULN. 20 pts received P binder for P>HC during VEN ramp up and all others resolved without additional medication. Most instances were isolated; 4 pts had concurrent calcium (Ca^{2+}) or uric acid (UA) changes, and P was not treated in these cases. 70% of pts received allopurinol and 20% allopurinol and rasburicase at VEN start. 6 pts received rasburicase in addition to ongoing prophylaxis. Though 144 pts had Ca^{2+}<lower limit of normal (LLN), only 6 were treated.

Table 1.

n of pts		TLS risk L n=61	TLS risk M n=61	TLS risk H n=46	All N=168
K	>ULN	41	40	33	114
	>6 mmol/L*	0	1	0	1
	Treated	4	10	7	21
P	>ULN	34	44	41	119
	>1.5 mmol/L*	18	27	34	79
	Treated	1	8	11	20
Ca^{2+}		45	58	41	144
	<0.3 µmol/L*	0	4	4	8
	Treated	0	3	3	6
UA	>ULN	3	4	6	13
	>476 µmol/L*	2	2	4	8
	Treated	2	1	3	6

**Howard criteria (Howard et al., N Engl J Med 2011).*

Summary/Conclusion: Despite the frequency of K >ULN, treatment was applied in 13% of pts for rising or sustained elevation, and most had pre-VEN K >ULN. Most cases of P >ULN were isolated, asymptomatic, and resolved without intervention. VEN causes rapid tumor cytoreduction consistent with analyte changes, though clinical sequelae are rare and mitigated by approved dose ramp up, TLS prophylaxis/monitoring, and timely intervention.

PB1866

DYNAMIC CHANGES IN HLA-DR EXPRESSION DURING SHORT-TERM AND LONG-TERM IBRUTINIB TREATMENT IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

G. Gabcova[1], Z. Mikulkova[1], G. Manukyan[2], T. Papajik[3], P. Turcsayni[3,*], R. Fillerova[1], T. Dyskova[1], V. Smotkova Kraiczova[1], S. Zehnalova[4], P. Gajdos[4], R. Urbanova[3], M. Kudelka[4], E. Kriegova[1]

[1]Department of Immunology, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic, [2]Laboratory of Molecular and Cellular Immunology, Institute of Molecular Biology NAS RA, Yerevan, Armenia, [3]Department of Hemato-Oncology, aculty of Medicine and Dentistry, Palacky University and University Hospital,, Olomouc, [4]Department of Computer Science, Faculty of Electrical Engineering and Computer Science VSB-Technical University of Ostrava, Ostrava, Czech Republic

Background: There is the first evidence of different BCR internalization kinetics of neoplastic cells from the patients with chronic lymphocytic leukemia (CLL) after short-term and long-term administration with ibrutinib.
Aims: We aimed to assess the influence of short-term and long-term ibrutinib treatment on the HLA-DR expression on CLL and T cells.
Methods: The immunophenotyping of CLL and immune cells in peripheral blood was performed in 16 high-risk CLL patients treated with ibrutinib using a flow cytometry.
Results: Ur data demonstrated the reduced expression of HLA-DR on CLL cells early after ibrutinib administration (P=0.038) accompanied by an increase in CLL cell counts (P=0.003). *In vivo* reduction in the HLA-DR expression was confirmed by *in vitro* culturing of CLL cells with ibrutinib at protein and mRNA levels (P<0.01). The decrease in HLA-DR on CLL cells after the first month of the treatment was followed by the gradual increase of its expression by the 12$^{th\ month}$ (P=0.016) returning to the levels before initiation of the treatment. The one-month follow-up resulted in elevated absolute counts of CD4+ (P=0.003) and CD8+ (P<0.001) cells which were associated with the increased number of CD4+ and CD8+ cells bearing HLA-DR marker (P<0.001). The long-term administration was associated with increased numbers of CD4+ bearing HLA-DR (P=0.047), along with no changes in CD8+ bearing HLA-DR, CD4+ and CD8+ cell counts, respectively.
Summary/Conclusion: Our results provide the first evidence of the time-dependent immunomodulatory effect of ibrutinib on CLL and T cells. The clinical consequences of time-dependent changes in HLA-DR expression in ibrutinib treated patients deserve further investigation.
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PB1867

SECOND LINE TREATMENT OUTCOMES IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) IN CLINICAL PRACTICE: RESULTS FROM A DECADE OF PRACTICE IN HELSINKI 2005-2015

V. Lindström[1], J. Mehtälä[2], R. Klement[3], A. Leval[4], T.M. Järvinen[5,*]

[1]Department of Hematology, Helsinki University Hospital Comprehensive Cancer Center and University of Helsinki, Helsinki, [2]EPID Research, Espoo, Finland, [3]StatFinn EPID Research, Tartu, Estonia, [4]Janssen-Cilag Ab, Solna, Sweden, [5]Janssen-Cilag Oy, Espoo, Finland

Background: Approximately 1800 individuals live with chronic lymphocytic leukemia (CLL) in Finland, with 350 diagnosed annually. Real-world treatment outcomes for this patient (pt) group have not been assessed previously in Finland and in the absence of direct head-to-head trials, robust historical control data have become important to ascertain the efficacy of novel therapies prior to introduction. The Finnish Hematology Registry (FHR) was created to allow for the collection of real-world practice outcomes in CLL and other hematological malignancies, with Finland's centralized nationwide healthcare system enabling reliable pt identification and comprehensive follow-up.
Aims: The aim of this registry study is to describe treatment and survival outcomes for CLL pts from routine practice settings, with special emphasis on second line treatment.
Methods: A non-interventional, retrospective study design was used to collect routine clinical practice data from the FHR. Eligible pts aged ≥18 years diagnosed with CLL and receiving 1 or more treatment lines during 2005-2015 were identified. As the registry had limited CLL pt follow-up data at study start, clinical investigators accessed medical records to retrieve and validate data. We report on 124 pts who met inclusion criteria from the Helsinki University Hospital, Helsinki, Finland, a hospital region accounting for 30% of the total national CLL incidence. Second line pts (n=64) were further stratified into those starting their treatment in 2006-2010 and in 2011-2015 time period, and treatment outcomes are described per time period and select treatments. For this initial analysis of 2nd line outcomes, crude overall survival (OS) and time-to-next-treatment (TTNT) were calculated per treatment line using Kaplan–Meier methods, with log-rank to estimate p-values.
Results: Median time to second line treatment was 31 months (m). During the follow-up, subtle changes in overall treatment practice were observed with fludarabine-cyclophosphamide-rituximab (FCR) and bendamustine-rituximab (BR) becoming the mainstay over fludarabine-cyclophosphamide (FC) after 2008. Across second line treatments, B/BR (36%), FCR (17%), chlorambucil-based therapies (20%) and (FC) (8%) were the most frequently used treatments. Novel agents ibrutinib, idelalisib+rituximab and obinutuzumab were not used prior to 2015. Median OS and TTNT from the start of any given 2nd line treatment were 37 m and 19 m, respectively. Pts receiving B/BR had shorter, though non-significant, median OS (37 m) compared to FCR (39 m) (p=0,51) (Table 1). TTNT was longer for pts receiving FC (33 m, p=0,42) and FCR (28 m, p=0,80) compared to B/BR (22 m). Despite apparent changes in treatment practice and increased use of chemoimmunotherapy over the years, improvements in 2nd line median OS (30 *vs* 42 m, p=0,19) and TTNT (10 *vs* 20, p=0,43) for the period 2006-2010 *vs* 2011-2015 did not reach statistical significance.

Table 1.

Table 1. Median second line overall survival (OS) and time to next treatment (TTNT) by select treatment regimens and treatment start time periods. NA; not reached.

	Treatment regimen (n)			Treatment start time period (n)	
	FC (5)	FCR (11)	B/BR (23)	2006-2010 (22)	2011-2015 (42)
Median OS (m), (95 % CI)	NA (36 – NA)	39 (36 – NA)	37 (22 – NA)	30 (18 – 47)	42 (26 – NA)
P-values	FC vs. BR = 0,14 FCR vs. BR = 0,51			0,19	
Median TTNT (m), (95 % CI)	33 (27 – NA)	28 (10 – NA)	22 (10 – NA)	10 (5 – 34)	20 (14 – 35)
P-values	FC vs. BR = 0,42 FCR vs. BR = 0,80			0,43	

Summary/Conclusion: We describe real-world CLL treatment trends and outcomes from a decade of practice in the Helsinki hospital region focusing on 2nd line regimens. These results are consistent with reports from Sweden (Asklid *et al.* 2016). Changes in treatment practice were observed between different time periods, however, results do not show significant improvements for OS and TTNT during this decade of follow-up. In the Nordics, real world evidence has become essential when introducing novel targeted therapies into clinical practice settings, with comprehensive disease registries expanding to allow for more detailed follow-up.

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MULTICENTRIC REAL LIFE COHORT OF UNSELECTED CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS (CLL): MOLECULAR ANALYSIS, CLINICAL INTEGRATION AND PROGNOSTIC IMPLICATIONS

R. Murru[1,*], S. Uda[1], G. Piras[2], S. Oppi[1], R. Asproni[2], F. Culurgioni[1], M. Monne[2], A. Uras[2], A. Bianchi[2], A.D. Palmas[2], G. Latte[2], G. La Nasa[1]

[1]SC Ematologia E Ctmo, Ospedale Oncologico A. Businco Ao Brotzu, Cagliari, [2]uoc Ematologia, Ospedale San Francesco Assl Nuoro-Ats, Nuoro, Italy

Background: Clinical heterogeneity of chronic lymphocytic leukemia (CLL) ranges from an indolent course to a very aggressive disease, while some patients never need treatment whilst many others require multiple lines of therapy during their lifetime and often die from disease. Integration between biological and clinical findings, with particular reference to innovative prognostic markers, is an increasingly pressing need for a risk stratification tool, useful for predicting disease outcome. Translation on real life setting and into everyday care is a real proposal in helping physicians to make better decisions.
Aims: To determine and analyze the most relevant biological and clinical characteristics of a multicentric real life CLL cohort and their impact on overall survival (OS) and time to progression (TTP), comparing with those reported in the literature.
Methods: A multicentric real life cohort of 273 unselected CLL patients in different clinical Binet stage were retrospectively analyzed for IGHV status, CD38 expression, *NOTCH1* mutation (mut), FISH (*del13q, tris12, del11q, del17p*), *TP53* mutational status. A large part of patients (n°190, 70%), have been included in a clinical research project currently in progress, apply-

ing ultra-deep Next Generation Sequencing (NGS), with a major aim to point out new possible prognostic or predictive biological markers (data in progress). All patient, 114 untreated (42%) and 159 treated patients (58%), have been diagnosed from 01/1988 to 01/2018, with a long term follow up (median: 89.2 months; range: 2-343). Biological and clinical patient characteristics are summarized in table 1. All data have been statistically analyzed using Prism 4.

Results: We investigated OS in patients with stage A (107.7 months), stage B (75.5 months) and C (70.1 months), defined at diagnosis ($p<0.001$). Median time to progression (TTP) was 52 months for all treated patients, regardless of Binet stage at diagnosis. Moreover, IGHV mutational status was analyzed at diagnosis in 171 (63%) patients (unmutated: 22%; mutated: 78%), reporting statistically significant differences in median OS, regardless of treatment (95.3 months and 89.2 months, respectively; $p<0.001$). CD38 expression was evaluated in 191 patients (70%), analyzed and integrated with FISH aberrations and IGHV status. FISH analysis was overall performed in 238 samples (before first line therapy: n°200; relapsed/refractory: n° 38), identifying a small group (7%) with multiple aberrations and unfavorable prognosis. *NOTCH1* c.7544_7545delCT mutations were investigated in 207 DNA samples (76%). *NOTCH1*-mut was detected in 23 patients (11%) and correlated with *tris12* and IGVH status, analyzing impact on overall survival (OS). Only 9% of NOTCH1 mut developed Richter syndrome with fatal outcome, confirming a worse prognostic role characterized by chemoresistance and rapid disease kinetics.

Table 1.

BIOLOGICAL AND CLINICAL CHARACTERISTICS OF PATIENTS			
	N° of patients	%	Median (OS in months)
Cohort of unselected patients	273		89.2
Binet stage A	156/227	69	107.7
Binet stage B	49/227	21	75.5
Binet stage C	22/227	10	70.1
Females	112	41	
Males	161	59	
Median age at diagnosis			64 yrs (36-90)
IGHV mutated status	134/171	78	89.2
IGHV unmutated status	37/171	22	95.3
CD38 expression	191/273	70	
CD38+	44/191	23	
CD38+/IGHV mut	27	61.3	
CD38+/IGHV unmut	11	25	
CD38+/ IGHV not evaluated	6	13.7	
CD38+/negative FISH	13	29.5	
CD38+/del13q	8	18.3	
CD38+/tris12	7	15.9	
CD38+/del11q	2	4.5	
CD38+/TP53 mutation	1	2.3	
CD38+/FISH not evaluated	13	29.5	
NOTCH1 mutated status	25/207	12	
FISH	238	87.2	
del13q	76	32	88.6
Tris12	35	15	85.37
del11q	13	6	75.3
del17p/TP53 mutation	8	3	80.9
Complex caryotipe (del 13q/del11q, del 13q/del17q, del13q/TP53mutation)	17	7	70.8
TP53 mutation	5	2	55.4
FISH negative	84	35	82.3

Summary/Conclusion: We choose to emphasize our real life data related to IGHV, FISH aberrations and *NOTCH1 mut*, not yet widely applied in everyday care, confirming the need for early identification of well-known biological and clinical findings. Interesting results related to IGHV and OS must be validated through large prospective cohort study, suggesting a clue for possible other biological factors and encouraging the introduction of innovative genetic lesion analysis (ie, NGS), with the aim of selecting high risk *vs* low risk patients.

PB1869

HIGH DOSE METHYLPREDNISOLONE AND RITUXIMAB IN RELAPSED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

R. Pileckyte[1,2,*], V. Valceckiene[2], J. Sejoniene[3], L. Griskevicius[1,2]

[1]Clinics of Internal, Family Medicine and Oncology, Faculty of Medicine, Vilnius University, [2]Hematology, Oncology and Transfusion Medicine Center, [3]Radiology Center, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania

Background: BTK and BCL2 inhibitors have changed the treatment paradigms of high risk and/or elderly patients with chronic lymphocytic leukemia (CLL), but long term efficacy and toxicity are still unknown and the costs are relevant. Rituximab (RTX) and high dose methylprednisolone (HDMP) can be an effective and safe treatment option for relapsed CLL. Here, we present the long term results of two phase II studies of HDMP and RTX combination given in different schedules.

Aims: LT-CLL-001: to evaluate the efficacy and safety of HDMP and RTX in relapsed (R) high risk CLL patients.LT-CLL-2S: to evaluate the efficacy and safety of HDMP and RTX in relapsed elderly or unfit CLL patients

Methods: In LT-CLL-001 R CLL patients with high risk features (17p del, *TP53* mut, 11q del and/or trisomy 12 or fludarabine refractory) were included. The treatment schedule was described elswere.[1]In LT-CLL-2S study, relapsed patients with CIRS >6 and / or ≥65 years were included. HDMP was administered at 1 g/m^2 iv daily for 3 days of each course for 4 or 6 courses. RTX was administered at 1000 mg/m^2 on day 1 of each course for 4 courses. Treatment cycles were repeated every 3 weeks.

Results: 29 patients were enrolled in LT-CLL-001. Demographic data have been described elswere[1]. The median observation time was 31 (1-76) month. The follow up for alive patients was 66.5 (60-76) months. Overall response rate (ORR) was 62%, all partial remissions (PR). The median progression free survival (PFS) and overall survival (OS) were 12 and 31 months. Five-year OS was 21% for all the patients; for patients with 17p deletion/*TP53* mutation 3-year OS was 38%, 5-year OS was 8%. Grade III-V infections were observed in 2% of all AEs.3 patients died during treatment phase. There were 3 cases (10.3%) of secondary malignancies: 1 Richter transformation, 1 polycythaemia vera and 1 breast cancer.25 patients were included in LT-CLL-2S. The median age was 73 years (range 65-80), 6 (26%) had 17pdel/*TP53* mutation, 3 (12%) had 11q del. 9 patients were given >4 courses 16 patients were given ≤4 courses. ORR was 28%, all PR, mainly due to residual lymphadenopathy. All 18 patients with B symptoms had their symptoms resolved. Significant improvement in anemia *($p<0,001$)* was noted. The median follow-up was 44 (11-69) months. The median PFS was 11 months (range 10-12). Median OS was 68 (62-74) months. No difference in ORR or PFS was noted between patients who received ≤4 or >4 courses, but a tendency for better OS was observed for the patients with more treatment (36 months *versus* not reached, *$p=0.062$*). AE were mainly I-II° (82%), no deaths occurred during treatment.After their respective study, the relapsed patients with treatment indications recieved ibrutinib in notable difference between the studies: one (5%) out of 20 LT-CLL-001 patients compared to 7 (32%) out of 22 LT-CLL-2S patients.

Summary/Conclusion: Lower ORR was observed in LT-CLL-2S compared to LT-CLL-001 mostly because of residual lymphadenopathy. However, median PFS between the studies were similar and toxicity of LT-CLL-2S was lower.The different ORR in two studies could be explained by the higher dose of HDMP in LT-CLL-001. The longer median OS in LT-CLL-2S could be explained by lower number of high risk patients and the availability for BTK inhibitors as salvage therapy. HDMP and RTX combination can still be applied in some relapsed high risk and elderly or unfit CLL patients without the significant risk of concomitant diseases exacerbation if new therapies are contraindicated or unavailable.

PB1870

THE VALUE OF RITUXIMAB-CONTAINING CHEMOTHERAPY IN SECOND-LINE WITH OR WITHOUT PREVIOUS RITUXIMAB EXPOSURE: A POPULATION-BASED STUDY AMONG PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA IN THE NETHERLANDS

L. van der Straten[1,2,*], M.-D. Levin[2], E. van den Broek[3], O. Visser[4], W. Posthuma[5,6], J. Doorduijn[7], A. Kater[8], A. Dinmohamed[1,7,9]

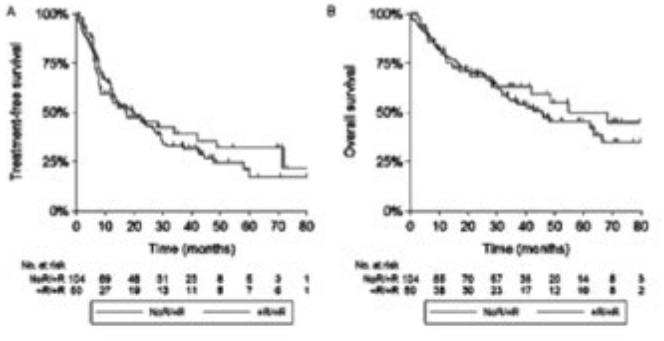
[1]Research, Netherlands Comprehensive Cancer Organisation (IKNL), Utrecht, [2]Internal Medicine, Albert Schweitzer Hospital, Dordrecht, [3]PALGA Foundation, Houten, [4]Registration, Netherlands Comprehensive Cancer Organisation (IKNL), Utrecht, [5]Internal Medicine, Reinier de Graaf Group, Delft, [6]Hematology, Leiden University Medical Center, Leiden, [7]Hematology, Erasmus MC Cancer Institute, Rotterdam, [8]Hematology, Academic Medical Center, Amsterdam, [9]Public Health, Erasmus University Medical Center, Rotterdam, Netherlands

Background: In an era with novel agents, chemoimmunotherapy with rituximab (R) still generally constitutes the cornerstone of first-line chronic lymphocytic leukemia (CLL) treatment. International clinical practice guidelines recommend to repeat first-line chemoimmunotherapy if the treatment-free interval between first- and second-line treatment is over 24 to 36 months. However, the effectiveness of R-based regimens in second-line—with or without previous R-based regimens in first-line—has virtually not been evaluated at the population level.

Aims: The aim of this population-based study was to assess the effectiveness of R-based chemotherapy for the second-line treatment of CLL patients in relation with first-line treatment with or without R-based chemotherapy.

Methods: We selected all CLL patients diagnosed between 2004-2008 who initiated second-line treatment with R-containing chemotherapy from the PHAROS CLL registry that encompasses ~40% of the Dutch population and is notified by the nationwide Netherlands Cancer Registry. Patients who initiated second-line treatment with R-based chemotherapy were categorized into two groups, namely those who received first-line chemotherapy with (+R/+R) or without R (NoR/+R). The primary and secondary endpoint was treatment-free survival (TFS) and overall survival (OS), respectively (see definitions in Fig 1). Multivariable Cox regression was used to assess covariates (listed in Table 1) associated with TFS and OS. A $P<0.05$ indicates statistical significance.

Results: A total of 154 rituximab-treated CLL patients were included in this analysis, of whom 104 (68%) and 50 (32%) were in the NoR/+R and +R/+R group, respectively. Patients in the NoR/+R group were significantly older than patients in the +R/+R group (median age 71 [range 37-88] *vs* 67 [range 40-83] years; $P=0.046$). Most patients in the NoR/+R and +R/+R group received first-line treatment with alkylating agents (86% *vs* 84%; $P=0.797$), followed by purine analogues (PA; 13% *vs* 14%; $P=0.795$). In the second-line setting, 54% and 20% of patients received R with alkylating agents, 22% and 28% with PA, and 24% and 53% with other modalities (*i.e.* R monotherapy or DHAP) in the NoR/+R and +R/+R group, respectively ($P<0.001$). At a median follow-up of 30.7 months (range 0.2-115), the median TFS was 19.6 (95% confidence interval [CI], 12.6-27.3) and 17.1 months (95%CI, 7.9-48.6) for the NoR/+R and +R/+R group, respectively ($P=0.627$). Third-line treatment was started in 48% and 36% and deaths occurred in 51% and 42% in the NoR/+R and +R/+R groups, respectively. The median OS was 45.8 (95% CI, 31.9-66.5) and 54.9 months (95% CI, 27.7-not reached) for the NoR/+R and +R/+R group, respectively ($P=0.433$). In multivariable analysis, first-line treatment with R±PA, as compared with R±alkylating agents, was associated with lower TFS ($P=0.009$) lower OS ($P=0.003$). In addition, second-line treatment with R-based regimens without alkylating agents or PA ($P=0.049$), as compared with R with PA, and time between first- and second-line treatment per one month increase ($P=0.023$) was associated with better TFS. Furthermore, age per one increase was associated with lower OS ($P=0.004$).



- Treatment-free survival was defined as the time for start of second-line treatment until institution of third line treatment or death resulting from any cause, or last follow-up (December 31, 2014), whichever occurred first.
- Overall survival was defined as the time for start of second-line treatment until death resulting from any cause or until the last date the patient was known to be alive (December 31, 2014), whichever occurred first.

Table 1. Multivariable Cox regression for treatment-free survival and overall survival

Covariate	Treatment-free survival				Overall survival			
	HR	95% CI		P	HR	95% CI		P
Age, years (per one unit increase)	1.02	0.99	- 1.03	0.212	**1.04**	**1.01**	- **1.07**	**0.004**
Female sex	0.84	0.55	- 1.29	0.422	0.78	0.47	- 1.28	0.328
R in pretreatment	1.17	0.73	- 1.91	0.504	1.00	0.56	- 1.78	0.995
Type of pretreatment								
(R)Alkylating agents	ref	1			ref	1		
(R)Purine analogues	**2.62**	**1.23**	- **4.30**	**0.009**	**3.08**	**1.45**	- **6.50**	**0.003**
(R)other	1.16	0.27	- 5.00	0.839	0.60	0.11	- 6.07	0.833
Type of retreatment								
R-Alkylating agents	ref	1			ref	1		
R-Purine analogues	0.81	0.50	- 1.31	0.397	0.89	0.49	- 1.59	0.685
R-other	**0.55**	**0.31**	- **1.00**	**0.049**	0.81	0.42	- 1.57	0.529
Time between first and second line	**0.98**	**0.96**	- **1.00**	**0.023**	0.98	0.95	- 1.00	0.058

Figure 1.

Summary/Conclusion: In this population-based study, we demonstrated that TFS and OS among rituximab-treated CLL patients in second-line was not influenced by first-line chemotherapy with R. This novel finding illustrates that R-based chemotherapy in second-line is a viable treatment option among particular patient subsets in an era with novel, expensive agents.

PB1871

SECOND CANCERS IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS TREATED WITH BCR INHIBITORS-RETROSPECTIVE ANALYSIS OF THE POLISH ADULT LEUKEMIA STUDY GROUP (PALG)

B. Puła[1], E. Iskierka-Jażdżewska[2], M. Długosz-Danecka[3], A. Szymczyk[4], M. Hus[4], A. Szeremet[5], J. Rybka[5], J. Drozd-Sokołowska[6], A. Waszczuk-Gajda[6], J.M. Zaucha[7], J. Hołojda[8], W. Piszczek[9], P. Steckiewicz[10], M. Wojciechowska[11], M. Osowiecki[12], W. Knopińska-Posłuszny[13], A. Kopacz[14], M. Dudziński[14], D. Zawirska[3], M. Piotrowska[3], E. Subocz[15], J. Hałka[15], B. Kumiega[16], L. Gil[17], L. Szukalski[18], R. Wichary[19], B.K. Budziszewska[1], E. Lech-Maranda[1,20], W. Jurczak[3], K. Giannopoulos[21], T. Robak[2], K. Warzocha[1], K. Jamroziak[1,*]

[1]Department of Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, [2]Department of Hematology, Medical University of Lódź, Copernicus Memorial Hospital, Lódź, [3]Department of Hematology, Jagiellonian University, Kraków, [4]Department of Hematooncology and Bone Marrow Transplantation, Medical University of Lublin, Lublin, [5]Department of Hematology, Blood Neoplasms and Bone Marrow Transplantation, Wrocław Medical University, Wrocław, [6]Department of Hematology, Oncology and Internal Medicine, Medical University of Warsaw, Warsaw, [7]Department of Hematology and Transplantology, Medical University of Gdańsk, Gdańsk, [8]Department of Hematology, Specialist District Hospital, Legnica, [9]Department of Hematology, Copernicus Hospital, Toruń, [10]Department of Hematology, Holycross Cancer Center, Kielce, [11]Department of Hematology, Specialist District Hospital, Olsztyn, [12]Department of Lymphoid Malignancies, Maria Sklodowska-Curie Memorial Institute and Oncology Centre, Warsaw, [13]Department of Hematology, Gdynia Oncology Centre, Gdynia, [14]Department of Hematology, Specialist District Hospital, Rzeszów, [15]Department of Hematology, Military Institute of Medicine, Warsaw, [16]Department of Hematological Oncology, Regional Specialist Hospital, Brzozów, [17]Department of Hematology and Bone Marrow Transplantation, Karol Marcinkowski University of Medical Sciences, Poznań, [18]Department of Hematology, Nicolaus Copernicus University, Bydgoszcz, [19]Department of Hematology and Bone Marrow Transplantation, Katowice Medical Department, Silesian Medical University, Katowice, [20]Department of Hematology and Transfusion Medicine, Centre of Postgraduate Medical Education, Warsaw, [21]Department of Experimental Hematooncology, Medical University of Lublin, Lublin, Poland

Background: Chronic lymphocytic leukemia (CLL) patients are at a significantly increased risk of developing a second malignant neoplasm. Recent introduction of B-cell receptor inhibitors (BCRi) ibrutinib and idelalisib was a major breakthrough in the treatment of relapsed and refractory disease. However, knowledge on incidence of second cancers in the context of BCRi therapy is limited.

Aims: To study the incidence and pattern of second cancers and Richter transformation (RT) in relapsed and refractory CLL patients treated with ibrutinib or idelalisib.

Methods: Retrospective analysis was performed concerning diagnosis and outcome of second malignancies occurring during BCRi treatment in Polish hematology centers of the Polish Adult Leukemia Study Group (PALG).

Results: Clinical data of 211 heavily pretreated relapsed and refractory CLL patients, of whom 157 received ibrutinib and 54 were treated with idelalisib, were included in the analysis. Median ibrutinib treatment duration was 33.5 (range 0.4-46.8) months. Eleven (7%) second malignancies developed under ibrutinib therapy including 7 cancers of epithelial origin (2 lung cancers, 2 skin cancers, 1 peritoneal cancer, 1 urinary bladder and 1 breast cancer) and 4 cases of RT (3 histologically confirmed Hodgkin variant of RT and 1 clinically suspected RT of unknown histology). Median time from CLL diagnosis to the diagnosis of second malignancy was 83.1 (38.3-298.0) months, while median time from ibrutinib initiation to second cancer reached 19.63 (3.9-31.9) months. Idelalisib alone or in combination with anti-CD20 antibodies was administered for a median of 13.8 (0.1-23.5) months. In the group of idelalisib treated patients 7 (12.9%) malignancies occurred including 5 epithelial cancers (2 thyroid cancers, 1 renal cancer, 1 penile cancer and 1 gastric cancer) and 2 RT. Median time from CLL diagnosis to second cancer and median time from idelalisib start to diagnosis of second cancer were 47.7 (13.1-178) months and 21.3 (2.3-31.5) months, respectively. Comparing the frequency of second malignancies in ibrutinib and idelalisib treated patients, we did not reveal significant differences between both cohorts (p=0.25). The analysis of clinical decisions following the diagnosis of second malignancies in this real-life patient population showed different strategies including transient or permanent discontinuation of BCRi or dose modifications. Regarding the outcome of patients with second cancer, 6 out of 11 patients in the ibrutinib cohort and 2 out of 5 patients treated with idelalisib died. Importantly, in all these cases the second malignancy was the primary cause of death.

Summary/Conclusion: In this retrospective analysis we show that second cancers are not an uncommon clinical problem during BCRi therapy of

CLL, at least in relapsed refractory patients, heavily pretreated with standard alkylating agents and/or purine nucleoside analogs based therapies. Interestingly, we observed more epithelial cancers than RT. The potential influence of specific type of BCRi on incidence of second cancers as well as optimal dosing of BCRi during systemic treatment of second malignancies needs further research.

PB1872

INFLUENCE OF INTENSITY CHEMOTHERAPY ON THE FREQUENCY OF MRD NEGATIVE REMISSIONS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

M. Mikhaleva[1,*], A. Kuvshinov[1], S. Voloshin[1], E. Belyakova[2], E. Krasnikov[3]

[1]Clinical Department of Chemotherapy of Hemoblastosis, Depression of Hemopoiesis and Bone Marrow Transplantation, Federal State Budget Institution "Russian Scientific and Research Institute of Hematology and Transfusiology of Federal Medical Biological Agency", [2]North-western State Medical University named after I.I. Mechnikov, Saint-Petersburg, Russian Federation, [3]St. Petersburg State Budgetary Healthcare Institution City Hospital №40, Saint-Petersburg, Russian Federation, Saint-Petersburg, Russian Federation

Background: Minimal residual disease (MRD) is a powerful prognostic factor in the course of chronic lymphocytic leukemia (CLL). The achievement of MRD negative (MRD^{neg}) remissions is associated with a longer survival of patients with CLL. In addition, it is known that increasing of intensity the induction chemotherapy regimens allows to achieve the maximum eradication of the tumor clone and, thereby, to increase the duration of progression free survival (PFS).
Aims: To assess influence of intensity the induction chemotherapy on the frequency of achievement MRD^{neg} status and duration of PFS.
Methods: In this study were included 118 patients with CLL (male/female ratio 1.3:1), age 31 to 80 years (median 61 years). The median of follow-up duration is 36 months (range 5-84). We have used the revised NCI guidelines (Hallek M, *et al*., 2008) for diagnosis, initiation of therapy, evaluation of response and MRD (10^{-4}). Patients were treated with rituximab-containing chemotherapy regimens (RFC, RB, RChl, R-CHOP). Median of chemotherapy lines-1 (range 1-3). Patients were divided into three groups depending on the number of cycles of chemotherapy: group 1-4 cycles (n=18), group 2-6 cycles (n=78), group 3-8 cycles (n=22). The evaluation of MRD status was carried out using 5-color flow cytometry on bone marrow samples after 2 months of completion chemotherapy. In addition, intermediate evaluation of MRD status after 4 treatment cycles was carried out in group 2 (n=36) and group 3 (n=6) underwent.

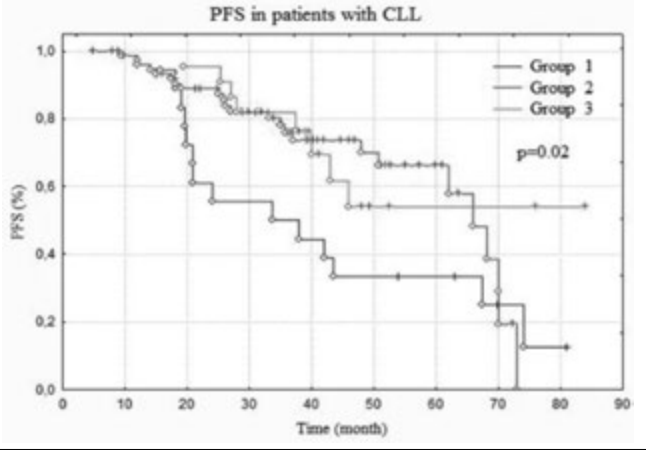


Figure 1.

Results: Group 1: complete remission (CR)-4 (MRD^{neg}-2), partial remission (PR)-14 (MRD^{neg}– 2); group 2: CR-16 (MRD^{neg}-6), PR-62 (MRD^{neg}-26); group 3: CR-6 (all MRD^{neg}), PR-16 (none MRD^{neg}). The frequency of achievement of MRD^{neg} status at the completion of chemotherapy regimen was: group 1-22.2% (4/18), group 2-41.0% (32/78), group 3-27.3% (6/22) (p>0.05). The frequency of achievement of MRD^{neg} status after 4 cycles of chemotherapy in group 2-22.2% (8/36) and in group 3-33.3% (2/6). Median PFS in group 1 was 33.7 months, in group 2-66.0 months, and in group 3-not achieved (p=0.02). When comparing PFS in patients among all groups who achieved MRD^{neg} status after 4 cycles of chemotherapy no significant differences were found, which is probably due to a small number of events and short observation period. However, it was noted that progression/relapse of the disease was occurred only in the group 1-11.1% (2/18), while in groups 2 and 3, progression/relapse of the disease was not detected in any patient who achieved MRD^{neg} status after 4 cycles of treatment (p=0.016).
Summary/Conclusion: Consider that intensity of chemotherapy does not affect on the frequency of achievement MRD^{neg} status, but affects on PFS, we can assume that patients with a large number of cycles of chemotherapy achieved a deeper MRD^{neg} status (less than 10^{-4}). Intensification of chemotherapy regimens can increase the risk of significant hematologic toxicity and infectious complications, that requires a personified management strategy for patients with CLL.

PB1873

ADVANCED DATA-MINING METHODS REVEALED FOUR SUBGROUPS AMONG HIGH-RISK CLL PATIENTS

P. Turcsanyi[1,*], M. Kudelka[2], M. Radvansky[2], E. Kriegova[3], L. Kruzova[1], R. Urbanova[1], P. Schneiderova[3], H. Urbankova[1], T. Papajik[1]

[1]Hemato-Oncology, Department of Hemato-Oncology, Faculty of Medicine and Dentistry, Palacky University Olomouc and University Hospital Olomouc, Olomouc, Czech Republic, Olomouc, [2]Department of Computer Science, Faculty of Electrical Engineering and Computer Science, VSB-Technical University of Ostrava, Ostrava, [3]Department of Immunology, Faculty of Medicine and Dentistry, Palacky University Olomouc and University Hospital Olomouc, Olomouc, Czech Republic, Olomouc, Czech Republic

Background: The clinical course of chronic lymphocytic leukemia (CLL), the most common leukemia in western countries, is highly heterogeneous. Further stratification of CLL patients who may profit from novel targeted therapies is critically needed, particularly in high-risk patients.
Aims: To subdivide the high-risk CLL patients based on the clinical course, overall survival (OS) and treatment response.
Methods: Study cohort consisted of 116 high-risk CLL patients (F/M 36/80, median age 63 yrs, min-max 34-87 yrs) diagnosed between 2000 to 2015 in single reference centre. Besides genetic aberrations (del(11q), del(17p), *TP53* mutation and complex karyotype, CK), age, gender, Binet stage, blood counts, beta-2-microglobulin, thymidinkinase, LDH, splenomegaly, bulky lymphadenopathy (>5 cm) at the time of diagnosis and clinical course, treatment response and OS were evaluated. Advanced data-mining methods were used to analyse the data.
Results: The network analysis revealed four subgroups of patients differing in their profiles of genetic aberrations, bulky lymphadenopathy, splenomegaly and gender. The best prognosis was observed for Profile (P)-I (n=47, predominantly men with del(11q) + no CK + no TP53 disruption) and P-II (n=29, men/women + del(11q) with CK + no TP53 disruption) with OS 70 and OS 54 mo, respectively. The ultra high-risk patients were those with P-III (n=19, predominantly women with del(17p)/*TP53* mutation) and P-IV (n=21, men/women with CK + TP53 disruption) with OS 38 and OS 40 mo, respectively. The OS differed between (P-I and P-II) and (P-III and P-IV) (p<0.002). Of 54 patients treated with fludarabin, 33% were refractory (10.6% P-I, 13.8% P-II, 31.0% P-III, 14.3% P-IV). Adding rituximab to therapy did not improve OS in our patients regardless of profiles (p=0.98). Patients who required treatment shortly after diagnosis (<3 mo) had the shortest OS (48 mo) *versus* those treated later, evident in all profiles (OS 84 mo, p=0.037).
Summary/Conclusion: Our data showed that patients within P-III (women with del(17p)/*TP53* mutation) and P-IV (men/women with CK and TP53 disruption) have the worst prognosis among high-risk patients. These ultra high-risk patients are candidates for novel upfront treatment algorithms.
Grant support: MZ R VES16-32339A.

PB1874

PROSPECTIVE REAL WORLD DATA OF AN ONGOING POST-AUTHORIZATION SAFETY STUDY ON IDELALISIB IN PATIENTS WITH CLL AND REFRACTORY FL

W. Knauf[1,*], M. Hoechstetter[2], P. Eissmann[3], N. Hucke[3], A. van Troostenburg[3], H. Ramroth[3], M. Rummel[4]

[1]Centrum Haematologie / Onkologie Bethanien, Frankfurt a. M., [2]Klinikum Schwabing, Munich, Germany, [3]Gilead Sciences Inc., Foster City, United States, [4]Universitätsklinikum Giessen, Giessen, Germany

Background: Idelalisib, a first-in-class PI3Kδ inhibitor, is indicated in combination with rituximab or ofatumumab for the treatment of adult patients with chronic lymphocytic leukemia (CLL) who have received at least one

prior therapy or as first-line treatment in the presence of 17p deletion or *TP53* mutation in patients who are not eligible for any other therapies and as monotherapy for the treatment of adult patients with follicular lymphoma (FL) that is refractory to two prior lines of treatment.
Aims: To prospectively collect safety and efficacy data on idelalisib use in routine clinical practice in Germany.
Methods: A prospective, two-cohort, multicenter, non-interventional post authorization safety study (PASS); inclusion of patients is based on the physician's decision to initiate treatment with idelalisib in accordance with the German Summary of Product Characteristics; primary objectives are progression-free survival, overall response rate and overall survival; secondary objectives include the incidence and severity of adverse drug reactions and fatal events; descriptive statistics are used for data analysis.
Results: The second pre-planned interim analysis includes results of 93 patients (83 patients with CLL, 10 patients with FL) in 49 active sites in Germany that had completed at least 3 months after initiation of idelalisib treatment. Patients with CLL had a median age of 74 years and were predominantly male (69%) while patients with FL had a median age of 66 years and were predominantly female (90%). Median time from diagnosis to start of idelalisib therapy was 93 (range 0.4-307.8) months for CLL and 37 (range 21.5-360.1) months for FL. 68% of patients had a Karnofsky index of ≥80 and 86% of patients presented with one or more co-morbidities. 35% (n=88) of documented co-morbidities (n=249) were diseases of the circulatory system according to ICD-10 classification. Among these the most frequently reported were hypertensive disease (n=41), cardiac arrhythmias (n=15) and chronic ischemic heart disease (n=10). In the CLL cohort deletion 17p and/or *TP53* mutation was detected in 21%, deletion 11q in 19% and deletion 13q in 23% of patients. The median observation time was 6 months for the entire study population, 7 months for the CLL and 5 months for the FL cohort. By the time of data extract 63% of patients had permanently discontinued idelalisib treatment with a median time to permanent treatment discontinuation of 5 months. A total of 75 treatment interruptions were reported.
Summary/Conclusion: Post-authorization safety and efficacy data are important to assess the benefit/risk profile of therapies in routine clinical practice. Our prospective PASS on the use of idelalisib in Germany is therefore reporting safety and efficacy data on an ongoing basis.

PB1875

MAINTENANCE DOSING OF IBRUTINIB FOR CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL) / SMALL LYMPHOCYTIC LYMPHOMA PATIENTS

W. Alexander, R. Ramakrishna*

Southern Haematology and Cancer Research Institute, Wollongong, Australia

Background: Ibrutinib is a BTK inhibitor, indicated for use in CLL/SLL patients as a second line therapy when purine analogues are either infective, or contraindicated. Standard dosing regime is 420 mg daily. Complications associated with this level of dosing include skin lesions or infections, cardiac arrhythmias, hypertension, bleeding, secondary malignancies and other infections. It has been suggested that reduced dosing may be equally efficacious in this patient group.
Aims: To consider the efficacy of reduced dose of ibrutinib (maintenance dosing) for retaining remission in CLL/SLL patients who have achieved CR or VGPR on full dose therapy.

Table 1.

Table 1.
Number of patients affected by complications of full dose ibrutinib therapy

Side Effects prior to dose reduction	Number affected
Skin lesion (acne-like)	3
Skin abscess and/or cellulitis	3
Nail changes	1
AF	2
Hypertension	1
Bruising and/or epistaxis	3

Methods: A retrospective data audit was conducted on all patients who had received maintenance dosing of ibrutinib at Southern Sydney Haematology. Criteria being assessed included total time on therapy, time on maintenance dosing, effects of reduced dosing on any side effects, evidence of relapse, and basic demographic data.
Results: Out of 40 patients treated with ibrutinib at the facility, a total of 13 patients had received reduced dosing with ibrutinib: 8 males and 5 females. Median age was 69 (range 63-77). Total time on therapy was a median of 35 months (range 31-36 months) while time on reduced dosing was a median of 14 months (range 6-24 months). Seven patients had developed various side effects on full dose therapy (table 1) but none of these side effects recurred on reduced dosing. One patient developed new onset atrial fibrillation seven months after starting reduced dosing of ibrutinib that is currently medication controlled. No patients have relapsed on reduced dosing and this entire cohort remains on maintenance therapy.
Summary/Conclusion: Based on findings from this cohort, reduced dosing of ibrutinib 980 mg -1260 mg/week for patients who have achieved VGPR or CR and have been stable on therapy for 12-24 months would appear to be sufficient to maintain remission and a good way for managing skin lesions or other minor side effects. This confirms the results of other small-scale studies conducted at other institutions.

PB1876

OBINUTUZUMAB IN FIRST-LINE TREATMENT OF ELDERLY/ COMORBID PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA- THE REAL-WORLD RESULTS OF POLISH ADULT LEUKEMIA GROUP (PALG)

M. Długosz-Danecka[1,*], W. Jurczak[1], E. Łątka[1], M. Morawska[2], K. Gawroński[3], A. Wiśniewska[4], M. Dudziński[5], E. Wąsik-Szczepanek[6], E. Chmielowska[7], A. Łabędź[8], K. Wdowiak[9], E. Paszkiewicz-Kozik[10], I. Hus[11]

[1]Department of Hematology, Jagiellonian University, Krakow, [2]Experimental Hematooncology Department, Medical University of Lublin, Lublin, [3]Department of Hematology, Military Institute of Medicine, Warszawa, [4]Department of Oncology and Chemotherapy, Kopernik Memorial Specialist District Hospital, Koszalin, [5]Department of Hematology, Clinical District Chopin Memorial Hospital, Rzeszów, [6]Department of Hematooncology and Bone Marrow Transplantation, Medical University of Lublin, Lublin, [7]Department of Clinical Oncology, Oncology Centre, Bydgoszcz, [8]Department of Hematology, Rydygier Memorial Hospital, Krakow, [9]Department of Internal Medicine and Oncology, Silesian Medical University, Katowice, [10]Department of Lymphoid Malignancies, Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology, Warsaw, [11]Department of Clinical Transplantology, Medical University of Lublin, Lublin, Poland

Background: The current first-line treatment for fit chronic lymphocytic leukemia (CLL) patients remains fludarabine-based therapy. Elderly patients with numerous comorbidities, poorly tolerate such regimen and pose a huge challenge.
Aims: In our analysis, we investigated the efficacy and safety obinutuzumab-chlorambucil combination in elderly and unfit patients.

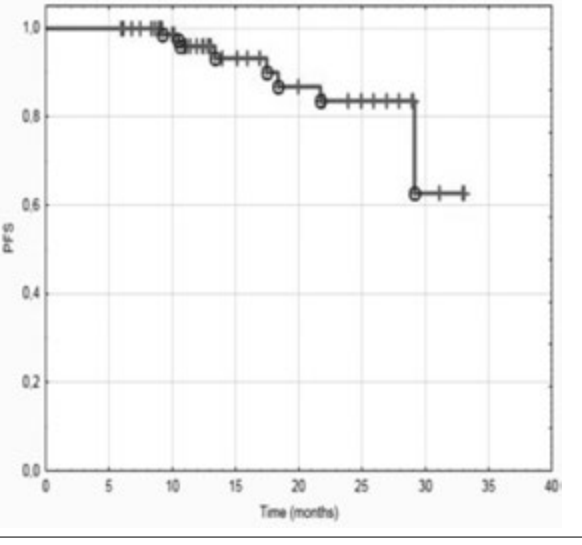


Figure 1.

Methods: We include in our analysis 86 treatment-naïve CLL patients (median age 74 years, range 51-86 years) with significant burden of coexisting comorbidities. All patients presented the Cumulative Illness Rating Scale (CIRS) score greater than 6 and/or creatinine clearance (CrCl) of 30–69 ml/min. Most patients (94,19%) had four or more coexisting comorbidities, with cardiovascular, endocrine or metabolic, respiratory and genitourinary disorders being the most frequent. Obinutuzumab was infused intravenously

at 1000 mg on days 1, 8 and 15 of cycle 1 and on day 1 of cycles 2–6 (28-day cycles) with first infusion split over 2 days for patients' safety. Chlorambucil was administered orally at a dose of 0.5 mg per kilogram of body weight on days 1 and 15 of each cycle.
Results: Overall response rate (ORR) at 2 months after treatment completion was 95.35% including complete remission (CR) in 37 patients (45.12%) and partial remission (PR) in 45 patients (54.88%). Stable disease was noted in 4 patients (4.65%) and progressive disease (PD) was not observed after the end of therapy. The median progression free survival (PFS) was 13,05 months. The relapses occurred in 6 patients (7%) with 3 patients (3.5%) completing treatment with CR and 3 (3.5%) with PR. The median number of treatment cycles was 6. The most frequent adverse events (AE) were infusion-related reactions (IRR) and neutropenia. Grade 3 IRR occurred in 2.3% of patients, grade 2 in 12.8% (in 81.8% of patients only during the first infusion of monoclonal antibody). Grade 3 neutropenia was reported in 11.6% of patients, grade 2 in 22.1% with no incidence of febrile neutropenia. There were no AE of grade 4 or 5.
Summary/Conclusion: Our data confirm that obinutuzumab-chlorambucil is an effective and well-tolerated regimen in untreated CLL patients with comorbidities.

PB1877

ATRIAL FIBRILLATION IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA RECEIVING TREATMENT WITH IBRUTHINIB

G. Gendlin*, E. Emelina, I. Nikitin

Hospital Therapy N 2, Pirogov Russian National Research Medical University, Moscow, Russian Federation

Background: Atrial fibrillation (AF) is a frequent comorbidity in patients with chronic lymphocytic leukemia (CLL) receiving treatment with ibrutinib (Ib).
Aims: To study the incidence of AF and the possibility of using various anticoagulants in patients with CLL in the background of treatment with Ib.
Methods: We examined 174 patients with CLL who received Ib 420 mg as the first, second, third and fourth lines of therapy. All patients underwent electrocardiography, echocardiography and 24-hour ECG monitoring.
Results: The study included patients aged 32 to 95 years (66.0 (59.0-72.0) years), 111 men aged 32 to 95 years (66.0 (60.0-72, 0) years) and 63 women aged 39 to 83 years (64.0 (54.0 -71.0) years), p=0.34. AF was detected in 25 patients (14.7%) receiving Ib. In 11 patients (6.4%) AF diagnosed before the onset of treatment with Ib. In 14 patients (8.2%) AF occurred during the treatment with Ib in the period from 1 to 24 months (4.5 (2.0-16.0) months). 6 patients with AF (24%) had a permanent form, 19 had paroxysmal AF (76%). In 2 patients the paroxysmal form changed to a constant one during the Ib treatment period. In accordance with the recommendations of the ESC on diagnosis and treatment of AF in 2016 and the scale CHA_2DS_2-VASc, anticoagulants received 14 patients (56%), of whom 3 patients received dabigatran etexilate (dabi) at a dose of 150 mg * 2 times a day, 7 patients received rivaroxaban (riva) at a dose of 20 mg per day, 4 patients was treated apixaban (api) at a dose of 5 mg * 2 times a day. In 3 patients (12%) receiving anticoagulants occurred non-major bleeding: in one patient taking riva 20 mg / day there was hematuria during occurred thrombocytopenia and anticoagulant treatment was canceled; one patient receiving dabi 150 * 2 times a day had repeated nasal bleeding (to him was prescribed api at a dose of 2.5 mg * 2 times a day); in one patient treating with api 5 mg * 2 times a day there was a hematoma with a small external bleeding (the dose was reduced to 2.5 mg * 2 times a day). Riva was abolished in 3 patients (12%) because of severe thrombocytopenia.
Summary/Conclusion: Careful monitoring of patients with CLL and AF receiving treatment with Ib and anticoagulant therapy is necessary to early detect thrombocytopenia, hemorrhagic complications and timely dose adjustment or anticoagulant withdrawal.

PB1878

THE NUMBER OF PREVIOUS TREATMENT LINES AND COMORBIDITIES ARE ASSOCIATED WITH SERIOUS INFECTIONS AND RISK FOR ASPERGILOSIS IN CLL PATIENTS TREATED WITH BCR INHIBITORS IN REAL LIFE

J. Badiola*, P. González-Sierra, J.M. Puerta-Puerta, P. García-Martín, F. Hernández-Mohedo, C. Alarcón-Payer, M. Jurado-Chacón

Hematology, UGC intercentros Hospital Universitario Virgen de las Nieves-Nuevo San Cecilio, Granada, Spain

Background: In addition to blocking signals of cell proliferation, BCR tyrosine kinases inhibitors (BCR inhibitors) also inhibit other signalling pathways, such as toll-like receptors, which are essential for the control of infections. There are few real-life data on the infectious risk associated to ibrutinib and idelalisib.
Aims: To analyse the incidence of the infections in patients receiving treatment with BCR inhibitors and to identify predisposing factors for the development of infections in these patients.
Methods: Data were collected retrospectively, including patients diagnosed with chronic lymphocytic leukemia (CLL) who have received BCR inhibitors for more than 1 month, in Virgen de las Nieves University Hospital in Granada (Spain), from June 2015 to February 2018. Patients received ibrutinib in monotherapy and/or idelalisib-rituximab, and prophylaxis with aciclovir and trimethoprim/sulphamethoxazole.
Results: 22 patients were treated with BCR inhibitors: 11 with ibrutinib, 6 with idelalisib-rituximab and 5 with both treatments, sequentially. Patient characteristics are shown in Table 1. During the follow-up period, 33 episodes of infections occurred, with 20 of them (60.6%) requiring hospital admission and occurring in 11 patients. The focus and etiology are shown in Graph 1. The mean number of days of treatment prior to the first episode of infection (grade ≥3) was 101 days, 54.5% and 27.3% of them after 3 and 6 months, respectively. We observed a positive correlation between the probability of infection (grade ≥3) and the CIRS score ($p<0.05$), and also an almost statistically significant and positive correlation between the probability of infection and the number of previous treatment lines ($p=0.051$). Age, cytogenetic status, type of BCR inhibitor and the diagnosis-to-treatment time, have were not predictive factors of hospital admission. Pulmonary aspergillosis was diagnosed in 4 patients (18.2%), with a trend for a correlation between the risk for this fungal infection and the CIRS score ($p=0.08$) and the number of previous treatment lines ($p=0.054$). Finally, infection-related global mortality was 27.3% (6 patients) due to pulmonary aspergillosis (2 patients), bloodstream infection by *Enterococcus faecium* (1 patient), varicella-zoster virus (1 patient), flu (1 patient) and respiratory infection without clear etiology (1 patient). The average number of days from the start of treatment to death was 298 days.

Table 1. Clinical characteristics of the patients (average).

Male 54.5%	Age 67 years	CIRS score 8.3	CIRS score 8.3
Treatment with ibrutinib and idelalisib-rituximab 72.7% and 50%	Nº of previous treatment lines 2.1	17p-/TP53 mutated 54.5%	

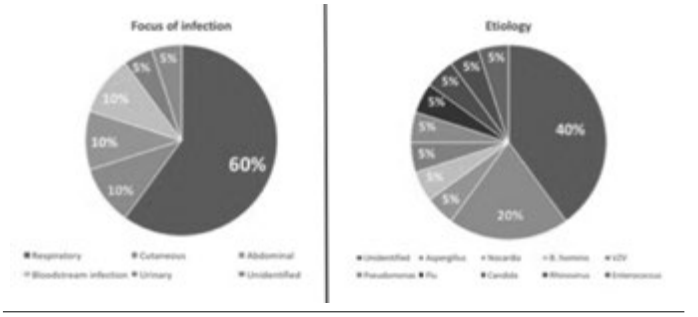


Figure 1.

Summary/Conclusion: Infectious complications were greater than expected, being the main cause of discontinuation. Furthermore, they required hospital admission in more than half of the cases with a considerable mortality. CIRS score and the number of previous treatment lines were predictors of serious infections. Therefore, it seems advisable for patients with multiple treatments and comorbidities not to delay the treatment with BCR inhibitors to avoid infectious complications that may cause treatment discontinuation. With the high incidence of pulmonary aspergillosis in our series, specific strategies for the early detection, treatment and prophylaxis should be adopted. More studies in real-life settings are needed.

PB1879

IMMEDIATE AND LONG-TERM OUTCOMES OF SPLENECTOMY IN PATIENTS WITH NON-HODGKIN LYMPHOMA AND CHRONIC LYMPHOCYTIC LEUKEMIA COMPLICATED BY AUTOIMMUNE HEMOLYTIC ANEMIA

Y. Yevstakhevych[1,*], I. Yevstakhevych[1], Y. Vygovska[1], M. Semerak[1], O. Petronchak[2], V. Loginskiy[1]

[1]SI "Institute of Blood Pathology and Transfusion Medicine NAMS of Ukraine", L'viv, [2]Lviv State Oncology Regional Treatment and Diagnostic Center, L/viv, Ukraine

Background: Autoimmune hemolytic anemia (AIHA) is associated with non-Hodgkin lymphoma (NHL) and with chronic lymphocytic leukemia (CLL) in 2-3% and 5-10% of cases, resp. The most effective treatment for these diseases is chemotherapy with corticosteroid hormones. However, the presence of AIHA in combination with massive splenomegaly may significantly reduce the efficacy of cytostatics. In such cases, splenectomy becomes a reasonable treatment of choice.
Aims: To conduct a retrospective analysis of immediate and long-term outcomes of splenectomy in patients with CLL and NHL, complicated by AIHA.
Methods: 10 patients with NHL and 14 patients with CLL, complicated by AIHA (warm-type) underwent splenectomy.
Results: Splenectomy (SE) proved effective in 12 (85.7%) patients with CLL and AIHA: a big tumor mass was resected, hemolysis was suppressed, hemoglobin level normalized or increased, and platelet count normalized in patients with Rai IV stage. One patient with Rai III stage died of acute adrenal insufficiency three days following operation. As shown by the evaluation of long-term outcomes of SE, median event-free survival (EFS) reached 12.0 months (duration of follow-up was 1-26 months), and median overall survival (OS) reached 25.5 months (duration of follow-up was 2-256 months); there were four (33.3%) patients with a 2-year survival, one (8.3%) patient with a 5-year survival, and two (16.7%) patients with a 10-year survival. Mean post-splenectomy survival in patients with Rai III and IV stage reached 77.6 and 54.5 months, resp. Eight (57.1%) patients were initiated on chemotherapy following 1-14 months after SE, and one patient has been alive for 256 months after surgery with no supportive therapy but complaining of frequent acure respiratory infections. Eight (57.1%) patients with CLL and AIHA died following 2-32 months after SE. Seven patients died due to progression of underlying disease, whereas the relapse of hemolysis was observed just in a single patient. One patient died following 26 months after operation of OPSI-syndrome with a clinical manifestation of severe septic shock. SE was effective in 100% patients with NHL and AIHA: hemolysis was suppressed, hemoglobin level increased; in patients with leukopenia, their leukocyte count normalized, and their abdominal discomfort due to an increased spleen weight disappeared. During the first-year postoperatively, just one patient required cytostatic treatment. As shown by the evaluation of long-term outcomes of SE in patients with NHL and AIHA, EFS reached 29.0 months [12.0-49.0 months], and OS reached 31.0 months [18.1-52.2 months] with a 3-year survival in 45% patients and a 5-year survival in 22% patients. The shortest postoperative survival (3 months) was observed in a patient with nodal marginal zone lymphoma, and the best outcome was achieved in splenic marginal zone lymphoma (SMZL): the patient has been living for 206 months after SE in the condition of a complete clinical remission without any treatment. One patient with SMZL died following 9 months after SE of OPSI-syndrome. Patients with diffuse large B-cell lymphoma (DLBCL, 6 cases) showed a low median of overall survival (26 months), despite of promising immediate outcomes of SE.
Summary/Conclusion: Splenectomy remains to be a leading treatment of choice in the management of chronic lymphoid neoplasias, complicated by AIHA and massive splenomegaly. Long-term outcomes are worse in patients with CLL and Rai IV stage of AIHA. In patients with NHL, complicated by AIHA, the efficacy of operation depends on the type of lymphoma.

PB1880

COPY NUMBER ALTERATIONS DETECTED BY A MULTIPLEX PCR GENOTYPING METHOD AND FISH IN CHRONIC LYMPHOCYTIC LEUKEMIA

L. Fan*, S. Qin, Y. Miao, X. Du, H. Yang, H. Qiu, L. Wang, W. Xu, J. Li

Department of Hematology, the First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital, Nanjing, China

Background: Copy number alteration (CNA) is related with the occurrence and progression of chronic lymphocytic leukemia (CLL) patients. The acquisition of these genomic aberrations is mostly by fluorescence *in situ* hybridization(FISH).
Aims: The purpose of this study was to introduce the AccuCopy method in detecting the CNA in CLL and to compare the usefulness with FISH.
Methods: One hundred chemotherapy-naïve CLL patients were enrolled in this study. Both FISH and AccuCopy were applied on all of them.
Results: AccuCopy was able to identify all del(17p) patients confirmed by FISH and patients with high frequencies of del(11q) (by FISH and patients with high frequencies of del(11q) (40%), respectively). Furthermore, CNA identified 1 sample with a low number of del(17p) cells below the positive cut-off value by FISH, which was accompanied by TP53 mutation.
Summary/Conclusion: Our results suggest that AccuCopy is equally efficient in assessing del(17p) and high frequencies of del(11q) compared to FISH. Besides, we detected 2 deletions and 3 insertions of SETD2 in the patient cohort. Patients carrying SETD2 aberrations showed a higher level of thymidine kinase 1($p<0.001$), C-reactive protein($p<0.001$) and Erythrocyte Sedimentation Rate($p=0.005$). The SETD2 aberrations also correlates with more complexkaryotype(20.0% *vs* 1.8%)($p=0.012$), more MYD88 mutation(33.3% *vs* 2.9%)($p=0.008$) and a higher ZAP70 percentage($p=0.011$). More CNAs need to be validated using this method.

PB1881

IMMUNOLOGICAL RECONSTITUTION AND PLATELET DISFUNCTION DURING IBRUTINIB IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

I. Innocenti[1,*], F. Autore[1], M.A. Alberelli[2], R. Pasquale[3], A. Corbingi[1], B. Di Giacomo[4], S. Bellesi[4], F. Corrente[4], S. Sica[4], E. De Candia[5], L. Laurenti[4]

[1]Department of Hematology, Catholic University of Sacred Heart, Rome/ Institute of Hematology, [2]Departement of Oncology and Hematology, Catholic University of Sacred Heart, Rome, Rome, [3]Department of Hematology, IRCCS Ca' Granda Ospedale Maggiore Policlinico and University of Milan, Milan, [4]Department of Hematology, Catholic University of Sacred Heart, Rome/ Institute of Hematology, [5]Department of Oncology and Hematology, Catholic University of Sacred Heart, Rome, Italy

Background: Chronic Lymphocytic Leukemia (CLL) is characterized by immune disregulation, often including hypogammaglobulinemia, which contributes to a high rate of infections. Ibrutinib (IBR) seems to generate partial reconstitution of normal B cells and humoral immunity, expecially increase of IgA, in CLL. Frequent IBR associated side-effects are bleeding events, usually mild (Common Toxicity Criteria (CTC) grade1-2), rarely severe (grade3-4). A defect of platelet function, namely an inhibition of Btk-and Tec-mediated signaling downstream platelet glycoproteins GPVI and GPIb, has been hypothesized to cause these bleedings.
Aims: To investigate immune reconstitution and to characterize clinical and laboratory features of platelet dysfunction during IBR.
Methods: We report our monocentric retrospective study on immunological recovery and bleeding in CLL patients (pts) treated with IBR. From May 2014 to December 2017 we treated 27 pts with IBR for progressive naïve CLL if pts harboring del17p/TP53 mutation or with relapsed or refractory disease. In these pts before and 1, 3, 6, 9 and 12 months after beginning of therapy immunological reconstitution was investigated by measuring IgG, IgM, IgA levels, CD3 T lymphocytes, CD4 and CD8 T-helper and T-suppressor subset, B lymphocytes CD19+ and NK lymphocytes CD16/56+ by flow cytometry and platelet dysfunction was investigated by light transmission aggregometry using platelet-rich plasma and ADP, PAR1-AP, Collagen, Arachidonic Acid, ristocetin as platelet agonists. At each time point the grade of bleeding was measured by CTC score. No pts received concomitant antiplatelet or anticoagulant therapy.
Results: Immunoglobulin levels did not show any change during the study period. Median values were constantly below the normal range during IBR. Immunological reconstitution showed a rapid increse of CD19+ lymphocytes above the normal range after 1 month of IBR and rapidly decreased to normal values. CD3+ lymphocytes remained into the normal from baseline to 12 months during IBR, the subset CD4+ decreased from months 3 to 12 with median values below the normal range, on the contrary CD8 showed a progressive increase during the same period with median values into the normal range. CD16/56+ were into the normal range during the study period. We recorded CTC grade 1 or 2 bleedings (bruising, petechiae, conjunctival and retinal hemorrhage, rectal bleeding) in 18 pts; no pts needed IBR interruption or dose reduction. All pts displayed severe impairment of collagen induced aggregation during IBR. On the contrary, the aggregation by low-dose ADP significantly improved. The aggregation by PAR1-AP, ristocetin and arachidonic acid was unchanged before and under IBR. In 18 pts the vWF:Ag and RiCo were high at the onset of the disease and reduced up to normal values under IBR.
Summary/Conclusion: There was no improvement of humural immunity in our pts even after 12 months of IBR, after response to therapy. As expected, B-lymphocytes rapidly decreased up to the normal range after 1 month; T-cell compartment remained within normal values, with a trend toward an increase of T-Suppressor and a reduction of T-Helper after 12 months of treatment, which contributes to cellular immune reconstitution. Our study showed minor bleedings in pts treated with IBR. A severe impairment of collagen-induced aggregation was caused by IBR, which was counteracted by amelioration of ADP-induced aggregation. Finally, pts under anticoagulant or antiplatelet treatment might need be carefully monitored by clinical and laboratory evaluation.

PB1882

METHOD COMPARISON OF A NOVEL SLIDE-BASED INTEGRATED HEMATOLOGY ANALYZER AND A FLOW CYTOMETRY-BASED SYSTEM USING SAMPLES WITH TARGETED MEDICAL CONDITIONS

H. Russcher[1,*], T. Khartabil[2], D. Bracco[2], T. Allen[2]

[1]Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands, [2]Roche Diagnostics, Westborough, United States

Background: The **cobas m** 511 integrated hematology analyzer (**cobas m** 511 system) is a novel slide-based system that performs a CBC, WBC differential, reticulocyte count, and nucleated RBC count using digital analysis.
Aims: This single-center study investigated whether the **cobas m** 511 system delivered comparable results to the Sysmex® XN-10 Automated Hematology Analyzer using samples from patients with medical conditions.
Methods: Laboratory hematology results were reviewed to identify subjects with 23 targeted medical conditions (including hematological malignancies and disorders of cell numbers and function). Residual whole blood samples (n=130) were processed on both systems within 8 hours of venipuncture. Consistent with CLSI EP09-A3, a method comparison was used to assess the correlation and bias between the systems for all parameters. Individual patient parameter results that were valid on both instruments were included.
Results: All 26 reportable parameters evaluated showed good-to-excellent correlation between the automated results of the **cobas m** 511 system and Sysmex Analyzer, with no significant bias (**Table 1**).

Table 1. Cobas m 511 *vs* Sysmex Analyzer results.

Parameter [units]	Sample range	Pearson's R	Intercept	Slope
WBC [10^3/µL]	(0.11-95.41)	0.999	-0.02	0.995
RBC [10^6/µL]	(1.79-7.68)	0.996	-0.01	0.992
HGB [g/dL]	(6.28-17.24)	0.995	-0.31	1.064
HCT [%]	(18.60-56.00)	0.982	-0.53	1.034
MCV [fL]	(69.50-107.80)	0.879	5.35	0.975
MCH [pg]	(20.22-36.48)	0.977	2.87	0.946
MCHC [g/dL]	(28.20-36.42)	0.548	14.88	0.559
RDW [%]	(11.50-27.10)	0.929	2.81	0.850
RDW-SD [fL]	(34.10-93.00)	0.908	7.46	0.910
PLT [10^3/µL]	(9.00-1379.00)	0.994	-2.02	0.943
MPV [fL]	(8.40-13.00)	0.843	0.82	0.915
#NRBC [10^3/µL]	(0.00-4.44)	0.980	N/A	N/A
#NEUT [10^3/µL]	(0.46-36.82)	0.999	0.03	1.008
#LYMPH [10^3/µL]	(0.16-4.89)	0.985	0.01	0.976
#MONO [10^3/µL]	(0.14-7.65)	0.991	-0.03	1.013
#EO [10^3/µL]	(0.00-1.23)	0.978	0.00	1.031
#BASO [10^3/µL]	(0.00-2.51)	0.962	-0.08	1.829
#RET [10^6/µL]	(0.00-0.34)	0.971	-0.01	0.982
HGB-RET [pg]	(14.68-42.04)	0.934	-2.75	1.193

Data for%NRBC,%NEUT,%LYMPH,%MONO,%EO,%BASO, and%RET not shown

Summary/Conclusion: The **cobas m** 511 system and Sysmex Analyzer produce comparable results for samples with targeted medical conditions. This demonstrates the robustness of the **cobas m** 511 system when abnormal samples are encountered.

PB1883

ANALYSIS OF PERCENT IDENTITY OF IGHV MUTATION AS PROGNOSTIC FACTOR IN CLL PATIENTS TREATED WITH FLUDARABINE, CYCLOFOSFAMIDE AND RITUXIMAB: A SINGLE CENTRE EXPERIENCE

A. Giordano*, P. Curci, R. Rizzi, M. Delia, T. Perrone, M.A. Urbano, S. Mallano, V. Carluccio, A. Mestice, E. Minervini, F. Albano, G. Specchia

Haematology, University, Bari, Italy

Background: The mutation status of the immunoglobulin heavy chain variable region gene (IGHV) is an established prognostic factor in patients with chronic lymphocytic leukaemia (CLL). The degree of somatic hypermutation, determined as percent sequence identity to germline in IGHV (IGHV%) is analyzed in clinical practice. Currently CLL with<98% IGVH identity are considered "mutated" and CLL patients with >98% IGHV identity are considered "unmutated. " Recent data have assessed the prognostic role of IGHV% as a continuous variable in CLL patients treated with fludarabine, cyclophosphamide and rituximab (FCR).
Aims: In our study we investigated the prognostic significance of absolute percent identity of somatic of IGHV mutation on Progression Free Survival (PFS) and Overall Survival (OS) in unmutated CLL patients (pts) treated with frontline FCR in our Institution.
Methods: We retrospectively evaluated 73 pts with CLL treated with frontline FCR at the University Hospital of Bari (Italy) with a median of 5 years follow-up. The mutational status of the IGHV was studied in all pts and the degree of somatic hypermutation of IGHV was determined as percent identity from the germline sequence. Pts were divided in mutated and unmutated using a cut-off of 98% sequence identity. Among unmutated pts (identity sequence >98%) we selected two groups: the first one between 98% and 99% (IGHV-98-99%) and the second one with identity range between 99% and 100% (IGHV-99-100%), respectively. PFS and OS were calculate and compared between the two groups.
Results: Among the 73 CLL pts treated with frontline FCR 48 pts (65%) with unmutated IGHV CLL were identified; among them, 20 pts (41%) and 28 (59%) belonged to IGHV 98-99% and IGHV 99-100% group, respectively. No significant differences were observed (p=ns) in terms of PFS and OS between the two groups.
Summary/Conclusion: In our study no difference in terms of survival was observed in unmutated IGHV CLL pts on the basis of the percent identity of IGHV mutation for distinguishing two classes of risk. Further studies and more consistent cohorts of pts are warranted to confirm these data.

PB1884

DOES THE DOSE MODIFICATION OF FLUDARABINE, CYCLOPHOSPHAMIDE, RITUXIMAB (FCR) IMPACT TREATMENT OUTCOME IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA? ANSWER BASED ON SINGLE CENTER "REAL-LIFE" DATA

V. Vukovic[1,*], D. Antic[1,2], V. Djurasinovic[1,2], Z. Bukumiric[3], M. Dencic-Fekete[1], T. Dragovic-Ivancevic[1], M. Todorovic-Balint[1,2], J. Bila[1,2], A. Sretenovic[1], M. Smiljanic[1], J. Jelicic[1], B. Mihaljevic[1,2]

[1]Clinic for Hematology, Clinical Center of Serbia, [2]Medical Faculty, University of Belgrade, Belgrade, Serbia, [3]Institute for Medical Statistics and Informatics, Medical Faculty, University of Belgrade, Belgrade, Serbia

Background: There are little published …real life" data about fludarabine, cyclophosphamide, and rituximab (FCR) combination in patients with chronic lymphocytic leukemia (CLL).
Aims: Herein, we present single-center experience based on long-term follow up of 170 CLL pts treated with FCR with focus on side effects and their impact on the outcome.
Methods: In this retrospective study, we analyzed data from 170 pts with CLL treated with FCR, mostly as a first-line treatment pointing at the correlation between side effects frequency, dosage, and treatment outcome.
Results: Median follow-up was 49 months (range, 2-180). Male/female ratio was 2.8:1 and median age before treatment commencement was 61 year, while 72% of pts were younger than 65 years. Unfavourable cytogenetic profile (del17p and/or del11q) carried 25% of patients. Most of the pts (72%) received FCR as a first-line treatment. Thirty-five percent of patients had a dose reduction of FC from the beginning (82%) or during the treatment (18%), most of them (87%) of >25% of the expected full dose. More than a half dose reductions were due to decreased creatinine clearance, in 18% the reason was neutropenia and/or infections, 13% physician's decision and 15% for other reasons. A hundred and ten (67%) pts completed their treatment with 6 FCR cycles, but only 72 (42%) pts received 6 cycles of full-dose FCR. Fifty-six percent of pts at least once received granulocyte colony-stimulating factor (GCSF) for neutropenia grade 3 or 4, 45% of pts had at least one episode of prolonged neutropenia, 16% exhibited late-onset neutropenia, while 28% of pts had infection that caused treatment delay and/or interruption, and/or hospitalization. Neutropenia occurrence was not related to sex, age, comorbidity status, leukocyte count, or cytogenetic profile, but it was significantly more frequent in pts who had already been treated with some chemotherapy regimen (p= 0.035). It occurred significantly more often in pts who did not complete their treatment with 6 cycles (67% *vs* 50%; p=0.046). Treatment delay was observed in almost half of pts (48%), mostly due to severe neutropenia and infections, and in 90% of pts, it happened for the first time after some of the first 4 cycles. Thirty-four percent of pts experienced treatment discontinuation, 60% of them due to cytopenia(s) and/or infections and 22% due to the resistant or progressive disease. The overall response rate was 70,6%, equally split between complete (CR) and partial response (PR). When observing untreated pts, CR was achieved in 46,6% in contrary to 10,9% of previously treated pts (p<0.001). In our group, cytogenetic profile predicted treatment response only in treatment-naïve pts (p<0.001 *vs* p=0.376 for previously treated). Pts who received 6 cycles of full-dose FCR experienced significantly better treatment response (p<0.001). Treatment delay had not been shown to change the outcome when we observed the whole group and pts with

favorable cytogenetic, but when the group was further restricted on pts received full-dose 6 FCR cycles, the adverse impact of treatment delay on outcome was statistically significant (p=0.024). Treatment interruption significantly decreased CR rate (12% *vs* 46%; p<0.001).
Summary/Conclusion: In "real-life" setting treatment with FCR protocol is severely compromised with myelosuppression and infective complications which can considerably influence its therapeutic effect. Further studies are warranted to define risk-factors for myelosuppression after FCR.

PB1885

SAFETY AND EFFICACY OF BENDAMUSTINE AND RITUXIMAB COMBINATION CHEMOTHERAPY AS FIRST LINE TREATMENT IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA: A SINGLE CENTRE EXPERIENCE FROM NORTH INDIA

A. Kumar Tripathi[1,*], S. Verma[1], R. Kushwaha[2], N. Kumar[1], S. Kumar[1], S. Srivastawa[1], V. Singh[3]

[1]Clinical haematology, [2]Pathology, [3]Community Medicine, K.G. Medical University Lucknow India, Lucknow, India

Background: Chronic Lymphocytic Leukemia (CLL) is one of the most common chronic lymphoproliferative disorder (CLPD) in India and the most common CLPD in the west. Treatment of CLL has changed over 2 decades from single agent alkylating compound to combination chemo like Fludarabine, Cyclophosphamide, and Rituximab (FCR) or, Bendamustine and Rituximab based treatment depending on comorbidities and diseases cytogenetic risk. BR chemotherapy has shown impressive results in CLL patient in many phase 2 studies. FCR regimen has many side effects including severe immunosuppression and profound cytopenias. BR has been proven to be better than FCR in CLL10 trial especially in elderly patients with comorbidities. We analysed our "real world" response data of B+R chemotherapy in Indian patients with CLL outside a clinical trial.
Aims: The aim of this study was to analyse safety and efficacy of Bendamustine and Rituximab (B+R) chemotherapy in patients with previously untreated CLL
Methods: We retrospectively analysed the departmental data of all CLL patients who received B+R as the first line treatment. Thirty six patients receiving at least IV cycles of B+R chemo between December 2012 to September 2017 were included in the study. Bendamustine was administered at a dose of 90 mg/m2 on days 1 and 2 combined with 375mg/m2 Rituximab on day 1 in a 28 days cycle. Baseline IGHV mutational status and FISH analysis was not done and MRD was not assessed. Responses were analysed at end of IV-VI cycles of chemo by clinical examination and haematological parameters. Response rates were defined based on IWGCLL response criteria for CR, PR and stable disease. Progression free survival and Overall survival was analysed using Kaplan Meier survival analysis.
Results: Thirty six patients were included in the study with the median age of 55 years (34-75 years). Only 3 patients (8.3%) were above 70 years of age. Most patients belonged to Rai stage III /IV (80%) and Binet C (80%) stage. Mean Hb was 104g/L (26-154), mean total WBC count 144x10^9/L (66-390) and platelet count 50x10^9/L (30-200).Median number of chemotherapy cycles administered were 6 (range 4-12). Overall response rate was 100% with CR and PR rates of 41% & 59% at the end of 4-6 cycles of chemotherapy. Major side effects were cytopenias of any grade in 56% (grade 3 in 25%), febrile neutropenia in 16% and skin toxicities 36% (11% having grade 3 skin toxicity). At the median observation period of 24 month median PFS was 40 months and 70% of patients were surviving. Most common cause of death was progressive disease in 10 patients (27%) and febrile neutropenia in 2 patients (5.5%).
Summary/Conclusion: B+R chemotherapy is well tolerated by previously untreated Indian patients of CLL with impressive response rates, long progression free survival, and manageable side effects in a real world scenario outside a clinical trial.

PB1886

IBRUTINIB IN THE TREATMENT OF EARLY RELAPSE OF CHRONIC LYMPHOCYTIC LEUKEMIA

N. Timofeeva*, E. Stadnik, V. Strugov, A. Zaritskey

Almazov National Medical Research Centre, Saint-Petersburg, Russian Federation

Background: Despite the success of CLL treatment with fludarabine and bendamustine-containing regimens, about 25% of patients had relapsed within the first 24 monthsafter the end of the first-line therapy.In this group of patients PFS after standard "salvage therapy" is 6-18 months,overall survival is extremely low-13-47 months.???The lack of effective approaches to second-line therapy for patients with earlyrelapses of CLL has led to the application of new agent in clinical practice -ibrutinib. Despite the success of CLL treatment with fludarabine and bendamustine-containing regimens, about 25% of patients had relapsed within the first 24 monthsafter the end of the first-line therapy. In this group of patients PFS after standard "salvage therapy" is 6-18 months, overall survival is extremely low-13-47 months. The lack of effective approaches to second-line therapy for patients with earlyrelapses of CLL has led to the application of new agent in clinical practice-ibrutinib.
Aims: To estimate ibrutinib efficacy in the treatment of early CLL relapses and in patients with ≥2 lines of preceding therapy. Analysis of treatment results in patients with del(17p) and monitoring of minimal residual disease (MRD) and ibrutinib safety profile.
Methods: The analysis included the results of ibrutinib treatment in 31 patients with CLL. Twenty eight patients were treated by bendamustine and fludarabine containing regimens. The median prior treatment lines were 2 (range 1–10). The indications for the treatment initiation were the fi rst early relapse in 51% of cases (n=16) and a relapse after 2 and more lines of therapy in 49% of cases (n=15). Ibrutinib was administered in mono- (n=15) and combined therapy (n=14) as well as in the R-BAC scheme (n=2). Using FISH analysis del(17p) was found in 9 patients (34%).
Results: Within the median follow up of 18 months (range 7-42+) the overall survival (OS) rate was reported to be 87%, and the progression-free survival (PFS) rate was 7%. The maximum MRD after a year of ibrutinib treatment was observed in case of combination with immunochemotherapy (e.g., R-BAC). Within the period of 18 months OS rate was 100%, in the patient group with early relapses and 66% in the group with a relapse after 2 and more therapy lines (p=0.02). Within the same examination period PFS was signifi cantly higher (94%) in the patient group with early relapses compared to the previously treated patients (60%) (p=0.034). The most common adverse events were grade 1–2 purpura (30%), grade 1–2 diarrhea (10%), atrial fi brillation paroxysms (10%) and arterial hypertension (10%). Severe infectious complications registered in 6% (n=3) patients were successfully solved in the course of combined antibacterial and antimycotic treatment.

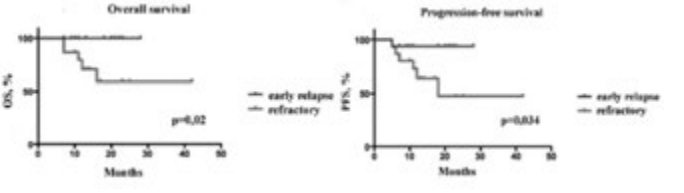


Figure 1.

Summary/Conclusion: Ibrutinib was shown to be effective drug for treatment of relapsed CLL. The OS and PFS values were more favourable in patients with early relapses compared to the patients with relapses after ≥2 lines of therapy prior to ibrutinib treatment. The maximum elimination of the tumor clone was observed after combined ibrutinib/immunochemotherapy treatment. The tolerance of ibrutinib was reported to be satisfactory with acceptable toxicity profile. No mortality due to infection complications was observed.

PB1887

COMPARISON OF CLINICAL FEATURES AND SURVIVAL BETWEEN YOUNG AND OLD CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS: A SINGLE CENTRE STUDY FROM TURKEY

R. Eren[1,*], A. Karışmaz[1], D. Karataş[2], M.H. Doğu[1], S. Altındal[1], O. Yokuş[1], E. Suyanı[1]

[1]Hematology, Univeristy of Health Sciences, İstanbul Training and Research Hospital, [2]İnternal Medicine, Univeristy of Health Sciences, Bağcılar Training and Research Hospital, İstanbul, Turkey

Background: The average age of the patients at the time of diagnosis is 72 and the incidence of CLL increases with age. Comorbidities, which increase by age, including coronary artery disease, heart failure, diabetes mellitus, chronic obstructive pulmonary disease and cognitive geriatric syndromes influence the performance status of the elderly patients leading to negative impact on treatment continuation and survival.
Aims: We aimed to investigate the clinical characteristics, the rate of treatment demand, time to treatment and mortality rates differ between c CLL patients<65 years and ≥65 years, which is the cut-off age for the definition of elderly by World Health Organization

Methods: A hundred and thirty-seven patients who were diagnosed and followed with the diagnosis of CLL were included in this retrospective analysis. The patients were diagnosed according to International Workshop on Chronic Lymphocytic Leukemia (iwCLL) criteria and Rai staging system was used for disease-staging. Patients were divided into two groups based on the World Health Organization (WHO) definition of elderly, which starts at age 65. Clinical characteristics, treatment demand, time to treatment and mortality rates of the two groups were compared. In a subgroup analysis, patients receiving treatment were also analysed according to age.
Results: There were 63 (46%) patients aged<65 years and 74 (54%) patients ≥65 years. Two groups were comparable in term gender, Rai stage, WBC, ALC, Hgb level, Plt count, presence of splenomegaly, lymphadenopathy, Del 13q14, Del 11q22, Del 17p and Trisomy 12, time to treatment and treatment demand (p>0.05). Even though the difference was statistically insignificant, 28.6% (18/63) of the patients<65 required treatment, while this rate was 44.6% (33/74) for patients ≥65. We also analysed the patients, who received treatment, according to the age groups When we divided patients who received treatment into two groups as<65 years and ≥65 years the two groups were similarly comparable in terms of gender, age, WBC, ALC, Hgb, plt, Rai stage, presence of splenomegaly, lymphadenopathy, Del 13q14, Del 11q22, Del 17p and Trisomy 12, and time to treatment (p>0,05)
Summary/Conclusion: We found no difference in CLL patients who are younger and older than 65 in terms of clinical characteristics, disease stage, time to treatment and treatment demand. Mortality rates were higher in older patients with the upshot of comorbid diseases. The major limitations of our study were the limited number of patients and conducting with retrospective chart review.

PB1888

SAFETY PROFILE OF IBRUTINIB: A RETROSPECTIVE ANALYSIS OF 31 PATIENTS

J. Uriarte*, S. Herraez, C. Barrenetxea, A. Blanco, J.A. Marquez, Z. Díez Gallarreta, I. Leal, C. Alonso, L. Hernandez, F. Marco

Hematology, Hospital Universitario Basurto, Bilbao, Spain

Background: Ibrutinib is an irreversible molecular inhibitor of Bruton's tyrosine kinase (ITK), which has altered the treatment paradigm for hematological diseases such as Chronic Lymphocytic Leukemia (CLL), Mantle Cell Lymphoma (MCL), and Waldeström's Macroglobulinemia (WM). Due to its recent incorporation, long-term safety profile has not yet been defined, although the most frequently reported adverse effects are the following: diarrhea, neutropenia, mild bleeding, exanthema and musculoskeletal pain.
Aims: Our aim is to analyze Ibrutinib's adverse effects in our group of patients, paying special attention to cardiological, infectious and hemorrhagic ones.
Methods: It is a descriptive, observational and retrospective study on the use of Ibrutinib in our hospital between February 2016 and February 2018. We analyzed patient's profile (age, sex, previous cardiological disease and anticoagulant and antiplatelet therapy, hematological diagnosis, and previous treatment lines), duration of Ibrutinib-based therapy and level of response achieved, adverse effects, treatment interruptions and dose reduction (if needed).

Table 1.

Adverse effects		Management
No adverse effects	18	
Cardiac toxicity		
Isolated atrial fibrillation de novo	1	Temporary treatment discontinuation (TTD)
Atrial fibrillation + Congestive heart failure	2	TTD, admission, ASA TTD
Atypical chest pain	1	Definitive treatment
Congestive heart failure worsening	1	discontinuation (DTD)
Infectious toxicity		
Mild respiratory (not documented)	1	TTD, admission DTD, admission
Pneumocytis jirovecii pneumonia	1	No therapy modifications (NTM)
Mild testicular infection	1	
Other toxicities		
HTA impairment	1	NTM
Diarrhea	2	NTM
Muscle cramps	1	NTM
Peripheral edema	1	Dose reduction

Results: Thirty-one patients were treated with Ibrutinib, of which 74% were male and 26% female, with an average age of 70.5 years (between 48-84). Of the total, four had previous heart disease: Atrial Fibrillation (2), ischemic heart disease (1), and aortic valve insufficiency (1); three were being treated with acetylsalicylic acid (ASA), one with ASA and Clopidogrel, and two with oral anticoagulants (Acenocoumarol/Apixaban). ASA was suspended on the first three cases and Clopidogrel on the fourth one. Treatment indications were the following: CLL (17), MCL (7), WM (4), B-Cell Prolymphocytic Leukemia (2) and Diffuse Large B Cell Lymphoma (1). Of the 31 patients, 8 received Ibrutinib as first-line treatment, 17 as second-line, 4 as third-line and 2 as fourth-line. The average duration of treatment was 7 months (between 0.25-21.5). Inicial dose had to be reduced in one patient due to mild hepatic failure (Child-Pugh A). In terms of response level, 10 achieved complete response, 4 partial response (of which one later progressed), 7 progressed during treatment (4 MCL -as second line-, 3 CLL -as second line-, and 1 B Cell Prolymphocytic Leukemia -as first line-). In the 9 remaining patients, the level of response has not yet been evaluated since they have recently started therapy with Ibrutinib. Adverse effects observed during the treatment period are detailed in the chart (see attached file 1).
Summary/Conclusion: Based on our experience, the use of Ibrutinib has an acceptable toxicity profile, with adverse cardiac effects being the most frequent and serious. For this reason, we consider fundamental previous cardiological assessment of the patients and their close monitoring during the treatment. However, it seems important to note that the majority of adverse effects have occurred in patients in second and third lines of treatment.

PB1889

ASSOCIATION OF INITIAL PROGNOSTIC PARAMETERS AND REQUIREMENT FOR TREATMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA

A. Senturk Yikilmaz[1,*], S.M. Bakanay[1], S. Akinci[2], M. Gündüz[2], S. Küçükyurt Kaya[1], S. Doğan[1], M. Falay[3], I. Dilek[1]

[1]Department of Hematology, Yıldırım Beyazıt University, [2]Department of Hematology, Atatürk Education and Research Hospital, [3]Department of Hematology, Numune Education and Research Hospital, Ankara, Turkey

Background: B-cell chronic lymphocytic leukemia (CLL) is the most common haematological malignancy in advanced age. The clinical course of the disease is highly variable, therefore there is a need to investigate the various prognostic factors. The CLL cell typically expresses CD5, CD19, CD23 and a monoclonal surface Ig (K or λ) while CD20 is moderately/weakly expressed.
Aims: We aimed to analyze the clinical, genetic and immunophenotypic features which might have prognostic value in CLL.
Methods: Between February 2010 and June 2018, 87 cases diagnosed with CLL were retrospectively analyzed. Patients who were followed without treatment (T0 group) and who required at least one line treatment (T) were compared. Patients who required 1 line treatment (T1) were also further compared with patients who required >1 line treatment (T2). Statistical analyzes were performed using chi-square test using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). A value of less than 0.05 was considered significant.
Results: The mean age of our patient population was 65 (±SD12,8) with Male/Female 56/31. At diagnosis, 68(78.2%) patients were at early stage (0, I, II) and 19 (21.8%) were at advanced stage (III,IV). Del17p, del13q and trisomy 12 were evaluated in 49, 41 and 31 patients and 5, 13 and 4 patients were found out to be positive, respectively. Anemia and thrombocytopenia were present in 25 (28.7%) and 16 (18.4%) patients, respectively. Twenty one (24.1%) patients had B symptoms. Splenomegaly, lymphadenopathy and hepatomegaly were present in 34(39%), 67(77%) and 21(24.1%) patients, respectively. Four of the 15 patients who had direct coombs positivity also had clinical evidence of hemolytic anemia. Four patients had immune thrombocytopenia, 2 of them had concurrent direct coombs positivity and one also had hemolytic anemia. The median Hb, leukocyte and platelet counts were 13.2 gr/dl (4.4-17.5 gr/dl), 23.6x10^9/L (1,7-527x10^9/L) and 200x10^9/L (10-345 10^9/L), respectively. Follow up period was median 38 months (3-180 months). Twenty five (28.7%) patients were treated at the time of diagnosis. Thirty three (37.9%) patients had T1 and 6 (6.9%) patients required T2. The ratio of male patients in group T were significantly higher than female patients (p=0.038). All patients in group T2 were male. More patients in group T had CD38 expression than group T0 (p=0.04). There was no significant difference between the groups in terms of FMC7 and CD11c expressions. Of the 5 patients with del17p, 2 patients required treatment at diagnosis, 2 patients required treatment after 13 and 48 months of follow up, respectively.
Summary/Conclusion: In our CLL patients, requirement for treatment was associated with CD38 expression, del17p positivity at diagnosis and male gender.

PB1890

CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL): AN AUDIT OF COMPLIANCE WITH KEY ASPECTS OF BRITISH SOCIETY FOR HAEMATOLOGY (BSH) GUIDELINES ON DIAGNOSTIC WORK UP

D.S.R. Vohra*, D.H. Patel

Haematology, Royal Albert Edward Infirmary(Wrightington, Wigan and Leigh NHS Trust), Wigan, United Kingdom

Background: Chronic lymphocytic leukaemia (CLL) is a malignant clonal disorder of B lymphocytes with levels greater than $5x10^9$/L ($5x10^3$/microliter) involving the bone marrow and peripheral blood. The incidence of CLL increases with age. Upon diagnosis, British Society for Haematology(BSH) recommends that all patients should have specific tests such as full blood count, reticulocyte count, direct antiglobulin test (DAT), immunophenotype, and serum immunoglobulins. In addition to this, screening for hepatitis B and C, TP53 deletion and a baseline CT scan should be done prior to treatment.
Aims: The aim of the audit is to assess the compliance with key aspects of BSH guidelines on diagnostic work up for CLL.
Methods: Electronic records for 32 patients diagnosed with CLL between January 2016 and May 2017 in Royal Albert Edward Infirmary (England) were analysed.
Results: The demographics show a higher proportion of males 59% (19 of 32 patients) compared to females 41% (13 of 32 patients). Similar studies show a male-to-female ratio of 2:1 which is comparable to our results. The mean age at diagnosis was 72 years. 3 patients (9.4%) had haemoglobin levels below 100g/l of which no patients had a reticulocyte count and direct antiglobulin test done. All 32 patients had an immunophenotype typical of CLL. 8 patients (25%) patients did not have their serum immunoglobulins checked. 3 of 32 patients (9%) received treatment with FCR (Fludarabine, Cyclophosphamide, Rituximab) as first line therapy. Before treatment, all 3 patients had screening for hepatitis B and C, TP53 deletion and baseline CT scan.
Summary/Conclusion: We suggest that patients with low haemoglobin levels should have a direct antiglobulin test and reticulocyte count performed. All patients should have their serum immunoglobulins checked. A diagnostic work up and pre-treatment checklist should be designed to guide specialist teams.

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PB1891

SALIVARY PROTEOMIC PROFILE OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA DISTINGUISHES TKIS RESPONDERS FROM NON-RESPONDERS

F. Perutelli[1,*], A. Cecchettini[2], E. Polizzi[3], S. Grassi[1,4], L. Mattii[5], F. Guerrini[1], E. Ciabatti[1], A. Di Paolo[6], M. Petrini[1], S. Galimberti[1]

[1]Clinical and Experimental Medicine, Hematology, [2]Clinical and Experimental Medicine, Biology, [3]Surgical, Medical, Molecular and Critical Pathology, Biotechnologies, University of Pisa, Pisa, [4]Medical Biotechnologies, University of Siena, Siena, [5]Clinical and Experimental Medicine, Histology, [6]Clinical and Experimental Medicine, Pharmacology, University of Pisa, Pisa, Italy

Background: Today the gold standard analyses to characterize patients affected by chronic myeloid leukemia (CML) require bone marrow samples, however peripheral blood may be used to monitor the molecular response during tyrosine kinase inhibitors (TKIs) treatment. Recent studies have shown that saliva could be an alternative substrate for biomarker detection in many diseases. To date, there are no preemptive studies that investigate the salivary proteomic profile to identify non-responders to TKI therapy in CML.
Aims: The aim is to identify putative response biomarkers in the saliva of CML patients.
Methods: We investigated the salivary expression profile of 176 proteins at two different time points in 2 groups of patients with CML. The first group was represented by 4 patients in stable deep molecular response during TKIs treatment, while the second one included 4 sex and age-matched TKI-failure cases. Analyses were performed at baseline and after 6 months of treatment with TKIs. Proteomic analysis was performed by using albumin- and IgG-depleted saliva samples through a nano-HPLC system coupled with a TripleTOF™ 5600 mass spectrometer. Statistical comparative analysis was performed using PeakView™ Software with SWATH™ Acquisition MicroApp 2.0 and MarkerView™.
Results: Overall, 64 proteins resulted differently expressed in resistant *versus* sensitive cases. As shown in the image, myeloperoxidase (PERM), thymosine beta-4 (TYB4), matrix metalloproteinase-9 (MMP9), peroxiredoxin-2 (PRDX2), catalase (CATA) and macrophage migration inhibitory factor (MIF) were down-regulated, while MMP-9-inhibitor (TIMP1), SPARC-like protein-1 (SPRL1), transgelin-2 (TAGL2), leucocyte elastase inhibitor (ILEU), carbonic anhydrase-6 (CAH6), kallikrein-1 (KLK1) and -11 (KLK11), cadherin-1 (CADH1) and beta-2-microglobulin (B2MG) were over-expressed in resistant patients compared to sensitive ones.

Figure 1.

Summary/Conclusion: Our results show that in resistant patients, proteins implicated in hypoxia of the niche are over-expressed. This may be implied in the induction of resistance to TKIs because of the lower BCR-ABL1 expression. In details, these proteins are involved in differentiation of the osteoblasts (PRDX2, MIF), which are main cellular components of the niche; in myeloid differentiation (TYB4, ILEU, PERM), which is a sign of response to TKIs therapy; in fibrosis of the niche (MMP-9, TIMP1), thus preventing the oxygenation of the leukemic stem cell (LSC) and consequently its protein expression; in tumor-induced angiogenesis (MIF, TAGL2); in LSC quiescence (CATA); in components of the niche microenviroment (SPRL1, CAH6); in

factors that support the adhesion of LSC to the stroma (TAGL2, CADH1). In addition, some of these proteins have been already described as prognostic factors in solid tumors (MIF, KLK1, KLK11) and leukemias (B2MG, MIF, CADH1), while CADH1 seems to be correlated to a better response to imatinib in CML patients. Our data show that proteins traditionally identified in peripheral blood only, can be detected also in the saliva, thus indicating that use of this compartment for characterization of CML patients may be feasible. Moreover, the salivary expression profile in resistant *versus* sensitive cases is significantly different and coherent with their response to TKIs treatment, thus suggesting salivary proteomic profile as a possible method to identify the occurrence of resistance to TKIs. However, further studies are needed to indicate saliva as a valid alternative to peripheral blood and bone marrow to monitor response to TKIs treatment in CML patients.

PB1892

ASSOCIATION OF PLK1 AND AURORA KINASE INHIBITORS WITH WEE1 INHIBITORS: A NOVEL THERAPEUTIC APPROACH FOR BLAST CRISIS CML

M. Mancini[1,*], S. De Santis[2], C. Monaldi[2], F. Castagnetti[2], L. Bavaro[2], M. Martelli[2], G. Gugliotta[2], G. Rosti[2], E. Tenti[2], M.A. Santucci[2], M. Cavo[2], G. Martinelli[3], S. Soverini[2]

[1]Department of Experimental Diagnostic and Specialty Medicine-DIMES, Department of Experimental Diagnostic and Specialty Medicine-DIMES, University of BolognaInstitute of Hematology "L. e A. Seràgnoli", [2]Department of Experimental Diagnostic and Specialty Medicine-DIMES, Istituto di Ematologia Seràgnoli-DIMES, Bologna, [3]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (I.R.S.T.), Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (I.R.S.T.), Meldola, Italy

Background: Polo-like kinases (PLKs) and Aurora kinases (AKs) act as key cell cycle regulators in healthy human cells. In cancer, these protein kinases are often overexpressed and deregulated, thus contributing to uncontrolled cell proliferation, growth and consequent genomic instability. Chronic myeloid leukemia (CML) is a myeloproliferative disorder of hematopoietic stem cell. Despite the striking success of tyrosine kinase inhibitor (TKI) therapy, a small but significant proportion of CML patients may still progress from the chronic phase (CP) to the accelerated phase (AP)and eventually to the blast crisis (BC). This is likely due to cooperating molecular events additional to the initial t(9;22) translocation. Even in the TKi era, CML patients who progress to BC have a poor outcome, hence the urgent need to identify novel therapeutic strategies, targeting alternative signaling pathways important for leukemic cell proliferation are required.
Aims: In this study, a new therapeutic strategy based on AKA or Plk1 inhibition with PHA-739358 (Danusertib) or BI6727 (Volasertib), associated with Wee1 inhibition with AZD1775 was evaluated in K562 cells sensitive (K562-S) and resistant (K562-R) to imatinib and in primary cells from CML patients in BC.
Methods: Protein expression and activation was assessed by Western Blotting. Apoptotic cell death was quantified by annexin V/propidium iodide staining and flow cytometry. Cell cycle progression were evaluated by flow cytometry. Drug cytotoxicity in *ex vivo* experiments was evaluated in clonogenic assays in 1 healthy donor and in 3 CML patients in BC.
Results: Both Danusertib and Volasertib (0.5 μM for 24h) showed cytostatic and cytotoxic effects in CML cells by inducing G2/M-phase arrest and apoptosis. Moreover, they caused a dose- and time-dependent reduction of the G0/G1 cell fraction and an increase of the G2/M fraction. Cell cycle arrest was associated with increased levels of phospho (p)-Chk1 and p-Chk2, p-cyclin B1, p-cdc2 and p-Wee1. Using a Wee1 inhibitor (AZD1775) after 24h treatment with Danusertib and Volasertib 0.5 μM, when cells were arrested in G2 phase and Wee1 was overexpressed and hyper-activated, resulted in a synergistic inhibition of cell viability in both K562-S and -R. AZD1775 combined with either Danusertib or Volasertib caused a time-dependent increase of annexin-V-positive cells by activating the mitochondrial apoptotic pathway as reflected by an increment of Bax expression and induction of the cleavage of caspase-3, -9 and PARP. Moreover, both drug combinations induced a significant increase of the DNA double-strand break marker γH2AX, suggesting that Wee1 inhibition promotes mitosis and propagates genomic instability by forcing the cells through successive replication cycles, ultimately resulting in apoptosis from mitotic catastrophe. Finally, clonogenic assays performed by using CD34+ progenitors from three BC CML patients, showed that PLK1 or AKs inhibition, associated with Wee1 inhibition, reduce the clonogenic activity of the CD34+ compartment in a synergistic way compared to the same drugs administrated alone.
Summary/Conclusion: Taken together, our findings indicate that PLK1 and AKs inhibitors associated with WEE1 inhibition display the potential for being further explored in innovative clinical trials aimed to improve the outcomes of patients with CML in blast crisis, or resistant to multiple lines of TKI therapy.

PB1893

ALTERED EXPRESSION OF JAK-STAT PATHWAY AS POSSIBLE PREDICTIVE MARKER IN PATIENTS AFFECTED BY CHRONIC MYELOID LEUKEMIA RECEIVING TYROSIN KINASE INHIBITORS (TKIS)

S. Grassi[1,*], S. Palumbo[2], V. Mariotti[2], E. Ciabatti[3], F. Guerrini[4], F. Perutelli[4], C. Baratè[3], M. Petrini[4], S. Pellegrini[2], S. Galimberti[4]

[1]Medical Biotechnologies, University of Siena, Siena, [2]Clinical and Experimental Medicine, Clinical Biochemistry and Molecular Biology, University of Pisa, [3]Clinical and Experimental Medicine, Hematology, AOUP, [4]Clinical and Experimental Medicine, Hematology, University of Pisa, Pisa, Italy

Background: The JAK-STAT pathway is involved in the transduction of signals mediated by cytokines, interferons and growth factors with consequent support of neoplastic cell growth and invasion in many types of cancer. Additional implications in inflammation and immunity in the tumor microenvironment have been recently recognized, because this pathway seems to sustain the stem cell maintenance. Moreover, persistent STAT3 activation would confer resistance to therapy with tyrosine kinase inhibitors in chronic myeloid leukemia (CML) by controlling the leukemia stem cell (LSC) self-renewal and favoring its hiding in the bone marrow niche (Groner, 2017). Inhibition of this pathway might represent a potential way to ameliorate the molecular response in warning or failed CML patients or to sustain deep responses in cases tempting the discontinuation of therapy.
Aims: Our purpose was the evaluation of some BCR/ABL1-independent molecular predictive markers of response in CML patients. Thus, we analyzed the expression of 86 genes belonging to the JAK-STAT pathway in 10 cases assessed at diagnosis and after 6 months of therapy with TKIs.

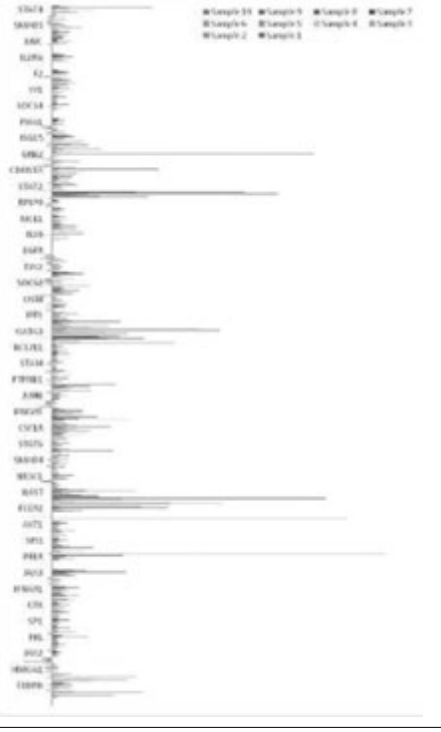


Figure 1.

Methods: 10 patients received TKIs as first-line treatment (7 imatinib, 2 nilotinib, 1 dasatinib). Concomitantly to the BCR/ABL1 transcript, we quantitated the expression level of 86 JAK-STAT genes by RT-qPCR (PrimePCR SYBR® Green assay, Biorad©, Milan, Italy) at baseline and after 6 months of therapy. Expression values were calculated by the Vandesompele method using four housekeeping genes.
Results: According to European Leukemia Network guidelines, after six months of treatment 9 patients were in optimal response and 1 was in failure, and the gene expression analysis showed a relevant deregulation of the pathway. Indeed, 79 genes resulted up-regulated, while only 7 were down-

expressed. To evaluate a possible clinical role of the JAK-STAT pathway, we correlated the gene expression results with the achievement of MR3. At 6 months of treatment, we identified correlation of MR3 with up-regulation of LRG1 (p=0.030), a gene belonging to the *leucine-rich repeat* (LRR) family that is overexpressed during the granulocyte differentiation, and down-regulation of IL2RA (p=0.030) and MPL (p=0.029). IL2RA is involved in the LSC growth and MPL is linked to persistent activation of JAK-STAT. Moreover, the up-regulation in responsive patients also involved the immunity signaling linked to interferon (IFN) receptors complex, and induced interferon-related factors with anti-proliferative and pro-apoptotic effects, such as CSF1R, IRF1, IRF9, and ISG15. The increased expression of GATA3, SOCS3, JAK3 is involved in NK and T cell recruitment as immunology protection in responsive patients. Finally, in responsive cases we observed a significant up-regulation of: 1) OSM, involved in bone remodelling and reduction of fibrosis damaging the LSC survival in the niche and 2) IFN receptor type 1 that is usually expressed in stromal cells as a protection factor against cancer progression.

Summary/Conclusion: In this work, we demonstrated that the JAK-STAT pathway is really implicated in the resistance to TKIs and, on the other hand, that the de-regulation of some genes of this family might be related to the achievement of better molecular responses. This observation could have a practical clinical output, suggesting the effectiveness of the combination of JAK-STAT inhibitors (ruxolitinib or methotrexate) with TKIs in resistant CML patients.

PB1894

BCR-ABL EXON 7 DELETION CAUSES TKI RESISTANCE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

I. Mikhailov*

Faculty of fundamental medicine, Lomonossov Moscow State University, Moscow, Russian Federation

Background: One of the most controversial causes of TKI resistance in patients with CML is the pathogenic BCR- ABL del. c.1086-1270 transcript. This transcript was first described in 2008 in TKI resistant patients (Curvo *et al*., 2008). BCR-ABL del. c.1086-1270 transcript encodes truncated fusion protein BCR-ABL p.R362fs*21 which is an alternative splicing product. It has been shown that such truncated protein is unlikely to have tyrosine kinase activity because of the disfunction of the ATP-binding site (Meggyesi *et al*., 2012). However, we suggest that pathogenic effect of BCR-ABL p.R362fs*21 expression is possible due to the dimerization with a typical protein BCR-ABL p210. Such mechanism has already been proved for the splice variant of serine-threonine kinase BRAF (Poulikakos *et al*., 2011).

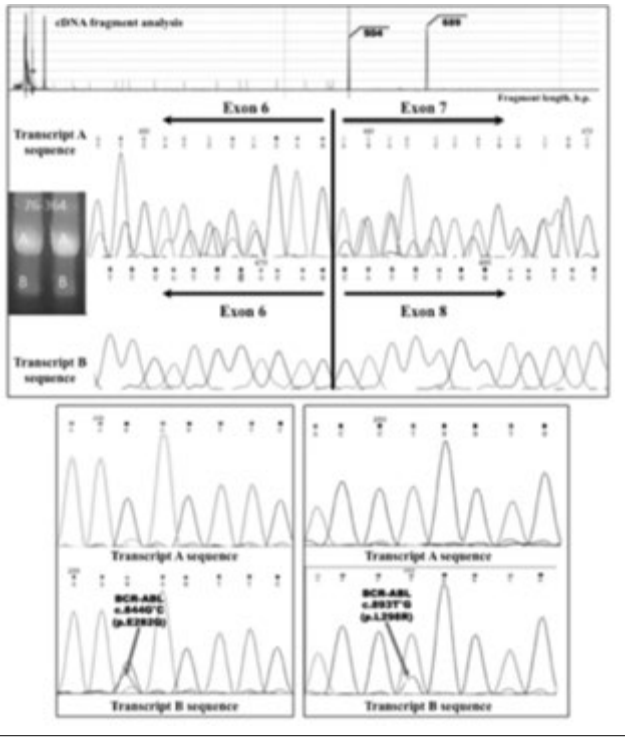


Figure 1.

Aims: To evaluate the pathogenic effect of BCR-ABL del. c.1086-1270 (p.R362fs*21) on TKI resistance formation in Russian patients with CML.

Methods: 33 male and 50 female CML patients (age 24-80) with BCR-ABL transcript level >0.1% were included in the study. BCR-ABL exon 7 deletion was analyzed with two round (nested) PCR followed by Sanger sequencing. Initial screening for deletions was performed by means of fragment analysis (Applied Biosystems 3130).

Results: Fragment analysis detected BCR-ABL del. c.1086-1270 transcript in 32 patients (38%). The ratio of TKI-sensitive to TKI-resistant among these 32 patients was approximately 47% (15 patients) to 53% (17 patients) respectively. Direct sequencing showed the tumor clone, which contains the BCR-ABL1 del.c.1086-1270 always paired with a normal clone of BCR-ABL p210, in all TKI-resistant patients. This fact was verified by the results of agarose gel electrophoresis. We suggest, that these results show an indirect evidence of the dimerization of the protein BCR-ABL del. c.1086-1270 with chimeric protein Bcr-Abl p210. Furthermore, the deletion clone of each TKI-resistant patient always has a point mutation (F317V, F317L, E282Q, M351T, T315I) which is unlikely for the normal clone (Figure 1). This fact contradicts the alternative splicing theory.

Summary/Conclusion: Our results show a definite correlation between the presence of the deletion and TKI-resistance. BCR- ABL1 del.c.1086-1270 is likely to play a trigger-role in TKI-resistance formation: clones with the exon 7 deletion are extremely prone to the accumulation of combined point mutations, which can increase the risk of TKI-resistance formation.

PB1895

DOWN-REGULATION OF MUSASHI2 AFFECTS ON MTOR SIGNALING AND INDUCES APOPTOSIS IN CD34+ CHRONIC MYELOID LEUKEMIA CELLS

M. Mossahebi-Mohammadi[1,2,*], S. Babashah[3]

[1]Hematology and Blood Banking, Faculty of Medical Sciences, Tarbiat Modares University, [2]Sarem Cell Research Center (SCRC), Sarem Woman's Hospital, [3]Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran, Islamic Republic Of

Background: Chronic myeloid leukemia (CML) is a clonal myeloproliferative disease. The high levels of Musashi2 (Msi2) is associated with down-regulation of Numb, a cell-fate determinant gene, in CML cells.

Aims: In current study, we knocked down Msi2 using RNA interference (RNAi) strategy and investigated the effects of this knock down on the expression of mTOR signaling.

Methods: Synthetic double stranded siRNAs designed to target human Msi2 were transfected to CD34 CML cells. Changes in the expression levels of Msi-2, Numb, TOR, and Bcl-2 genes 48h after transfection were evaluated by real-time PCR. Induction of apoptosis in transfected leukemic cells was determined using Annexin-PI staining and flowcytometry analysis.

Results: We found that upon Msi2 suppression, the expression levels of the cell-fate determinant Numb showed significantly increase in CD34+ CML cells. Msi2 down-regulation and subsequent increase in Numb expression levels caused reduction in expression of mTOR, as an oncogenic regulator. In addition, Msi2 down-regulation promoted cell apoptosis via the down-regulation of Bcl-2 expression. We observed that Msi2 downregulation resulted in decreased cell proliferation and elevated rate of apoptiosis in CD34+ CML cells.

Summary/Conclusion: It seems that Msi2 could be an option for targeting CD34+ CML cells and its down-regulation through RNAi strategy may lead to induction of apoptosis in leukemic stem cells. This approach may open up new opportunities for leukemia therapy.

PB1896

WNT PATHWAY IS INVOLVED IN BCR-ABL1-INDEPENDENT RESISTANCE TO TKIS IN CHRONIC MYELOID LEUKEMIA PATIENTS

S. Grassi[1,*], S. Palumbo[2], V. Mariotti[2], E. Ciabatti[3], F. Guerrini[3], C. Baratè[3], M. Petrini[3], S. Pellegrini[2], S. Galimberti[3]

[1]Medical Biotechnologies, University of Siena, Siena, [2]Clinica and Experimental Medicine, clinical biochemistry and clinical molecular biology, [3]Clinica and Experimental Medicine, Hematology, University of Pisa, Pisa, Italy

Background: After the introduction in the clinical practice of tyrosine kinase inhibitors (TKIs),the overall survival of CML patients is really improved, but several mechanisms of resistance have been reported. In addition to BCR-ABL1-related mechanisms (amplification, ABL1 mutations), the persistence of the leukemic stem cell (LSC) in the bone marrow niche is a very

relevant problem. The hypoxia and the presence of immunosuppressive cells in the microenvironment are well-known mechanisms that sustain the LSC; nevertheless, an increasing interest is also put today into the WNT/Beta-catenin pathway, that is necessary to self-renewal of normal cells, but its deregulation also causes leukemogenesis and progression in several types of cancers (Zhao, 2007).

Aims: we decided to assess the expression of 86 genes of the beta-catenin/WNT pathway at diagnosis and after 6 months of treatment with TKIs in a cohort of 10 patients with different responses to treatment.

Methods: Buffy coats obtained from peripheral blood samples of 10 patients (7receiving imatinib, 2 nilotinib, and 1 dasatinib) have been used for the total RNA extraction. In addition to the quantification of the BCR-ABL1/ABL1 ratio%IS, we used RT-q PCR to measure the expression of 86 genes from the WNT pathway (PrimePCR pathway kit, Biorad, Milan, Italy) at diagnosis and after 6 months of therapy. Expression values were calculated by the Vandesompele method using four housekeeping genes. Data have been analyzed by the "Gene Study" PrimePCR analysis software (Biorad).

Results: Five patients achieved an optimal response and five were no responders, according to the European Leukemia Network guidelines. Interestingly, after 6 months of treatment, we observed a de-regulation of 36 genes. Down-expression occurred in 14% of genes, while 79% of genes were up-regulated. When we compared the change of expression with the quality of response to TKIs, a differential expression between patients with or without an optimal response was observed: in the cases without optimal response, we found as up-regulated: 1) FZD7, already known to be responsible for the protection of leukemic CML cell, proliferation and drug resistance in K562 cells; 2) WNT6, that predicts unfavorable survival in solid cancer and whose expression is inversely correlated to the responseto ECF (Epi, cisplatin, 5-fluorouracil) chemotherapy in human gastric cancer cells; 3)WISP1, with anti-apoptotic activity, associated to poor prognosis and advanced stage in glioblastoma. On the other hand, the most frequently down-regulated gene was CSNK1A1, whose aploinsufficiency has been shown to result in a more probable transformation of myelodysplastic syndrome (MDS) in acute myeloid leukemia (AML) and to induce proliferation, invasion and metastasis in multiple myeloma (MM), lymphoma (DLBCL) and AML.

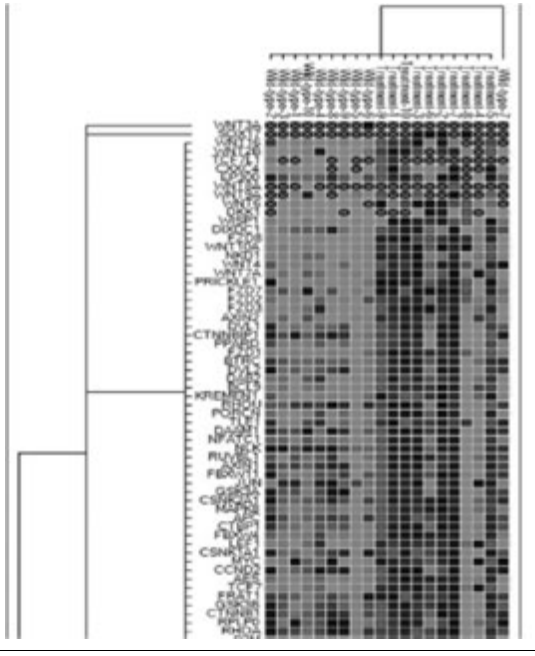


Figure 1.

Summary/Conclusion: With these experiments of gene expression profiling we demonstrated, although in a small group of CML patients, that the beta-catenin /WNT pathway might be relevant to condition the response to TKIs. Obviously, the analysis of a larger number of patients will improve the biological suggestions coming from these preliminary data

PB1897

EFFECTS OF DIFFERENT TKIS ON CHRONIC MYELOID LEUKEMIA STEM CELLS AND TKIS DISCONTINUATION

L. Jiang[1,*], Q. Li[2], J. Cui[1], Y. You[1], Z. Zhong[1], X. Zhu[3], P. Zou[1]

[1]Institute of Hematology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, [2]Department of Hematology, Wuhan No.[1] Hospital, [3]Department of Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Background: BCR-ABL tyrosine kinase inhibitors (TKIs) are selective therapies for patients with chronic myeloid leukemia (CML) and induce deep molecular response (DMR). However, many clinical trials of discontinuing TKIs have reported that sustained treatment-free remission (TFR) could only be observed in about 40% of patients for two years. Failure to discontinuation results from the inability of TKIs to eradicate CML leukemia stem cells (CML-LSCs). Among the several factors predict relapse after discontinuation of TKI therapy, only the duration of TKI therapy and DMR closely related to which generation of TKIs was used have been associated with TFR. Furthermore, in our previous observation, 12/22 patients maintained a stable DMR after TKIs withdrawal, and we found patients with second generation TKIs relapsed less than those with Imatinib. Thus we suppose second generation TKIs and Imatinib may have different effects on chronic myeloid leukemia stem cells and affect the outcome of TKIs discontinuation.

Aims: The aim of this study is to investigate how different TKIs affect CML-LSCs in the study of TKIs discontinuation.

Methods: Fresh bone marrow samples were obtained from patients with newly diagnosed chronic phase CML and isolated for CD34+ cells by magnetic cell sorting. Then treated CD34+ cells cultured in growth factors supplemented serum-free medium with Imatinib and Dasatinib for continually three days and stopped for one day. Colony-forming cell (CFC) assays, apoptosis measurement by Annexin-V staining, and cell proliferation detected by WST were performed. Also the three groups of samples were performed proteomic analysis.

Results: After treating CML CD34+ cells with Imatinib and Dasatinib for 72h, then stopped for 24h, the number of CFU in dasatinib group was less than imatinib group, as proliferation. On the contrary, there was much apoptosis in dasatinib group. Proteomic analysis identified 160 upregulated and 151 downregulated proteins differentially expressed between imatinib group and dasatinib group, which marked enriched in mitochondrial oxidative phosphorylation, including NADH dehydrogenase, cytochrome c oxidase and ATP synthase.

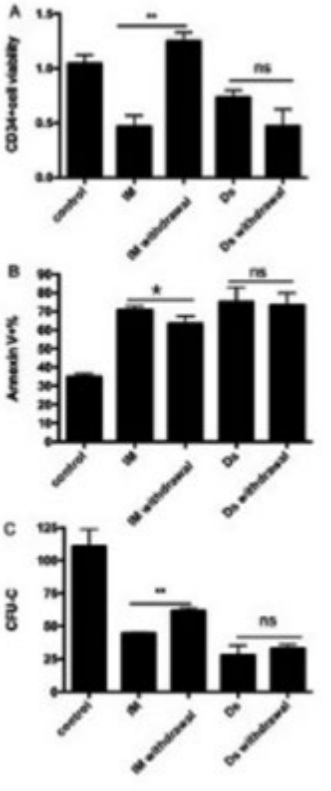


Figure 1.

Summary/Conclusion: We demonstrated that Imatinib and Dasatinib have obviously different effects on CML-LSCs through regulating metabolism process, specifically promoting oxidative phosphorylation to increase proliferation and avoid apoptosis, which may provide new target for eliminating CML-LSCs in the study of successful TKIs discontinuation.

PB1898

DESIGN, SYNTHESIS, ANTI-PROLIFERATIVE, CELL CYCLE INHIBITORY AND IN-VIVO ANTILEUKEMIC ACTIVITY OF NOVEL THIAZOLIDINEDIONES IN COMBINATION WITH IMATINIB

H. Joshi*, R. CS

Pharmaceutical Chemistry, Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai, India

Background: Imatinib, the most affordable and effective tyrosine kinase inhibitor for chronic phase chronic myeloid leukaemia is becoming resistance in many patients. Efforts are being made to combine the imatinib with other molecules to dodge the resistance and to get deeper molecular responses. This concept has been substantiated by recent phase 2 trial of imatinib in combination with well-known thiazolidinedione (TZD), pioglitazone. TZDs like rosiglitazone, pioglitazone once known for their potent antidiabetic action were withdrawn from the market due to their ability to cause cardiotoxicity and bladder cancer respectively and these side effects have been attributed to their full agnostic activity towards peroxisome proliferative activated receptor γ (PPAR γ).
Aims: To circumvent the PPARγ activation and thus toxicity, we design and synthesized novel TZDs (3a-3y) and studied their in-vitro and in-vivo antiproliferative activity in combination with imatinib.
Methods: Synthetic scheme was standardized and molecules 3a-3y were synthesized as per standard scheme. Molecules were characterized using IR, 1H NMR, 13C NMR and mass spectrometry. Antiproliferative activity was carried out using SRB assay on various leukemic cell lines. Moieties were also screened for their toxicity on non-transformed hepatocyte using MTT assay. Cell cycle inhibition assay was carried out using propidium iodide and FACS. Inhibition of proliferative and cell cycle markers, PCNA and cyclin D1 inhibition was checked by western blot analysis. PPARγ activation assay was carried out using ELISA test. In-vivo activity of 3t and 3x in combination with imatinib was carried in mice xenograft model with the permission from institutional ethical committee.
Results: The IR, 1H NMR, 13C NMR and mass spectra data confirmed the structure of molecules. The anti-proliferative activity of molecules was in the range of 0.19-70 µM on k-562 cell line. The MTT assay confirmed the safety of all moieties on untransformed hepatocytes. Molecules bearing significant anti-proliferative activity were also found to arrested the cells in G0/G1 phase in dose and time dependent manner. Two molecules 3t and 3x emerged as a lead entities. Maximum tolerated dose analysis revealed that molecules were tolerated up to 2000 mg/kg. These two lead compounds found to significantly reduce the tumour volume in combination with imatinib.
Summary/Conclusion: In the developing countries like India, Imatinib is the only affordable tyrosine kinase inhibitor (TKI). Compared to 2nd generation TKIs, imatinib is well tolerated and has a proven efficacy. These novel agents when combined with imatinib will help to get deeper responses and overcome resistance to imatinib. Further investigation on leukemic stem cell and long term toxicity of these molecule are under investigation.

PB1899

PGP AND BCRP INHIBITOR AS A MODULATOR OF IMATINIB RESISTANCE

R.S. Alves[1,2], A.C. Gonçalves[1,2], J. Jorge[1,2], A. Almeida[3,4], A.B. Sarmento-Ribeiro[1,2,5,6,*]

[1]Laboratory of Oncobiology and Hematology, [2]Coimbra Institute for Clinical and Biomedical Research (iCBR)-Group of Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra (FMUC), Coimbra, [3]Hospital da Luz, [4]CEDOC- Chronic Diseases Research Center, Lisboa, [5]Clinical Hematology Department, Centro Hospitalar e Universitário de Coimbra (CHUC), [6]Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra, Portugal

Background: In chronic myeloid leukemia (CML), the most relevant mechanisms associated with the acquisition of resistance to tyrosine kinase inhibitors (TKIs) are those dependent on the therapeutic target, the BCR-ABL oncoprotein. Point mutations and overexpression are the mechanisms take into considerations for therapeutic selection according to guidelines. However, intracellular drug concentration has proved to be very important in therapy response and in the acquisition of drug resistance. Imatinib (IMA), as other TKI, to achieving the intracellular compartment need influx transporters like OCT1 and OCTN2. In opposition, the presence of efflux transporters, like P-gP and BCRP, remove the TKIs from the cell conditioning the achievement of the therapeutic dose. Based on this, the modulation/inhibition of efflux transporters may contribute to the higher efficacy of TKIs.
Aims: The objective of this study was to evaluate the therapeutic potential of Elacridar (P-gP and BCRP inhibitor) in monotherapy and in combination with Imatinib, trying to overcome resistance in *in vitro* models of CML.
Methods: To achieve this goal, we used three CML cell lines: K562 cells (sensitive to Imatinib), K562-RC (8x resistant to IMA) and K562 RD (18x resistant to IMA). P-gP and BCRP activity was evaluated by flow cytometry (FC). The therapeutic potential of Elacridar was assessed in cells incubated in the absence and presence of Elacridar, in monotherapy and in combination with increasing doses of Imatinib, by the resazurin method. Cell death was evaluated by optical microscopy (May-Grünwald-Giemsa staining) and by FC (Annexin V/7-AAD). The *Apoptosis, DNA Damage, and Cell Proliferation* Kit was used to analyze the mechanism of cell death and proliferation. The cell cycle was evaluated by FC (PI/RNAse). The data were analyzed statistically, and the differences were considered significant when $p<0.05$.
Results: Resistant cell lines show higher expression and activity of P-gP and BCRP compared to the sensitive one. Elacridar in monotherapy, in tested concentrations, did not reach the IC_{50} in any cell line. However, the association of 250 nM of Elacridar with Imatinib modulated the resistance and re-sensitized resistant cells to Imatinib. In mechanistic terms, Elacridar in monotherapy induced cell death by apoptosis/necrosis, showing no effect in cell cycle progression. In combination with Imatinib was observed cell death by apoptosis, accompanied by increased caspase-3 activation, cleaved PARP, and DNA damage (phosphorylated H2AX). This effect was accompanied by a cell cycle arrest in S phase.
Summary/Conclusion: In conclusion, our results suggest that Elacridar in therapeutic combination with Imatinib re-sensitize resistant cell lines to Imatinib, namely in cell lines which the main mechanism of drug resistant was associated with efflux transporters. These results, if translated into clinical practice, may contribute to therapy response improvement in patients resistant to Imatinib.
This work was supported by Center of Investigation in Environment, Genetics, and Oncobiology (CIMAGO) and FCT (SFRH/BD/51994/2012).

PB1900

MYOFIBROBLASTS DERIVED FROM CHRONIC MYELOGENOUS LEUKEMIA PATIENTS EXPRESSED BCR-ABL TRANSCRIPT WHEN CULTURED WITH INTERLEUKIN-1 BETA

N. Shirafuji*, T. Matsuo, R. Sumiyoshi, H. Tashiro

Department of Hematology/Oncology, Teikyo University School of Medicine, Tokyo, Japan

Background: We previously reported that human interleukin 1-beta (IL-1-b) stimulated bone-marrow stromal myofibroblasts to express a hematopoietic molecule CD34. We also reported that a some fraction of myofibroblasts from bone marrow of acute myelogenous leukemia (AML) patients and from chronic myelogenous leukemia (CML) ones showed characteristics of original AML blasts and CML cells, such as chromosomal translocations and the expression of myeloid markers. However, when myofibroblasts were not separated and cultured totally, that is, bulk cultures mixed with normal and leukemic myofibroblasts, leukemia-specific markers were detected in DNA level, but not expressed in RNA levels for a long term cultures.
Aims: We showed in this report that myofibroblasts positive for leukemic markers was dormant and G0 phase in the mixed normal and leukemic myofibroblasts, and when IL-1-beta was added in the cultures, the fusion transcript was observed.
Methods: Bone marrow samples were collected from informed CML patients, which were separated with density centrifugation method. Cells were cultured in DMEM with 20% FCS to prepare myofibroblasts. The obtained myofibroblasts were cultured for one month, and FISH was analyzed with bcr and abl probes. Cells were further cultured in DMEM/F12 medium supplemented with 20% KSR and with recombinant human IL-1-b for one week. The morphological changes and the expression of specific genes were observed.
Results: When bone marrow-derived stromal myofibroblast obtained from CML patients were cultured in an ordinary DMEM with 10% FCS medium, FISH analysis detected 1-5% BCR-ABL fusion chromosome in the cultures; however, RT-PCR analyses revealed that leukemia-specific Bcr-Abl transcript was not detected. When CML-derived myofibroblasts were cultured in DMEM/F12 medium with IL-1-beta for one week, Bcr-Abl transcript was detected with RT-PCR analysis. And, CD41 molecule and GATA-2 transcription factor were also detected in the cultures.
Summary/Conclusion: Serum-free culture with IL-1-beta induced activation of CML-derived myofibroblasts that were dormant in the ordinary culture. In this system hematopoietic progenitor-cells may promote to grow.

PB1901

BEX1 GENE EXPRESSION IS A PREDICTOR FOR THE RESPONSE TO NILOTINIB IN CHRONIC MYELOGENOUS LEUKEMIA

D.-Y. Shin[1,2,*], I. Kim[1,3], S.-K. Sohn[4], Y. Koh[1,2], J. Shin[1], D.-Y. Kim[5],

J.-H. Lee[5], K.-H. Lee[5], H.J. Kim[6], J.-S. Ahn[6], Y.-K. Kim[6], J. Park[7], J.H. Moon[4], Y.J. Lee[4], J.-O. Lee[8], S.-M. Bang[8], J.-W. Cheong[9], K.H. Kim[10], S.H. Kim[11], H.G. Kim[12], H. Kim[13], S.H. Nam[14], Y.R. Do[15], S.K. Park[16], H.H. Song[17], D. Oh[18], M.K. Kim[19], C.W. Jung[20], S. Park[21]

[1]Internal Medicine, Seoul National University Hospital, [2]Biomedical Research Institute, Seoul National University Hospital, [3]Cancer Research Institute, Seoul National University College of Medicine, Seoul, [4]Internal Medicine, Kyungpook National University Hospital, Daegu, [5]Internal Medicine, Asan Medical Center, University of Ulsan College, Seoul, [6]Internal Medicine, Chonnam National University Hwasun Hospital, Hwasun, [7]Internal Medicine, Gachon University Gil Medical Center, Incheon, [8]Internal Medicine, Seoul National University Bundang Hospital, Seongnam, [9]Internal Medicine, Yonsei University Severance Hospital, [10]Internal Medicine, Soonchunhyang University Seoul Hospital, Seoul, [11]Internal Medicine, Dong-A University Hospital, Busan, [12]Internal Medicine, Gyeongsang National University Hospital, Jinju, [13]Internal Medicine, Ulsan University Hospital, Ulsan, [14]Internal Medicine, VHS Medical Center, Seoul, [15]Internal Medicine, Keimyung University Dongsan Medical Center, Daegu, [16]Internal Medicine, Soonchunhyang University Bucheon Hospital, Bucheon, [17]Internal Medicine, Hallym University Medical Center, Seoul, [18]Internal Medicine, Bundang Cha General Hospital, Seongnam, [19]Internal Medicine, Yeungnam University Medical Center, Daegu, [20]Internal Medicine, Samsung Medical Center, Seoul, [21]Internal Medicine, Inje University Haeundae Paik Hospital, Busan, Korea, Republic Of

Background: The selective targeting of ATP binding pocket in ABL gene by 1st generation tyrosin kinase inhibitor (TKI) imatinib led a huge success in the nearly complete control of chronic myeloid leukemia (CML). However, the inevitable development of resistant mutation bypassing the inhibitory effect of imatinib in CML cells initiated the development of 2nd generation TKI (2G-TKI) of nilotinib and dasatinib. With the introduction of 2G-TKI, the standard frontline treatment of CML is now changed into 2G-TKI.
Aims: We investigated the molecular predictor for the treatment response in patients with newly diagnosed CML and treated with 2nd generation TKI, nilotinib.
Methods: We performed mRNA sequencing (>30M reads) in peripheral blood samples from 12 patients who achieved $MR^{3.0}$ at 6 months (n=3) or not (n=3) and achieved $MR^{2.0}$ at 3 months (n=3) or not (n=3) for candidate gene discovery, and then conducted real-time quantitative PCR (RQ-PCR) for validation in 72 patients who were treated with nilotinib as frontline treatment. Gene expression from follow-up samples at 3 months was also measured.

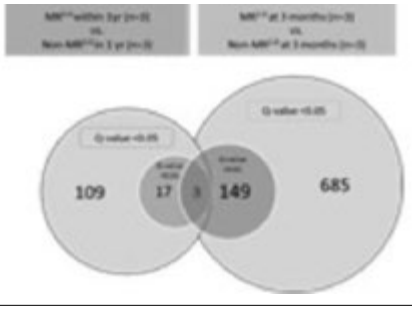


Figure 1.

Results: According to the criteria of fold change >2.0 and q-value<0.01, a total of three genes of AOC1 (Amine oxidase, copper containing 1), BEX1 (The Brain-Expressed X-linked (BEX) 1 gene), and PRSS57(Protease, Serin, 57) were extracted from mRNA-sequencing data as candidate genes for the molecular predictor for the response to nilotinib (Figure 1). The results of RQ-PCR for validation of these 3 genes demonstrated that baseline expression level of AOC1 and PRSS57 were not associated with $MR^{2.0}$ at 3 months (p=0.95 and 0.81, respectively). However, low baseline BEX1 gene expression ($2^{-\Delta Ct}$ X 100<12.09) at baseline was significantly associated with good early molecular response at 3 months. Baseline high BEX1 expression was significantly associated with the reduced cumulative incidence of deep molecular response of $MR^{5.0}$ (HR=0.41, p=0.022), and showed a trend toward the decreased incidence of $MR^{4.5}$ (HR=0.53, p=0.052). BEX1 gene expression was decreased in most cases (84.3%, 59/70 patients), while it was increased in 11 patients. The median change of BEX1 expression ($2^{-\Delta Ct}$ X 100) was -11.9 (range, -286.2 ~ 27.5). However, $MR^{2.0}$ at 3 months since the nilotinib treatment was not associated with the direction of change of BEX1 expression (χ^2=0.54, p=0.46)
Summary/Conclusion: Our study showed that lower BEX1 gene expression can predict early (3 months) molecular response and deep molecular response of $MR^{5.0}$ in newly diagnosed CML treated with nilotinib. Further larger scale confirmatory study is warranted.

PB1902

HEAT SOCK PROTEIN 90 INHIBITION AS A POTENTIAL THERAPEUTIC TARGET IN CHRONIC MYELOID LEUKEMIA

D. Santos[1], R. Alves[1,2], A.C. Gonçalves[1,2], J. Jorge[1,2], H. Girão[3], S. Catarino[3], J. Barbosa de Melo[2,4], A.B. Sarmento-Ribeiro[1,2,5,6,*]

[1]Laboratory of Oncobiology and Hematology and University of Hematology, [2]Coimbra Institute for Clinical and Biomedical Research (iCBR)-Group of Environment Genetics and Oncobiology (CIMAGO), [3]Coimbra Institute for Clinical and Biomedical Research (iCBR), [4]Cytogenetics and Genomics Laboratory, Faculty of Medicine University of Coimbra, [5]Clinical Hematology Department, Centro Hospitalar e Universitário de Coimbra (CHUC), [6]Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra, Portugal

Background: HSP90 belongs to the heat shock protein family, a functional class of chaperone molecules that are transcriptionally upregulated by heat and other stressors. HSP90 facilitates the maturation, stability, activity, and intracellular folding of more than 200 proteins, called 'client proteins'. In cancer cells, HSP90 helps to overcome multiple environmental stresses, including genomic instability/aneuploidy, proteotoxic stress, increased nutrient demands, reduced oxygen levels, and to prevent destruction by the immune system. One of these client proteins of HSP90 is BCR-ABL, the oncoprotein responsible for Chronic Myeloid Leukemia (CML). Cancer cells that depend on this oncoprotein for survival are sensitive to HSP90 inhibition. Hsp90 inhibitors, by preventing nucleotide-dependent cycling interfere with the chaperone activity of HSP90, resulting in targeting of client proteins to proteasome degradation. Alvespimycin (17-DMAG) is an HSP90 inhibitor that has better pharmacokinetic properties and fewer side-effects compared to others benzoquinone ansamycins.
Aims: This work aims to study the effect of alvespimycin in chronic myeloid leukemia cell lines (sensitive and resistant to imatinib) and to explore the role of HSP family in the sensitivity to imatinib.
Methods: In this context, we used 3 CML cells lines: the K562 cells, sensitive to Imatinib, and the K562-RC and K562-RD cells resistant to Imatinib. Cells were incubated in the absence and presence of increasing concentrations of 17-DMAG (from 1 to 1000 nM), in a single dose. The dose-response curves were determined by resazurin assay. Cell death was determined by microscopy (May-Grunwald Giemsa staining) and by flow cytometry (FC), using Annexin V and Propidium Iodide (PI) double staining. The Apostat Probe was used to evaluate caspase expression levels and JC-1 probe to determine the mitochondrial membrane potential, by FC. Cell cycle was evaluated by FC, using PI/RNase assay. The protein expression levels of HSP family were analyzed by western blot.
Results: Our results showed that 17-DMAG induce a reduction in cell lines viability, with an IC50 of 50 nM for K562 and K562-RD cells and lower than 50 nM for the K562-RC cell line, after 48 hours of treatment. This compound induces cell death predominantly by apoptosis, confirmed by morphological analysis, FC and by the increase of JC-1 Monomers/Aggregates ratio. Furthermore, 17-DMAG induces cell cycle arrests in K562 in G0/G1 phase. The HSP protein analysis showed that K562-RC have slightly increased in HSP90 expression comparing with K562 cells.
Summary/Conclusion: In conclusion, our results suggest that inhibition of HSP90 by alvespimycin (17-DMAG) could be used as a new potential approach in the treatment of CML, even in case of Imatinib resistance.
This work was supported by Center of Investigation in Environment, Genetics, and Oncobiology (CIMAGO).

PB1903

KILLER IMMUNOGLOBULIN-LIKE RECEPTOR GENOTYPES IN TURKISH PATIENTS WITH ACUTE AND CHRONIC MYELOID LEUKEMIA

M. Erkoç[1], E. Gürkan[2,*], H.D. Dinçyürek[2], S. Dinkçi[3], Ö.G. Öztürk[4], E. Erken[5]

[1]Dept of Internal Medicine, [2]Dept of Hematology, [3]HLA Laboratory, [4]Central Laboratory, [5]Dept of Rheumatology/Immunology, Çukurova University Medical School, Adana, Turkey

Background: It is a well-known fact that natural killer (NK) cells have anti-tumour activities. This activity of NK cells is regulated by specific receptors called KIR (Killer Immunoglobulin-like receptors). The proportion between the activatory and inhibitory receptors, binding strength of receptors to the given ligands and the balance between the composite signals determine the health and ailment attitudes of NK cells. Previous studies have shown decreased expression of HLA class 1 molecules on leukemic cells. It has been postulated that lack of clearance of the leukemia cells could be caused

by the lack of activity of the NK cells, which may be the result of the genetically determined KIR/HLA ligand content.
Aims: In this study we investigated the association of KIR genes with acute (AML) and chronic myeloid leukemia (CML) patients in Turkish population and also tried to correlate with the clinical outcomes.
Methods: Patient group consisted of patients with AML (n=34) (M/F: 31/15); mean age 56 years, and CML (n=46) (M/F: 18/16; mean, 52 years. Age and sex matched 100 healthy controls were also included. DNA from venous blood samples was extracted by DNA isolation kit (QIAGEN Vertriebs GmbH, Vienna, Austria). Genotyping of KIR genes was performed using the multiplex KIRSSO typing kit from Tepnel Lifecodes Corporation (CT, USA). The statistical significances were assessed through Pearson's homogeneity chi-squared test.
Results: The presence of KIR2DL3 was significantly increased in the leukemia patients relative to the controls (91.3% *vs* 78%, OR=0.340, P=0.016). In CML patients, KIR2DL3 gene frequency was significantly higher compared to controls (93.5% *vs* 78.0%, OR=0.247, P=0.031). In addition, presence of KIR2DL2 and KIR2DS2 was significantly decreased in the CML patients compared with controls with the same level of significance (34.8% *vs* 52.0%, OR=2.031, P=0.053). In CML patients who achieved MMR (BCR-ABL1≤0.1% at 12 months of treatment), gene frequency of KIR2DL2 and KIR2DS2 was lower compared to the control group (33.3% *vs* 52.0%, P=0.054, for both analysis); whereas in those without MMR gene frequency was 40%, P>0.05). CML patients without MMR had a higher frequency for KIR2DS1 compared to controls (70.0% *vs* 35.0%, P=0.041) which was 36.11% for those patients with MMR. There were no associations of KIR AA and Bx genotypes between leukemia patients and controls. The genotypic associations for AML patients were not significant.
Summary/Conclusion: KIR2DL2 and KIR2DS2 were suggested in previous studies as being markers for the much more activating haplotype for NK cells. Decreased gene frequencies of KIR2DL2 and KIR2DS2 in CML patients in our study support these findings as such that their reduction would presumably lead to more inhibition with tyrosine kinase inhibitors. Better treatment outcomes observed in this study as reflected by a higher frequency of MMR in patients who had lower KIR2DL2 and KIR2DS2 frequencies suggest that prognostic information could be available by further understanding the role KIR interactions in leukemic patients. In addition our results suggest that increased frequency of a weak inhibitory receptor KIR2DL3 in leukemic patients might contribute decreased NK cell activity against leukemia cells.

PB1904

THE ROLE OF LEUKEMIC STEM CELLS AND EARLY PROGENITOR CELLS IN CHRONIC MYELOGENOUS LEUKEMIA RECURRENCE AFTER THE DISCONTINUATION OF TREATMENT WITH TKI

T. Perekhrestenko[1,*], I. Sviezhentseva[2], D. Bilko[2], G. Starodub[1], D. Irina[3]

[1]Blood disease, State Institution «Institute of Hematology and Transfusiology of NAMS of Ukraine», [2]The Center of Molecular And Cell Research, The National University of Kyiv Mohyla Academy, [3]Hematology department, State Institution "National Research Center for Radiation Medicine of NAMS of Ukraine", Kyiv, Ukraine

Background: Leukemic stem cells (LSCs) and myeloid progenitor cells are believed to play an important role in the pathogenesis of chronic myeloid leukemia (CML). LSCs are able to remain at hemopoietic niches for a long time, remaining insensitive to TKI. It is very difficult to identify using cytogenetic and molecular diagnostic methods. Therefore, in a long-term remission, after the discontinuation of the TKI therapy, LSCs can cause relapse of CML in patients' bone marrow.
Aims: The aim of our study is to compare the proliferative activity of hemopoietic stem and progenitor cells of patients with CML who had a long-term remission as a result of TKI treatment.
Methods: We studied bone marrow samples of 17 patients with CML who had a long-term remission as a result of the TKI therapy. Experiments were conducted *in vitro*, with a use of liquid culture. In order to accumulate the bone marrow stem cells, patients' hemopoetic cells were cultivated for 28 days with bone marrow stromal cells in DMEM culture medium (SIGMA, USA) with addition of 10% fetal calf serum (SIGMA, USA). Subsequently, patients' hemopoietic stem cells were cultivated in a liquid culture *in vitro* in RPMI medium (SIGMA, USA) with an addition of a granulocyte-macrophage colony stimulating factor (SIGMA, USA) and a 20% fetal calf serum. We counted the number of cells in the liquid culture using a Goriaev's chamber. We also prepared hematopoietic cell samples after cultivation, using cytocentrifuge. We stained samples using Pappenheim method and counted different hemopoetic cells under a microscope. We compared the results of cultivation with the presence of CML remission in patients after 6 months of discontinuation of TKI.
Results: After cultivation of patient's bone marrow cells in a liquid culture *in vitro* solely with bone marrow stromal cells, there was a small number of hematopoietic precursor cells (15-37,000 per ml) on 28th day of cultivation. Following the cultivation, these cells were removed and cultivated with an addition of the granulocyte-macrophage colony stimulating factor. The results showed that in patients who had CML relapse six months after the discontinuation of the TKI therapy, the proliferative activity of hemopoietic progenitor cells increased by 17%, compared to Patients who had a stable remission as a result of a TKI therapy. In addition, patients' with unstable remission as a result of TKI therapy showed the prevalence of early forms of cells of the granulocyte-macrophage series (blast cells, promyelocytes and myelocytes).
Summary/Conclusion: Consequently, long-term cultivation with stromal cells allows to accumulate the stem cells and the early progenitor bone marrow cells from patients with CML. LSCs seem to be the reason of CML relapses when the TKI therapy is discontinued. They remain in a state of rest for a long time, and are able to restore the leukemia clone under favorable conditions.

PB1905

LW-213 TRIGGERED G2/M CELL CYCLE ARREST AND APOPTOSIS OF CHRONIC MYELOGENOUS LEUKEMIA CELLS THROUGH SUPPRESSION OF CDK9-RELATED SIGNALING PATHWAYS

J. Xu*, H. Hui

State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing, China

Background: LW-213, a derivative of wogonin derived from traditional Chinese medicine plant *Scutellaria baicalensis Georgi*, has been shown anticancer activities on several breast cancer cell lines and xenograft models. Our previous results have reported the anti-leukemia effects of wogonin while the therapeutic effect of LW-213 in chronic myelogenous leukemia (CML) has been barely known.
Aims: To clarify whether LW-213 has better inhibitory effect on CML and its possible mechanism.
Methods: we explore the potency of this compound on K562, imatinib-resistant K562, and primary CML cells.
Results: These results showed that LW-213 inhibited proliferation of CML cells and induced cell cycle arrest in G_2/M phase, leading to cell apoptosis. Meanwhile, LW-213 down-regulated cyclin dependent kinase 9 (CDK9) and inhibited phosphorylation of Stat3, down-regulating Mcl-1and cyclin B1. All results suggest that LW-213 triggered G2/M cell cycle arrest and apoptosis through the suppression of CDK9-related signaling pathways. In vivo, LW-213 prolonged survival of NOD/SCID mice inoculated with primary CML cells without any organ toxicity.
Summary/Conclusion: In conclusion, LW-213 might be a potential candidate compound on clinical treatment in imatinib-resistant CML patients.

PB1906

A MATHEMATICAL APPROACH TOWARDS IMMUNOLOGICAL CONTROL OF MINIMAL RESIDUAL DISEASE IN CML PATIENTS

I. Roeder[1,2], A.C. Fassoni[3], T. Haehnel[1], C. Baldow[1], I. Glauche[1,*]

[1]Institute for Medical Informatics and Biometry, Faculty of Medicine, Technische Universität Dresden, [2]Data and Trial Management Unit, National Center for Tumor Diseases (NCT), Partner Site Dresden, Dresden, Germany, [3]Instituto de Matemática e Computação, Universidade Federal de Itajubá, Itajubá, Brazil

Background: There is increasing evidence in patients with Chronic Myeloid Leukemia (CML) pointing to the role of the immune system in the sustained control of residual leukemic cells after tyrosine-kinase inhibitors (TKI) treatment cessation. It has been speculated that, once the treatment has reduced the leukemic cell burden below a certain threshold, the immune cells are capable to control the disease, or even to completely eliminate the leukemic clone. However, at the moment these mechanisms are poorly understood.
Aims: It is our aim to contribute to a quantitative, mechanistic understanding of the immune response in TKI-treated CML. Specifically, we address the questions if and why the immune response appears to be effective only at a low level of leukemic cells. Here it is of particular interest to understand the interaction of two dynamic processes: (i) the stimulation of immune cells by the presence of leukemic cells and (ii) the immune system-mediated elimination of these cells.
Methods: We developed a mathematical framework describing CML progression and treatment in terms of ordinary differential equations. Within

this model framework we consider different assumptions about the mechanisms (i) by which immune cells are stimulated by leukemic cells and (ii) of how leukemic cells are targeted by immune cells. The combination of the different assumptions leads to several structurally different models, which are characterized by different systems dynamics (such as unavoidable relapse, low-level control of leukemic cells, potential cure, etc.).
Results: We compare our conceptual results with available data sets on the BCR-ABL1 levels from CML patients after TKI cessation, thereby allowing us to assess and critically discuss the plausibility of the particular assumptions. Specifically, we show that both the recruitment of specific immune cells as well as the immune response-related kill of leukemic cells need to be determined by non-constant and even non-linear regulation processes to consistently explain clinically observed outcomes after TKI cessation, as there are, early molecular relapse, fluctuating but extremely low BCR-ABL1 levels, and long-term BCR-ABL1 negativity.
Summary/Conclusion: The model analyses suggest that the shape of the dose-response relationship of leukemic cell burden and immune response is essential to understand and predict the molecular relapse dynamics after TKI cessation. To identify these relationships, one needs to *define* relevant readouts of immune response and to *quantify* these over time and in relation to the tumor load. Our results highlight the necessity to understand the mechanisms of immune control in leukemia therapy to employ this effect for optimal treatment results and to identify clinical measures that allow to derive better predictions about the outcome of treatment cessation.

PB1907

AVOIDING BCR/ABL EXPRESSION IN CHRONIC MYELOID LEUKEMIA CELLS BY CRISPR/CAS9 SYSTEM GENOME EDITION TOOL.

V. Alonso-Pérez[1,*], M. Herrero-García[2], L. Méndez-Sánchez[2], E. Vuelta[2], M. Abáigar[3], A. Montaño-Brioso[3], A. Marin-Quilez[1], M. Quijada-Álamo[1], J.L. Ordóñez[1], F. Sánchez-Guijo[4], J.M. Hernández-Rivas[1], M. Sánchez-Martín[2], I. García-Tunón Llanio[1]

[1]Molecular genetics in onco-hematology Research Group, Institute for Biomedical Research of University of Salamanca (IBSAL)-University of Salamanca, [2]Servicio de Transgénesis, Nucleus, Universidad de Salamanca, [3]Institute for Biomedical Research of University of Salamanca (IBSAL)-University of Salamanca, Salamanca, Spain, [4]Hematology Department, Institute for Biomedical Research of University of Salamanca (IBSAL)-University of Salamanca, Salamanca, Spain

Background: CML is a disorder driven by hematopoietic stem cells that acquire the t(9;22)(q34;q11) traslocation resulting in the expression of the BCR-ABL oncogenic fusion protein, which increase cell survival, proliferation and induces an aberrant myeloid commitment. CML treatments are predominantly based on the use of tyrosine kinase inhibitors (TKI). However, CML eradication is still hindered by the emergence of TKI resistant cells. A definitive and effective therapeutic strategy is therefore still needed for these patients. Few studies have used genome edition CRISPR-based techniques to eliminate fusion oncogenes generated by chromosome translocations. BCR-ABL fusion is an ideal therapeutic target to be eliminated at the genomic level by using these new genome edition tools. We demonstrate that CRISPR/Cas9 system efficiently disrupts, at a genomic level, the BCR-ABLp210 gene fusion in an *in vitro* model of human BCR-ABL, avoiding its oncological effects.
Aims: To evaluate the ability of CRISPR/Cas9 technology to induce frameshift mutations on the BCR/ABL sequence avoiding its expression in K562 cell line.
Methods: The K562 cell line derived from a chronic myeloid leukemia (CML) patient and expressing B3A2 BCR-ABL fusion gene, is known to be particularly resistant to apoptotic cell death. Through CRISPR/Cas9-mediated genomic edition, we set to edit the coding sequence of BCR-ABL in K562 cells, inducing indels that modify the oncogene ORF, and therefore, the protein expression. We designed 3 sgRNAs against tyrosine kinase domain sequence of ABL1 and were introduced in a plasmid containing the Cas9 nuclease coding sequence. K562 cells were electroporated with the mix of sgRNAs. The genetic edition on ABL1 tyrosine kinase domain sequence was checked by PCR and Sanger sequencing. The BCR/ABL expression was analyzed by qPCR. To assess the edited cells viability and apoptosis, flow cytometry, measuring Annexin V staining and DNA content was used.
Results: We achieved to edit the coding sequence of BCR-ABL in K562 cells by CRISPR/Cas9 system, inducing mutations that modified the oncogene ORF. We found a 100 bp recurrent induced deletion in the sequence corresponding to the TK domain of ABL1. This deletion leads to a premature stop codon, which prevents the correct translation of the protein. As a result of this deletion, the edited K562 cells showed a decreased expression of ABL1, higher levels of apoptosis and an altered expression of downstream ABL1 targets, proof of lack of expression of the BCR-ABL oncoprotein.
Summary/Conclusion: We demonstrate the ability of CRISPR/Cas9 system to disrupt at genomic level, the coding sequence of BCR/ABL fusion oncogene resulting from translocation t (9;22) in established cell lines derived from patients of CML, avoiding its oncological effects. The use of CRISPR-Cas9 technology could be a promising new therapeutic option for CML patients who have developed TKI resistances. Bone marrow CML leukemic stem cells could be *ex vivo* edited by CRISPR-Cas9 technology and specifically selected to transplantation treatment.

PB1908

OUTCOME OF FRONTLINE TREATMENT WITH GENERIC OF IMATINIB IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

S. Menif*, N. Ben Romdhane, Z. Manai, H. Ghedira, S. Zriba, F. Msaddek

Hematology, Pasteur Institut, Tunis, Tunisia

Background: The development of oral well tolerated bcr-abl tyrosine kinase inhibitor (TKI) has revolutionized the treatment of chronic myeloid leukemia (CML) and allowed thousands of patients over the past 15 years to live healthy and productive. Generic imatinib is already available in a number of countries, the use of generic is expected to lower the cost of CML therapy however the data regarding their efficacy and tolerability in larger populations of CML patients are lacking. In January 2015 Health Tunisia approved CEMIVIL, a generic of imatinib, manufactured by HIKMA Pharmaceutical.
Aims: The aim of this study was to evaluate the efficacy and tolerability of imatinib generic in Tunisian patients suffering from chronic phase CML.
Methods: In this retrospective analysis data from patients with frontline cemivil between January 2015 and December 2017 is included .we report the rate of bcr-abl/abl reduction to<10% at 3 months and to<1% at 6 months of therapy, the rate of optimal response and failure according to current ELN guidelines, the rate of MMR, MR4 and MR4.5 achieved at 12 months of therapy and the rate of patients switched to second generation TKI have been assessed.
Results: Between January 2015 and December 2017, 51 patients started do novo treatment with generic. Early molecular remission defined by bcr-abl/abl ratio <10% at 3 months was achieved in 83%, bcr-abl/abl ratio <1% at 6 months in 89% of patients. At 12 months of therapy 13 patients (25%) achieved RM^4 and 5 patients (9%) achieved $RM^{4.5}$. 14 Patients (27%) have been switched to second generation TKI, 2 patients due to intolerance, 8 patients due to resistance and 4 patients due to progression.
Summary/Conclusion: The findings of the present study showed comparable efficacy and safety of Cemivil in the treatment of patients with CML. The arrival of generic imatinib at a reduced price has the potential to markedly impact the cost of care for CML. It may also increase the access of patients to this remarkable drug that has previously been unaffordable to some.

PB1909

MTORC2 COMPLEX SUSTAINS LEUKEMIC STEM CELL SELF-RENEWAL BY NUCLEAR RELOCALIZATION OF ITS COMPONENTS

C. Panuzzo*, L. Prioni, J. Petiti, V. Rosso, G. Carrà, C. Calabrese, E. Signorino, E. Bracco, D. Cilloni, A. Morotti

San Luigi Hospital, Orbassano, Italy

Background: The effect of hypoxia on protein translation is mediated in part through inhibition of mammalian target of rapamycin complex 1 (mTORC1), but the principle mechanism for hypoxia-induced mTORC1 inhibition however was not elucidated until recently.
Aims: In order to elucidate leukemic stem cell behaviour including self-renewal, apoptosis, proliferation and molecular pathway involved in these mechanisms we analysed K562 under hypoxic condition. We focused our studies on mTOR pathaway, expecially on mTORC2, which ability to activate directly AKT by Ser473 phosphorylation makes it an interestly candidate in cell self-renewal scenario.
Methods: K562 erythroleukemia cells were maintained in RPMI 1640, supplemented with 10%FCS. Hypoxia exposure was performed by incubation in 5% CO2, 1% Oxygen, 94% N2 condition for different time point (20 and 40 hours). Cell viability and death were performed by MTT assay and Annexin V identification by FACS analysis. Total cell lyses, nuclear and cytoplasm fractions were used to carry out western blot to analyse mTORC1 and mTORC2 pathway and to investigate cellular delocalization of TOR component in hypoxia condition. Immunofluorescence were performed on K562 slices after permeabilization and subsequent primary and FITC-secondary antibody incubation to detect mTOR component localization. RNA

were extract by TRIzol method and used to analyse genes known to be involved in hypoxia pathway.
Results: In agreement with previous works, the hypoxic culture of k562 led to decreased in proliferation and increased in CD34 expression, suggesting the ability of cell line to take a "stem phenotype" under hypoxic condition. Moreover we have observed a reduction of the S phase of the cellluar cycle, and subsequent accumulation on G2/M phase. We observed that after hypoxia exposition mTORC1 pathway is strongly abrogated, as documented by reduction in 4EBP, ribosomal protein S6 and mTOR phosphorylation. Conversely mTORC2 resulted significantly activated, with increased in AKT (ser473), NDRG and SGK phosphorylation, reduction in GSK phosphorilation and increased in total Rac protein. This phenomenon is accompanied by a strong and significant relocation of mTOR, Rictor and Akt in the nucleus. Interesting these peculiar localization has been observed by immunofluorescence on CD34+ CML cells suggesting therefore that it could be a typical behaviour of cells localizing in hypoxic environment. The quantitative analysis on different hypoxic treatments showed a significant increase in VEGF (known to be regulated by HIF1) and SOX genes (SOX2 and SOX17) which would appear to be negatively regulated by mTORC1. Furthermore the computational analysis on HIF promoter showed the presence of many HMG-box motifs, typical of SOX genes, suggesting a possible link between mTORC1 inactivation, mTORC2 and AKT nuclear activation and HIF expression in hypoxic K562 cells.
Summary/Conclusion: We observed that hypoxic treatment leads to a strong mTORC1 decreased activity, inhibits proliferation and promotes survival and stem cell phenotype in K562, with an increased in mTORC2 activity and its relocalization in nucleus. How Rictor and mTORC2 complex act in nucleus is still unknown. A recent work performed on S. Pombae showed that Rictor is able to rilocalize in nucleus by direct link to AKT and E2F transcription factor, favouring its transcription activiy. Our future propose will be to investigate the role of E2F, which have emerged as essential regulators of stem cell fate control in a number of lineages, in maintaining leukaemia stem cells.

PB1910

NILOTINIB *VS* IMATINIB IN ALBANIAN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE

A. Cili*, P. Xhumari, P. Pulluqi, T. Caja, E. Calliku, A. Perolla, A. Ivanaj

Hematology, Central University Hospital Mother Tereza, Tirana, Albania

Background: The superior efficacy of nilotinib over imatinib as frontline therapy for CML-CP was first demonstrated in the international phase 3 study Evaluating Nilotinib Efficacy and Safety in Clinical Trials–Newly Diagnosed Patients (ENESTnd).
Aims: Because the efficacy and safety of TKIs may vary depending on ethnic background or genetic factors, focused investigations within well-defined patient populations are crucial in order to better understand the relative benefits and risks of each treatment option for individual patients. We did conduct this study in Albanian patients with newly diagnosed chronic myeloid leukemia in chronic phase
Methods: Adult patients of Albanian ethnicity with Ph+ CML-CP within 6 months of diagnosis and with an Eastern Cooperative Oncology Group performance status ≤2 were eligible. Patients were randomized 1:1 to nilotinib 300 mg twice daily or imatinib 400 mg once daily.The study was conducted according to the ethical principles of the Declaration of Helsinki
Results: Treatment with a tyrosine kinase inhibitor (TKI) targeting BCR-ABL1 is currently the standard of care for patients with chronic myeloid leukemia (CML) in chronic phase (CML-CP). A total of 121 patients were randomized (nilotinib, n=61; imatinib, n=60) from May of 2011 to August 2014 at Hematology clinic ,University Hospital Center "Mother Teresa". In this study, we present the results of a 48 months follow-up data of a study that was conducted to investigate nilotinib 300 mg twice daily *vs* imatinib 400 mg once daily in Albanian population. This study met its primary end point with a statistically significant higher rate of major molecular response (MMR; BCR-ABL1 ≤0.1% on the International Scale) at 12 months in the nilotinib arm *vs* the imatinib arm (50.2% *vs* 26.3; *P*<.0001), and MMR rates remained higher with nilotinib *vs* imatinib throughout the follow-up period.. Estimated 60 months rates of freedom from progression to AP/BC on study were significantly higher on nilotinib (95.1% [*P*=.0497] compared with imatinib (91.2%).
Summary/Conclusion: For the first time we are presenting a geographical distribution of CML patients in Albania. In conclusion, rates of MMR at 12 months were superior with nilotinib *vs* imatinib in Albanian patients with newly diagnosed Ph+ CML-CP. These results suggest that treatment with frontline nilotinib may allow more patients with Ph+ CML-CP to achieve early, deep molecular responses and attain improved long-term outcomes. Estimated rates of 5-year OR on study were 96.7%, and 93.3% for nilotinib 300 mg twice daily and imatinib 400 mg once daily. The safety profiles of both drugs were similar to those from previous studies e international phase 3 study Evaluating Nilotinib Efficacy and Safety in Clinical Trials–Newly Diagnosed Patients (ENESTnd). Because the efficacy and safety of TKIs may vary depending on ethnic background or genetic factors, focused investigations within well-defined patient populations are crucial in order to better understand the relative benefits and risks of each treatment option for individual patients. We did conduct this study in Albanian patients with newly diagnosed chronic myeloid leukemia in chronic phase

Chronic myeloid leukemia – Clinical

PB1911

LONG-TERM OUTCOMES OF EARLY CP CML PATIENTS WHO HAVE ACHIEVED CCYR BUT NOT MMR AFTER 24 MONTHS ON FRONTLINE IMATINIB THERAPY

S.-H. Kim[1,*], S.-Y. Choi[2], S.-E. Lee[3], W.-S. Lee[4], Y.R. Do[5], J.-Y. Kwak[6], S. Oh[7], J.-A. Kim[8], D.Y. Zang[9], D.-W. Kim[10]

[1]Internal Medicine, Dong-A University College of Medicine, Busan, [2]Leukemia Research Institute, The Catholic University of Korea, [3]Hematology, Seoul St. Mary's Hosipital, Seoul, [4]Internal Medicine, Inje University Busan Paik Hospital, Busan, [5]Hematology-Oncology, Keimyung University, Daegu, [6]Hematology-Oncology, Chonbuk National Universtiy Medical School, Jeonju, [7]Internal Medicine, Kangbuk Samsung Hospital, Seoul, [8]Hematology, St. Vincent's Hospital, Suwon, [9]Internal Medicine, Hallym University Hospital, Anyang, [10]Hematology, Leukemia Research Institute, Seoul St. Mary's Hospital, Seoul, Korea, Republic Of

Background: The BCR-ABL1 tyrosine kinase inhibitors (TKIs) became standard therapy for chronic phase (CP) chronic myeloid leukemia (CML) since imatinib mesylate (IM) was approved as frontline therapy. Complete cytogenetic response (CCyR) has been established as a predictor for disease outcome by the International Randomized Study of Interferon and STI571 (IRIS), and based on many clinical studies. The National Comprehensive Cancer Network (NCCN) and the European Leukemia Net (ELN) have established treatment guidelines that include molecular response milestones. Although failure to achieve these milestones means that switching to a different TKI is needed to limit the risk of progression and death, there is insufficient data to show long-term outcomes in the patients who were in a molecular failure after 24 months (CCyR but not MMR).
Aims: The aim of this study was to evaluate long-term survival end points and identify predictive factors for an achievement of overall major molecular response (MMR) in the patients who have achieved CCyR but not MMR after 24 months on frontline IM therapy, as additional information to guide clinical decisions on selecting patients.
Methods: Among 604 newly diagnosed CP CML patients who received IM for at least 2 years with no prior treatment, 288 (47.7%) patients achieved CCyR but not MMR after 24 months on frontline IM therapy. Finally, to evaluate molecular responses, 280 patients with transcript type of b2a2 and b3a2 were included in this analysis. All qRT-PCR were tested with at least 4.5-log sensitivity in a single laboratory (Leukemia Research Institute, The Catholic University of Korea, Seoul, Korea).
Results: 280 newly diagnosed CP CML patients (including 190 men and 90 women) were evaluated. With a median age of 38.1 years (range, 5-77 years), the distribution of low, intermediate, and high Sokal risk scores were 22%, 35% and 43%, respectively, excluding 30 patients with unknown risk. With a median follow-up of 134.2 months (range, 25.6–195.0 months), 163(58%) patients continued IM and maintained at least a complete cytogenetic response (CCyR), while 117 patients permanently discontinued IM due to intolerance (n=31), warnings according to ELN 2013 recommendations with adverse events (n=43), and treatment failure (n=17), progression (n=9), death (n=1), enrollment of treatment-free remission (TFR) study (n=10), and others (n=6). Among them, 98 (84%) switched to second-generation tyrosine kinase inhibitors (2G TKIs). The CI rates of MMR by 3, 4, and 5 years were 27.9±2.8%, 53.6±3.3%, and 71.8±3.0%, respectively, and the CI rates of CMR by 3, 4, and 5 years were 1.9±0.8%, 5.0±1.4%, and 10.8±2.1%, respectively. The 10-year FFS, PFS, and OS were 89.3±2.2%, 96.0±1.3%, and 98.5±0.9%, respectively. The patients with transcript type of b3a2, compared to b2a2 (RR of 0.22, *P*=0.001) and high Sokal risk, compared to low Sokal risk (RR of 0.67, *P*=0.040) showed a lower rate of overall MMR achievement on IM therapy.
Summary/Conclusion: This study demonstrated long-term survival outcomes in the patients who have achieved CCyR but not MMR after 24 months on frontline IM therapy. In addition, we identify the factors related to an achievement of overall MMR. It provides additional information to guide clinical decisions on selecting high-risk patients. Based on our findings, clinical investigations considering what the treatment goals are, overall survival with a low risk of toxicity or achieving of a deeper molecular response with a hope for TFR, are needed.

PB1912

COMORBIDITY INDEX RELATED OUTCOME IN CHRONIC MYELOID LEUKEMIA PATIENTS

I. Cojbasic[1,2,*], L. Macukanovic-Golubovic[1,2], M. Vucic[1,2]

[1]Faculty of Medicine, University of Nis, [2]Clinic of Hematology and Clinical Immunology, Clinical Center Nis, Serbia, Nis, Serbia

Background: Recent studies have indicated that comorbidity may impact the treatment response and survival of chronic myeloid leukemia (CML) patients treated with tyrosine inhibitors (TKIs). The Charlson Comorbidity Index (CCI) was originally introduced as a tool to measure the influence of relevant comorbid diseases in terms of reduced life expectancies. The score is well established and validated and it is widely used for both hospitalized patients and outpatients.
Aims: The objective of this study was to evaluate the influence of baseline comorbidities on treatment response rate and survival in patients with CML.
Methods: We reviewed the records of patients with newly diagnosed CML between 2006 and 2018, who were receiving imatinib as first line and nilotinib as second line therapy. For this retrospective analysis, patients were divided into two groups with none (CCI 2) or one and more present comorbid conditions (CCI ≥3). The CCI has been used to evaluate concomitant underlying disease at diagnosis as the most extensively studied comorbidity index. Complete cytogenetic response (CCyR) and major molecular response (MMR) have been defined according to the European LeukemiaNet criteria. Overall survival (OS) was calculated from the date of imatinib initiation until death at any time and for any reason.
Results: At the time of analysis, 106 patients could be analysed, of which 57 (53.8%) were female, with median age of 56.4 (18-78) years at imatinib initiation. According to the Euro score, 51.9% patients were classified as low risk, 38.7% as intermediate risk and 9.4% as high risk. EUTOS score was low for 83% of patients. Median follow-up time was 71.5 (6-132) months, and 45 (42.5%) patients had documented comorbidities, 30 (28.3%) with CCI relevant diseases. The distribution of CCI comorbidity risk categories were: diabetes mellitus (n=9), chronic pulmonary disease (n=5), moderate to severe renal insufficiency (n=4), cerebrovascular disease (n=3), peripheral vascular disease (n=3), myocardial infarction (n=2), congestive heart failure (n=2) and peptic ulcer (n=2). The most common comorbidities not considered with the CCI were: arterial hypertension (n=6), angina pectoris (n=3), arrhythmia (n=2), thyroid disfunction (n=2), etc. At 5 years, cumulative incidence of achieving CCyR according to the CCI was similar in both analysed groups: 84.9% in CCI 2 and 78.3% in CCI ≥3. Likewise, no differences in cumulative incidence of MMR has been observed between patients in different CCI groups: 75.5% in CCI2 and 68.9% in CCI≥3. Median time to optimal response for patients with CCI2 or CCI≥3 did not differ within each arm, for CCyR 10.5 *vs* 11.8 months and for MMR 18.3 *vs* 19.1 months. However, it was found that patients with comorbidities had significantly shorter OS. Probabilities of OS at 5 years for patients with CCI2 and CCI≥3 were 92.1% (95%CI: 89.5%>95.3%) and 68.9% (95%CI: 62.8%>72.7%), respectively. There were 18 patients in both groups who died during the follow-up, of which 10 patients died due to non-CML related causes while 8 deaths were resulting from progression of CML.
Summary/Conclusion: This study indicated that in CML patients' comorbidities do not affect achievement of therapeutic response, since the possibility of achieving CCyR and MMR was similar regardless of presence or absence of comorbidity. However, comorbidities have significant negative impact on overall survival, as CML patients' survival is influenced more by comorbidities than by CML itself.

PB1913

EFFICACY AND SAFETY OF SWITCHING FROM BRANDED TO GENERIC IMATINIB IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA: A STUDY OF THE GRUPPO TRIVENETO LMC ON 294 PATIENTS

M. Bonifacio[1,*], M. Tiribelli[2], G. Binotto[3], M.C. Miggiano[4], M. Danini[5], C. Minotto[6], L. Scaffidi[1], D. Griguolo[2], L. Marin[2], L. Frison[3], F. D'Amore[3], M. Basso[7], R. Sartori[7], M. Tinelli[8], M. Stulle[9], S. Fortuna[10], G. Bertoldero[6], E. De Biasi[5], M. Ruggeri[4], G. Semenzato[3], R. Fanin[2], G. Pizzolo[1], M. Krampera[1]

[1]Department of Medicine, Section of Hematology, University of Verona, Verona, [2]Department of Medical Area, Division of Hematology and BMT, University of Udine, Udine, [3]Department of Medicine, Hematology and Clinical Immunology, Padua School of Medicine, Padua, [4]Hematology Department, San Bortolo Hospital, Vicenza, [5]Hematology Departmental Unit, P.Cosma Hospital, Camposampiero (Padua), [6]Department of Medical Specialities, Oncology and Onco-Hematology Unit, Mirano Hospital, Mirano (Venice), [7]Hematology Department, Castelfranco Veneto Regional Hospital, Castelfranco Veneto (Treviso), [8]Department of Internal Medicine, G.Fracastoro Hospital, San Bonifacio (Verona), [9]Hematology Division, Azienda Sanitaria Universitaria Integrata, Trieste,

[10]Department of Internal Medicine, Alto Vicentino Hospital, Santorso (Vicenza), Italy

Background: Imatinib is the most commonly used drug in chronic myeloid leukemia (CML) patients (pts) worldwide. In early 2017 a generic formulation was introduced in Italy and CML pts have switched from branded (*i.e.* Glivec®, Novartis) to generic imatinib upon requirement of regional health authorities. Since the use of generic drugs represents a novelty in cancer field, some concerns exist about efficacy and safety in comparison to their originators.
Aims: To analyze the outcome of CML pts switched from branded to generic imatinib for changes in adverse event (AE) profile or efficacy.
Methods: We analyzed a cohort of 294 chronic phase CML pts treated in 10 hematological centers with branded imatinib for at least 6 consecutive months before switching to a generic formulation. After switching, RQ-PCR and biochemical exams were performed at least every 3 months and the assessment of AE was continuously performed. Molecular responses were defined according to the ELN2013 recommendations. The severity of AE was assessed according to the CTCAE 4.0 scale.
Results: Median age at diagnosis was 57 years (range 19-87 years). Sokal score was L/I/H in 162 (55%), 93 (32%) and 24 (8%) pts, respectively (15 cases were not evaluable). Median duration of branded imatinib treatment was 7.4 years (range 0.5-16.7 years). Imatinib dose at switch was 400 mg, less than 400 mg and more than 400 mg daily in 71%, 27%, and 2% of pts, respectively. Imatinib dose was not changed at the time of switch. The majority of pts (171/294, 58%) had experienced at least one AE while on branded imatinib, most commonly muscle cramps, fluid retention, diarrhea and anemia (table). Grade 3-4 non hematological AE were uncommon and included infections (n=4), arrhythmia (n=2) ischemic stroke (n=1) and cardiac failure (n=1). Of note, 9 pts had a secondary neoplasm diagnosed while on branded imatinib. At the time of switch molecular responses were as follows: less than MR3 in 25 (8%), MR3 in 75 (26%), MR4 in 87 (30%) and MR4.5 or better in 107 (36%) pts. At a median follow-up of 7.5 months after switch to generic imatinib (range 0-12.2 months), 49 pts (17%) reported new or worsening AE (table), most commonly nausea, diarrhea and muscle cramps. Grade 3-4 non hematological AE included increase of lipase (n=3), infections (n=2), vomiting (n=1), muscle pain (n=1), and severe allergic reaction at the first intake of generic imatinib (n=1). Twelve pts (4%) interrupted generic imatinib for >30 days and 20 pts (7%) had the dose permanently reduced, most commonly to 200 mg daily. Twenty-two pts (7.5%) discontinued generic imatinib treatment for intolerance (n=9), treatment-free remission attempt (n=8), lack of molecular response (n=3) and death (n=2, both unrelated to CML). Overall, 6 pts (2% of the whole population) switched back to branded imatinib, with improvement in the AE profile, and 4 pts moved to bosutinib (n=3) or nilotinib (n=1). The efficacy of switch was evaluable in 282 pts. Molecular responses remained the same in 229 pts (81%), improved in 37 pts (13%) (from less than MR3 to MR3 or better: n=13; from MR3 to MR4 or better: n=24) and worsened in 16 pts (6%) (from MR3 to less than MR3: n=3; from MR4 or MR4.5 to MR3: n=13).

Table 1.

Table. Frequency of adverse events upon treatment with branded and generic imatinib

Adverse event term	Branded imatinib	Generic imatinib *persistant AE* (severity unchanged from branded imatinib)	Generic imatinib *new or worsened AE* (severity increased from branded imatinib)
Fluid retention	58 (19.7%)	8 (2.7%)	5 (1.7%)
Muscle cramps	71 (24.1%)	23 (7.8%)	9 (3.1%)
Arthralgia	28 (9.5%)	8 (2.7%)	6 (2.0%)
Ocular symptoms	31 (10.5%)	6 (2.0%)	6 (2.0%)
Skin rash / pruritus	24 (8.2%)	6 (2.0%)	3 (1.0%)
Nausea / vomiting	24 (8.2%)	5 (1.7%)	7 (2.4%)
Diarrhea	46 (15.6%)	5 (1.7%)	10 (3.4%)
Increased creatinine	10 (3.8%)	7 (2.4%)	1 (0.3%)
Anemia	37 (12.6%)	14 (4.8%)	4 (1.3%)
Leukopenia	18 (6.1%)	5 (1.7%)	3 (1.0%)
Thrombocytopenia	25 (8.5%)	7 (2.4%)	1 (0.3%)
Overall*	**171 (58.2%)**	**64 (21.8%)**	**49 (16.7%)**

* this value is not the sum of the column since a patient may have experienced more than one AE

Summary/Conclusion: Switch to generic imatinib for pts who have been receiving branded imatinib for at least 6 months appears to be effective and safe. Molecular responses may continue to improve over time. Some pts experienced new or worsened AE but in our cohort only 3.4% of pts needed to switch back to branded imatinib or move to other TKIs.

PB1914

THE SIGNIFICANCE OF VERY EARLY MOLECULAR RESPONSE WITH FRONTLINE DASATINIB TREATMENT IN CHRONIC MYELOID LEUKEMIA PATIENTS AS A STRONG PREDICTOR OF LONG-TERM OUTCOME: FINAL ANALYSIS OF PCR-DEPTH STUDY

W.S. Lee[1,*], H.-J. Kim[2], J.H. Kong[3], H. Kim[4], Y.R. Do[5], J.-Y. Kwak[6], S. Oh[7], S.H. Kim[8], J.-A. Kim[9], D.Y. Zang[10], Y.-C. Mun[11], Y.-W. Won[12], S.-E. Lee[13], S.Y. Choi[14], S.-H. Kim[14], M.-Y. Choi[14], D.-W. Kim[13]

[1]Int. Medicine, Hemato-Oncology, INJE UNIVERSITY BUSAN PAIK HOSPITAL, Busan, [2]Hematology-Oncology, Chonnam National University Hwasun Hospital, Gwangjoo, [3]Hematology-Oncology, Wonju College of Medicine, Yonsei University, Wonju, [4]Hematology, Gacheon University Gil Hospital, Incheon, [5]Hematology-Oncology, Keimyung University Hospita, Daegu, [6]Hematology-Oncology, Chonbuk National University Hospital, Jeonju, [7]Int. Medicine, Hemato-Oncology, Sungkyunkwan University, Kangbuk Samsung Hospital, Seoul, [8]Internal Medicine, Dong-A University Hospital, Busan, [9]Hematology, The Catholic University of Korea, St. Vincent's Hospital, Suwon, [10]Internal Medicine, Hallym University Hospital, [11]Hematology, Ehwa Womans University Hospital, Seoul, [12]Internal Medicine, Hanyang University Guri Hospital, Guri, [13]Hematology, Seoul St. Mary's Hospital, Leukemia Research Institute, The Catholic University of Korea, [14]Hematology, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea, Republic Of

Background: In BCR-ABL1 tyrosine kinase inhibitor (TKI) treated chronic phase chronic myeloid leukemia (CP-CML), early molecular response (EMR) at 3 months is currently identified as being one of the most important prognostic factors. Sokal risk score and dose intensity during first 3 months were strongly associated with achievement of EMR. As dasatinib is a novel, oral tyrosine kinase inhibitor with improved potency, identification of very early molecular response (VEMR) would be beneficial.
Aims: We evaluated the possibility of the VEMR at 1 month predicting long-term outcomes in newly diagnosed CP-CML patients treated with dasatinib.
Methods: In this multi-center, observational, open-label study, 102 patients with CP-CML were enrolled to receive dasatinib at a dose of 100 mg once daily. The primary end point was complete molecular response (CMR) by 18 months. Secondary end points including molecular response (MR) by 1, 3, 6, 12, 18, 24, 36 months, time to and duration of MMR and CMR, and safety were tested. A receiver operating characteristic (ROC) curve from BCR-ABL1 transcript level on Day+28 was calculated to predict EMR and MMR at specific timepoints.
Results: Median age was 49 years (19-81 years) and 61 patients were male. With median follow-up duration of 33.6 months (0.9-38.6 months), 71 (69.6%) out of 102 patients were still on dasatinib treatment and 31 patients discontinued due to disease progression (n=2) or treatment failure (n=3) or adverse events (n=18) or other reasons (n=8). The BCR-ABL1 mutations, assessed in 10 patients after dasatinib discontinuation, were detected in 3 patients which were all T315I mutation. The cumulative CMR by 18 months and MMR by 36 months were 20.5% and 84.8% respectively. In safety analyses, grade 3/4 thrombocytopenia (30.3%) was most common. Pleural effusion occurred in sixteen (15.6%) patients which were mostly grade 1/2. The cut-off value of BCR-ABL1 transcript on Day+28 was 40% by ROC curve analysis. Among 95 patients who had available molecular data of both D+28 and 12 months, fifty nine (62.1%) patients had less than 40% of BCR-ABL1 transcript (VEMR) on Day+28. Among them, 49 (83.1%) patients achieved MMR at 12 months. However, only 27.8% (10 out of 36 patients) of patients without VEMR achieved MMR (p<0.0001). Among 72 patients who had available molecular data of both D+28 and 36 months, forty-six (63.9%) patients achieved VEMR. In 46 VEMR patients, 43 (93.5%) patients achieved MMR at 36 months. However, 80.8% (21 out of 26 patients) of patients without VEMR achieved MMR (p=0.10). Three-year overall survival (OS) & progression-free survival (PFS) rates were 98.0% and 95.1%, respectively. Three-year PFS rates for VEMR and no VEMR group were 98.4% *vs* 88.8% respectively (p=0.04).
Summary/Conclusion: Our study shows that VEMR at 1 month can be a strong predictor for further molecular responses as well as long-term outcome. Therefore, it would be helpful to monitor BCR-ABL1 transcript level at 1 month in patients who treated with more potent TKIs.

PB1915

THE RESTORING OF DEEP MOLECULAR RESPONSE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA AND MOLECULAR RELAPSE AFTER TREATMENT DISCONTINUATION

E. Chelysheva[1,*], A. Turkina[1], V. Shuvaev[2], A. Bykova[1], G. Gusarova[1], O. Shukhov[1], A. Petrova[1], M. Vakhrusheva[1], S. Goryacheva[1], L. Kolosova[1], M. Fominykh[2], I. Martynkevich[2], A. Abdullaev[1], A. Sudarikov[1], V. Savchenko[1]

[1]National Research Center for Hematology, Moscow, [2]Russian Research Institute of Hematology and Transfusiology, Saint-Petersburg, Russian Federation

Background: Discontinuation of tyrosine kinase inhibitors (TKI) treatment in patients (pts) with chronic myeloid leukemia (CML) having deep molecular response (DMR) now is moving from clinical trials into routine clinical practice. The loss of major molecular response (MMR) with *BCR-ABL*>0.1% by IS is generally defined as a molecular relapse and a trigger to restart therapy. The *BCR-ABL* level at molecular relapse and the impact of the delay in treatment resuming after MMR loss which may happen in routine clinical setting are rarely evaluated.
Aims: To evaluate the restoring of DMR in CML pts after resuming of TKI therapy in accordance with the time of therapy restart.
Methods: We observed 70 pts with CML (68 in chronic phase, 2 in accelerated phase at diagnosis) who stopped TKIs being in stable DMR>1 year. DMR was considered as at least MR4 (*BCR-ABL*<0.01%). The follow-up was done by quantitative PCR detection of *BCR-ABL* level. The data were collected retrospectively and prospectively in 2 central clinics of Moscow (n=66) and St.Petersburg (n=4) during years 2008-2016 outside of clinical trials. The reasons to stop TKI were toxicity (n=30), pregnancy (n=18) and patients' decision (n=22). Median (Me) time of observation after TKI cessation was 29 months (range 3-120). The low/intermediate/high Sokal risk group was in 45(64%)/ 14(22%)/ 9(14%) of pts respectively. Imatinib/nilotinib/dasatinib/bosutinib were used before treatment cessation in 45 (64%)/ 15(21%)/ 9(13%)/ 1(2%) of pts. Me time of TKI therapy was 6 years (IQR 4-9). Me time of DMR duration was 1.8 years (IQR 2.8-5). TKI were resumed after MMR loss or by physician's decision. The delays in treatment restart were related to drug access, patients' decisions or pregnancy. Event free survival (EFS) was evaluated considering MMR loss, TKI resuming and death as the events. Cumulative incidence (CI) of DMR restoring was evaluated in accordance with the time since MMR loss to TKI restart.

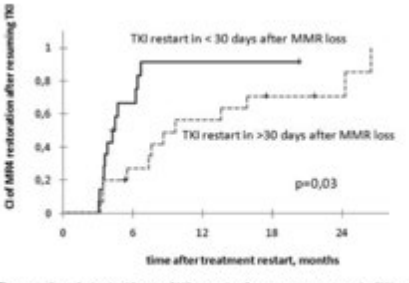


Figure 1. Cumulative incidence of MR4 regain after treatment restart in CML patients

Figure 1.

Results: The EFS after TKI discontinuation was 69%, 50% and 39% at 6,12 and 24 months(mo) accordingly. Two patients with DMR died from cardiovascular disease. TKIs were resumed in 38 (54%) pts, no MMR loss was in 6 pts at treatment restart. The MMR loss occurred in 32 (46%) of pts with the Me of *BCR-ABL* level 0.54% by IS (range 0.11% -13%). Me time from MMR loss detection to TKI resuming was 38 days (range 3-276). Treatment was restarted within<30 days in 14 pts and later than 30 days in 18 pts. MR2 loss (BCR-ABL>1%) without hematologic relapse (HR) was in 16 pts at TKI resuming. No HR occurred in 4 pts with MR2 loss and treatment delay of 111-276 days. A HR was observed in 2 pts who did not restart treatment in 122 and 155 days after MMR loss. The same TKI was reinitiated in 30 pts. TKI change due to previous toxicity or by administrative reasons was in 7 pts and 1 pt accordingly. TKI was also changed in 1 pt without MR2 after 6 mo of treatment restart and a DMR was achieved thereafter. Me time of observation after TKI resuming was 24 mo (range 2-116). CI of DMR regain was 73% and 100% after 12 and 24 months of treatment restart. DMR regain was observed later in pts who resumed TKIs later than 30 days after loss of MMR (figure 1).
Summary/Conclusion: A leukemic clone in CML patients may remain sensitive to TKI during prolonged treatment interruptions. However the delays in treatment restart may lead to a HR and a switch to other TKI may be required. CML patients should have regular molecular monitoring during off-treatment period and after TKI resuming.

PB1916

SURVIVAL AFTER 1, 3 AND 5 YEARS OF TYROSINE KINASE INHIBITOR THERAPY IN SIMPLICITY, AN OBSERVATIONAL STUDY OF CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CP-CML) PATIENTS IN ROUTINE CLINICAL PRACTICE

M. Michallet[1,*], J. Cortes[2], C. Gambacorti-Passerini[3], R. Hehlmann[4], L.A. Williams[2], S.L. Goldberg[5], C. Davis[6], F. De Solda[6], G.P. Sen[7], S. Gajavelli[6], M. Mauro[8]

[1]Centre Hospitalier Lyon Sud, Lyon, France, [2]MD Anderson Cancer Center, Houston, United States, [3]San Gerardo Hospital di Monza, Monza, Italy, [4]ELN-Foundation, Weinheim, Germany, [5]John Theurer Cancer Center at Hackensack University Medical Center, Hackensack, [6]Bristol-Myers Squibb, Princeton, [7]ICON Plc, San Francisco, [8]Memorial Sloan-Kettering Cancer Center, New York, United States

Background: Data on survival in CP-CML outside clinical trials are limited. SIMPLICITY (NCT01244750) is an ongoing observational study of CP-CML patients (pts) in routine clinical practice receiving first-line (1L) imatinib (IM), dasatinib (DAS) or nilotinib (NIL) in the US and Europe.
Aims: To describe survival in SIMPLICITY pts with 1, 3 and 5 years of follow-up after start of 1L IM, DAS or NIL.
Methods: Demographics and clinical characteristics are given for pts alive at 5 years and pts who died by 5 years for the overall prospective population and 1L tyrosine kinase inhibitor (TKI) cohorts. Descriptive p-values were generated using Chi-squared tests (categorical variables) and t-tests (continuous variables). Mean survival rate at 1, 3 and 5 years was calculated using the Kaplan-Meier estimator. Mean survival rates are presented for the overall population, for pts who remained on 1L TKI (by 1L TKI) and for pts who switched to 2L TKI (by most recent TKI at follow-up). P-values comparing mean survival rates were based on log-rank test.
Results: By September 07 2017, 1240 pts were enrolled prospectively in SIMPLICITY. Numbers of pts followed for 1, 3 and 5 years were 1192, 1027, and 526 respectively. By Year 3, 50 pts had died, 142 discontinued and 21 pts not reached 3-year follow-up. By Year 5, 78 pts had died, 240 discontinued and 396 pts had yet to reach 5-year follow-up. Median (interquartile range; min–max) age of pts was 55 (47–65; 19–87) years in pts alive at 5 years and 71 (65–78; 19–90) years in pts who had died by 5 years. Other demographics were comparable, but pts alive at 5 years had a mean (±standard deviation) number of comorbidities of 3.2 (±2.6) *vs* 4.6 (±3.3) in pts who had died by 5 years (p<0.001). Patient characteristics were similar across 1L TKI cohorts. Mean survival rate at 1, 3 and 5 years was 99%, 96%, and 93% for the overall population; 98%, 94%, and 91% for 1L IM pts; 99%, 98%, and 96% for 1L DAS pts; and 99%, 95%, and 91% for 1L NIL pts. Mean 5-year survival rates were higher in 1L DAS pts *vs* 1L IM or 1L NIL, pending consideration of confounding variables. The 5-year survival rate was higher for pts remaining on 1L DAS *vs* pts on 2L DAS (97% *vs* 91%; p=0.02); similarly for pts who remained on 1L NIL *vs* pts switched from 1L NIL (95% *vs* 85%; p=0.005). At 5 years 78 pts had died in the overall population over a mean follow-up of 4 years. In the IM, DAS, and NIL cohorts, 36, 13 and 29 pts had died over a mean follow-up of 4–4.25 years. In the overall population, 15 deaths were related to CP-CML, 41 unrelated to CP-CML and 22 had unknown relationship to CP-CML. Of deaths in the IM, DAS, and NIL cohorts, 8, 4 and 3 were related to CP-CML, and 16, 5 and 20 were unrelated to CP-CML.

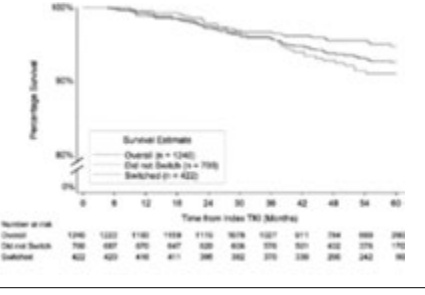


Figure 1.

Summary/Conclusion: Survival rates in the overall SIMPLICITY population, and in pts receiving IM, DAS, and NIL, are high. Survival rates were higher in pts who remained on 1L DAS and 1L NIL than pts who switched.

Survival rates and the proportions of pts who died by 5 years differed between TKI cohorts. Due to the descriptive nature of the statistics, comparisons should be made cautiously. Advanced survival modeling will consider other confounders such as age, gender, etc. These preliminary findings suggest use of TKIs is associated with long-term survival benefit in routine clinical practice. In future analyses with longer median follow-up differences may be discernible between different TKI cohorts and analysis groups.

PB1917

BOSUTINIB *vs* IMATINIB FOR NEWLY DIAGNOSED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA: LEARNINGS FROM 2 PHASE 3 TRIALS (BFORE AND BELA) CONDUCTED IN DIFFERENT ERAS

M. Deininger[1,*], J. Cortes[2], C. Gambacorti-Passerini[3], M. Mauro[4], C. Chuah[5], D.-W. Kim[6], V. Kota[7], J. Lipton[8], R. Crescenzo[9], E. Leip[10], F. An[11], A. Viqueira[12], N. Bardy-Bouxin[13], A. Hochhaus[14], T. Brümmendorf[15]

[1]University of Utah, Salt Lake City, [2]University of Texas MD Anderson Cancer Center, Houston, United States, [3]University of Milano-Bicocca, Monza, Italy, [4]Memorial Sloan Kettering Cancer Center, New York, United States, [5]Singapore General Hospital, Duke–NUS Graduate Medical School, Singapore, Singapore, [6]Seoul St. Mary's Hospital, Leukemia Research Institute, The Catholic University of Korea, Seoul, Korea, Republic Of, [7]Winship Cancer Institute of Emory University, Atlanta, United States, [8]Princess Margaret Hospital, Toronto, Canada, [9]Pfizer Inc, Collegeville, [10]Pfizer Inc, Cambridge, [11]Pfizer Inc, New York, United States, [12]Pfizer Spain, Madrid, Spain, [13]Pfizer International Operation-Oncology, Paris, France, [14]Klinik für Innere Medizin II, Universitätsklinikum Jena, Jena, [15]Universitätsklinikum RWTH Aachen, Aachen, Germany

Background: Two randomized phase 3 trials compared first-line bosutinib *vs* imatinib in patients (pts) with chronic phase chronic myeloid leukemia (CP CML). In BELA (NCT00574873; 2008–2015), Philadelphia chromosome–positive (Ph+) pts received bosutinib 500 mg once daily (QD) or imatinib 400 mg QD; the primary endpoint was complete cytogenetic response (CCyR) rate at 12 mo. In BFORE (NCT02130557; 2014–ongoing), Ph+ or Ph–/*BCR-ABL*+ pts received a lower bosutinib starting dose (400 mg QD); the primary endpoint was major molecular response (MMR) rate at 12 mo in the modified intent-to-treat (ITT) population (Ph+ pts with e13a2/e14a2 transcripts).
Aims: Efficacy of bosutinib (400 and 500 mg QD) and imatinib (400 mg QD in both trials) was assessed after ≥12 mo of follow-up from BFORE and BELA, respectively; the safety profile of bosutinib 400 and 500 mg QD was also evaluated.
Methods: Efficacy outcomes were reported for the ITT population, except where noted for the BFORE study. Treatment-emergent adverse events (TEAEs) were assessed in all pts who received ≥1 bosutinib dose, regardless of Ph+ or transcript status.

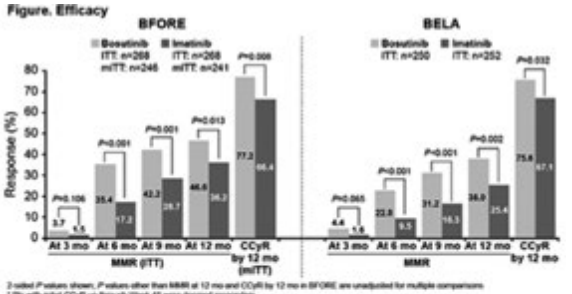


Table. Safety

n (%)*	BFORE Bosutinib 400 mg QD n=268		BELA Bosutinib 500 mg QD n=248	
	Any grade	Grade 3/4	Any grade	Grade 3/4
Any TEAE	263 (98)	151 (56)	238 (96)	162 (65)
Diarrhea	188 (70)	21 (8)	172 (69)	27 (11)
Thrombocytopenia	94 (35)	37 (14)	78 (31)	34 (14)
Alanine aminotransferase increased	82 (31)	51 (19)	78 (31)	44 (18)
Aspartate aminotransferase increased	61 (23)	26 (10)	65 (26)	20 (8)
Anemia	50 (19)	9 (3)	58 (23)	19 (8)
Vomiting	48 (18)	3 (1)	79 (32)	8 (3)

* Select TEAEs in ≥20% of pts in either study

Figure 1.

Results: Clinically meaningful and consistent improvements in MMR rates at 6, 9, and 12 mo with bosutinib *vs* imatinib, as well as in CCyR rate by 12 mo, were seen in BFORE and BELA (**Figure**). Consistent with the ITT population in BFORE, the MMR rate at the 12-mo visit was higher with bosutinib than imatinib (47.2% *vs* 36.9%; *P*=0.02) in the modified ITT population. Despite improved CCyR rate by 12 mo, BELA did not meet the primary endpoint of CCyR rate at 12 mo (bosutinib 70% *vs* imatinib 68%; *P*=0.601); in BFORE, CCyR rate at 12 mo was not assessed, as pts with a CCyR but no MMR prior to the 12-mo visit may not have had a cytogenetic assessment at this time point per protocol. Transformation to accelerated/blast phase was similar with bosutinib *vs* imatinib in BFORE (1.5% *vs* 2.2%) but less frequent in the bosutinib arm in BELA (1.6% *vs* 4.8%). Median bosutinib dose intensity (relative dose intensity) was 392 mg/day (98%) in BFORE and 482 mg/day (96%) in BELA; corresponding values for imatinib were 400 mg/day (100%) in both studies. The safety profile of bosutinib was generally similar in BFORE and BELA, except for a lower rate of vomiting (18% and 32%, respectively; **Table**). No new safety signals were identified for bosutinib in BFORE. Lower percentages of bosutinib-treated pts in BFORE *vs* BELA had grade 3/4 TEAEs (56% *vs* 65%; study drug–related 49% *vs* 57%), serious TEAEs (20% *vs* 25%), dose delays due to TEAEs (57% *vs* 63%), and dose reductions due to TEAEs (34% *vs* 37%). A higher percentage of bosutinib-treated pts remained on study drug after ≥12 mo of follow-up of BFORE *vs* BELA (78% *vs* 71%) with lower discontinuation rates due to TEAEs (14% *vs* 21%).
Summary/Conclusion: Increased physician experience in TEAE management with the lower bosutinib starting dose in BFORE *vs* BELA (400 *vs* 500 mg QD) likely contributed to the success of BFORE. Despite differences in study endpoints, bosutinib dose, and era of tyrosine kinase inhibitor therapy, the improved efficacy profile with bosutinib *vs* imatinib in newly diagnosed pts with CP CML was consistent between BFORE and BELA.

PB1918

MYELOID-DERIVED SUPPRESSOR CELLS IN CHRONIC MYELOID LEUKEMIA

A. Ahn[1,*], C.-J. Park[1], Y.-U. Cho[1], S. Jang[1], E.-J. Seo[1], M.-H. Bae[2], D.-H. Lee[3], J.-H. Lee[4], J.-H. Lee[4], K.-N. Koh[5], H.-J. Im[5], J.-J. Seo[5]

[1]Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, [2]Department of Laboratory Medicine, Hanyang University College of Medicine, Guri Hospital, Seoul, [3]Department of Laboratory Medicine, Gyeongsang National University Hospital, Jinju, [4]Department of Internal Medicine, [5]Department of Pediatrics, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea, Republic Of

Background: Myeloid-derived suppressor cells (MDSC) represent phenotypically heterogeneous populations of myeloid cells at different stages of maturation, which are able to suppress tumor-specific T cell response. MDSC are produced from normal granulocytic precursors in emergent states such as carcinoma and inflammation. Increases in MDSC have been reported in several hematologic malignancies including plasma cell myeloma, chronic lymphocytic leukemia, and acute myeloid leukemia.
Aims: This is a prospective study that measured frequencies of MDSC and we evaluated the significance and disease correlation of MDSC in patients with chronic myeloid leukemia (CML).
Methods: Peripheral blood (PB) (n=77) and bone marrow (BM) (n=76) aspirates were obtained from 6 pediatric and 60 adult patients diagnosed with CML. The status of CML included chronic (n=30), accelerated (n=3), blastic phases (n=4), and complete hematologic response (CHR) after treatment (n=40). A total of 40 PB samples were collected from age and sex-matched healthy controls (HC). Thirty eight patients who underwent staging work-up of lymphoma, but without BM involvement, were recruited for BM negative controls (NC). We measured the number of MDSC by flow cytometry using the FACSCanto II flow cytometer and FacsDIVA software (Becton-Dickinson Inc, CA, USA). We acquired 50,000 cells and quantified the frequencies of 2 subsets of MDSC, granulocytic MDSC (gMDSC, HLA-DRlowCD11b+CD14–CD33+CD15+) and monocytic MDSC (mMDSC, HLA-DRlowCD11b+CD14+) in PB and BM.
Results: The correlation between BM-gMDSC% and PB-gMDSC% was good (r=0.712, *P*<0.001) and BM-gMDSC% was linear to PB-gMDSC% (y=0.960x+4.101, r^2=0.507). BM-gMDSC% and PB-gMDSC% showed negative correlation with *BCR-ABL1* quantitation (r=–0.478 and r=–0.547, *P*<0.001, respectively). BM-gMDSC% at diagnosis (mean±SD; 5.8±13.8%) were significantly lower than that of the CHR (40.6±16.4%) and BM NC group (37.8±11.3%) (*P*<0.001, respectively) (Fig.1). BM-gMDSC% was lower in the *BCR-ABL1* positive CHR group (23.4±18.3%) as compared to the *BCR-ABL1* negative CHR group (31.4±14.1%), but not statistically significant (*P*=0.123). PB-gMDSC% at diagnosis (8.7±14.4%) was also significantly lower than that of the CHR (22.8±11.2%) and HC groups (27.7±10.9%) (*P*<0.001, respectively). However, there was no statistical difference in the mMDSC% in both BM and PB. The overall survival rate of

the high BM-gMDSC% group (≥20%) was higher than that of the low BM-gMDSC% group (<20%) in both diagnosis and CHR groups (*P*=0.430 and *P*=0.011, respectively). However, both BM-mMDSC% and PB-mMDSC% did not show significant differences between each group.

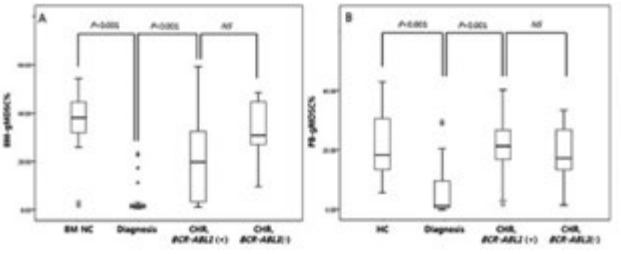


Figure 1.

Summary/Conclusion: BM-gMDSC% and PB-gMDSC% at diagnosis were significantly lower than those of BM NC and HC, respectively. Higher gMDSC% at CHR might be related to good prognosis in CML patients. These findings are contrary to the known MDSC findings in other malignancies. Granulocytes in CML are differentiated from leukemic stem cells, and probably the emergent myelopoietic condition does not affect the granulopoiesis of CML. The increased MDSC in CHR might reflect the regeneration of normal granulopoietic precursors.

PB1919

INTOLERANCES AND HOSPITALIZATIONS AFTER 1, 3 AND 5 YEARS OF TKI THERAPY IN SIMPLICITY, A STUDY OF CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA PATIENTS IN ROUTINE CLINICAL PRACTICE

M. Mauro[1,*], S.L. Goldberg[2], C. Gambacorti-Passerini[3], B. Simonsson[4], C. Davis[5], F. De Solda[5], G.P. Sen[6], S. Gajavelli[5], J. Cortes[7]

[1]Memorial Sloan-Kettering Cancer Center, New York, [2]Hackensack Meridian Health, New Jersey, United States, [3]University of Milano Bicocca, San Gerardo Hospital di Monza, Monza, Italy, [4]Uppsala Universitet, Uppsala, Sweden, [5]Bristol-Myers Squibb, Princeton, [6]ICON Plc, San Diego, [7]MD Anderson Cancer Center, Houston, United States

Background: Deeper insight is needed into the safety of tyrosine kinase inhibitors (TKIs) in chronic-phase chronic myeloid leukemia (CP-CML) outside clinical trials. SIMPLICITY (NCT01244750) is an ongoing observational study of CP-CML patients (pts) in routine clinical practice receiving first-line (1L) imatinib (IM), dasatinib (DAS) or nilotinib (NIL) in the US and Europe.
Aims: This analysis describes intolerances and reasons for hospitalizations (collectively defined as 'events') in the retrospective/prospective SIMPLICITY cohorts, in pts with 1, 3 and 5 years (yrs) of follow-up (FU) after start of 1L TKI.
Methods: Events collected consisted of intolerances on TKI change (dose change, discontinuation, or switching) and reported hospitalizations and associated reason. Demographics are given for pts with ≥1 event, and those with no events, by 5 yrs. Mean (±SD) number (no.) of events during 1, 3 and 5 yrs of FU are presented for the overall population, by 1L TKI, and in pts remaining on 1L TKI *vs* those switching in the FU period. Pts discontinuing 1L TKI without switching to 2L TKI were excluded from the analysis. Common adverse events (AEs; all grades) are given by system organ class (SOC) for the overall population and will be given for the specified sub-cohorts.
Results: By September 07 2017, 1492 pts were enrolled in SIMPLICITY, of which 454, 666 and 720 pts had ≥1 events by 1, 3 and 5 yrs (of which 446, 586 and 357 pts had ≥1, 3 and 5 yrs of FU), and 990, 736 and 637 pts had no events (of which 961, 627 and 293 pts had ≥1, 3 and 5 yrs of FU). The median (interquartile range [IQR]; min–max) age of pts was 61 (50–71; 19–95) yrs in pts with ≥1 event and 52 (43–64; 18–91) yrs in pts with no events by 5 yrs. Pts with ≥1 event by 5 yrs had a higher mean (±SD) no. of comorbidities (3.8 [±2.9] *vs* 2.5 [±2.4]) than pts with no events (p<0.001). There was a mean (±SD) of 2.6 (±2.2) events in 454 pts with ≥1 event by 1 yr: 3.4 (±2.6) events for pts who switched *vs* 2.1 (±1.7) events in pts remaining on 1L TKI. There were 3.3 (±3.2) events in 666 pts with ≥1 event by 3 yrs: 4.2 (±3.6) events for pts who switched *vs* 2.3 (±2.4) events in pts remaining on 1L TKI. There were 3.7 (±3.8) events in 720 pts with ≥1 event by 5 yrs: 4.7 (±4.3) events for pts switching *vs* 2.6 (±2.6) in pts remaining on 1L TKI. There were differences between cohorts, with pts receiving 1L NIL having the highest no. of events at 1, 3 and 5 yrs (3.0, 3.9 and 4.1 *vs* 2.3, 3.0 and 3.4 for DAS [p=0.02, p=0.009, p=0.08], 2.5, 3.2 and 3.7 [p=0.06 p=0.04, p=0.29] for prospective IM, and 2.8, 3.2 and 3.7 [ns] for retrospective IM). Across TKIs, common AEs by SOC were gastrointestinal disorders (27.8%, 29.4% and 31.5% by 1, 3 and 5 yrs), blood/lymphatic disorders (24.0%, 19.8%, and 19.4% by 1, 3 and 5 yrs), respiratory disorders (15.9%, 20.7% and 24.2% by 1, 3 and 5 yrs), and skin/subcutaneous disorders (14.5% and 14.1% by 1 and 3 yrs). No. and reasons for death will be presented.
Summary/Conclusion: Events were more frequent in pts switching *vs* those remaining on 1L TKI, and inter-cohort comparisons showed the no. of events to be highest in 1L NIL pts at all time points. The no. of pts with events increased with longer FU. Common AEs are similar to those reported for TKIs in pivotal trials. SIMPLICITY is not designed to comprehensively capture these events, and thus presented data may offer a snapshot of TKI profiles. Our findings suggest, in routine clinical practice, events accrue with time, are associated with TKI switch, and vary by TKI; further analysis is ongoing.

PB1920

TARGET: A SURVEY OF REAL-WORLD MANAGEMENT OF CHRONIC MYELOID LEUKEMIA ACROSS 33 COUNTRIES

A. Turkina[1,*], J. Wang[2], V. Mathews[3], G. Saydam[4], C.W. Jung[5], H. Al Hashmi[6], M. Yassin[7], T. Hughes[8]

[1]Scientific and advisory department of hemotherapy of myeloproliferative disorders, National Research Center for Hematology, Moscow, Russian Federation, [2]Chinese Academy of Medical Sciences and Peking Union Medical College, Institute of Hematology and Blood Disease Hospital, Tianjin, China, [3]Department of Haematology, Christian Medical College, Vellore, India, [4]Department of Hematology, Ege University Hospital, Izmir, Turkey, [5]Samsung Medical Center, Seoul, Korea, Republic Of, [6]Adult Hematology and Hematopoietic Stem Cell Transplant Department, Oncology Center King Fahad Specialist Hospital, Dammam, Saudi Arabia, [7]National Center for Cancer Care and Research, Doha, Qatar, [8]South Australian Health and Medical Research Institute, Adelaide, Australia

Background: Despite the availability of multiple guidelines for the management of chronic myeloid leukemia (CML), technical, financial and educational challenges, among others, may prevent some physicians from implementing these recommendations. In this context, the TARGET survey was developed by a Steering Committee consisting of 8 international hematologists (from Australia, China, India, Qatar, Russia, Saudi Arabia, South Korea, and Turkey).
Aims: The aims of the TARGET survey were to: I. Assess the current care of patients with CML in the participating countries compared with international guideline recommendations; II. Identify the challenges faced by physicians in implementing these recommendations; III. Develop practical solutions to support physicians in optimizing the management of CML patients.
Methods: Data were collected via a self-administered questionnaire, completed online, from 1-Apr-17 to 31-Aug-17. It included 23 questions divided into 7 sections (physician's profile, CML diagnosis, molecular monitoring and mutation analysis, treatment objectives, treatment efficacy, treatment toxicities, treatment discontinuation) and 7 clinical cases. The survey covered multiple regions worldwide (Africa, Asia, Australia, Middle East, Russia & Turkey) with 33 participating countries and was available in English, Russian, Turkish and Chinese. The analysis was performed using MODALISA software. Results are reported descriptively.
Results: Of the 1008 physicians contacted, 614 completed the questionnaire. The majority (59%) were male and practiced in Russia (19%), China (13%), India (12%), Turkey (8%) & S.Korea (7%). Most respondents (67%) had been treating CML for >10 years; 30% had personally seen 20-50 CML patients in the previous year. Molecular monitoring: 26% did not have access to a standardised PCR test and a further 52% were unaware of when the last standardization had occurred. Despite 89% of respondents knowing that BCR-ABL levels should be assessed every 3 mths in the first 12 mths of treatment, this was only achieved in clinical practice for 51% of respondents with cost being the main barrier (48%). Treatment toxicities: 4% respondents would change treatment for persistent Grade 1 adverse events (AEs). In contrast, 81% & 90% would do so for Grade 3/4 hematological & nonhematological AEs. Opinions were divided in the case of persistent Grade 2 AEs (59% would switch for nonhematological AEs). Treatment Free Remission (TFR) was considered by few (6%) as their primary treatment goal. Achieving deep molecular response was recognized by 47% as a prerequisite for TFR. The major hurdles for attempting TFR in current practice were lack of guidelines (30%), and insufficient molecular monitoring capability (21%-frequency and/or sensitivity). Management of

patients with a "warning" response also yielded mixed responses based on the clinical cases: 53% followed the ELN guideline recommendation of 'watch & wait' whilst 47% adjusted the hypothetical patient's treatment. Similarly, in the clinical case study of a patient receiving imatinib 400mg/day with an optimal response (MMR at 12 mths) but not in deep molecular response at 24 mths, 41% would change the patient's treatment.
Summary/Conclusion: The TARGET survey identified several gaps in current CML management when compared with international guidelines. Practical solutions to address these gaps are currently being developed.

PB1921

HIGH DIVERSITY IN THE SEQUENTIAL TREATMENT WITH TKI'S OF CML. A LONG-TERM, REAL LIFE ANALYSIS OF THE SPANISH REGISTRY OF CML (RELMC)

L.F. Casado Montero[1,*], N. García Ormeña[2], C. Mba[3], J.V. García Gutierrez[4], P. Giraldo[5], S. Osorio[6], M. Pérez Encinas[7], R. De Paz[8], J. Martínez López[9], G. Bautista[10], M.J. Requena[11], L. Palomera[12], M.J. Peñarrubia[13], C. Calle[14], J.A. Hernández Rivas[15], J.L. Steegmann[16]

[1]Hematology, Hospital Virgen de la Salud, [2]Registro Español de LMC, Toledo, [3]Registro Español de LMC, [4]Hematology, Hospital Ramón y Cajal, Madrid, [5]Traslational Research Unit, Instituto de Investigación Sanitaria Aragón (IIS Aragón), Zaragoza, [6]Hematology, Hospital Gregorio Marañón, Madrid, [7]Hematology, Hospital Clínico Universitario de Santiago de Compostela, Santiago de Compostela, [8]Hematology, Hospital La Paz, [9]Hematology, Hospital-[12]-de-Octubre, [10]Hematology, Hospital Puerta de Hierro, Madrid, [11]Hematology,, Hospital Universitario Severo Ochoa, Leganés, [12]Hematology, Hospital Clínico Universitario Lozano Blesa, Zaragoza, [13]Hematology,, Hospital Clínico Universitario Valladolid, Valladolid, [14]Hematology, Hospital General de Ciudad Real, Ciudad Real, [15]Hematology, Hospital Universitario Infanta Leonor, [16]Hematology, Hospital Universitario de la Princesa, Madrid, Spain

Background: Several tyrosine kinase inhibitors (TKIs) are available for treatment of patients with chronic myeloid leukaemia in chronic phase (CML-CP).
Aims: We analysed different TKI modalities used as therapy for CML-CP in a long-term analysis.
Methods: In a retrospective cohort analysis, we included data from patients with CML-CP treated in clinical practice with TKI modalities at Spanish Registry of CML (RELMC) (17 hospitals around all the country) between 2000 to 2014. The main aim of the study was to describe the sequence of TKI treatment, in real life practice and the rate of last deep molecular response (DMR) (MR^4, $MR^{4.5}$ or undetectable transcript) in each scheme.

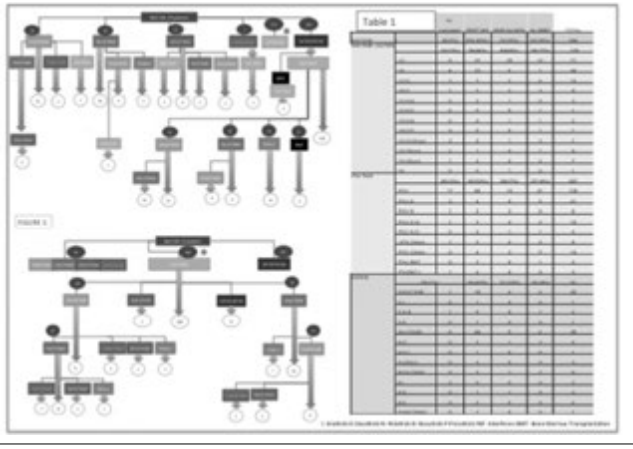


Figure 1.

Results: Our analysis included 862 patients with CML in first chronic phase treated with TKIs in first line or after Interferon alpha. **Demographics** 517 M, 345 F, median age: 52 y (14-94). Sokal Index distribution (low 49% Inter 38% High 13%), EURO Index distribution (low 50% Inter 45% High 5%), EUTOS Index distribution (low 93% High 7%), LT-EUTOS Index distribution (low 68% Inter 25% High 7%). **Schemes of treatment:** Table 1 summarizes all the schemes used and the last molecular response. Patients were divided in 4 groups depending of TKIs treatment. Group 1: Only treated wit Imatinib 394(45,7%) Group 2: Imatinib and then 2ºGTKIs due to intolerance or failure 170 p (19,7%) (12 schemes of sequential TKIs treatment) Group 3 2ºGTKs in first line 91 p (13 sequential schemes) (10,5%) Group 4 Interferon alpha and then ITKs: 207 (24%)(9 sequential schemes). Figure 1 summarizes evolution of different treatments around 14 years. **Last deep molecular response:** With a median of follow up of 82 months (1-351 months) from diagnosis, 77 months (1-311 months) from first treatment and 70 months (1-191 months) from first TKI treatment. The rates of deep molecular response for each group were (G1: DMR 65% MMR 13% No MMR 15%, G2: DMR 46% MMR 24% No MMR 17% G3: DMR 62% MMR 13% No MMR 12% G4: DMR 53% MMR 17% No MMR 18%). **Long-term survival (PFS or OS):** We did not find statistical differences between groups of treatment, either from diagnosis, first treatment or first TKI. Reaching a deep response guarantees better outcomes. **Predicting variables:** SOKAL, EUTOS, EURO and LT-EUTOs indexes continue to be useful in predicting long-term outcome.
Summary/Conclusion: In the setting of a multicentric, hospital-based CML registry, treatment with TKIs is very variable, resulting in a great number of sequential combinations of TKI. The rate of deep molecular response is roughly 60% in patients treated with imatinib and not needing change of TKI, and in those treated upfront with 2ºGTKI, but appears lower in patients treated with imatinib upfront, but who need to switch to 2ºGTKIs. Survival outcomes were not different, although it is worth to point out the shorter follow-up of patients treated upfront with 2ºGTKIs.

PB1922

IMPACT OF BCR-ABL TYROSINE KINASE INHIBITORS ON THE SURVIVAL AND FUNCTIONS OF ENDOTHELIAL CELLS

H. Haguet[1,2,*], J. Douxfils[1], C. Chatelain[1], C. Graux[3], A. Wannez[1], F. Mullier[2], J.-M. Dogné[1]

[1]Pharmacy department, University of Namur, Namur, [2]Hematology Laboratory, [3]Hematology department, CHU UCL Namur, Yvoir, Belgium

Background: BCR-ABL tyrosine kinase inhibitors (TKIs) are the mainstay of the treatment from chronic myeloid leukemia (CML). However, new generation TKIs (dasatinib, nilotinib, bosutinib and ponatinib) increase the risk of arterial occlusive events compared with the first generation (imatinib). To date, the mechanism(s) of these side effects is poorly understood but clinical data suggest that new generation TKIs might accelerate atherogenesis, in which endothelial cells play major function (e.g. lipid deposition, leukocyte recruitment).
Aims: This research aims to determine the effect of BCR-ABL TKIs on endothelial cell survival and major functions (endothelial cell migration and expression of adhesion molecules) using an *in vitro* model.
Methods: All experiments were performed on endothelial cells derived from human umbilical vein (HUVEC). Viability after 24h of TKI treatment was assessed using MTS and LDH assays following standard protocols. Reactive oxygen species (ROS) generation was quantified using a ROS detection reagent (CM-H2DCFDA) following the manufacturer instructions. The cell-surface expression of 3 adhesion molecules (ICAM-1, VCAM-1 and E-selectin) after 4-hour TNF-α activation (10ng/mL) was measured by on-cell ELISA and flow cytometry. Migration of endothelial cells was evaluated by a scratch assay (*i.e.* scratch of a confluent monolayer with a pipette tip and monitoring of the wound closure by inverted microscopy).
Results: Dasatinib, nilotinib and ponatinib at high concentration reduce cell metabolism indicating an inhibition of proliferation or induction of apoptosis by these 3 TKIs. Additionally, high-dose ponatinib (0.5µM) induces necrosis as demonstrated by increased LDH release. The analysis of ROS generation demonstrates that the 5 TKIs do not induce oxidative stress and do not alter cell viability by this way. Leukocyte recruitment is also an important process in atherogenesis and requires the expression of adhesion molecules by activated endothelial cells. The expression of ICAM-1, VCAM-1 and E-selectin was quantified by on-cell ELISA in HUVEC which demonstrated a decreased of ICAM-1, VCAM-1 and E-selectin expression with dasatinib, nilotinib and ponatinib at high concentration (0.5µM, 2µM and 0.5µM respectively) and no or little impact of imatinib and bosutinib. This reduction correlates with the decreased viability of endothelial cells with these 3 treatments, an hypothesis that has been confirmed by flow cytometry. Finally, we evaluate the migration ability of endothelial cell after treatment by scratch assay. High dose dasatinib inhibits endothelial cell migration whereas other TKIs do not have any impact. Therefore, dasatinib might induce arterial occlusive events through impaired vascular wound healing.
Summary/Conclusion: Over the 5 commercialized BCR-ABL TKIs, dasatinib, nilotinib and ponatinib possess the most impact on endothelial cells. They reduce viability by inducing apoptosis, necrosis or inhibiting cell proliferation, which possibly help to the development of atherosclerosis through impaired endothelium permeability, enabling migration and trapping of lipoprotein into the intima. Additionally, dasatinib reduces endothelial cell migration, which might contribute to formation of arterial thrombosis. Determination of mechanism(s) by which TKIs promote cardiovascular events is required to implement appropriate risk minimization measures and select patients to whom the prescription of these drugs should be avoided.

PB1923

COMPARISON OF MOLECULAR RESPONSES AND OUTCOMES IN CHRONIC MYELOID LEUKEMIA-CHRONIC PHASE PATIENTS TREATED WITH NILOTINIB INITIALLY FOLLOWED BY IMATINIB, COMPARED TO TREATMENT WITH IMATINIB UPFRONT

A. Jain*, A. Irom, M. Mahapatra, T. Seth, R. Saxena, S. Sazawal

Clinical Hematology, AIIMS, New Delhi, Delhi, India

Background: In CML-CP, Imatinib remains the drug of choice for frontline therapy in resource constrained countries, despite newer Tyrosine Kinase Inhibitors. However, Nilotinib/Dasatinib although costlier, have the advantage of earlier and deeper molecular responses. Giving Nilotinib for the first 3 months and then switching to Imatinib to capitalise on the quicker and deeper responses without adding substantially to the cost of treatment has never been studied before.
Aims: To compare the molecular responses and long term outcomes in treatment naïve CML-CP patients treated with Nilotinib for the initial 3 months followed by Imatinib compared to treatment with Imatinib upfront.
Methods: Newly diagnosed treatment naïve CML –CP patients affording therapy, who gave consent were included in the study. Accelerated phase/blast crisis patients were excluded. Patients were divided into 2 groups. Group 1 received Imatinib 400 OD upfront. Group 2 received Nilotinib 300BD for 1st 3 months and then were switched over to Imatinib 400 OD after 3 months. Clinical and lab follow up with complte blood counts and quantitative RT-PCR for Bcr-abl was done at 3, 6 and 12 months.
Results: 21 patients in group 1 and 18 patients in Group 2 were enrolled and followed up. Mean age was 37.4 and 38.16 yrs respectively. Group 2 had more males than group 1 (77.77% *vs* 33.33%, p value 0.006). Sokal score profile was not statistically different in both the groups (low risk: 33.33%vs 22.22%, intermediate risk: 28% *vs* 44.44%, high risk: 38% *vs* 33.33%). The mean RQPCR for bcr-abl was significantly higher at 3 months in patients in Group 1 compared to Group 2 (Table 1). A warning response was also more common in Group 1. The 6 and 12 month responses were however not significantly different in both the groups. In Group 2, there were 5 patients (1 Sokal low, 3 intermediate and 1 high risk) who achieved optimal response at 3 months, however had loss of response at 6 months (3) and 12 months (2). Only 1 patient on Imatinib had loss of respose at 6 months.

Table 1. RQPCR for bcr abl at various time points in both groups.

	3 months			6 months			12 months		
	Group 1	Group 2	P	Group 1	Group 2	P	Group 1	Group 2	P
N (data available for)	18	17		16	16		9	13	
Mean	21.67	2.424	0.018	2.182	3.703	0.47	1.333	3.05	0.5745
>10%	6	0	0.01	2	3	0.63	0	1	0.48
>1 to 9.99 %	9	9	0.86	3	4	0.67	2	2	0.49
>0.1 to 0.99%	3	5	0.37	4	4	0	0	3	0.20
<0.1%	0	3	0.06	7	5	0.47	7	7	0.07
AP/BC	1 (AP)	1 (BC)		0	0		0	0	

Summary/Conclusion: Nilotinib shows significantly better responses than Imatinib at 3 months. This early advantage with Nilotinib is not sustained over time with both groups having similar responses at 6 and 12 months. Furthermore, patients may lose the initial reposnse on Nilotinib and progress when switched to Imatinib. However achieving early molecular responses almost always results in good long term outcomes. Thus the duration of Nilotinib may have to be increased to benefit the majority of the patients before switching over to Imatinib.

PB1924

IS THE TIME TO CYTOGENETIC RESPONSE PREDICTIVE FOR SURVIVAL IN CHRONIC MYELOID LEUKEMIA? POPULATION DATA FROM RUSSIAN CML REGISTRY AND SIMULATION MODEL

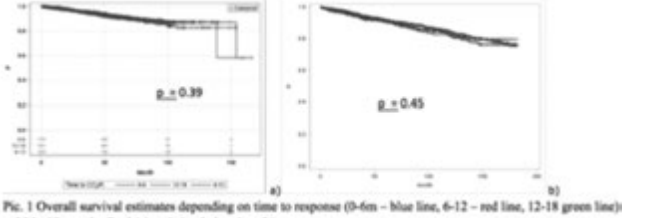
S. Kulikov[1,*], A. Kulikovsky[1], O. Lazareva[2], A. Turkina[2], A. Golenkov[3], T. Pospelova[4], S. Kutsev[5], J. Shatokhin[6], T. Konstantinova[7], A. Zaritzkey[8], E. Parovichnikova[9], V. Savchenko[9]

[1]Laboratory of Biostatistics and Information Systems, [2]Department for chemotherapy of myeloproliferative disorders, National Research Center for Hematology, [3]Regional Scientific Research Clinical Institute named after M.F. Vladimirsky, Moscow, [4]Hematology Center of Novosibirsk, Novosibirsk, [5]Research Centre for Medical Genetics, Moscow, [6]State Medical University, Rostov, [7]Sverdlovsk Regional Clinical Hospital No. [1], Ekaterinburg, [8]Almazov National Medical Research Center, St.-Petersburg, [9]National Research Center for Hematology, Moscow, Russian Federation

Background: The belief that "not only the response, but also the early response to therapy" predicts the best long term clinical outcome is common in chronic myeloid leukemia (CML). The latest data do not confirm that time to response is relevant for overall survival.
Aims: The aim of this study was to check it on the data of the Russian CML Registy and on simulation model.
Methods: Russian CML Registry include more than 10 thousand patients (pts) data. In the analysis 8326 CML pts in chronic phase(CP) with first line TKI therapy were included: 91% of pts were treated by Imatinib and 9% by other TKIs. Mean age was 47.3 years, 4607 f / 3705 m. Date of Complete Cytogenetic Response (CCyR) was assessed as the date of first test with 0% of Ph'+ cells or date of molecular test with BCR/ABL ≤0.1% IS. Overall survival (OS) was estimated starting land-mark (LM) time point, event was death from any reason, date of last contact was censored for alive pts. Survival analysis and simulation was performed by SAS statistics. Distribution of time to response and to death was modeled as mixture of exponents with parameters fitted to real data.
Results: Firstly we followed traditional way and compared overall survival estimates (OS) depending upon the response status (yes/no) at several LM time points (table 1,a).There are significant differences in OS at all LMs as expected. Then in the analysis we included only responded pts at a LM time =18 months and compared OS in 3 groups with different times to response: 0-6 months, 6-12 months, 12-18 months (pic.1,a). There are no significant differences in OS. The COX regression analysis also does not find significant influence of time to response on the OS. The distribution of time to response was fitted by the mixture of 3 exponents related 3 groups of responders: fast-runners, moderate-runners and resistant pts. The parameters was following: 1 group: 6.5m, 60%, 2 group: 35 m, 32%, 3 group: 220 m, 8%, where fist value is mean time to respond, second-portion in cohort. Then we suppose that mean life duration in 1 and 2 groups equal to 50years, and for 3 group-9 years. The simulation results for n=5000 pts is displayed in table 1, b and picture 1, b.*:* The deal is that in LM analysis you compare the group of fast-runner with mixture of slow runners and "never" responders. The result depends upon the proportion of compounds in the second group. So, our analysis does not confirm significant correlation of the survival and speed of respond.

Table 1. LM OS estimates of responder *vs* non-responders, all p<0.001. Real (a) and simulation (b) data.

LM	a) Real registery data	b) Simulation data
	10years OS, CCyR vs nonCCyR	10years OS, CCyR vs nonCCyR
6m	82% vs 73%	84% vs 74%
12m	85% vs 67%	84% vs 69%
18m	86% vs 63%	83% vs 67%



Pic. 1 Overall survival estimates depending on time to response (0-6m – blue line, 6-12 – red line, 12-18 green line) at LM =18 month. Real (a) and simulation data (b).

Figure 1.

Summary/Conclusion: The population of CML pts is a mixture of "any time" responders and "never"-responders. Tradition LM analysis output is wrong treated as evidence that survival depends of the time to respond. More specific analysis does not confirm that. This was demonstrated on big population data and explained by simulation model.

PB1925

GASTRIC CONDITIONS AS PROXY FOR HELICOBACTER PYLORI INFECTION AND RISK OF CHRONIC MYELOID LEUKEMIA

G. Larfors[1,*], J. Richter[2], A. Själander[3], L. Stenke[4], M. Höglund[1]

[1]Department of Medical Sciences, Uppsala university, Uppsala, [2]Dep of Hematology, Oncology and Radiation Physics, Skåne university hospital, Lund, [3]Department of Public Health and Clinical Medicine, Umeå university, Umeå, [4]Department of Medicine, Karolinska institutet, Stockholm, Sweden

Background: Although the pathophysiological mechanisms in chronic myeloid leukemia (CML) have been elucidated to a large degree, no etio-

logical risk factors have been clearly established. Slightly simplified, we know a lot about what happens, but we know little of why. Peptic ulcers in CML patients have been reported for long, but it was only recently that a study found an increased incidence of ulcers before onset of CML, indicating a common etiological mechanism, rather than ulcers being a consequence of the malignancy. As Helicobacter pylori is both a known risk factor for ulcers, and a known carcinogen, it could serve as a candidate for such a common risk factor.
Aims: To evaluate a possible relationship between chronic Helicobacter infection and risk of CML by using previous gastric conditions or medication as proxy variables for Helicobacter infection.
Methods: In a matched case-control design, 980 patients registered in the Swedish CML Register and diagnosed between 2002 and 2012 were compared to 4960 control subjects, randomly selected from the Swedish population and matched on age, sex, and region of residence. Records of previous medical conditions and prescribed medications were retrieved from the National Patient Register and the Swedish Prescribed Drug Register, respectively. From these and other registers, data were further collected on potential confounders such as co-morbidities and socio-economic status.
Results: Compared to controls, CML cases significantly more often had a previous diagnosis of dyspepsia, gastritis, or peptic ulcer, with odds ratios between 1.8–2.0. Adjustments for socio-economic factors and co-morbidity did not substantially change these estimates. Furthermore, previous prescription of proton pump inhibitors was more frequent among CML cases than controls (OR 1.5, p=0.0005). Meanwhile, neither inflammatory bowel disease nor the intake of non-steroid anti-inflammatory drugs were associated with CML, indicating that it is not gastrointestinal ulcer or inflammation per se which influences risk.
Summary/Conclusion: The present study provides epidemiological evidence consistently linking certain previous gastric conditions or medications to CML risk. All of these conditions have known correlations to Helicobacter pylori infection. Although far from decisive, the data indicate that H pylori could be involved in CML carcinogenesis. H pylori is a known carcinogen, but has previously not been implicated in myeloid malignancies. The findings call for further studies with biological material from CML patients, to validate the possible relationship.

PB1926

EVOLUTION OF PLASMA LEVELS, RESPONSE AND TOLERANCE TO IMATINIB AFTER SWITCHING FROM GLIVEC® TO GENERIC IMATINIB IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP)

A. Wojnicz[1], J. Cornago[2], V. Vaitekovich[3], C. Mba[3], E. Sola[2], C. Martínez[4], J.L. Steegmann[2,3,*]

[1]Clinical Pharmacology Dpt and IIS-IP, [2]Hematology Dpt. Hospital de la Princesa, [3]IIS-IP. Hospital de la Princesa, [4]Pharmacy Dpt. Hospital de la Princesa, University Hospital La Princesa, Madrid, Spain

Background: Although switching to generic imatinib is increasingly used across the world, mainly because of economic reasons, there is a paucity of information regarding the effects of this switch on plasma levels, response and treatment tolerance.
Aims: Primary: Determine and compare plasma levels of imatinib after switching from Glivec to generic imatinib. Secondary: To assess if response or tolerance are changed after switching.
Methods: Imatinib plasma levels, BCR-ABL (IS) values and tolerance were measured before switching (while the patient was taken Glivec), and after 1 month and 3 months of treatment with generic imatinib (all patients took the same brand of generic imatinib). The study was approved by the Ethics committee, and all patients gave their informed consent. Plasma levels were measured using liquid chromatography tandem mass spectrometry method as previously published by our group. BCR-ABL values are expressed in IS. Cmin, Cmax, BCR-ABL values and standard hematologic and biochemistry values (including PTH and CK values) were entered in the statistic model. Descriptive statistics, means comparison and correlations were done using SPSS package.
Results: All patients treated in our institution with Glivec were included. As most of our patients with CML are treated upfront with 2G TKI, only 15 patients have been included. The median duration of Glivec treatment before switching was 109 months (11-191). All patients but one (ID:1961) were at MR4 or better at baseline. At baseline, all the patients had adequate Cmin values(≥1µg/mL), except one (ID:1181), who has indetectable transcript at that time. After change to generic Imatinib, all samples showed adequate Cmin values, except two (ID:752, +1m and ID 1961, +3m). When comparing baseline with 1 month and 3 month values, there were no significant differences between the mean values of Cmin, BCR-ABL values and standard hematologic values. The only significant difference was between phosphate baseline and 1-month values (3±0.43 *vs* 2.77±0.27 mg/dl; p=0.039). As regard to response, all patients with MR4 or better maintained this kind of response, and the same occured with the patient with MMR. Concerning tolerance, there was no change in adverse events (AE) in any patient. In patient 1252, as plasma levels at baseline, 1m and 3m were higher than 1.8µg/mL, and considering that he had multiple low-grade toxicity, dose was reduced at 3m, with amelioration of AE.

Table 1.

ID PAC	Dose Glivec	BCR-ABL Baseline	Glivec Cmin*	BCR-ABL 1m	Ima. Cmin. 1m	BCR-ABL 3m	Ima. Cmin. 3m
752	400	0,005	2,17	0,006	0,69	0,007	1,83
1740	400	0,008	1,94	0,0018	1,17	0,002	1,93
1181	400	0	0,88	0	1,54	0	1,87
1071	300	0	1,43	0	1,48	0	
1233	300	0	1,27	0	1,39	0	1,55
629	300	0	1,56	0	1,47	0,0008	1,62
1252#	400	0,003	1,84	0,007	1,91	0,004	1,89
1302	400	0	1,60	0	1,61	0	1,97
1258	400	0	2,30	0	2,16	0	2,69
720	400	0,008	1,61	0,003	1,55	0,01	1,38
1961	400	0,03	1,61	0,03	1,13	0,03	0,40
708	300	0	1,05	0	1,49	0	1,59
726	400	0	1,06	0	1,16	0	1,08
1840	400	0,008	1,94	0,006	2,40	0,002	2,58
15	400	0	2,05	0,001		0,005	2,92

* Cmin values are expressed as µg/mL. #Dose of Pt 1252 was diminished to 300 mgld at 3m. Table 1.

Summary/Conclusion: Taking into account the limitation of our study (small sample size), it seems that changing from Glivec to generic Imatinib is not associated with significant changes in Cmin Imatinib values, BCR-ABL values or adverse events. The prompt and simultaneous measurement of plasma levels and molecular response has allowed us to reassure patients about the change, and to adjust the dose in one patient, diminishing the toxicity. Based on these results, we conclude that this approach is useful in patients whose treatment is switched from Glivec to generic imatinib.

PB1927

REAL-WORLD EVIDENCE OF MOLECULAR RESPONSE TO TYROSINE KINASE INHIBITORS FROM CHINA SUPPORTS EUROPEAN LEUKEMIANET 2013 RECOMMENDATIONS FOR THE MANAGEMENT OF CHRONIC MYELOID LEUKAEMIA

H. Yin[1,*], Y. Chen[1], L. Chen[1], Y. You[1], P. Zou[1], L. Meng[2], W. Li[1]

[1]Hematology, Union Hospital, Tongji Medical College of Huazhong University of Science and Technology, [2]Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science And Technology, Wuhan, China

Background: Currently, there lacks available real-world clinical efficacy data on different strategies, such as comparing the efficacy of frontline nilotinib with imatinib and the efficacy of second-line nilotinib in Pts resistant or intolerant to imatinib. Meanwhile, when to apply the second generation of TKIs instead of the first generation of TKIs as well as their clinical efficacy hasn't been defined.
Aims: The present study was aimed to explore these issues and optimize clinical therapy through evaluating molecular response at specific milestones according to ELN guidelines.
Methods: We have enrolled 319 Pts from our hospital from January 2000 to December 2017 who were newly diagnosed with CML. 29 Pts received frontline nilotinib and the other 290 received imatinib .The study was descriptively analyzed and evaluated the efficacy of imatinib and nilotinib.
Results: At data collection, CML-CP was confirmed in 309 of 319 Pts who were newly diagnosed, and CML-AP was confirmed in 10 of 319 the Pts. 210 (65.8%) Pts were male. Median follow-up was 45 (range 3 to 199) months and median age was 39 (range 2 to 76) years. The molecular responses of frontline therapy at ELN2013 milestones during the observation period are shown in Table 1. During the observation period, 84/290(29%) Pts with frontline imatinib switched to nilotinib; 32/84 switched early (within 12 months) and 52/84 switched later.Rates of CCyR at any time for Pts who switched early and later were 90.6%(29/32) and 88.5%(46/22) (*P*=0.756).Rates of MMR at any time for Pts who switched early and later were 84.4%(27/32) and 76.9%(40/52) (*P*=0.409). The documented main reason for first switch was treatment failure[(50/84(59.5%) Pts, medium time was 19.5 months] while 12/84 Pts (median time was 11 months) were switched for "warning", 15 (17.9%) Pts were switch for intolerance(median time was 21 months), 6/84 (7.1%) Pts were switched for

willing to withdrawal (medium time was 25.5 months) and one for unexplained eosinophilia(time was 35 months). Rates of CCyR and MMR at any time for Pts who switched TKI following a prior "warning " response were 83.3%(10/12) and 75%(9/12). Rates of CCyR and MMR at any time for Pts who switched TKI following a prior "failure " response were 86%(43/50) and 72%(36/50). 206/290 Pts remained on frontline imatinib with no observed switch. In the 54/206 Pts following "warning" response, 22/54 had a "failure" response at one or more ELN2013 milestones. In the 133/206 Pts following "optimal" response, 4/133 had a "failure" response and 1/133 had a "warning" response. Rates of CCyR and MMR at any time for Pts who remained on frontline imatinib following a prior "warning" response were 87.0%(47/54) and 66.7%(36/54). Rates of CCyR and MMR at any time for Pts who remained on frontline imatinib following a prior "failure" response were 78.9%(15/19) and 63.2%(12/19). Rates of CCyR and MMR at any time in Pts were 93.1%(270/290) and 82.8%(240/290) separately, significantly higher than that in the frontline imatinib group and similar to the rate of CCyR and MMR(100% and 86.2%) in the frontline nilotinib group.

Table 1.

Table 1. Comparison of frontline therapy outcomes

Response	first-line TKI (n = 319)		p value
	Nilotinib (n=29)	Imatinib(n=290)	
EMR or PCyR by 3 months	96.6% (28/29)	51.3% (138/269)	0.00C
BCR-ABL1IS≤10% or CCyR by 6 months	92.9% (26/28)	58.2% (164/282)	0.00C
MMR rate by 12 months	85.2% (23/27)	68.0% (176/259)	0.064
MMR rate by 18 months	95.2% (20/21)	77.3% (184/238)	0.054
CCyR rate overall	100% (29/29)	84.1% (244/290)	0.02C
MMR rate overall	93.1% (27/29)	77.9% (226/290)	0.054
CCyR rate at any time	100%(29/29)	83.8%(243/290)	0.025
MMR rate at any time	86.2%(25/29)	65.9%(191/290)	0.025
PFS rate	96.6% (28/29)	97.2% (282/290)	0.831
EFS rate	82.8% (24/29)	40.7% (118/290)	0.00C

Abbreviations:CCyR,Complete Cytogenetic Response;PCyR,Partial Cytogenetic Response; EMR,Early molecular response;MMR,Major molecular response;EFS,event-free survival; PFS,survival free from progression to AP or BP.

Summary/Conclusion: As the first-line drug for newly diagnosed CML Pts, Nilotinib can achieve a deeper molecular response in a shorter time than imatinib. According to ELN's suggestion, the clinical efficacy of Pts who switch early to nilotinib for resistant or intolerant to imatinib can reach similar efficacy as the first-line nilotinib. Overall, the results support the use of ELN2013 recommendations to guide TKI management.

PB1928

LONG- TERM SURVIVAL OF PATIENTS WITH CHRONIC MYELOID LEUKAEMIA IN RUSSIA: THE ANALYSIS OF 607 PATIENTS

O.V. Lazareva[1,*], E. Chelycheva[1], A. Kulikovsky[2], G. Safuanova[3], L. Gavrilova[4], L. Napso[5], E. Vasil'ev[6], N. Glonina[7], E. Ovsyannikova[8], V. Skatova[9], G. Milutina[10], T. Klitochenko[11], O. Senderova[12], L. Yalunina[13], A. Luchinin[14], L. Visotskaya[15], A. Golenkov[15], S. Volkova[16], A. Lyamkiva[17], E. Burnasheva[18], G. Kuchma[19], M. Koz'mina[20], M. Pepelyaeva[21], S. Meresiy[22], E. Fedorova[23], T. Konstantiniva[24], E. Volodicheva[25], E. Dasheeva[26], V. Yablokova[27], V. Shuvaev[28], E. Lomaia[29], A. Zaritskey[29], S. Kulikov[2], A. Turkina[1]

[1]Department for chemotherapy of myeloproliferative disorders, [2]Laboratory of Biostatistics and Information Systems, National Research Center for Hematology, Moscow, [3]Bashkir State Medical University, Ufa, [4]Republican Clinical Hospital #4, Saransk, [5]Clinical Oncological Dispensary No. [1], Krasnodar, [6]Regional Clinical Hospital, Krasnoyarsk, [7]Regional Clinical Hospital No. [1] named after prof. S.I. Sergeev, Khabarovsk, [8]Astrakhan State Medical University, Astrakhan, [9]Regional Clinical Hospital of St. Joasaph, Belgorod, [10]Regional hospital №1, Bryansk, [11]Clinical Oncology Dispensary, Volgograd, [12]Irkutsk Order "Sign of Honor" Regional Clinical Hospital, Irkutsk, [13]Regional Clinical Hospital, Kemerovo, [14]Scientific Research Institute of Hematology and Blood Transfusion of the Federal Medical and Biological Agency, Kirov, [15]Regional Scientific Research Clinical Institute named after M.F. Vladimirsky, Moscow, [16]Regional Clinical Hospital named after N.A. Semashko, Nizhny Novgorod, [17]State Medical University of Health Ministry of Russian Federation, Novosibirsk, [18]State Medical University of Health Ministry of Russian Federation, Rostov, [19]State Medical University, Orenburg, [20]Polyclinic №2 of the Department of Health of the Orel Region, Orel, [21]Order of the "Badge of Honor" Perm Regional Clinical Hospital, [22]State Clinical Hospital "Clinical Health Care Unit #1", Perm, [23]Regional Clinical Hospital named after V.D. Seredavin, Samara, [24]Regional City Hospital #1, Ekaterinburg, [25]Regional Clinical Hospital, Tula, [26]Regional Oncology Center, Chita, [27]State Medical University, Yaroslavl, [28]Russian Research Institute of Hematology and Transfusiology, [29]Almazov National Medical Research Center, St.-Petersburg, Russian Federation

Background: The results of long-term follow-up of patients with chronic myelogenous leukemia (CML) do not lose their importance. The use of TKI (imatinib, IM) resulted to dramatic improvement in survival, so probability of CML-related death for patients (pts) could be significantly lower than chance of dying due to common mortality causes besides than CML.
Aims: To analyze overall survival (OS) and causes of mortality in CML pts treated in routine clinical practice in Russian Federation for a long period (>12 years) of time.
Methods: The analyzed cohort consisted of 607 Ph/*BCR-ABL*-positive CML pts from 29 regions of Russia (ELN OSP EUTOS) diagnosed in 2002- 2006 with IM therapy initiation ≤6 months (mo) after diagnosis established. Median (Me) of age was 48(18-82) years (y), 47% males. Pretreatment regimens were as follows: hydroxyurea 454(76%) pts; chemotherapy 25(4%) pts, IFN-α 37(6%) pts. Chronic phase (CP) of CML was diagnosed in 557 (93%) pts, accelerated phase (AP)-38(6%) and blast crisis (BC)-in 6(1%) pts. The number of patients with CML according to years of diagnosis were: 2002-15pts, 2003-38pts, 2004-46pts, 2005-206pts, 2006-302pts. Last database update had been made on Nov. 2017 for 473 pts; in addition, 134 pts from 2 regions were updated in 2015.
Results: The median follow-up was 92,4 (1- 170,3) mo. At 12 years, OS was 50%(figure 1); OS by age groups were: 20-40yy- 68%, 40-60yy- 69%, 60-80yy- 40%. Mortality in the whole cohort of 607 pts was 27% (168 pts). Of these 168 pts, 91(54,2%)pts deaths were as results of CML progression to AP or BP including non-complaent cases; 3pts (1,7%) were reported as died in CP CML, 3pts (1,7%)-after allogeneic stem cell transplantation (2 due to infection complications), in 19 (11,4%) cause of death was unknown. Deaths caused by concomitant diseases were in 52 cases (31%): coronary artery disease/myocardial infarction/heart failure in 18 cases, acute ischemic stroke in 8 cases, second malignancies (Cr) in 8 cases (lung tumor, metastatic esophageal Cr, stomach Cr, brain tumor, Cr of the sigmoid colon, Cr of rectal and colon, melanoma, renal Cr), accidents-2 cases, liver cirrhosis in 2 pts, in 2 cases-respiratory virus infections complicated with pneumonia and others.

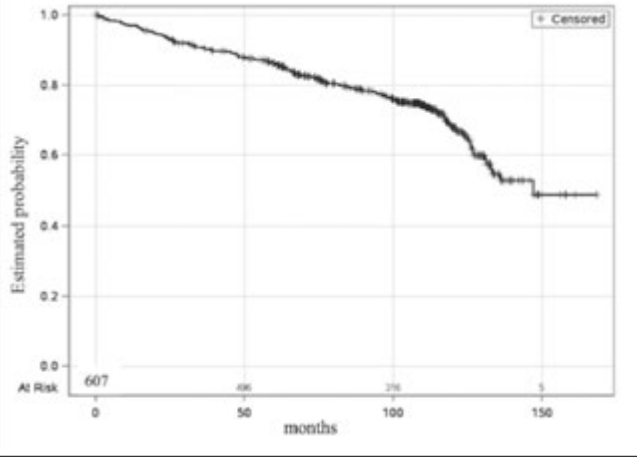


Figure 1.

Summary/Conclusion: Long-term follow-up of patients participated in the tracked cohort of 607 patients in the OSP EUTOS study allowed to characterize the results of OS and the cause of death according to age and comorbidity. The cause of death in our study in 34% of cases was unrelated to CML, which corresponds to the reference data (Castagnetti 2013, Hehlmann 2014, Pfirrmann 2015). Mortalities related to cardiovascular causes and second malignancies in pts with CML have significant proportion (50% and 15% from the whole mortality, respectively). A substantial reduction of OS from 78 to 50% is observed after 8 to 12 years of TKI therapy due to cohort aging and dying out for ordinary reasons.

PB1929

IMATINIB TREATMENT IN ELDERLY PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) IN THE REAL-LIFE SETTING: UPDATED DATA OF THE CERRAHPASA CML COHORT

D. Ozmen[1,*], M. Oztas[2], F. Bektas[2], A.E. Eskazan[1], M. Pehlivan[1],

S. Sadri[1], D. Keskin[1], N. Ozgur Yurttas[1], T. Elverdi[1], A. Salihoglu[1], M.C. Ar[1], S. Ongoren[1], Z. Baslar[1], Y. Aydin[1], T. Soysal[1]

[1]Department of Internal Medicine Division of Hematology, [2]Department of Internal Medicine, Istanbul University Cerrahpasa Medical Faculty, Istanbul, Turkey

Background: Tyrosine kinase inhibitors (TKIs) are the mainstay of chronic myeloid leukemia (CML) treatment. Median age of CML at diagnosis is 50 years, but a significant proportion of the patients (pts) are diagnosed after age 60. Pts with CML in chronic phase (CML-CP) nowadays live near-normal lifespans, thus, the number of elderly CML pts with comorbidities started to increase.This brings out the issues regarding TKI toxicities, medication adherence and responses to TKI therapy, and we have previously presented the real-life data regarding toxicity and efficacy of imatinib (IM) in our elderly CML cohort [Blood. 2016;128:1905].
Aims: The aim of this study is to update the previously presented data showing the efficacy and safety of IM in the elderly population (pts≥60 years; Group A) with CML and to compare these data with younger patients (pts<60 years; Group B).

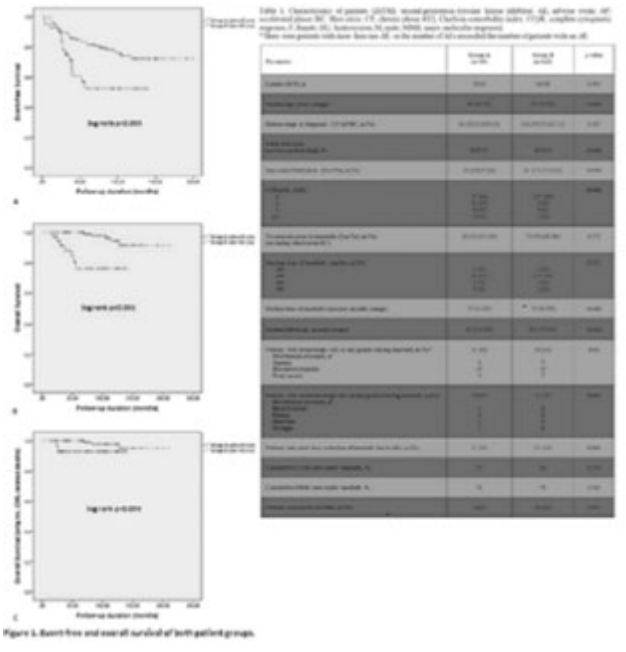


Figure 1.

Methods: Patient demographics, dose and duration of IM therapy, disease risk scores, cytogenetic and molecular responses, comorbidities, adverse events (AEs), follow-up durations and outcomes were evaluated from pts' files, retrospectively. The Charlson Comorbidity Index (CCI) of each patient was calculated as stated before [Haematologica. 2011;96(10):1457-61].
Results: Our cohort consisted of 181 pts with a median age of 46 years (range, 18-83 years). Group A consisted of 39 pts, and there were 142 pts in Group B (Table 1). The two groups were balanced regarding gender, disease stage, therapies prior to TKI, and the starting dose of IM. There were more pts with intermediate and high Sokal risk scores in Group A (p<0.001), and pts in Group A had significantly more comorbidities (p<0.001) with higher CCI scores (p<0.001). Median time of IM exposure (p<0.001) and follow-up durations (p<0.001) were significantly longer in Group B than those of Group A. There were significantly more hematologic AEs among pts in Group A than those of Group B (26% *vs* 10%, p=0.01). Nonhematologic AEs were significantly more common in Group A (20% *vs* 7%, p=0.022), and the rates of pts with IM dose reduction due to AEs were significantly higher in Group A than that of Group B (28% *vs* 11%, p=0.006). Cumulative complete cytogenetic and major molecular response rates and the percentage of patients who switched to second-generation TKIs were similar in both groups (Table 1). Event-free (Fig.1A) and overall (OS) (Fig.1B) survival rates were significantly higher in Group B than those of Group A (p=0.005 and p<0.001, significantly). There were 7 and five pts who died during the follow-up in Groups A and B, respectively. Among these deaths, 5 pts in Group A and two pts in Group B died due to non-CML related causes, and the OS rates were comparable when non-CML related deaths were excluded (Fig.1C) (p=0.096).
Summary/Conclusion: Although TKI therapy is relatively safe and effective in elderly pts with CML-CP, TKI-related AEs are more common in this population and comorbidities may play a role in the generation of TKI toxicities and outcomes. Prompt and timely management of these AEs and TKI dose modifications may lead to better outcomes. In our patient cohort, inferior OS rates were observed among elderly pts, but OS rates were similar in both groups when non-CML related deaths were excluded.

PB1930

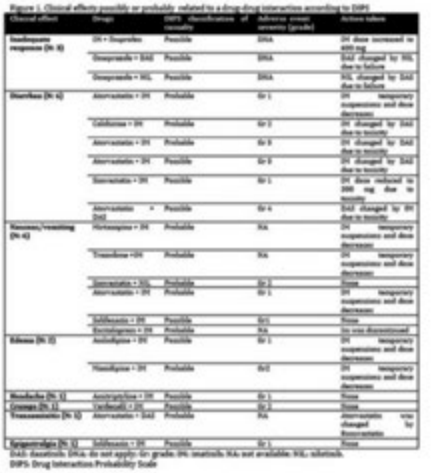
AN APPROACH TO DRUG-TO-DRUG INTERACTIONS IN CML PATIENTS TREATED WITH TYROSINE KINASE INHIBITORS. AN OBSERVATIONAL STUDY OF THE SPANISH CML GROUP (GELMC)

I. Gómez-Centurión[1,*], S. Osorio Prendes[1], V. Escudero Vilaplana[2], R. Pérez-López[3], R. Ayala[4], F. Vall-Llovera[5], V. García-Gutierrez[6], M.T. Gómez Casares[7], J.D. González San Miguel[8], J.-Á. Hernández-Rivas[9], F. Sánchez-Guijo[10], M. Romera[11], L. Villalón[12], V. Conesa[13], A. Rodriguez[14], F. Casado[15], M.N. Sáez Perdomo[7], Ú. Baños[14], J.L. Díez-Martín[1], J.L. Steegmann[16]

[1]Hematology, [2]Pharmacy, Hospital General Universitario Gregorio Marañón, Madrid, [3]Hematology, Hospital Universitario Clínico Virgen de la Arrixaca, Murcia, [4]Hematology, Hospital Universitario XII de Octubre, Madrid, [5]Hematology, Hospital Universitari Mútua Terrassa, Terrassa, [6]Hematology, Hospital Universitario Ramón y Cajal, Madrid, [7]Hematology, Hospital Universitario de Gran Canaria Dr. Negrín, [8]Hematology, Complejo Hospitalario Universitario Insular Materno Infantil de Canarias, Las Palmas de Gran Canaria, [9]Hematology, Hospital Universitario Infanta Leonor, Madrid, [10]Hematology, Complejo Asistencial Universitario de Salamanca, Salamanca, [11]Hematology, Hospital Santa Lucía de Cartagena, Cartagena, [12]Hematology, Hospital Universitario Fundación Alcorcón, Madrid, [13]Hematology, Hospital General Universitario de Elche, Elche, [14]Hematology, Hospital Universitario Virgen Macarena, Sevilla, [15]Hematology, Hospital Virgen de la Salud, Toledo, [16]Hematology, Hospital Universitario de la Princesa, Madrid, Spain

Background: Imatinib and the newer BCR-ABL tyrosine kinase inhibitors (TKIs) are the standard therapy for chronic myeloid leukemia (CML). With these drugs, CML patients are achieving similar survivals than the general population, thus classical aspects of chronic diseases, such as treatment adherence and drug-to-drug interactions (DDI) are becoming more important in patients management. DDIs between TKIs and some concurrent medications could lead to toxicity or inadequate response. Although this is a well known effect, and different guidelines include DDIs as a potencial cause of toxicity or resistance, the information about its frequency and its clinical impact is limited.
Aims: To determine the potential DDIs in CML patients treated with TKIs and its clinical impact.
Methods: This was a retrospective, collaborative study performed in 15 centers within the framework of the Spanish CML Group (GELMC). Each participating center included data from all new CML patients that were diagnosed between 1st January 2014 and 31st December 2015, treated with TKI as first-line therapy for CML in chronic or accelerated phase. Concurrent medications, adverse events (AEs), potential DDI and its potencial effects were analyzed.

Table 1.



Results: A total of 134 TKI treatments, in 105 patients were included. The mean number of concomitant medications was 4,8 (0-19). The mean number of AEs during the first year of treatment was 2 (SD: 1.9, range 0-11). The

AEs severity, according to common terminology criteria for adverse events (CTCAE) 4.03 version, was: grade 1, 40,7%; grade 2, 35,2%; grade 3, 16,1%; and grade 4, 4,8%. Sixtythree patients (60%) had at least one DDI. The mean number of DDIs by TKI treatment was 1,2 (0-8). It was significantly associated with the number of concomitant medications and age. A total of 159 DDIs were detected, involving 55 different drugs, being the most common types, proton pump inhibitors, statins and antidepressants. Clinical or analitical effects of DDIs were suspected by the investigators in only five patients (4,7%). This number increased to 20% in a central review. When such an association was suspected, we applied the DIPS scale (Drug Interaction Probability Scale) to try to estimate causality. We detected 21 clinical effects in 21 patients (20%), that according to the DIPS scale were possibly or probably related to a DDI: 18 (86%) of these effects were related to toxicity, and 3 (14%) to inadequate response (Figure 1). Most of these AEs attributed to DDIs were mild. The most common were diarrhea, vomiting, edema, cramps and transaminitis, and 78,5% were grade 1-2. We did not find significant differences in the frequency of AEs, or in the molecular response, in patients with or without DDIs.

Summary/Conclusion: Potential DDIs are present in most of patients treated with TKIs. A clinical effect was suspected by the treating phisycians in only 4.7% of the patients, but increased to 20% in the central review. Nevertheless most of this possible or probable clinical effects were mild, and could possibly had appeared with the individual drugs, thus it is difficult to be sure to what extent the DDIs have caused or worsened the AEs. We did not see a clear effect of DDI in response as a group, although 3 patients with inadequate response were taking drugs that could decrease TKI effectivity. Thus, due to DDIs high frequency, and the possibility of clinical relevant effects, we consider that physicians treating CML patients should consider this aspect in their patients management.

PB1931

Abstract withdrawn.

PB1932

ANALYSIS OF SERUM LIPIDS, CARDIOVASCULAR RISK AND INDICATION FOR STATIN THERAPY AT THE START AND DURING IMATINIB AND NILOTINIB THERAPY IN *DE NOVO* CML PATIENTS RESULTS FROM REAL-LIFE PROSPECTIVE STUDY

T. Horňák*, L. Semerád, D. Žáčková, B. Weinbergerová, Z. Šustková, J. Procházková, J. Mayer, Z. Ráčil

Department of Internal Medicine, Hematology and Oncology, Masaryk University and University Hospital Brno, Brno, Czech Republic

Background: Nilotinib (NILO) administration in CML patients (pts) is known to be associated with impairment of lipid metabolism, which is one of the possible risk factors leading to cardiovascular (CV) adverse events (AE) of NILO. On the contrary, these abnormalities were not observed during imatinib (IMA) treatment. A routine protocol for lipid and CV risk assessment at the start and during both TKIs administration is still not established.

Aims: 1) To analyse the serum lipids levels and CV risk in *de novo* CML pts treated with NILO and IMA at baseline and during the therapy. 2) To identify CML pts who meet the criteria for a hypolipidemic treatment according ESC/EAS guidelines at diagnosis and during the TKI therapy.

Methods: Consecutive pts diagnosed with CML between 6/2014-7/2017 treated with NILO or IMA in the 1st line were included in this prospective study. The selection of TKI was based on treating physician's decision. Patients received clinical and laboratory diagnostic workup and CV risk assessment according to ESC/EAS at the start of TKI therapy and then every 3 months for two years. Changes in serum lipids levels were analysed only in pts without hypolipidemic therapy at the study visit.

Results: Thirty- four pts treated with IMA and 23 with NILO were included in this trial (median follow-up: IMA=12m, NILO=9m). Pts in NILO group were significantly younger compared to IMA (median age-45 *vs* 67 yrs; p=0.0006) and had significantly less comorbidities (diabetes mellitus 0% *vs* 32%, p=0.0018; ischemic heart disease-0% *vs* 24%; p=0.0163). The baseline CV risk SCORE value of NILO pts was also significantly lower (SCORE-median-6% *vs* 1%; p=0.009). Moreover, there were significantly more pts already or newly indicated for statins at the start of IMA [IMA 68% (7/34 pts on statin, 16/34 pts newly indicated) *vs* NILO 30% (3/23 pts on statin, 4/23 newly indicated); p=0.0076]. These differences between groups reflect common selection of TKIs by treating physicians based on their known AEs. Baseline levels of total- and LDL- cholesterol (CHOL) between groups were not different, but this could be affected by different percentage of patients already on statins in both groups. During the first 6 months of treatment the level of total- and LDL- CHOL in NILO pts significantly increased. Total- and LDL- CHOL did not change in IMA pts. HDL cholesterol significantly increased in both groups (Table). During the first 6 months 2/34 (6%) IMA pts and 3/23 (13%) NILO pts previously classified at low CV risk moved to high or very high CV risk category (p=ns). No new CV event occurred in both groups. The number of pts additionally indicated for statin therapy during the first 6 months of TKI treatment was similar in both groups [IMA *vs* NILO-2/34 (5,9%) *vs* 2/23 (8,7%); p=ns].

Table 1.

Table. Baseline characteristics and changes of serum lipids during TKI therapy. (Baseline- M0, month 6- M6)

BASELINE CHARACTERISTICS			
	IMATINIB (n= 34)	NILOTINIB (n= 23)	p
Age median (yrs)	67	45	0.0006
Diabetes mellitus (n)	11	0	0.0018
Ischemic heart disease (n)	8	0	0.0163
CV risk SCORE median (%)	6	1	0.0009
Already on statins/ newly indicated	23	7	0.0076

CHANGES OF SERUM LIPIDS DURING TKI THERAPY				
	IMATINIB (M0 n= 21, M6 n= 16)	p	NILOTINIB (M0 n= 19, M6 n= 18)	p
Total CHOL M0 median (mmol/l)	5.2	0.0966	4.5	0.0001
Total CHOL M6 median (mmol/l)	4.5		5.5	
HDL- CHOL M0 median (mmol/l)	1.2	0.0013	1.0	0.0001
HDL- CHOL M6 median (mmol/l)	1.45		1.5	
LDL- CHOL M0 (mmol/l)	2.9	0.1456	2.7	0.0001
LDL- CHOL M6 median (mmol/l)	2.45		3.0	
Triacylglycerols M0 median (mmol/l)	2.04	0.0004	1.95	0.0045
Triacylglycerols M6 median (mmol/l)	1.28		1.32	

* only data of pts without hypolipidemic therapy at the time of study visit were analysed

Summary/Conclusion: Our prospective real-life study clearly showed the routine selection of TKI by treating physician in *de novo* CML based on comorbidities and age. Importantly, there is high percentage of patients with *de novo* CML in central European population who fulfil criteria for statins already at the start of TKI therapy (53% in our study). Even though NILO increases the total/LDL- CHOL, the increase of CV risk and number of pts newly indicated for statin therapy does not differ from the IMA group, at least not during the first 6 months of therapy. However, this may be influenced by mentioned routine selection of TKI in CML patients and also longer follow-up is needed.

PB1933

IMPACT OF KINASE DOMAIN MUTATIONS ON SECOND LINE NILOTINIB THERAPY IN CHRONIC MYELOID LEUKEMIA CHRONIC PHASE-REAL WORLD DATA FROM A DEVELOPING COUNTRY PERSPECTIVE

S. Francis Thekkudan*, A. Sharma, A. Gupta, S. Nityanand

Hematology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India

Background: Nilotinib is a second generation tyrosine kinase inhibitor (TKI) approved for adults with newly diagnosed chronic myeloid leukemia (CML) in chronic phase (CP) and those with Imatinib-resistant or intolerant CML-CP and CML in accelerated phase (AP). The occurrence and impact of baseline as well as evolving kinase domain (KD) mutations on Nilotinib therapy were assessed in this study. Also, no Indian data is available till date on the efficacy of second line nilotinib and this study reflects a real world data from a developing country with resource-constrained setting.

Aims: Primary objectives were to assess the molecular response as well as the prevalence, pattern and evolution of KD mutations before and during second line Nilotinib therapy in CML- CP patients.

Methods: Patients of CML-CP aged ≥18 years, who developed Imatinib-resistance or Imatinib-intolerance and thus switched to second line Nilotinib therapy, were included. KD mutation analysis was done retrospectively in the stored cDNA of samples extracted for BCR ABL analysis, by Sanger's direct sequencing. Mutation analysis was repeated when there was a sub-optimal response/loss of response at 6 months and 12-month time points.

Results: Of the total 674 newly diagnosed CML patients presented from January 2013-June 2016, 85 (12.6%) patients were started on Nilotinib, of which 11 patients were non compliant/irregular in follow up and thus excluded from further analysis. 18 patients (24.3%) had BCR-ABL KD mutations at the time of switch over to Nilotinib. Median duration of Nilotinib therapy was 32.4 months, with a minimum follow up of at least 12 months. Optimal response was achieved in 70.27% patients at 6 months of Nilotinib therapy, of which major molecular response (MMR) rate was

24.3%. All 18 patients who achieved MMR at 6 months continued to maintain MMR till last follow up (median duration-28.6 months). Of the suboptimal responders, 54.5% patients had evolution of new mutations at 6 months. At 1-year end point, 45.9% patients achieved MMR and 42.8% of the suboptimal responders had evolution of new mutations, especially in P-loop and A-loop domains. Number of mutations per patient increased with time, with more of double and triple mutants evolving at 6 month and 1-year time points. When stratified according to presence or absence of any mutations at switch over, significantly better MMR rates at 1 year were seen in mutation negative group (20% *vs* 55.5%, p= 0.014). Estimated 4-year overall survival and EFS of the cohort was 93.3% and 38.6%. Only 4 (5.4%) patients developed Grade III-IV hematologic toxicity. 2 patients who developed blast crisis died.

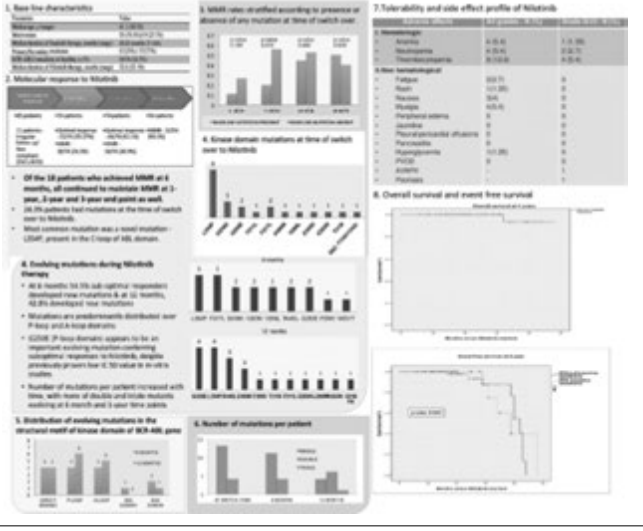


Figure 1.

Summary/Conclusion: Nilotinib is an effective second line TKI with 24.3%, 45.9% and 59.2% MMR rates at 6 months, 1 year and 2 year, respectively. Achievement of MMR at 6 months predicts long-term stable molecular remissions.So, earlier and deeper molecular kinetics can be used as a prognostic marker to identify low-risk patient subsets. Evolving new unfavourable mutations are major causes of treatment failure. In contrary to ELN guidelines, all new mutations need not necessarily imply to label a TKI failure and does not warrant a change in therapy, especially in a developing country like India. Side effects are usually well tolerated, most common being cytopenias. Large-scale studies with long-term follow up are required for assessing the durability of sustained MMR with Nilotinib and to broaden the spectrum of clinically significant mutations influencing the TKI therapy.

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IMATINIB RESPONSE AND MICRORNAS PROFILE IN CML-A BIOMARKER FOR DRUG RESPONSE

R.S. Alves[1,2], A.C. Gonçalves[1,2], J. Jorge[1,2], D. Luís[3], A. Ribeiro[3], G. Marques[4], L. Jorge[4], P. Rodrigues-Santos[5,6], P. Freitas-Tavares[3], A. Almeida[7,8], A.B. Sarmento-Ribeiro[1,2,3,9,*]

[1]Coimbra Institute for Clinical and Biomedical Research (iCBR)-Group of Environment Genetics and Oncobiology (CIMAGO), [2]Laboratory of Oncobiology and Hematology, Faculty of Medicine University of Coimbra (FMUC), [3]Clinical Hematology Department, [4]Clinical Pathology Department, Centro Hospitalar e Universitário de Coimbra (CHUC), [5]Immunology Institute, Faculty of Medicine University of Coimbra (FMUC), [6]Immunology and Oncology Laboratory, Center for Neurosciences and Cell Biology (CNC.IBILI), Coimbra, [7]Hospital da Luz, [8]CEDOC-Chronic Diseases Research Center, Lisboa, [9]Center for Neurosciences and Cell Biology (CNC.IBILI), University of Coimbra, Coimbra, Portugal

Background: The introduction of Imatinib and other Tyrosine Kinase inhibitors (TKIs) in the treatment of Chronic Myeloid Leukemia (CML) changed the course of this disease dramatically. Despite the higher therapeutic efficacy of different TKIs, CML cells develop mechanisms that overcome the therapeutic effect becoming resistant. The actual challenge is to predict which patients will develop resistance to TKIs, in order to improve therapeutic selection. Several mechanisms are described as necessary for resistant phenotypes, like BCR-ABL point mutation or overexpression, influx/efflux drug transporters expression (such as P-gP and OCT1), and alternative signaling pathways activation (like PI3K/Akt/mTOR). All these mechanisms are extremely controlled in cells, and microRNAs (miRs) are essential regulators of gene expression. In this context, the miRs profile could play a critical role in Imatinib response/resistance.
Aims: The aim of this work was investigated the role of miR-21, miR-519c, miR-451 and miR-26 expression levels in CML patients at diagnosis and correlated the expression levels of this miRs with Imatinib response.
Methods: For that, we assessed the expression levels of miR-203, miR-21, miR-519c, miR-451, miR-26 and miR-16 (endogenous control) by TaqMan MicroRNA Assays, in 29 CML patient samples at diagnosis. The study population presented a median of 53 years old, with 52% of males, and 86% of the patients were diagnosed at chronic phase. The proper statistical analysis was performed and was considered a significance levels of 95% ($p<0.05$).
Results: : The miR-451 was the miR with higher expression levels (median: 7.3), the miR-26 show a median of expression of 0.086, and the miR-21 presented the lowest levels (median: 0.0003). The expression of miR-519c was not detected in any sample. The expression levels of the evaluated miRs was independent of the phase of disease at diagnosis. Moreover, we evaluated the potential use of miRs expression as a biomarker of response to Imatinib at 6 and 12 months of treatment follow-up. The ROC analysis show that patients with miR-451 expression levels higher than 5.69 and lower expression levels of miR-21 than $1.16x10^{-4}$ have an optimal response to Imatinib at 12 months [AUC 0.77 (CI95% 0.58-0.95); Sensibility=92%; Specificity=64%; p=0,017; AUC 0.77 (CI95% 0.57-0.97); Sensibility=55%; Specificity=100%; p=0,021, respectively]. The patients with this profile combining miR-451 and miR-21 according to the cut-off levels have 44.8x higher probability of achieving an optimal response at 12 months. In opposition, patients with miR-451 expression levels lower than 5.69 and higher expression levels of miR-21 than $1.16x10^{-4}$ present 21.6 times lower probability to achieve an optimal response at the same time point.
Summary/Conclusion: Our preliminary results suggest that miRs profile in CML patients at diagnosis could constitute a new biomarker of Imatinib response, particularly the levels of miR-451/miR-21. However, more studies are necessary with a higher number of patients. The work was supported by FMUC and Banco Santander Totta (FMUC-BST-2016-214), CIMAGO (Project 10/14) and FCT (SFRH/BD/51994/2012).

PB1935

IMPACT OF AGE ON THE CLINICAL RESPONSE, TOXICITY AND SURVIVAL IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB IN THE FIRST-LINE: AN ANALYSIS OF CAMELIA REGISTRY

P. Belohlavkova[1], K. Steinerova[2], M. Karas[2], I. Skoumalova[3], P. Rohon[3], K. Indrak[3], J. Voglova[1], E. Cmunt[4], T. Necasova[5], Z. Kristkova[5], M. Trneny[4], P. Zak[1], T. Papajik[3], E. Faber[3,*]

[1]4th Department of Internal Medicine-Hematology, University Hospital and Charles University, Hradec Kralove, [2]Department of Hematology and Oncology, University Hospital and Charles University, Pilsen, [3]Department of Hemato-Oncology, University Hospital and Faculty of Medicine, Olomouc, [4]1st Department of Internal Medicine, Faculty Hospital and Charles University, Prague, [5]Institute of Biostatistics and Analyses, Masaryk University, Brno, Czech Republic

Background: Treatment of chronic myeloid leukemia (CML) patients with imatinib (IMA) significantly prolongs survival. Age is still considered as an important prognostic factor, as it is included in Sokal and ELTS risk scores too. However, several previous studies have demonstrated that the treatment with IMA eliminated the negative effect of the age on the response and survival in CML patients.
Aims: The aim of the analysis was to evaluate optimal response, toxicity, overall survival (OS), progression free survival (PFS), event free survival (EFS) and failure free survival (FFS) according to the age of patients at the time of diagnosis (<65 years *vs* ≥65 years).
Methods: A total of 372 patients (170 women, 202 men) treated with IMA 400 mg/day in the first line were retrospectively evaluated from CAMELIA registry. The median age of diagnosis was 54 (range: 18-88) years. The median follow up was 82.3 (18-177.3) months (M). The patients were divided into two groups: age<65 years (292 patients) and age ≥65 years (80 patients). ELN definitions for treatment response were used. For statistical analysis were used Mann-Whitney and Fisher tests, Kaplan-Meier method with the log-rank test was used for survival analysis.
Results: Elderly patients at the time of diagnosis CML had a statistically higher ECOG score (p=0.001), however leukocyte count and spleen size were statistically lower compared to the younger patients (p=0.028; p=0.034). There were no statistically significant differences in achievement of complete hematologic (CHR), complete cytogenetic (CCyR) and major

molecular responses (MMR) at the optimal time-points between the two groups. Median time to CHR, CCyR and MMR was not different in both groups (3; 12; 35M in younger *vs* 3; 10 and 35M in elderly patients). In the elderly group the incidence of adverse events was higher (p<0.001) as well as more frequent dose reduction of IMA was required in elderly patients (26.3% *vs* 10.3%). There were 35 deaths in the younger group and 18 deaths in the elderly group (12% *vs* 22,5%) but the incidence of CML-related death was comparable in both groups (7.5% *vs* 6.2%). 10-year OS was 86% *vs* 68% (p<0.001) but OS with CML-related death was 96% *vs* 89% without the statistically difference (p=0.07). Non-significant trend to inferior PFS 68% *vs* 84% (p=0.07) was observed in elderly patients. FFS and EFS showed no the statistical difference between both groups (50% *vs* 35%; p=0.43 and 40% *vs* 30%; p=0.39). Patients with a sustained dose of IMA 400 mg/die have better survival in both patient groups but without the statistically differences (p=0.06; p=0.20) (Figure A,B). At present, 173 (67.8%) younger patients and 44 (74.6%) older patients are still treated with IMA.

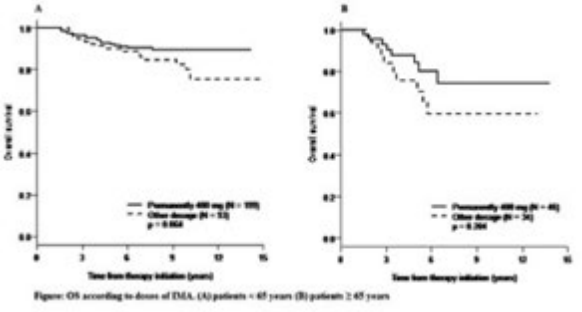

Figure 1.

Summary/Conclusion: Our analysis has shown that the achievement of optimal CHR, CCyR and MMR responses is comparable in both groups. Worse overall survival in the group of patients with age ≥65 is due to the other causes of death than CML. Currently, age at the time of diagnosis CML has lost its negative prognostic significance. The analysis was supported by a research grant from Novartis.

PB1936

RESULTS OF GENERIC IMATINIB TREATMENT IN SOKAL INTERMEDIATE & HIGH RISK CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS IN RESOURCE-CONSTRAINED SETTING: A SINGLE CENTER EXPERIENCE FROM NORTH INDIA

U. Nath[1,*], N. Nagar[2], R. Chetia[2], A. Baveja[2], J. Nanda[2]

[1]Medical Oncology Haematology, [2]Department of Medical Oncology Haematology, All India Institute of Medical Sciences (AIIMS), Rishikesh, Rishikesh, India

Background: The advent of the tyrosine kinase inhibitor (TKI) Imatinib has drastically improved survival in Chronic myeloid leukemia (CML). Recent evidence suggests that patients with Sokal intermediate or high risk CML-CP may preferentially benefit from upfront 2nd generation TKI therapy.[1]
Aims: Our study reports the complete hematological response (CHR) & early molecular response rates at 3 months of treatment with generic Imatinib in newly diagnosed Sokal intermediate & high-risk CML-CP patients in the severely resource-constrained setting of a tertiary care institution in north India.
Methods: The study included 40 newly diagnosed CML-CP patients with either Sokal intermediate or high risk disease, who were diagnosed between March 2016 & October 2017. All the patients in the study hailed from very poor socio-economic background with severe financial constraint, and none of them had any medical insurance. All the patients were treated with generic Imatinib mesylate 400 mg/day which was available free of cost at the hospital. None of the patients could afford one of the two 2nd generation TKI drugs (Nilotinib & Dasatinib) which are available in India, despite adequate counseling & information regarding the efficacy of 2nd generation TKIs. Treatment response was monitored and defined as per European LeukemiaNet recommendations.[2] Hematological response was assessed at 3 months for CHR. Molecular response was assessed at 3 months, 6 months & 12 months of Imatinib treatment if patients could afford BCR-ABL Q-PCR (IS) test.
Results: The median age of patients was 30 years (range 18-75 years). Male:female ratio was 4:3. The median interval from symptom onset to diagnosis was 4 months (range 2-12 months). The median spleen size was 10 cm below costal margin (range 3-28 cm). The median total leukocyte count at diagnosis was 257x10^9/L (range 36-700x10^9/L, and the median platelet count was 338x10^9/L (range 102-1160x10^9/L). At 3 months, CHR was achieved by 90% (36/40) patients; rest had no CHR (Imatinib failure). Molecular response at 3 months could be assessed in 33 out of the 40 patients. Optimal molecular response at 3 months (BCR-ABL ≤10% I.S.) was achieved in 21 out of these 33 patients (63.6%); the rest had BCR-ABL >10%. Imatinib failure was documented at 6 months in 5 patients (BCR-ABL >10% after 6 months). On follow up, major molecular response (BCR-ABL ≤0.1% I.S) at 12 months has been documented in four patients. None of the patients with Imatinib failure could afford BCR-ABL kinase domain mutation analysis. Only one patient switched to Nilotinib therapy after Imatinib failure at 6 months; Imatinib dose has been increased to 800 mg/day in the rest. Five patients have been lost to follow up.
Summary/Conclusion: The real scenario of CML treatment in developing countries with resource-constrained settings is very much different from that in the developed countries. The response rates to generic Imatinib therapy in Sokal intermediate & high risk CML-CP patients are not impressive. There is scope for improvement in treatment response with upfront 2nd generation TKI therapy, if these drugs can be made available at lower costs which each & every CML patient can afford.

Reference

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PB1937

HLA CLASS I AND CLASS II ALLELE FREQUENCIES IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA AND THEIR EFFECT ON SURVIVAL

I. Kaygusuz Atagunduz[1,*], T. Toptas[1], F. Gecgel[1], T. Memis[2], F. Ture Ozdemiroglu[3], R. Deniz[2], T. Elibol[1], Y. Ipek[1], C. Adiguzel[4], T. Firatli Tuglular[1]

[1]Hematology, [2]Internal Medicine, [3]Immunology, Marmara University Hospital, [4]Hematology, Bahcesehir University Hospital, Istanbul, Turkey

Background: Human Leukocyte Antigens-HLA molecules present pathogens and tumor-derived peptides to T-cells, which initiates an adaptive immune response. Besides the immune response, many studies have shown a correlation between HLA and different etiologies of many diseases, including autoimmune, infectious, neoplastic, and idiopathic diseases. Chronic Myeloid Leukemia (CML) is a clonal myeloproliferative disorder characterized by t (9; 22) translocation. This translocation results in the formation of the chimeric bcr/abl gene encoding the p210 fusion protein. p210 is thought to be a potential endogenous immunogenic tumor-specific antigen. Because these fusion proteins contain peptide sequences that are foreign to the immune system, they may produce a tumor-specific cytotoxic T cell response when presented by the patient's HLA molecules. Some *in vitro* studies indicate that HLA molecules may play a key role in the immune response to CML cells. Many clinical studies have reported a relationship between CML and specific HLA alleles that differ from race to race. In these studies, some HLA alleles are suggested to decrease the occurrence of CML and some HLA alleles are found to be associated with the onset of the disease. However, despite intensive research and several Meta-analyses, the role of HLA in the pathogenesis of CML is still unclear.
Aims: In this study, we aimed to investigate the frequency of class I and class II HLA alleles and to evaluate their effect on survival in the CML patients.
Methods: One hundred and seventy patients with CML diagnosis were included in our study. The age range of the patient group was 19-79 and the median age was 45 years and 50% of the patients were female. The control group (n=426) consisted of healthy donors and 49.5% were female. A comparison of the class I and class II HLA allele types between the patient and control groups was done. Patients and donors in the control group underwent HLA typing by PCR-SSP before the year 2010 and by PCR-SSO after the year 2010.
Results: HLA- B*52, B*55, DRB1*03, DRB1 16 alleles had higher frequencies in the CML patients when compared with the control group and, HLA-A*68, B 35, C 04, C*16, DRB1*04, DRB1*11 ve DQB1*03 allele frequencies were decreased. After the Bonferroni correction, the decrease in the frequency of DQB1*03 allele was found to be statistically significant. (p<0.008; after Bonferroni correction) Overall survival rates for a median follow-up of ten years in patients with and without HLA-DQB1-03 alleles, were 87.5% and 88.5%, respectively, and there was no statistically significant difference between the two groups in terms of overall survival (P=0.57)

Summary/Conclusion: The decrease in the frequency of DQB1*03 alleles in CML patients evaluated in our study suggests that the expression of this allele may have a protective effect for the development of CML. Possibly, the immune response occurring in the presence of the presentation of bcr/abl-derived peptides via DQB1-03 is protective against the development of CML. However, DQB1-03 expression had no effect on the duration of survival in patients receiving tyrosine kinase inhibitors. In light of these results, larger studies addressing the effects of HLA allele expression on the disease course and response to treatment in CML patients are necessary for our population.

PB1938

THE HIGHER THE DOSE OF DASATINIB, THE LOWER THE MOLECULAR RESPONSES AS WELL AS THE GREATER THE DOSE-LIMITING TOXICITIES IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

J. Lee[1,*], S. Lee[1], S.Y. Jung[1], H. Noh[2], H.-Y. Song[3], S.Y. Choi[3], S.-H. Kim[3], D.Y. Zang[4], D.-W. Kim[5]

[1]Department of Pharmacy, Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul, [2]College of Pharmacy, Yonsei University, Incheon, [3]Leukemia Research Institute, The Catholic University of Korea, [4]Department of Internal Medicine, Hallym University College of Medicine, Hallym University Hospital, [5]Department of Hematology, Seoul St. Mary's Hospital, Leukemia Research Institute, The Catholic University of Korea, Seoul, Korea, Republic Of

Background: A fixed dosing regimen of BCR-ABL1 tyrosine kinase inhibitors (TKIs) can lead to under- and over-exposure to the drug in heavy and light-weight patients, respectively.
Aims: The aim of this analysis is to assess the effects of the body surface area(BSA)-adjusted doses of dasatinib on molecular responses (MRs) and dose-limiting toxicities (DLTs) in patients with chronic myeloid leukemia (CML).
Methods: A clinical study was conducted in newly diagnosed adult patients with CML in chronic phase (CP-CML). After adjusting the dose using each patient's body surface area (Dose/BSA), the effects of Dose/BSA were evaluated on the achievement of MRs and the occurrence of DLTs. The MRs were the MR2 defined as BCR-ABL1 transcript<1% on the international scale (IS) at 6 months, equevalent to the complete cytogenetic response, and the major molecular response (MMR) defined as the transcript<0.1% IS at 12 months of dasatinib treatment. A DLT was defined as an interruption or reduction of dasatinib dose owing to any grade 3+ adverse reaction associated with dasatinib treatment by 12 months. Logistic regression analyses were performed to determine the association between the Dose/BSA and the achievement of MRs or the occurrence of DLTs. Chi-square tests were used to compare the MRs and DLTs between the patients divided into quartile groups of Dose/BSA.
Results: The clinical data were collected from all 101 patients enrolled in the study between the year of 2013 and 2017. They were receiving a fixed initial dose of dasatinib 100 mg once daily. The higher the Dose/BSA of dasatinib, the lower the degree of MR achievement was. The rates of MR2 and MMR achievement were 80% and 66%, respectively. The higher Dose/BSA was associated with the lower achievement of MR2 (logit [P] = -0.085 × [Dose/BSA] + 6.61, p=0.024). Regarding DLTs, the rate of DLT occurrence was 46%. The higher the Dose/BSA of dasatinib, the greater the occurrence of DLTs was (logit [P] =-0.12 × [Dose/BSA]-7.20, p<0.001). From the first to the fourth quartile groups of Dose/BSA, the rates of MR2 achievement were 84, 92, 76 and 65%, respectively, which demonstrated a decreasing trend as Dose/BSA increases (chi-square test, p=0.016). In addition, the rates of DLT occurrence from the first to the fourth quartile groups of Dose/BSA were 13, 39, 76 and 59%, respectively, which demonstrated an increasing trend as Dose/BSA increases (chi-square test, p<0.001).
Summary/Conclusion: The higher Dose/BSA of dasatinib not only increased the chance of DLT occurrence but also decreased the probability of MR achievement. It appears necessary to administer a lower initial dose of dasatinib to an individual patient in order to achieve a higher MR as well as to keep a lower rate of DLTs in the treatment of patients with CP-CML.

PB1939

QUALITY OF LIFE IN CHRONIC MYELOID LEUKEMIA PATIENTS RECEIVING A TYROSINE KINASE INHIBITOR

G. Guclu[1], B. Aras[2], E. Gunduz[3,*]

[1]Internal Medicine, [2]Genetics, [3]Hematology, Eskisehir Osmangazi University, Eskisehir, Turkey

Background: Chronic myeloid leukemia (CML) is a disease frequently diagnosed during routine examination or blood tests and patients may remain asymptomatic for many years. Recently the standard therapy is imatinib mesylate which provides both hematological, cytogenetic and molecular remission. Second generation tyrosine kinase inhibitors (TKIs) are effective in the first line therapy but often used in resistant/intolerant patients. Treatment requires tight compliance and regular blood checks. The patient's lifetime treatment as well as regular controls affect the quality of life. Although a good quality of life with imatinib has been shown in many studies, generic imatinib is used in many countries and the data about second generation TKIs are scarce.
Aims: The aim of this study was to evaluate the quality of life of CML patients who are treated with tyrosine kinase inhibitors (generic imatinib, dasatinib and nilotinib).
Methods: A hundred and twenty patients and a healthy control group (n=20) were included after obtaining written informed consent. EORTC QLQ-CML24 and EORTC QLQ-C30 (version 3.0) scales were used to assess the quality of life. Mean age of the patients was 55±14.4 years and 68 (56%) of the patients were female. The mean duration of illness during the questionnaire was 6.7±4.5 years. Imatinib group included 72, nilotinib group included 25 and dasatinib group included 23 CML patients.
Results: Scale of satisfaction with the care received and the scale of satisfaction with the amount of information received in imatinib group were lower than dasatinib group (p=0.007). Scale of insomnia in nilotinib grup was lower than imatinib group (p=0.027). No difference was found between imatinib, nilotinib, dasatinib groups and healthy controls for all other scales in the questionnaire.
Summary/Conclusion: The quality of life in CML patients under generic imatinib, nilotinib and dasatinib were evaluated in this study. The results give information about the quality of life with generic imatinib which is used in the first line therapy of CML in many countries and add new data on the quality of life with second generation TKIs. According to the results obtained it can be concluded that the quality of life is mostly similar with healthy controls and also similar between second generation TKIs.

PB1940

CARDIOVASCULAR EVENTS IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH NILOTINIB

A. Bykova*, G. Gusarova, E. Chelysheva, O. Shukhov, A. Petrova, N. Tsyba, I. Nemchenko, O. Lazareva, A. Turkina

Scientific Advisory Department of Chemotherapy of Myeloproliferative disorders, National Research Center for Hematology, Moscow, Russian Federation

Background: The first reports on peripheral arterial occlusive diseases (PAOD) in Chronic Myeloid Leukemia (CML) patients (pts) treated by nilotinib appeared in 2011. Some experimental data showed direct proatherogenic actions of nilotinib (inhibition of DDR1 receptor, possible participation in pathogenic blockage of KIT and PDGFR kinases), possibility to create angiospasm. However detailed mechanisms of cardiovascular events (CVEs) emergence after exposure to nilotinib are not described.
Aims: Evaluate frequency and risk factors of CVEs emergence in CML pts treated by nilotinib.
Methods: 119 CML pts treated by nilotinib and monitored in the National Research Center for Hematology of Russia were included in the study 2007 to 2017. Male:female ratio was 45:74. Median age at the start of nilotinib treatment was 43 years (range 23-60). 67 (56.3%) pts were transferred to nilotinib due resistance to imatinib, 26 (21.8%)-due to imatinib toxicity, 8 (6.7%)-due to suboptimal response to imatinib, other-9 (7.6%); 12 pts (10%) were treated by nilotinib as first line therapy, 98 pts (82.3%) were treated by nilotinib as second line therapy after imatinib. 74 pts received 800 mg/day dose of nilotinib, 42 pts-600 mg/day, 3-400 mg/day. We evaluated risk factors (RF) of CVEs, including age, smoking, arterial hypertension presence, overweight, family history of early cardiovascular diseases, type 2 diabetes, glucose level, Lipemic index (total cholesterol and fractions, triglycerides). SCORE scale was used to evaluate RF of death due to CVEs.
Results: Before nilotinib treatment the following RF of CVEs were found: males >55 years-8 (17.7%), females >65 years-7 (9.4%), smoking-28 (23.5%), increased level of total cholesterol –39 (32.7%), overweight-34 (28.6%), hyperglycemia-12 (10%), family history of early CVEs-6 (5%), arterial hypertension-29 (39.5%), type 2 diabetes-8 (6.7%). SCORE distribution was as follows: low risk-64, moderate-18, high-8, very high-11, no data-18. In nilotinib treated pts at 14 (11.7%) (9 males (20%) and 5 females (6.8%)) the following CVEs were observed: Acute Cerebrovascular Event (ACE)-7 (5.8%), PAOD-2 (1.7%), first time observed angina pectoris-5 (4.2%). 13 of 14 of the pts mentioned above (92.8%) had a high risk according to SCORE scale before nilotinib treatment. Transfer to other tyrosine

kinase inhibitor (TKI)-18 (15%): dasatinib-12, bosutinib-2, ponatinib-1, imatinib-1, PF114-2; observation without treatment-29 (24%). 12 pts (10%) died, of which 8 (6.7%)-due to disease progression, 4 (3.4%)-due to CVEs, 2 (1.7%)-due to nilotinib treatment. 60 pts (50.4%) continue nilotinib treatment with observation median of 52.6 months (1-135.8).

Table 1.

Table 1. Distribution of patients with cardiovascular events by gender, age, risk of SCORE, and duration of therapy with nilotinib

Patients	Gender	Age, years		SCORE	Duration of treatment with nilotinib before CVS, months
		Start of nilotinib treatment	CVEs		
O.A.D.	M	55	58	high	58
S.O.P.	M	49	50	high	15
M.A.Z.	F	25	30	low	60
S.N.B.	M	54	54	high	1
V.C.D.	M	47	49	high	21
V.K.M.	F	68	69	high	12
A.N.G.	M	28	37	high	108
A.M.F.	M	66	68	high	19
I.E.M.	F	62	63	high	15
E.P.K.	F	50	52	high	24
V.M.T.	M	43	49	high	72
I.T.B.	F	78	83	high	60
A.A.S.	M	67	71	high	48
Y.S.L.	M	52	59	high	74

males - 9 (> 55 years - 3), females - 5 (> 65 years - 2)

Summary/Conclusion: Combination of obtained data allows to propose that nilotinib can be an additional factor of accelerated development of atherosclerosis leading to various CVEs. It cannot be excluded that CVEs emergence can be linked to nilotinib dose as most pts with CVEs received maximum possible dose-800 mg/day, apart from 3 pts taking lesser dose (2-600 mg/day, 1-400 mg/day). All pts with observed CVEs, excluding one, had a high risk of CVEs in accordance with SCORE scale. Taking into account possible correlation of CVEs frequency in nilotinib treated pts with high risk in accordance with SCORE scale it is recommended to estimate RF during screening to prevent CVEs emergence. At increased risk of CVEs emergence it is recommended to correct RF, which can be modified, decrease nilotinib dose or transfer to other TKI.

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MORE THAN 10-YEARS SURVIVORS WITH CHRONIC MYELOID LEUKEMIA WITHOUT ANY MOLECULAR RESPONSE-REAL LIFE EVIDENCE FROM A SINGLE INSTITUTION

G. Balatzenko[1,*], K. Ignatova[2], B. Spassov[2], S. Ivanova[1], J. Stoyanova[2], A. Lilova[2], V. Stoeva[2], Y. Petrov[3], E. Hadjiev[4], G. Tsvetkova[5], M. Guenova[6]

[1]Laboratory of Cytogenetics and Molecular Biology, [2]Department of Clinical Hematology, [3]Department of hematopoietic stem cells transplantation, National Specialized Hospital for Active Treatment of Hematological Diseases, [4]Clinic of Hematology, Medical University Sofia, Faculty of Medicine, Department of Internal Diseases, UMHAT Alexandrovska, [5]Clinic of Hematology, Medical University Sofia, Faculty of Medicine, Department of Internal Diseases, UMHAT Alexandrovska, [6]Laboratory of Hematopathology and Immunology, National Specialized Hospital for Active Treatment of Hematological Diseases, Sofia, Bulgaria

Background: The extreme effectiveness of tyrosine kinase inhibitors (TKI) has dramatically changed the clinical course and outcome of chronic myeloid leukemia (CML) patients (pts). Generally, the achievement of optimal molecular response (MR) predicts close to normal survival. In contrast, pts who fail to respond to TKI have an inferior prognosis. Whether long-term survival is possible in pts resistant to all used TKIs, and if the absence of MR is inevitably associated with a fatal outcome is not well determined.
Aims: To determine the proportion of CML pts treated with TKIs without any molecular response among more than 10-years survivors.
Methods: We retrieved 340 CML pts (168 females and 172 males; mean age of 521±16.4 years), who were diagnosed between 1998 and 2008, with a follow-up of at least 10 years after the diagnosis in the hospital records of the National Specialized Hospital for Active Treatment of Hematological Diseases-Sofia, Bulgaria. Pts were treated with chemotherapy, interferon alpha and/or by TKI. Response to therapy was evaluated according to the standard criteria. Molecular monitoring was carried-out initially by nested primers RT-PCR, afterwards by manual quantitative RT-PCR, and since 2012 by GenExpert (Cepheid) platform.
Results: In total 88/340 pts had ≥10-years survival, including 24 who achieved MR5.0 (27.3%); 13 MR4.5 (14.8%); 8 MR4 (9.1%); 19 MR3.0 (21.6%). No MR was found in the remaining 24 pts (27.3%). All refractory pts received at least 3 different TKIs with or without preceding chemotherapy or interferon alpha. All pts expressed typical *BCR-ABL1* mRNAs (13 with b3a2; 11 with b2a2), and all were diagnosed in chronic phase except one who presented in accelerated phase. The mean leukocyte count was 153.9±82.7x10^9/l; platelet count 629.9±405.8x10^9/l; and hemoglobin 125.5±30.1 g/l. All pts with available records were EUTOS score low risk. No significant differences in the main clinical and laboratory features between pts with or without MR were seen. All 24 pts achieved complete hematological response without any MR, with only a transient 1 log reduction of the *BCR-ABL1* level after the 2nd line TKI initiation in 2 of them. T315I *ABL1* mutation was found in only 1/24 (4.2%) case. During the whole period of observation, 6 (27.3%) nonresponders developed ≥1 blast crisis, while none of the pts with an achieved MR progressed. In terms of survival, 8 of the non-responders died 10-18 years after diagnosis. The remaining 16 are still alive 10-19 years after the onset.
Summary/Conclusion: Our results suggest that long-term survival might be observed in some low risk CML pts who achieved hematological response, in the absence of any molecular response to several lines of TKIs. A possible role of variations in the biology of the disease and/or a certain sensitizing effect of the preceding therapy at least in some of the pts can be speculated. Additional studies are warranted to elucidate the mechanisms underlying this observation as well as to search for additional indicators for prediction of outcomes.
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MATHEMATICAL MODELLING REVEALS THE POTENTIAL FOR CONSIDERABLE DOSE REDUCTIONS IN TYROSINE KINASE INHIBITOR TREATED CHRONIC MYELOID LEUKEMIA

I. Roeder[1,2,*], A.C. Fassoni[3], C. Baldow[1], I. Glauche[1]

[1]Institute for Medical Informatics and Biometry, Faculty of Medicine, Technische Universität Dresden, [2]Data and Trial Management Unit, National Center for Tumor Diseases (NCT), Partner Site Dresden, Dresden, Germany, [3]Instituto de Matemática e Computação, Universidade Federal de Itajubá, Itajubá, Brazil

Background: The availability of tyrosine kinase inhibitors (TKIs) has revolutionized Chronic Myeloid Leukemia (CML) therapy. However, permanent control of the disease requires continuing and potentially life-long TKI therapy. While TKI cessation appeared as a safe option for about half of the optimally responding patients, a systematic assessment of the long-term effects of TKI dose de-escalation is missing.
Aims: It is our aim to theoretically study quantitative effects of TKI dose de-escalation as a potential alternative treatment option for patients with good treatment response.
Methods: We use a mathematical model (applying ordinary differential equations) to analyze and consistently describe response data of TKI-treated CML patients from independent clinical trials. The model describes CML as a clonal competition process of normal and leukemic cells that is modulated by the TKI effect. It allows us to estimate patient-specific parameters that describe cell cycle activation and de-activation of leukemic stem/progenitor cells as well as the TKI-induced kill of leukemic cells.
Results: Our analysis reveals that the TKI-induced long-term decline in CML tumor load is limited by the activation of quiescent leukemic stem cells. Based on this finding we suggest dose de-escalation schedules in which the treatment intensity can be substantially reduced without altering the long-term leukemic stem cell response. We also suggest a step-wise dose alteration to identify optimal, patient-specific TKI doses.
Summary/Conclusion: Our analysis provides strong theoretical evidence that TKI dose de-escalation does not lead to a reduction of long-term treatment efficiency in most patients. We demonstrate that continuous *BCR-ABL1* monitoring allows to provide patient-specific predictions of an optimal (reduced) TKI-dose that does not decrease the anti-leukemic effect on residual leukemic stem cells. We make the predictions that dose halving might be safe for the majority of patients and that a longer treatment with a reduced dose is more efficient than the same cumulative dose applied in a shorter period. The model results are consistent with the interim analysis of the DESTINY trial, which studies dosage-halving in CML patients in sustained remission, and it provides clinically testable predictions. Our results reveal a currently unutilized clinical potential of dose de-escalation in long-term CML treatment to reduce treatment-related side effects and therapy costs.

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EVALUATION OF NK CELLS IN CHRONIC MYELOID LEUKEMIA PATIENTS: CORRELATION WITH RESPONSE TO TREATMENT

I. Attolico*, E. Pacciante, L. Impera, A. Minervini, L. Anelli, A. Zagaria,

P. Carluccio, A. Ricco, A.V. Russo Rossi, F. Albano

Ematologia con Trapianto, A.O. Policlinico di Bari, Bari, Italy

Background: Chronic myeloid leukemia (CML) is characterized by a reciprocal chromosomal translocation between chromosomes 9 and 22 [t(9;22)], producing the Bcr-Abl oncogene. Tyrosine kinase inhibitors (TKIs) represent the standard of care for CML patients (pts) through a direct oncokinase inhibition and well characterized immunomodulatory effects on T and NK cells. The main treatment goal is the achievement of deep molecular response (DMR), which makes disease progression highly unlikely and may even trigger discontinuation of TKIs. In recent years immunological factors are increasingly acknowledged as important variables being linked to achievement of DMR as well as maintenance of treatment-free remission in patients upon TKI discontinuation. NK cells are dysfunctional in CP CML patients at diagnosis and NK cell numbers among lymphocytes are reduced, worsening with disease progression to advanced and blast crisis phase CML.
Aims: We studied lymphocytes subsets, particularly NK cells number, in CML pts at diagnosis and at attainment of molecular response, to evaluate variations in NK cells number during the course of the disease.
Methods: 81 patients (54 M, 27 F; median age: 59, range: 19-86) were evaluated. 16 pts were in Chronic Phase (CP) at diagnosis, 23 in MR3, 40 in MR4/MR4.5, 2 were resistant to TKIs. 21 pts were studied at diagnosis and at achievement of Major or Deep Molecular Response (MMR/DMR). Flow cytometry analysis was performed on peripheral blood samples using the following antibodies: CD45, CD3, CD16, CD56, CD4, CD8, CD19. Samples were acquired on the FACS CANTO II (BD Bioscences) cytometer and analyzed with BCANTO software using an immunological gate (SSC/CD45) to select lymphocytes.
Results: When we studied lymphocytes subsets at a single timepoint in all the pts, we observed a significantly lower number of NK cells in pts evaluated at diagnosis (median: 13,5%, range: 3-35%) than in pts who were in MMR (median: 17%, range: 9-45%) or DMR (median:19%, range: 12-49%). In 2 pts resistant to TKIs NK values were respectively 2% and 3%. In 21 pts analyzed at diagnosis and at achievement of MMR (12 pts) and DMR (8 pts), we observed a progressive increase in NK number during the course of the disease, along with a progressive reduction of BCR-ABL transcript.
Summary/Conclusion: An increased percentage of NK at diagnosis seems to correlate with a faster achievement of MMR or DMR. Our data suggest a better prognosis for all patients maintaining an higher percentage of NK during the course of the disease. Further analysis should be made considering the effect of different TKIs on the immunological state of each patient.

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NILOTINIB IN 161 PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE AFTER IMATINIB RESISTANCE OR INTOLERANCE: "LA RAZA" NATIONAL MEDICAL CENTER EXPERIENCE

M. Ayala[1,*], D. Moreno-Lorenzana[2], J. Domínguez[3], E.J. Cordova[4], X. Aquino[3], A. Chavez-Gonzalez[2]

[1]Hematology & BMT Unit, Specialties Hospital, Nacional Medical Center "La Raza", [2]Oncology Research Unit, Oncology Hospital, National Medical Center, Mexican Institute for Social Security, [3]Grupo Consulmed, [4]Immunogenomics and Metabolics Disease Laboratory, Instituto Nacional de Medicina Genómica, México, Mexico

Background: The arrival of the second-generation (2ndG) tyrosine kinase inhibitors (TKIs) improved a significant percentage of patients with chronic myeloid leukemia in chronic phase (CML-CP) after imatinib resistance or intolerance, increasing disease survival.
Aims: The primary endpoint of this open study was to determine complete cytigenetic response (CCyR) and major molecular response (MMR) rate in patients treated with nilotinib, at 6 and 12 months respectively. Secondary objectives were to determine the OS, PFS, and EFS, as well as establishing nilotinib safety profile.
Methods: A prospective, open label, single-center study was undertaken to examine the clinical characteristics, cytogenetic and molecular response rates, overall survival, progression-free survival, and event-free survival of patients with CML-CP treated with nilotinib as a second or third-line therapy at "La Raza" National Medical Center.
Results: Of the 161 CML-CP patients included in this analysis, 98.47% were imatinib resistant and 1.53% were imatinib intolerant. The median age of the individuals was 46 years, with a median CML duration of 76 months, and a median duration of prior imatinib therapy of 29.5 months. Overall, 81% of the patients treated with nilotinib achieved a durable complete cytogenetic response (CCyR), which dropped to 71% by excluding patients who had a CCyR at baseline cytogenetic analysis. Moreover, 62% of the individuals achieved a major molecular response (MMR) at 18 months. Among the 161 nilotinib-treated patients; 88.2% were alive and 11.8% died. The median duration of nilotinib therapy for all treated patients was 46 months. The overall survival at 48 months was 92%. Adverse events were mostly moderate, transitory, and manageable.

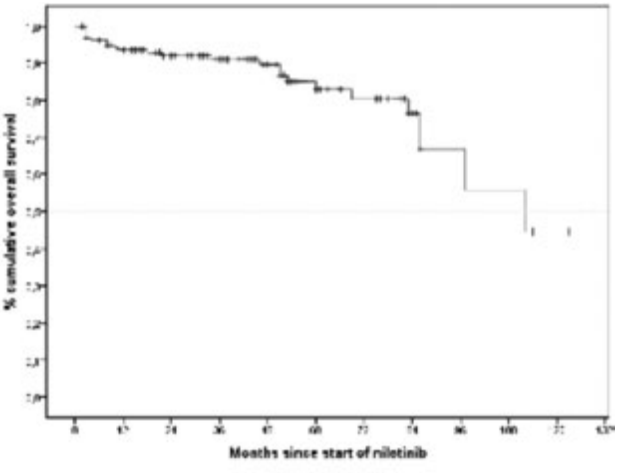

Figure 1.

Summary/Conclusion: This study shows that nilotinib is effective and can provide favorable long-term benefits for CML-CP patients who are resistant or intolerant to imatinib. Furthermore, nilotinib had a manageable safety profile.

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EVALUATION OF THE CEPHEID XPERT® BCR-ABL ULTRA ASSAY× IN CLINICAL SPECIMENS IN COMPARISON TO THE QIAGEN® IPSOGEN BCR-ABL1 MBCR IS-MMR ASSAY

S. Amar[1], G.-J. Day[2,*], E. Manor[1]

[1]Human Genetic Institute, Soroka University Medical Center, Beer-Sheva, Israel, [2]Oncology, R&D, Cepheid, Sunnyvale, United States

Background: Monitoring BCR-ABL transcript levels in peripheral whole blood (WB) of patients on tyrosine kinase inhibitor (TKI) therapy using real-time quantitative PCR (RT-qPCR) is standard of care in the management of Chronic Myeloid Leukemia (CML). Xpert® BCR-ABL Ultra (Ultra) ¥, a cartridge-based assay for use on the GeneXpert Instrument system, automates and standardizes the RT-qPCR process by integrating RNA extraction, cDNA synthesis, target amplification, detection and quantification directly from specimens in less than 2 hours. Using a lysate prepared from 4mL whole blood (WB) resulting in an effective WB input volume of 600µL, Ultra reproducibly achieves molecular response (MR) sensitivity to 4.5 logs below baseline (MR 4.5, defined as BCR-ABL1 *IS* ≤0.0032%) per the WHO International Scale (*IS*). Sample prep procedures were developed for cases where a high white blood cell count (WBCC) is known or is suspected, or when repeat testing is needed for Invalids with ABL Ct<10, allowing the use of Ultra with various input sample volume for WB or bone barrow (BM) in a wide variety of clinical situations.
Aims: We sought to evaluate the automated Ultra assay with various input sample volume. The Qiagen® psogen BCR-ABL1 Mbcr *IS*-MMR manual real-time PCR assay (*IS*-MMR), a copy number based standardization assay, was compared with Ultra in terms of assay sensitivity, concordance and classification of molecular responses.
Methods: Thirty CML specimens with%BCR-ABL/ABL (*IS*) between 100% and 0% were evaluated in WB or BM. Based on the EUTOS criteria for method comparison, acceptable concordance between two assays is defined as achieving 2 out of 3 required benchmarks: 1) ≥50% of samples within 2-fold range, 2) ≥75% within 3-fold range, and/or 3) ≥90% within 5-fold range. A linear regression analysis and an overall reporting bias analysis using the Bland-Altman model were evaluated for comparison.
Results: Among 30 CML specimens evaluated, 25 were detected by both assays with 13 identified between 100% *IS* and 0.1% *IS*, and 12 between 1% *IS* and ≤0.01% *IS* with 100% agreement to the *IS*-MMR assay (Table 1). Five specimens determined as negative by Ultra were also identified as negative by *IS*-MMR. The concordance analysis between the two assays exceeded the EUTOS criteria with 19/25 (76%), 24/25 (96%), and 24/25 (96%) falling within a 2-fold range, a 3-fold range and a 5-fold range, respectively (Table 1). Linear regression analysis showed good correlation

between two assays (R^2=0.9811; N=25) (Fig.1A). In addition, an overall bias of -0.06 (~1.15 fold) was derived using the Bland-Altman model, suggesting high concordance in% *IS* reporting between two assays across the assay dynamic range (Fig.1B).

Table 1. Concordance analysis between Ultra and *IS*-MMR per the EUTOS Criteria

Transcript Level %IS (MR)	Number of Specimens		Fold Difference (Ultra/IS-MMR)				Total
	IS-MMR	Ultra	≤2-fold (0.5-2)	≤3-fold (0.33-3)	≤5-fold (0.2-5)	>5-fold (<0.2 or >5)	
100 - 0.1 (MR0 – MR3)	13	13	11	13	13	0	13
0.1 - ≤0.01 (MR3 – <MR4)	12	12	8	11	11	1	12
Agreement	25/25 (100%)		19	24	24	1	25
Percent			76% (19/25)	96% (24/25)	96% (24/25)	4% (1/25)	100% (25/25)
Benchmarks per the EUTOS Criteria			≥50%	≥75%	≥90%	NA	NA

Fig. 1. High concordance observed between Xpert® BCR-ABL Ultra and Qiagen IS-MMR with various whole blood or bone marrow sample input volume

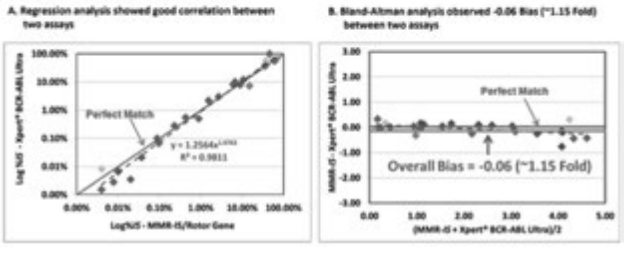

Figure 1.

Summary/Conclusion: Good concordance between Ultra and *IS*-MMR assays was achieved in WB and BM for all three benchmarks per the EUTOS criteria with good correlation observed in the linear regression analysis and using the Bland-Altman model. The Ultra assay demonstrated the ability to detect BCR-ABL1 transcripts in patients with MR 4.5 or greater reduction, thus achieving sufficient sensitivity for use in monitoring CML patients under TKI treatment. ˣCE-IVD; *in vitro* diagnostic medical device. May not be available in all countries. Not available in the United States.

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CHRONIC MYELOID LEUKEMIA WITH ISOLATED THROMBOCYTOSIS WITHOUT SIGNIFICANT LEUKOCYTOSIS IS A SUBTYPE OF CHRONIC MYELOID LEUKEMIA WITH DISTINCT DISEASE CHARACTERISTICS

Y. Sugimoto[1,*], K. Ohishi[1,2], M. Masuya[1], N. Katayama[1]

[1]Hematology & Oncology, Mie University, [2]Blood Transfusion Service, Mie University Hospital, Tsu, Mie, Japan

Background: Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm which commonly manifests with neutrophilic leukocytosis in a chronic phase of the disease. We have experienced two young female CML with more than 3,000x10^9/L platelet count without significant leukocytosis. Although their clinical features suggested essential thrombocythemia (ET), they were diagnosed as CML due to the presence of Philadelphia (Ph) chromosome. Our cases together with other cases in the literature indicated that CML with remarkable thrombocytosis without significant leukocytosis (CML-IT) may have some common clinical characteristics.
Aims: We attempted to define common clinical characteristics in CML-IT, comparing with typical CML and ET.
Methods: We reviewed the literature to compile and summarize reported cases of CML-IT. We defined marked thrombocytosis as a platelet count more than 1,000x10^9/L, and normal or slightly elevated white blood cell (WBC) count as a WBC count less than 12.0x10^9/L. Clinical data of ET patients with remarkable thrombocytosis without significant leukocytosis in our hospital was also collected and compared with those of CML-IT.
Results: Our CML-IT cases were both in their mid-thirties at diagnosis. Basophils were increased, but immature myeloid cells were not detected in PB. Their von Willebrand factor ristocetin cofactors were below 30%. *JAK2* V617F, *CALR* exon 9, and *MPL* exon 10 mutation analyses were performed and confirmed to be unmutated. Their bone marrow histology exhibited marked megakaryocytic hyperplasia, and most of megakaryocytes were small and hypolobulated or unilobulated. These features were dissimilar to bone marrow histopathological findings in ET. Although Sokal score was high in both of them, deep molecular response was obtained within 2 years with tyrosine kinase inhibitor (TKI) treatment. We found 26 cases of CML-IT in the literatures and analyzed total 28 cases of CML-IT including our two cases. Most of them were female (3 males and 25 females), and the median age was 43 years old. Neutrophil alkaline phosphatase (NAP) score was decreased in chronic phase CML in general, but it was normal or increased in all 12 CML-IT. Immature myeloid cells were not detected in peripheral blood (PB) in 18 out of 20 CML-IT as opposed to those in typical CML. G-banding karyotype of bone marrow cells showed that Ph clone was below 19/20 in 10 cases out of 12 CML-IT. 8 cases were treated with TKI, and 7 out of 8 cases obtained complete cytogenetic response at 3 months. 25 cases of ET with the similar laboratory data with CML-IT cohort were found in our hospital. This ET cohort included 11 males and 14 females. Mutational analyses revealed 16 cases with *CALR* mutation, 4 cases with *JAK2* V617F mutation, and 1 case with *MPL* S505N mutation. As was the case with CML-IT, severe splenomegaly and immature myeloid cells in PB were not seen in this ET cohort. However, basophilia beyond the upper limits of normal range was not detected in ET cohort. NAP score was low in all of tested cases. These two clinical findings were different from clinical characters observed in patients with CML-IT.
Summary/Conclusion: Our cases together with other cases in the literature suggest that there is a subgroup of CML which exhibits hematologic findings similar to those in ET. Although they might be misdiagnosed as ET, laboratory findings with normal or high NAP score and basophilia may be useful to differentiate between CML-IT and ET. Further studies are warranted to better understand the clinical characteristics and molecular mechanism of CML-IT.

PB1947

EARLY AND SUSTAINED DEEP MOLECULAR RESPONSE OF NILOTINIB AS A FRONTLINE THERAPY IN HIGH SOKAL RISK CML PATIENTS

U. Zaidi*, B. Kaleem, M. Borhany, S. Maqsood, N. Fatima, G. Sufaida, T. Shamsi

Haematology, National Institute of Blood Diseases and Bone Marrow Transplantation, Karachi, Pakistan

Background: Nilotinib, a second-generation tyrosine kinase inhibitor, has shown faster and deeper molecular responses when used as initial therapy in chronic phase chronic myeloid leukemia (CML). Data on efficacy and safety of Nilotinib in Asian population, particularly from Pakistan is scarce.
Aims: We aimed to determine the molecular response to Nilotinib and its safety profile in our population of chronic phase CML patients.
Methods: This single arm, non-randomized clinical trial was conducted among 138 newly diagnosed CML patients presented in chronic phase. All patients received Nilotinib 600 mg per day. The haematological and molecular response was assessed at 3 and 6 months respectively and thereafter at 6 monthly intervals. Moreover, event free survival (EFS), transformation free survival (TFS), overall survival (OS) and adverse events were also observed at long term analysis.
Results: The cumulative incidence of major molecular response (MMR) was 86% and deep molecular response (DMR *i.e.* MR 4.0 and MR 4.5) was 39%. Early MMR and DMR at 6 months of therapy were achieved by 71% and 32% patients respectively. Two- year EFS, TFS and OS rates for the whole group were 93%, 95%, and 97%, respectively. At median follow up of 29 months, 75% and 45% of patients were able to sustain MMR and DMR respectively. 50% patients had a high Sokal score at diagnosis. Adverse events were mainly weight gain in 29% and myelosuppression in 15% of patients.
Summary/Conclusion: Our results show high efficacy and safety of Nilotinib in both high and low Sokal risk CML patients.

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REAL-WORD DATA FROM LONG TERM FOLLOW-UP OF IMATINIB INITIALLY TREATED CHRONIC MYELOID LEUKEMIA PATIENTS

I. Cojbasic[1,2,*], L. Macukanovic-Golubovic[1,2], I. Tijanic[1,2]

[1]Faculty of Medicine, University of Nis, [2]Clinic of Hematology and Clinical Immunology, Clinical Center Nis, Serbia, Nis, Serbia

Background: The introduction of the tyrosine kinase inhibitors(TKIs) into clinical practice has strikingly improved survival and remission rates for chronic phase-chronic myeloid leukemia(CP-CML) patients. An emerging goal of TKIs therapy is to achieve sustained deep molecular response. It has been consistently shown that early molecular response is associated with

positive long-term outcome concerning overall survival and progression-free survival in CML patients.
Aims: The aim of this study was to investigate long-term outcomes regarding remission rates and survival in unselected group of CP-CML patients initially treated with imatinib.
Methods: This is a retrospective analysis single centre database of CP-CML patients treated with first-line imatinib 400mg daily since diagnosis and followed between August 2006 and August 2017. Patients have been analysed in intention to treat. The chronic, accelerated or blastic disease phase(CP, AP, BP) were defined according to the ELN criteria. The risk scores were calculated according to the Sokal, Euro and EUTOS formulations. Cytogenetic and molecular responses were defined according to ELN definitions and followed the procedures as described elsewhere. Cumulative incidences of treatment response were calculated under consideration of competing risks defined as progression of disease to AP, BP or death. From date of imatinib initiation, overall survival(OS) was calculated until death at any time and for any reason; progression-free survival(PFS) was calculated until progression to AP or BC at any time; event-free survival(EFS) was calculated until death, progression to AP or BP, ELN failure on imatinib or imatinib treatment discontinuation for any cause.
Results: In total 102 patients were eligible for analysis, with a median duration of therapy of 58.8(6-132) months, of which 54(52.9%) were female, with median age of 55.3(18-78) years at imatinib initiation. EUTOS score was high in 16.7% of patients. The cumulative incidence of CCyR was 69.6%(95% Cl: 66-75%) at 12 months and 82.5%(95% Cl: 79-85%) at 5 years. The cumulative incidence of MMR was 48%(95% Cl: 45-52%) of evaluable patients at 12 months, and 73.3%(95% Cl: 70-76%) at 5 years. The cumulative incidence of MR4 was 27.5%(95% Cl: 24-32%) at 24 months and 56.9%(95% Cl: 53-59%) at 5 years. Median-time to achieve MMR and MR4.0 was 1.7 and 2.8 years respectively. In the multivariate Cox model analysis, MMR at 12 months was predictive for PFS(p=0.001) and OS(p=0.001) but not for EFS(p=0.271), unlike that MR4 at 24 months was predictive for EFS(p=0.003) and PFS(p=0.011) but not for OS(p=0.505). The estimated EFS rates were 76% at 2 years, 60% at 5 years and 45% at 8 years, PFS rates were 97.1% at 2 years, 95.1% at 5 years and 92.2% at 8 years, OS rates were 98% at 2 years, 87.3% at 5 years and 82.4% at 8 years. At last follow-up, after a median of 64.5(6-132) months, 68(66.7%) patients were still on imatinib, while 25(24.5%) have been switched to 2nd TKIs nilotinib for resistance in 19(76%) or intolerance in 6(24%) cases. Overall, at latest follow-up, 16.7% patients died, of which 47.1% because of CML progression and 52.9% from other causes.
Summary/Conclusion: Analysis of this CML patients' cohort initially treated with imatinib, consistently has been confirming high rates of remission and survival and low rates of disease progression. Subsequently, imatinib has been continuing to provide an excellent long-term treatment responses and survival outcome because more than half of patients achieved a stable deep molecular response that gives the opportunity for treatment-free strategy.

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EFFECTIVENESS IN REAL LIFE OF TKI TREATMENT ON CML RESULTS FROM THE ANDALUSIAN REGISTRY OF CHRONIC MYELOID LEUKEMIA (RALMC) 2002-2016

J.M. Puerta-Puerta[1,*], A. Jiménez-Velasco[2], M.I. Mata-Vázquez[3], M.J. Ramírez-Sánchez[4], M.Á. Portero-Frías[5], M.J. García-Pérez[6], C. Ruiz-Nuño[2], M.S. Durán[7], A. Paz-Coll[8], A. Rosell-Mas[9], M.D.C. Fernández-Valle[10], I. Simón-Pilo[11], C. Ferrer-Chaves[12], I. Ballesteros[13], Y. Moatassim[14], M.C. Avellaneda[15], J. Oliveros[16], P. López-Garrido[17]

[1]Hematology, UGC intercentros Hospital Universitario Virgen de las Nieves-Nuevo San Cecilio, Granada, [2]Hematology, Hospital Regional Universitario Carlos Haya, Málaga, [3]Hematology, Hospital Costa del Sol, Marbella, [4]Hospital de Jerez, Jerez de la Frontera, [5]Hematology, Hospital Universitario Virgen Macarena, Sevilla, [6]Hematology, Hospital Torrecárdenas, Almería, [7]Complejo Hospitalario de Jaén, Jaén, [8]Hematology, Hospital de Puerto Real, Puerto Real, [9]Hematology, Hospital Universitario Virgen de la Victoria, Málaga, [10]Hopital Puerta del Mar, Cádiz, [11]Hospital Virgen de Valme, Sevilla, [12]Hematology, Hospital San Juan de la Cruz, Úbeda, [13]Hospital de la Axarquía, Vélez Málaga, [14]Hematology, Hospital Santa Ana, Motril, [15]Hospital San Agustín, Linares, [16]Hematology, Hospital La Inmaculada, Huércal Overa, [17]Grupo Andaluz de LMC, Granada, Spain

Background: Normally, information regarding efficacy of treatments is obtained through clinical trials which are characterized by their strict inclusion and exclusion criteria. In real life, results depend on the relationship between the physician and the patient and on the selection of first and subsequent lines of treatment for patients who show intolerance or resistance to TKI treatment.
Aims: TKI treatment description results among TKI patients with CML from the RALMC in real life: Overall survival rates (OS) as it relates with leukemia (LRS), progression and event free survival (PFS and EFS). Rates of discontinuation due to inefficacy and intolerance. To compare effectiveness and security among imatinib and 2GTKI (nilotinib and dasatinib).
Methods: Retrospective descriptive analysis of patients from the RALMC with clinical and therapeutic follow-up since January 2002 to December 2016. Analysis of survival rates through Kaplan-Meier method, distinguishing among OS: time span since diagnosis until death due to any cause; LRS: time span since diagnosis until dead due to a cause connected to CML; PFS: time span since diagnosis until progression to AP or BC or death and EFS: time span since diagnosis until death due to any cause, progression of illness or change of treatment on first line due to inefficacy or intolerance.
Results: 505 patients, 279 males (55.2%), 104 (20.6%) of whom were over 70 years. 427 (84.6%) began on Imatinib, 46 (9.1%) on Nilotinib, and 32 (6.3%) on Dasatinib. 131 patients (30.7%) with imatinib in first-line changed treatment: 46 (10.8%) due to intolerance and 85 (19.9%) due to inefficacy. 11 patients with 2GTKI (14.1%) changed treatmente too: 6 (7.7%) due to intolerance and 5 (6.4%) due to inefficacy. 21 patients (4.2%) progressed to advanced phases: 14 (2.8%) to BC and 7 (1.4%) to AP. 63 patients (12.5%) died during the follow-up. 15 (23.8%) due to causes related to CML and 48 (76.2%) due to causes not directly related to CML. 49 patients over 70 (47.1%) died during the follow-up: 41 (83.7%) due to causes not related to CML.

Table 1.

Global series	OS	LRS	PFS	EFS
5 years	92.9%	98.3%	91.2%	69.7%
10 years	85.4%	97.2%	82.9%	57.6%

OS, PFS and EFS are higher among patients treated with 2GTKI vs Imatinib to 5 years (98.6% vs 81.6%; 97.2% vs 80.5%; 84.7% vs 59.8%, p=0.002).

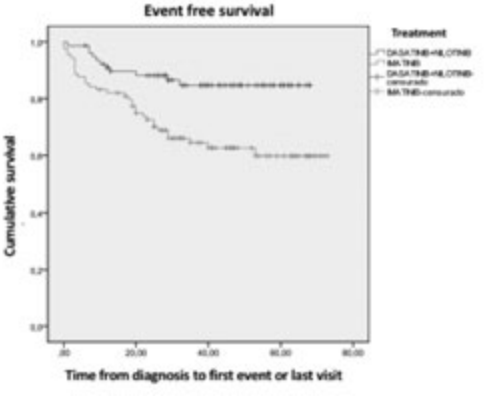

Figure 1.

Summary/Conclusion: Patients from RALMC present high OS rates and death causes are not related directly with the illness, independently of time of diagnosis. Patients treated on first line with 2GTKI present better OS, PFS and EFS rates than those treated with Imatinib. While these results are limited in our series: young patients and high risk patients begin their treatment on 2GTKI whereas older and with low-risk prognostication begin their treatment on Imatinib. Mean age for patients treated with Imatinib was almost 10 years older than that of patients treated with 2GTKI, with three times more patients over 70 years old being treated with Imatinib than with 2GTKI (27% *vs* 10.3%).

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INTERDISCIPLINARY APPROACH FOR THE MANAGEMENT OF CARDIOVASCULAR RISK FACTORS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

J. Badiola[1,*], J.M. Puerta-Puerta[1], R. Roa-Chamorro[2], F. Jaén-Águila[2], J.D. Mediavilla-García[2], M. Jurado-Chacón[1]

[1]Hematology, [2]Internal Medicine, Hospital Virgen de las Nieves, Granada, Spain

Background: The introduction of the tyrosine kinase inhibitors (TKI) for the treatment of chronic myeloid leukaemia (CML) has been a paradigm shift in the prognosis of the patients who now achieve near-normal life expectancy. This formidable improvement notwithstanding, there is concern

about TKI-associated vascular adverse events (VAE) and the management of cardiovascular risk factors (CVRF) still remains to be a challenge in these patients. We report on the creation of an interdisciplinary workgroup between the departments of Haematology and Internal Medicine for the comprehensive assessment of CVRF in CML patients treated with TKI.
Aims: To describe the protocol used in the unit, to identify previous and post-assessment CVRF and to detect vascular subclinical disease and VAE.
Methods: Since September 2016, 61 CML patients with TKI treatment were evaluated in the cardiovascular risk unit. The following data were collected: personal and familiar history, clinical history and physical exploration, basic analytical tests and specific complementary tests (ankle-brachial index - ABI), pulse wave velocity (PWV) and ambulatory blood pressure monitoring (ABPM).
Results: Of the 61 patients, 54.4% received imatinib, 42.6% nilotinib and 24.6% dasatinib, with an average treatment duration of 92, 52 and 35 months respectively. The average age was 58 years and 63.9% were males. The CVRF identified before the first evaluation in the cardiovascular risk unit are shown in the Table 1. We found that 28.5% of the patients had poor control of blood pressure as measured with ABPM, 10.7% had poor glycaemic control (glycated haemoglobin greater than 6.5%) and 21.6% ≥stage 3 renal insufficiency. In 35% of the patients, total cholesterol was greater than 200mg/dl, in 36.6% LDL cholesterol was >130mg/dl, in 23.3% triglycerides levels were >150mg/dl and in 17.2% homocysteine was >20mmol/l. With regard to vasculopathy, 10.8% of the patients had pathologic ABI. Suprinsingly, a subclinical vascular damage, as measured with PWV, was detected in a far greater percentage of the patients (24.5%). During the follow-up, we observed VAE in 18.6% of the patients, more specifically 8 peripheral arterial disease events, 2 strokes and 1 acute myocardial infarction.

Table 1. Previous CVRF.

Family history of CVRF	51.7%
Arterial hypertension	36.1%
Diabetes	13.1%
Dyslipidemia	21.3%
Previous VAE	6.6%
Renal insufficiency (≥stage 3)	3.3%

Summary/Conclusion: For an adequate control of the CVRF in CML patients, an integral multidisciplinary evaluation is mandatory. In a significant number of the patients, after the initial evaluation in the cardiovascular risk unit is carried out, there are new diagnosis of CVRF and/or poor control of such factors. The identification of these cases allows to optimize the management of CVRF and to decrease VAE. The PWV could be an optimal tool to detect subclinical endothelial damage prior to VAE and to identify those patients who might benefit from a more intensive treatment of their CVRF.

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PROGNOSTIC ROLE OF REBOUND BASOPHILIA DURING CYTOREDUCTION IN PATIENTS WITH CHRONIC MYELOID LEUKAEMIA

L. Vrablova[1,*], L. Benesova[1], B. Maiwaldova[1], T. Furst[2], I. Skoumalova[1], P. Rohon[1], K. Indrak[1], H. Urbankova[1], M. Divoka[1], E. Faber[1]

[1]Haemato-oncology department, Faculty Hospital Olomouc, [2]Faculty of Science, Palacky University, Olomouc, Czech Republic

Background: Basophilia is a frequently observed condition in chronic myeloid leukemia (CML) but its etiopathogenesis is not completely clear. Basophilia may not only be an accompanying phenomenon of CML but it may play a pathogenic role in the disease development. The role of CCL3 inflammatory chemokine produced by basophils was described in CML. We have observed **rebound basophilia (RB)**-an increase in the relative proportion of basophils in WBC differential counts (WDC) in some CML patients within the first month of cytoreduction treatment with hydroxyurea and/or imatinib. For the purpose of this study, RB was defined as an increase of one or more percent of basophils in WDC (assessed by light microscopy or counter in cases where light microscopy WDC was not available) with respect to the initial assessment before cytoreduction.
Aims: The aim was to analyze the incidence and possible prognostic role of RB.
Methods: We have retrospectively analyzed the WDC of CML patients at our institution during the first month of treatment. Overall and progression-free survival (OS, PFS) were calculated according to the Kaplan-Meier methodology, the log-rank test was used to assess the difference in survival between RB positive and RB negative patients, and the Kruskal-Wallis test were used to assess the difference in continuous variables between the two groups.
Results: We have analyzed 41 female and 63 male patients of median age of 54 years (range 20–88), the median follow-up was 3.1 years (range 3.5 months-10.9 years). There were 65 cases of RB including 7 patients who were not in the accelerated phase at diagnosis but fulfilled the criteria for CML acceleration with isolated RB over 20% during cytoreduction. Statistically significant association of RB with younger age (p=0.02) and the Sokal score (p=0.02) was documented. Spleen size (in cm below the costal margin) was significantly larger (p=0.02) in patients with RB (median=5 cm) than in patients without RB (median=0 cm) Younger patients with RB died more often after the progression of CML, while elderly patients without RB died more often due to comorbidity without CML progression. RB positive patients more frequently required the treatment with second generation tyrosine kinase inhibitors (TKI) due to the imatinib failure (38.9%) in comparison with the RB negative group (22%; p=0.06), however their response to the treatment was not compromised. We found no statistical difference in progression free survival between the RG positive and RB negative groups (63% *vs* 75%, p=0.346). The overall complete cytogenetic response rates at 6 months after treatment initiation (CCyR) were not different between the RG positive and RB negative (29% *vs* 16%, [FEpMC1] p=0.14). Patient with and without RB had similar rates of major molecular response after 12 months after treatment initiation (25% *vs* 34%, p=0.31).
Summary/Conclusion: RB may be encountered in about half of CML patients. Although RB may has been observed by many physicians, its cause is unknown. RB is associated with other CML risk features but it probably lacks an independent prognostic value. However, RB may have a role in the prognostic stratification of younger CML patients. Further experiments and studies in larger cohorts of patients are needed to reveal the causes of RB and to evaluate its possible prognostic role.
Supported by the grant of IGA_LF_2018_04.

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TREATMENT- FREE REMISSION UP TO 12 YEARS. INTERIM ANALYSIS OF A REGIONAL GERMAN CML-REGISTER. (A CONTRIBUTION OF GERMAN CML-ALLIANCE)

D. Kämpfe[1,*], J. Brenn[2], T. Haverkamp[3], C. Schulte[4], H. Tesch[5], H. Stein[6]

[1]Praxis f. Hämatologie u. Onkologie, Lüdenscheid, [2]Incyte Biosciences Germany GmbH, Planegg/Martinsried, [3]Leitung Analysenbereich Molekulargenetik II, MVZ Dr. Eberhard & Partner Dortmund (GbR), Dortmund, [4]Institut für Hämatopathologie Hamburg, Dres. Tiemann, Schulte Holding GmbH, Hamburg, [5]Centrum für Hämatologie und Onkologie Bethanien, Frankfurt / M., [6]Pathodiagnostik Berlin, Berliner Referenzzentrum für Lymphom- und Hämatopathologie, Berlin, Germany

Background: Treatment-free remission (TFR) is an achievable goal for patients with chronic myeloid leukemia (CML) in the tyrosine kinase inhibitor (TKI) era. Median follow-up time in studies up to 60 month are documented very well, and stable molecular remissions (MR)>3 in approximately 50% of patients during this period are reported. Treatment conditions differ in primary therapy (type of TKI; IFNa ?) and in duration of deep molecular remission (mostly 1 to 2 years in current studies with 2nd GenTKI).
Aims: To enhance the current debate: "How to achieve TFR?" we describe 7 patients of our "real world" register, who are in TFR for at least 2 years after Imatinib (IMA) as their 1st line TKI- therapy.
Methods: Our register includes 52 CML-patients, newly diagnosed between 01/1998 and 12/2017. Median age (62,1y, 22-84y), sex ratio (m : f=1,7 : 1) and raw incidence ($1{,}1/10^5$) correspond to literature. Diagnoses are based on morphological and genetic findings of EUTOS-accredited laboratories. Ending the TKI-therapy was considered after 3 years of continuous genetic remission (MR >MR 4,5 and / or nested PCR negativity). 8 of 14 candidates finished therapy that way. 1 more patient finished 6 months after IMA in 2nd molecular remission after previous allo-transplant.
Results: After stopping TKI-therapy 7 of 9 patients (77,8%) remained in complete molecular remission. Median OS from diagnosis is 187,1 months (119-240 mo). Median duration of TFR is 59,5 months (24,5-144,5 mo). Median age (at diagnosis) was 56,9 years (40,8-69,4 ys), sex ratio (m : f) is 5 : 2. BCR-ABL transcript type E14a2 was found in 4 *vs* E13a2 in 2 patients (1x unknown), which may be different from the expected ratio in CML-CP. EUTOS risk was "high" in 3 patients; 1 of 7 was in primary accelerated phase (pAP). 1 patient got TKI after initially aggressive conventional chemotherapy and consecutive HU / IFNα ("OSHO 1995"-study) for 174 months, with interruption of IFNα for 24 months because of autoimmune thyreoiditis. Also 2 other patients received IFNα prior to TKI, 1 in combination ("CML IV" study, later in the "NICOLI" study-immediately finished

because of GUILLAIN-BARRE-like-syndrome). TKI was changed from IMA to DASA in 1 and to NILO in another case. Body mass index (BMI) of 2 patients was >30, 1 of them got IMA mainly 300 mg per day. Remarkably in 2 patients JAK2-mutation was detected (low level, but consistently), one in combination with KIT-mutation and one in a patient with splenomegaly and suspected 2nd neoplasia (CMML? MDS?) who acquired additional KRAS & CSMD1 mutations in addition to a presumably germline variant of JAK3 (p.V722I).
Summary/Conclusion: Despite limited number of patients our real life experience illustrates: 1.) TFR more than 10 years is an achievable aim, also in patients treated with IMA as a 1st line TKI and under real life conditions. 2.) Most common features of our TFR-patients were: 3-year continuing deep mol.-genetic remission (6/7) and after using IFNα (4/7). Both common features are discussed thoughtfully in the literature. Most other characteristics were heterogeneously. 3.) As recently discussed (GALE and HOCHHAUS 2018) TFR may be achieved by one or in combination of different mechanisms. This could correspond to the heterogeneous characteristics of our patients. 4.) Furthermore TFR stabilizing effect could be growth-inhibition by non-BCR-ABL-mutated subclones implying passenger mutations.

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FREQUENCY OF GATEKEEPER MUTATION IN THE NON-RESPONDING CHRONIC MYELOID LEUKEMIA PATIENTS

B. Kaleem*, S. Shahab, M. Borhany, U. Zaidi, T. Farzana, D. Zahid, T. Shamsi

Haematology, National Institute of Blood Diseases and Bone Marrow Transplantation, Karachi, Pakistan

Background: Chronic Myeloid Leukemia is a myeloproliferative disorder that results due to reciprocal translocation t(9;22)(q34.1;q11.2) leading to the formation of an oncogene. Although Imatinib proved to be revolutionary treatment wise but 20% showed resistance. Point mutations in the ABL Kinase Domain were one of the causes of the resistance of which T315I was found to be the most resilient against all Tyrosine Kinase Inhibitors (TKIs).
Aims: This study observes the frequency of T315I mutation in TKI non-responders in our population.
Methods: Patients labeled as non-responders according to European Leukemianet guidelines to the first line therapy *i.e.* Imatinib or Nilotinib at our centre were included. The blood sample was collected in an EDTA vacutainer. DNA was extracted and then analyzed for the presence of both wild type and mutant via PCR. Negative controls were healthy individuals and demographically similar to the patients.
Results: Out of 150 patients, 44 were found to be non-responders. Patients were divided into three groups on the basis of TKI administered. Imatinib (n=3), Nilotinib (n=22) and those shifted from one TKI to another (n=19). Of these, 37/44(84.1%) patients had the presence of T315I.
Summary/Conclusion: Mutational analysis in the event of resistance and prior to the shift to next generation is mandatory. The presence of mutations will tailor the further treatment plan of the patient. As in our case the presence of T315I mutation will help in counseling the patients for the stem cell transplantation and prevent their further exposure to TKIs which would be an unwanted financial and psychological burden for them.

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CHRONIC MYELOID LEUKEMIA (CML) MAY CO-EXPRESS THE P210 BCR-ABL1 AND P195 BCR-ABL1

S. Gonçalves Pereira*, F. Saraiva, L. Monteiro, M. Silva, R. Vinhas, P. Viana Baptista, S. Costa Santos, A. Lourenço, P. Ribeiro, A.R. Fernandes, A. Botelho De Sousa

Hematologia Clínica, CHLC, [2]UCIBIO, Dep. of Life Science, FCT-UNL, Lisboa, Portugal

Background: Around 95% CML pts have breakpoints in the M-bcr, which result in b2a2 (e13a2) and/or b3a2 (e14a2) fusion mRNAs, both of which are translated into the p210 BCR-ABL protein, which in turn functions as a constitutively active Tyrosine Kinase (TK) leading to alterations in cell proliferation, differentiation, adhesion, and survival. Rarely, other breakpoints can occur-p190 BCR-ABL1 protein (m-bcr) or p230 BCR-ABL1 protein (μ-bcr), respectively, e1a2 and e19a2. There are other atypical breakpoints outside these cluster regions, usually associated with an aggressive clinical course.
Aims: To document a confirmed case of the co-expression of the p210 BCR-ABL1 and p195 BCR-ABL1 proteins in a CML patient, in order to draw attention to this rare diagnosis and its aggressive clinical course.
Methods: We report the case of a previously healthy 36 year-old man, with no relevant past diseases and a one-month history of fever and night sweats, on physical examination no hepatosplenomegaly or lymphadenopathy were found. Haemoglobin was 12.9g/dl, WBC were 58300/μL (90.8% neutrophils, 1.4% eosinophils, 0% basophils, 6.5% lymphocytes, 1.3% monocytes) and platelet count was 507000/μL. Bone marrow aspiration showed 1% blasts and FISH for BCR-ABL1 was 98% positive. A diagnosis of CML was made, with a Sokal score of 0.57, Hasford 58 and EUTOS 0. The patient initiated imatinib 400mg/day and a haematological response was achieved, with no cytogenetic response at 3 months of treatment, despite an increase of the imatinib dose to 600mg at 4 months and 800mg two weeks later. Treatment was switched to bosutinib at month 9. Cytogenetic response was achieved at 7 months after bosutinib initiation.
Results: The molecular analysis showed a rare co-expression of the p210 BCR-ABL1 and p195 BCR-ABL1, attributable to an alternative splicing of the transcript arising from the M-BCR chimeric oncogene. While waiting for related-donor survey for subsequent allogeneic transplantation, at month 11 of bosutinib, the disease progressed to a lymphoid blastic crisis, presenting with pancytopenia with 61% blastemia, and 96% bone marrow lymphoblasts; p210 remained in cytogenetic response yet p195 remained FISH positive. R-HyperCVAD+dasatinib+intra-thecal chemotherapy was initiated. At the end of the 1st A cycle a complete response was documented.
Summary/Conclusion: Different transcript co-expression has been reported by others in rare cases of CML, but with p210 and p190 BCR-ABL1. This is the first report of CML co-expressing p210 and p195 BCR-ABL1. Possibly, because of the shorter transcript lacking regulatory domains, p195 is associated with a dismal prognosis. Even though this patient initially presented in a chronic phase, the p195 clone remained positive in FISH and ultimately the disease progressed to a lymphoid blastic crisis.

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DEVELOPMENT OF SAMPLE PREPARATION PROCEDURE FOR TESTING SPECIMENS WITH HIGH WHITE BLOOD CELL COUNTS FOR XPERT® BCR-ABL ULTRA×

V. Mahaffey[1], S. Amar[2], G.-J. Day[3,*], E. Manor[2], D. Viswanatha[1]

[1]Molecular Hematopathology Lab, Mayo Clinic, Rochester, United States, [2]Human Genetic Institute, Soroka University Medical Center, Beer-Sheva, Israel, [3]Oncology, R&D, Cepheid, Sunnyvale, United States

Background: Monitoring BCR-ABL transcript levels in peripheral whole blood (WB) of patients on tyrosine kinase inhibitor (TKI) therapy using real-time quantitative PCR (RT-qPCR) is standard of care in the management of Chronic Myeloid Leukemia (CML). A successful RT-qPCR reaction requires isolation of high quality RNA and optimization of the input quantity for the conversion of RNA to cDNA for accurate quantification. GeneXpert® BCR-ABL V2[YY] or Xpert® BCR-ABL Ultra (Ultra), a cartridge-based assay for use on the GeneXpert Instrument system, automates and standardizes the process in less than 2 hours. The Ultra calibration and quantification are standardized by monitoring the input RNA quality and quantity based on the cycle threshold (Ct) of the control gene ABL, which is highly correlated to the input white blood cell (WBC) number in whole blood (WB). Using a lysate prepared from 4mL WB, resulting in an effective WB input volume of 600μL, Ultra reproducibly achieves molecular response (MR) sensitivity to 4.5 logs below baseline (MR 4.5, defined as BCR-ABL1 *IS* ≤0.0032%) per the WHO International Scale (*IS*). There are, however, clinical situations where the total RNA isolated from high numbers of WBC circulating in the patient's blood or in bone marrow (BM) samples can overload the Ultra cartridge and other quantitative BCR-ABL assays, requiring subsequent dilution of the sample and retesting.
Aims: In this set of experiments, we sought to define the WBC input limits for Ultra that would predict cartridge overload, and thereby provide guidance regarding when to dilute the patient's sample in the presence of high WBC.
Methods: Serial dilutions of high or normal WBC counts (WBCCs) specimens were tested in Ultra to determine the upper and lower WBC input limits corresponded to the valid ABL Ct cutoffs of 10 and 18, respectively. Sample prep procedures were developed to allow using 50μL or lower input volume from WB, BM, or their 1st lysate with or without the WBCCs information,, and validated by testing in CML specimens with various WB or BM input volume, compared to the Qiagen® BCR-ABL1 Mbcr *IS*-MMR assay (*IS-MMR*) (Table 1).
Results: The WBC input number of ~20 million cells/mL WB corresponded to the upper limit, and ~150K cells/mL WB corresponded to the lower limit of the valid ABL Ct range for Ultra, respectively. For CML specimens with WBCCs<20 million cells/mL WB, the standard initial 4mL WB sample input yielded ABL Ct results within acceptable limits. For the WBCCs ≥20 million

cells/mL WB, reaching as high as >500 million, sample prep procedures were developed to use 50µL or lower input volume (Table 1). When the WBCC information is not available but with suspicion of high WBC yielding a very viscous 1st lysate that is hard to pipette, a 1st lysate dilution procedure was developed to allow for testing with 50µL or lower WB or BM input volume. However, for the situation where a low input sample volume, for example 50µL, was used but still yielded ABL Ct<10, then an even lower input sample volume (10µL or lower) might be used to re-test the sample (Table 1). This was validated in Ultra by testing CML specimens with various WB or BM input volume and showed high concordance when compared to the Qiagen® *IS*-MMR.

Table 1.

Table 1. Recommended input whole blood volume relative to the White blood cell counts for Ultra

		White blood cell count (million/mL whole blood)				
		<20	≥20 & < 50	≥50 & <100	≥100 & <300	≥300
Input whole blood volume	Initial testing	600µl*	50µl	25µl	10µl	5µl
	When repeat testing is needed due to an initial invalid call with the ABL Ct < 10	10 µl - 50µl	2µl** - 10µl	2µl** - 5µl	2µl**	1µl**

*Calculated WB input volume using the 1st lysate prepared from 4mL whole blood
**Calculated WB input volume using 10X pre-diluted WB with 1X PBS

Summary/Conclusion: In summary, sample prep procedures were developed in cases where high WBCC is known, or is suspected, or when repeat testing is needed for Invalids with ABL Ct<10, allowing the use of Ultra with various input sample volume for WB or BM in a wide variety of clinical situations.

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IMPROVING EUTOS SCORE SENSITIVITY BY USING SERUM ALPHA/BETA TRYPTASE-1 LEVEL IN NEWLY DIAGNOSED EGYPTIAN PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA IN CORRELATION TO THE MOLECULAR MILSTONES

A. Youssef[1,*], M. Elsorady[1], M. Ayad[2], A. Abdelrahman[1]

[1]Internal medicine, hematology and BMT unit, [2]Clinical pathology department, Faculty of Medicine Alexandria university, Alexandria, Egypt

Background: The European treatment and outcome study (EUTOS), a chronic myeloid leukemia (CML) prognostic score based only on the percentage of basophils in the blood and on spleen size, was formulated and shown to have improved predictive power. The EUTOS score marks a significant advance because it provides better positive predictive values than those obtained with either of the previous scores (Sokal and Hasford) and its easy application. Moreover, it is specifically based on Imatinib-treated patients, and does not need the use of the other factors that have not been found to affect the response to Imatinib. Serum alpha/beta tryptase 1 is a novel biomarker with a prognostic significance that can be used to differentiate clonal from non clonal causes of basophilia that may affect EUTOS score sensitivity.
Aims: Testing EUTOS score in newly diagnosed CML in chrnoic phase and evaluating the addition of serum alpha beta tryptase one on the score sensitivity in correlation with the established prognostic scores (Sokal and Hasford) and molecular milestones.
Methods: 48 CML cases to 19 controls were studied with a male : female ratio 1:1 and mean age of 43.94 years. Serum α/β tryptase 1 level was measured at presentation using quantitative sandwich ELISA technique (EIAab kit catalog no: E1070h, China). Patients were recruited from hematology outpatient clinic, Alexandria faculty of medicine excluding patients in acceleration or blastic transformation. All patients were subjected to the routine clinical examination, abdominal ultrasonography, viral hepatitis and HIV screening, peripheral blood smear examination, bone marrow examination and BCR ABL 1 at diagnosis, 3 and 6 months using RQ PCR. Sokal, Hasford and EUTOS scores were calculated for each patient at presentation. EUTOST score was calculated based on the formula (serum tryptase in mg + subcostal splenic span in cm). All patients included were eligible to start frontline Imatinib treatment 400 mg daily during molecular monitoring.
Results: The results show the EUTOS score has lower sensitivity and specificity at the cut off 87 compared to 154 suggesting the higher splenic meaurements and basophilia in our study, when applying the higher cut off better predictive power was seen. With the addition of serum tryptase (EUTOST) there is a noticed increase in the sensitivity and specificity compared to EUTOS score (increased area under ROC curve). This may be explained by higher prevalence of liver disease in our sample (5 schistomiasis and 7 chronic HCV cases).

Table 1.

	EUTOST	
	rs	P
BCR-ABL at 3	0.736*	<0.001*
BCR-ABL at 6	0.830*	<0.001*
Sokal	0.829*	<0.001*
Hasford	0.715*	<0.001*
EUTOS	0.972*	<0.001*

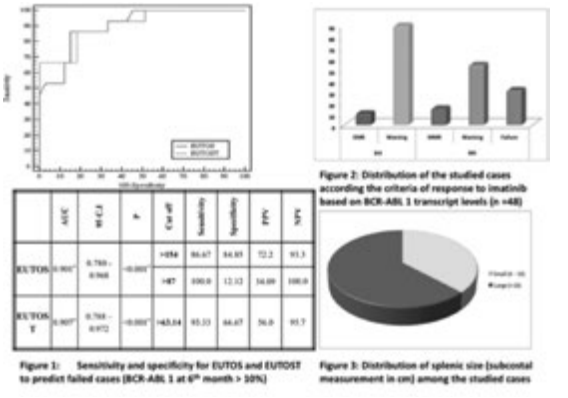

Figure 1: Sensitivity and specificity for EUTOS and EUTOST to predict failed cases (BCR-ABL 1 at 6th month > 10%)

Figure 2: Distribution of the studied cases according the criteria of response to imatinib based on BCR-ABL 1 transcript levels (n =48)

Figure 3: Distribution of splenic size (subcostal measurement in cm) among the studied cases

Figure 1.

Summary/Conclusion: EUTOS score showed high sensitivity when used with a higher cut off value 154 suggesting that the non clonal causes of basophilia and splenomegaly in the Egyptian population play a role in high risk prognostication. Besides identifying the non clonal causes of high EUTOS risk prognostication, applying serum tryptase level has improved its prognostic significance and predictive power.

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EVALUATION OF LOW LEVEL DETECTION OF MAJOR BCR-ABL1 MRNA IN CHRONIC MYELOID LEUKEMIA (DOMEST ADDITIONAL STUDY)

H. Kitamura[1,*], Y. Tabe[2], K. Tsuchiya[2], M. Yuri[2], S. Misawa[2], T. Horii[2], A. Ohsaka[2], S. Kimura[1]

[1]Division of Hematology, Respiratory Medicine and Oncology, Department of Internal Medicine, Faculty of Medicine, Saga University, Saga, [2]Department of Clinical Laboratory Medicine, Juntendo University School of Medicine, Tokyo, Japan

Background: Chronic myeloid leukemia (CML) is caused by a reciprocal translocation between chromosome 9 and 22 forming an oncogenic *BCR-ABL1* fusion gene. A *BCR-ABL1* Tyrosine kinase inhibitor (TKI) is a therapeutic agent in patients with CML. Although highly potent TKIs have been developed for deeper molecular response, about 50% of sustained DMR achieved CML patients relapse after TKI cessation. To predict the suitable time point for TKI treatment cessation, highly sensitive and specific molecular real-time quantitative polymerase chain reaction (RQ-PCR) is required to monitor minimal residual disease (MRD) levels.
Aims: In this study, we aimed to evaluate the newly developed RQ-PCR as an additional study of "The Delightedly Overcome CML Expert Stop TKI (DOMEST) trial" conducted by the DOMEST group in Japan.
Methods: In the DOMEST study, after stopping imatinib DMR was assessed by RQ-PCR in a central laboratory (BML, Tokyo, Japan). For this analysis we used residual total RNAs from 102 patient samples of DOMEST trial, which recruited CML patients in chronic phase received imatinib therapy,

reduced *BCR-ABL1* levels to undetectable levels (MR4.0) by transcription-mediated amplification (TMA), reverse transcriptase-polymerase chain reaction or RQ-PCR for over 2 years. The median age of the patients was 62 years (27-88). The percentage of the patients who had low Sokal risk scores was 57 of 102 cases (56%) and the median duration of imatinib treatment was 99.5 months (13.0-160.0).
Results: *BCR-ABL1* negativity has been confirmed by the central laboratory RQ-PCR assay at the starting time point of the DOMEST study (n=102). In 6 of these samples (6%) the new RQ-PCR assay detected *BCR-ABL1* transcripts which were confirmed by the sequencing analysis. During the MRD monitoring after imatinib cessation, the new RQ-PCR assay detected *BCR-ABL1* positivities at the same timing with the central laboratory RQ-PCR in 15 of 102 cases (15%). Correlation of IS% *BCR-ABL1*/*ABL1* between the new RQ-PCR and the central laboratory RQ-PCR assays showed a strong correlation (r=0.74, *P*=0.003). The new RQ-PCR assay detected MRD in 21 cases (21%) at the earlier timing than the central laboratory RQ-PCR assay. We further examined *BCR-ABL1* mRNA levels in these 21 samples by a commercially available ODK-1201 RQ-PCR assay kit (Otsuka Pharmaceutical Co., Tokyo, Japan), which detected the low level of *BCR-ABL1* mRNA in 14 samples (74%) and negative in 5 samples (26%) with 2 samples of not available due to the insufficient amount. No sample with *BCR-ABL1* positive by the central laboratory RQ-PCR assay and negative by the new RQ-PCR assay was observed.
Summary/Conclusion: The newly developed RQ-PCR assay has been shown to be more efficient for monitoring MRD in TKI-treated CML comparing the conventional PCR based assays. Further clinical studies of monitoring MRD in TKI-treated CML are required.

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TWELVE YEARS RESULTS OF FIRST-PHASE CHRONIC MYELOID LEUKEMIA (CML) TREATMENT WITH IMATINIB 400 MG (IMATIB*) IN 222 PATIENTS

N. Abdennebi*, F. Harieche, F. Boukhemia, M. Benakli, R. Ahmed Nacer, R.-M. Hamladji

Hematology and Bone Marrow Transplantation, Pierre and Marie Curie Center, Algiers, Algeria

Background: The classic evolution of 3-phase CML (chronic, accelerated and blast) has been revolutionized by the advent of the tyrosine kinase inhibitors leading the way to Imatinib: we are now talking about a chronic disease. From April 2005 to October 2017, 222 patients (pts) with CML in the first phase were treated with Indian Imatinib: Imatib*.
Aims: Evaluation of Imatib (Indian Imatinib).
Methods: There are 115 men and 107 women (sex ratio: 1.07) with a median age of 44 years (13-77). The molecular study by qualitative RT-PCR specified the type of transcript in 219 pts (98.6%): b2a2 at 98 pts (45%), b3a2 at 117 pts (53%), a double transcript b2a2-b3a2 at 2 pts and variant BCR-ABL at 2 pts (c3a2 and e1a2). According to Sokal classification, 129 pts (58%) are at intermediate score, 57 pts (26%) at high risk and 36 pts (16%) at low risk. According to the Eutos score, 170 pts are at low risk (77%) and 52 pts at high risk (23%). All patients were treated with Imatib *400mg after treatment with Hydroxy-Urea in 210 pts (94.5%) and *de novo* in 9 pts. It should be noted that 3 pts received pegylated interferon because of pregnancy diagnosis in the first trimester.
Results: In October 2017, 204 pts are evaluable with a median follow-up of 76 months (3-163). The response is complete hematologic in all pts and major molecular (residual disease<0.1% by quantitative RT-PCR) at 6, 12 and 24 months respectively in 80/172 pts (46.5%), 101/155 pts (65%) and 99/124 pts (80%). Primary or secondary resistance is observed in 53 pts / 204 (26%) with no RMM at least at 12 months in 26 pts, loss of RMM already obtained in 8 pts, ratio>10% at 6 months in 5 pts and hematologic relapse in 14 pts. A progression to acute leukemia is observed in 12 pts (6%) but in no pt beyond 6 years. Sixteen pts / 204 (8%) died: 11 of acutization, 4 of a probable Imatib complication and one of a non-CML cause. At 12 years old, 142pts (69.5%) are still treated with Imatib * 400 mg including 72 in RMM4 and 7 in RMM3 more than 3 years of treatment. The actuarial overall survival (OS) and event free survival (EFS) are respectively 88.6% and 77% comparable to those of the IRIS study. According to Sokal score, the OS are 100, 88 and 84.3% and the EFS are 92.8; 76.3 and 71.6% respectively for low, intermediate and high risk with only a significant difference between low and high risk. According to Eutos, the OS and EFS are not significantly different for low and high risk, they are 91 and 85.3% for OS and 78.7 and 72.6% for EFS.
Summary/Conclusion: With a 12-years decline in use, we were able to confirm that the efficacy of Imatib is comparable to that of the originator molecule with a cost ten times lower, which made it possible to treat all the pts of the Algerian territory.

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EPIDEMIOLOGY IN CHRONIC MYELOID LEUKEMIA (CML) IN ANDALUSIA (SPAIN). RESULTS FROM THE ANDALUSIAN REGISTRY OF CML (RALMC) 2002-2016

J.M. Puerta-Puerta[1,*], A. Rosell-Mas[2], C. Ferrer-Chaves[3], M.S. Durán[4], M.J. Ramírez-Sánchez[5], M.Á. Portero-Frías[6], M.J. García-Pérez[7], C. Ruiz-Nuño[8], A. Paz-Coll[9], M.I. Mata-Vázquez[10], M.C. Avellaneda[11], I. Simón-Pilo[12], I. Ballesteros[13], Y. Moatassim[14], J. Oliveros[15], A. Jiménez-Velasco[8], P. López-Garrido[16]

[1]Hematology, UGC intercentros Hospital Universitario Virgen de las Nieves-Nuevo San Cecilio, Granada, [2]Hospital Universitario Virgen de la Victoria, Málaga, [3]Hospital San Juan de la Cruz, Úbeda, [4]Hematology, Complejo Hospitalario de Jaén, Jaén, [5]Hematology, Hospital de Jerez, Jerez de la Frontera, [6]Hematology, Hospital Universitario Virgen Macarena, Sevilla, [7]Hospital Torrecárdenas, Almería, [8]Hospital Regional Universitario Carlos Haya, Málaga, [9]Hematology, Hospital de Puerto Real, Puerto Real, [10]Hospital Costa del Sol, Marbella, [11]Hematology, Hospital San Agustín, Linares, [12]Hospital Virgen de Valme, Sevilla, [13]Hospital de la Axarquía, Vélez Málaga, [14]Hospital Santa Ana, Motril, [15]Hospital La Inmaculada, Huércal Overa, [16]Grupo Andaluz de LMC, Granada, Spain

Background: Despite clinical and therapeutic advances on CML, little is known about its epidemiology and there are not many publications showing results on patients' health in real life. Population registries allow to obtain information on incidence, prevalence and effectiveness of treatments; this information is usually obtained through clinical trials characterized by their strict inclusion and exclusion criteria.
Aims: To describe the epidemiological characteristics of patients with CML CP in Andalusia and to calculate the rates of incidence among patients older than 20, adjusted to the European population in order to allow for comparison with other registries.
Methods: Population-based retrospective descriptive study on real life, which analyzes the epidemiological features among patients included in RALMC since January 2002 to December 2016. In order to calculate the accumulated incidence new diagnostics were taken since January 2005 until December 2012 and in order to compare with other incidence rates, adjustments via direct rates adjustment was made, taking as reference the standard a European population sample from IARC. RALMC and its associated projects are carried out according to current Spanish legislation as well as recommendations for research projects given in the Helsinki Declaration.
Results: With a number of new diagnostics at 288 patients older than 20 in Andalusia between 2005 and 2012, accumulated incidence rates as it is adjusted to the European standard population for CML in Andalusia is of 0.97 cases out of every 100.000 inhabitants/year. Until December 2016, 505 patients have been included, 279 males (55.2%). Mean age is 55 (20-90). 104 patients (20.6%) older than 70 with a mean of global follow-up of 86 months (11-194). 275 patients (54.5%) low-risk Sokal, 166 (32.9%) intermediate risk and 63 (12.5%) high risk. Low Hasford Euro score 248 patients (49.1%), intermediate 184 (36.4%), high 54 (10.7%) and 19 unknown (3.8%) and low EUTOS score in 383 patients (75.8%), high 103 (20.4%) and 19 unknown (3.8%).

Table 1.

BASELINE CHARACTERISTICS OF THE STUDY RALMC POPULATION (N=505 PATIENTS)

	Sex	Age		Sokal			Euro score				EUTOS			1st line Treament		
	M	M_e		L	I	H	L	I	H	Un	L	H	Un	Im	Nil	Das
n	279	55	104	275	167	63	248	184	54	19	383	103	19	427	46	32
(%)	55.2		20.6	54.5	33	12.5	49.1	34.6	10.7	3.8	75.8	20.4	3.8	84.6	9.1	6.3

Summary/Conclusion: CML incidence in Andalusia is comparable to that described in the scientific literature and is in keeping with results from the European registry EUTOS from 2015. Epidemiological characteristics from our patients are homogeneous to those described in other registries in terms of sex, age of diagnosis and prognostication indices distribution. The creation of RALMC on population basis allows to obtain information regarding CML in real life, which is in turn needed for health planning and resource management in clinical praxis and support outside of clinical trials. However, methodological limitations in our registry must be taken into consideration: in Spain, CML patients are not obliged to declare their status, participation in RALMC is voluntary and inclusion is done under written consent.

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A STUDY TO IDENTIFY THE POPULATION VARIATION IN THE RESPONSE AND THE SIDE EFFECT PROFILE IN CHRONIC MYELOID LEUKEMIA-A NATIONAL EXPERIENCE FROM SRI LANKA

O. gamage[1,*], K. Perera[2], I. Wijesiriwardena[3]

[1]Haematology, Royal wolverhampton NHS Trust, Wolverhampton, United Kingdom, [2]Oncology, National Cancer Institute, [3]Haematology, University of Sri Jayawardhenapura, Colombo, Sri Lanka

Background: Imatinib, is the first tyrosine kinase inhibitor of the BCR-ABL protein. Imatinib was introduced for the treatment of with Chronic Myeloid Leukaemia (CML) in 2001.The discovery of the wonder drug, has changed the treatment paradigm of CML, now moving towards for the "treatment free remission". Appearance of kinase domain mutations and non-adherence are the main current challenges. Imatinib has proven its safety and efficacy in long term 10 year follow up data of IRIS study with overall survival of 83.3% without any cumulative or late toxic effects. The paucity of such studies analyzing the expanding Asian patient population, is a significant drawback in clinical practice, especially in explaining some side effects in them such as skin manifestations.
Aims: To look at the demographics in CML patients across the country in Sri Lanka and analysis of the response rates to Imatinib as the first line treatment in CML. To identify the variation of the side effect profile in the Sri Lankan population.
Methods: A total of 86 patients were treated with Imatinib as the first line TKI,who had completed minimum of 2 years of treatment were included. The data set was analyzed for basic demographics, Haematological and cytogenetic response. The side effect profile was analyzed, in relation to the time from starting treatment and the severity and graded the severity of cytopenias according to the WHO grading.
Results: Of the 86 patients, consist of 48 (56%) males and 38(44%) females, within the age of 15- 72 years at diagnosis, representing whole country. 38% of the patients were in high sokal risk category and 62% low or IM risk group. Analysis of the response to therapy according to the ENL response criteria 2016, revealed 89% hematological and 56% complete cytogenetic response achieved within optimal time frame. A detailed analysis of 36 patient's revealed 51% achieved a Major molecular response (MMR) by the completion of one year treatment. In terms of side effect profile, commonest during the first 6 months were of grade1 and 2, specifically gastrointestinal effects (86%), Skin hypopigmentation (69%), Muscular skeletal (63%), derangement of the liver enzymes(58%),fluid retention (32%) and other skin manifestations accounted in 20%. Of the haematological toxicities, neutropenia (57%) and thrombocytopenia (52%) were the commonest, and mostly grade 1 and 2 while, Grade 3 and 4 cytopenias were in 9 patients (10%). Most of the off target effects were settled after 6 months of the treatment. Most importantly, skin manifestations were the commonest which was persisted beyond 6 months of treatment. Interestingly, it was persisted in all the patients while on the treatment and was reversible on withdrawal. Skin hyperpigmentation was observed in 4 (7%).
Summary/Conclusion: Imatinib has induced optimal response in majority of patients. Therefore, Imatinib has shone it's efficacy in real life conditions, in our population. Reversible skin hypopigmentation is significantly higher than reported. There is higher events of transient liver derangement and cytopenias, could be explained by the smaller body built of the population.

PB1961

LATE MYOCARDIAL TOXICITY IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS ON LONG-TERM TYROSINE KINASE INHIBITOR (TKI) THERAPY

L. Genge[1,2,*], S. Narayanan[2], D. Hogge[2], M. Power[2], D. Sanford[2], D. Forrest[2], S. Nantel[2], M. Barnett[2], Y. Abou Mourad[2], A. Gerrie[2], K. Song[2], C. Toze[2], R. Broady[2], H. Sutherland[2], J. White[2], T. Nevill[2]

[1]Department of Internal Medicine, University of British Columbia, [2]Division of Hematology, Leukemia/BMT Program of BC, Vancouver, Canada

Background: Imatinib mesylate is a small-molecule TKI that dramatically changed the treatment landscape for patients with CML. Other TKIs have since been developed (e.g. Dasatinib) as second-line agents for intolerance or refractory disease. TKIs are generally well-tolerated with common side effects being nausea, diarrhea, fluid retention, myalgias and rash. TKIs were first implicated in cardiomyocyte toxicity following a report of ten CML patients who developed early congestive heart failure following treatment with Imatinib (Kerkela, 2006). Several *in vitro* and *in vivo* studies have since shown that Imatinib and Dasatinib can induce cardiotoxic effects via both on-target and off-target mechanisms, although the incidence in humans remains unclear (Force, 2011; Savi, 2018). There are no studies to date specifically examining myocardial toxicity in humans on long-term TKI treatment.
Aims: To describe a series of patients (pts) on prolonged TKI therapy for CML with documented myocardial toxicity without another identifiable etiology for the cardiac complication.
Methods: A retrospective chart and data base review was performed on pts with CML treated with TKIs by the Leukemia/BMT Program of BC over a 15-year period (2002-2017). Basic demographic data was collected along with date of commencement of TKI, disease status, TKI dose and response, adverse effects, duration of therapy at diagnosis of myocardial toxicity, investigation and management of cardiac complication and outcome. Specific attention was paid to the presence of traditional cardiac risk factors (RFs) and the presence of alternative etiologies for the myocardial toxicity.
Results: Five pts with chronic-phase CML were identified (Sokal score good-risk in 4 pts, intermediate-risk in 1 pt), 3 males and 2 females, with a median age of 52.6 years (yrs) (range 43.3-56.2) at diagnosis. Myocardial toxicity was identified at a median age of 61.9 yrs (range 56.5-71.1) after median total TKI therapy duration of 12.5 yrs (range 3.3-15.3). All pts had exposure to Imatinib for a median total of 12.5 yrs (range 0.5-15.3) and three had exposure to Dasatinib for median 0.5 yrs (range 0.3-2.8). Four pts had evidence of cardiomyopathy with reduced left ventricular ejection fraction, median 35% (range 20%-45%) by echocardiography, two with associated pericardial effusion. The fifth patient had a left atrial myxoma. RFs for atherosclerotic disease included obesity (2 pts), type II diabetes mellitus (1 pt) and remote (>25 yrs) smoking (1 pt). Two pts had remote exposure to cardiotoxic agents (Doxorubicin 26 yrs and Cyclophosphamide 15 yrs previously, respectively). Two pts had cardiac angiography and two pts had MIBI scan with no evidence of ischemic heart disease; all pts were assessed by a cardiologist with no other identifiable explanation for the cardiotoxicity aside from chemotherapy exposure. The atrial myxoma pt underwent surgical resection with no cessation of Imatinib while TKI therapy was held in all pts with cardiomyopathy. All pts commenced medical therapy, improved clinically and echocardiographically and were restarted on TKI therapy.
Summary/Conclusion: Despite strong mechanistic evidence of cardiomyocyte toxicity of TKIs, clinical reports in humans are rare. We present a series of pts with delayed cardiotoxicity in the form of cardiomyopathy with reduced systolic function and the first reported case of atrial myxoma presumed secondary to prolonged TKI therapy. Early screening of symptomatic individuals for cardiotoxicity from TKI therapy is strongly recommended.

PB1962

ADDITIONAL CYTOGENETIC ABNORMALITIES IN CHRONIC MYELOID LEUKEMIA; EXPERIENCE FROM PAKISTAN

N. Anwar*, S. Khurram, M. Nadeem, S. Shan, N. Fatima, M. Borhany, T. Shamsi

Hematology, National Institute of blood diseases and bone marrow transplantation, Karachi, Pakistan

Background: Chronic myeloid leukemia (CML) is characterized by formation of Philadelphia (Ph) chromosome as a result of reciprocal translocation between chromosome 22 and 9 *i.e.* t(9;22)(q34;q11.2). Additional cytogenetic abnormalities ACAs are reported internationally in 5-12% newly diagnosed CML.
Aims: The study was done to observe the frequency of cytogenetic abnormalities in CML in addition to Ph chromosome at the time of diagnosis, their baseline hematological characteristics and correlate their outcome on follow up.
Methods: This was a cross sectional study carried out at the department of Cytogenetics and Molecular Pathology of National Institute of Blood Diseases and Bone Marrow Transplant Karachi Pakistan from May 2010 to September 2016.All the patients diagnosed with CML during the study period on the basis of morphological and cytogenetic analysis were included and observed for ACAs. Baseline cytogenetic analysis was performed on overnight, 24-hrs unstimulated and 72-hrs stimulated bone marrow cultures using standard procedures. The GTG (G-bands via trypsin using giemsa) banding technique was applied, karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN) 2013, karyogram were made using Metasystem®. *BCR-ABL1* by real-time quantitative PCR was done by QIAGEN kits on Rotor-Gene Q 5plex HRM instrument with 72-tubes rotor, at baseline and at 18 months of treatment performed on peripheral blood and bone marrow. MMR was defined as 03 log reduction from the baseline.
Results: Out of total 222 cases of CML,18(8.1%) patients revealed ACAs; we found –Y, double Ph chromosome and trisomy 8, each in 01(6%) patient, complex karyotype in 5(28%) patients, del7q and hyperdiploidy each in 2(11%) patients. Two of the patients (11%) exhibited 3 way variant Ph translocations. We found lower median hemoglobin and platelet count and higher median eosinophil, basophil count and spleen size in patients with ACAs as compared to previously reported data. Our study also included the outcome of such patients followed at 6 and 18 months post treatment. Only 03 patients achieved major molecular response (MMR).

Summary/Conclusion: The main aim of the study was to highlight the importance of detecting ACAs in patients with CML at diagnosis. We also observed baseline characteristics and correlation of treatment response in such patients. As ACAs are reported to be associated with adverse outcomes and disease progression in literature, in this context our study would be valuable in adding additional information in local data

PB1963

DISCONTINUATION OF TYROSINE KINASE INHIBITORS IN PORTUGUESE CML PATIENTS: A SINGLE INSTITUTE EXPERIENCE

N. Cerveira[1,*], B. Loureiro[2], S. Bizarro[1], C. Correia[1], L. Torres[1], S. Lisboa[1], J. Vieira[1], D. Pereira[2], C. Moreira[2], S. Chacim[2], N. Domingues[2], A. Espírito-Santo[2], I. Oliveira[2], I. Moreira[2], L. Viterbo[2], Â. Martins[2], M.R Teixeira[1,3], J.M Mariz[2]

[1]Department of Genetics, [2]Department of Onco-Haematology, Portuguese Oncology Institute, [3]Institute of Biomedical Sciences (ICBAS), University of Porto, Porto, Portugal

Background: In the last few years, several clinical discontinuation trials have demonstrated that 40-60% of chronic phase CML patients (CP-CML) who have achieved a stable deep molecular response (DMR), defined as a sustained molecular response of at least 4.5 ($MR^{4.5}$), can stop therapy without relapsing. In addition to DMR, other variables that have been associated with a successful treatment-free remission (TFR) include low Sokal risk group at diagnosis, chronic-phase disease, optimal response to TKI therapy, longer duration of TKI therapy (>8 years), and longer duration of DMR (>2 years). In all published trials, the majority of patients who experienced relapse did so within 6 months of TKI cessation and, with the exception of one case that progressed to blast crisis, relapsed patients remained responsive to retreatment and regained at least a major molecular response (MMR). However, most information on treatment cessation was obtained from clinical trials with strict recruiting criteria.
Aims: In this study, we aimed to assess persistence of TFR in 25 CML patients treated at our institution that discontinued therapy due to several causes including DMR and TKI intolerance, and to identify factors that could be associated with TFR.
Methods: The medical records of all CP-CML patients who were treated with TKIs in our institution between 1997 and 2015 were reviewed and clinical and laboratory data was collected. The eligibility criteria were CP-CML, treated with any of the first-line approved TKIs (Imatinib, Nilotinib, and Dasatinib), that discontinued TKI therapy due to any reason. Only patients with typical transcripts [that is, b3a2 (e14a2) and/or b2a2 (e13a2)], determined by RT-PCR at the time of diagnosis were included.
Results: We evaluated the outcome of 25 patients with CML that discontinued TKI therapy in our institute due to any reason. Of them, 76% discontinued therapy in sustained deep molecular response (SDMR) and 24% were in unsustained DMR (UDMR). Discontinuation of therapy due to adverse effects was observed in 5% and 50% of the patients in the SDMR and UDMR groups, respectively. After TKI discontinuation patients were followed for a median of 20 months. At the time of this analysis, 56% patients had a molecular relapse after a median of 4 months. SDMR and longer treatment duration were associated with lower probability of molecular relapse: 25% in SDMR patients with TKI treatment >96 months patients and 85% in UDMR patients with TKI treatment ≤96 months. All relapsed patients promptly resumed TKI therapy and regained at least a major molecular response (MMR).
Summary/Conclusion: Our results suggest that TKI discontinuation is safe outside clinical trials and particularly effective in CML patients who are in SDMR with longer duration of TKI treatment.

PB1964

THE EVALUATION OF THE TOTAL ANTIOXIDANT CAPACITY IN RELATION TO THE TREATMENT WITH TYROSIN-KINASE INHIBITORS IN CHRONIC MYELOID LEUKEMIA

M.-A. Gaman[1,*], *e.g.* Pascu[2], A.M. Gaman[2,3]

[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Bucharest, [2]Pathophysiology, University of Medicine and Pharmacy of Craiova, [3]Hematology, Filantropia City Hospital of Craiova, Craiova, Romania

Background: Chronic myeloid leukemia (CML) is a chronic myeloproliferative neoplasm arising from the reciprocal translocation t(9;22) which is the source for the bcr-abl P210 protein that determines the development and perpetuation of the leukemic granulocytic clone. Increased levels of oxidative stress, defined as an imbalance of the redox status of the cell induced by overproduction of reactive oxygen species or decreased antioxidant defense, lead to chromosomal abnormalities, promote genomic instability and blastic transformation, and are possibly responsible for the development of resistance to tyrosin-kinase inhibitors (TKI). The involvement of oxidative stress has been studied in aging and other physiological processes, and also in a couple of hematological disorders: essential thrombocythemia, chronic lymphocytic leukemia or primary immune thrombocytopenia[1-8].
Aims: To evaluate the levels of oxidative stress and the total antioxidant capacity in order to establish a possible link between oxidative stress, TKI therapy and molecular response in patients with CML.
Methods: We evaluated 26 patients with chronic phase CML, diagnosed according to the ELN/WHO criteria, hospitalised in the Clinic of Hematology, Filantropia City Hospital Craiova, Romania, compared to healthy controls. Informed consent was obtained from all recruited participants. The patients were treated with first or second generation TKI. Oxidative stress was evaluated at diagnosis and when switching from first to second generation of TKI was decided, and correlated with the molecular response (MR). We evaluated the total antioxidant capacity (TAC) using a multidetection microplate reader FLUOstar Omega and a Sigma-Aldrich antioxidant assay kit. The BCR-ABL transcript was detected by RT-PCR. The statistical analysis was performed using the student T-test and a p-value ≤0.05 was considered significant.
Results: The study group included 14 males and 12 females (age range 23-84 years). The patients were treated with first generation TKI as first line therapy. Due to intolerance or failure to first generation TKI, 7 patients required second generation TKI as second line therapy. All patients with CML had low levels of TAC compared to healthy controls ($p \leq 0.05$), with a lower level in patients with intolerance or failure to first generation TKI and switched to second generation TKI ($p \leq 0.05$). We found a significant correlation between the low level of TAC, types of TKI, BCR-ABL transcript and MR.
Summary/Conclusion: In our study group, low levels of TAC were found in CML patients compared to healthy controls. Patients that were switched from first to second generation of TKI had significantly lower TAC levels. Our study is ongoing, but we suppose that oxidative stress plays a role in genomic instability and self-mutagenesis, causing TKI resistance via a bcr-abl independent mechanism.

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Enzymopathies, membranopathies and other anemias

PB1965

STANDARDIZATION AND VALIDATION OF A NOVEL AUTOMATED DIGITAL ANALYSIS OF RED BLOOD CELLS MORPHOLOGY

S. Payán Pernía*, R. Awol, M. Serra Ferrer, J. Sánchez García, V. Criado Casas, X. Sales García, Á.F. Remacha Sevilla

Hematology, Hospital De La Santa Creu I Sant Pau, Barcelona, Spain

Background: Assessment of erythrocyte morphology is essential in the study of anaemia. However, microscopic evaluation of peripheral blood smears is a time-consuming procedure. Automated digital cell morphology analysers could simplify and standardize this evaluation. These platforms have recently incorporated red blood cells (RBC) morphology evaluation.
Aims: Standardization of the morphological analysis of RBC performed by the CellaVision Advanced RBC Software Application.
Methods: Standardization: The values of macrocytosis and microcytosis proposed by Cellavision were recalibrated according to those obtained with XN analysers (Sysmex Corporation, Chuo-ku, Kobe, Japan) in several pathological samples. Subsequently, the reference interval for each of the different morphological anomalies of RBC was calculated in 67 subjects with normal mean corpuscular volume, RBC and reticulocyte count, and iron metabolism. Three levels of intensity were defined for each morphological anomaly according to the 2015 ICSH recommendations: 1 (mild), 2 (moderate) and 3 (intense). The intra-day and inter-day reproducibilities were assessed. For the intra-day study, 5 smears of the same blood sample were analysed along the same day. For the inter-day study, three different blood smears were obtained for each sample and analysed at 0, 24, and 48 hours. Validation: 100 patients with anaemia were selected. The results of the automated system were compared with those obtained through microscopic evaluation of direct blood smears (without anticoagulant). For each patient, morphology was evaluated by the automated digital system, by an experienced technician, and by a hematologist (the reference). According to results, morphology was divided into three categories: normal or without clinical relevance, compatible with the type of anaemia (e.g. hypochromia in iron deficiency anemia), and relevant for the diagnosis (e.g., schistocytes). STATISTICS: In the reference population, intervals were calculated and morphological findings were compared using the Student's t-test. In the validation study, concordance (agreement) between results was analysed by the *kappa index statistic*. METHODOLOGY: Blood was collected in EDTA-anticoagulated tubes. The automated morphological analysis was performed with the CellaVision Advanced RBC Application on the DM96 analyser (CellaVision AB, Lund, Sweden). Blood smears for the automated analysis were performed and stained using the SP-10 (*Sysmex Corporation, Chuo-ku, Kobe*, Japan), an automated slide maker and stainer.
Results: Statistically significant differences were found for macrocytosis and dacryocytes with regard to sex. In the analysis of the intra-day reproducibility, no differences were observed. A decrease in stomatocytes and an increase in equinocytes were detected in the inter-day analysis. VALIDATION: Results obtained by the technician using the direct blood smear coincided with those retrieved by the haematologist in 92.5% of cases (κ=0.918). The automated analysis agreed with the hematologist's results in 93.7% of the cases (κ=0.879).
Summary/Conclusion: The automated evaluation of RBC morphology using the CellaVision Advanced RBC Software Application offered reproducible intra-day but not inter-day results. Its standardization allowed the evaluation of the RBC morphology in a faster way and with decreasing variability. There was an excellent concordance between the morphological assessment by CellaVision and by optical microscopy in the setting of anaemia.

PB1966

THE PEAK REGISTRY, A GLOBAL LONGITUDINAL OBSERVATIONAL STUDY OF PATIENTS WITH PYRUVATE KINASE DEFICIENCY

P. Bianchi[1], B. Glader[2], R.F. Grace[3], H. Kanno[4], D.M. Layton[5], C. Rose[6], E.J. van Beers[7], J.-L. Vives Corrons[8], L. Hua[9], B. Jones[9,*]

[1]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, Milan, Italy, [2]Stanford University School of Medicine, Palo Alto, [3]Dana-Farber Boston Children's Cancer and Blood Disorders Center, Boston, United States, [4]Faculty of Medicine, Tokyo Women's Medical University, Tokyo, Japan, [5]Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom, [6]Hôpital Saint Vincent de Paul, Lille, France, [7]University Medical Centre Utrecht, Utrecht, Netherlands, [8]Josep Carreras ICO-Germans and University of Barcelona, Barcelona, Spain, [9]Agios Pharmaceuticals, Inc., Cambridge, United States

Background: Pyruvate kinase (PK) deficiency is a rare hereditary glycolytic enzymopathy caused by mutations in the *PKLR* gene, which lead to reduced red blood cell PK (PK-R) enzyme activity, resulting in defective red blood cell glycolysis and hemolytic anemia. It is the most common cause of glycolytic hemolytic anemia. Over 300 causative *PKLR* mutations have been identified to date. Patients with PK deficiency may experience symptoms of hemolytic anemia, most commonly fatigue (sometimes extreme), jaundice, dyspnea and weakness. Current treatment is limited to supportive care options. Affected neonates may need phototherapy or exchange transfusions for severe hyperbilirubinemia, while treatment strategies for adults can include red blood cell transfusions, splenectomy/cholecystectomy, and iron chelation, each of which is associated with some risk to the patient. No disease-specific therapy currently exists. The observational PK deficiency Natural History Study (NHS) conducted by Boston Children's Hospital (ClinicalTrials.gov NCT02053480; N=258) initiated the longitudinal analysis (2-year follow-up) and reporting on PK deficiency-related signs, symptoms and treatment outcomes to better understand the natural history and clinical burden of the disease. The Peak Registry, a global, longitudinal, non-interventional study of PK deficiency, aims to build upon the NHS with additional patients and longer follow-up.
Aims: To report the design of the Peak Registry.
Methods: The Peak Registry is a global, longitudinal, observational registry for adult and pediatric patients with PK deficiency. The 9-year study will enroll approximately 500 patients over 7 years at an estimated 60 study centers in up to 20 countries. All enrolled patients will be followed prospectively for at least 2 years, and up to 9 years. Patients of all ages with a diagnosis of PK deficiency confirmed by genetic testing are eligible to enroll. Each patient or their parent/guardian must be willing and able to give written informed consent and/or assent. Patients who are actively enrolled in any Agios-sponsored clinical trial involving treatment with a PK activator will be excluded. Demographic, clinical, and treatment data, and other data relevant to the management of patients with PK deficiency, will be collected from participating registry physicians via electronic case report forms. The primary objective of the registry is to develop an understanding of the longitudinal clinical implications of PK deficiency, including natural history, treatment and outcomes, variability in clinical care, and disease burden. Secondary objectives include: to understand the prevalence, incidence, and severity of complications associated with PK deficiency; examine phenotype-genotype correlation; evaluate pregnancy outcomes; and provide longitudinal data to assist physicians with the clinical management of individual patients. Site and patient recruitment are ongoing. Study conduct, data analyses and reporting is governed by a steering committee comprised of experts involved in the research, diagnosis, and/or care of patients with PK deficiency in cooperation with the sponsor (Agios Pharmaceuticals, Inc.).
Results: Not yet available.
Summary/Conclusion: This non-interventional study aims to extend the work of the NHS with additional patients from an expanded geographic distribution and longer follow-up, to further improve the understanding of the complex clinical burden of PK deficiency, its natural history, and outcomes of current treatment practice patterns.

PB1967

A FEW WORDS ABOUT APLASTIC ANEMIA IN ARMENIA

S. Daghbashyan*, N. Melkikyan, L. Sahakyan, A. Tovmasyan

Armenian Haematology center aft.prof.R.Yeolyan, Yerevan, Armenia

Background: Aplastic anemia is a kind of disease which occurs in the result of bone marrow stem cell damage which causes a deep inhibition of the hematopoiesis' three germs in the bone marrow. Aplastic anemia is a quite rare disease, but at the same time it is one of the most severe disorders of hemopoiesis with a mortality rate exceeding 80%. In most cases it occurs among young people.
Aims: We aimed to quantify the incidence, prevalence and survival rate aplastic anemia patients in Armenia and their variation with gender, age, year of diagnosis.
Methods: In this work we included aplastic anemia patients diagnosed within 2005 to 2017. The initial data for this survey have been derived from ambulance/dispensary cards, hospitalization journals, and clinical data from the Registry of Blood Diseases at the R.Yeolyan Hematology Center, Yerevan, Armenia. The data has been supplemented by the data from the Registry of Oncological Diseases of the V. Fanarjyan NCO, as well as from death certificates. The demographic data has been obtained from the National Statistics Board of Republic of Armenia. The obtained data has been statistically analyzed using EPI INFO-2002 program.

Results: A total of 69 cases of aplastic anemia were identified, 41 (59.4%) of whom were male. The overall incidence of aplastic anemia was 1.8 per million inhabitants per year (95%CI 0.9–2.9) and the incidence increased with age. The higher incidence rates were noted in 2008 and 2014 (accordingly 0.25 and 0.29). The sex-specific incidence rates were 2.2 for males and 1.7 for females (ratio 1.29). Patients can be affected at any age, although there is a biphasic age distribution with peaks from 10 to 25 (2.9 per million per year) years and >60 years (1.7 per million per year). Most of the cases were classified as severe or very severe aplastic anemia. Currently 46.4% of studied patients are alive. Survival rates at 5 and 10 years after the diagnosis were 50% and 37%, respectively. Age and disease severity at the time of diagnosis were associated with a lower survival rate.
Summary/Conclusion: This is the first general population study to describe the incidence of AA in Armenia during 2005-2017. It forms the basis for quality assessment of aplastic anemia treatment in Armenia and offers a unique opportunity for population-based research. The incidence of aplastic anemia in Armenia is low but the case fatality rate is high. Advanced age and severe disease at the time of diagnosis were associated with decreased survival.

PB1968

UNVEILING THE UTILITY OF AUTOMATED RBC AND PLATELET PARAMETERS IN THE EVALUATION OF VITAMIN B12 DEFIFIENCY ANEMIA

R. Rajashekar*

Hematopathology, JSS Medical College, MYSORE, India

Background: Vitamin B12 deficiency induced megaloblastic anemia is diagnosed by complete blood count (CBC) parameters including RBC indices such as mean corpuscular volume (MCV), red cell distribution width-coefficient of variation and standard deviation (RDW-CV, RDW-SD).Platelet indices such as mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR) have been evaluated in thrombocytopenias of various etiologies.The present study aimed at exploring the reliability, diagnostic accuracy and utility of automated RBC and platelet parameters in evaluating vitamin B12 deficiency induced anemia and thrombocytopenia.
Aims: 1.To evaluate MCV, RDW-CV and RDW-SD for their ability to detect vitamin B12 deficiency and related anemia.2.To evaluate the diagnostic accuracy of RDW-SD *versus* RDW-CV.3.To evaluate the discriminative function of platelet indices in thrombocytopenia of megaloblastic etiology in comparison with hypoproductive and hyperdestructive causes of thrombocytopenia and to correlate platelet indices with bone marrow megakaryocyte cellularity.
Methods: This retrospective analytical study included 100 cases with serum levels of vitamin B12<200 pg/ml. The hemoglobin, MCV, RDW-CV and RDW-SD were estimated by Sysmex XN-1000. Pearsons correlation was used for calculating the correlation of hematological parameters. Among 100 cases, 32 had thrombocytopenia and underwent bone marrow biopsy. Platelet indices in these 32 cases of thrombocytopenias of megaloblastic etiology were compared with platelet indices of 31 cases of marrow proven hypoproductive thrombocytopnias (aplastic anemia, hypoplastic anemia, acute leukemia) and 32 cases of hyperdestructive thrombocytopnias (Immune thrombocytopenia).Descriptive analysis was used and comparison of means in all the groups was done with one way ANOVA using Scheffe's test. Categorical data was analyzed using Chi-square test.Platelet indices and bone marrow megakaryocytes were analyzed and correlated in each group. A p-value of less than 0.05 was considered statistically significant.
Results: Among 100 cases of vitamin B12 deficiency, elevated RDW-SD, RDW-CV and MCV were seen in 90%, 72% and 64% cases respectively. RDW–SD showed a strong negative correlation with serum vitamin B12 levels (p-value= 0.029) (p<0.05).RDW-CV showed a weak negative correlation with serum Vitamin B12 levels with an insignificant p-value of 0.58 (p>0.05). The sensitivity of RDW-SD, RDW-CV and MCV were 95%, 81% and 69.1% respectively for detection of anemia. Mean values of platelet indices were higher (p<0.05) in hyperdestructive group [PDW(16.6fL), MPV(12.1fL), P-LCR(42.3%)] compared to the hypoproductive group [PDW(11.8fL), MPV(10.9fL), P-LCR(31.5%)].Mean values of PDW (14.7fL) and MPV (11.6fL) in megaloblastic group showed a higher value (p-value<0.05) than hypoproductive group but no significant difference was seen compared to hyperdestructive group (p-value>0.05).The mean P-LCR (37.4%) in megaloblastic group was intermediate from the other two groups (p-value<0.05).
Summary/Conclusion: RDW-SD is the most sensitive discriminant function even when MCV is normal despite vitamin B12 deficiency, since so far all the attention is being given to MCV as the earliest hematologic indicator of vitamin B12 deficiency. Platelet indices are of significant discriminative value in differentiating the various causes of thrombocytopenias. Both hypoproduction and ineffective thrombo-poiesis are the underlying pathomechanisms in megaloblastic thrombocytopenia as evidenced by the marrow findings and platelet indices.

PB1969

ROLE OF IRON METABOLISM, INTERLEUKIN-6, INTERLEUKIN-10, TUMOR NECROSIS FACTOR ALPHA IN PROGRESSION OF ANEMIA IN PATIENTS WITH SOLID TUMORS

V. Sakhin[1,*], M. Pugachev[2], E. Madzhanova[3], A. Sotnikov[4], A. Gordienko[4], S. Kazakov[5], E. Kryukov[6], O. Rukavitsin[7]

[1]Cardiology department, 1586 military hospital of the Ministry of Defense of Russia, Moscow region, Podolsk, [2]Therapeutic department, 1602 military hospital of the Ministry of Defense of Russia, Rostov-na-Donu, [3]Immunochemical laboratory, 1586 military hospital of the Ministry of Defense of Russia, Moscow region, Podolsk, [4]Department of Hospital Therapy, Russian Military Medical Academy of the Ministry of Defense of Russia, St. Petersburg, [5]Center for Clinical Laboratory Diagnostics, [6]Head of the hospital, [7]Hematology Center, Main Military Hospital n.a. N. Burdenko, Moscow, Russian Federation

Background: A significant number of cancer patients need correction of anemia. Anaemia has a negative impact on quality of life as well as survival. Iron metabolism disorder and cytokines plays important roles in the pathogenesis of this anaemia. The mechanism of their action needs specification.
Aims: To study the aspects of iron transfer and influence of interleukin-6 (IL-6), interleukin-10 (IL-10) and tumor necrosis factor-alpha (TNF-) on progression of anemia in patients with solid tumors
Methods: Sixty-three patients (forty one patients with anaemia/ twenty two patients without anaemia) with Stage II-IV cancer were examined. RBC count, WBC count were performed, levels of HB, HCT, MCV, MCH, MCHC were determined using Sysmex XS-500i analyser (Japan). Serum iron concentration, total iron binding capacity (TIBC), ferritin level, C-reactive protein (CRP) level, transferrin saturation index (TSI) were determined using Olympus Au 480 analyser, (Beckman Coulter, the USA). Concentration of transferrin was determined by Siemens Admia 1200 analyser (Diamond Diagnostics, the USA). Concentrations of IL-6, IL-10 and TNF-α were determined using Stat Fax 2100 analyser (Awareness Technology Inc., the USA). Mann-Whitney U Test was applied to check for statistically significant differences in study samples. In order to evaluate the relation between the variables Spearman correlation coefficient (r) was calculated.
Results: Patients with anaemia show lower concentrations in comparison with patients without anaemia: serum iron (5.5 [IQR, 2.9-7.7] *versus* 10.9 [IQR, 7.9-14.7]; p<0.05), TSI (11.4 [IQR, 5.1-14.3] *versus* 17.7 [IQR, 12.5-23.7]; p<0.05), TIBC (50.2 [IQR, 39-60] *versus* 64.2 [55.5-73.1]; p<0.05), lower levels of HCT (30.1 [IQR, 26.9-33.8] *versus* 40 [36.5-44.5]; p<0.05), MCH (26.9 [IQR, 24.8-29] *versus* 29.4 [27.2-31.6]; p<0.05), MCHC (318.9 [IQR, 302.5-331] *versus* 338.8 [327.5-350]; p<0.05), and higher concentrations of CRP (103.7 [IQR, 32.1-155] *versus* 34.5 [IQR, 9.3-65.7]; p<0.05), IL-6 (41.5 [IQR, 3.8-31.1] *versus* 7.1 [IQR, 0.00-9.4]; p<0.05), IL-10 (18.3 [IQR, 4.5-14.4] *versus* 0.9 [IQR, 0.3-5.5]; p<0.05) and TNF-α (58.6 [IQR, 36.1-81.1] *versus* 8.25 [IQR, 1.3-13.6]; p<0.05). Concentrations of ferritin, transferrin and MCV level in both groups were the same (p>0.05). As for iron, there was a correlation with HB level (r=0.37) and no correlation with RBC level was found (r<0,3). A correlation with levels of RBC (r=-0.55), HB (r=-0.52), HCT (r=-0.51) and WBC (r=0.45) was found for IL-6. A correlation with level of HB (r=-0.64) was found for IL-10. A correlation with levels of RBC (r=-0.74), HB (r=-0.69), HCT (r=-0.63), WBC (r=0.39) was found for TNF-α. A correlation with WBC (r=0.77), HB (r=-0.74), HCT (r=-0.71), RBC (r=-0.6), MCH (r=-0.32), and MCHC (r=-0.54) levels was found for IL-10.
Summary/Conclusion: Data obtained prove the fact that anaemia in cancer patients is associated with progression of functional deficit of iron. A correlation between IL-6, IL-10, TNF-α and hemogram items indicates their significant influence on anaemia progression and evidences its complicated nature, which is not limited by iron deficit only. Mechanism and intensity of cytokines influence on progression of anaemia in cancer patients need further clarification

PB1970

HLA-DR ALLELES ASSOCIATED WITH THE DEVELOPMENT OF AUTOIMMUNE HEMOLYTIC ANEMIA

S. Smirnova*, E. Khamaganova, Y. Sidorova, A. Abakumova, I. Urybin, A. Sudarikov

National research center for hematology, Moscow, Russian Federation

Background: The association of HLA I and II class antigens with the development of autoimmune diseases (AD) had been reported for the Ankylosing spondylitis, rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes mellitus, acquired form of idiopathic thrombotic thrombocytopenic purpura, etc. HLA complex responsible for antigen presentation to T-lymphocytes play an important role in immune response, however the mechanisms underlying the association of HLA antigens with the development of AD are not fully understood. According to the literature, there is a negative correlation between the presence of HLA-DQ6 and the positive direct Coombs test in the patients with idiopathic immune hemolytic anemia (IHA) and hemolysis secondary to other neoplastic or viral diseases. Possible association of HLA antigens with the development of autoimmune hemolytic anemia (AIHA) has not been studied yet.
Aims: Identify the association of HLA-DR antigens with the development of AIHA.
Methods: The study included 24 patients with AIHA (diagnosed according to WHO 2016 classification) and 1507 healthy donors. Male to female ratio was 1:2.4. The median age was 37. Haplotypes of the DRB1 locus were assessed by SSP-HLA-typing for patients with AIHA (48 haplotypes) and healthy donors (3014 haplotypes).
Results: Comparison of control and study groups revealed DRB1 alleles with altered frequency of occurrence. Frequencies of DRB1*03 alleles (7 out of 48 examined haplotypes; 14% *vs* 8% in the control group), DRB1*09 (3 of the 48 haplotypes studied; 6% *vs* 0.86% in the control group), DRB1*16 (4 of 48 examined haplotypes; 8% *vs* 3% in the control group) were increased in AIHA group. While DRB1*11 (2 of the 48 haplotypes studied; 4% *vs* 13% in the control group) and DRB1*15 (2 of the 48 haplotypes studied; 4% *vs* 13% in the control group) occur more often in the control cohort.
Summary/Conclusion: We have observed an increased incidence of the DRB1*03 allele among patients with AIHA. It should be noted that an increased incidence of this allele among patients with type 1 diabetes, celiac disease, diffuse toxic goiter, myasthenia gravis had been reported previously for European population. Incidence of DRB1*09 allele, which is frequent for Asian population and is very rare for European population, was also increase in Russian patients with AIHA. No association of DRB1*11 or DRB1*15 alleles with any AD had been reported so far. The altered frequencies of these alleles could be associated with the severity of the disease, resistance to therapy, or with other factors. Further studies with extended patients cohorts and long-term dynamic monitoring of patients are required for the definite interpretation of these observations.

PB1971

ROLE OF HEPCIDIN AND SOLUBLE TRANSFERRIN RECEPTOR IN PATHOGENESIS OF ANAEMIA IN PATIENTS WITH SOLID MALIGNANT TUMORS

V. Sakhin[1,*], M. Pugachev[2], E. Madzhanova[3], A. Sotnikov[4], A. Gordienko[4], S. Kazakov[5], E. Kryukov[6], O. Rukavitsin[7]

[1]Cardiology department, 1586 military hospital of the Ministry of Defense of Russia, Moscow region, Podolsk, [2]Therapeutic department, 1602 military hospital of the Ministry of Defense of Russia, Rostov-na-Donu, [3]Immunochemical laboratory, 1586 military hospital of the Ministry of Defense of Russia, Moscow region, Podolsk, [4]Department of Hospital Therapy, Russian Military Medical Academy of the Ministry of Defense of Russia, St. Petersburg, [5]Center for Clinical Laboratory Diagnostics, [6]Head of the hospital, [7]Hematology Center, Main Military Hospital n.a. N. Burdenko, Moscow, Russian Federation

Background: A significant number of cancer patients need correction of anemia. Iron metabolism disorder and play important roles in the pathogenesis of this anaemia. Hepcidin is the main circulating in the blood regulator of iron absorption and its distribution in tissues. Soluble transferrin receptor binds transferrin and the resulting complex transports iron into the cell. The importance of hepcidin and soluble transferrin receptor in pathogenesis of anemia in patients with solid malignant tumors requires clarification.
Aims: To study the aspects of hepcidin and soluble transferrin receptor (sTfR) release in patients with solid malignant tumor and anaemia, and its relation with values of iron metabolism, interleukin-6 (IL-6), interleukin-10 (IL-10) and tumour necrosis factor-alpha (TNF-α).
Methods: Sixty-three patients (41 patients with anaemia/ 22 patients without anaemia) with Stage II-IV cancer were examined. RBC count was performed, levels of HB, HCT, MCV, MCH, MCHC were determined using Sysmex XS-500i analyser (Japan). Serum iron concentration, total iron binding capacity (TIBC), ferritin level, C-reactive protein (CRP) level, transferrin saturation index (TSI) were determined using Olympus Au 480 analyser, (Beckman Coulter, the USA). Concentration of transferrin was determined by Siemens Admia 1200 analyser (Diamond Diagnostics, the USA). Concentrations of IL-6, IL-10 and TNF-α were determined using Stat Fax 2100 analyser (Awareness Technology Inc., the USA). Concentration of sTfR was determined using ACCESS analyser (BeckmanCoulter, the USA). Hepcidin concentration was determined using Charity photometer (Promnauchpribor, Russia). Mann-Whitney U Test was applied to check for statistically significant differences in study samples. In order to evaluate the relation between the variables Spearman correlation coefficient (r) was calculated.
Results: Patients with anaemia show higher concentrations in comparison with patients without anaemia: hepcidin (47.8 [IQR, 50-57.8] *versus* 33.6 [IQR, 21.1-50]; $p<0.05$), sTfR (30.7 [IQR, 16.4-63.3] *versus* 17.3 [IQR, 14.9-19.2]; $p<0.05$). A correlation with levels of RBC (r=-0.41), HCT (r=-0.35), CRP (r=0.49), TIBC (r=-0.51), ferritin (r=0.61), transferrin (r=-0.55), IL-6 (r=0.52), TNF-α (r=-0.41) was found for hepcidin. A correlation with levels of HB (r=-0.57), HCT (r=-0.5), transferrin (r=0.41), IL-10 (r=0.57) was found for sTfR.
Summary/Conclusion: higher concentration of hepcidin and sTfR in patients with solid malignant tumor and anaemia in comparison with patients without anaemia, as long as its correlation with values of RBC, HB and HCT evidence the participation of hepcidin and sTfR in progression of anaemia in these patients. Correlations between hepcidin, sTfR and values of iron metabolism indicate that these proteins effect on progression of anaemia through its influence on iron metabolism. The correlations between hepcidin, IL-6 and IL-10, as well as correlations between sTfR and IL-10 values evidence a regulating role of cytokines in synthesis of reviewed transferrin receptors.

PB1972

PREVALENCE OF PERNICIOUS ANEMIA IN PATIENTS WITH LOW B12 LEVEL IN AN INDIAN TERTIARY CARE HOSPITAL

A. Dubey*, B. Palate

Haematology, Haemato-oncology and Bone Marrow Transplant, P D hinduja Hospital, Mahim, Mumbai, Mumbai, India

Background: Pernicious anemia is a chronic illness where body fails to produe normal red blood corpuscles because of Vitamin B12 deficiency caused as a result of impaired absorption due to lack of intrinsic factor, which may be due to the presence of intrinsic factor antibody or gastric parietal cell antibody leading to gastric atrophy. It is the most common cause of Vitamin B12 deficiency in the west, however in a study conducted by Desai *et al.* the incidence of pernicious anemia was found to be very low in Indian subjects. Moreover, majority of these vitamin B12 deficient patients do not get investigated for pernicious anemia as the deficiency is attributed to the vegeterian diet of the patients. This retrospective study shows that the incidence of pernicious anemia in the Indian population may be higher than reported till now.
Aims: To evaluate the prevalence of pernicious anemia in patients with documented low serum vitamin B12 levels in Mumbai.
Methods: A total of 133 patients with clinical suspicion of Vitamin B12 deficiency who were not recently transfused within the last one week or were not on vitamin B12 supplements were tested for serum vitamin b12 levels. Out of these, 38 patienst found to have severely low levels of levels of vitamin b12 of less than 150pg/ml were then tested for gastric parietal cell antibody and intrinsic factor antibody.
Results: A total of 8 patients from the 132 patients tested were found to have Pernicious Anemia. Out of these, 2 were positive for only intrinsic factor antibody, 5 were positive for only gastric parietal cell antibody while one patient was positive for both the antibodies. The latter patient was also found to be Anti TPO antibody positive and referred for further endocrinological evaluation. Of note is the factt hat 2 patients had equivocal intrinsic factor antibody levels while 3 patients had equivocal gastric parietal cell antibody levels within the values falling on the upper limit of the reference range and for all practical purposes, these patients were treated as having pernicious anemia.
Summary/Conclusion: The prevalence of pernicious anemia is reported to be lower in Chinese, Blacks and the Indian population. Till date, studies reported in India have further emphasized this fact. However, this retrospective study shows that the incidence of pernicious anmeia is higher than reported and has important implications in the treatment of the patients.

PB1973

MOLECULAR CHARACTERIZATION OF RED CELL PYRUVATE KINASE DEFICIENCY IN INDIA: A STUDY OF 22 CASES

P. Kedar*, R. Dongerdiye, R. Devendra, A. Chiddarrwar, V. Gupta

V. Thorat, P. Warang, M. Madkaikar

Haematogenetics, National Institute of Immunohaematology (ICMR), Mumbai, India

Background:-Pyruvate kinase (PK) deficiency is one of the most common causes of hereditary hemolytic anemia around the world with clinical manifestations varying from mild to severe hemolysis. Here we investigate PK-deficient cases referred to our laboratory for biological analysis of the unknown cause of hemolytic anemia.
Aims: To diagnose Pyruvate kinase deficiency (PKD) in hereditary non spherocytic hemolytic anemia cases in India and Genotype-phenotype correlations in PKD
Methods: Test for aaemoglobinopathies, RBC membrane protein defects and red cell enzyme activities were measured by standard methods; Molecular characterization of the PK gene mutations included restriction enzyme analysis, mutation scanning by DNA sequencing. In silico prediction using bioinformatics tools (SIFT, Polyphen2) was done and the molecular modeling were performed using PYMOL software.
Results: Total 21 families were identified in which 17 homozygous PKD cases identified and eighteen different PKLR mutations were found among which six are described for the first time (V294M, I282S, M373V, A423E, W525X and R559X) and 11 known mutations which includes (E315K, D331G, G332S, G358R, N393S,E407K, R486W, R479H, R498C, V506I, R510Q and IVS9 ds A-G +3) are associated with severe clinical presentation. All missense mutations were located in crucial domains of the molecule (catalytic site, cleft between the A and C domains, A/A' interface and have severely damaging effect leading to need of regular blood transfusion. The most frequent mutations in the Indian population appear to be V294M, followed by R479H, R510Q and D331G. Genotype-phenotype correlations for the novel mutations were investigated by three-dimensional structure analysis.
Summary/Conclusion: In India, the genetic heterogeneity of PKLR is still high but differs from that observed in the previous study carried out in 2009 and 2013. DNA sequencing was helping for genetic counseling and prenatal diagnosis.

PB1974

HB KIRKLARELI (AH58L): SECOND DESCRIPTION IN A BRAZILIAN PATIENT WITH DYSPNEA AND O2 SATURATION FALL

M.D.F. Sonati[1,*], G. Pedroso[1], P. Nascimento[1], D. Albuquerque[2], M. Grigoleto[1], M. Santos[1], F. Costa[2], S. Jorge[1]

[1]Clinical Pathology, [2]Hematology and Hemotherapy Center, State University of Campinas-UNICAMP, Campinas, Brazil

Background: Hemoglobin (Hb) Kirklareli [$\alpha_1$58(E7)His®Leu;HBA1: c.176A®T] was first described by Bissé *et al*. in 2016 as a new α-globin variant associated with iron deficiency and increased CO binding. It was found in a Turkish female patient with mild anemia and in her father, who was not anemic but was a smoker with high levels of HbCO. Studies with recombinant human Hb Kirklareli showed that the αH58L subunits autoxidize and lose hemin more rapidly than native α subunits causing rapid denaturation of the oxygenated variant under physiological conditions. On the other hand, the mutant α subunits showed a much higher affinity for CO than O2, which prevents denaturation and explains the phenotypic differences between the patient and her father.
Aims: We found the Hb Kirklareli mutation in the α_2-globin gene (HBA2:c.176A®T) of a Brazilian patient admitted to the university hospital with severe dyspnea and O2 saturation fall and in his father, who presented with normocytic and normochromic moderate anemia. Our patient is an 11-year-old White male of Portuguese descent with history of respiratory problems since he was 6-y-o. Cardiac and pulmonary functions, inhalation and perfusion scintigraphy and CT scan of thorax were normal as well as the bone marrow and peripheral blood examination (patient's red blood cell parameters: RBC=4.62, Hb=12.2, Hct=37.8, MCV=81.8, MCH=26.4, RDW=12.7; his father: RBC=2.87, Hb=8.4, Hct=25.6, MCV=89.2, MCH=29.3, RDW=14.5).
Methods: Hb Kirklareli could not be distinguished from Hb A by electrophoresis at alkaline pH and cation exchange high-performance liquid chromatography (HPLC); at acidic pH it migrated in between Hbs S and C and in isoeletricfocusing in between Hbs A and F. The reversed phase HPLC revealed a slower extra peak (15% of the total, heme+globins) corresponding to the mutant α-chain.
Results: Direct sequencing of the α-genes revealed heterozygosis for the CAC®CTC substitution at codon 58 of the alpha$_2$-globin gene in both, patient and his father
Summary/Conclusion: To our knowledge, this is the second family reported with Hb Kirklareli. In this case the variant is not associated with iron deficiency. Neither the patient nor his father is a smoker; the father is anemic but the patient has normal Hb values. However, the patient has a long history of respiratory problems without any other cause being detected, suggesting that other studies are needed to better understand the clinical behavior of this variant and the compensatory mechanisms elicited by it.
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PB1975

RED BLOOD CELL'S RHEOLOGICAL PROPERTIES IN PATIENTS WITH CHOREA-ACANTHOCYTOSIS

J. Leal[1,2,*], D. Lazari[1,3], B. Neu[3], R. Brock[1], M. Adjobo-Hermans[1], G. Bosman[1]

[1]Biochemistry, Radboud University Medical Center, Nijmegen, Netherlands, [2]COBIO, National Council for Scientific and Technological Development, Brasilia, Brazil, [3]Faculty of Life Sciences, Rhein-Waal University of Applied Sciences, Kleve, Germany

Background: Functional deficits and morphological abnormalities of red blood cells (RBC) become apparent when they are subjected to biochemical or mechanical stress in vitro, or to pathological conditions in vivo. Chorea-acanthocytosis (ChAc) is a rare disease characterized by degeneration of neurons in the basal ganglia and by the presence of misshapen RBC distinguished by their thorny protrusions, named acanthocytes.
Aims: Verify the rheological properties (deformability and aggregation) of ChAc samples, comparing them to healthy controls.
Methods: The deformability and aggregation of the samples were analyzed using Lorrca®. The aggregation was also accessed via microscopy by verification of rouleaux formations.
Results: Our data on the misshapen RBC of patients with ChAc suggest that their abnormal morphology is associated with alterations in rheological properties. We have observed a decrease in the capacity to aggregate with other RBC, however, it was noticed an increase in the force needed to tease these aggregates apart. Moreover, we have measured a decrease in deformability as compared to RBC from healthy subjects.
Summary/Conclusion: The clinical implications of such alterations, in addition to oxidative and physical stress, likely not only affect the red blood cell itself but may additionally cause neuronal damage of susceptible areas in the brain due to a possible reduction in oxygen supply or clogging of the microcapillaries.

PB1976

A MUMMY EMERGES FROM THE GRAVE: SCURVY CONFOUNDING THE CLINICAL PRESENTATION OF A CHILD WITH FANCONI ANEMIA

A. Ozcan[1], S. Saracoglu[2], J.M. Verboon[3], M. Karakukcu[1], T. Patiroglu[4,*], A. Coskun[5], V.G. Sankaran[3], E. Unal[1]

[1]Division of Pediatric Hematology, Oncology & HSCT Center, Department of Pediatrics, [2]Division of Pediatric Radiology, Department of Radiology, Erciyes University, Faculty of Medicine, Kayseri, Turkey, [3]Division of Hematology/Oncology, Boston Children's Hospital, Harvard Medical School, Boston, United States, [4]Division of Pediatric Hematology, Oncology & HSCT Center, Erciyes University, Faculty of Medicine, [5]Division of Pediatric Radiology, Department of Radiology, Erciyes University, Faculty of Medicine, Kayseri, Kayseri, Turkey

Background: Scurvy is a rare disorder that can be seen in patients with severely restricted diets.[1] The last case of scurvy was reported nearly thirty-years ago in the Ethiopian refugees.[2] Scurvy diagnosis may prove challenging for physicians unfamiliar to its features, particularly in the presence of other hematologic disorders.
Aims: Here, we report a case of scurvy in a child with Fanconi Anemia.
Methods: A 10 years old female Syrian refugee was referred to Erciyes University for hematopoietic stem cell transplantation (HSCT) for Fanconi aplastic anemia (FAA) with platelet refractoriness. Blood count analyses revealed a hemoglobin of 6.3 g/dL, mean corpuscular volume of 103 fL, leukocyte count of 810x10^9/L, and platelet count of 2x10^9/L. Bone marrow aspiration and biopsy showed hypoplastic marrow with 10% cellularity. A chromosomal breakage test with diepoxybutane was consistent with FAA. Using whole exome sequencing, we identified a rare, predicted pathogenic missense variant (M-CAP score: 0.16; FANCA: p.Gly1009Asp;) According to clinical features, laboratory findings and genetic analysis she had FAA, but MRI revealed that she may also have scurvy. The serum vitamin C level was very low (0.1 mg/dL,
Results: FAA is an inherited disease characterized by bone marrow failure, short stature, skeletal abnormalities, and a high relative risk of myeloid, and epithelial malignancies due to genomic instability. Scurvy is a rare disorder that can be seen in patients with severely restricted diets. The last case

of refugees with scurvy was reported nearly thirty-years ago. The features of scurvy can confound a diagnosis of another genetic blood disorder, such as FAA. It may be speculated that the scurvy should complicate the clinical course by worsening the pancytopenia.
Summary/Conclusion: Hematologists managing patients originating from low income countries should consider factors that may confound various blood diseases such as FAA. Nutritional deficiencies can be a significant contributor to such complicated presentations

PB1977

COMPARISON OF THE EFFICACY OF PARENTERAL AND ORAL TREATMENT FOR NUTRITIONAL VITAMIN B12 DEFICIENCY IN CHILDREN

R.G. Sezer[1], H. Akoğlu[1], A. Bozaykut[1], N.N. Ozdemir[2,*]

[1]Pediatrics, Zeynep Kamil Maternity and Children's Diseases Training and Research Hospital,, [2]Pediatric Hematology Oncology, Kanuni Sultan Suleyman Education and Research Hospital, Istanbul, Turkey

Background: Nutritional vitamin B12 deficiency in children has an increasing prevalence in developing countries. Vitamin B12 deficiency is classically treated with parenteral therapy, although oral replacement is proven to be safe and effective in adult studies. There are few studies on oral replacement therapy of vitamin B12 in children.
Aims: We aimed to compare the efficacy of oral administration of vitamin B12 *versus* intramuscular vitamin B12 injections in pediatric population.
Methods: Children with serum cobalamin concentrations less than 300 pg/mL, aged between 1 months to 18 years were included in this prospective study. Children were treated either with the parenteral or oral vitamin B12. Serum samples for complete blood count and vitamin B12 levels were collected at baseline and day 30. The primary and secondary outcomes of the study were the normalization of serum vitamin B12 and hemoglobin at first month, respectively.
Results: Pre-treatment vitamin B12 increased from 183.5±47 pg/mL and 175.5±42.5 pg/mL to post-treatment value of 482±318.9 pg/mL and 838±547 pg/mL in the oral and parenteral treatment arms, respectively (p-value<0.001). Post-treatment mean values for vitamin B12 was nearly two folds higher in the parenteral group than the oral group (p-value<0.001). The number of patients who still have anemia at the 1st month of treatment were not significant in the parenteral and oral arms (p-value=0.44).
Summary/Conclusion: In this study, oral and parenteral formulations were both effective in normalizing vitamin B12 levels. We suggest that oral formulations may be considered safely as a first line treatment for vitamin B12 deficiency in children.

PB1978

INFLUENCE OF INFLAMMATORY CYTOKINES ON GENESIS OF ANEMIA IN LYMPHOPROLIFERATIVE DISORDER'S PATIENTS

T. Glazanova[1], O. Rozanova[1], N. Romanenko[2,*]

[1]clinical immunology, [2]clinical hematology, Russian research institute of hematology and transfusiology, Saint-Petersburg, Russian Federation

Background: The inflammatory cytokines (IC) play an important role in immune response to inflammation and tumor growth. Increase of their systemic level suggests the active status of immune system. However, these cytokines not only inhibit tumor growth, but can also exert myelosuppressive effect on hemopoiesis leading to development of cytopenia, including anemia.
Aims: To evaluate the influence of some IC on the development of anemia in patients with lymphoproliferative disorders (LPD).
Methods: We have examined the following groups: 1) patients with anemia (Hb 89,5±15,9 g/L; n=39) age 22-82 years, of them with II and III st. multiple myeloma-20 patients, III-IV st. Non-Hodgkin's lymphoma-8, st. chronic lymphocytic leukaemia-11; 2) healthy volunteers (Hb ≥120 g/L; n=15) age 19-76 years, as a control group. We have evaluated serum levels of the following IC: tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and interferon-γ (INF-γ).
Results: We have revealed that the level of studied IC in the group of patients was several times higher than in the control group: 1) TNF-α level in patients with anemia was 130,8±36,2 pg/ml (n=39) *vs* 42,1±13,2 pg/ml in control group (n=15), p<0,01; 2) IL-1β-484,1±66,5 pg/ml (n=30) *vs* 177,0±43,7 pg/ml (n=15), respectively, p<0,01; 3) IL-6-466,2±42,9 pg/ml (n=19) *vs* 128,0±36,5 pg/ml (n=15), respectively, p<0,01; 4) INF-γ-604,5±57,4 pg/ml (n=30) *vs* 47,7±6,6 pg/ml (n=15), respectively, p<0,0001. Correlation analysis have revealed significant associations between serum levels of TNF-α and INF-γ (r=+0,41; p<0,05; n=25), TNF-α and IL-1β (r=+0,62; p<0,01; n=18), and INF-γ and IL-1β (r=+0,49; p<0,01; n=30), suggesting their synergistic action. At the same time, there were no correlations between serum IL-6 and other listed above cytokines (p>0,05). Also there was significant negative association between Hb (reflecting the severity grade of anemia) and such cytokines as IL-1β (r=-0,46; p<0,01; n=30) and TNF-α (r=-0,58; p<0,01; n=21), indicating their negative influence on erythropoiesis, and genesis of anemia. In control group there have not been revealed any associations-either between different IC and Hb (p>0,05).
Summary/Conclusion: This study have revealed the synergistic action of TNF-α, IL-1β and INF-γ, suggesting their role in genesis of anemia of LPD.

PB1979

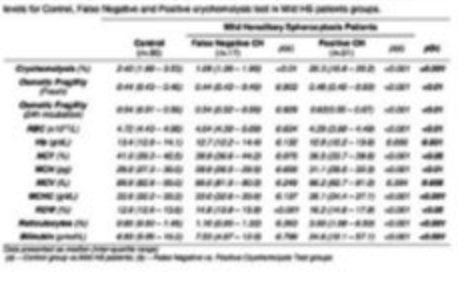
RELIABILITY OF CRYOHEMOLYSIS TEST IN HEREDITY SPHEROCYTOSIS-A PORTUGUESE POPULATION STUDY

S. Rocha[1,*], E. Cleto[2], I. Couto-Guerra[2], E. Costa[2], M.-J. Valente[1], C. Catarino[1], S. Coimbra[1,3], E. Bronze-da-Rocha[1], L. Belo[1], A. Santos-Silva[1]

[1]UCIBIO, REQUIMTE, Laboratório de Bioquímica, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal, [2]Sector de Hematologia Pediátrica, CHPORTO-Centro Materno-Infantil do Norte (CMIN), Porto, [3]CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias Saúde (IINFACTS), Gandra PRD, Portugal

Background: Hereditary Spherocytosis (HS) is the most common non-immune congenital hemolytic anemia in individuals of northern European ancestry, ranging from an asymptomatic condition to a severe life-threatening anemia (1). HS severity is classified as Mild, Moderate and Severe according to reticulocyte count, hemoglobin (Hb) and bilirubin levels (1). Mild HS cases are more difficult to diagnose, once the complementary tests, Osmostic Fragility (OF) and cryohemolysis (CH) tests, often fail to identify less severe cases. CH was proposed as a more specific/sensitive diagnostic tool for HS however, it still gives false negative results (2).
Aims: Our aim was to evaluate the reliability of CH test in Portuguese patients previously diagnosed with HS, and to search for other biomarkers.
Methods: We studied 30 healthy individuals (matched for age and gender with patients) and 82 unsplenectomized HS patients (48 with mild, 27 with moderate and 6 with severe anemia). We performed the OF and CH tests (3); evaluated the red blood cell (RBC) count, Hb concentration, hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and red cell distribution width (RDW) (3); reticulocyte count and bilirubin levels were also assessed (3). To evaluate differences between groups we performed the Mann–Whitney U test, as most variables presented a non-Gaussian distribution. A *p* value<0.05 was considered as statistically significant.

Table 1.



Results: For Moderate and Severe HS, CH test was an excellent diagnosis tools; however, it gave false negative results in 17 out of the 48 Mild HS patients [using the cutoff value for normal CH test (2)]. We compared data (Table 1) from control and two sets of Mild HS: False Negative and Positive CH test groups. When compared to Control, the Positive mild HS group showed significant differences for all variables, except Hb and MCV; the False Negative group presented no significant differences to control group, except a significant increase in RDW. All studied parameters were significantly different, when comparing False Negative and Positive groups, except for Hb and MCV.
Summary/Conclusion: Our data show CH test failed HS diagnosis in 35.4% of Mild HS cases, while only 10.4% of mild HS patients had a RDW lower than the cut-off reference value of 14.0% (1), suggesting that RDW deserves further studies as a useful marker for detection of Mild HS cases.

Acknowledgments: Financial support from FCT/MEC through national funds and co-financed by FEDER, under the Partnership Agreement PT2020 (UID/MULTI/04378/2013-POCI/01/0145/FERDER/007728) and Norte Portugal Regional Coordination and Development Commission (CCDR-N)/NORTE2020/Portugal 2020 (Norte-01-0145-FEDER-000024).

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PB1980

DIFFERENTIAL DIAGNOSIS BETWEEN UNCLEAR HEMOLITIC ANEMIA AND WILSON'S DISEASE: USE OF MULTIPLEX ALLELE SPECIFIC PCR FOR THE MOST COMMON ATP7B GENE MUTATIONS

I. Fevraleva[1,*], E. Sysoeva[2], N. Tsvetaeva[2], O. Nikulina[2], A. Sudarikov[1]

[1]Department of Molecular Hematology, [2]Department of Orphan disease, National Research Center for Hematology, Moscow, Russian Federation

Background: Wilson's disease is autosomal recessive disorder caused by mutations in copper-transporting ATPase (ATP7B). The clinical variants of the disease include isolated neurological and hepatic forms, 15% of disease cases manifest with hemolysis in the absence of autoantibodies. Confirmation of homozygous or compound heterozygous carriage of ATP7B gene mutations is essential for Wilson's disease diagnostics. Direct sequencing of a complete ATP7B gene the most reliable method of detecting mutations is still quite expensive for screening. Here we present alternative approach to the detection of polymorphisms in the ATP7B gene.
Aims: To develop screening method effective for the detection of the most common ATP7B gene mutations.

Table 1.

aa subst.	mutation	Primers and Probes	
HIS1069GLN	rs76451636	H1069Q W	5' GGAGGCCAGCAGTGAACTC 3'
		H1069Q Mt	5'GGAGGCCAGCAGTGAACTA 3'
		H1069Q com	5'CTAGGAGAGAAGGACATGGTGA 3'
		FAM_H1069Q	5' FAM-CTTGGGCGTGGCAGTCACCAAATACTG -BTQ1-3'
ARG778LEU	rs28942074	R778L W	5'TGCCAAGTGTTCCAGCCAGC 3'
		R778L Mt	5' TGCCAAGTGTTCCAGCCAGA 3'
		R778L com	5'GTC ATC CTG GTG GTT GCT GT 3'
		R&G_R778L	5' R&G-TGTCGAAGAATGTCACAGGGCTCCTCTC –BTQ1- 3'
3400delC	rs137853289	delC W	5' TGAGGCTGGCAGCCTTCAC 3'
		delC Mt	5'TGAGGCTGGCAGCCTTCAG 3'
		delC com	5' CAGCAGAGGCAATCACTGCT 3'
		Cy5_delC	5' Cy5-CAGCTGGTTAAAAGTAGAGGTGGGTCAAACC – BTQ2-3''
ARG969GLN	rs121907996	R969Q W	5' CCAGACAGAGGTGATCATCTG 3'
		R969Q Mt	5' CCAGACAGAGGTGATCATCTA 3'
		R969Q com	5' CTT GCCTCC CTTGATGAGGAT 3'
		ROX_R969Q	5' ROX -CTTTCCAGACGTCCATCACGGTGCTGT – BTQ2-3'
GLY1267ARG	rs121907992	G1267R W	5' GAAAGTCGCCATGGTCG 3'
		G1267R MT	5'GAAAGTCGCCATGGTCAA'
		G1267R com	5'GACGTCGGCTGCCTGAT 3'
		Cy5 G1267R	3' Cy5-CAGACATGGGTGTGGCCATTGGCAC- BTQ2-3'

Methods: Allele-specific real-time polymerase chain reaction was optimized for the detection of common ATP7B mutations. The primers were chosen so that the last nucleotide in the forward primer chain corresponded to the normal or mutant nucleotide of the detected polymorphism. TaqMan probes were synthesized to anneal inside the amplicon behind the mutation point. PCRs on DNAs isolated from PBMC were run on Rotor-Gene instrument. Initial denaturation 300 sec. at 95°C was followed by 45 cycles of 63°C (50 sec.) and 95°C (15 sec.). The raw data was analyzed by instrument software.
Results: Primers and TaqMan probes were designed to measure HIS1069GLN, ARG778LEU, 3400delC, ARG969GLN and GLY1267ARG polymorphisms in the ATP7B gene. Probes were labeled by non-overlapping fluorescent dyes (FAM, R & G, ROX and Cy5) to facilitate multiplex detection of 5 mutations (see fig.1 for details). About 500 mutations of ATP7B gene are described so far, however, only a few of them are found frequently. HIS1069GLN homozygous mutation is responsible for 38-49% cases of Wilson's disease in the European population. Incidence of remaining 4 mutations reported not to exceed 2-3% for each. 260 patients with hemolysis or liver pathology of unknown origin admitted to National Research Center for Hematology between 2010 and 2017 years were tested using the method described and 28 patients carrying ATP7B gene mutation were found. Thirteen were heterozygous for HIS1069GLN; one heterozygous for ARG778LEU and 14 were homozygous for HIS1069GLN.
Summary/Conclusion: The test system developed is effective for the detection of most common ATP7B gene mutations and may be beneficial for the differential diagnosis between unclear hemolytic syndromes, liver pathology of unknown origin and Wilson's disease of various clinical manifestations.

PB1981

POTENTIAL UTILITY OF N-ACETYLCYSTEINE IN HEMOGLOBIN LOUISVILLE

S. De La Iglesia*, J. Gonzálvez Fernandez, C. Acosta Fleitas, L. Pérez Ortiz, L. González Pinedo, A. Sáez Salinas, T. Molero Labarta

Hematology, HUGC Doctor Negrin, Las Palmas De Gran Canaria, Spain

Background: Hemoglobin (Hb) Louisville is an unstable hemoglobin that was first described in 1971 in a Caucasian family by Keeling et at, and simultaneously in a Romanian family by Bratu *et al.*, who named this variant Hb Bucuresti. The Hb Louisville heterozygotes suffered from mild anemia with hemolytic crisis and showed a decreased oxygen affinity. In some cases, severe hemolytic reactions were produced by infections and oxidant drugs. It has been demonstrated that the antioxidant action of N-acetyl cysteine (NAC) helps improve markers of oxidative stress and hemolysis in sickle cell disease (SCD).
Aims: Since NAC is a safe, inexpensive drug, that has been used for many years for numerous indications and, as there is no other treatment that can be offered at the moment, it has been decided to try this drug in a family of patients with Hb Louisville.
Methods: Four members of the same family, who suffered from Hb Louisville, treated with NAC for the last three months (1-5), and who are still receiving the treatment till the present time. Hemoglobin, LDH and bilirubin levels were analyzed before and after the treatment started. In every visit to the hospital, the patients were asked about their quality of life and the presence of any symptoms.
Results: No adverse effects related to the drug's administration were observed. Not a single change in either hemoglobin levels or in hemolytic parameters was detected until now. Nonetheless, the patients did report a significant improvement in relation to their symptomatology. This analysis will keep on and further updated results will be presented at the European Congress.
Summary/Conclusion: Given this hemoglobinopathy's low frequency, it is extremely difficult to assemble an adequate number of cases in order to carry out a systematic study. Nevertheless, taking into consideration the absence of NAC's side effects, it seems appropriate to conclude that this drug can be offered to patients suffering from the heterozygous trait of unstable hemoglobinopathies.

PB1982

HEMOGLOBINA D-OULED RABAH, TWO CASES REPORTED IN A SINGLE CENTER

A. Pérez-Ortega*, L.M. Juárez-Salcedo, J. Arbeteta-Juanis, H. Guillén-García, A. Gil-Pérez, A. Vázquez-Ramo, D. De Miguel-Llorente

Haematology, Guadalajara University Hospital, Guadalajara, Spain

Background: Hemoglobinopathies are the most frequent monogenetic alterations. The implantation of the "heel test" as a screening method in Spain has allowed the increase of new cases diagnostics. Hemoglobin (Hb) D most frequent variant is Hb D-Punjab. Hb D-Ouled Rabah, has been only studied as an anthropological entity with few studies of clinical significance. We present the case of two family members, the father in a homozygous state for Hb D and the son in a double heterozygous state (Hb D-Ouled Rabah/Hb S).
Aims: To describe the diagnosis process and clinical features of two Hb D-Ouled Rabah carrier patients, in homozygote state and Hb D-Ouled Rabah/Hb S double heterozygote state, in our hospital.
Methods: In March 2017, a newborn is referred to our outpatient clinic, with high suspicion of an abnormal hemoglobin (Hb) diagnostic in the heel-screening test (results were compatible with a B-Thalassemia/HbS in heterozygote state). A blood test confirmed the absence of acute hemolysis signs, with complete blood counts (CBC) and biochemistry tests in normal ranges. High-performance liquid chromatography (HPLC) was executed for the patient and both parents as the first study. Electrophoresis of hemoglobin in both, acid and alkaline media was also performed with no conclusive results. In order to achieve the diagnosis, molecular biology studies were necessary. The final reports were double heterozygote for the newborn (Hb D-Ouled Rabah [β19 Asn>Lys] and Hb S [β6 Glu>Val]) and Hb D-Ouled Rabah in homozygote state [β19 Asn>Lys] for the father.
Results: The CBC reveals Hb of 12.0 g/dL, hematocrit: 32.4%, mean corpuscular volume: 90.9 fL, reticulocyte count: 1.16%. Peripheral smear showed dyanocytes. The HPLC study in the patient showed Hb A2: 45%, Hb F: 5.7% and Hb S: 39%. Patient's parents HPLC results: Father Hb A2: 77.3%, Hb F: 0.8%. Mother Result: Hb F: 0.8%, Hb A2: 3.6%, Hb S: 35%. In order to complete the study, an acidic and alkaline medium hemo-

globin electrophoresis was accomplished. In the index case, we observed an Hb S in heterozygosis without being able to determine if he was also heterozygous for Hb G or Hb D. *(Image 1)* In the father case, we observed a band corresponding with a probable Hb G or Hb D in the homozygous state without being able to discern which of the two corresponding. *(Image 2)* The mother was carrying a Hemoglobin S in a heterozygous state.Given the inconclusive results, we conducted a molecular characterization of both, newborn and father abnormal hemoglobin. In the index case, it was described as a double heterozygous for a Hemoglobin D-Ouled Rabah [β19 Asn>Lys] and a Hemoglobin S [β6 Glu>Val], in the case of the progenitor, a Hemoglobin D-Ouled Rabah was found in homozygous state [β19 Asn>Lys]. Since there is no clinical information on the management of this type of cases, we decided to use the same prevention that we use in patients with a sickle cell disease.

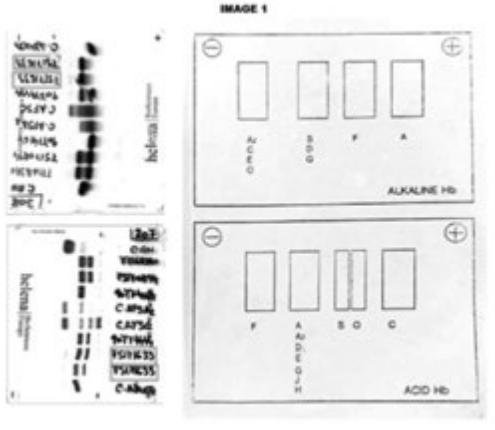


Figure 1.

Summary/Conclusion: The clinical behavior of Hb D-Punjab and Hb S in a double heterozygote state is well known but there is little knowledge about the hemolytic risk shared by Hb D-Ouled Rabah and Hb S when they occur together as in our patient. An adequate study of both electrophoresis and molecular biology in cases that are inconclusive will allow accurate diagnosis as well as an exhaustive description of the case. The monitoring of the evolution is of vital importance to predict the risk of hemolysis and acquire descriptive models to be able to use them as new cases appear.

PB1983

REVIEW OF REFERENCE VALUES OF EMA-BINDING TEST

I. Hernández-Rodríguez[1,*], C. Morales-Indiano[2], J. Juncà[1], S. Vergara[3], M. Raya[3], E. Viñets[3], C. Juan[3], M. Sorigué[1], E. Orna[1], J. Grau[1], M.-A. Llopis[2], J.-T. Navarro[1], E. Feliu[1]

[1]Hematology Department., Institut Català d'Oncologia, Hospital Germans Trias i Pujol. Institut de Recerca contra la Leucèmia Josep Carreras (IJC). Universitat Autònoma de Barcelona., [2]Clinical Analysis Department, Laboratori Clínic del Barcelonés Nord i Vallès Oriental, [3]Hematology Department., Hospital Germans Trias i Pujol., Badalona, Spain

Background: Measuring the fluorescence intensity of eosin-5-maleimide (EMA) labelled red cells has been shown to be an effective screening for the detection of hereditary spherocytosis (HS), particularly in combination with acidified glicerol test or osmotic fragility test. EMA predominantly binds covalently to the ε-NH2 group of the lysine-430 in the band 3 protein of the red cell cytoskeleton. The red cells of patients with hereditary spherocytosis have a lower EMA mean channel fluorescence intensity (MFI) than normals and other haemolytic anaemias. Reference ranges according to local population must be calculated. In our experience, absolute values of MFI undergo small changes on each reagent preparation, so the difference regarding a mean of many controls acquire more signification. Reference ranges of neonatal population have not been well established.
Aims: The aim of this study was to establish the reference ranges of EMA-test in the neonatal and paediatric population, and to review the current values expected in the adult population
Methods: Twenty-three venous blood samples from 21 newborn patients (10 women and 11 men), aged from 0 to 30 days (n=19), 60 days (n=1) and 90 days (n=1) were taken from patients attended in the emergency. Only 4 of the cases were anaemic. Moreover, 33 adult patients with an EMA test performed were reviewed. Other tests were considered, such as acidified glycerol test, osmotic fragility test and RBC membrane protein quantification by SDS-PAGE electrophoresis, to establish a final diagnosis of HS. Five microliters of whole blood from each sample was washed with 0.9% NaCl solution and incubated in darkness at room temperature for 1 h with 25 μL of EMA dye (0.5 mg/mL, in phosphate-buffered saline, PBS), with intermittent mixing. Stained red blood cells were washed three times using 0.5%/FBS/PBS and centrifuged after each wash. Cells were suspended in 500 μL of 0.5% FBS/PBS solution, and 100 μL of labelled cell suspension was diluted in 1.4 mL of FBS/PBS. Flow cytometric analysis was carried out using Cytomics FC 500 flow cytometer (Beckman Coulter, Fullerton, CA, USA). Blood samples from six blood donors were used as normal controls for each assay. The reference interval was calculated according to the CLSI guidelines, C28-A3 and a robust method was used.
Results: Reference 95% interval for newborn patients was -33.5 to 17.9, ranging from a very negative value to an almost normal adult value. Reference intervals for adult individuals were changed from those recommended in the literature (difference >16% suspected HS, and difference >21% compatible with hereditary spherocytosis) to 11% and 16%, respectively. Details of each case will be provided.
Summary/Conclusion: Reference interval for newborn patients has a wide range. Studies on HS newborns must be conducted to establish the range of suspected HS. Adult reference interval for older children and adults should be calculated for each local population, as it may differ significantly from the original recommendations.

PB1984

CEFTRIAXONE-INDUCED HEMOLYTIC ANEMIA IN A CHILD SUCCESSFULLY MANAGED WITH INTRAVENOUS IMMUNOGLOBULIN: CASE REPORT

U. Aygüneş*

Pediatric Hematology-Oncology, Cumhuriyet University Faculty of Medicine, Sivas, Turkey

Background: Ceftriaxone is a frequently used empiric antibiotic in children. Acute hemolysis is a rare side effect of ceftriaxone therapy associated with a high mortality rate.
Aims: Here, we report successful management of life-threatening ceftriaxone-induced hemolytic anemia (CIHA) in a previously healthy 5-year-old girl. We also reviewed the literature of hemolytic anemia. Careful observation is required for pediatric patients receiving ceftriaxone.
Methods: A 5-year-old female was transferred to our hospital for further treatment of a sudden episode of macroscopic hematuria, hemoglobinuria, and hemolytic anemia that developed after being given fifth dose of ceftriaxone. The previous week, she had complained of a cough and fever and had been diagnosed with tonsillopharyngitis. She had been treated with ceftriaxone (100 mg/kg/day intravenously) for 2 days as an outpatient at another center. Her symptoms worsened and the fever persisted for 3 days. Her blood tests were checked on the third day. The complete blood count included hemoglobin 11.1 g/dl, hematocrit 30%, white blood cell count 18180/μl, and platelet count 519000/μl. She was hospitalized and continued on IV ceftriaxone. She had sudden-onset pallor, fatigue, and hemoglobinuria within about 30 minutes of starting the fifth dose of IV ceftriaxone. On admission to our hospital, she was unconscious and was transferred to our pediatric intensive care unit. Her vital signs were heart rate 154/min, respiratory rate 26/min, temperature 37.0°C, and blood pressure 90/60 mm Hg. The spleen was palpable 1 cm below the costal margin. She was given IV fluids and the initial laboratory tests revealed severe anemia with hemoglobin (Hb) 3.8 g/dl, hematocrit 11.1% and reticulocyte count was 3.82%. The peripheral blood smear was consistent with 4 (+) hemolysis, having schistocytes. The blood chemistry revealed indirect bilirubinemia (3.89 mg/dl; normal range: 0.3-1.1) and elevated lactate dehydrogenase (1257 U/l; normal range: 125-250). The haptoglobin level was<6.54 (normal 30–200) and Coombs' direct antiglobulin test was positive for IgG (3+) and for C3d (3+). The reddish urine was 4 (+) for hemoglobin. CIHA was diagnosed and the ceftriaxone stopped. The patient was treated with red blood cells (10 ml/kg), high-dose immunoglobulin (1 g/kg). She improved quickly and her Hb increased from 3.8 to 7.3 g/dl. Her Hb did not drop again nor did she develop acute tubular necrosis. She was discharged on the tenth hospital day with Hb 12.7 g/dl and normal blood chemistry.
Results: The patient had no further hemolytic episodes. Her hematocrit remained stable over the following 3 months and direct antiglobulin test is still 1 (+).
Summary/Conclusion: Ceftriaxone is used very frequently in children; an early diagnosis and proper treatment of hemolytic anemia are essential to improve the patient outcome. The pathophysiological mechanism is the same as for non-drug autoimmune hemolytic anemia. However, there is still

no consensus treatment for CIHA. Intravenous immunoglobulin can be used in clinical emergencies, such as our case, or in refractory cases.

PB1985

SERUM AND RED CELL FOLATE LEVELS AMONG LOW BIRTH WEIGHT NEONATES IN WAD MADANI OBSTETRICAL AND GYNAECOLOGICAL TEACHING HOSPITAL,GEZIRA STATE,SUDAN (2016)

A. Khadir*, A.E. Mustafa

Haematopathology, Faculty of medicine, Gezira university, MEDANI, Sudan

Background: A central feature of embryonic and fetal development is widespread cell division; folate is central because of its role in nucleic acid synthesis. During gestation folate deficiency can impair cellular growth and replication in the fetus or placenta. Low birth weight is closely associated with inhibited growth and cognitive development, and chronic diseases later in life. Many researches found a relationship between umbilical cord folate status and intrauterine growth restriction. In sudan no similar study was found.
Aims: It was a case-control study aimed to measure folate levels among groups of normal and low birth weight neonate to evaluate the correlation between umbilical cord folate levels and birth weight.
Methods: Haematological parameters were measured using automated cell counter,microscopic examination of peripheral blood smears and reticulocytes preparations. Serum and red cell folate were measured by electrochemiluminscence technology (cobas e 411 analyzer). Statistical analysis was done using SPSS program version 20.
Results: From the study it was found that umbilical cord RBC folate status to be an important predictor of neoborn birth weight, with increasing cord RBC folate being associated with increasing neoborn birth weight. Statistically significant association was found between red blood cell folate levels and birth weight (P value of 0.047 in case group). Thirteen out of 43 cases had low red cell folate levels. This indicates the presence of relationship between folate levels in fetus and birth weight. No statistically significant association was found between levels of serum folate and birth weight in the case group (P Value 0.59 in case group). This may be due to fact that serum folate is a marker of recent dietary intake and it is subjected to prandial variation.
Summary/Conclusion: Red blood cell folate is considered the most reliable biomarker of folate status, as it reflects tissue folate stores. A single measurement of serum/plasma folate reflects only the time of blood collection and cannot differentiate between occasional low dietary intake of the vitamin and folate deficiency. Our study revealed that there is clear association between folate deficiency and increase incidence of low birth weight and preterm delivery.

Gene therapy, cellular immunotherapy and vaccination – Biology & Translational Research

PB1986

UMBILICAL CORD-DERIVED MESENCHYMAL STROMAL CELLS PROCESSED WITH SERUM FREE CULTURE AND CRYOPRESERVATION FOR CLINICAL APPLICATION OF SEVERE ACUTE GVHD

T. Nagamura-Inoue[1,*], A. Takahashi[1], T. Shimazu[1], A. Hori[1], T. Mukai[1], M. Okada[1], F. Nagamura[2], A. Tojo[3]

[1]Department of Cell Processing And Transfusion, Research Hospital, The Institute of Medical Science, The University of Tokyo, [2]Division of Advanced Medicine Promotion, [3]Division of Molecular Therapy, Advanced Clinical Research Center, The Institute of Medical Science, The University Of Tokyo, Tokyo, Japan

Background: Recently, umbilical cord (UC) has become attracted source of mesenchymal stromal cells (MSC) for the treatment of severe acute graft *versus* host disease (aGVHD), because of abundant sources and ease of collection without invasive process for the donor and low immunogenicity with immunosuppressive ability. In addition, because supplemented fetal bovine serum (FBS) in the medium with which UC-MSCs are cultured introduces the possibility of xenogeneic antigens and infections including bovine spongiform encephalopathy (BSE), lower antigenic and safer medium is needed for clinical use.
Aims: We recently established the serum-free culture and cryopreservation in UC-MSC processing. Objectives of this study were to evaluate UC-MSC cultured with serum-free medium and cryopreserved in serum-free cryoprotectant for the treatment of severe acute GVHD.
Methods: UC tissue was cut and cryopreserved with serum-free cryoprotectant, STEM-CELLBANKER. Master UC-MSCs (P1) were isolated from frozen-thawed UC by an improved explant method. The master UC-MSCs were cryopreserved once and thawed and expanded until P4 in serum-free RM medium (Rohto Pharmaceutical Co.,Japan). Product cells were collected followed by automated concentration and washing instrument produced by Kaneka Co., Japan. The product cells were cryopreserved in DBA-D solution consisted of dextran-40, Bicarbonate ringer, Citrate buffer, and 10v/v% DMSO. Mixed lymphocyte reaction (MLR) assay co-cultured with or without UC-MSCs was carried out using responder mononuclear cells stained with CFSE, and analyzed by flowcytometry.
Results: UC-MSC cultured with RM medium showed significantly higher proliferation ability compared with those with 10% FBS and MEM. The UC-MSCs were positive for CD105, CD73, CD90, and negative for CD45 and HLA-DR. HLA-DR, CD80, CD86, and CD40 were negative even in the high concentration of IFN-γ, while BM-MSCs became positive for HLA-DR. PD-L2 was constitutively expressed in UC-MSC, while PD-L1 was induced by the addition of IFN-γ. In MLR, responder T cell proliferation triggered by allogeneic dendritic cells was inhibited efficiently by 3rd party derived UC-MSCs.In the presence of the transwell insert, the inhibitory effect of UC-MSC was not the same degree as when there was direct contact between UC-MSCs and activated T cells. Realtime-PCR revealed the induction of IDO only in the co-cultured with UC-MSC and MLR.
Summary/Conclusion: These results demonstrated that UC-MSCs cultured with serum-free medium and cryopreservation, have high proliferation potency with immunosuppressive effects. UC-MSC may be a feasible alternative source to BM-MSC for immunosuppressive therapy in severe aGVHD.

PB1987

A MATHEMATICAL MODEL DESCRIBING THE RELATIONSHIP BETWEEN GENETICALLY-MODIFIED HEMATOPOIETIC STEM CELL TRANSPLANT AND RESULTING BLOOD HEMOGLOBIN (HB) LEVELS IN BETA THALASSEMIA

T.W. Ho[1,*], A.S. Lundberg[1], R. Blanchard[1], W. Denney[2]

[1]CRISPR Therapeutics, [2]Human Predictions, LLC, Cambridge, United States

Background: Beta thalassemia is a genetic blood disorder characterized by reduced red blood cell (RBC) lifespan, low blood hemoglobin and the need for lifelong transfusion. CTX001 is a gene-edited cellular therapy for the treatment of beta thalassemia, in which autologous hematopoietic stem cells (HSCs) have been edited to disrupt the erythroid enhancer region (EER) of BCL11a on chromosome 2. Treatment with CTX001 is intended to increase expression of fetal hemoglobin (HbF) in RBCs. CTX001 is being co-developed by CRISPR Therapeutics and Vertex Pharmaceuticals.

Aims: A mathematical model was developed to describe the relationship between a dose of genetically modified HSCs and resulting blood Hb following engraftment.
Methods: Literature and original research data were used to develop a model comprised of distinct biological processes characterizing the effect of gene editing on HbF in the RBC, the proportion of modified HSC administered, bone-marrow engraftment of HSCs, surviving RBC in circulation, and, finally, an estimate of blood Hb levels. Individual components included the ratio of gamma/alpha globin mRNA, RBC survival post ablation, lifespan of RBCs as a function of globin protein ratios (alpha-partner/alpha globin) and extrapolation of RBC lifespan to Hb. The relationship between dosing and Hb was evaluated across a range of input variables. Data were manually extracted from literature sources with individual-level data reported on endpoints relevant to the models (Ferrari, 2017; Vigi *et al.*, 1969; Clegg *et al.*, 1979). The input data were independently quality controlled. R with the rstanarm library was used to generate mathematical models using Bayesian inference and minimally-informative priors. Models were selected to represent the data available and were a combination of linear fixed effect, linear mixed effect, and nonlinear fixed effect models. The best model was determined by statistical properties and visual checks of the analysis results.
Results: RBC lifespan, as measured by ^{51}Cr pulse, appears directly and linearly related to alpha-partner/alpha globin ratio (Vigi *et al.* 1969). Original research data indicated that CRISPR modification of the BCL11a EER increased the gamma/alpha globin mRNA ratio. Efficiency of transfection (% cells transfected) was modeled as a variable process without covariates. A linear mixed effects model estimated that the percent of leukocytes in blood emanating from transfused HSCs is 77.3% [50.1, 105, 95% credible interval] x the efficiency of gene modification in the HSCs. When the alpha-partner/alpha globin ratio is 1, the RBC half-life is estimated to be 29.4 [27.3, 31.6] days consistent with the normal ^{51}Cr pulsed RBC half-life in subjects without beta thalassemia. When no alpha partner is present, the estimated lifespan is 0.522 [-0.935, 1.80] days, aligning with expectations that alpha partners are required for RBC survival. Using these models and internal data on the distribution of alpha-partner/alpha mRNA and protein ratios in reticulocyte-like cells, the model estimate for the alpha-partner/alpha ratio is 0.746 following bi-allelic genetic modification of HSC cells (CTX001). The estimated ratio would suggest an average ^{51}Cr pulsed RBC lifespan of 21 -34 days, depending on assumptions for maximum RBC lifespan constraints.
Summary/Conclusion: Simulations across the model suggest that the proportion of biallelically modified HSCs must be 20 to 50% for notable improvement in blood Hb levels (target 10 g/dL) following treatment with CTX001.

Hematopoiesis, stem cells and microenvironment

PB1988

DETECTION AND STUDY OF MOSAIC RUNX1 MUTATION IN POST AML CR SAMPLES USING HIGHLY SENSITIVE DDPCR

K.D. Johansen[1,*], H. Hasle[2], L.H. Ebbesen[1], A. Aggerholm[1]

[1]Department of Hematology, [2]Department of Pediatrics and Adolescent Medicine, Aarhus University Hospital, Aarhus, Denmark

Background: The RUNX1 gene encodes a transcription factor and is a key regulator in the development of normal hematopoiesis. Somatic *RUNX1* mutations are recurrent in malignant diseases including myelodysplastic syndromes (MDS), acute myeloid leukemia (AML) and germline *RUNX1* mutations are associated with Familial platelet disorder with predisposition to AML (FPD/AML). In this study we analyzed serial samples from a 10-year-old girl with prolonged thrombocytopenia post AML treatment for a constitutional *RUNX1* mutation. A remission PB sample 510 days post AML diagnosis was investigated by targeted NGS and showed a *RUNX1* c.347T>C (p. F116S) mutation which was detected at a variant allele frequency (VAF) of 6%.
Aims: Establishment of a ddPCR assay for detection of a mosaic *RUNX1* mutation and exploration of its longitudinal evolution and presence in different hematopoietic cell lineages.
Methods: A droplet digital PCR (ddPCR) assay was designed for detection of the *RUNX1* c.347C>T mutation. Primers and HEX/FAM labeled probes were designed for detection of both mutant and wild type DNA. The assay sensitivity was experimentally validated to be 0.1%. For sequential studies DNA was isolated from peripheral blood (PB) and bone marrow (BM) remission samples. T-cells, B-cells, and myeloid cells were isolated from PB with CD3, CD19, and CD15 antibodies respectively using EasySep™ reagents from Stem Cell Technology. Fibroblasts and keratinocytes from a skin biopsy were cultivated for further analysis, and DNA from the patient's Guthrie card was extracted.
Results: Fibroblasts and keratinocytes from the skin biopsy were found to be negative for the *RUNX1* mutation. While positive for *GATA2* and double *CEBPA* mutations at AML diagnosis no *RUNX1* mutation could be detected in the diagnostic sample. In sequential analyses of PB and BM post AML the mutation was seen for the first time in PB 419 days post AML with a VAF of 0.2%, increasing to 13% and 23%, over the next 400 days (figure). Analyses of T-cells, B-cells, and myeloid cells isolated from a PB remission sample 845 days post AML diagnosis showed presence of the *RUNX1* mutation in all three lineages with allele frequencies of 4%, 32%, and 8% respectively.

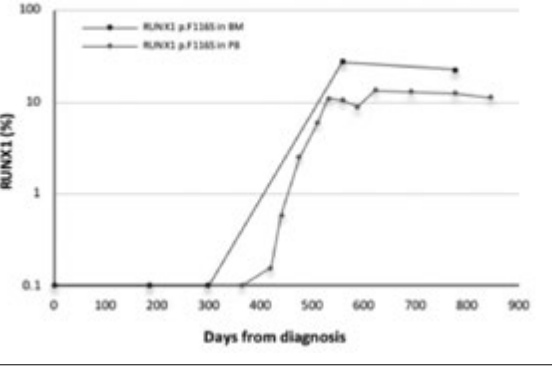


Figure 1.

Summary/Conclusion: The *RUNX1* mutation identified was excluded as being germline due to its absence in fibroblast and keratinocytes from skin. Using ddPCR we were able to detect the mutation in T-cells, B-cells, and myeloid cells meaning that the mutation has originated in a hematopoietic stem cell. The patient was reported to have had a lifelong tendency to easy bruising and it is tempting to speculate that the mutation occurred in the hematopoietic system during embryonic development. However, we were not able to detect the mutation in DNA from patient's Guthrie card and have no other PB samples prior to the AML diagnosis. If the *RUNX1* mutation is responsible for the reported easy bruising and post AML thrombocytopenia, it must arise from another hematopoietic stem cell than those leading to the AML. Another possibility is that the *RUNX1* mutation is therapy induced and possesses a proliferative advantage compared to normal stem cells. Future fluorescence activated cell sorting of normal and

leukemic hematopoietic stem cells from the diagnostic AML sample and subsequent *RUNX1* ddPCR analysis will hopefully aid us in determining the occurrence of the mutation.

PB1989

CORD BLOOD STEM CELLS EXPANSION IN THE MICROFLUIDIC DEVICE UNDER THE INFLUENCE OF NICOTINAMIDE

M. Asgari*, M. Nikougoftar

Cord Blood Bank, Iran Blood Transfusion Organization, Tehran, Iran, Islamic Republic Of

Background: The low number of umbilical cord blood cells is an important barrier to successful bone marrow transplantation. Therefore, the growth of these cells while maintaining their functional characteristics is of great importance. Using microfluidic technology as well as culturing cells on 3D scaffolds like DBM has greatly contributed to the simulation of the mechanical and chemical micro-environment of the bone marrow tissue. There are also specific compounds for proliferation without differentiation of cells, one of them is nicotinamide
Aims: In the study we aimed to expand hematopoietic stem cells by nicotinamide and in the 3D scaffold (DBM) under the effect of microfluidic device.
Methods: hematopoietic stem cells(HSCs) were cultured 7 days in two groups: 1. In DBM in the microfloid device 2. In DBM in static state (control), under the following conditions: 1.negative control 2.cytokine 3.nicotinamide 4.nicotinamide+cytokine 5.negative control with feeder 6.cytokine+feeder 7.nicotinamide+feeder 8.nicotinamide+cytokine+feeder After 7 days, cell count, their purity using flow cytometry, Colony forming unit assay and apoptosis were evaluated. The status of the cells on the scaffold was observed using electron microscopy
Results: Our results indicated that more cells were counted in static culture than microfluidic culture (perfusion), while the ability of cell colonization in microfluidic culture was far higher. The purity of HSCs was higher in microfluidic culture, and fewer cells entered the apoptotic phase. In all cases, the combination of nicotinamide and cytokine in the presence of feeder, was accompanied with more proliferation, more CD34 + cells, less apoptosis, and higher colonyforming ability than other groups.
Summary/Conclusion: Nicotinamide by preventing epigenetic changes resulting from laboratorial cell culture and microfluidic device associated with DBM by simulating bone marrow niche is a very suitable compound for the development of these cells.

PB1990

DIFFERENT CLINICAL PRESENTATION OF 3 PATIENTS WITH FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS WITH TWO NOVEL MUTATIONS

S. Akyol[1], A. Ozcan[1], T. Sekine[2], S.C. Chiang[2], E. Yilmaz[1], M. Karakurkcu[1], T. Patiroglu[1,*], Y. Bryceson[2], E. Unal[1]

[1]Pediatric Hematology, Erciyes University Medical Faculty, Kayseri, Turkey, [2]Center for Hematology and Regenerative Medicine, Department of Medicine, Karolinska University Hospital Huddinge, Stockholm, Sweden

Background: Although familial hemophagocytic lymphohistiocytosis (FHL) can manifest with combination of unremitting fever, hepatosplenomegaly and pancytopenia, FHL should be considered in unusual initial presentations. Although familial hemophagocytic lymphohistiocytosis (FHL) can manifest with combination of unremitting fever,hepatosplenomegaly and pancytopenia, FHL should be considered in unusual initial presentations.
Aims: Familial hemophagocytic lymphohistiocytosis should be considered in unusual initial presentations.
Methods: We present three cases with FHL with two novel mutations with different initial presentations.
Results: The first patient has a homozygous UNC13D stop-gain (c.2650C>T.p.Gln884Ter) mutation and presented at the age of 21 months with seizures and hyperintense signal changes on T2-weighted and T2-fluid attenuated inversion recovery magnetic resonance imaging (MRI) which was thought to be central nervous system involvement. Also she had macular widespread body rush and fever which was ongoing for six months. The patient responded to the HLH-2004 protocol, and allogenic hematopoetic stem cell transplantaon was planned from her healthy sister. The second and third patients were siblings that carried a homozygous STXBP2 splice-site mutation (c.430-1G>A), and presented in infancy with fatal sepsis and hyperferritinemia, yet without full clinical features of HLH. The first patient has a homozygous UNC13D stop-gain (c.2650C>T.p.Gln884Ter) mutation and presented at the age of 21months with seizures and hyperintense signal changes on T2-weighted and T2-fluid attenuated inversion recovery magnetic resonanceimaging (MRI) which was thought to be central nervous system involvement. Also she had macular widespread body rush and fever-which was ongoing for six months. The patient responded to the HLH-2004 protocol, and allogenic hematopoetic stem celltransplantaon was planned from her healthy sister. The second and third patients were siblings that carried a homozygous STXBP2splice-site mutation (c.430-1G>A), and presented in infancy with fatal sepsis and hyperferritinemia, yet without full clinical featuresof HLH.
Summary/Conclusion: Hematologists should be vigilant regarding the varied presentations of FHL in different ages, with different mutations. Hematologists should be vigilant regarding the varied presentations of FHL in different ages, with differentmutations.

PB1991

NEONATAL LYMPHOPENIA SCREENING FOR DIAGNOSIS OF COMBINED IMMUNODEFICIENCY: SINGLE CENTER STUDY

T. Patiroglu[1,*], M. Cansever[2], A. Poyraz[3], I. Muderris[3]

[1]Pediatric Hematology, [2]Pediatric Immunology, [3]Pediatric, Erciyes University Medical Faculty, Kayseri, Turkey

Background: Combined immunodeficiency (CID) is a primary immunodeficiency diseases with severe loss of T and B lymphocyte function. Lymphopenia is an important finding in diagnosis of CID.
Aims: We planned the screening of lymphopenia in cord blood samples during labor for diagnosis of CID.
Methods: Complete blood count (CBC) was measured in cord blood sample of every baby born in our hospital from january to december 2018. If lymphocyte count was below 3000/mm3, it was accepted as neonatal lymphopenia. Immunological investigations including serum immunoglobulin levels, lymphocyte subgroups and chest radiography for thymus shadow were evaluated in newborns who had lymphopenia.
Results: In this study, CBC was measured in 1500 cord blood samples during labor and lymphopenia was found in 39 of them. Mean lymphocyte count was calculated as 2250/mm3 (range:1550-4400). Two of 39 newborns with lymphopenia had CID according to immunological investigations. One patient with CID had RAG 1 defiency. It is expected the result of genetical analysis of other patient with CID.
Summary/Conclusion: The lymphopenia can occur may included infectious,genetic, systemic and iatrogenic causes. Early diagnosis of CID can be life saving, so that neonatal screening of lymphopenia is important for early diagnosis of CID.

PB1992

WRIST BONE CHANGES IN 78 CASES WITH FANCONI ANEMIA

S. Akarsu[1,*], H. Artas[2], C. Albayrak[3], D. Albayrak[4], S. Akbayram[5], M. Soker[6], B. Yilmaz[7], S. Karaman[8], Z. Karakas[8], T. Fisgin[9], Y. Oymak[10], C. Vergin[10], E. Zengin[11], N. Sarper[12], E. Turkkan[13], M. Akin[14], U. Kocak[15], Z. Kaya[15], I. Yenicesu[15]

[1]University of Firat, Faculty of Medicine, Department of Pediacric Haematology, [2]University of Firat, Faculty of Medicine, Department of Radiology, Elazig, [3]Ondokuz Mayıs Üniversitesi, Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları AD, Çocuk Hematoloji Onkoloji BD, [4]Medical Park Hastanesi, Çocuk Hematoloji BD, Samsun, [5]Gaziantep Üniversitesi, Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları AD, Çocuk Hematoloji Onkoloji BD, Gaziantep, [6]Dicle Üniversitesi, Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları AD, Çocuk Hematoloji Onkoloji BD, Diyarbakır, [7]Marmara Üniversitesi, Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları AD, Çocuk Hematoloji Onkoloji BD, [8]İstanbul Üniversitesi, Cerrahpaşa Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları AD, Çocuk Hematoloji Onkoloji BD, [9]Bahçeşehir Üniversitesi, Çocuk Sağlığı ve Hastalıkları AD, Çocuk Hematoloji Onkoloji BD, Istanbul, [10]Behçet Uz Çocuk Hastanesi, Çocuk Sağlığı ve Hastalıkları AD, Çocuk Hematoloji Onkoloji BD, Izmir, [11]Kocaeli Üniversitesi, Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları AD, Çocuk Hematoloji Onkoloji BD, [12]Kocaeli Üniversitesi, Kocaeli Üniversitesi Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları AD, Çocuk Hematoloji Onkoloji BD, Kocaeli, [13]Okmeydanı Eğitim ve Araştırma Hastanesi, Çocuk Sağlığı ve Hastalıkları AD, Çocuk Hematoloji Onkoloji BD, Istanbul, [14]Denizli Devlet Hastanesi, Çocuk Sağlığı ve Hastalıkları AD, Çocuk Hematoloji Onkoloji BD, Denizli, [15]Gazi Üniversitesi, Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları AD, Çocuk Hematoloji Onkoloji BD, Ankara, Turkey

Background: Fanconi anemia (FA) is an autosomal recessive genetically transmitted hereditary disease which predisposes the patient to progressive bone marrow failure, growth retardation, leukemia, and solid tumors. In nearly

50% of the children with FA, skeletal anomalies are found. Approximately 70% of them are upper extremity anomalies. The most frequently encountered problems are observed along the thumb-side, and radial edge of the forearm. Besides, much information is not available on wrist bones (proximal, central, and distal rows) and relevant anomalies in patients with FA.
Aims: We wanted to present wrist bone anomalies which we evaluated in 78 cases together with literature information.
Methods: Seventy-five cases with the diagnosis of FA followed up by 12 Departments of Hematology-Oncology from 9 different cities were evaluated by a single radiologist based on criteria defined in *Greulich Pyle Hand Wrist Atlas Radiology*, and changes of hand-wrist bones of all cases aged between 15 months, and 27 years were determined.
Results: Demographic characteristics, and hand-wrist bone anomalies of all cases were determined, and tabulated. Wrist bones belong to a group of short bones. Congenital anomalies may be embryologically classified in the "underdevelopment group." Embryogenesis of the upper extremity develops from lateral edge of the embryo at 4. week after fertilization. At 8. week of the fertilization, formation of all extremities is completed.
Summary/Conclusion: In the radiologic, endocrinologic, and orthopedic evaluation, and interpretation of wrist radiograms, the abovementioned signs, and symptoms of this disease should be also investigated.

Hodgkin lymphoma – Biology & Translational Research

PB1993

Abstract withdrawn.

PB1994

PHENOTYPIC CHARACTERIZATION OF CIRCULATING T AND NK CELLS IN HODGKIN LYMPHOMA (HL) PATIENTS IN RELATION TO TUMOR BURDEN

T.A. Mulder[1,*], K. Heimersson[1], B. Näsman-Glaser[1], L. Hansson[1,2], M. Palma[1,2]

[1]Department of Oncology-Pathology, Karolinska Institute, [2]Department of Hematology, Karolinska University Hospital, Stockholm, Sweden

Background: In classical Hodgkin lymphoma (cHL), the malignant Reed-Sternberg (HRS) cells usually represent only 0.1% to 2% of cells in the affected lymph nodes. These cells are embedded in an inflammatory microenvironment, dominated by lymphocytes and other immune cells that support their survival and growth. Conversely, the HRS cells orchestrate the immune cell infiltrate in their own favour through complex interactions.
Aims: To characterize the phenotype of lymphocytes in the peripheral blood of cHL patients with different clinical characteristics and in comparison to healthy individuals.
Methods: Peripheral blood samples were obtained from 36 cHL patients at diagnosis. Eighteen patients had limited-stage (I-IIA) and 18 had advanced-stage (IIB-IV) disease. Twenty sex- and age-matched healthy individuals were included as controls. Patients with nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL), ongoing acute Epstein-Barr virus infection and positive serology for human immunodeficiency virus were excluded. Cells from whole blood and the peripheral blood mononuclear cell (PBMC) fraction were analysed by flow cytometry. Absolute numbers of lymphocyte subsets were derived by dual-platform counting technologies.
Results: No significant difference was observed in absolute lymphocyte counts between cHL patients and healthy controls (median 1.60 $x10^9$/L and 1.45 $x10^9$/L in limited and advanced disease pts, respectively, *vs* 1.70 $x10^9$/L in controls). Lower numbers of naïve CD8+ T cells were observed in cHL pts compared to controls (median 0.11 $x10^9$/L in limited disease *vs* 0.10 $x10^9$/L in advanced *vs* 0.23 $x10^9$/L in controls, p=0.02 and p=0.001, respectively). Limited-stage patients had higher numbers of the antigen-experienced CD8+ T cell subsets CD45RA-CCR7- (median 0.07 *vs* 0.05 $x10^9$/L, p=0.01) and CD45RA+CCR7- (median 0.22 *vs* 0.14 $x10^9$/L, p=0.04). Limited-stage patients also had higher numbers of CD69+ (median 0.054 *vs* 0.028 $x10^9$/L, p=0.001) and PD-1+ (median 0.13 *vs* 0.08 $x10^9$/L, p=0.007) CD8+ T cells. Limited-stage (n=3) as well as advanced-stage patients (n=4) also had higher numbers of PD-1+TIM-3+ double-positive T cells than controls (n=7) (median 0.082 (p=0.02) *vs* 0.12 (p=0.006) *vs* 0.077 $x10^9$/L, respectively). Moreover, both limited-stage and advanced-stage patients had higher numbers of Ki67+ CD4+ T cells (median 0.015 (p=0.008) *vs* 0.015 (p=0.04) *vs* 0.008 $x10^9$/L, respectively) and CD8+ T cells than controls (median 0.009 (p=0.01) *vs* 0.010 (p=0.005) *vs* 0.004 $x10^9$/L, respectively). As for the NK cells, the total number in peripheral blood of advanced-stage patients was lower than that of controls (median 0.14 *vs* 0.20 $x10^9$/L, p=0.01). On top of that, advanced-stage patients (n=5) had a smaller fraction of NK cells that were double-positive for NKG2D and DNAM-1 than controls (n=7) (median 71.6 *vs* 93.4%, p=0.01).
Summary/Conclusion: In addition to the tight control that HRS cells seem to have over the immune cells in their tumour microenvironment, they might also influence circulating lymphocytes. Indeed, cHL patients had reduced amounts of naïve CD8+ T cells. This, in patients with limited disease, was associated with an increase in antigen-experienced and chronically activated CD8+ T cells. Moreover, cHL patients had an increased amount of proliferating CD4+ and CD8+ T cells irrespective of disease stage. Finally, advanced-stage cHL patients also had lower numbers of NK cells which seemed to express activating receptors to a lower extent than healthy controls. Further studies are ongoing to characterize these findings in depth.

PB1995

EPSTEIN-BARR VIRUS EXPRESSION IN HODGKIN'S LYMPHOMA IN SUDAN

E. Hussein[1,2,*], A.M. EL Hassan[2], S.M. Bakhiet[2]

[1]Haematology and Immunohaematology, Faculty of Medical Laboratory Sciences, University of Khartoum, Sudan, [2]Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan

Background: Hodgkin's lymphoma (HL) is a potentially curable malignant lymphoma with distinct histology, biologic behavior, and clinical characteristics. Epstein Barr- virus (EBV) has remained the main candidate suggested as the infectious agent causing Hodgkin's lymphoma for many years. To our knowledge, in The Sudan, no previous study was performed to associate Hodgkin's lymphoma to infectious agents. In particular, no data available about the prevalence of EBV in HL, nor the variability among different HL pathological subtypes. It is important to stress that infectious agents are so common in in The Sudan as one of the developing countries.
Aims: To screen the paraffin embedded lymph nodes for the presence of Epstein Barr virus genome in Hodgkin's lymphoma cases compared to reactive lymphadenitis controls.
Methods: This is a retrospective case control study. All cases of HL were first confirmed histologically and the difficult ones were further immunohistochemically confirmed. Genomic DNA was extracted from formalin fixed paraffin embedded lymph nodes using commercial DNA extraction Kits, followed by PCR amplification of three different genes (LMP 1, EBNA1 and IR3 region), using three different sets of specific primers. Amplified DNA was then visualized after agarose gel electrophoresis.
Results: Forty-one cases were enrolled, 25 (61%) of them were males and 16 (39%) were females with 1.6/1 male to female ratio. The mean of age was 30 years for both males and females. Fifty-nine percent of patients were either children or young adults of less than thirty years old. HL cases were examined for histology and immunohistochemistry and accordingly, classified as 23 (56%) cases of mixed cellularity (MC) (**Fig.1**), 16 (39%) cases of nodular sclerosis (NS) (**Fig.2**), one case (2.4%) of each of lymphocyte predominance (LP) and lymphocyte depletion (LD). EBV genome was detected in approximately half (51%) of HL patients (P value=0.035) and 18.8% of reactive lymphadenitis controls (**Fig.3**, **Fig,4**). EBV virus was much more expressed in Mixed cellularity HL subtype compared to other subtypes as the virus was detected in 13 cases of Mixed Cellularity and 7 cases of Nodular Sclerosis (**Fig.5**).
Summary/Conclusion: EBV virus is associated with approximately half of Hodgkin's lymphoma cases in Sudanese enrolled in this study and particularly associated with the Mixed Cellularity subtype. Further perspective study is highly recommended to investigate the involvement of EBV in pathogenesis of Hodgkin's lymphoma in Sudanese and whether it can be used for future targeted therapeutics.

Hodgkin lymphoma – Clinical

PB1996

PROGNOSTIC SCORES IN HODGKIN LYMPHOMA (HL): COMPARISON OF THE IPS AND THE GHSG SCORE IN AN UNIFORMLY TREATED COHORT

M. Fevereiro*, C. Gaspar, S. Gonçalves Pereira, M. Lemos, A. Botelho De Sousa

Hospital dos Capuchos, Lisboa, Portugal

Background: The International Prognostic Score (IPS), which was introduced and validated 2 decades ago by Hasenclever and Diehl for advanced stages, is the most widely used risk stratification tool in HL. However, it has been criticised for its low validity in early bulky disease and modest predictive power in unfavourable disease. In the present era, with more than 80% of patients (pts) cured by first-line treatment, we need an easy-to-apply and accurate index to help deciding risk-adapted approaches. The German Hodgkin Study Group (GHSG) proposed an alternative score, which has not yet gained broad acceptance.
Aims: We retrospectively compared the influence of the IPS and the GHSG score on overall survival (OS), event-free survival (EFS) and relapse rate (RR) in an uniformly treated cohort in our center.
Methods: From Jan/97 to Dec/16, 292 consecutive pts with classical HL (80% nodular sclerosis) were treated with Stanford V. Median age was 32 (15-72), 35% had advanced stage disease and 39% had bulky mediastinal disease (all of whom received radiotherapy). The IPS was low-risk in 56%, intermediate-risk in 33% and high-risk in 11%. By the GHSG classification, 18% were early favourable, 33% early unfavourable and 49% advanced; the differences in distribution were significant ($p<0.01$).
Results: Overall response rate was 95%. With a median follow up of 9.5 years, median OS is not yet reached. OS at 12 years varied from 89 to 47% in IPS groups, and from 87 to 54% in GHSG groups ($p<0.05$ for all comparisons). EFS varied from 74 to 34% for IPS and from 75 to 58% for GHSG ($p<0.05$). RR was similar in low and intermediate-risk IPS (23 *vs* 26%) and was 35% in high-risk IPS; in GHSG groups it was 15%, 22% and 31% respectively. The IPS was a better discriminator than the GHSG score for OS (AUC 0.69 *vs* 0.63, p=0.005) and EFS (AUC 0.61 *vs* 0.60, p=0.006).
Summary/Conclusion: The distribution of pts by IPS and GHSG score showed significant differences in our cohort, with an inversion of low and high-risk groups. This discrepancy is attributable to the high proportion (39%) of bulky mediastinal disease in our series, which is also the probable cause of a paradoxically high RR in the low-risk IPS group. The prognostic performance of the GHSG score was not superior to the IPS, reinforcing the need to evolve beyond clinical scores and to incorporate novel biomarkers into clinical practice, in order to accurately identify pts wirh the poorest risk.

PB1997

PROGNOSTIC SIGNIFICANCE OF NEUTROPHIL LYMPHOCYTE RATIO (NLR) IN NODULAR LYMPHOCYTE PREDOMINANT HODGKIN LYMPHOMA (NLPHL)

S. Paydaş[1,*], I. Barista[2], M. Dogan[3], M. Pehlivan[4], G. Seydaoglu[5], S. Lacin[6], A. Unal[7], B. Yildiz[8], O. Arslan[9], M. Ozcan[9], I. Gullu[6], S. Bilgin[4], N. Keni[7], Z. Baslar[4], N. Karadurmus[8], H. Ozsan[10], M. Yagci[11], H. Akbulut[12]

[1]Medical Oncology, Cukurova University, Adana, [2]Medical oncology, Hacettepe University, [3]Medical Oncology, Ankara Numune Hospital, Ankara, [4]Hematology, Istanbul University, Istanbul, [5]Biostatistics, Cukurova University, Adana, [6]Medical oncology, Hacettepe Univesrity, Ankara, [7]Hematology, Erciyes University, Kayseri, [8]Medical Oncology, GATA, [9]Hematology, Ankara University, Ankara, [10]Hematology, Dokuz Eylül University, Izmir, [11]Hematology, Gazi University, [12]Medical Oncology, Ankara University, Ankara, Turkey

Background: NLPHL accounts for 5% of HL cases and its biology is different from classical HL (cHL). It has an indolent course, propensity for multiple and late relapses and high grade lymphomas. For this reason prognostic parameters are very important for risk adapted treatment strategy.
Aims: The aim of this study is to determine the prognostic significance of neutrophil lymphocyte ratio (NLR) which is an important inflammation parameter beside the known prognostic parameters such as sex, age, stage IV disease, serum albümin, low hemoglobin, leukocytosis, and lymphocytopenia used in cHL.
Methods: 75 cases were retrospectively evaluated. Female/male ratio was 20/55. Albumin level<4g/dl, hemoglobin<12g/dl, high WBC >10.10^9/L and lympho-

cytopenia <1x10^9/L were evaluated as poor prognostic indicators. Eleven cases had been treated by radiation alone, 32 had been treated by chemotherapy, 27 cases had been treated by chemotherapy and radiotherapy, two cases had been treated rituximab and three cases were followed without therapy.
Results: Seventy three percent of the cases had early stage disease, B symptoms and bulky disease were seen in in 15 and 6 cases, respectively. Bone marrow involvement was detected in 6 cases. Leukocytosis was found in 14 cases, lymphocytopenia in 11 cases and NLR was >4 in 26 cases. According to the relapse status mean and median progression free survival (PFS) were longer in cases with early stage disease (p:0.101) and with bulky disease (p:0.119). Mean and median PFS were significantly shorter in cases who had B symptom, low serum albumin and hemoglobin, leukocytosis, lymphocytopenia and high NLR(>4) groups (p:0.034, p:0.0001, p:0.033, p:0.0001, p:0.014; respectively). Figure 1 shows PFS curve according to NLR groups. According to multiple Cox regression analyses, among age, hemoglobin, albumin, stage, B symptom and NLR, NLR (OR:3.3; 95%CI:1.2-8.9; p:0.017) and hemoglobin (OR:4.8; 95%CI:1.4-16.7; p:0.050) were found to be independent risk factors related with PFS.

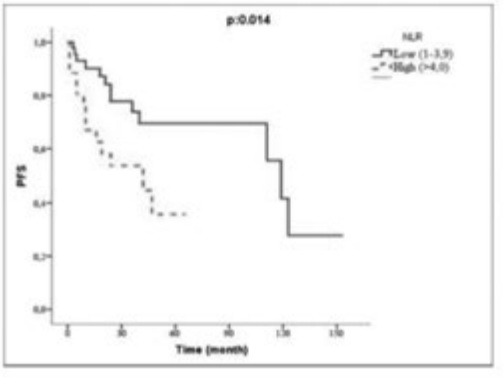


Figure 1.

Summary/Conclusion: Higher NLR (>4) was found to be an important prognostic factor in cases with NLPHL. In relapse setting, developing an alternative scoring system including B symptoms, bulky disease and NLR will be more informative in determination of risk groups and risk adapted treatments in cases with NLPHL. Note: Ethical approval has been taken from Cukurova University

PB1998

Abstract withdrawn.

PB1999

PATIENT CHARACTERISTICS, TREATMENT PATTERNS, AND CLINICAL OUTCOMES IN THE FRONTLINE TREATMENT OF ADVANCED-STAGE CLASSICAL HODGKIN LYMPHOMA IN THE UNITED KINGDOM, FRANCE, AND GERMANY

T. Illidge[1], M. Hentrich[2], A. Stamatoullas-Bastard[3], E. Zagadailov[4], A. Gautam[4], N. Way[5], J. Cambron-Mellott[5], M. Dalal[4,*]

[1]University of Manchester, Christie Hospital, Manchester, United Kingdom, [2]Rotkreuzklinikum Munich, Munich, Germany, [3]Henri Becquerel Center, Rouen, France, [4]Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Ltd, Cambridge, [5]Health Outcomes Practice, Kantar Health, San Mateo, United States

Background: Classical Hodgkin lymphoma (cHL) is a hematologic malignancy with poor prognosis for advanced-stage patients who do not respond well to frontline (FL) therapy.
Aims: This study examines patient characteristics and clinical outcomes associated with FL systemic regimens used to treat advanced-stage cHL in the United Kingdom (UK), France (FRA), and Germany (DE).
Methods: Hematologists and oncologists (N=57) from the UK, FRA, and DE retrospectively identified patients diagnosed with advanced stage cHL and treated with FL systemic therapy: doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD); doxorubicin, vinblastine, and dacarbazine (AVD); dose-escalated bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone (BEACOPP$_{escalated}$); BEACOPP. Descriptive statistics examined patient characteristics, FL regimens, and associated adverse events (AEs). Bivariate analyses (ANOVA, chi-square, or Fisher's exact test) compared patient characteristics by regimen. Univariate analyses and bivariate analyses (chi-square or Fisher's exact test) compared clinical outcomes by regimen. Progression-free survival (PFS) was assessed with Kaplan Meier curves.
Results: Mean (SD) age at initial cHL diagnosis was 46.2 (16.2) for the aggregate sample (N=297), which was mostly male (65.0%), evenly distributed across the UK (32.3%), FRA (34.3%), and DE (33.3%), comprised of patients initially diagnosed with advanced-stage cHL (Stage IIb 19.2%, Stage III 35.1%, Stage IV 45.7%), and patients treated in FL with ABVD (57.6%), AVD (7.1%), BEACOPP (13.1%), or BEACOPP$_{escalated}$ (22.2%). Among patients who received ABVD, AVD, BEACOPP, and BEACOPP$_{escalated}$, administration of FL systemic regimen type differed by patient mean [SD] age at initial cHL diagnosis (50.1 [16.3], 60.5 [10.2], 37.2 [14.2], 36.8 [10.1], respectively), p<.001, and by country (UK 45.0%, FRA 35.1%, DE 19.9%), (UK 52.4%, FRA 9.5%, DE 38.1%), (UK 10.3%, FRA 43.6%, DE 46.2%), (UK 6.1%, FRA 34.9%, DE 59.1%), respectively, p<.001. Among patients who received ABVD, AVD, BEACOPP, and BEACOPP$_{escalated}$, AEs included, but were not limited to alopecia (46.2%, 38.1%, 48.7%, 81.8%, respectively), neutropenia (22.2%, 4.8%, 28.2%, 51.5%, respectively), infection (7.0%, 9.5%, 5.1%, 18.2%, respectively), and peripheral neuropathy (4.1%, 4.8%, 7.7%, 1.5%, respectively). Response at end of FL therapy differed within the aggregate sample (complete response [CR] 66.3%; partial response [PR] 22.2%; stable disease 7.4%; progressive disease 1.4%; "no response" [refractory] 2.7%), p<.001. Responses by FL regimen are presented in **Table 1**. Median follow-up was 8.8 months (range: 0-64 months). Unadjusted 12-month PFS by FL regimen is presented in Table 1.

Table 1.

Table 1. Clinical outcomes by FL systemic regimen

	ABVD (N=171)	AVD (N=21)	BEACOPP (N=99)	BEACOPP$_{escalated}$ (N=66)
Complete Response (CR)	70.8%	38.1%	59.0%	68.2%
Partial Response (PR)	19.3%	23.8%	35.9%	21.2%
Stable Disease	5.3%	28.6%	5.1%	7.6%
Progressive Disease	1.2%	4.8%	0.0%	1.5%
No Response (Refractory)	3.5%	4.8%	0.0%	1.5%
Mean (SD) Patient Age	50.1 (16.3)	60.5 (10.2)	37.2 (14.2)	36.8 (10.1)
Unadjusted 12-Month PFS (95% CI)	67.4% (59.3%- 75.5%)	31.9% (4.3%- 59.6%)	65.8% (48.0%- 83.6%)	53.9% (38.4%- 69.4%)

Note: responses (CR, PR, stable disease, progressive disease, no response [refractory]) were observed and recorded at the end of FL therapy; responses differed by frontline regimen, p=.02. ABVD: doxorubicin, bleomycin, vinblastine, and dacarbazine; AVD: doxorubicin, vinblastine, and dacarbazine; BEACOPP$_{escalated}$: dose-escalated bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone; BEACOPP: bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone; FL: frontline; PFS: progression-free survival; CI: confidence interval; N: sample size; SD: standard deviation.

Summary/Conclusion: ABVD and AVD were more commonly administered for older patients, whereas BEACOPP and BEACOPP$_{escalated}$ were more commonly administered for younger patients. Alopecia, neutropenia, and infection were most commonly observed in BEACOPP$_{escalated}$ patients, whereas peripheral neuropathy was most commonly observed in BEACOPP patients. CR at end of FL therapy was more common in ABVD and BEACOPP$_{escalated}$ patients than in AVD and BEACOPP patients in this retrospective analysis. These findings demonstrate that treatment outcomes in the real-world practice setting may be different than those observed in clinical trials. These findings underscore the unmet need in patients with cHL and the importance of novel treatments.

PB2000

INTERIM POSITRON EMISSION TOMOGRAPHY AND CHILDHOOD HODGKIN INTERNATIONAL PROGNOSTIC SCORE CAN PREDICT SURVIVAL OF CHILDREN WITH HODGKIN LYMPHOMA IN DEVELOPING COUNTRIES

R. Khedr[1,2,*], S. mahfouz[2], L. Shalaby[3]

[1]Pediatric Oncology, Children's cancer hospital Egypt 57357, [2]Pediatric Oncology, National Cancer Institute, Cairo University, [3]Pediatric Oncology, National Cancer Institute, Cairo, Egypt

Background: Although "interim" positron emission tomography (PET) may inform therapeutic decisions, Risk stratification at diagnosis could may allow earlier and potentially more efficacious treatment modification during treatment of HL.

Aims: In this study we aimed to identify the prognostic role of both the IPET and the CHIPS in predicting the prognosis of HL in our center.
Methods: This is a retrospective, single center study where a total of 140 patients with newly diagnosed Hodgkin lymphoma were enrolled. Only 83 patients were eligible for analysis of both IPET and CHIPS scoring. PET scan was performed at baseline and after two cycles of chemotherapy. Treatment was not changed according to the results of the interim scan. PET scans reports was used using the Deauville five-point scale, blinded to treatment outcome. Childhood Hodgkin International Prognostic Score (CHIPS),was evaluated [included age (<13), number of involved sites (<3), hemoglobin (<10.5), albumin (<3.5), and erythrocyte sedimentation rate (ESR) (<20). ESR<50].Log rank testing was used to compare EFS for each CHIPS (0–3).
Results: Eighty-three scans out of 140 patients were eligible for analysis of both IPET and CHIPS scoring. Twenty six patients were scored positive (31.3%) and 57 (68.7%) as negative. The 5- years overall survival (OS) was 94%, 83% for patients with interim positive scans and 97% for patients with interim negative scans (P<0.01). The 5-year Event-free survival (EFS) rate was 86.7% for the whole study population, 76% for patients with interim positive scans and 91% for patients with interim negative scans (P<0.04). Stage 4 disease, large mediastinal mass, albumin (<3.5), and fever were independent predictors of EFS that were each assigned one point in the CHIPS. 3-year EFS was 95.7% for patients with CHIPS=0, 84.2% for patients with CHIPS=1, 75% for patients with CHIPS=2, and 80% for patients with CHIPS=3 (mostly due to a very limited number of patients) .

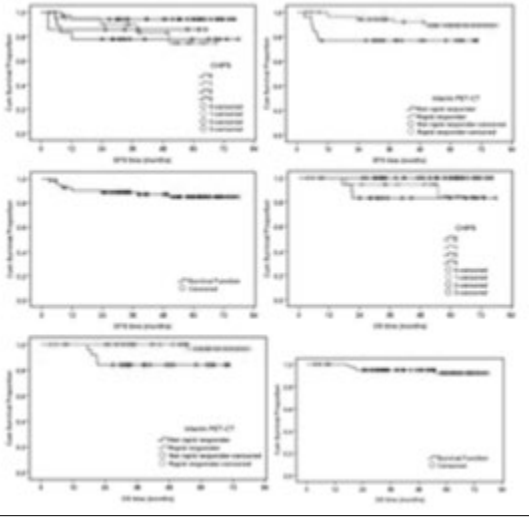


Figure 1.

Summary/Conclusion: The prognostic role and validity of using the interim PET scan response have been confirmed to be strongly related to treatment outcome by the present study. However the use of CHIPS scoring CHIPS is a good inexpensive approach to predicting risk in patients with HL that may improve ability to tailor therapy to risk factors known at diagnosis.

PB2001

PET-CR +2 METABOLIC RESPONSE GUIDED TREATMENT IN PEDIATRIC HODGKIN LYMPHOMA PATIENTS

V. Papadakis[1,*], K. Antoniadi[1], C. Kelaidi[1], M. Ampatzidou[1], V. Lyra[2], I. Sevaslidou[2], J. Nikas[3], M. Gavra[3], A. Alexopoulou[4], K. Stefanaki[5], S. Polychronopoulou[1]

[1]Pediatric Hematology Oncology, [2]Nuclear Medicine, [3]Computerised and Magnetic Resonance Imaging, Agia Sofia Children's Hospital, [4]Radiation Therapy, P&A Kyriakou Children's Hospital, [5]Pathology, Agia Sofia Children's Hospital, Athens, Greece

Background: The prognostic value of post 2-3 chemotherapy blocks PET-CT scan metabolic response has established validity for adult Hodgkin Lymphoma (HL) patients (pts). The Pediatric Protocol EuroNet-PHL-C1 STUDY evaluates patients with CT/MRI imaging and PET-CT-Scan following 2 OEPA chemotherapy blocks in an effort to avoid irradiation.
Aims: To present preliminary imaging and outcome results of a Greek pediatric pt cohort.
Methods: From 2/2009-4/2017 22 pts (13 boys) with median age of 13,1 years (range 3,9-16,3) with newly-diagnosed HL were treated according to the EuroNet-PHL-C1 STUDY. PET-CT-Scan was performed at diagnosis, following 2 OEPA cycles (PET+2) and at the end of treatment. Three Therapy-Groups (TG) were are defined: TG-1: pts stage IA/B, IIA, without mases ≥200 ml, Erythrocyte Sedimentation Rate (ESR)<30mm/hr; TG-2: pts stage IEA/B, IIEA, IIB, IIIA and pts stage IA/B and IA with≥200 ml masses and/or ESR ≥30mm/hr; TG-3: pts stage IIEB, IIIEA/B, IIIB, IV A/B. Treatment plan: Initially 2 OEPA for all pts; 2 or 4 COPP or COPDAC blocks followed for TG2 and TG3 pts, respectively. Irradiation of the initially involved sites 20-30Gy was prescribed only for pts with inadequate response (MRI/CT and/or PET+2) following 2 OEPA blocks, for all TGs.
Results: A total of 22 pts stage IIA (7), III (4/ 1A) and IV (11/ 9B, 8S, 6E) diagnosed with nodular sclerosis (19) or mixed cellularity (3) were treated according to groups TG1 (3), TG2 (3) and TG3 (16). Seven pts had bulky-disease (stage II:5, stage IV:2) and 15 pts ESR≥30mm/hr (stage II:1, III:4, IV:10). Adequate overall response following 2 OEPA cycles was observed in 2/3, 2/3 and 5/16 pts group TG1, TG2 and TG3, respectively. Good metabolic response (PET+2 with Dauville-score ≤3) was documented in3/3, 3/3 and 10/16 pts, respectively. Irradiation was prescribed to 11/15 pts following appropriate TG chemotherapy (TG1:1, TG3:10), while 4 TG3 pts had disease progression (3) or no complete response following 6 chemotherapy blocks (1).All 4 received protocol-allocated second line treatment, (IEPx2-ABVDx2) and with responsive disease they underwent autologous stem cell transplantation (ASCT) with BEAM cytoreduction, without irradiation in 2/4, due to good response. Of these, only 1 pt relapsed (4,3 from diagnosis) with single pulmonary nodule; she remains in complete remission following surgical resection only, for another 4,6 years. Another 2 pts relapsed 3,8 and 0,7 years from diagnosis and were treated accordingly (IEPx2-ABVDx2 and ASCT), without irradiation.
Summary/Conclusion: PET+2 inadequate metabolic response was observed in pts who had resistant disease or subsequent relapse. To the contrary, it appeared that pts with adequate mass regression and metabolic response can be managed without irradiation, while maintaining CR. Pts with resistant or relapsed disease can be rescued with second line chemotherapy and ASCT. Good response following ASCT allows for elimination of irradiation, too. The effectiveness of intensification of chemotherapy following the initial 2 OEPA cycles, in PET+2 scan positive pts while diminishing the number of pts receiving irradiation in children, is under investigation, in an effort to improve overall outcome, while diminishing the number of pediatric pts receiving irradiation.

PB2002

EFFICACY AND SAFETY OF HIGH DOSE BENDAMUSTINE PLUS BRENTUXIMAB VEDOTIN IN REFRACTORY OR RELAPSED CLASSICAL HODGKIN LYMPHOMA: A SINGLE CENTER EXPERIENCE

M. Raimondo*, R. Della Pepa, C. Giordano, N. Pugliese, C. Cerchione, F. Trastulli, C. Mortaruolo, I. Zacheo, M. Picardi, F. Pane

Hematology, Federico II, University of Naples, Naples, Italy

Background: The management of patients with refractory or relapsed Hodgkin lymphoma (HL) remains controversial. A standard salvage therapy is DHAP chemotherapy treatment (dexamethasone, high-dose cytarabine, cisplatin) followed by autologous stem cells transplant (ASCT) which obtains an ORR of about 50%. Bendamustine has demonstrated efficacy in several lymphoproliferative disorders but limited data are available regarding the schedule in patients with HL, in particular its dosage and possible combinations. Brentuximab Vedotin is a CD30-directed antibody-drug conjugate, currently approved for the treatment of relapsed or refractory HL.
Aims: The objective of this prospective study is to evaluate the efficacy and safety of the salvage cytotoxic regimen with Bendamustine and Brentuximab association in patients with refractory and/or relapsed HL.
Methods: Patients with relapsed or refractory classical HL from September 2013 to November 2017 were enrolled in the prospective study receiving Brentuximab 1.8 mg/kg every 21 days at day 1 associated with Bendamustine 120 mg/mq every 21 days at day 1 and 2 (Bv+B schedule). Dose reductions and/or treatment delay were recorded. The treatment efficacy was evaluated according to the Revised Response Criteria for Malignant Lymphoma. The Overall Response Rate (ORR), Overall Survival (OS) and EFS (Event Free Survival) were analyzed. Any adverse event occurred was recorded and classified for type and grade using NCI-CTCAE criteria (v 4.0).
Results: Twelve consecutive patients (5 M/7 F) with a median age of 32 years (21-44) received this salvage regimen treatment (Bv+B). All patients had an advanced stage disease, with 3 patients having stage IV disease. The median number of prior therapies was 3 (range 2-6) and all patients received ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) as upfront therapy. All patients received Bv+B schedule as salvage therapy and achieved a complete response (ORR 100%). Ten patients underwent a stem cell trans-

plant (8 autologous and 2 haploidentical), while two patients are currently in follow-up. All patients received primary prophylaxis for chemotherapy related neutropenia with G-CSF. Three patients (25%) had a grade 1 infusion reaction with fever and a skin rash, managed with corticosteroid injections and a successful antihistamine plus corticosteroid prophylaxis in the subsequent cycles of treatment; 4 patients (33%) had a citomegalovirus reactivation treated with Valganciclovir (450 mg twice a day); 2 patients had a grade 1 peripheral neuropathy. A dose reduction of 20% for bendamustine and 10% of brentuximab vedotin was required for 4 patients (33%). No patient had to delay chemotherapy treatment and all patients respected the treatment time schedule. EFS was 90% at 24 months, for a median follow up of 12 months, as one patient did not maintain the complete response and received subsequent salvage treatment. OS was 100%.

Table 1.

Table. Patients characteristics	Patients (%) (n=12)
Age	
Median, y (range)	32 (21-44)
Sex	
Male	5 (42)
Female	7(58)
Hematological malignancy	
HL	12 (100)
NS	8 (67)
MC	3 (25)
LD	1 (8)
LR	0
Ann Arbor stage*	
Stage III	8 (67)
Stage IV	4 (33)
International Prognostic Score*	
0-1	3 (25)
2-3	6 (50)
4-7	3 (25)
Number of prior therapy†	
Median (range)	3 (2-4)
ABVD	12 (100)
IGEV	11 (90)
DHAP	4 (33)
BEACOPP	1 (9)
Autotransplant	2 (16)
Response to primary therapy‡	
RC	0 (0)
RP	4 (34)
PD	5 (42)
SD	3 (25)
BvrB cycles, n	
Median (range)	4 (4-6)

*Stage III, multiple lymph node groups on both sides of diaphragm; stage IV, multiple extranodal sites or lymph nodes and extranodal disease.
†seven factors were included: Serum albumin concentration <4 g/dL, hemoglobin concentration <10.5 g/dL, Male sex, age older than 45 years, Ann Arbor stage IV, white cell count > 15.000/µL, lymphocyte count <8% and/or absolute lymphocyte count <600 cells/ µL. Each factor gets 1 point and possible scores range from 0 to 7. A higher score indicates poorer prognosis.
‡ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine), IGEV (Ifosfamide, gemcitabine, vinorelbine), DHAP(dexamethasone, high-dose cytarabine, cisplatin), BEACOPP (bleomycin, etoposide, adriamycin, procarbazine, prednisone, vincristine, cyclophosfamide);
‡RC (Remission Complete); PR (Partial Response); PD (Progressive Disease); SD (Stable Disease)

Summary/Conclusion: High-dose bendamustine plus brentuximab have shown relevant efficacy and a relatively good safety profile in a setting of heavily pretreated patients with HL. Adequate monitoring of CMV reactivation is recommended. This combination could be considered as a bridge to first or second autologous or allogenic SCT. However, these results should be validated by controlled and prospective studies involving larger number of patients.

PB2003

ABSOLUTE LYMPHOCYTE COUNT AND LYMPHOCYTE-MONOCYTE RELATIONSHIP AS A PROGNOSTIC CRITERIA IN HODGKIN'S LYMPHOMA

A. Rukavitcyn[1,*], O. Rukavitcyn[2]

[1]Hematology, [2]Gospitalnaya ploshad' III, Main Military Hospital, Moscow, Russian Federation

Background: Decline the absolute lymphocyte count in the peripheral blood of less than 8% for Hodgkin's lymphoma is an unfavorable prognostic factor, but with the development of induction chemotherapy, it lost significance in the revised IPS-3 scale. To determine the clinical significance of the absolute lymphocyte count, lymphocyte value and the ratio with monocyte we correlated this with present clinical and laboratory features and Progressive-free survival (PFS) after treatment by ABVD (adriamicyn, bleomicyn, vinblastine, dacarbazine).
Aims: Determine the clinical significance of the absolute lymphocyte count, and the ratio lymphocyte with monocyte's to the progression-free survival of patients with Hodgkin's lymphoma treated with the ABVD, according to the other prognostic criteria
Methods: The study included 124 untreated patients with classic Hodgkin's lymphoma from 2002 to 2016, and a median of 29.6 years of age. Inclusion criteria were: a histologically confirmed diagnosis of classical Hodgkin's lymphoma, the time from the onset of the first symptoms of the disease before the start of therapy was 1-2 months. The hemogram examinations till 1-2 days prior to chemotherapy, lymphocyte-monocyte ratio (LMR) was determined by dividing the absolute lymphocyte count on the absolute monocyte count. The minimum duration of follow-up after the last cycle of chemotherapy was not less than 36 months.
Results: In the population of patients (n=124), 74% (n=92) patients achieved and maintained a remission status within 36 months-favorable group. 26% (n=32) of patients did not achieve remission after induction chemotherapy or developed a relapse within 36 months-unfavorable group. In an unfavorable group, 21 patients (64%) had an LMR level less than 2, while in a favorable group in 14 patients (20%) the LMR level was below 2 and 76 patients (80%) had LMR levels in the range 2-5. The disease-free survival (DFS) during 3 years in patients with 3-4 and 4-5 LMR was 92% and only 35% and 61% in groups with LMI<1 and 1-2 respectively (p=0.038 log rank test). Using the ROC curve, the LMR-1.8 threshold level was determined which had the maximum effect on disease-free survival. With 74% specificity and 54% sensitivity, the ROC curve area was 0.73. Multivariate analysis using the Cox proportional hazard model with the inclusion of decrees level of LMR<2, age 45 and older, B-symptoms, IV stage, anemia, decreased serum albumin, increased LDH, leukocytosis, lymphocytopenia revealed that the decreased of level LMR<2 was an independent factor of poor prognosis (p=0.028; RR=1,8, CI=0.4-5.2).
Summary/Conclusion: The decreased of level lymphocyte-monocyte ratio (LMR)<2 can be an independent prognostic factor of patients with classic lymphoma Hodgkin's

PB2004

HODGKIN LYMPHOMA IN ADOLESCENTS TREATED WITH ABVD

B. Sidi Mohamed El Amine*, H. Asma, O. Fouzia, S.A. Nadjet, C. Malika, Z. Naima, Z. Zahia

Universitary hospital of Sidi Bel Abbés, Sidi Bel Abbes, Algeria

Background: Hodgkin lymphoma (HL) is a most common malignancy affecting adolescents and young adults, but the standard treatment of adolescent patients is not well defined, particularly in the choice between pediatric and adult protocols.
Aims: To compare risk factors and therapeutic outcomes of adolescents and young adults with HL treated with ABVD.
Methods: 98 patient patients treated in our department between 1994 and 2010 were analyzed: 33 adolescents between 15 and 21 years old, and 65 young adults between 22 and 39 years. All patients received as initial chemotherapy the ABVD protocol, followed in 65 (66.3%) by radiotherapy. We compared the patient characteristics of the two populations using Fisher's exact test. Survival was estimated using the Kaplan and Meier method, and compared by the log rank test. Cox proportional hazard regression was used to evaluate prognostic factors in adolescent patients.
Results: Histological type 2 and hypoalbuminemia were more frequent in adolescent patients (P=0.05, P=0.02 respectively). The incidence of other risk factors were without significant difference between the two populations. With a median follow-up of 67 months, overall survival (OS) and event-free survival (EFS) at 5 years were in the order of 79% and 72% for all patients. The overall rate of complete remission (CR) was 87.8% : 84.8% in adolescents and 89% in young adults (P=0.53). The log rank test showed no significant difference between the two age groups, in terms of overall survival (P=0.31) or EFS (P=0.51). At 5 years, the estimate of OS and EFS are in the order of 77% and 74% for young adults, 73% and 68% for adolescents, respectively. After cox regression analysis, two factors were crucial for the survival of adolescent patients: the presence of bulky disease (P=0.008) or an extranodal lymphoma involvement (P=0.04).
Summary/Conclusion: The prognosis and risk factors of adolescent patients and young adults with HL treated with ABVD are similar. These data suggest that adult protocols can offer a safe and effective treatment option for adolescent patients.

PB2005

LONG-TERM ENDOCRINOLOGIC SIDE EFFECTS OF CHILDHOOD HODGKIN'S LYMPHOMA TREATMENT

G. Aydogan*, M. Eltan, F. Akici, Z. Salcioglu, D. Tugcu, N. Aktay

Pediatric Hematology, Kanuni Sultan Suleyman Education And Research Hospital, Istanbul, Turkey

Background: Childhood Hodgkin's lymphoma has been treated successfully

for decades now. Long-term survivors of Hodgkin lymphoma (HL) are at an increased risk for a range of late complications. Raising awareness, close follow-up, and adoption of selected early-detection and risk-reduction strategies may help to reduce the adverse impact of these late effects on patients.
Aims: The aim of this study is the evaulation of long-term endocrinologic side effects on thyroid functions and bone mineral density due to childhood Hodgkin's lymphoma treatment.
Methods: This prospective study was held with 40 patients (34 male, 6 female), who had been treated between 1994-2013 in Pediatric Hematology and Oncology Department of Kanuni Sultan Suleyman Education and Research Hospital. Demographics, histopathological characteristics of tumor, stage, age at the time of radiotherapy, dose of radiations, time period after radiotherapy protocol, TSH, free T4, antithyroglobulin, anti-thyroid peroxidase, calcitonin, thyroglobulin, thyroid ultrasonography, any thyroid nodules, levels of calcium, phosphate, magnesium, ALP, 25OH-vitamine D, PTH and z-scores were all recorded for each patient on a study form. Whenever a nodule was detected in thyroid ultrasonography, sintigraphy was carried out followed by a biopsy, if indicated.
Results: The ages of patients were ranging between 4-31 years (mean 14.13±6.08). All patients were treated with chemotherapy combined with radiotherapy. Average dose of radiotherapy was 21.487± 4.487 Gy. In 72.5% of patients the primary disease was localised on head and neck. Seven patients had B symptoms. Eleveted TSH levels were detected in twelve of forty patients (30%). Thyroid hormon therapy was given to five patients, who have clinical symptoms of hypothyroidism. Nodule in thyroid gland was detected in five patients by ultrasonography. Three patients were directed to biopsy according to scintigraphic and ultrasonographic evaluations. Hypothyroidism was detected 3.10±2.02 years and nodules were detected 8.90±7.90 years after treatment. Five (12.5%) out of 40 patients had osteoporosis detected by dual-energy X-ray absorbsiometry. Mean age of patients with osteoporosis was 16.4±6.88 while mean age of patients was 13.8±6. In 25 patients 25-OH D vitamin levelswere below normal. Among 7 patients having B symptoms 3 (42.8%), 11 patients under combined chemotherapy with steroid 3 (27.5%) had osteoporosis.
Summary/Conclusion: Patients with Hodgkin lymphoma under treatment may have abnormal thyroid functions and osteoprorsis as long term complications. Their evaluation for thyroid functions and osteoporosis are needed. Timely replacement therapies diminish the appearance of these complications and improve the quality of life of the patients.

PB2006

MULTICENTRE STUDY OF QUANTITATIVE PET VALUES IN PRIMARY PATIENTS WITH HODGKIN LYMPHOMA (HL): UPDATE RESULTS

I. Kriachok[1,*], O. Novosad[1], I. Pastushenko[1], T. Skrypets[1], T. Kadnikova[1], I. Tytorenko[1], K. Ulianchenko[1], O. Gorbach[1], V. Kozlov[2], L. Mykhalska[3], V. Kosinova[4], N. Kostiukova[5], O. Karnabeda[6], V. Stratienko[7], M. Novikov[4], O. Oliinichenko[8], I. Kmetyuk[9], O. Karpova[10], A. Ashykhmin[11], O. Lukjanec[12]

[1]Oncohematology, National Cancer Institute, Kiev, [2]Department of Internal Medicine #[1] with additional course of cardiovascular diseases, National Medical University, Odessa, [3]Center of Hematology, Clinical Hospital "Feofaniya", Kiev, [4]Oncology Hospital "LISOD", [5]Kyiv centre of bone marrow transplatation, [6]Oncology Hospital "Innovacia", Kyiv, [7]Regional Clinical Hospital, Kherson, [8]Center of Nuclear Medicine KMCOC, [9]Clinical Hospital «Feofaniya", [10]Clinical Hospital «Feofaniya, [11]Radiology, National Cancer Institute, Kyiv, [12]Cherkassy Regional Oncology Center, Cherkasy, Ukraine

Background: PET, combined with computed tomography (PET-CT), performed during and after therapy, has a high prognostic value for predicting first-line therapy results in Hodgkin lymphoma (HL) patients. Quantitative PET parameters (qPET) as a predictive factor for HL progression are still not well established.
Aims: The primary endpoint was to evaluate influence qPET values of event-free survival (EFS) after treatment. We analyzed absolute value of PET parameters, such as negative predictive value (negative PET scan and no treatment failure, NPV), positive predictive value (positive PET scan and treatment failure, PPV) and qPET (SUVmean, SUVmax, MTV and TLG) which might associate with EFS.
Methods: Quantitative PET parameters at the baseline (PET-1), interim (PET-2, after 2-4 cycles) and end of chemotreatment (PET-3) PET-CT scans were investigated. Metabolic PET-CT imaging were performed at participating PET centers according to routine protocols, compatible with EANM guidelines for FDG PET-CT imaging in malignant tumors. MTV was computed by using the 41% maximum standardized uptake value thresholding method, and the optimal cutoff for survival prediction was determined.
Results: In this retrospective analysis 96 patients with HL with a stage I-II (58.3%), III-IV (41.7%) who were admitted between Aug 2012 and Feb 2018 in 9 Ukrainian hematological centers were analyzed. Patients were treated with ABVD or BEACOPP-14/esc, based on risk group. The ORR of 96 patients (CR ,PR) was 85.4%. A negative PET-2 had 76% pts, while 9.4% had a positive PET-2. Patients with negative PET-2 and positive PET-2 had CR rates of 75% and 4.16%, respectively, which yielded a PPV of 11.4% and NPV of 88.6%. ROC analysis revealed that PPV is an important marker which is associated with poor EFS in primary patients with HL (Se=100%; Sp=100%; AUC=1.0, p<0.0001). 5-year EFS was 100% for NPV patients and 10% for PPV patients, which was statistically different (p<0.05). Multivariate analysis confirmed NPV and PPV significant variables on EFS (p<0.0001). In our study we found two new strong associations between qPET at PET-3 and EFS (PET-3 negative in 77% cases): SUVmax [Se=63%; Sp=100%; AUC=0.88, *p*<0.0001], SUVmean [Se=75%; Sp=90%; AUC=0.86, *p*<0.0001] at PET-3 and EFS by ROC analysis. Patients with SUVmax<1.84 had higher level of 5-year EFS *vs* SUVmax >1.84 (93% *vs* 15%, p<0.05). Also, 5-year EFS was 85% *vs* 25% in patients with SUVmean<3.19 *vs* SUVmean >3.19, respectively (p<0.05). ∑MTV [HRs1.4; 95% (CI) 1.0–2.0, *p*<0.0001 by Cox regression] and TLG [Se=75%; Sp=100%; AUC=0.97, *p*<0.0001 by ROC analyses] parameters at PET-3 were confirmed as important markers to predict survival in patients with primary HL. 5-year EFS was 85% and 20% in patients with ∑MTV<0 and ∑MTV >0, respectively (*p*<0.05). Unfortunately, qPET at PET-1 and PET-2 were not statistically significant in predicting clinical outcomes, due to the small sample size of our study.
Summary/Conclusion: Quantitative PET parameters is an important tool for clinicians in the diagnosis and management of patients with HL. The prognostic role and validity of the qPET for interpretation of PET should be confirmed prospectively.

PB2007

ANTI-PD1 INHIBITOR FOLLOWED BY ALLO STEM CELL TRANSPLANTATION IN 6 HEAVELY PRETREATED LYMPHOMA'S PATIENTS: EXPERIENCE OF JULES BORDET INSTITUTE

A. Salaroli*, P. Lewalle, S. Wittnebel, M. Maerevoet

Hematologie, Institut Jules Bordet, Brussels, Belgium

Background: Anti–programmed cell death protein 1 (PD-1) antibodies are tested in patients (pts) with advanced lymphoma. Following treatment, many of those ps are likely to be candidates for allogeneic hematopoietic stem cell transplant (HSCT). Armand and Merryman demonstrated that PD-1 blockade in relapsed Hodgkin-lymphoma (cHL) followed by allo-HSCT appears to be highly efficacious but frequently complicated (22-44%) by rapid onset of severe and treatment-severe and treatment-refractory graft *versus* host disease (GVHD). One-year overall and progression-free survival rates were 89%.
Aims: We report here the Institut Jules Bordet's experience of 5 pts, 3 Hodgkin lymphoma (cHL) and 2 primary mediastinal B cell lymphoma (PMBCL) and which achieved a complete remission (CR) after antiPD1 inhibitor and or chemotherapy consolidated with allo-HSCT. All of these patients have been heavily pretreated. A sixth pt is just admitted for this procedure.
Methods: Table 1. Pts characteristics and outcome. The data incuded the data from the sixth pt just admitted will be detailled and uptdated.
Results: The time from the last Nivolumab from allo-HSCT is of 25 to 70 days. One pt died 21 days after allo-HSCT of RSV lung infection and septicemia. This patient has been treated by radiotherapy in the lung and gemcitabine few months before HSCT and that could be explained the pulmonary failure. All pts have viral and bacterial infectious complications, mainly grade I-III in severity, Two pts developed a grade II acute and one of them pts a chronic cutaneous GVHD, managed by corticoid alone.
Summary/Conclusion: AntiPD1 therapy could be represent a new opportunity to achieve a complete remission and bridge to an Allo-HSCT and may be cure heavely pretreated cHL and PMBCL pts. In our experience, all pts have presented viral and bacterial infections, mainly grade I-III in severity, as expected for these population. Two pts have a GVHD resolved with systemic and local corticoid .Four of these 5 patients are still alive and in complete remission respectively at 21, 175, 280 and 336 days after Allo-HSCT. A sixth pt is still ongoing for Haplo-ASCT

PB2008

PREGNANCY RATE AND RISK OF RELAPSE IN PATIENTS IN COMPLETE REMISSION AFTER HODGKIN'S LYMPHOMA

F. Gaudio[1,*], C. Nardelli[2], T. Perrone[1], F.E. Laddaga[3], S. Paola[1], M.L. di Noi[1], S. Mallano[1], R. De Palo[2], G. Specchia[1]

[1]Department of Emergency and Organ Transplantation (D.E.T.O.), Hematology Section, [2]Unit of Pathophysiology of Human Reproduction and Gametes

Cryopreservation, [3]Pathology, Bari, Italy

Background: As the number of survivors of young female Hodgkin's lymphoma (HL) increases, it is becoming more common to manage the pregnancies of women who have a history of exposure to chemotherapies and radiation therapy. Little is known about the success rate of women attempting pregnancy. Many patients and clinicians are worried that pregnancy after the diagnosis of HL may increase the risk of relapse, despite a lack of empirical evidence to support such concerns
Aims: In the present study we included 61 women who received a diagnosis of HL between 2007 and 2014 and who were younger than 40 years of age and were in complete remission and alive without relapse >or=3 years after treatment.
Methods: Of these patients, 45 (74%) patients had had stage I-II, 16 (26%) patients stage III-IV; 18 (30%) patients suffered from constitutional symptoms; 35 patients (57%) had bulky disease and 12 (20%) had extranodal involvement. Before treatment, 58 (95%) patients reported a regular menstrual cycle. We determined the pregnancy rate, and time-to-pregnancy among survivors who had become pregnant, or tried to become pregnant for over 2 months following chemotherapy.
Results: Among the 61 women with HL, 29 (48%) were nulliparous throughout follow-up, 25 (41%) were parous but had no pregnancies during follow-up, and 7 (11%) had a pregnancy during follow-up. After a median follow-up of 5 years, 11 (18%) women had reached menopause before the age of 40 years (range: 19-39 years; median: 33.5 years). A regular menstrual cycle was reported by more than 90% of female survivors of early-stage HL (recovery time mostly 12 months). Ten tried to become pregnant; 3 without success; 7 women became pregnant with the birth of 6 healthy children. The median time-to-pregnancy among HL survivors was 2.0 months. The 12-month pregnancy rate was 70%. The median time from the end of the therapy to pregnancy was 40 months. No recurrence occurred. In one case, 3 months after the birth of the child acute myeloid leukemia occurred, and the patient died.
Summary/Conclusion: We found no evidence of significant impairment of the fertility of female HL long term survivors We found no evidence that a pregnancy after diagnosis increases the relapse rate among women whose HL is in remission. Survivors of HL need to consider a range of factors when deciding about future reproduction.

PB2009

TREATMENT OUTCOMES FOR HODGKIN LYMPHOMA WITH BULKY MEDIASTINAL DISEASE (2BX): HOSPITAL AMPANG'S EXPERIENCE

S. Hon*, T.S. Leong, S.M. Tan, P.K. Liew, T.C. Ong, J. Sathar, J. Tsen Chuen Tan

Haematology Unit, Hospital Ampang, Selangor, Malaysia

Background: Hodgkin Lymphoma (HL) with bulky mediastinal disease represents a unique cohort of patients which pose specific challenges in diagnosis, treatment, and prognosis.
Aims: We aim to analyse the treatment outcome of these patients in Hospital Ampang.
Methods: This is a retrospective analytical study. Data was obtained from Hospital Ampang electronic database. Recruitment started from January 2006 and December 2015.
Results: A total of 77 adult patients with HL with bulky mediastinal disease were identified. All patients were followed up from January 2006 till December 2017. The median age of diagnosis was 25 years (range 14 to 61 years), and 56% (n=43) were males. 47% (n=36) are of nodular sclerosing (NS) subtype. 95% (n=73) were treated with standard protocol, ABVD, whereas 5% (n=4) were treated with BEACOPP protocol. 51% (n=39) of them have received upfront consolidative radiotherapy. The median follow-up duration was 54 months (range 1 to 137 months). 42% (n= 32) attained complete remission whereas 30% (n= 23) had progression of disease in all patients treated with chemotherapy. Overall survival (OS) rate was 88.9% at 48 months and 83.7% at 60 months in all patients treated with chemotherapy. Progression free survival (PFS) was 75.1% at 24 months and 61.7% at 48 months. Amongst the ABVD cohort, the relapse rate was 23% (n=17), 45% (n=33) needed salvage chemotherapy and 25% (n=18) underwent stem cell transplantation. Among the patients who underwent salvage chemotherapy, 39% (n=13) eventually passed on. Amongst the transplanted patients, the mortality rate was 27% (n=5) due of progression of disease.
Summary/Conclusion: Bulky mediastinal HL is a peculiar condition with the outcome determined by the primary treatment. From our cohort, 45% of patients undergoing conventional therapy with ABVD require salvage chemotherapy. This suggest that more intensive chemotherapy should be employed as an initial treatment for better tumour load control and downgrade accordingly based on the interim PET-CT report.

PB2010

PROGNOSTIC FACTORS OF SURVIVAL IN ADOLESCENT AND YOUNG ADULT (AYA) PATIENTS WITH EARLY-STAGE CLASSICAL HODGKIN LYMPHOMA ACCORDING TO SUBTYPE: A 15-YEAR STUDY IN A PERUVIAN SINGLE CANCER CENTER

J. Vasquez*, J. Moreno, J. Bravo, L. Mas, H. Gomez

Medical Oncology, Instituto Nacional de enfermedades neoplásicas, Lima, Peru

Background: Hodgkin lymphoma is a good prognostic hematological malignancy, early disease has been evaluated in population regardless age, however prognostic factors were not evaluated in adolescents and young adults according to subtype.
Aims: To describe prognostic factors for progression-free survival and overall survival according histologic subtype in AYA population.
Methods: Retrospectively, we reviewed medical records from epidemiology department of Peruvian National Cancer Institute for all classical Hodgkin lymphoma patients aged between 15 and 39. Patients were treated from 2000 to 2014 and received at least 2 cycles of chemotherapy. Survival curves were estimated by Kaplan Meier, the difference was performed by the log-rank test, Cox proportional hazard models were used to identify prognostic factors of survival. Statistical analysis was based on IBM SPSS software version 24.

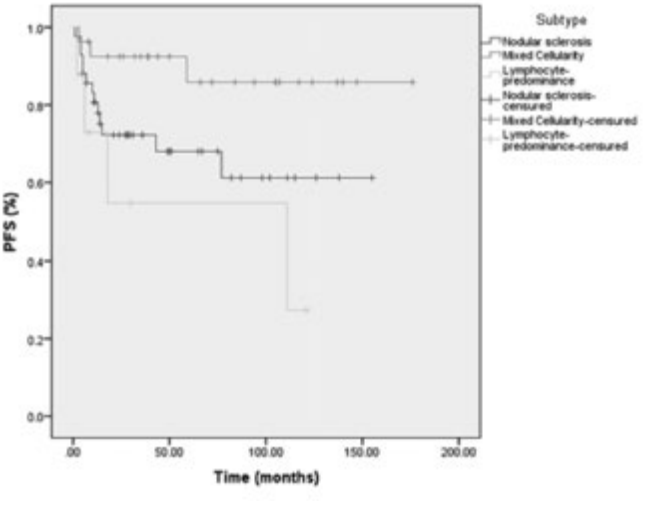


Figure 1.

Results: 1037 patients were admitted in the study period, 387 were AYAs, 77 (19.8%) m*et al.*l criteria. Median age was 25 years (15-39). 39 (50.6%) were male. Nodular sclerosis was the most common subtype with 42 (54.5%) followed by mixed cellularity with 27 (35.1%) and lymphocyte-predominance with 8 (10.4%). According to stages, II stage was more frequent with 69 (89.6%), B symptoms was present in 33 (40.3%). The primary site more common was cervical lymph node with 49 (63.65%). Erythrocyte sedimentation rate (ESR) of 1-30 was seen in 17 patients, 31-50 in 7 patients and 51 or more in 16 patients. Extra lymph node was present in 5 patients, lymph node involvement >2 in 41, bulky disease was present in 8 primary mediastinal lymphomas and in the same number for cervical lymphadenopathy. Regarding the treatment all patients received ABVD chemotherapy with a median of 6 cycles. 56 patients (72.7%) patients received radiotherapy subsequently to chemotherapy. 49 patients (63.6%) achieved complete response, 24 (31.2%) had partial response, 4 (5.2%) had progressive disease. The median progression-free survival was not reached, five-year PFS was 73.3%, univariable analysis (UA) showed statistical significance (p=0.02) for histologic subtype and not achieving a complete response with p<0.01. In the bivariable analysis (BA) the only poor prognostic factor was not achieving a complete response (Hazar ratio (HR) =4.76, 95% confidence interval (CI)=2.87-7.91, p=<0.01). The median overall survival (OS) was not reached, five-year OS was 95.7%. In the UA not achieve a complete response was the only poor prognostic factor with p=<0.01.
Summary/Conclusion: Classic Hodgkin lymphoma in early stage has a long-term overall survival. Radiotherapy do not impact neither OS nor PFS. Histologic subtype is a poor prognostic factor for PFS in UA but not in BA. The only poor prognostic factor for both PFS and for OS was not achieving a complete response.

PB2011

EXPRESSION OF CD20 DOES NOT IMPACT OUTCOME IN CLASSICAL HODGKIN LYMPHOMA IN THE CONTEMPORARY ERA OF THERAPY

R.A. Alshammari*, M. Snnallah, B. Alahmari, A. Alhejazi, A. Al Askar, M. Alzahrani, M. Damlaj

King Abdulaziz Medical City, Riyadh, Saudi Arabia, Riyadh, Saudi Arabia

Background: Prevalence of cluster of differentiation 20 (CD20) in classical Hodgkin Lymphoma (cHL) has been reported to range from 10-20%. However, the prognostic impact of such expression has been variable in the literature. Earlier reports indicated improved progression free survival (PFS) and overall survival (OS) (Tzankov *et al*., 2003) but such findings were later refuted where it was found to confer an adverse outcome (Portlock *et al*., 2004).
Aims: The primary aim of this project is to retrospectively study the prevelance of expression of CD20 in Hodgkin Lymphoma, its prognostic impact on response and overall outcome and to ascertain the role of Rituximab in managing such cases. The aim of this study is to examine the prevalence and prognostic impact of CD20 expression in cHL in a patient population from the Middle East and North Africa Region (MENA).
Methods: After due IRB approval, patients diagnosed with cHL who received frontline therapy at our institution from 2008-2016 were identified and included for further analysis. All variables were retrospectively extracted. CD20 expression of 10% or higher was considered positive. Patients with nodular lymphocyte predominant HL were excluded. Cohorts were stratified per expression of CD20 and compared using Chi-squared and Wilcoxon tests, as appropriate. PFS was defined as time from diagnosis until progression or death due to any cause. Time to end point analysis was performed with the method of Kaplan and Meier with log ranks. Statistical analysis was computed using JMP software.
Results: A total of 199 patients were identified and included for further analysis. Prevalence of CD20 expression was noted in 41 (20.6%) of patients. The cohort was subsequently stratified per CD20 expression. Baseline age, stage, chemotherapy and radiotherapy delivered was similar between the strata. The monoclonal antibody Rituximab was used more frequently in those with CD20 expression (34% *vs* 0.6%; p<0.0001). There was also a trend of significance for higher proportion of male gender in the CD20 expressing cohort (34% *vs* 0.6%; p<0.0001). The estimated 2 year PFS was 71.2% *vs* 69.5% for CD20 positive and negative, respectively (p=0.52). The estimated 2 year OS for those with or without CD20 expression was 87.4% and 90.8%, respectively (p=0.96).

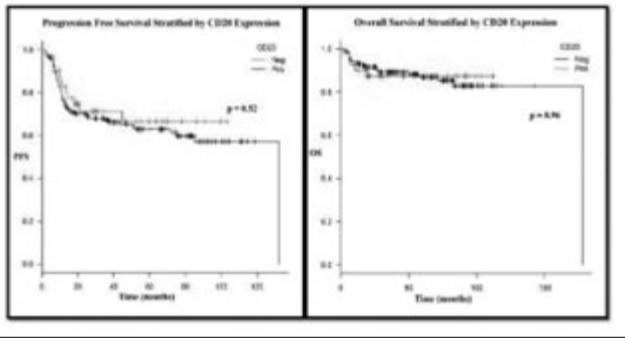


Figure 1.

Summary/Conclusion: We observed a comparable prevalence of CD20 expression in patients from the MENA region with cHL. However, in contrary to reported literature, CD20 expression did not confer an altered prognosis. It is possible that such expression is rendered insignificant in the contemporary era of therapy.

PB2012

CLASSICAL HODGKIN LYMPHOMA TREATMENT PATTERNS, MOLECULAR PROFILING, AND CLINICAL OUTCOMES IN US ONCOLOGY PRACTICES: A PROSPECTIVE OBSERVATIONAL STUDY

C. Yasenchak[1,*], P. Armand[2], S. Larson[3], D. Stevens[4], T.K. Le[5], F. De Solda[5], S. Samakoglu[5], S. Gajavelli[5], J. Svoboda[6]

[1]Willamette Valley Cancer Institute and Research Center/US Oncology Research, Eugene, [2]Dana-Farber Cancer Institute, Boston, [3]University of California, Los Angeles, Los Angeles, [4]Norton Cancer Institute, Louisville, [5]Bristol-Myers Squibb, Princeton, [6]University of Pennsylvania, Philadelphia, United States

Background: Newly diagnosed classical Hodgkin lymphoma (cHL) is highly curable with multi-agent chemotherapy (CTx) +/- radiotherapy. However, standard-of-care treatment (Tx) for relapsed/refractory (R/R) cHL fails in ~50% of patients (pts). Recent clinical trials utilizing novel agents, including the PD-1 inhibitors nivolumab and pembrolizumab and the antibody–drug conjugate brentuximab vedotin (BV), have shown efficacy in R/R cHL. However, real-world clinical practice data describing effictiveness, safety, and pt experiences are limited. Better understanding of tumor biology and pt factors may impact Tx choices in cHL.
Aims: To describe current Tx patterns, evaluate clinical and pt-reported outcomes (PROs), and characterize tumor/pt molecular profiles in cHL pts.
Methods: This is an ongoing, observational, multicenter, prospective study (NCT02856646) involving ~80 US oncology practices with a target enrollment of 500 pts and a planned follow-up of ≤5 y. Eligible pts were ≥18 y with histologically confirmed cHL. At enrollment, pts were Tx naïve or within 2 wk of beginning any line of anticancer therapy. Informed consent was obtained for all pts. Index therapy was defined as therapy received at or within 2 wk of enrollment. Data were collected from existing medical records, by pt interaction using PRO questionnaires (FACT-Lym assessment), and by physician assessment. PRO data were collected every 3 mo during the 1st y of enrollment and every 6 mo thereafter. Blood and tissue samples were collected for biomarker analysis.

Table 1.

Demographic and baseline cHL characteristics

Characteristic	All patients (N=116)
Age, median (range), years	39 (18–74)
Male	68 (59)
Disease stage at initial diagnosis	
I	7 (6)
II	53 (46)
III	32 (28)
IV	19 (16)
cHL risk class	
Early favorable	34 (29)
Early unfavorable	12 (10)
Advanced favorable	18 (16)
Advanced unfavorable	17 (15)
International Prognostic Score	
Albumin <4 g/dL	50 (43)
Hemoglobin <10.5 g/dL	15 (13)
Male	68 (59)
Age ≥45 y	43 (37)
Stage IV disease	19 (16)
Leukocytosis	10 (9)
Lymphocytopenia	7 (6)
ECOG status evaluable at study entry	105 (91)
0	42 (40)
1	50 (48)
≥2	10 (10)
≥1 prior systemic therapies	28 (24)
ABVD	25 (89)
BV	7 (25)
ICE	7 (25)

Data reported as n (%) unless specified otherwise. Unknown or missing data are not presented; therefore, percentages in each category may not sum to 100%
ECOG, Eastern Cooperative Oncology Group

Results: At data cut-off (Sept 2017), 116 pts were enrolled; 59% were male, median (range) age was 39 (18–74) y, and 46% had stage II disease at initial diagnosis (**Table**). At enrollment, 76% were Tx naïve and 24% had received prior therapy; index therapies were immunotherapy (3%) and CTx (94%). The most common index CTx were doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD; 73%); ifosfamide, carboplatin, and etoposide (ICE; 9%); and BV (9%). In total, 78%, 8%, and 3% of index therapies were induction, consolidation/intensification, and maintenance regimens, respectively. Complete response was achieved in 73% of pts who had completed index therapy. Of pts who discontinued index therapy, 33% and 17% discontinued due to relapse/progression and toxicity, respectively. Among pts with Tx-related adverse events (TRAEs), the most common were nausea (41%), fatigue (34%), neutropenia (31%), and constipation (25%). The most common concomitant medications received for TRAEs were ondansetron, filgrastim, and levofloxacin. At baseline, FACT-Lym v4 well-being assessments were available for 115 (99%) pts, (mean) scores were: physical (13.2), social (25.2), emotional (13.3), functional (19.8) and other concerns (25.9). Further PRO analyses will focus on differences between pt groups, stratifying by line of Tx and clinical characteristics over time. Biomarker analyses (9p24.1 amplification, PD-L1 and CD68 expression, Epstein-Barr virus status) for current pts are in process.
Summary/Conclusion: Initial data from the largest prospective observational study in cHL to date suggest that most pts receive multi-agent CTx as 1st-line Tx, consistent with the current Tx paradigm. Effectiveness, safety, and PRO analyses by pt subgroup and Tx line will continue as more pts are

enrolled to identify any tumor biomarkers and pt factors which may correlate with clinical outcomes. Pt recruitment is ongoing.
Study support: BMS.

PB2013

RISK FACTORS FOR SECOND MALIGNANCES IN HODGKIN LYMPHOMA TREATED WITH ABVD: A SINGLE CENTER EXPERIENCE

I. Herraez[1,*], R. Del Campo[1], A. Cladera[1], M. Mascaró[1], J.M. Guerra[1], D. Gómez[1], E. Gónzalez[1], N. Amer[1], J.B. Borrás[1], A. Gutierrez[2], S. Franco[1], J. Bargay[1]

[1]Hematology, Hospital Son Llatzer, [2]Hematology, Hospital Universitario Son Espases, Palma de Mallorca, Spain

Background: Hodgkin lymphoma (HL) is a lymphoproliferative malignancy that represents approximately 10% of all cases of lymphoma. More than 90% of patients with favorable disease and 70-80% of patients with unfavorable disease are cured with an initial therapy that consists, above all, in two to four cicles of Doxorubicin, Bleomicin, Vinblastine and Dacarbazine (ABVD) for favorable disease or four to six cycles of ABVD for unfavorable disease, with or without radiotherapy. Late therapy-related effects in HL survivors include hypothyroidism, fertility issues, secondary cancers and vascular disease. Second malignancy was first recognized as a problem in the early 1970s. Increasing attention has focused on the more common individual tumor types, including lung cancer and breast cancer. The risk of secondary breast cancers is associated with young age in woman (particularly<30 years) and young age at the time of radiation. In the other hand, it is described that lung cancer risk is increased in who received mediastinal radiation and particularly in smokers.
Aims: We aim to evaluate the risk factors that increase the incidence of secondary malignances in patients con HL treated with ABVD +/- radiotherapy.
Methods: We retrospectively selected from the pathology registries of Son Llatzer Hospital (HSLL) patients with HL treated with ABVD +/- RT. Kaplan–Meier was used for time-to-event variables and the log-rank test was used to compare groups as well as Cox Regression for multivariate survival analysis.

Table 1.

	Incidence of 2nd malignacy	p
Age: <14-65 >65	1 (2%) 9 (19%)	0.001
Sex: -Male -Female	7 (15%) 3 (6%)	0.51
B symptoms: <no <sp	6 (14%) 4 (9%)	0.9
Bulky: <no <sp	0 (0%) 10 (13%)	0.99
ECOG: <0-1 <2-4	7 (9%) 3 (15%)	0.12
Smoker: <no <no	2 (4%) 8 (18%)	0.019
IPS3: <0-3 >3	3 (5%) 6 (17%)	0.002
Albumin: <normal <low	2 (6%) 7 (18%)	0.29
Lymphocytes (/mm3 or %): <0-1000 >1010	7 (26%) 3 (5%)	0.008
BT: <no <sp	1 (5%) 9 (12%)	0.68
Hemoglobin (g/dl): >10.5 <10.5	4 (6%) 6 (19%)	0.01
AA stage: <II III-IV	1 (2%) 9 (17%)	0.039
Number of treatments: <1 <More than 1	9 (11%) 1 (10%)	1

Table 1

Results: From 2001 to 2017, 94 patients fulfilled inclusion criteria. Median age was 42 years (14-84), 41% with >45 years, 58% males, 53% AA (Ann Arbor stage II-IV), 13% with ECOG performance status >1, 20% with IPS >3, 13% with low lymphocytes (<8% or<600/mm^3. Response was as follows: 77 (82%) achieved complete response (CR), 5 (5%) partial response and 8 (8%) progression. 86% of global survival at 5 years and 82% of progression free survival at 5 years. With a median follow-up of 65 months, 10 second cancers and one concomitant malignancy were diagnosed in 94 patients: 7 respiratory tract malignancies, 2 gynecological cancers and 1 digestive tract cancer. The concomitant cancer was a breast cancer. Different factors at the time of diagnosis were associated in our patients with an increased incidence of secondary malignances. Univariate analysis showed that age >45 years, being smoker, IPS>3, low lymphocytes and advanced stage (III-IV) increased significantly the risk of secondary neoplasms (Table I). Multivariate analysis showed that only having >45 years (relative risk 14; 95 percent confidence interval, 1.6-125.9), lymphocytes <1010/mm^3 (relative risk 6.4; 95 percent confidence interval, 1.3-31.7) and being smoker (relative risk 4.1; 95 percent confidence interval, 0.7-24.2) retained independent significance. The number of cycles of ABVD received and the radiotherapy were not related with an increased incidence of secondary malignancies.
Summary/Conclusion: Patients with risk factors at diagnosis such as: smoking history, low lymphocytes and<45 years had an increased incidence of secondary neoplasms during the next years after ABVD. We conclude that it is important to emphasize, in this type of patients, the establishment of optimal prophylactic and screening strategies.

PB2014

PD-1 INHIBITORS IN THE TREATMENT OF RELAPSED AND REFRACTORY CLASSICAL HODGKIN'S LYMPHOMA

M. Kichigina*, G. Tumyan, E. Demina, Z. Pervin, I. Monin, O. Yakimovich, D. Tupitsina, Z. Shikhabova

Hematology, National Medical Research center of oncology named after N.N. Blochina, Moscow, Russian Federation

Background: The main problems in the modern treatment of classical Hodgkin's lymphoma (cHL) are patients with refractory disease and relapses after ASCT. The most promising for this category of patients seems to be the use of a new class of monoclonal antibodies-PD-1 (nivolumab, pembrolizumab)/ They block the interaction between the receptor of PD-1 on activated T-lymphocytes and its ligands (PD-L1, PD-L2) on tumor cells, simulate the antitumor immune response.
Aims: evaluate the effectiveness and safety of therapy of a new class of monoclonal antibodies-PD-1 (nivolumab).
Methods: According to the NPP program" in N.N. Blokhin National Cancer Research Center 12.2015 to 09.2017 treatment with Nivolumab was given to 18 patients with relapses\refractory cHL. The median ages are 35 years. The median line of the therapy before the nivolumab was 4 (2-8): 13 (72%) patients underwent ASCT, 4 were not candidates for autograft, 3 received treatment brentuximab vedotin (1 with autograft, 2-only BV). ECOG was predominantly 2, in 3 patients-3. Nivolumab was administered at a dose of 3 mg/kg every 2 weeks before the progression or realization of adverse events (AEs). The median number of treatment courses is 25 (1-44). The effect of therapy was assessed in 17 patients, 1 patient was excluded from the evaluation due to the development of serious AE after the first administration.
Results: Treatment was effective in 53% of patients (9 patients), of whom full remissions (PR) were achieved in 29% (5 patients). Stabilization with a median duration of the effect of 15 months was found in 7, and in 1 patient-progression after 9 injections of nivolumab. By 09.2017 16 out of 17 patients are alive, in whom the effect is estimated, 9 of them remain without progression, including all 5 patients with PR. From the progression of LH died 1 patient. With median follow-up of 18 months (from 1 to 27 months), the median survival to progression was 16 months, overall survival was 94%. It should be noted that all but the patient with progression and the patient withdrawn from treatment in connection with the development of AE, noted a significant clinical improvement and an increase in general status to ECOG 0-1. Of the complications of treatment, Herpes zoster (1), community-acquired pneumonia (3), transient elevation of transaminase levels>4 norms (1) were noted. One patient had a serious immune-mediated reaction (demyelination) after the first administration of nivolumab, which caused the drug to be discontinued.
Summary/Conclusion: Immunotherapy with PD-1 inhibitors (nivolumab) is highly effective in relapses and refractory cHL, it has moderate toxicity, however, the possibility of developing immune-mediated reactions requires further accumulation of material and additional attention of the physician.

PB2015

A CLINICAL CASE OF IMMUNE THROMBOCYTOPENIA ASSOCIATED WITH PD-1 INHIBITOR (NIVOLUMAB) TREATMENT IN A PATIENT WITH RECURRING HODGKIN'S LYMPHOMA

M. Platonov[1,*], S. Moiseev[1], N. Michailova[2]

[1]Hematology, ARCERM EMERCOM of Russia, [2]Hematology, Raisa Gorbacheva Memorial Institute of Children's Hematology and Transplantation, Sankt-Petersburg, Russian Federation

Background: PD-1 ligand inhibitors are widely used nowadays for treating melanoma, non-small cells lung cancer, head and neck cancer, Hodgkin's

lymphoma. Severe hematological side effects of treatment with PD-1 inhibitors (Nivolumab, Pembrolizumab) have only been described in the form of third or fourth degree neutropenia. No cases of isolated thrombocytopenia in connection with PD-1 inhibitor therapy have been reported before. Presented here is the clinical case of a Hodgkin's lymphoma patient successfully treated with Nivolumab while having developed isolated reversible immune thrombocytopenia.
Aims: The case traces the dynamics of severe immune thrombocytopenia in a female patient with Hodgkin's lymphoma who, despite having developed fourth degree thrombocytopenia, was continually treated with Nivolumab undergoing dose adjustment and Cyclosporine therapy.
Methods: Blood cells count assessment was used to evaluate a single patient's clinical case within a sixteen-month period of consecutive Nivolumab treatment.
Results: A thirty-eight year-old female diagnosed with stage IIB chemoresistant Hodgkin's lymphoma received PD-1 inhibitor Nivolumab starting September 2016 as immune therapy for her primary condition, at a dose of 3 mg/kg every two weeks. In January 2017, fourth degree thrombocytopenia at count of $3x10^9$/l was detected after 10^{th} injection, accompanied by petechial skin rash on lower extremities. No anemia or leukopenia was found. Prednisolone pulse therapy with continued administration at a dose of 2 mg/kg per day had no effect within a month. After that, the therapy was supplemented with Cyclosporine at a dose of 3 mg/kg per day, which, at the end of March 2017, was followed by the Nivolumab injection renewal at a dose of 1 mg/kg at the same interval. Cyclosporine was used as an immune suppressor with ability to prevent cytokine gene transcription in activated T-cells. Further on, the patient demonstrated thrombocyte count increasing to normal and subnormal levels despite continuous treatment with Nivolumab. By the end of September 2017, baseline thrombocyte value was restored, and in accordance with that, the Cyclosporine dose was gradually reduced to a total withdrawal and the Nivolumab dose elevated up to 2 mg/kg per day. To this date (January 22, 2018) the patient remains in full PET-negative remission of Hodgkin's lymphoma. Her thrombocyte count is $167x10^9$/l.
Summary/Conclusion: From that clinical case of Hodgkin's lymphoma patient, we saw that isolated immune thrombocytopenia could not be a sole reason to stop PD-1 inhibitor therapy if adequate measures to mitigate autoimmune activity were taken. Addition of Cyclosporine for our patient suppressed activated T-lymphocytes without reducing the clinical effect of Nivolumab therapy.

PB2016

REAL WORLD DATA ON THE EFFICACY AND SAFETY OF BRENTUXIMAB VEDOTIN IN RELAPSED/REFRACTORY HODGIN LYMPHOMA IN SLOVAKIA

M. Ladicka[1,*], L. Sopko[2], J. Chudej[3]

[1]Hematooncology, National Cancer Institute, [2]Hematology, University Hospital, Bratislava, [3]Hematology, University Hospital, Martin, Slovakia

Background: Brentuximab vedotin (BV) is an antibody-drug conjugate (ADC) that selectively delivers monomethyl auristatin E, an antimicrotubule agent, into CD30-expressing cells. A phase I and II studies with BV demonstrated significant activity with favourable safety profile in patients with relapsed and refractory (R/R) CD30 positive lymphomas.
Aims: To evaluate epidemiological data and the impact of BV on survival in R/R Hodgkin lymphoma (HL) patients.
Methods: This is a retrospective analysis of 51 patients with R/R HL treated at three Slovak centers from 1/2014 to 12/2016. Fifty one patients received BV treatment in the period from 07/2012 to 02/2017. Bretuximab vedotin was administrated intravenously in the dose of 1.8 mg/kg every 3 weeks.
Results: In brief, the median age at the time of diagnosis was 33 years. In addition, the median age of participants at the time of initiation of our study was 38.8 years. Median time from the first-line therapy to BV treatment was 28 months. In total, 35 patients (70.0%) had R/R HL within 1 year after the end of last treatment. Based on clinical judgment, the treating physician categorized participants as eligible (n=39) or ineligible (n=11) for autologous stem cell transplantation (ASCT). The median number of previous lines of the therapy was 5 in ASCT-eligible patients and 3 in ASCT-ineligible patients. The progression free survival (PFS) was 7.0 months for 32 patients who did not undergo SCT. In patients (n=8) who achieved complete (CR) or partial remission (PR), the PFS was 34 months. On the other hand, 24 patients with stable disease (SD) or disease progression had PFS only 4.9 months. The median PFS for patients (n=13) treated with BV therapy and immediately followed by allogeneic stem cell transplant (aloSCT) has not been reached. In patients (n=5) who had undergone chemotherapy followed by aloSCT, PFS was only 5.8 months (Figure 1). We have identified 29 patients who underwent ASCT with at least one risk factor for relapse or disease progression. The most common risk factor was early progression after first-line treatment. The patients underwent BV treatment with a median of 8 cycles, and 4 patients (8%) completed 16 cycles of the treatment. BV dose was reduced in 16% of patients; most common reasons were anemia, neutropenia and thrombocytopenia existing prior to initiating BV treatment, toxicity during the treatment was reason for reduction only in one patient.

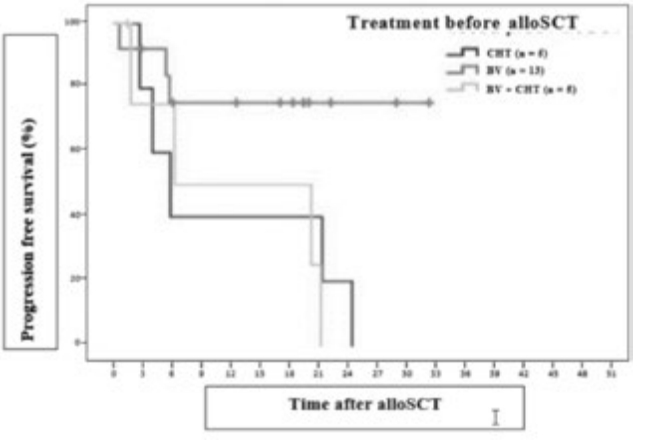


Figure 1.

Summary/Conclusion: Our study included patients in second or further line of therapy. Overall, 70% of patients had documented R/R HL within 1 year after the end of last treatment. No severe toxicity related to BV regimen was observed. Improved outcomes were observed in patients who achieved a complete remission on BV. BV use pre-allogeneic SCT is associated with a rapid response in a majority of patients with R/R HL compared with standard chemotherapy. Therefore, BV may represent also an optimal therapeutic option as a bridge to aloSCT in R/R HL patients. Our analysis confirmed BV efficacy in heavily pretreated R/R HL patients in real clinical practice.

PB2017

EFFECT OF THE INITIAL TREATMENT ON THE MUSCLE MASS AND RESPONSE TO THERAPY IN HODGKIN'S LYMPHOMA

S. Kucukyurt Kaya[1,*], B. Bilgin[2], M. Metin Rusen[3], A. Yikilmaz Senturk[1], S. Dogan[1], M. Gunduz[4], S. Akinci[4], S.M. Bakanay[1], I. Dilek[1]

[1]Department of Hematology, [2]Department of Oncology, Ankara Yildirim Beyazit University, [3]Department of Radiology, [4]Department of Hematology, Ankara Ataturk Training and Research Hospital, Ankara, Turkey

Background: Loss of muscle mass has been reported to be an independent predictor of clinical outcome in multiple gastrointestinal cancers.
Aims: The decrease in the muscle mass may have an effect on the response to treatment in Hodgkin's lymphoma(HL). We aimed to investigate the effect of chemotherapy on muscle mass in HL patients.
Methods: This study included 37 newly diagnosed HL patients who were referred to our clinic between 2010 and 2017. Patients were diagnosed after lymph node biopsy and initial evaluation was done by either PET-CT or computarized tomography (CT) and were staged according to Ann-Arbor Staging System and Cotswolds modification. Body surface areas were calculated. All patients received Adriamycin 25 mg/m2, Bleomycin 10 mg/m2, Vinblastine 6 mg/m2, Dacarbazine 375 mg/m2 (ABVD) chemotherapy administered intravenously on Day 1 and Day 15. Following 4 courses of the ABVD, an interim evaluation was carried out with CT. The computed tomographic scans of the patients before the treatment and after 4 courses of ABVD were available in the Picture Archive and Communication System (PACS) of the hospital. The L3 vertebra was identified on the axial CT and the posterior paravertebral muscle was manually contoured bilaterally to determine the skeletal muscle index. L3 skeletal muscle index and fat-free mass were calculated. The decrease in the muscle mass was defined by the presence of the posterior paravertebral area less than the median of the cohort.
Results: The median age of the patients was 41 (19-76) and Male/Female =26/11. There were 18 early-stage (stage I-II) and 19 advanced-stage (stage III-IV) patients. Partial and complete responses were observed in 24 and 10 patients, respectively. Three patients progressed despite treatment. When the treatment-based change of the paravertebral muscle area was examined, there

was a statistically significant decrease in muscle area after 4 courses of treatment in both early and advanced stage disease (Table 1). There was no statistically significant difference between the early and advanced stage patients in terms of the pre-treatment paravertebral muscle area (p=0.46). When the subgroups were examined in terms of response to the treatment, there was no statistically significant difference in the basal muscle measurements of the patients with complete and partial responses and progression (p=0.44). However, a statistically significant decrease was determined in the paravertebral muscle area following the chemotherapy in patients with complete and partial response to the treatment; while there was a distinct but statistically insignificant decrease in the muscle mass in patients with progression (p values 0.028, 0.002 and 0.18, respectively). The ratio of decrease in the paravertebral muscle area are shown in Figure 1.

Table 1.

Table 1: Difference in paravertebral muscle area with 4 cycle chemotherapy

	Pre-treatment Paravertebral Muscle Area (cm^2)	Post-treatment Paravertebral Muscle Area (cm^2)	P
Entire Group	48.38	46.92	< 0.001
Early Stage (I-II)	49.55	48.08	0.002
Advance Stage (III-IV)	46.51	43.74	0.011

Summary/Conclusion: There was a significant decrease in the muscle mass after the chemotherapy for HL. However, more data is required to determine its association with response to treatment.

PB2018

HODGKIN LYMPHOMA: THE MOFFITT CANCER CENTER EXPERIENCE

A. Rose[1,*], A. Lim[1], A. Tungesvik[1], L. Isenalumhe[2], L. Sokol[2]

[1]Internal Medicine, University of South Florida, [2]Hematology/Oncology, H. Lee Moffitt Cancer Center, Tampa, United States

Background: Hodgkin lymphoma (HL) is a clonal disease of B-cell origin comprising about 10% of all malignant lymphomas. Classical HL (cHL) includes 4 histological subtypes: lymphocyte rich, nodular sclerosis, mixed cellularity and lymphocyte depleted. Nodular lymphocyte predominant (NLPHL) type is currently a separate entity. Greater than 75% of patients (pts) are cured with current treatment modalities. Prognosis is based on clinical stage, presence of systemic symptoms, presence of bulky disease and treatment rendered.
Aims: In this large single institution database of HL pts, we analyzed demographics, prognostic features, and survival over a 27 year period.
Methods: We evaluated 761 pts with Hodgkin Lymphoma diagnosed between 1990-2017 at Moffitt Cancer Center in this retrospective study. We estimated overall survival (OS) and various clinical and laboratory factors with a potential impact on survival by using the Kaplan Meier curve as well as Cox Proportional Hazards regression. The relationship between clinical features and prognosis was tested using frequencies and either the Chi-square test, Fisher's Exact test or the Cochran-Armitage trend.
Results: Among all patients, 55.3% were male and the majority were Caucasians (79.2%). Mean age at diagnosis was 38.5 years. 60% of pts were<39 years of age, 31% were 40-64 years old and 9% were 65 and older. Histological subtypes include nodular sclerosis 72.2%, mixed cellularity 19%, lymphocyte rich 3%, lymphocyte depleted 0.7%, and nodular lymphocyte predominant 5.1%. Stage at diagnosis; I-6.9%, II- 47.5%, III 22.7%, IV 22.9%. B-symptoms were present in 50.4% of pts, and 22.6% had bulky disease. 67.2% of pts with advanced stage disease (≥stage III) presented with B symptoms compared to 36.6% with early stage disease (≤stage II). Bulky disease revealed no correlation with the stage. There was a significant difference in mean age at diagnosis according histological subtypes; with nodular sclerosis more prominent at younger ages (35.7yrs) compared to lymphocyte depleted (50yrs) (p-value<0.05). The mean time of follow-up was 7.2 years and median survival was 17.8 years with a 5-year OS of 83.1% (CI: 80, 85.6%) and 10-year OS of 70.9% (CI: 66.4, 74.8%). Survival was significantly different among the age cohorts<39yrs, 40-65 and >65 years with 10-year OS survival of 73.4%, 71.7% and 47.3% respectively (p-value<0.001). Survival was longer in pts with NLPHL histology in comparison to the cHL subtypes. NLPHL had a median survival of 37.4 years with a 5-year OS of 96.9% (CI: 79.8, 99.6%) compared to cHL histology with a median survival of 16.8 years and a 5-year OS of 82.8% (CI: 79.4% *vs* 85.9%).
Summary/Conclusion: Our data suggests that pts with Hodgkin's lymphoma have very good long-term outcomes. Significant indicators of poor prognosis include classical histology type, advanced stage at diagnosis, presence of B symptoms, and advanced age. Outcomes based on treatment regimen are in progress.

PB2019

HODGKIN LYMPHOMA OF THE GASTROINTESTINAL TRACT IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

M. Barzilai[1,*], I. Avivi[1], Y. Herishanu[1], C. Perry[1], R. Ram[1], Y. Ron[2], N. Sarid[1]

[1]Hematology, [2]Gastroenterology, Sourasky medical center, Tel Aviv, Israel

Background: Patients with inflammatory bowel disease (IBD) on immunosuppression are at risk of developing lymphoma, particularly primary gastrointestinal (GI) Non-Hodgkin lymphoma. Primary GI Hodgkin lymphoma (HL) in this setting is a rare and poorly defined clinical entity.
Aims: We report on a patient with Crohn disease (CD) who developed GI HL and review the available literature.
Methods: An electronic search of Medline, updated to December 2017, was performed .Each paper was reviewed and duplicate reports describing the same patients were included just once.
Results: Ten single-case studies and 6 case-series, published between 1978-2016, involving 23 patients ,were identified. Twenty-one (91%) patients had CD, whilst two others had ulcerative colitis. Eighteen (79%) patients were male, with a median age of 39 at lymphoma diagnosis. Diagnosis of HL occurred at a median of 8 and 4.4 years after the detection of IBD and commencing immunosuppression, respectively. HL had a predilection (80%) to involve the inflamed GI site. The histological subtype was mixed cellularity in 65% of cases and in-situ hybridization for EBV-encoded RNA, when documented, was positive in all cases. Thirty-five, 20%, 5% and 40% of patients were diagnosed in stage 1 to 4, respectively. Interestingly, 66% of patients with advanced disease had liver involvement. Immunosuppression was stopped in most (70%) patients upon lymphoma diagnosis. HL treatment consisted of chemotherapy only, surgery followed by chemotherapy or surgery only in 50%, 33% and 16% of cases, respectively. Four patients had IBD flare during HL remission.
Summary/Conclusion: Patients with IBD who develop HL with GI involvement have distinct characteristics: male predominance, a predilection for inflamed GI sites, mixed cellularity histological subtype, EBV positivity, and a unique pattern of spread to the liver.

PB2020

LONG-TERM FOLLOW-UP OF 2 PATIENTS TREATED WITH 90Y-RITUXIMAB RADIOIMMUNOTHERAPY FOR RELAPSE OF PREDOMINANTLY LYMPHOCYTIC NODULAR HODGKIN'S LYMPHOMA

S. Golstein[1,*], K. Muylle[2], M. Vaes[1], M. Vercruyssen[1], C. Spilleboudt[1], A. de Wind[3], D. Bron[1]

[1]Hematology, [2]Nuclear Medicine, [3]Anatomo-Pathology, Institut Jules Bordet, ULB, Brussels, Belgium

Background: Predominant lymphocytic nodular Hodgkin's lymphoma (NLPHL) is a rare lymphoma.
Aims: Life expectancy being more than 10 years, short term and long term life-threatening toxicities should be avoided. However, there are no evidence-based guidelines for initial treatment or relapse.
Methods: Two patients relapsing 12 and 17 months after initial treatment with rituximab were included in a prospective phase II trial of radioimmunotherapy (RIT). Yttrium 90 was coupled to rituximab (^{90}Y-rituximab) and administered according to the following protocol : a first infusion of rituximab 250mg/m^2, repeated one week later and directly followed by the injection of ^{90}Y-rituximab (14,8 MBq/kg). The rational of using unlabeled ("cold") antibodies before injecting ^{90}Y-rituximab is to reduce tissue toxicity of RIT by providing a more favorable biodistribution profile of the radiolabeled antibody (Vaes M. *et al.* J Cancer Sci Ther 2012).
Results: Case # 1: A 27 years old Mediterranean man noticed in April 2008 a bulky right axillary mass, CS IA.A biopsy was performed and the diagnosis of NLPHL, CD20+ was established. The first line treatment consisted in radiation therapy (RT) and 4 weekly administrations of Rituximab. The ^{18}FDG-PET/CT performed 6 months after the treatment showed a complete metabolic response but after 18 months a moderate hypermetabolic activity in infracarinal lymphadenopathies and beside the spleen is described. In June 2010, the relapse is confirmed showing lymph node involvement in the right pelvic region, the splenic hilus and the left axillar region. The patient accepted to be enrolled in a RIT protocol in October 2010. This treatment induced a complete remission which is still ongoing after 7 years. No adverse events were reported. **Case #2:** A 38-year-old Caucasian man

presented with small bilateral cervical and supraclavicular lymphadenopathies. A biopsy was performed and showed a NLPHL (CD30 +, CD20 +) stage IIIA. ^{18}FDG-PET/CT confirmed cervical but also juxta-centimetric retroperitoneal lymph nodes and rituximab treatment (4 weekly courses) was started in August 2007. Response assessment by^{18}FDG-PET/CT, performed 4 months after the end of the treatment, showed a complete metabolic response. Eleven months after the end of treatment, ^{18}FDG-PET/CT revealed a 2cm diameter retroperitoneal lymph node which increased to 5 cm in diameter at 21 months concomitantly with hypermetabolic retroperitoneal lymph nodes. RIT was started in September 2009, according to our study protocol. No side effects were reported during and after treatment. Six months after RIT, ^{18}FDG-PET/CT showed a complete metabolic remission, persisting until today, 8 years later.
Summary/Conclusion: There are no prospective randomized studies to guide the therapy in NLPHL. Radiotherapy plays an essential role in early stages and chemotherapy combined with anti-CD20 antibody is very effective in advanced diseases. We opted for salvage treatment with RIT to treat two young patients with early relapsed NLPHL. A complete response was maintained for 7+ years compared to less than 2 years after rituximab alone. To our knowledge, RIT has never been reported for this indication.

PB2021

SUCCESSFUL TREATMENT WITH NIVOLUMAB OF HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS SECONDARY TO REFRACTORY HODGKIN LYMPHOMA. A CASE REPORT

C. Cantò*, E. Finolezzi, S. Luciani, E. Ranucci, F. Angrilli

Dipartimento di Ematologia, Biotecnologie e Medicina Trasfusionale, PO "Spirito Santo", Pescara, Italy

Background: Hemophagocytic lymphohistiocytosis (HLH) is an infrequent but potentially life-threatening hyperinflammatory syndrome that occurs in about 6% of patients affected by Hodgkin Lymphoma. Treatment of secondary HLH includes corticosteroids, cyclosporine A, etoposide and/ or malignancy-directed therapies. Anecdotal cases report successful results with Alemtuzumab, Infliximab, Daclizumab, Blinatumomab and Ruxolitinib.
Aims: We describe a case of HLH complicating refractory classical Hodgkin lymphoma (cHL) that resolved only after treatment with Nivolumab. At our knowledge, this is the first case reported.
Methods: A 21-year-old man was diagnosed with cHL, CS IIIB, in September 2014. He was refractory to five lines of treatment (ABVD, IGEV, Brentuximab Vedotin, Bendamustine and Beacopp). In April 2016 he developed fever, marked splenomegaly and grade 4 pancytopenia. Other laboratory findings showed hypoalbuminemia, hypertransaminasemia and hyperbilirubinemia; antibody tests for HCV, HBV, HIV, HSV, CMV and EBV infections were negative. The bone marrow biopsy showed the presence of numerous macrophages embedding hematopoietic elements, without lymphoma infiltration. Consequently, HLH was diagnosed. Treatment with corticosteroids and etoposide was ineffective. In May 2016 he started treatment with Nivolumab (in the setting of the Italian Extented Access Program) at the dose of 3 mg/kg every 14 days.
Results: Until Nivolumab treatment was started the patient required transfusion support with 14 PRBC Units and 19 Plt Units. After the first administration of Nivolumab he developed an episode of respiratory insufficiency infusion related that resolved with mild corticosteroid therapy. The patient became transfusion independent after the third dose of Nivolumab. After 32 doses of Nivolumab, in November 2017 treatment was stopped because of a severe pancreatitis treatment related. Unfortunately in February 2018 the patient died in complete remission in a road accident.
Summary/Conclusion: HLH is a potentially fatal hyperinflammatory disease induced by a pathologic immune activation, with proliferation of well-differentiated macrophages/histiocytes and increased phagocytic activity. The tumor in cHL is mainly composed of inflammatory cells providing a protective niche to the sparse CD30-positive malignant HRS cells. Intriguingly, an increased number of tumor-associated macrophages (TAM) was strongly associated with treatment failure and shortened survival in patients with cHL. TAM can express PD-1 and this suggests that anti-PD-1 therapies may also function through a direct effect on macrophages. Left untreated, the prognosis of HLH is poor and generally fatal. Therefore, prompt recognition and timely treatment are critical. Usually, in patients who present with secondary HLH, treatment of the underlying cause can lead to control and resolution of HLH. At the onset of HLH, our patient had a chemorefractory cHL and conventional treatment of HLH was totally ineffective. The anti-PD1 antibody nivolumab induce a high overall response rate in relapsed/ refractory cHL. In our patient nivolumab induced a prompt control of HLH and a subsequent complete remission of cHL. More knowledge is warranted about interactions between macrophages and PD-1/PDL-1 inhibitors.

PB2022

THE PERIPHERAL BLOOD ILC, AND TH-17 RELATED CYTOKINE PRODUCTION ARE IMPAIRED IN A CHILD WITH A NOVEL MUTATION IN ITK WHO PRESENTED WITH RELAPSED HODGKIN LYMPHOMA

M. Cansever[1], A. Eken[2], I. Somekh[3], N. Zietara[3], A. Ozcan[4], M. Karakukcu[5], M. Rohlfs[3], E. Unal[4], C. Klein[3], T. Patiroglu[4,*]

[1]Department of Pediatrics, Division of Pediatric Immunology, [2]Department of Medical Biology, Erciyes University, Faculty of Medicine, Kayseri, Turkey, [3]Dr von Hauner Children's Hospital, University Hospital, Ludwig Maximilians University, Munich, Germany, [4]Erciyes University, Faculty of Medicine, [5]Erciyes University, Faculty of Medicine,, Department of Pediatrics, Division of Pediatric Hematology-Oncology, Kayseri, Turkey

Background: Common immunological features in patients with interleukin-2-inducible T-cell kinase (ITK) deficiency include: CD4 + T cell loss along with absolute lymphopenia and progressive hypogammaglobulinemia. *iNKT* cells are severely reduced in the peripheral blood of ITK deficient patients; hence a critical role has been postulated for *iNKT* cells in the response to EBV infection.
Aims: Herein we report a child with a novel ITK mutation who showed impaired peripheral blood innate lymphoid cells, and Th-17 related IL-17A, IL-22, and GM-CSF cytokine production.
Methods: A Previously healthy (5 year old ?) patient, from a consanguineous family was admitted to the hospital with complaints of unilateral swelling on of the neck. Biopsy was compatible with Hodgkin lymphoma and she was treated with ABVD/COPP protocol, but she experienced an early relapse just one year after completing chemotherapy. ICE regimen followed by autologous transplantation was performed. After transplantation she had hypogammaglobulinemia and persistent and fatal EBV infection. Next generation sequencing returned a homozygous frame-shift variant in ITK (LRG_189t1: c.328delA: p. Thr110Argfs Ter1551.) The variant was confirmed by Sanger sequencing and segregated with the disease. This variant has not been previously described. but variants in ITK are described to cause lymphoproliferative syndrome with splenomegaly and hepatomegaly, as well as anemia, thrombocytopenia and pancytopenia. We report impaired CD3/CD28 induced proliferation by T cells. Itk mutant cells were more apoptotic without or upon TCR activation. Additionally, T cells produced less IL-17A, IL-22, and GM-CSF. Conversely, IFN-γ production was increased. Lastly, we analyzed peripheral ILC populations and observed reduced ILC3.
Results: Although it has previously been reported that TH-17 cytokine levels are impaired in ITK deficient mice, we report for the first time that peripheral blood innate lymphoid cells, and Th-17 related cytokine production are impaired in a child with a novel ITK mutation.

PB2023

THE PROGNOSTIC SIGNIFICANCE OF EXTRANODAL DISEASE IN HODGKIN'S LYMPHOMA

B. Sidi Mohamed El Amine*, H. Asma, O. Fouzia, S.A. Najet, B. Soraya, Z. Zahia

Universitary hospital of Sidi Bel Abbés, Sidi Bel Abbes, Algeria

Background: Hodgkin's lymphoma (HL) is a highly curable disease. Usually confined to the lymph nodes, extranodal involvement can occur and has an independent prognosis value.
Aims: To analyze the presenting features and the prognostic significance of extranodal disease in HL.
Methods: We performed a retrospective single institution study of 155 HL cases; from January 1992 to December 2010. The median age was 27 years (range, 15-80 years), Stages III-IV were present in 85 (54.8%) patients. Combined radio-chemotherapy was administered to 95 (61, 2%) patients and chemotherapy alone to 60 (38.8%). We analyzed the prognostic relevance of extranodal involvements and their significance was tested according to response rate an overall survival (OS).
Results: Extranodal disease was documented in 52 patients (33.5%) and 49 (44%) had bulky disease. Extranodal sites included the liver in 21 (13.5%), bone marrow in 11 (7%), pleura in 5 (3%), pharynx in 5 (3%), bone in 10 (6%), lung in 4 (1, 7%) and eye in 1 (0, 6%). The 52 patients with extranodal disease had poor prognosis compared with the nodal group (5 year OS, 51% *versus* 75%; p<0.001). Compared with the nodal subset, the extranodal patients presented more frequently with advanced stage disease (92% *vs* 08%; p<0.0001), B symptoms (90% *vs* 10%; p=0.002), a significantly low serum albumin (63.4% *vs* 24%; p<0.001) and a higher ESR (77% *vs* 23% p=0.005). Complete remission rates in the extranodal and the nodal subsets of patients were 65% *vs* 84% (p<0.001).

Summary/Conclusion: In our study, extranodal disease in patients with HL is frequent, especially in advanced stages, associated with a poor outcome and might be eligible for more effective treatment approach.

PB2024

A FAMILIAL REPORT OF MIXED CELLULARITY HODGKIN'S DISEASE(HD): THE SAME SUBTYPE HD, EBV NEGATIVE, WITH DIFFERENT OUTCOME

M. Moschovi*, A. Lagos, C. Tsipou, N. Tourkantoni

Hematology-Oncology Unit, [1]st Department of Pediatrics, National and Kapodistrian University of Athens, Athens, Greece

Background: Mixed cellularity Hodgkin's disease(HD) is a subtype of classic HD constituting the second most common type and 15-30% of all cases of HD. It is mostly observed in patients with either Epstein-Barr virus (EBV) or Human Immunodeficiency Virus (HIV) infection, having less inheritance rate comparing to other subtypes.
Aims: Presentation of two identical cases among siblings with different outcome.
Methods: Two siblings, a girl and a boy, were presented with mixed cellularity Hodgkin disease. Both, had the same histological subtype, the same stage(IIIs), the same location of the disease and both, at the biopsy specimen, where negative for EBV infection. The only apparent difference between the two cases was the presenting age. The girl was diagnosed at the age of 15 years old and the boy at the age of 25 years old. Both of them received chemotherapy. The girl achieved remission with chemotherapy only. The boy received initially only chemotherapy. Due to the relapsed disease during chemotherapy, radiotherapy was added but he succumbed from refractory disease.
Results: The two cases were siblings. They had the same histological subtype of the disease, the same location, different age of onset but eventually different outcome. The girl received chemotherapy according to the SIOP HD protocol, and till now she is in first remission, 10 years after the diagnosis. By contrast, the boy had a poor outcome.
Summary/Conclusion: Considering that both cases where identical on their anatomical and histological characteristics and also both of them where negative for EBV infection, we conclude that the different age of onset of this subtype of HD, and the different gender, play a crucial role on the outcome. Local genetic predisposing factors may contributed to these familial HD cases.

Indolent Non-Hodgkin lymphoma – Clinical

PB2025

RISK FOR REACTIVATION OF HEPATITIS B INFECTION-IMPROVING TESTING PRE-IMMUNOCHEMOTHERAPY

C.L. Neoh*, M. Rogers

Haematology, Royal Surrey County Hospital, Guildford, United Kingdom

Background: Immunochemotherapy is an important treatment modality in haematological malignancies and hepatitis B virus (HBV) reactivation is known to occur following this. Although it is more commonly seen in patients with positive hepatitis B surface antigen (HBsAg), it can also occur in patients who are negative HBsAg but have detectable anti-HBV core antibody (anti-HBcAb), indicating past 'resolved' infection. Hence, patients should be screened for both HBsAg and anti-HBcAb prior to commencing immunochemotherapy that are known to increase risk for HBV reactivation. Patients with positive anti-HBcAb can often still be treated with immunochemotherapy but require antiviral prophylaxis, joint-care with a gastroenterologist and close monitoring of viral blood tests. An audit was initially conducted to assess the rates of HBV screening among patients being commenced on rituximab-containing regimens in the haematology department at Royal Surrey County Hospital (RSCH). This audit demonstrated that significant numbers of patients were not being screened fully and remedial measures were put in place. Following that, a re-audit was conducted to assess compliance rates post-intervention. Some studies have also shown HBV reactivation during treatments with ibrutinib, lenalidomide and obinituzumab, therefore we have included this cohort of patients in the re-audit. This re-audit showed that haemato-oncologists' practice on appropriate HBV screening can be improved to minimise risk of HBV reactivation.
Aims: To assess compliance with full HBV screening (HBsAg and anti-HBcAb) in patients receiving rituximab, ibrutinib, lenalidomide or obinutuzumab containing regimens at Royal Surrey County Hospital following remedial measures after the previous audit.
Methods: Patients who had been commenced on regimens including rituximab, ibrutinib, lenalidomide or obinutuzumab under the haemato-oncology unit were identified from the ARIA chemotherapy e-prescribing system. This included patients on regimens commencing 01/01/2017 to 24/11/2017. Winpath system was used to check for HBV testing over the last 10 years.
Results: 64 patients were identified; of which 54 patients on rituximab-containing regimens, 6 on ibrutinib, 3 on lenalidomide and 1 on obinutuzumab. This re-audit has clearly shown that there has been an improvement in rates of full HBV testing prior to treatment with immunochemotherapy. This was evidenced by an improvement from 7% to 56%. However, there were still a quarter of patients who did not have any HBV testing at all (only showing a modest improvement from 29% to 25%).

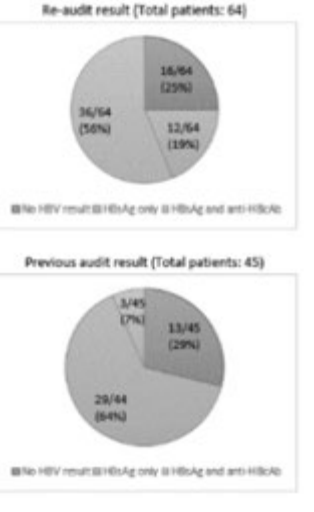


Figure 1.

Summary/Conclusion: This result has clearly shown that there is a need to assess haemato-oncologists' practice in ensuring a full HBV testing is done prior to commencing immunochemotherapy agents which are recognised as risks for HBV reactivation. Appropriate remedial measures have shown

to improve compliance rates. In our cohort, of all the patients who had a complete screening of both HBsAg and anti-HBcAb, 2 were found to be positive anti-HBcAb and received appropriate viral prophylaxis and regular HBV DNA viral load monitoring. This highlighted the importance of a proper screening prior to subjecting the patients to reactivation risks. In conclusion, a significant improvement in HBV testing rates has been achieved. However, ongoing efforts are required to bring about further improvement to ensure patient risks are minimised.

PB2026

CASTLEMAN DISEASE WITH THE EXPERIENCE OVER 17 YEARS

F.D. Atilla[1], N. Soyer[2], M. Tombuloglu[1], F. Vural[2], F. Sahin[2], M. Tobu[2], D. Demir[3], N. Ozsan[3], M. Hekimgil[3], G. Saydam[1,*]

[1]Hematology, [2]EGE University, Izmir, Turkey, [3]Pathology, Ege University, Izmir, Turkey

Background: Castleman disease (CD) is a non-clonal lymphoproliferative disorder as a common cause of non-neoplastic lymphadenopathy. CD encompasses several distinct clinicopathological disorders at the intersection of haematology, immunology, oncology, rheumatology and virology that share a spectrum of histopathological features. An international collaborative working group has reached consensus definitions and classification, defining diagnostic criteria for CD which enables to clinicians reaching the proper diagnose.
Aims: The aim of this study is to review our CD patients from a single centre according to newly established diagnostic criterias.
Methods: All patients with a biopsy proven histopathological characteristics of CD diagnosed at Ege University Hospital between 2000-2017 years were reviewed for analysis. Clinical and laboratory datas were collected retrospectively. The patients were divided into two main groups based on the anatomical distribution of the disease: Unicentric CD (UCD) and multicentric CD (MCD). Also MCD were divided into two groups: HHV8 positive MCD and idiopathic MCD.
Results: A total of 64 patients were reviewed. Among the study group; 34 patients were excluded because two were diagnosed with synchronous Hodgkin lymphoma, one was diagnosed with POEMS, and the rest were unable to access all data. Detailed clinical and laboratory datas were summarized in Table 1. The mean age at diagnosis of 30 patients with adequate data was 48.8 (26-82). After histopathological evaluation, majority had hyaline vascular type (n=19), followed by mixed type (n=8) and plasma cell type (n=3). There were 16 patients in UCH group, none of them had HHV-8 and HIV positivity. After a median follow up of 54 months, the estimated 2-year OS was 87.7% [95% confidence interval (CI): 72.1–103.3]. There were 7 patients in the HHV-8+MCH group and two of them had Kaposi sarcoma. All of whom received chemotherapy and/or immunotherapy treatments. Clinical manifestations in the HHV-8+MCH group were; fever, splenomegaly, skin lesions, acute renal failure, oedema, effusion, respiratory symptoms, CRP/ferritin and LDH elevation. After a median follow up of 38.2 months, the estimated 2-year OS was 46% [95% confidence interval (CI): 17.1–74.9]. There are 7 patients in the iMCH group with HHV-8 negative and the clinical findings are very similar to the HHV-8+MCH group. Two-year overall survival in iMCH group was higher than HHV-8+MCH with 82.7% [95% confidence interval (CI): 58.6–106.8].
Summary/Conclusion: CH is a very rare lymphoproliferative disease which should be kept in mind in the differential diagnosis with asymptomatic and localized lymphadenopathies or widespread lymphadenopathies with severe systemic symptoms. Future studies should be multicentred and collaborative in order to evaluate significant numbers of patients and to establish up to date and effective treatment protocols for this rare but potentially life-threatening disorder.

PB2027

RITUXIMAB MAINTENANCE IMPROVES SURVIVAL IN FOLLICULAR LYMPHOMA: A RETROSPECTIVE NATIONWIDE REAL-WORLD ANALYSIS FROM TAIWAN CANCER REGISTRY

H.-H. Huang[1,*], Y.-C. Wen[2], H.-M. Chen[2], F.-Y. Hsiao[2,3,4], B.-S. Ko[1]

[1]Division of Hematology, Department of Internal Medicine, National Taiwan University Hospital, [2]Graduate Institute of Clinical Pharmacy, [3]Health Data Research Center, [4]School of Pharmacy, National Taiwan University, Taipei City, Taiwan, Republic of China

Background: Follicular lymphoma (FL) is the most frequent type of indolent lymphoma in western countries, but it is less frequent in Asia. Several trials have demonstrated the progression-free benefit of rituximab maintenance (R-maintenance) in FL in western countries. However, the overall-survival benefits of R-maintenance in Asian FL patients remain uncertain.
Aims: We utilized the Taiwan Cancer Registry Database (TCRD), the National Death Registry Database (NDRD), and the National Health Insurance Research Database (NHIRD) to investigate the clinical importance of R-maintenance for newly diagnosed FL patients in Taiwan.
Methods: From TCRD, we identified 836 patients with newly diagnosed FL during 2009 to 2012. We retrieved the clinical information from NHIRD, and the survival status from NDRD. We enrolled patients with stage II-IV diseases and receiving 4-8 cycles of rituximab containing frontline chemotherapies. We excluded those who died or received chemotherapies again within 180 days after the end date of the frontline therapies. Total 396 patients were included. Their demographics, clinical parameters, overall survival (OS), and time to next treatment (TTNT) were subjected for analysis.
Results: Among the 396 patients, 260 underwent R-maintenance, and 136 served as the observation group. Compared with the observation group, the R-maintenance group had similar distribution in age, gender, Ann Arbor stages, Charlson Comorbidity scores and the sites of the practice setting. However, those with R-maintenance underwent less intensive frontline chemotherapies (less R-CHOP receiving rate; 53.5% in R-maintenance, *versus* 66.2% in observation; *p* value 0.0150) and less cycles of rituximab-containing frontline therapies (the rate of receiving 7-8 cycles frontline therapies, 25.8% in R-maintenance, *versus* 41.9% in observation; *p* value 0.0010). The patients receiving R-maintenance had a significantly better OS in univariate analysis (hazard ratio (HR), 0.43; 95% confidence interval (CI), 0.20-0.91), and even in multivariate analysis (HR, 0.42; 95% CI, 0.19-0.91). In the multi-variate analysis, age older more than 60 years and stage IV disease also showed negative impacts on OS. On the other hand, the TTNT was similar in the R-maintenance and observation groups (multivariate analysis, HR, 0.96; 95% CI, 0.65-1.42).

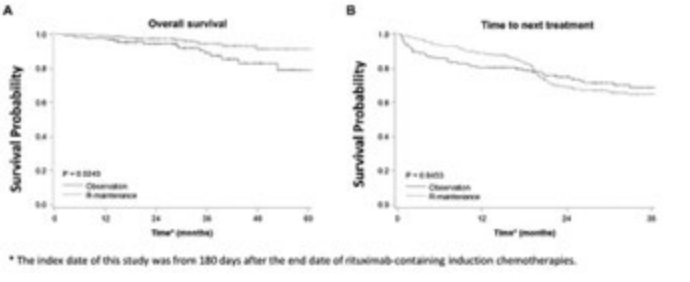


Figure 1.

Summary/Conclusion: Our study is the first real-world study to demonstrate the OS benefit of R-maintenance after the frontline therapies in newly diagnosed FL patients of Asian population.

PB2028

Abstract withdrawn.

PB2029

THE IMPACT OF FIRST COMPLETE REMISSION BY PET-CT AND TIME TO NEXT TREATMENT ON OVERALL SURVIVAL OF FOLLICULAR LYMPHOMA PATIENTS

T.-D. Tan[1,*], L.-W. Chiou[1], J.-S. Wu[2], M.-Y. Lee[3], Y.-Y. Huang[4], S.-S. Chen[5], J.-W. Lee[6], P.-Y. Chen[1], M.-T. Sung[1]

[1]Hematology and Medical Oncology, [2]Radiation Oncology, [3]Pathology, [4]Nuclear Medicine, [5]Radiology, [6]Epidemiology and Biostatistics, Koo Foundation Sun Yat-Sen Cancer Center, Taipei City, Taiwan, Republic of China

Background: Rituximab alone or combined with chemotherapy is the treatment of choice for follicular lymphoma and we retrospectively analyzed the impact of PET-CT complete remission (CR) and time to next treatment (TTNT) on outcome of follicular lymphoma patients.
Aims: To investigate the importance of PET-CT CR and TTNT on overall survival.
Methods: Between 2002 and 2014, we have 174 follicular patients treated at our institute and 150 patients can be evaluated the treatment response and long-term outcome.
Results: The CR after first line treatment with either R-COP or R-CHOP is 89% and PR 7% and 10-year overall survival is 62.6%. Eleven percent of patients died of lymphoma and 3% died of other causes. Forty seven patients

(31%) underwent second line of treatment with 19 (40%) TTNT shorter than 24 months and 28 (60%) longer than 24 months. There is no difference of overall survival between R-COP (86%) *versus* R-CHOP (77%) in 5 years, but there is trend to have more next treatment event in R-COP group as compared with R-CHOP group (60% *vs* 35% on 8-year follow up). There is no difference of overall survival between with or without rituximab maintenance. For PET-CT response, there is significant overall survival difference between CR and PR patients (88% *vs* 70%, p<0.001), and longer TTNT is seen in initial CR patients. TTNT longer than 24 months have better overall survival as compared with shorter than 24 months patients (93% *vs* 54% on 5-year).

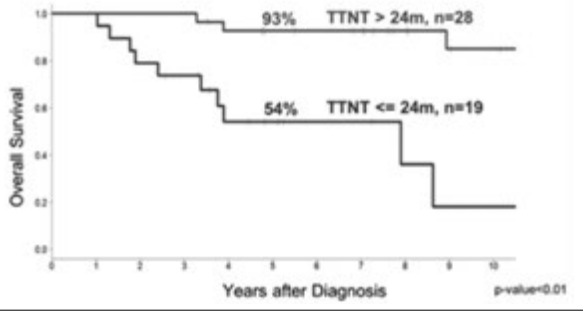


Figure 1.

Summary/Conclusion: Initial PET-CT CR patients have better overall survival as compared with PR patients and PET-CT CR should be the treatment goal on initial treatment. Besides, TTNT longer than 24 months patients have better outcome as well.

PB2030

EFFICACY OF RITUXIMAB PLUS BENDAMUSTINE REGIMEN AS FIRST OR SECOND LINE TREATMENT IN WALDENSTRÖM'S MACROGLOBULINAEMIA

L. Poetsch[1], F.-X. Gros[2,*], M.-S. Dilhuydy[2], C. Gary[3], N. Milpied[2], K. Bouabdallah[2]

[1]Hematology, F-3000, CHU Bordeaux, [2]Hematology, [3]Pharmacy, F-33000, Bordeaux, Bordeaux, France

Background: Waldenström's macroglobulinaemia (WM) is a rare lymphoid disorder that represents 1-2% of hematological neoplasms. There is no standard frontline therapy for WM although Rituximab and an alkylating agent are recommended (*Buske, Ann Oncol 2013*). Bendamustine plus Rituximab (BR) is an effective regimen for relapsed WM (*Tedeschi, Leukemia Lymphoma 2015* ; *Rummel, Lancet 2013*) and is routinely used in our center since 2010.
Aims: To evaluate the efficacy of BR regimen in Wladentrom's Macroglubulinemia compared to RCD.
Methods: We conducted a retrospective, monocentric study from January 2010 to January 2018 to assess the efficacy and safety of the BR regimen. Twenty-six patients (pts) ≥18 years were analyzed and 23 of them had previously been treated including 13 pts who received the RCD regimen (Rituximab+Cyclophosphomide+Dexamethasone). BR regimen consisted in 375 mg/m2 of rituximab on day 1 and 90 mg/m2 of Bendamustine on days 1 and 2 every 4 weeks, with a maximum of 6 cycles. All patients were considered for response as well as short and long-term complications. Only pts who received BR as salvage treatment were analyzed for survival.
Results: Median age at initiation of BR was 68 (54-84) and 16 pts (62%) were male. At BR initiation, patients were classified according to the IPSS-WM score as low (16%) intermediate (42%) and high risk (42%). Treatment criteria for BR therapy were anemia (42%), high tumor burden (30%), thrombocytopenia (19%), B-symptoms (11%), hyper viscosity (7%), neuropathy (3%), and renal failure (3%). Twenty two (84%) patients completed the planned 6 courses and 89% of them received the full doses. Four patients had to stop for progressive disease (n=1), toxicity (n=2) and death from multi-organ failure 45 days after the first cycle (n=1). Among patients evaluable for response (n=25), the overall response rate (ORR) was 61% including complete response (CR, 19%) and partial response (PR, 42%). When focusing only on patients who received BR as salvage therapy (n=23), 22 pts were evaluable for response with an ORR of 56% (CR=22%, PR=34%). With a median follow-up of 32 months, median progression free survival (PFS) was 40 months (Fig. 1). Apart from the patient who died early after the first cycle, 3 patients died from progressive disease after 3, 7 and 8 months after the end of treatment. Among patients who received previously RCD, median PFS was 31, 5 months and 23 months for BR and RCD respectively (not statistically significant). Hematologic and gastrointestinal (mainly nausea) toxicities were the most frequent adverse events with 23% grade 3-4 neutropenia, 22% grade 2 febrile neutropenia, 11% grade 3-4 thrombocytopenia and 3% grade 3-4 anemia. Six patients (23%) had grade 1 nausea. Twenty six percent of pts had prolonged lymphopenia and 15% had hypogammaglobulinemia with infectious complications leading to gammaglobulin infusions. Only 1 pts had prolonged neutropenia 6 months after the completion of BR regimen.

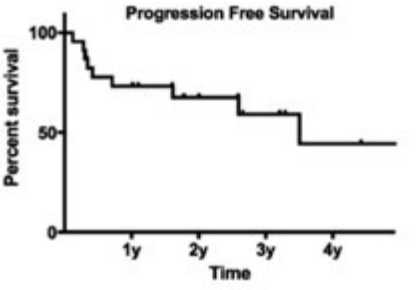


Figure 1.

Summary/Conclusion: BR regimen is efficient in patients previously treated for WM. Although not significant, there is a trend for longer PFS among patients who received previously RCD. BR regimen is well-tolerated even in elderly patients but frequently involved in long-lasting cytopenias which increases the risk of infection and supports prophylactic measures.

PB2031

THE ROLE OF 18F-FDG PET/CT IN THE DIAGNOSIS AND FOLLOW-UP OF GASTRIC LYMPHOMA: A MONOCENTER EXPERIENCE

F. Cacace[1,*], S. Leone[1], M. Memoli[1], C. Mortaruolo[1], N. Pugliese[1], C.G. Mainolfi[2], S. Del Vecchio[2], A. Cuocolo[2], G. Ciancia[2], A. De Renzo[1], M. Picardi[1], F. Pane[1]

[1]Dipartimento ad Attività Integrata di Ematologia, Oncologia, Anatomia Patologica e Diagnostica per Immagini, [2]Dipartimento di Scienze Biomediche Avanzate, AOU Federico II Naples, Naples, Italy

Background: The role of 18F-FDG PET/CT in the diagnosis and follow-up of gastric lymphomas is still controversial. As 18F-FDG PET/CT staging role in gastric DBLC is well established, several studies haven't still clarified the detection rate of PET/CT imaging in gastric MALT lymphomas.
Aims: The aim of our retrospective study is to evaluate the role of 18F-FDG PET/CT in the diagnosis and follow-up of gastric lymphoma.
Methods: Thirty-two patients with histological confirmed gastric lymphoma (MALT: 19; DLBCL: 13) underwent a 18F-FDG PET/CT for initial staging and post therapy evaluation. The PET images were analyzed visually and semi-quantitatively by measuring the maximum standardized uptake value (SUV MAX) and compared with Ann Arbor stage, Lugano staging system for gastrointestinal lymphomas, epidemiological (age, sex), histological /morphological (presence of gastritis, ulcers, H. pylori infection, tumor size, superficial lesions or mass-forming) characteristics.
Results: From January 2007 to November 2016, in our institution, we analyzed 32 gastric lymphoma: 19 patients had histological diagnosis of MALT lymphoma, whereas 13 patients received histological diagnosis of DLBCL. At diagnosis, 15 patients with MALT lymphoma had positive PET/CT-mean lesion SUV max of 6.14 (4.0-18.2) at the corresponding gastric lesion, the remaining 4 were not 18F-FDG avid. Nine patients were H. pylori positive. On the other hand, all 13 patients with DLBCL had positive PET/CT, as expected, with mean lesion SUV max of 17.1 (4-17.1). At post-treatment evaluation, all the patients had histological and radiological complete remission. The overall sensitivity of 18F-FDG PET/CT was 87% (CI 95%: 71-96.5) and specificity 100% (CI 95%: 89.1-100) (p value<0.0001) in our cohort. In MALT lymphoma PET sensitivity was 78% (CI 95%: 54.4-93.5) and specificity 100% (CI 95%: 82.3-100) (p value<0.0001), whereas in DLBCL the sensitivity (CI 95%: 75.3-100) and specificity (CI 95%: 75.3-100) were 100% (p value<0.0001).
Summary/Conclusion: Based on our data, 18F-FDG PET/CT appears to be accurate for initial staging and post treatment follow up in patients with MALT lymphoma and DLBCL. According to our observations, 18F-FDG PET/CT might be used to detect early relapse together with the histological evaluation. Our results should be considered as a preliminary study, limited at our cohort of 32 patients, which will be enlarged with new data.

PB2032

FC RECEPTOR SNP CAN BE A PREDICTOR OF EFFICIENCY OF TARGET THERAPY OF NON-HODGKIN'S MALIGNANT LYMPHOMAS IN RUSSIAN POPULATION

O. Berezina[1,2], T. Pospelova[1,2], F. Maksim[3], V. Ovchinnikov[2,*]

[1]Therapy, hematology and transfusiology, Novosibirsk state medical university, [2]Hematological department, City Clinical Hospital №2, [3]Laboratory of Pharmacogenomics, Institute of Chemical Biology and Fundamental Medicine Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russian Federation

Background: The effectiveness of targeted therapy of non-Hodgkin's malignant lymphomas with monoclonal antibody (MCA) rituximab has been showed in many randomized clinical trials. Nevertheless, a number of patients have resistance to this class of drugs, which may be associated with the inherent characteristics of the receptor of immune cells. Therefore, it seems relevant to study the genetic polymorphism of Fc -receptors, whose carriage affects this mechanism of action of MCA as an antibody-dependent cellular cytotoxicity.
Aims: To study the impact of Fc receptor SNP (Fc IIIRa) on the results of rituximab therapy in patients with B-cell non-Hodgkin's malignant lymphomas (NHL).
Methods: 191 patients from the City Hematology Center of Novosibirsk with a diagnosis of B-cell NHL were examined. Aggressive lymphomas were verified in 118 patients (61.8%), indolent lymphomas were diagnosed in 73 patients (38.2%). All patients received from 6 to 12 courses of polychemotherapy comprising rituximab. Genotyping of Fc receptor SNP was performed using Taq-man PCR. The significance of the differences was assessed using the $\chi 2$ criterion, the differences at $p<0.05$ were considered statistically significant.
Results: The distribution of the genotypes of the FcIIIRa gene in the aggressive lymphoma group was as follows: 53 patients (45%) have the wild T/T genotype, 57 persons (48.3%) have the T/G genotype, 8 (6.7%) patients have rare G/G genotype. In this group of NHL a complete or partial response to therapy was achieved in 78.6% of patients, relapse or progression of the disease was observed in 21.4% of the patients. During the statistical analysis the effect of this polymorphic locus on the results of therapy with aggressive lymphomas was not detected. In the group with indolent NHL 25 (35.6%) patients have the wild T/T genotype, 34 (46.6%) persons have the T/G genotype, 13 (17,8%) patients have the rare G/G genotype. In this group of NHL complete or partial response to therapy was achieved in 76.7% of patients, relapse or progression of the disease was noted in 23.3% of patients. It was revealed that the "rare" genotype FcγRIIIa was statistically significantly associated with an unfavorable outcome of the disease ($p<0.05$) in patients with indolent variants of NHL. The literature data on the effect of the FcγRIIIa polymorphic locus on the therapy effectiveness of indolent lymphomas are inconsistent, which may be due to genetic heterogeneity of populations and require a meta-analysis in which the results of this study may be included.
Summary/Conclusion: The correlation between the "rare" genotype of the Fc IIIRa polymorphic loci and the treatment failure in the group of patients with indolent lymphomas is shown, which allows considering this SNP as a potential marker of effeciency of treatment with rituximab.

PB2033

CLINICAL FEATURES AND TREATMENT OUTCOMES OF LIMITED STATE MANTLE CELL LYMPHOMA (CISL16-06)

J.-C. Jo[1,*], S.J. Kim[2], H.S. Lee[3], H.-S. Eom[4], S.I. Lee[5], Y. Park[6], J.-O. Lee[7], Y. Lee[8], H.-Y. Yhim[9], D.-H. Yang[10], J.M. Byun[11], H.J. Kang[12], H.J. Kim[13], H.-J. Shin[14], K.H. Yoo[15], Y. Choi[16], C. Suh[17]

[1]Hematology, Ulsan University Hospital, University of Ulsan College of Medicine, Ulsan, [2]Samsung Medical Center, Sungkyunkwan University School of Medicine,, Seoul, [3]Kosin University College of Medicine, Busan, [4]National Cancer Center of Korea, Koyang, [5]Dankook University College of Medicine, Cheonan, [6]Korea University College of Medicine, [7]Seoul National University Bundang Hospital, Seoul, [8]Kyungpook National University Hospital, Daegu, [9]Chonbuk National University Hospital, Cheonju, [10]Chonnam National University Hwasun Hospital, Gwangju, [11]Seoul National University Boramae Hospital, [12]Korea Cancer Center Hospital, Korea Institute of Radiological and Medical Sciences, Seoul, [13]Hallym Medical Center, Hallym University Sacred Heart Hospital, Anyang, [14]Pusan National University Hospital, Busan, [15]Gachon University Gil Hospital, Incheon, [16]Ulsan University Hospital, University of Ulsan College of Medicine, Ulsan, [17]Asan Medical Center, Seoul, Korea, Republic Of

Background: Limited stage (Ann Arbor stage 1 or 2) mantle cell lymphoma (MCL) is an extremely rare disease. Thus, there are little data about the clinical feature and treatment outcomes of patients with early stage mantle cell lymphoma.
Aims: The clinical features were reviewed, and the treatment outcomes were analyzed.
Methods: We consecutively collected stage 1 or 2 MCL cases diagnosed between 2000 and 2016 in 16 institutions in CISL group. All patients were pathologically confirmed and received systemic evaluation for staging work-up.
Results: The median age of patients was 66 years (range: 18-85 years), male (n=31, 75.6%) was predominant compared to female. The majority of patients (n=28, 68.3%) had stage 2 disease, 29 patients (70.7%) were symptomatic. The elevation of LDH (n=2, 4.9%) was not common, thus, 39 patients (95.1%) had low risk (0 or 1 score) of the International Prognostic Index (IPI), and 28 patients (68.3%) had low risk (1-3 score) of the MIPI. As the first therapeutic strategy, most patients (n=37, 90.1%) received chemotherapy, radiotherapy (n=2), surgical resection (n=1), and no treatment (n=1). Of patients who received chemotherapy, 23 patients (56.9%) were administered with rituximab containing regimen, and R-CHOP (n=17) and R-bendamustine (n=4) was commonly used. The best response rate was 97.4% (n=38) including 32 complete response (78%). In aspect of sites of relapse (n=16), local relapse rate was 31.3% (n=5), distant relapse rate 43.8% (n=7), and local & distant relapse rate 25% (n=4). With the median follow-up duration of 40.6 months, the median relapse free survival was 56.1 months, and the 5-year overall survival rate was 80.4%.
Summary/Conclusion: Limited state MCL showed indolent clinical and low risk prognostic features. Chemotherapy could be effective for controlling localized MCL lesion with high complete response rate. Less intensive chemotherapy may be preferred.

PB2034

DUODENAL-TYPE FOLLICULAR LYMPHOMA: WHICH IS THE BEST THERAPEUTIC OPTION?

F. Saraiva[1,*], A. Figueiredo[2], A. Botelho de Sousa[1]

[1]Hematology, HSAC-CHLC, [2]Patology, HCC-CHLC, Lisboa, Portugal

Background: Duodenal-type follicular lymphoma was firstly recognized as a distinct entity in the 2016 revision of the WHO classification. Most cases are asymptomatic, confined to the duodenum, dissemination being very rare and limited to the small bowel. Currently, the best therapeutic approach is unknown, due to its rarity and to the lack of large series. In the few cases that do present symptoms or are at risk of local complications intrinsic to the localization of the tumour (obstruction, haemorrhage, perforation), the option *watch and wait* is unsuitable and the difficulty increases of having to choose among the various options: surgery, radiotherapy, immunotherapy or immunochemotherapy.
Aims: Evaluate if Rituximab in monotherapy is an adequate treatment for patients with duodenal-type follicular lymphoma with symptoms or complications.
Methods: We report 3 cases (patients aged 57, 68 and 74) which were diagnosed due to dyspeptic complaints (2) and by chance (1). All of the patients mentioned were followed from the diagnosis and after treatment until the present day.
Results: Duodenal lesions were described as nodular in 2 cases and extensively ulcerated in the third, measuring from 3.6 to 6 cm. Histologically, all were low-grade (1/2). Two cases presented as isolated duodenal disease and one had biopsy-confirmed ileal involvement. No extra-intestinal involvement was documented. The *watch and wait* approach was deemed inadequate due to the extension and ulceration in one case and the presence of symptoms in the other two. Patients were treated with rituximab in monotherapy. All became asymptomatic (sustained after a 12-month period), and the 6-month endoscopic evaluation showed complete regression of the lesions in 2 of the patients and partial regression in the other (40% reduction).
Summary/Conclusion: Duodenal-type follicular lymphoma is very rare and data regarding treatment options are limited due to the small number of reported cases. Concerning the therapeutic approach, it seems to be a remarkably indolent variant of follicular lymphoma, which may have a *watch and wait* approach in most cases. Nonetheless, although randomized studies are difficult in this rare disorder, when the presentation implies treatment, whether due to the existence of symptoms or to a risk of local complications, immunotherapy with an anti-CD20 antibody is an appropriate option.

PB2035

A RETROSPECTIVE REVIEW OF EXPERIENCE IN DELIVERING MAINTENANCE RITUXIMAB FOR FOLLICULAR LYMPHOMA IN A DISTRICT GENERAL HOSPITAL

A. Pryce*, M. Shah, A. Babb

Haematology Department, West Middlesex University Hospital, London, United Kingdom

Background: The PRIMA study findings support the benefit of 2-years of Rituximab maintenance in patients with follicular lymphoma (FL) responding to immuno-chemotherapy treatment, with maintenance Rituximab delivering a sustained and persistent benefit with improved progression free survival without an unacceptable increase in long term toxicity. Therefore it is now routine practice to offer 2 years of maintenance Rituximab to this group of patients.
Aims: We reviewed our experience of rituximab maintenance in a district general hospital looking particularly at rate of infections and development of complications and toxicities.
Methods: Between January 2011 and January 2018 52 patients completed 2 years of Rituximab maintenance for Follicular lymphoma. We conducted a retrospective case note review, accessing clinical notes, laboratory results and radiological imaging to review for development of immunodeficiency, infection rates, and respiratory complications.
Results: The primary immuno-chemotherapy most frequently used prior to commencing maintenance was R-CHOP (42.3%). Maintenance Rituximab was planned for 24 months. Infective complications were common with patients receiving on average 2 courses of antibiotics during the maintenance period. Admissions were relatively frequent with 59.6% of patients requiring at least one admission, and 9.6% requiring three of more admissions, with infection being the most common reason for admission. During the maintenance Rituximab period 30.8% of the patients developed neutropenia and 28.8% developed significant hypogammaglobulinemia. 50% reported chest symptoms and 30.8% had new radiological findings of lung disease on CT compared with their end of treatment CT chest before commencing the Rituximab maintenance. Most common changes were atelectasis, but 11.5% developed new ground glass opacities or interlobular thickening, and 2% developed new diagnosis of bronchiectasis. One patient reactivated pulmonary tuberculosis. 3.8% of patients needed to discontinue treatment due to the frequency of respiratory tract symptoms.
Summary/Conclusion: This experience from treating patients at a district general hospital highlights that as maintenance Rituximab is increasing being offered due to it's beneficial affect on progression free survival, we need to be vigilant to the infective and respiratory complications that can develop. Therefore it is imperative that in order to reduce these complications and not adversely impact on the patients quality of life steps should be taken to monitor carefully for development of neutropenia, hypogammoglobulinemia, or chest symptoms or signs in order to take early interventions such as administration of G-CSF or monthly IVIg, and consideration of early imaging to prevent infectious and respiratory complications.

PB2036

HAIRY CELL LEUKAEMIA-SURVIVAL ANALYSIS AND PROGNOSTIC FACTORS-UNICENTRIC EXPERIENCE

D. Mota*, A. Roque, M. Santos, J.P. Carda, M.L. Ribeiro

Hematology, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

Background: Hairy cell leukaemia (HCL) is a rare condition characterized by pancytopenia and markedly susceptibility to infections. This lymphoproliferative disease presents high rates of response and survival. Nevertheless, treatment may not be curative, and there may be a need for re-treatment at the time of relapse. Thus, the identification of patients at risk of treatment failure remains an important question.
Aims: Characterization of the population with HCL diagnosed in a tertiary hospital. To identify in this group of patients factors predictors of survival.
Methods: Retrospective analysis of patients diagnosed with HCL at our institution between January, 1988 and December, 2017.
Results: Were included 51 patients; male gender 80.4% (n=41); median age at diagnosis 57 years (35-83). Had comorbidities 56.9% (n=29). Splenomegaly was present in 68% (n=34) and B symptoms in 33.3% (n=17). Had haemoglobin <11 g/dL 39.2% (n=20), platelets <100 G/L 58.8% (n=30), neutrophils<1G/L 58% (n=29). Median follow-up was 78.8 months (0.6-290.8). First line treatment was cladribine in 77.6% (n=38), pentostatin in 4% (n=2) and IFNα in 18.4% (n=9), remission (CR+PR) was obtained in 87.8% (n=43). Use of cladribine was associated with greater achievement of complete response (79.0 *vs* 22.2%, OR 0.28; p=0.004). Were re-treated 22 patients-72.7% of them with purine analogues (PA) –, 81.8% obtaining remission (CR+PR). Median overall survival (OS) was 232.8 months (232.7 with cladribine, 287.3 with IFNα). Median progression free survival (PFS) was 71.2 months (102.7 with cladribine, 67 with IFNα). Although there were no differences in OS between cladribine and IFNα (p=0.6892), in PFS there were significative differences (p=0.045). Seven patients presented second neoplasia, all of them treated with PA. Of the 51 patients, 27.5% died, 28.6% of them of infection. OS was inferior in age>65 years (p<0.001), ECOG >0 (p=0.0053) and serious infection at diagnosis (p=0.012). After multivariate analysis, age>65 years (HR 41.95; p=0.002) and ECOG>0 (HR 6.19; p=0.008) were independent predictors of lower survival at diagnosis.
Summary/Conclusion: Although the PA contributed to increase of complete response rate and PFS, there is still a need to optimize therapeutic strategies in order to increase OS, as decreasing adverse events and relapse / refractoriness in patients with HCL. Factors such as age at diagnosis >65 years and ECOG >0 are predictors of lower survival, however, more robust studies are needed to validate and identify other potential prognostic factors.

PB2037

SINGLE-CENTER EXPERIENCE WITH BENDAMUSTINE-BASED IMMUNOCHEMOTERAPY REGIMENS IN INDOLENT LYMPHOID MALIGNANCIES AND MANTLE-CELL LYMPHOMA

B. Dreta[1,*], S. Bašić-Kinda[1], D. Dujmović[2], I. Radman[1], M. Vodanović[1], P. Rončević[1], S. Dotlić[3], K. Dubravčić[4], M. Kralik[5], I. Aurer[1]

[1]Division of Hematology, Department of Internal Medicine, University Hospital Centre Zagreb, [2]Medical School, University of Zagreb, [3]Department of Pathology and Cytology, [4]Department of Laboratory Diagnostics, [5]Department of Radiology, University Hospital Centre Zagreb, Zagreb, Croatia

Background: Bendamustine-based regimens belong to most effective treatments for indolent lymphoid malignancies but reports on its toxicities vary.
Aims: To compare efficacy and toxicity of bendamustine-based regimens in a real-world situation to those reported in the literature and to identify possible risk factors for development of complications.
Methods: We performed a retrospective chart review of all our patients with CLL, indolent NHLs (iNHL) and mantle cell lymphoma (MCL) treated with bendamustine-based regimens between 2013 and 2017.
Results: 123 patients, 36-88 (median 67) years old, were included in this analysis. 65 were male and 58 female. 51 were younger than 65 y and 19 older than 75 y. 35 had chronic lymphocytic leukemia (CLL), 35 follicular lymphoma (FL), 19 marginal zone lymphoma (MZL), 14 lymphoplasmocytic lymphoma (LPL) and 20 MCL. 63 were treated in 1st, 42 in 2nd and 18 patients in subsequent lines of treatment. 103 patients received BR or similar, 19 R-BAC and 1 bendamustine monotherapy. Median follow-up was 14 mo. Response rates (RR), progression-free survival (PFS) and overall survival were similar to those reported in seminal studies. RR in 1 st line was 87% for CLL, 93% for indolent NHL (iNHL) and 80% for MCL. In later lines RR was 85%, 72% and 67% respectively. 18-mo PFS was 83% in 1st line CLL and 91% in iNHL, and 77% and 68% respectively in 2nd line. Hematological toxicity was also similar to that reported; in 1st line gr 3-4 neutropenia occurred in 20%, thrombocytopenia in 8% and anemia in 8% of patients. In later treatment lines gr 3-4 neutropenia occurred in 30%, thrombocytopenia in 11% and anemia in 10% of patients. Skin changes were mild, serious gastrointestinal toxicity occurred in 3% of patients. Infections were substantially more frequent than reported in the NHL1 and NHL2 studies, but not in CLL10. In 1st line of treatment gr 1-2 infections occurred in 38% and gr 3-4 in 22% of patients, and in later treatment lines in 30% and 30% respectively. Risk of serious infections was significantly higher in pts. previously treated with ≥2 lines (50% *vs* 23%, p=0,02) and those with reduced IgG levels at end of treatment (36% *vs* 14%, p=0.015). Risk of infections of all grades was higher in those with IgG<4 g/L (82% *vs* 59%, p=0.018); there was a trend in pts older than 75 (79% *vs* 57%, p=0.076). Sex, disease type, initial IgG levels and antimicrobial prophylaxis did not significantly influence infection risk. 5 1st line pts died of toxicity and 2 of lymphoma in comparison to 7 and 6 in later treatment lines. Infections clustered during first two cycles and after end of therapy. Four pts. developed secondary malignancies.
Summary/Conclusion: Efficacy of bendamustine-based regimens in routine clinical practice is similar to that reported in randomized studies, but infections are more frequent. Low IgG levels, advanced age and having received more than 2 lines of therapy increase the risk. Increased risk persists after end of therapy.

PB2038

HAIRY CELL LEUKEMIA: 20-YEAR SINGLE CENTER EXPERIENCE

M. Criscuolo*, L. Fianchi, E. Galli, C. Rotolo, F. D'Alò, L. Laurenti, A. Bacigalupo, L. Pagano

Hematology, Catholic University of Sacred Heart Fondazione Policlinico Universitario A. Gemelli, Rome, Italy

Background: Hairy cell leukemia (HCL) is a rare indolent disease which is curable in most cases. However, a small number of patients is still expected to relapse, or die, mainly due to infective complications.
Aims: The aim of the study is to analyze the course of the disease in a population of uniformly treated patients and identify early and late adverse events during a 20-year follow-up.
Methods: We retrospectively reviewed the charts and collected clinical and laboratory data of 37 patients diagnosed with HCL from October 1997 through May 2017. They were all treated with one course of Cladribine as first line therapy at our Hematology Department. Particularly, we focused on infective events, allergy, secondary neoplasms and relapse during the follow-up.
Results: Most patients sought medical evaluation because of constitutional symptoms and emerging abdominal discomfort. At disease onset, two-third of patients presented with white blood cells count lower than $4x10^9$/L and platelets count lower than $100x10^9$/L; massive splenomegaly was detected in 50% of patients. The median time to treatment was 1.4 months (range 0.2-20.6 months): cladribine were administered as a 7-day continue infusion in 89% of patients. The overall response rate was 97%, with 92% complete and 5% partial remissions. During neutropenia, 16 out of 23 patients (69.5%) experienced at least one infectious episode: one patients died, probably due to invasive aspergillosis, 2.7 months after treatment with no sign of hematologic recovery. Median time to recovery of neutropenia was 23 days (range: 5-720 days). Eight patients (22%) reported a diffuse cutaneous allergic reaction: all recovered after few days of antihistamine and steroid administration. Seven patients (19%) experienced a relapse, after a median time of 51.2 months (range: 17.3-146.1 months): 4 were retreated with Cladribine, 1 with Cladribine and Rituximab, 1 with Interferon α and 1 refused treatment. The overall response rate was 57.1% (4 patients, 2 complete and 2 partial responses); 1 patient died two weeks after treatment due to sepsis and 1 was treated with Pentostatin after failure of Interferon α. This patient experienced another relapse 2 years later, and died of septic shock 14 days after starting a BRAF inhibitor. After a median follow-up of 50.6 months (range: 3-219.5 months), overall mortality was 13%: 3 patients died from infection during neutropenia related to chemotherapy, and 2 patients died of causes unrelated to treatment (1 following a myocardial infarction and 1 from senescence). Overall survival and disease-free survival at 20 years were 78% and 81% respectively. Incidence of secondary neoplasms was 2.7%: one patient developed breast cancer 5 years after HCL onset. Age, white blood cells count, bone marrow fibrosis, splenomegaly, infections, allergy, duration of neutropenia and time to best response did not show any statistical significance for mortality at univariate analysis.
Summary/Conclusion: Due to the rarity of disease, multicentric survey is needed to identify factors predictive of response to treatment and ultimately survival. The patients who experienced a fatal infection during prolonged grade 4 neutropenia are of concern: reducing the duration of neutropenia using lower grade immunosuppressive and myelotoxic drugs, should be tested in order to improve the life expectancy of these patients.

PB2039

GASTRIC MALT LYMPHOMA : A MONOCENTRIC STUDY

M. Medhaffar[1,*], L. chtourou[2], I. Frikha[1], A. Amouri[2], M. Elloumi[1], N. Tahri[2]

[1]Clinical Hematology, [2]Gastro Enterology, Hedi Cahker hospital Sfax University, Sfax, Tunisia

Background: Gastric MALT lymphoma is one of low grade B lymphoma that can be cured in many cases by antibiotics targeting HP infection.
Aims: We report a study of 35 patients diagnosed and treated for Gastric MALT lymphoma.
Methods: This study concern all patients followed for Gastric MALT lymphoma between 2001 and 2016 at Sfax Hedi Chaker Hospital in the south of Tunisia. Upper endoscopy was performed for all patients, multiple biopsies served to Histo-pathologic analysis and HP detection. Physical examination, LDH dosage, echo-endoscopy, chest and abdominal scan and bone marrow biopsy were performed to Musshoff (Ann-Arbor modified) staging of lymphoma.First line treatment consists on HP eradication therapy (patrician choice: three therapy, sequential therapy or quadri-therapy Bismuth). A double endoscopic control was performed initially to verify HP eradication, and secondary to evaluate lymphoma response according to the GELA-Lysa criteria.Stomach Radiotherapy was proposed for HP negative localized lymphoma, HP resistant to two different eradication schemas or no responded lymphoma.
Results: A total of 35 patients had Gastric MALT lymphoma. Median of age was 51 years. Sex ratio=0.94. Pseudo-gastritis, ulcers, and pseudotumoral aspects were found in respectively 40%, 35% and 25%. HP was found in 32 patients (91%). Lymphoma had localized stage in all patients (stage IE= 80%, stage IIE=20%). 32 HP positive and 2 HP negative patients received first line eradication therapy. Eradication was obtained in 68% of cases and in all HP positive cases (100%) after two switched schemas. According to GELA-Lysa criteria: complete response was obtained in 27 cases (CR=25, pMRD=2), partial response in three cases, AND no change response in four cases. Five patients (2HP negative and 3 HP positive) underwent Radiotherapy achieving a CR. During follow up, no patient have lymphoma relapse, two cases with NC response showed a progression to diffuse B large cell lymphoma.
Summary/Conclusion: In our region, Gastric MALT lymphoma is frequently associated to HP (more than 90%) and presenting in localized stage. Eradication schemas therapy leads to the disappearance of HP in 100% of cases and regression of lymphoma in 80% of cases even in HP negative patients. Radiotherapy stills a second arm for non responded patients.

PB2040

CONTRIBUTION OF FLOW CYTOMETRY BY FINE NEEDLE ASPIRATION COUPLED WITH CYTOLOGY IN THE DIAGNOSIS AND CLASSIFICATION OF SMALL-CELL NHL B AND ITS CORRELATION WITH HISTOLOGY AND IMMUNOHISTOCHEMISTRY

A. Mohamed Rafik*, A. Fatima Zohra, B. Salah Eddine

Hématology, HCA, Dr Mohamed Seghir Nekkache, Alger, Algeria

Background: In the new WHO 2016 classification of lymphomas, the relative importance of each of the morphological, immunophenotypic, genetic and clinical criteria varies from one lymphoma to another, but the morphological and immunophenotypic criteria remain the cornerstone of the diagnosis. As a result, cytology and CMF coupling by fine needle aspiration puncture are easy and valuable tools for the diagnosis and follow-up of lymphoproliferative disorders, especially small-cell NHL-B-cells.
Aims: To demonstrate the value of fine needle aspiration coupled with CMF for rapid outpatient diagnosis and follow-up of small-cell NHL B.
Methods: Retrospective and descriptive study over a period of 6 years (from January 2010 to December 2015). We collected 180 samples including 134 evaluable. The diagnosis of small cell lymphoma was made in 38 patients (28%), 33 lymphadenopathies (by fine needle aspiration (23 G)), 3 ascites fluid, 1 pleural fluid and 1 subcutaneous mass. skin. A cytological study (MGG) was performed on the various samples. Labeling with more than 10 Antibodies on average for each sample. Then we proceed to the acquisition and analysis by flow cytometry (FACS CALIBUR 3 colors of Becton Dickinson). The histological study with immunohistochemistry was performed in 33 patients: 21 biopsies of adenopathy (14 biopsies of ADP alone, 6 with PBO, 1 cutaneous biopsy), 2 PBO alone, 1 tonsillectomy, 1 transparietal biopsy associated with a PBO, 1 exeresis biopsy of a hepatic mass and 1 gastrectomy. The 5 unexplained biopsies for the following reasons: 3 cases of ascites whose biopsy is difficult and 2 cases of relapse.
Results: In our series, the average age of patients was 61 years (22-83 years), with a clear male predominance (26 men and 12 women, sex ratio 2.5). At the CMF, several diagnoses and types of NHL could be made: follicular NHL 10 cases (26%), NHL with small cell without being able to specify the exact type 9 cases (24%), lymphocytic NHL 8 cases (21%), NHL to coat cells 7 cases (18%), NHL marginal area 2 cases (5%), lymphoplasmocytic NHL 2 cases (5%). In the histology, the diagnostics performed in 33 patients are as follows: 3 cases of LGCDB (41%), small cell NHL 5 cases (8%), follicular NHL 8 cases (7%), NHL with mantle cells 6 case (5%), marginal zone NHL 1 case (5%), lymphocytic NHL 7 cases (2%), angioimmunoblastic NHL 1 case (4%), inflammatory (reactive) 2 cases (5%). After these results, the overall correlation between CMF and histology is 94% in the diagnosis of NHL B: 70% the CMF allowed to make the diagnosis and to type the NHL, in 24% one finds a concordance in the diagnosis without being able to specify the morphological type and 6% of discordance (2 false positives in CMF).
Summary/Conclusion: when the fine needle aspiration coupled with CMF is performed by experienced operators and for the correct indications, it is one of the safest technique of small cell NHL B diagnostic and less massive, more profitable and more accurate.

Infectious diseases, supportive care

PB2041

IMATINIB MESYLATE ON PREVALENCE OF FALCIPARUM MALARIA: A CASE CONTROL STUDY FROM A TERTIARY CARE CENTRE IN KOLKATA

A. Iqbal[1,*], J. Chakraborty[1], S. Choudhuri[1], A. Naik[2], M. Bhattacharyya[1]

[1]Clinical Hematology, Institute of Haematology and Transfusion Medicine, [2]Transfusion Medicine, Medical College and Hospital, Kolkata, India

Background: Rising evidence of artemisin resistant malaria in Indian subcontinent and South America has prompted researchers to search for alternative antimalarials. In the last few decades, few reports and subsequent *in vitro* results have emerged showing dependence of malarial parasites, particularly Pl. falciparum on RBC tyrosine kinases for its maturation and egress. As there were no clinical data to support the hypothesis, we conducted a case control study with patients of CML-CP on fixed dose Imatinib as cases and a representative healthy population as control.
Aims: To find the prevalence of asymptomatic malarial parasitemia in patients of CML-CP on Imatinib and compare with the prevalence in healthy blood donors. To internally validate the results of positive RDT samples with PCR in both cases and controls.
Methods: A standard questionnaire and Rapid Diagnostic Test (RDT) for plasmodium antigens from the blood of cases attending CML clinic were used as study tools. 191 CML patients on Imatinib 400mg/d for a minimum period of 1 year were analysed.
Results: Out of 54 febrile episodes during the study period, one patient had vivax malaria. RDT testing on non-febrile participants revealed two samples positive for dual vivax-falciparum antigens and the other two for vivax antigen. All positive results were internally validated with PCR using two species-specific forward primers which showed three cases positive for vivax and one positive for both vivax and falciparum. Among 205 controls selected from healthy voluntary blood donors, three had vivax infections, three had falciparum infections and four were found to have infections with both parasites. The prevalence of malarial infections in cases and controls were found to be 2.05% and 4.87% respectively while falciparum parasitemia in cases and controls were found to be 0.51% and 3.41% respectively. When analysed, the difference was found to be statistically significant.
Summary/Conclusion: In a malaria endemic region where asymptomatic parasitemia rates are high, imatinib mesylate given as a fixed daily dose provided protection to patients of CML-CP from falciparum malaria. However the association between the occurrence of vivax malaria and exposure to Imatinib was not statistically significant. The internal validation of positive samples by PCR showed a high, acceptable correlation.

PB2042

RESISTANCE OF THE HEPATITIS B VIRUS IN PATIENTS WITH LYMPHOMAS

S. Lepkov[1,*], I. Subortceva[2], E. Demina[3], G. Tumian[3], J. Ribuchina[3], O. Kolomeitsev[3], P. Zeynalova[3], I. Komarov[3], A. Semenova[3], N. Kokosadze[3], S. Borisovskay[4], T. Padjeva[4], R. Ivashenco[5], O. Urvanceva[5], Y. Kemih[5], O. Zaharov[6], I. Lazarev[6], V. Ivanova[6], O. Ettinger[1], I. Nikitin[1]

[1]Therapy, National Research Medical University named by N.I. Pirogov, [2]Haematology, National Medical Research Center for Hematology, [3]Oncology, N.N. Blokhin National Cancer Research Center, [4]Therapy, [5]Radiology, City Clinical Hospital. V.M. Buyanova, [6]Haematology, Hematologic Moscow City Center at the State Clinical Hospital named by S. Botkin., moscow, Russian Federation

Background: Reactivation of the hepatitis B virus (HBV) is a serious, and in some cases life-threatening, complication that occurs in patients receiving chemotherapy. Conducting target therapy (rituximab) in mono-regime or in combination with polychemotherapy (PCT) is a factor of high risk of reactivation of HBV. Patients with resolved HBV infection who have a hepatitis B surface antigen (HBsAg) in their blood have a HBV reactivity in more then 80%, and fatal liver failure is not unusual for this category of patients.
Aims: In study included 53 patients with malignant lymphomas who had reactivated HBV from 2002 to 2016 during the treatment of lymphoma. Male: female ratio was 1: 1. The age of the patients ranged from 19 to 82 years (median 35 years). Among patients there were 11 with Hodgkin's lymphoma(HL) and 42 non-Hodgkin's lymphoma(NHL) (18 with indolent and 24 with aggressive). Reactivation of HBV in 89% (47) patients developed after 4-6 courses. In 6 patients, the reactivation of HBV developed after the end of PCT for 3 months. In patients who had reactivation HBV the ALT level was from 90 to 600 IU/L, AST-70 to 560 UD/L, alkaline phosphatase ranged from 120 to 1700 UD/L, GGTP-from 88 to 960 IU/L. The level of HBV DNA was from $1x10^5$ to $1.6x10^9$ copies/ml.
Methods: All patients with HBV reactivation were prescribed antiviral therapy: 37 patients received lamivudine for 100 mg/day and 15-entecavir for 1 mg/day. In 70% (37) patients on antiviral therapy, complete remission of HBV was achieved. In 30% (15) patients, the level of enzymes decreased and was not higher than 2xUIN. However, the level of HBV DNA was from $3x10^3$ to $1x10^5$ copies/ml. Antiviral therapy changed to tenofavir 300 mg per day in all 15 patients. Complete remission was achieved in 11 patients. 4 patients (3 with NHL and 1 with HL) had a short period (3-5 month) of decreasing HBV DNA, then HBV DNA began to rise again. The level of HBV DNA was $1x10^5$- $1.4x10^7$copies/ml. The resistance of HBV to the therapy of tenofavir was verify.
Results: 3 patients with NHL received the therapy with alpha interferon. The remission was achieved in 2 patients on therapy with tenofavir+interferon. In 1 patient, the level of HBV DNA was $1x10^2$ copies/ml. In patient with HL and resistant HBV infection developed the relapse of the HL. The patient, according to vital indications, started PCT. After 2 courses of PCT complications with multi-organ disturbances developed and the patient died.
Summary/Conclusion: Therapy of reactivation of HBV in patients with lymphomas is very complex. This problem has not been resolved. We surmise new mutant forms of HBV infection. Chemotherapy may leads the mutations in the genome of the hepatitis B virus. Therapy of new identified mutant forms requires the creation of new molecules. At present, when a multiple resistance of virus B occurs to atypical nucleosides, the main drug in the treatment of this hepatitis B is only alpha interferon

PB2043

PRIMARY PROPHYLAXIS OF INVASIVE FUNGAL INFECTIONS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA: RESULTS FROM A SINGLE-CENTRE STUDY

N.S. Fracchiolla[1,*], M. Sciumè[1], F. Cavalca[1], N. Orofino[1], F. Guidotti[1], A. Grancini[2], A. Freyrie[1], M.C. Goldaniga[1], D. Consonni[3], A. Cortelezzi[1]

[1]Oncohematology Division, IRCCS Ca' Granda-Maggiore Policlinico Hospital Foundation and University of Milan, [2]UOS Microbiology, Central Laboratory, I.R.C.C.S. Foundation, Cà Granda Ospedale Maggiore Policlinico, [3]Unit of Epidemiology, Fondazione IRCCS Ca' Granda-Ospedale Maggiore Policlinico, Milan, Italy

Background: Immunocompromised patients (pts) are at high risk of invasive fungal infections (IFIs), in particular those affected by acute myeloid leukemia (AML) undergoing remission-induction chemotherapy. Posaconazole (PSZ) is now available in different formulations and its use as antifungal prophylaxis in this high-risk hematological pts is strongly recommended.
Aims: Our aim was to describe the efficacy of primary prophylaxis of IFIs with PSZ in a real-life cohort of AML pts during induction chemotherapy.
Methods: AML pts consecutively treated with remission-induction chemotherapy (up to 3rd line treatment) between January 2010 and July 2017 were selected from institutional database. We excluded acute promyelocytic leukemia, re-induction therapy performed for relapse after transplantation and patients treated with hypomethylating agent. Diagnosis of IFIs was carried out according to the revised European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) definitions published in 2008.
Results: We identified 123 AML pts who received 171 remission-induction treatments. Primary prophylaxis of IFIs with PSZ was made in 110 cases, while the remaining 61 cases did not received any systemic antifungal prophylaxis. Among patients who received PSZ, median age was 61 years (male/female ratio 64/34%), in the remaining population median age was 64 years (male/female ratio 49/51%). Seventy cases were first-line induction therapies, while 40 were re-induction treatments. PSZ was given as oral suspension in 104 cases; the tablet formulation has been introduced since 2017 and it was used in 6 cases. Over 90% of analysed cases developed a febrile event. An antifungal treatment was administered in 28 of 61 cases (46%) which did not received prophylaxis and in 26 (23%) cases which received PSZ (p=0.003). Thirty-five (57%) cases in no prophylaxis group and 45 (41%) among PSZ group underwent a chest CT scan (p=0.039). CT alterations suggestive for IFIs were found in 71% of no PSZ cases and in 42% of PSZ cases with a statistically significant difference (p<0,01). Serum galactomannan antigen was positive in 3% of the tested cases: 1 of 106 PSZ tested cases and 4 out of 58 no PSZ tested cases (p<0,01). Yeasts and moulds were identified respectively in 5 (8%) of patients who didn't

received an antifungal prophylaxis; all microorganisms were isolated from the respiratory tract, except for one liver mould and one yeast positivity on rectal swab. Seven (6%) PSZ patients had a positivity for yeasts, in the same group 3 (3%) patients were positive for moulds (p=0.04). The latter were isolated from the respiratory tract, while almost the whole yeasts from blood samples. Seven (4%) cases were classified as proven (2 no PSZ group, 5 PSZ group), 12 (7%) as probable (9 no PSZ group, 3 PSZ group) and 24 (15 no PSZ group, 9 PSZ group) as possible IFIs. Eleven (6%) cases could not be classified (2 no PSZ, 9 PSZ). The total number of death was 14 (8%). Among patients who received an antifungal treatment, 9 deaths were observed (3 PSZ group, 6 no PSZ group). In the prophylaxed group, the most used antifungal treatment was liposomal amphotericin B (77%), followed by voriconazole (15%); while among no PSZ cases voriconazole was used in about 50% of patients (p<0.001). Twenty-one cases received a sequential or a combination regimen.
Summary/Conclusion: This monocentric survey underlined the feasibility and efficacy of PSZ prophylaxis in clinical practice; PSZ use confirm to be associated to lower incidence of IFIs, less need for antifungal treatment and lower IFIs attributable mortality.

PB2044

LOW FREQUENCY OF PRIMARY PROPHYLACTIC USE OF G-CSF IN THE JSMO FEBRILE NEUTROPENIA GUIDELINES BY JAPANESE ONCOLOGISTS TREATING PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA USING R-CHOP THERAPY

I. Yoshida[1,*], K. Tamura[2], S.-I. Kimura[3], H. Fujita[4], H. Iwasaki[5], S. Yano[6], H. Kusaba[7], K. Takahashi[8], T. Okamura[9], M. Yoshida[10]

[1]Hematologic oncology, National Hospital Organization Shikoku Cancer Center, Matsuyama, [2]General Medical Research Center, Fukuoka University, Fukuoka, [3]Hematology, Saitama Medical Center, Saitama, [4]Hematology, Saiseikai Yokohama Nanbu Hospital, Yokohama, [5]Infection Control and Prevention, University of Fukui, Faculty of Medical Sciences, Fukui, [6]Clinical Oncology and Hematology, Jikei University School of Medicine, Tokyo, [7]Medicine and Biosystemic Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, [8]Respiratory Medicine, Aichi Cancer Center Aichi Hospital, Okazaki, [9] Breast and Endocrine Surgery, Tokai University School of Medicine, Isehara, [10]Fourth Department of Internal Medicine, Teikyo University School of Medicine, Kawasaki, Japan

Background: Maintenance of dose intensity in patients with a potentially curable disease, such as diffuse large B-cell lymphoma (DLBCL), who are treated with chemotherapy, such as R-CHOP (rituximab, doxorubicin, cyclophosphamide, vincristine, prednisolone), is very important. We conducted a nationwide questionnaire survey to see how DLBCL patients are treated by Japanese oncologists, and reported a part of the findings at the 15th Japanese Society of Medical Oncology (JSMO) annual meeting (Kobe, 2017), showing that the dose of R-CHOP therapy was easily reduced (<65 years old: 16.4%; ≥65 years old: 44.3%). One of the reasons for dose reduction was bone marrow suppression and febrile neutropenia (FN). The results of further analysis of FN are reported here in detail.
Aims: To survey Japanese real-world FN treatment and the prophylactic use of granulocyte-colony stimulating factor (G-CSF), oral anti-bacterials, anti-fungals, drugs against herpes simplex, and sulfamethoxazole-trimethoprim during curative treatment using R-CHOP therapy for patients with DLBCL.
Methods: The FN Study Group of the Japanese Association of Supportive Care in Cancer (JASCC) designed a questionnaire on FN during R-CHOP therapy, which consists of 65 questions addressing the management of DLBCL in different practice settings. The survey was conducted from July 25 to August 22, 2016. JSMO members were requested to answer the questions on an online survey (SurveyMonkey.com), and the results were collected and analyzed.
Results: The survey was answered by 336 (4%) of the 8,158 JSMO members. Accordingly, 93.1% and 54.7% of respondents said they would take blood cultures before the start of anti-bacterial agents for inpatients and outpatients, respectively. Primary G-CSF prophylaxis among the non-elderly (<65 years old) is used by 23.6% of respondents to prevent FN in inpatients and by 26.8% for outpatients. It is routinely used for elderly (≥65 years old) patients by 39.9% of respondents for inpatients and by 45.3% for outpatients. The most commonly used antimicrobials for FN at outpatient clinics are oral new quinolones (66.8%), while inpatient FN is treated using intravenous infusion of 3rd generation or 4th generation cephalosporins (76.8%). Prophylactic use of oral anti-bacterials, anti-fungals, drugs against herpes simplex, and sulfamethoxazole-trimethoprim to prevent development of FN, herpes simplex infection, and Pneumocystis jirovecii pneumonia was reported by 12.2%, 18.2%, 3.7%, and 67.7% of the respondents, respectively. Only 10.2% of respondents use Multinational Association of Supportive Care in Cancer score as a reference to estimate FN risk, while 93.7% of them are fully aware of JSMO FN guidelines and 73.2% of them treat FN patients accordingly.
Summary/Conclusion: Although the respondents' rate of adherence to the JSMO FN guidelines for treatment of FN is relatively high, primary prophylactic use of G-CSF is low, especially for elderly (≥65 years old) patients. It is suggested that appropriate G-CSF use would substantially reduce FN further, allowing maintenance of relative dose intensity of R-CHOP therapy and improving the treatment outcomes of patients with DLBCL.

PB2045

PURPLE URINE BAG SYNDROME IN TWO ELDERLY WOMEN WITH ACUTE MYELOID LEUKAEMIA

C. Moret[1,*], R. Coll[1], D. Cruz[1], C. Babià[2], R. Guàrdia[1], J.N. Kelleher[1], M. Sagüés[1], Y. Gonzalez[1], J.M. Roncero[1], N.Y. Santos[1], D. Gallardo[1]

[1]Hematology and hemotherapy, ICO Girona, [2]Palliative Care Unit, Hospital de Santa Caterina, GIRONA, Spain

Background: Purple urine bag syndrome (PUBS) is a rare disorder characterized by purple discoloration of urine inside the urine collection bag and tube. It's usually seen in elderly women with a history of chronic or acute renal failure and constipation during longterm urinary catheterisation. Recognition of this entity is important to minimize concern and distress for patients and their relatives and also to avoid overmanagement.
Aims: CASE 1. An 86 year old woman was hospitalized because of anorexia, fever and asthenia. Her past medical history included arterial hypertension and chronic constipation. Basic investigations were done Hb 91 g/L, platelet count $18x10^9$/L and leucocytes 41.6 K/mcL. The bone marrow smear was compatible with Acute Myelomonocytic Leukaemia (AML) with negative cytogenetic and molecular biology. Due to the elderly conditions of the patient, palliative support treatment was decided.After 72 hours she presented lower abdominal pain with acute urinary retention and a urinary catheter was introduced. During the hospitalization, a purple discoloration of the urine bag and tube was noted. Urine examination showed urine leucocytes without RBC's with multi sensitive *E.Coli* on the urine culture. She was started on parenteral Ciprofloxacin and the catheter was changed, the purple urine returned to its normal colour but she died 48 hours later. **CASE 2.** An 88 year old woman was admitted to the Palliative Care Unit because of fever and dysuria in the context of neutropenia grade IV and severe constipation during the preceding days. Her past medical history included AML with normal karyotype and high rated FLT3-ITD diagnosed 6 months apreviously. She was receiving palliative support due to her age. On admission, she was found to have bleeding diathesis and acute urinary retention. Pansensitive *E.Coli* was isolated from blood and urine cultures. She was treated with intravenous Ciprofloxacin, urine catheterisation and laxatives. 48 hours later, she developed severe sepsis with hemodynamic instability and refractory hypotension. The urine in the bag was strikingly purple in colour. Because of the refractory sepsis condition and a terminal AML diagnosis,finally died 72 hrs after admission.
Methods: PUBS is a rare phenomenon where urine in catheter bags and tubing turns purple. The prevalence is unknown, range estimate between 8.3% and 42.1%.

Figure 1.

Results: PUBS occurs when the pigments indigo and indirubin, products of bacterial sulphatase and phosphatase mediated tryptophan metabolism,

accumulate in the urine and cross react with the PVC of catheter tubing to produce a purple hue. These bacteria include *Providencia spp, Citrobacter spp, Klebsiella pneumoniae,Proteus spp, Escherichia coli, Enterococcus spp, Morganella morganii, Pseudomonas aeruginosa* and B Streptococci. The principal PUBS risk factors include elderly patients, renal disease, chronic catheterisation, dehydratation, severe constipation, increased dietary thryptophan, high urinary bacterial load and urine alkalinity. There are several causes that change the urine colour:food dyes, drugs,haematuria, myoglobinuria, nephrolithiasis and poisons so it's important to consider some details from history to avoid unnecessary diagnostic tests. PUBS presentation is generally benign, but is associated with high morbidity and mortality due to the backgrounds of the patients. The management involve regular changing of urinary catheters,antibiotics if UTI is demonstrated and laxatives.
Summary/Conclusion: In the two cases described,the diagnosis was not controversial because urinalysis,urine and blood culture were positive.

PB2046

HHV6 REACTIVATION AFTER AUTOLOGOUS HEMATOPOIETIC CELL TRANSPLANTATION IS MANAGEABLE WITH IMMUNO GLOBULIN TREATMENT A SINGLE CENTER STUDY

S. Desilets, L. Gilis, S. Assaad, Y. Guillermin, P. Rey, M. Michallet, A. Belhabri, A.-S. Michallet*, L. Lebras, E. Nicolas-Virelizier

Hematology, Centre Léon Bérard, Lyon, France

Background: HHV6 reactivation is a well characterized phenomenon occurring after allogeneic hematopoietic cell transplantation (HCT). Recently, it has been more often reported after autologous HCT (auto-HCT) and the optimal management is not well established.
Aims: Our objective was to characterize the clinical and biological setting associated with HHV6 reactivation and describe its management.
Methods: We describe here a total of 18 cases of HHV6 reactivations that occurred in patients who received auto-HCT between years 2015 and 2017. There was X (%) males and Y (%) females, the median age at transplantation was 59 years (range: 39-67), diagnosis was lymphoma in majority of cases (N=15, 83%, 6 diffuse large B cell, 3 follicular, 4 mantle cell and 2 T cell), while 3 patients had multiple myeloma (MM). Conditioning regimen was melphalan 200 mg/m² in MM patients and for lymphoma patients it was thiotepa, etoposide, cytarabine melphalan (TEAM), (N=7), BCNU-EAM (BEAM) (N=3), Bendamustin-EAM (N=3) and BCNU-AM (N=2). After transplantation, the median time to neutrophils recovery (neutrophils >0.5 x 10^9/L) was 11 days (range: 9-13).
Results: The predominant symptom that justified the detection of HHV6 was the apparition of fever. (in 14 patients, 78%). The other symptoms were isolated pancytopenia for 3 patients (17%), and one patient had an isolated erythema. The symptoms associated with fever were diarrhea for 3 patients, pneumopathy for 2 patients; erythema for 3 and 1 macrophagic activation syndrome. Sixteen patients received intravenous immunoglobulin after HHV6 reactivation. The other 2 patients had a spontaneous resolution of their symptoms. None of the patients required antiviral therapy for the treatment of the HHV6 reactivation. The HHV6 reactivation did not have an impact on engraftment and hematological recovery.
Summary/Conclusion: We report a retrospective analysis of 18 cases of HHV6 reactivation following autologous stem cell transplant. The majority of the patients was successfully managed with intravenous immunoglobulin, avoiding therefore the possible toxicities of antiviral therapies.

PB2047

CLINICAL AND LABORATORY FEATURES OF INVASIVE ASPERGILLOSIS IN PATIENTS WITH B-CELL LYMPHOMA

J. Chudinovskikh[1], T. Semiglazova[1], O. Shadrivova[2], S. Ignatieva[2], S. Alekseev[1], I. Zuzgin[1], N. Klimko[2,*]

[1]N.N. Petrov National Medical Research Center of Oncology, [2]I.I. Mechnikov North-Western State Medical University, Saint-Petersburg, Russian Federation

Background: Invasive aspergillosis is the predominant invasive fungal infection in patients with haematological malignancies, including patients with B-cell lymphoma.
Aims: To study clinical and laboratory features of invasive aspergillosis (IA) in patients with B-cell lymphoma.
Methods: The study included 57 patients with Hodgkin lymphoma (HL), age from 16 to 65 years (median-33), and 51 patients with non-Hodgkin lymphoma (NHL), age from 19 to 74 years (median-50). For the IA diagnosis criteria EORTS/MSG 2008 were used.
Results: Before the development of IA all patients in both groups received cytostatic chemotherapy, the average number of courses-6. The main risk factors for IA in patients with HL and NHL were: prolonged lymphocytopenia (70% *vs* 48%), neutropenia (64% *vs* 71%), glucocorticosteroids use (61% *vs* 85%), and B-symptoms (63% *vs* 48%). In most cases nosocomial IA was diagnosed in both groups (65% *vs* 83%). The main etiological agents were: A. fumigatus (50% *vs* 39%), A. niger (43% *vs* 33%), and A. flavus (7% *vs* 8%). The lungs were involved in 100% cases, 6% NHL patients had ≥2 organs involvement. Galactomannan test was positive in BAL fluid in 75% patients with HL and in 78% patients with NHL. The presence of septated mycelium was observed at microscopy of BAL in 13% *vs* 22% patients with IA. Aspergillus spp. in BAL culture was obtained in 27% *vs* 47% patients with IA. Clinical manifestation of IA was nonspecific in both groups: fever (83% *vs* 76%), cough (75% *vs* 59%), dyspnea (50% *vs* 40%), bronchial obstruction (4% *vs* 9%), and hemoptysis (2% *vs* 10%). "Probable" IA was diagnosed in 98% of cases, "proven"-in 2% in HL patients, 88% and 12% in NHL patients. Antifungal therapy received 100% patients. The main antifungal drug was voriconazole-88% *vs* 98% cases. Overall 12-weeks survival in patients with Hodgkin lymphoma was 84%, in patients with non-Hodgkin lymphoma-81%.
Summary/Conclusion: The main risk factors for IA in patients with HL and NHL were prolonged lymphocytopenia (70% *vs* 48%), neutropenia (64% *vs* 71%), steroids use (61% *vs* 85%), and B-symptoms (63% *vs* 48%). Etiology agents were A. fumigatus (50% *vs* 39%), A. niger (43% *vs* 33%), and A. flavus (7% *vs* 8%). Clinical symptoms were nonspecific. The overall 12-weeks survival rate in patients with HL was 84%, in patients with NHL-81%.

PB2048

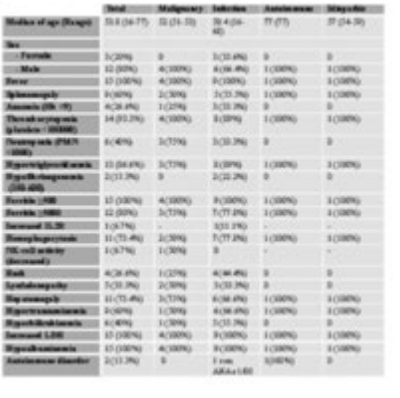
HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN THE HEMATOLOGY SERVICE OF A TERTIARY HOSPITAL. RETROSPECTIVE STUDY OF A SERIES OF CASES

R.M. Rodriguez Nuñez*, S. Suárez Ordóñez, A. Nieto Vázquez, C. Insua Vilas, M.D.C. Albo López

Hematology, Hospital Alvaro Cunqueiro, Vigo, Spain

Background: Hemophagocytic lymphohistiocytosis (HLH) is a serious, uncommon and underdiagnosed disease, characterized by an exaggerated inflammatory response due to the activation of macrophages and T lymphocytes; and it requires early diagnosis and treatment. HLH can occur as a family/genetic or sporadic/secondary disorder. It can appear spontaneously or triggered by neoplasms, inflammatory disorders or infections, with Epstein Barr virus (EBV) being the most frequent. Clinical manifestations are not specific, presenting these different signs and symptoms, the most common being high temperature, cytopenia and hepatosplenomegaly.
Aims: The aim of the study was to describe the clinical profile of HLH in a tertiary hospital, from 2011 to the present. We analyzed its etiology, clinical and laboratory characteristics, as well as its evolution and treatment.

Table 1.



Methods: Data were collected from patients diagnosed with HLH at the University Hospital of Vigo from October 2011 to the present. Patients who met the diagnostic criteria proposed by the International Hystiocyte Society in 2004 were included, and those treated for HLH who did not meet the criteria previously mentioned were excluded.
Results: We included 15 cases of HLH, corresponding to 10 patients of which 7 were male and 3 were female, with an overage age of 53.8 years

old (range, 16-77). 9 cases were secondary to infections, 4 to hematological diseases, 1 to autoimmune disorder and 1 was idiopathic. All of the patients were attended to with high temperature and high ferritin. Hemophagocytosis was evident in 11 of the 15 cases (in 2 of them a biopsy of the bone marrow was not performed and in 2 others it was inconclusive). NK-cell activity was studied twice being low in one case. Determination of IL-2R was only carried out in one of the cases being abnormal. In the majority, high temperature disappeared before 10 days, except in four cases in which it remained 12, 14, 16 and 33 days. 5 of them died: 2 cases were secondary to hematological disease, 2 to infection and 1 secondary to autoimmune disorder. Among the received treatments we find: HLH-2004 and HLH-1994 Protocols; conventional chemotherapy in cases related to hemopathy, and treatment of causal infection.
Summary/Conclusion: In our series, as in the literature, the most frequent cause of secondary HLH is EBV infection; being also the most prevalent diagnostic criteria high temperature and high ferritin. In most cases a diagnostic delay is observed, since in the initial moment they did not fulfill enough criteria, due in part to the fact that only in some episodes the determination of NK-cell activity and IL-R2 is done, because of the inaccessibility of the studies. Including HLH in the diagnostic algorithm of persistent febrile syndrome with cytopenias and elevation of acute phase reactants; it would allow an early diagnosis and an early treatment that would improve the prognosis. Likewise, correct etiology and microbiological identification would imply, in our experience, an adaptation of the treatment, avoiding the possible pharmacological toxicity.

PB2049

RISK FACTORS ASSOCIATED TO MORTALITY IN PATIENTS WITH HEMOPATHIES REQUIRING ENTRY IN THE INTENSIVE CARE UNIT

E. Morente Constantin[1,*], A.B. Rivera Gines[1], P. Romero Garcia[2], P.A. Gonzalez Sierra[1], F. Manzano Manzano[3], A. Garcia Ruiz[1], M. Jurado Chacon[1]

[1]Servicio de Hematología y Hemoterapia, Hospital Universitario Virgen de las Nieves, Granada, [2]UCI, Hospital De Santa Barbara, Soria, [3]UCI, Hospital Universitario Virgen de las Nieves, Granada, Spain

Background: Patients who present oncohematological pathology require admission to the ICU at times. Generally this is due to two situations that can sometimes overlap. First, to treat pathologies secondary to their underlying disease, and second, due to complications of the treatment they receive.
Aims: The main objective is to analyze the prognosis of hematological patients who have required admission to the ICU, as well as to study the factors that can modify this prognosis.
Methods: This is a descriptive study of 90 oncohematological patients admitted to the ICU of our center between 2010 and 2015. The following data have been collected: sex, age, date of admission to the ICU, reason for admission to the ICU, baseline homeopathy and if it was active at the time of admission, necessity of mechanical invasive ventilation (MIV) and the number of days, death during admission, haematopoietic progenitor cell transplantation and type, existence of GVHD and neutropenia.
Results: Out of the total of 90 patients, 62 men (68%) and 28 women (32%) were registered. The average age of the patients was 53 years, with an age range of 20 to 83. The overall mortality at admission was 67% (60 patients), and if we divide it by sex we observed that the mortality in men was 69% and in women of 64%. The most frequent hematological pathologies were: 32 patients had non-Hodgkin's lymphoma (35.5%); 19 acute myeloid leukemia, (21.1%); 9 Hodgkin's lymphoma (10%); 6 acute lymphoid leukemia (6.67%); 6 multiple myeloma (6.67%); 4 myelodysplastic syndrome (4.4%); 4 chronic lymphoid leukemia (4.4%); 2 chronic myeloid leukemia (2.2%); 2 polycythemia vera (2.2%). Finally, 1 patient with aplastic anemia, 1 with cardiac amyloidosis and 1 with intracranial plasmacytoma. There are 2 cases with unknown diagnosis. The most frequent causes that triggered the admission are (only or associated): acute respiratory failure, this pathology being the most frequent, since it is presented in 51 patients (56.67%), followed by infections in their different modalities corresponding to 26 patients (28.89%) and finally, heart failure, 9 patients (10%). The fact of requiring MIV was associated with a mortality of 71% (69 patients), a figure that rises to 74% if it is needed for a period of 7 or more days. Of the 26 patients who underwent transplantation of hematopoietic progenitors, 23 died, accounting for 88% of deaths (83% in autologous patients and 90% in allogeneic patients). Of the 61 patients who had their active homeopathy at the time of admission, 37 (60%) died, a figure that reaches 93% (14 out of 15) when they had a GVHD. A total of 34 patients presented neutropenia on admission to the ICU, and 82% died (28).
Summary/Conclusion: Patients with oncohematological diseases have a higher risk of presenting complications that require admission to the ICU. They also represent a type of patient with a high mortality after admission (overall mortality of 67%). According to our study, the situations that are linked to a higher mortality are: active GVHD (93% mortality), being a transplant recipient (88%), neutropenia (82%) and requiring MIV (71%).

PB2050

THROMBIN GENERATION MAY BE A PROGNOSTIC MARKER IN CHILDREN WITH FEBRILE NEUTROPENIA

A. Koca Yozgat[1], I. Eker[2,*], O. Gursel[3], M.F. Kılınckaya[4], D. Ozyoruk[1], A. Kara[5], N. Yaralı[5], N.Y. Ozbek[5]

[1]Pediatric Hematology and Oncolgy, Pediatrics Hematology Oncology Training and Research Hospital of Ankara Health Sciences University, Ankara, [2]Pediatric Hematology, Afyon Kocatepe University, Afyonkarahisar, [3]Pediatric Hematology, Gulhane Training and Research Hospital of Ankara Health Sciences University, [4]Biochemistry, Numune Training and Research Hospital, [5]Pediatric Hematology and Oncology, Pediatrics Hematology Oncology Training and Research Hospital of Ankara Health Sciences University, Ankara, Turkey

Background: Febrile neutropenia is a common occurrence in pediatric hematology patients due to myelosuppressive chemotherapy. Sepsis, which is an amplified, body-wide inflammatory response to an infection, is observed in 50% of patients with febril neutropenia. Effective management of sepsis during neutropenia stage could reduce the mortalities of cancer treatments. In addition to its direct role in promoting and regulating clot formation, it is documented in recent years that thrombin is a key component of inflammatory response also. It both regulates and enhances inflammatory responses against infections and tissue damage.
Aims: In our study, we aimed to observe the changes in thrombin formation in febrile neutropenia patients. Also we evaluate whether the amount of thrombin formation could be implified as a prognostic marker in febrile neutropenia.
Methods: Recruitment of patients took place at the Pediatric Hematology and Oncology Department of Pediatrics Hematology Oncology Training and Research Hospital of Ankara Health Sciences University between January to August 2016. Thrombin levels were measured by Thrombin Generation Test (TGT) by flourogenic method. Endogenous thrombin potentials (ETP; Total Thrombin Level in Samples) of 35 patients were evaluated. Results of patients were compared to results of 50 healthy children. Platelet Poor Plasma Samples of febrile neutropenia group were collected at initial admission, at 48.th hour and after recovery from neutropenia.
Results: Patient's mean day of recovery from neutropenia was 19.8±11.7 (min-max: 7-49). Mean ETP value at the 48th hour (1998±1037 nanomol x minute) was statistically higher than the mean ETP value of the initial admission (1471,8±582 nanomol x minute) (p:0.007), mean ETP value of control group (1260±267 nanomol x minute) (p:0.001) and mean ETP value of the time when neutropenia resolved (1492±530 nanomol x minute) (p: 0.008). Mean peak value of thrombin at the 48th hour (386±239 nmol/L) was statistically higher than the mean peak value of the initial admission (271±112 nmol/L) and the mean peak value of the time when neutropenia diminished (297±123 nmol/L) (p:0.02).
Summary/Conclusion: Thrombin is a multifunctional protein involves in coagulation, anticoagulation, platelet activation, endothelial activation, production of growth factors and proliferation of both smooth muscle cells and fibroblasts. Sepsis; is the main cause of mortality in the neutropenic phase following the treatment of malignancies. Our results support that thrombin formation may be used as a prognostic marker in pediatric patients with febrile neutropenia associated with chemotherapy, there is a need for further studies in larger subject groups.

PB2051

FACTORS CONTRIBUTING TO SEVERE PNEUMONIA IN PATIENTS AGAINST HEMOTOLOGICAL MALIGNACIES

Borisova*

Department of Medical and Social Expertise and Rehabilitation, State Establishment "Dnepropetrovsk Medical Academy of Health Ministry of Ukraine", Dnipro, Ukraine

Background: According to the World Health Organization, for the period from 2000 to 2012 among the 10 leading causes of death in the world, lower respiratory tract infections took 4th place. In patients with hemoblastomas, the problem of diagnosis and treatment of pneumonia is due to the high incidence, lack of clinical manifestations, severe course, frequent complications and rapid development of fatal consequences.

Aims: To determine the factors which cause the severity of pneumonia based on the study of a complex of clinical and laboratory, anamnestic and immunological parameters of patients with severe immunity disorders on a background of oncohematological diseases.
Methods: To solve this problem, a computer database was created from the results studies of retrospectively analyzed archival data of 605 cases of hospitalizations and the results of a prospective study of 276 cases of hospitalization of patients with oncohematological diseases in "City Multidisciplinary Clinical Hospital No. 4", Dnipro, Ukraine, 2010-2015.
Results: The results of the study proved that the severity of pneumonia and the unfavorable prognosis of the disease were most influenced by factors that can be conventionally grouped into groups: indicators characterizing oncohematological disease (age of the patient (p=-0.25, p<0.001), the form of oncohematological disease (p=0,29, p<0,001); the number of courses of HT (from 8) (p=0,33; p<0,001); anemia (p=0,61; p<0,001); presence of neutropenia (p=0, 46, p & lt; 0.001); indicators characterizing the inflammatory process in the lungs (Gp - pathogens (p=0.48, p<0.001); presence of complications: hemoptysis (p=0.36, p<0.001), pleurisy (p=0.58, p<0,001), respiratory failure (p=0,32, p<0,001); presence of cough (p=0,30; p<0,001); wet wheezes (p=0,48; p<0,001); ESR (p=0.38; p<0.001) and indicators of immune reactivity, characterizing the degree of immunodeficiency.
Summary/Conclusion: When the overall variance was determined, which determines the factors that cause the severity of pneumonia and the occurrence of an unfavorable prognosis in patients with oncohematological diseases, the contribution of the degree of immunodeficiency was about 52%.

PB2052

THE DYNAMICS OF PROTEIN C IN AN INFECTIVE CHRONIC SYSTEMIC INFLAMMATORY CONDITION: HUMAN IMMUNO DEFICIENCY VIRUS INFECTION

E. Chukwuka[1,2,*], O. Awodu[1]

[1]Haematology and Blood Transfusion, University of Benin Teaching Hospital, Benin, Nigeria, [2]Laboratory Department, Maternity and Children Hospital, Al Mubaraz, Saudi Arabia

Background: Human Immunodeficiency Viral (HIV) infection is a chronic systemic inflammatory and thrombogenic condition. Protein C has well defined anticoagulant functions, however, its anti-inflammatory functions which act distinctively from the 'anticoagulants', with regards to structure, receptor, signaling pathway and effect, is an exploit of current active research. Understanding the dynamics of this protein in a condition like HIV infection may give some insight to the functionality of this unique molecule particularly in the Nigerian setting.
Aims: To determine the levels of protein C, some inflammatory markers; Erythrocyte Sedimentation Rate (ESR) and C-reactive Protein (CRP) and d-dimer (a marker of ongoing coagulation) in HIV infected patients and evaluate the relationship between protein C and these parameters.
Methods: This is a cross-sectional study that assessed the activity of Protein C using chromogenic methods (Technochrom), ESR using Westergreen method, CRP and D-dimer using ELISA test kits (Agappe and Immunoclon respectively) in a total of 210 participants comprising of three groups; HIV seronegative participants (n=70), HIV seropositive therapy naive participants (n=70) and HIV seropositive participants on HAART (n=70). Informed consent from each participant and an approval from the medical ethical committee of the institution where study was conducted were obtained.
Results: The mean protein C level in the seronegative, HAART naïve and HAART experienced participants were 0.618±0.446 IU/ml, 0.548±0.473 IU/ml and 0.340±0.226 IU/ml respectively. There was a significant difference between the mean of the HAART naïve and HAART experienced participants (p<0.001). The mean ESR and CRP in the seronegative, HAART naïve and HAART experienced participants were 21.1±14.3mm/hr and 18.77±17.51mg/dl; 71.2±41.1mm/hr and 39.92±27.28mg/dl; and 40.8±35.3mm/hr and 27.99±21.54mg/dl respectively. There was a significant difference across the three groups of participants (p<0.001). The mean d-dimer levels for therapy naïve was 168.0±41.0 mg/L and 168.0±17.0 mg/L for the HAART experienced, the mean difference was statistically significant when compared with the seronegative participants (92.0±11.0 mg/L) p<0.001. The correlation between protein C and other parameters assessed only showed a significant correlation with ESR (r=0.36, p<0.001).
Summary/Conclusion: ESR and CRP levels were significantly higher in HIV infected participants, though participant on HAART were associated with a much lower level of inflammatory markers. D-dimer levels were significantly higher with HIV infection and the elevations persisted despite HAART. Protein C levels were significantly lower with HAART when compared with the other groups of participants. Hemostasis is grossly altered in HIV infection despite an associated reduced inflammatory state with HAART.

PB2053

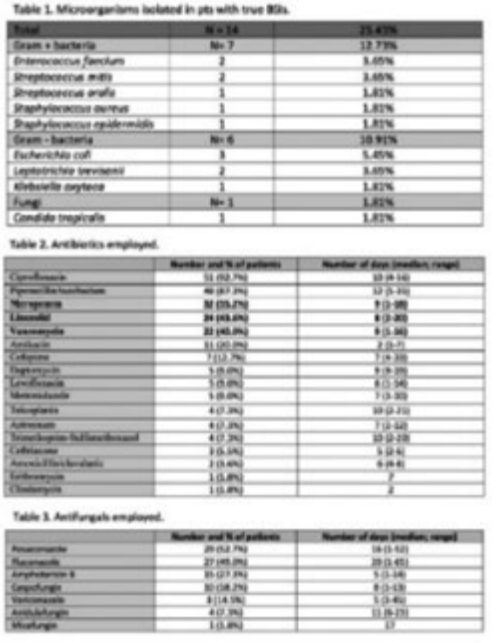
ANALYSIS OF INFECTIONS AND USE OF ANTIMICROBIALS IN PATIENTS UNDER-GOING INTENSIVE INDUCTION CHEMOTHERAPY FOR NEWLY DIAGNOSED ACUTE MYELOGENOUS LEUKEMIA

A. Aranbarri Larrañaga*, C. Lombardi Iglesias, L. Ondarra Segurola, I. Otero Longo, A.N. Bizjak Gómez, M. Araiz Ramirez, J.C. Vallejo LLamas

Hematology, University Hospital Donostia, San Sebastian, Spain

Background: It is known that patients undergoing intensive induction chemotherapy (IIC) for acute myeloid leukemia (AML) have a high risk for developing infectious complications.
Aims: To describe microbiologically documented infectious episodes, the use of antimicrobials and the outcome of all consecutive patients undergoing IIC for newly diagnosed AML during the last 4 years.
Methods: A total of 111 adult patients were diagnosed with AML at the Donostia University Hospital from 2014 to 2017. Patients with AML-M3 (8 pts) and those who did not receive IIC (48 pts) were excluded for this analysis. In total, 55 pts were treated with IIC: 50 with Ida + Ara-C (3 + 7) and 5 with Flag-Ida. The median age was 61 years (23-78). 30 were men and 25 women. Most of the pts (88%) stayed in HEPA-filtered rooms.
Results: Median hospital admission was 30 days (22-77). Five pts (9.1%) died during admission. Thirty one pts (56.4%) achieved complete remission after the IIC. A total of 336 blood cultures (BC) were performed (average: 6.11 BC/pt, range: 1-17). 21 BC (6.25%) were positive (false positives were excluded). Fourteen pts (25.45%) had at least 1 true bloodstream infection (BSI), whose characteristics are shown in Table 1. Other cultures performed were: urine (136), stool (98) and others (38). The antimicrobials used are shown in Tables 2 and 3. Only one of the 5 pts who died was due to infectious mortality (1.81%) (sepsis due to *Candida tropicalis*). No other IFI cases were documented.

Table 1.



Summary/Conclusion: 1) fungal BSIs, in general, and IFI incidence, in particular, were very low in this group of high risk pts (1.81%). This fact could be attributed to the combination of several factors: a) the majority of the pts stayed in HEPA-filtered rooms; b) the antifungal prophylaxis used; c) the use of empirical antifungal therapy for prolonged neutropenic fever. 2) Bacterial BSIs were relatively frequent (23.64%) among patients undergoing IIC for newly diagnosed AML. Gram + bacteria (12.73%) were slightly predominant over Gram - bacteria (10.91%). There was no mortality due to a bacterial infection. Our analysis shows, however, a high consumption of carbapenems (55.2% of pts), vancomycin (43.6% of pts) and linezolid

(22% of pts). Due to the growing concern and awareness of the problems of antibiotic resistance around the world, we recently implemented a new protocol for the management of fever, more conservative and restrictive, with the hope of reducing the use of unnecessary antibiotics, maintaining the low rate of infectious mortality.

PB2054

MOLECULAR DETECTION OF CYTOMEGALOVIRUS DNA IN ADULT EGYPTIAN PATIENTS WITH ACUTE LEUKEMIA RECEIVING CHEMOTHERAPY

M. Halaby[1,*], A. Nazir[1], N. Zaki[1], G. Helaly[2], F. Elsayed[1], S. Shawky[2]

[1]Internal Medicine Hematology unit, alexandria university faculty of medicine, [2]Microbiology, medical research institute, alexanderia, Egypt

Background: There are abundant data on the pattern and time course of CMV reactivation or primary infection in allogeneic stem cell transplant (allo-SCT) recipients, and well-defined strategies have been developed for prophylaxis, screening and preemptive treatment.(1) However, there is a remarkable paucity of data regarding CMV reactivation in adult patients with acute leukemia (AL) receiving chemotherapy. Re-activation can lead to prolonged cytopenias, fever and other manifestations, which are often misdiagnosed and treated empirically with antimicrobials.(2)
Aims: To investigate the prevalence of CMV DNA and its possible re-activation in adult Egyptian patients with AL receiving chemotherapy.
Methods: The study was on conducted on 63 Egyptian adult patients with newly diagnosed AL,admitted to the Hematology Unit,Internal Medicine Department, Alexandria Main University Hospital. The patients included 36 AML and 27 ALL cases. CBC, bone marrow aspiration and immunophenotyping were done at presentation. Patients were treated according to 3+7 standard protocol for AML and Larson protocol for ALL and were followed up clinically and by microbiological investigations on days 0, 14, 28 and 100 from the start of induction chemotherapy. CMV DNA was detected by real-time PCR on day 0 and day 100.
Results: AML patients included 16 males and 20 females with an age ranging from 18 to 57 years with a mean of 38.36±11.98 years. ALL patients were 17 males and 10 females and included 21 B-ALL and 6 T-ALL cases. Their ages ranged from 18 to 59 years with a mean of 26.19±10.24 years for B-ALL and from 19.0 to 43.0 years with a mean of 26.83±9.20 years for T-ALL. 100% of enrolled patients were neutropenic and febrile on day 14. Positive blood cultures for bacteria were obtained in 72.2% of AMLs and 76.9% of ALLs. Invasive sino-pulmonary fungal infection was diagnosed in 25% of AMLs, 30% of B-ALLs and 66.7% of T-ALL patients. By day 28, complete remission (CR) was achieved in 70.8% of AML and 80% of ALL patients, partial remission in 8.3% of AMLs and 5% of ALLs, while 20.8% of AML and 22.2% of ALL patients were refractory to induction chemotherapy. Mortality at day 28 was 33.3% and 22.2% in AML and ALL patients; respectively. None of the studied patients exhibited symptoms or signs related to CMV during the whole follow-up period. No CMV DNA was detected by real-time PCR neither on day 0 nor on day 100 among all AL patients enrolled in the study. Six out of patients in CR underwent allo-SCT from an HLA-related sibling and all of them were seropositive for CMV IgG and negative for CMV IgM throughoutand for 6 months following the transplant procedure.
Summary/Conclusion: Although CMV infection or re-activation is a serious problem in immunocompromised patients, yet it seems from ourresults that adult patients with de-novo AL do not have the same risk as neither they manifest nor CMV DNA was detected by PCR on days 0 and 100 from the initiation of standard induction chemotherapy. However,we recommend further studies with larger samples, different chemotherapy protocols and for longer follow-up period to confirm the status of CMV infection/re-activation in adult AL patients especially among those who are candidates for allo-SCT.

PB2055

ACUTE HEPATITIS E OCCURRING IN ONCOHEMATOLOGIC PATIENTS: A SINGLE CENTRE EXPERIENCE

N.S. Fracchiolla[1,*], V. Mattiello[1], L. Pettine[1], A. Freyrie[1], M.C. Goldaniga[1], L. Baldini[1], F.G. Rossi[1], A. I. Gregorini[1], V. Ferla[1], M. Sciumè[1], G. Lunghi[2], S. Piconi[3], A. Cortelezzi

[1]Oncohematology Division, IRCCS Ca' Granda - Maggiore Policlinico Hospital Foundation and University of Milan, [2]U.O.S Virology, IRCCS Fondazione Ca' Granda Ospedale Maggiore Policlinico di Milano, [3]Division of Infectious Diseases Unit, University of Milano, Ospedale L. Sacco, Milan, Italy

Background: Hepatitis E virus (HEV) is a zoonosis mainly transmitted via oral-fecal route. Recent reports confirmed that HEV infection can be also transmitted through the transfusion of blood products in immunocompromised patients. HEV infection varies from a simple acute hepatitis with spontaneous recovery to acute liver failure and to chronic infection in immunosuppressed patients.
Aims: To describe two cases of acute HEV infection occurring in two patients with hematologic malignancies.
Methods: Adult patients with Non-Hodgkin lymphoma who developed HEV infection were selected from the institutional database.
Results: A 67-year-old man (pt 1) diagnosed with advanced stage peripheral T-cell lymphoma underwent multiple cycles of chemotherapy (6 CHOEP and 4 GDP) and autologous stem cell transplantation (ASCT) in partial remission. At transplantation the patient was negative for anti-HEV immunoglobulins. Three months after ASCT abrupt onset of diarrhea, weight loss, jaundice and rising in liver tests, prompted evaluation and hospital admission. Laboratory studies revealed AST 832 UI/L, ALT 1128 UI/L, and raised cholestatic liver enzymes (gGT 456 UI/L, total bilirubin 9.4mg/dl, direct bilirubin 8,4mg/dl). A 65-year-old man (pt 2) diagnosed with IV-B stage DLBCL started HyperCVAD. Viral serology before chemotherapy was negative. The first cycle of therapy was complicated by the increase of transaminases and cholestasis levels: AST 347 UI/L, ALT 365 UI/L, gGT 407 UI/L, total bilirubin 4.9mg/dl, direct bilirubin 3.5mg/dl. Both pts underwent RBCs and platelets transfusions. Hepatitis A, B, C, EBV and CMV infections were ruled out, autoimmunity tests were negative. Abdomen ultrasound did not reveal biliary ducts dilation, nor focal hepatic lesions. In pt 1, anti-HEV IgM and HEV RNA in the serum were positive, while pt 2 was positive only for HEV-RNA ($1.3x10^6$ genomes/ml) but tested negative for IgM and IgG. Diagnosis of acute HEV infection was confirmed. No immunosuppressive therapy was ongoing. Possible routes of infection were investigated: no contact with animals, no travels to endemic countries and no additional cases of HEV infections were reported at our unit during their hospitalization. Close monitoring of RNA-titer of the first patient showed progressive decrease of serum copies (36720 copies/ml à 1538 copies/ml); HEV-RNA was no longer quantifiable during serological controls after one month. Symptoms and liver function tests improved during admission with the sole supportive care. Excluding virus persistence, no antiviral treatment with ribavirin was started. To pt 2 was given ribavirine therapy, 600 mg for six months, for prolonged detection of serum HEV-RNA. Progressive reduction of HEV-RNA titer together with decrease of hepatic enzymes was observed with close monitoring, and clearance after four months from diagnosis was detected.
Summary/Conclusion: Acute hepatitis E case reports have increased in recent years in developed countries. Systematic HEV RNA screening of all blood donation is under consideration and already implemented in some Western countries as the risk and importance of transfusion-transmitted HEV infections by contaminated blood products is currently a controversial topic in transfusion medicine. A model estimates that receiving blood components from 13 donors carries a similar risk to one year of dietary exposure. The present report suggests that patients with hepatitis who have received blood products should be screened for HEV, especially if immunosuppressed.

PB2056

INFECTIOUS COMPLICATIONS IN FIRST THREE CYCLES OF AZACITIDINE DID NOT NEGATIVELY IMPACT OVERALL SURVIVAL OF MYELODYSPLASTIC SYNDROME PATIENTS

Z.X. Ng[1,*], B.M.H. Keng[1], Z.Y. Myint[2], G.C. Wong[3]

[1]Yong Loo Lin School of Medicine, National University of Singapore, Singapore, [2]Singapore General Hospital, Singapore, Singapore, [3]Department of Haematology, Singapore General Hospital, Singapore, Singapore

Background: It is well known that infectious complications (ICs) may occur frequently in patients with myelodysplastic syndrome (MDS) on azacitidine treatment. These ICs may reduce the success rate of therapy. We retrospectively examined the clinical course of 49 patients treated with azacitidine at a large tertiary hospital in Singapore between 2010 and 2016, and evaluated if ICs affected their survival.
Aims: This paper aims to evaluate the incidence and mortality of infectious events in patients with MDS who receive azacitidine treatment, predisposing risk factors for infections, the value of antimicrobial and antifungal prophylaxis, and clinical parameters affecting survival.
Methods: Clinical data of MDS patients were obtained from our Institution Review Board-approved database. These included severity of MDS using the IPSS-R score, number of cycles and responses to azacitidine, number of days of hospitalisation, type of ICs, presence of comorbidities, absolute neutrophil count (ANC) at diagnosis, and overall survival (OS).

Results: A total of 307 cycles of azacitidine were administered to 49 patients (mean age 64.5±12.3 years). All patients received antibacterial prophylaxis, and all but 5 received antifungal prophylaxis. The median number of cycles was 5 (range 1-42). Thirty-two patients had at least one IC. In patients who received at least 6 cycles of azacitidine, IC rates were not higher in the first three cycles of treatment compared to the next three cycles. ICs in the first three cycles of treatment were not associated with lower OS as compared to no ICs within the first three cycles (p=0.069). IC rates were correlated with number of days hospitalised (p<0.001), but not correlated with age, response to treatment, IPSS-R score, or ANC at diagnosis. Median OS from date of diagnosis was 12 months (range 1-113 months). OS was correlated with age (p=0.007), IPSS-R score (p=0.01), number of cycles (p=0.027), and response to treatment (p=0.024), but not ANC at diagnosis. Infections were predominantly bacterial, with none of our patients experiencing any fungal infection.
Summary/Conclusion: The presence of infectious complications in the first three cycles of azacitidine therapy did not negatively impact overall survival. Age, response to treatment, IPSS-R score, and ANC at diagnosis did not predict for infectious complications. Antibacterial and antifungal prophylaxis may have beneficial effects.

PB2057

INFECTIONS IN PATIENTS WITH LYMPHOMA: AN ANALYSIS OF INCIDENCE, RELATIONSHIP AND RISK FACTORS

A. Yildiz[1,*], M. Albayrak[1], Ç. Pala Öztürk[1], O. Şahin[1], H.B. Afacan Öztürk[1], G. Güneş[1], S. Maral[1], H. Okutan[2]

[1]Hematology, University of Health Sciences, Diskapi Yildirim Beyazit Training and Research Hospital, [2]Hematology, Lösante's Children and Adult Hospital, Ankara, Turkey

Background: Bacterial infections and febrile neutropenia are major causes of morbidity and mortality in patients with hematological malignancy.
Aims: The aim of this study was to investigate the incidence and risk factors of infections in lymphoma patients.
Methods: This retrospective study was conducted on 200 lymphoma patients diagnosed and treated between January 2009 and December 2017 in Diskapi Yildirim Beyazit Training and Research Hospital, a tertiary referral hospital in Ankara, Turkey. A total of 65 patients developed at least one infection episode (IE) while 37 patients developed febrile neutropenia (FN).
Results: The mean follow-up period was 20.09±19.81 months. The incidence of IE was 32.5% (65/200) and FN was 18.5% (37/200). Analysis of the data revealed that the patients with IE had significantly higher diagnosis of central nervous system lymphoma (CNSL), lower baseline hemoglobin, lower baseline hematocrit, higher baseline lactate dehydrogenase levels, higher usage of central cathater, and a higher number of chemotherapy lines compared to patients with no IE. In logistic regression analysis, disease subtype of CNSL, usage of central catheter and LDH were found to increase the risk of infection. The OR for CNSL was 37.866 (p=0.003), 2.679 for central catheter (p=0.008) and 1.001 for LDH (p=0.011).
Summary/Conclusion: The risk of infection in patients with lymphoma was associated with central catheter usage, higher LDH levels and a diagnosis of CNSL. Baseline hematological parameters were not determined to have any impact on the occurrence of infection. Clinicians must be aware of these data soon after diagnosis as they will be required for the management of lymphoma patients through the whole period.

PB2058

INVASIVE SCEDOSPORIOSIS AND LOMENTOSPORIOSIS IN PATIENTS WITH HEMATOLOGICAL DISEASES IDENTIFIED IN THE LITERATURE AND THE FUNGISCOPE™ REGISTRY

D. Seidel[1,*], A. Meißner[1], M. Lackner[2], M. Stecher[1], S. Mellinghoff[1], L.A. Durán Graeff[1], J. Salmanton Garcia[1], A. Hamprecht[1], M.J. Vehreschild[1], P. Köhler[1], H. Wisplinghoff[3], J.J. Vehreschild[1], O.A. Cornely[1]

[1]University Hospital Cologne, Cologne, Germany, [2]Medical University of Innsbruck, Innsbruck, Austria, [3]Laboratory Wisplinghoff, Cologne, Germany

Background: Hematological malignancies and their treatment are main risk factors for invasive fungal infections (IFD). Less frequent IFD such as scedosporiosis and lomentosporiosis (IS/IL) are emerging complications in such patients, with mortality rates up to 90%. Efficient diagnostic and treatment approaches for IS/IL are not known or validated yet. Furthermore, intrinsic resistance to almost all available antifungals hamper appropriate treatment decisions.
Aims: Clinical data were collected, to describe clinical presentation and management of IS/IL in hematological patients and to identify effective treatment strategies.
Methods: Data of probable and proven IS/IL cases collected from the literature and FungiScope™, an international web-based registry, were analysed. Data collected include demographics, underlying conditions, sites of infection, antifungal treatment, susceptibility to antifungals and outcome.
Results: Clinical data of 29 patients with infections caused by *Scedosporium* spp. and 28 by *Lomentospora prolificans*) with a hematologic disease were collected. Most patients had acute leukaemia (52.6%), lymphoma (15.8%) or chronic leukaemia (8.8%); 29.8% received allogeneic HSCT. Blood stream infection was confirmed in 21 (75%) IL patients and 1 (3.4%) IS patient. Other most frequently affected organs were lung, eye and CNS (49.1%, 24.6%, 19.3%). All patients received antifungal drugs for treatment (median 22 days, IQR 5-92 days) and 28.1% patients were surgically treated in addition. *L. prolificans* isolates had MICs of 1 mg/L and higher for amphotericin B, voriconazole, posaconazole, and caspofungin. *Scedosporium* spp. isolates had voriconazole MICs of 1 mg/L and lower. Most patients were treated with voriconazole (68.4%) and/or amphotericin B (56.1%). Terbinafine in combination with voriconazole was frequently used for treatment of IL (35.7%). Day-42 and overall mortality was higher in IL (71.4% and 85.7%) than IS patients (27.6% and 55.2%).
Summary/Conclusion: IS/IL are rare, life threatening diseases in hematological patients. Despite aggressive antifungal treatment strategies, outcome remains poor. More effective treatment strategies are urgently needed to improve patient outcome.

PB2059

CLASSICAL OR ALTERNATIVE COMPLEMENT PATHWAY INHIBITION ALONE DOES NOT PREVENT WHOLE BLOOD KILLING OF ANTIBODY-COATED N. MENINGITIDIS OR S. PNEUMONIAE

L.A. Lewis[1], S. Panicker[2], R.B. De Oliveira[1], G.C. Parry[2,*], S. Ram[1]

[1]University of Massachusetts, Worcester, MA, [2]Bioverativ Therapeutics Inc., Waltham, MA, United States

Background: The complement cascade, responsible for the detection and clearance of pathogens, is activated by the classical (CP), lectin (LP) or alternative (AP) pathways, each of which can be independently activated by pathway-specific pattern recognition receptors. However, aberrant complement activation is observed in numerous diseases. While therapeutic complement inhibition at the level of C5 has proven to be a successful approach for treating various diseases, it is associated with an increased risk of infection, in particular, invasive meningococcal disease, even when vaccinating prophylactically. Targeting pathway-specific components provides the theoretical advantage of selectively inhibiting the pathway that triggers disease pathogenesis, while leaving the other pathways intact for immune surveillance.
Aims: Here we aimed to address the potential increased infection risk associated with complement pathway specific inhibition using a C1s inhibitor (TNT005; CP inhibitor), and a factor Bb inhibitor (anti-fBb; AP inhibitor). We performed *in vitro* bactericidal (complement-mediated) and whole blood killing assays (complement- and immune cell-mediated) to assess the relative contribution of the CP and AP in killing *N. meningitidis* and *S. pneumoniae.*
Methods: Experiments were performed using *S. pneumoniae* strain TIGR4 and group C *N. meningitidis* strain 4243. Flow cytometry was used to measure deposition of C3 and C4; pathway specific inhibitors were used to determine the relative contribution of the different pathways in depositing opsonizing complement fragments on bacteria. Bactericidal experiments in normal human plasma and whole blood killing assays containing both intact complement and phagocytes, were performed in the presence of CP and AP inhibitors (either alone or in combination). Experiments were performed in the presence or absence of capsular antibody to mimic vaccinated and non-vaccinated states, respectively.
Results: Inhibiting the CP alone using saturating concentrations of TNT005 prevented C4 deposition and killing of *N. meningitidis* in both normal human plasma and whole blood. However, in whole blood killing assays that contained specific anti-meningococcal antibodies, simultaneous inhibition of both the CP and AP was required to prevent killing of antibody-coated *N. meningitidis.* For antibody-coated *S. pneumoniae*, anti-fBb alone completely blocked C3 deposition, whereas TNT005 only partially inhibited (~40% decrease in fluorescence) C3 deposition. As expected, killing of *S. pneumoniae* was observed only in whole blood in the presence of phagocytes; blocking either the CP or AP alone did not impair killing of pneumococci in the presence of specific antibody (>90% killing at 3 h), but blocking both pathways resulted in >50% bacterial survival at 3 h.
Summary/Conclusion: The data presented here suggest that antibody-coated *N. meningitidis* can activate both the CP and AP of complement, and that

inhibition of either pathway alone would not significantly affect *N. meningitidis* killing in the presence of anti-*N. meningitidis* antibodies as membrane attack complex mediated killing could still occur via the unblocked pathway. Antibody-mediated killing of *S. pneumoniae*, which requires phagocytes, also proceeded in an unimpeded manner when the CP or AP were blocked individually. These data suggest that vaccination against *N. meningitidis* and *S. pneumoniae* is critical and likely to be effective when administering a therapeutic CP or AP inhibitor.

PB2060

PRESEPSIN (SOLUBLE CD14 SUBTYPE) AS A DIAGNOSTIC MARKER OF BACTERIAL INFECTIONS IN FEBRILE NEUTROPENIC PEDIATRIC PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

A. Abd El-Kreem[1,*], M. Hosam EL-Dein[1], A. Baraka[1], M. Zakaria[2]

[1]Clinical Pathology, [2]Pediatrics, Zagazig University Hospitals, Zagazig, Egypt

Background: Febrile neutropenia (FN) is one of the most common complications in patients with hematological malignancies which is caused by chemotherapy and the underlying disease. C-reactive protein (CRP) and procalcitonin (PCT) have been used as diagnostic markers of infections but they have some limitations. Recently, soluble CD14 subtype (sCD14-ST), also known as presepsin was reported as a novel and promising biomarker for the diagnosis of sepsis. However, its usefulness in identifying infections in patients with hematological malignancies during episodes of fever and neutropenia remains unclear.
Aims: The aim of this study was to evaluate the usefulness of presepsin compared to CRP and PCT as an early diagnostic marker of infections in FN patients with hematological malignancies.
Methods: In addition to blood cultures, we have measured presepsin, CRP and PCT, in 60 individuals. 30 of them were pediatric patients with hematological malignancies during episodes of fever and neutropenia (serving as patients group) and the other 30 were apparently healthy age and sex matched individuals (serving as control group), all of them participated in the study after consent of their relatives. patients were subclassified into 3 subgroups (patients with fever of unknown origin (FUO), patients with bacteremia, patients with clinically proved infections in the form of mucositis, pneumonia, urinary tract infection, neutrpenic enterocolitis). The sensitivity and specificity of the three biomarkers were compared in the 3 subgroups.
Results: Prespsin levels were significantly higher in bacteremia patients than those with FUO and clinically proved infections. In contrast CRP levels didn't show significant increase in bacteremia patients than those with clinically proved infections, also PCT levels were not significantly higher in clinically proved infections patients than FUO patients. Our study showed that there were slight elevation in presepsin levels in gram negative cultures than in gram positive cultures but this rise was not statistically significant. This study also showed that there was statistically significant negative correlation between presepsin and absolute neutrophil count (ANC) which also correlates with the severity of infections. The reliability of presepsin was higher than that of PCT and CRP in prediction of bacteremia in FN patients where the sensitivity of presepsin ,PCT and CRP was (100%, 100%, 77.8% respectively),and the specificity was (85.7%, 81%, 66.7% respectively). Presepsin was also superior to CRP and PCT in identification of bacterial infections either in the form of bacteremia or clinically proved infections with sensitivity (100%, 100%, 68.8% respectively) and specificity (100%, 64.3%, 85.7% respectively).
Summary/Conclusion: Presepsin can be used as a discriminator of infectious and non infectious origin of fever in FN patients with hematological malignancies even with very low total leucocyte count and ANC due to chemotherapy. The combination of presepsin and CRP may improve the sensitivity and specificity for prediction of bacterial infections in those patients.

PB2061

SALVAGING DISSEMINATED CUTANEOUS MUCORMYCOSIS WITH TRIPLE ANTI-FUNGAL THERAPY PLUS GRANULOCYTE INFUSION

K. Mullanfiroze[1,*], S. Tulpule[2], T. Singhal[1], S. Gupta[2], S. Sen[1]

[1]Dept of Paediatric Oncology & BMT, [2]Dept of Oncology & BMT, Kokilaben Dhirubhai Ambani Hospital, Mumbai, India

Background: Invasive fungal infections (IFI) needs a prompt identification of the fungus and aggressive anti-fungal treatment along with reversal of neutropenia and immunosuppression.
Aims: We describe a case of Mucormycosis where aggresive antifungal therapy & granulocyte infusions led to a successful outcome.
Methods: 13 year old child with Acute Lymphoblastic Leukemia (NCI-HR with CNS-2 status at presentation) was on UK-ALL 2003 high risk protocol. He presented in week 4 of induction with multiple tender skin lesions over the body. Lesions were ovoid with black discoloration and ulceration of the central region with a surrounding rim of erythema. On enquiry, parents reported the presence of a damp wall in their residence which was probably the source of his infection. He was initially afebrile with CBC - Hb 7.5 g%, TLC 310/mm^3 (0% neutrophils), platelet count 62000/mm^3. He was empirically started on parenteral antibiotics along with anti-fungal therap with liposomal amphotericin-B and Voriconazole. Bedside skin biopsy from the lesion was performed and sent for bacterial, fungal culture and histopathology. KOH mount on the biopsy was suggestive of budding yeast with hyphae. In view of progressive increase of the size of the lesions, deterioration of clinical condition with high grade fevers, inj.Caspofungin was also added. Dexamethasone was rapidly tapered and stopped. He was also given growth factors(inj. G-CSF at 10 mcg/kg/day subcutaneously q24 hourly) and granulocyte infusions(1x10^{10}/recipient body weight kg) until absolute neutrophil count (ANC) increased to more than 1000/mm^3 for 2 consecutive days. CT chest was suggestive of 8 mm nodule in the right side of the lung. With supportive care and above medications, his clinical condition and CBC started improving by day 6 of hospital stay. On day 7 of hospitalisation, he started having restlessness, anxiety, disorientation to time and person along with hallucinations and urinary and bowel incontinence followed by intermittent episodes of aphasia and aggressive behaviour. MRI brain was normal. In view of a normal MRI and worsening of CNS symptoms, voriconazole induced adverse reaction was suspected and it was withdrawn and Posaconazole was started. His GCS gradually started improving and he also regained bowel and bladder continence. The skin biopsy was reported as Mucor. His hallucinations and restlessness reappeared and hence posaconazole was stopped and amphotericin-B and caspofungin were continued. However, few days later he developed generalised erythematous, macular- papular, pruritic skin rash on his trunk region. Amphoericin-B induced hypersensitivity was suspected and hence it was also discontinued. He was then re-challenged with posaconazole and this time,he tolerated it well.
Results: Currently,the skin lesions have healed completely and in view of the final characterisation of the isolated fungus being reported as Mucormycosis, caspofungin has been withdrawn and he is on single agent posaconazole. Currently, the patient is well and skin lesions have healed completely.
Summary/Conclusion: There is extreme morbidity caused by IFI in neutropenic children. Combination anti-fungals, reducing immunosuppression and rapidly increasing the neutrophil count is essential, for the treatment of severe IFI. Avoidance of exposure to damp walls, seepage and construction areas must be emphasised to the care givers of neutropenic and immunosuppressed children.

PB2062

A CASE OF CYTOMEGALOVIRUS-INDUCED PANCYTOPENIA WITH CMV COLITIS

O. Apanasenko*

Kiev center of bone marrow transplantation, KIEV, Ukraine

Background: CMV infection is one of the important causes of morbidity and mortality after HSCT. It may manifest by involving multiple organs such as intestines, lungs, bone marrow, central nervous system involvement and others.
Aims: We describe a case of cytomegalovirus-induced pancytopenia with CMV colitis in patient after allogeneic stem cells transplantation from an unrelated donor for a secondary ALL.
Methods: A 19-year-old man underwent an allogeneic HSCT following conditioning with fludarabine, ATG, thiosulphane. He had received cyclosporine and corticosteroids for acute, then chronic GVHD with skin, liver and gastrointestinal involvement. On D+150 serum CMV PCR was positive with 6.22x10^2 copies of DNA/10^5 cells without evidence of CMV disease, and the patient was started on valganciclovir preventive therapy. After the cancellation of immunosuppression (five months after transplantation), the patient's clinical course had worsened - appeared frequent watery stool, abdominal cramps, weight loss. Colonoscopy with colon biopsy was performed, which showed the presence of ulcerative colitis. MV, EBV, human papillomavirus were negative, herpes simplex viruses 1 and 2 were positive in biopsy samples. Serum PCR CMV was not detected. He was started on antibiotics and antiviral therapy without any improvement. At D+270 PCR analysis showed that serum CMV was detectable, for which he again started treatment with valganciclovir 450 mg/day for 2 weeks. A gradual decrease in the number of leukocytes, platelets and hemoglobin level has been observed and watery liquid stool persisted. Differential diagnosis was conducted between CMV - enterocolitis and chronic intestinal GVHD.

Biopsies from repeated colonoscopy showed positive PCR CMV. For increased watery stool, febrile fever, epistaxis due to cytopenia, patient received antibacterial and thrombocyte replacement therapy, G-CSF. But despite that, cytopenia persisted. Monitoring PCR CMV in the blood showed low viral load ($4.26x10^2$ copies of DNA/10^5 cells). As possible causes of pancytopenia were considered recurrence of ALL, bone marrow failure, viral (CMV) infection. The patient's peripheral blood smear confirmed pancytopenia without any abnormal cells in bone marrow.
Results: Based on these findings, diagnosis of CMV infection affecting the small intestine and colon was made and IV ganciclovir 10 mg/kg was started (despite of cytopenia - WBC $1.6*10^9$/l, Hb 86 g/l, Pl $22*10^9$/l). The patient showed gradually improvement in pancytopenia, fever and frequency of stools. Quantitative PCR for CMV was repeated 2 weeks later and it became negative.
Summary/Conclusion: CMV infection could induce sustained and irreversible pancytopenia, despite normal findings in bone marrow aspiration. The pathogenesis is likely multifactorial, with both a central and peripheral effect. Several mechanisms have been proposed to explain the effect of CMV on human hematopoietic function. One indicates direct effect of CMV on bone marrow cells leading to cellular injury. Also alteration of accessory cell function by inducing the production of inhibitory cytokines resulting in a decreased production of hematopoietic factors or by altering cell surface adhesion molecule expression. Immunological mechanisms can be involved. The treatment of CMV viremia is not inconsequential and requires a close surveillance because first-line anti-CMV agents such as gancyclovir can further cause myelosuppression which may lead to superadded bacterial or fungal infection.

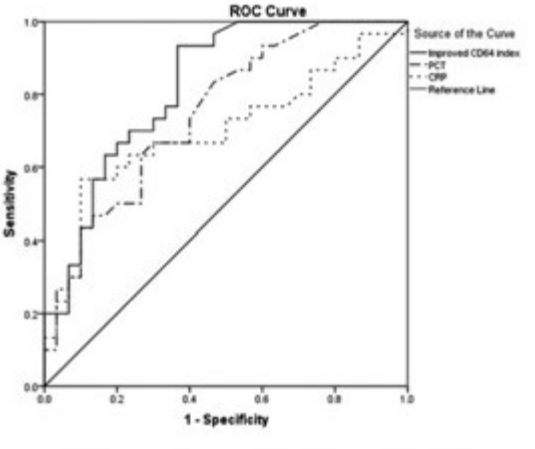
PB2063

THE DETECTION METHOD OF PERIPHERAL BLOOD CD64 INDEX IN PATIENTS WITH HEMATOLOGICAL MALIGNANCY

S. Yuxi*, W. liru

Fu-xing Hospital, Capital Medical University, 100038 - Beijing, China, Fu-xing Hospital, Capital Medical University - Beijing, China, Beijing, China

Background: Peripheral blood CD64 index contribute to diagnosing clinical bacterial infection and early sepsis, but some patients with hematologic malignancies in the process of CD64 index detection are interfered with abnormal cells, which caused mature neutrophils group-dividing difficult. Thus, it affects mature neutrophil CD64 index detection, leading to error or not detected. The application of CD64 index in blood system disease was affected.



ROC curve of Improved CD64 index, CRP, PCT.

Figure 1.

Aims: This cohort study include the patients with hematologic malignancies who are in the process of CD64 index test interference and influence the test results by abnormal cells. The antibody of CD45, CD15, CD10 was added to the original kit, the neutrophilic granulocytes were more accurately divided in order to measuring the improved CD64 index.
Methods: Try to improve the detection method of CD64 index of patients with hematologic malignancies.Comparing the diagnostic efficacy of improved CD64 index, PCT and CRP for sepsis with non-improved.
Results: The CD64 index detection was performed on patients with malignant hematologic disease with suspicious infection in the hematology ward. Results of 60 samples were disturbed by abnormal cells during the detection process. the CD64 index was higher than that in the non-sepsis group (P<0.0001). The improved CD64 index was better for sepsis diagnose than the before improved CD64 index, PCT and CRP.
Summary/Conclusion: For patients with malignant hematopathy who are disturbed by abnormal cells during the detection of CD64 index, the detection rate and the accuracy of detection can be improved by adding antibody.

PB2064

PERIOSTITIS - A RARE COMPLICATION OF VORICONAZOLE

S. Sen[1,*], S. Tulpule[2], S. Sivadasan[1], T. Singhal[1], K. Mullanfiroze[1]

[1]Dept of Paediatric Oncology & BMT, [2]Dept of Oncology & BMT, Kokilaben Dhirubhai Ambani Hospital, Mumbai, India

Background: Voriconazole is commonly used antifungal agent used in paediatric oncology patients for prophylaxis and treatment of fungal infections. We report here on one of our post transplant patients who presented with acute bone pain over the tibia.
Aims: To describe a uncommon complication of Voriconaole use that can be confused with relapse of disease.
Methods: A 6 year old boy with relapsed B cell ALL had a successful matched sibling donor transplant. Post transplant, patient was given presumptive therapy with liposomal amphotericin as his galactomannan screen was positive. Later his antifungal therapy was switched to voriconazole and he was discharged on the same. Voriconazole levels were monitored and kept at a therapeutic level. On Day + 45 patient presented with severe leg pain and difficulty in walking. There were no neurological findings and a X Ray of the legs were normal. Suspecting a relapse, a bone marrow aspiration and biopsy was done which showed no evidence of disease recurrence with MRD being negative and 100% donor chimerism. The pain increased in severity to an extent that the child stopped walking. A MRI scan was done which only showed periosteal thickening suggestive of mild periostitis over the tibia. Differential diagnosis of leukemic infiltration, osteomyelitis and drug induced periostitis was considered. As we had ruled out a relapse with a bone marrow examination and all inflammatory markers were negative, we deduced that drug induced periostitis was the most likely cause for the symptoms. Discontinuation of voriconazole led to complete resolution of pain within 48 hours.
Results: At one year post transplant, patient continued to be in remission and pain free.
Summary/Conclusion: Painful periostitis is a rare complication of long term voriconazole therapy which has only been recently reported in literature. Fluorosis and promotion of bone formation by osteoblast stimulation is the the proposed mechanism for voriconazole induced periostitis. Increased awareness of this rare condition can lead early correct diagnosis.

PB2065

PHAGOCYTOSIS BY PERIPHERAL BLOOD MONOCYTES IN A CHILD WİTH EBV ASSOCIATED LYMPHOMATOID GRANULOMATOSIS

S. Secil[1], S. Aytac[1,*], D. Cagdas[2], M. Cetin[1], F. Gumruk[1]

[1]Department of Child Health and Diseases, Hematology Unit, Hacettepe University Faculty of Medicine, [2]Department of Child Health and Diseases, Immunology Unit, Hacettepe University, Faculty of Medicine, Ankara, Turkey

Background: Lymphomatoid granulomatosis is a rare, Epstein Barr Virus (EBV) associated systemic angiodestructive disorder which is considered a part of the spectrum of lymphoproliferative disorders. It usually presents in adulthood and uncommon in children. The incidence is higher in patients with immunodeficiency disorder. Pulmonary involvement occurs in over 90% cases and most of the time respiratory symptoms are initial sign of manifestation. The most common secondary HLH cause in pediatric population is EBV associated HLH.
Aims: Herein, we present a peripheral hemophagocytosis in a child with EBV associated lymphomatoid granulomatosis. Phagocytosis by peripheral blood monocytes occasionally be found on peripheral smears which may alert the physcian for evaluating bone marrow.
Methods: Case: A sixteen year-old female with an initial dignosis of sarcoidosis was admitted to the hospital with a complaints of cough, fever and fatigue. On her physical examination there were ecchymoses both at her arms and legs and pallor with hepatosplenomegaly. Laboratory evaluation revealed Hb:6,9g/dl, Hct:20,8%, platelet:$64x10^9$/L, WBC:$0.9x10^9$/L and examination of the peripheral blood by light microscopy revealed striking phagocytic activity by the monocytes with a differentiation of neu-

trophil%62, monocyte%12, lymphocyte%18, stab neutrophil%8 (figure 1). Her ferritin level was found to be increased at 963,6 ng/mL. Bone marrow examination showed prominent hemophagocytic histiocytes (HS), which is consistent with HLH. She received chemotherapy according to the HLH-2004 protocol, which includes etoposide, steroids, cyclosporin and ganciclovir as an antiviral treatment. In her follow up her pulmonary symptoms did not resolve and due to suspicions related to the previos diagnosis of sarcoidosis, a new Contrast Enhanced CT scan was done which showed pleural effusion at left hemithorax, bilateral mediastinal, paratracheal, subcarinal, hilar lymphadenopathy and boundless, centrilobular nodular opacities in both lungs. The EBV DNA in blood was 4208×10^3copy/mL. A previous lung biopsy was reevaluated and found that EBER was positive in granulamatous reaction which is compatible with lymphomatoid granulomatosis.

Results: The patient was treated with anti-CD20 immunotherapy (Rituximab, 375 mg/m^2 intravenous infusion, four doses over a four week period). She got third doses of Rituximab, pulmonary lesions and HFS were resolved.

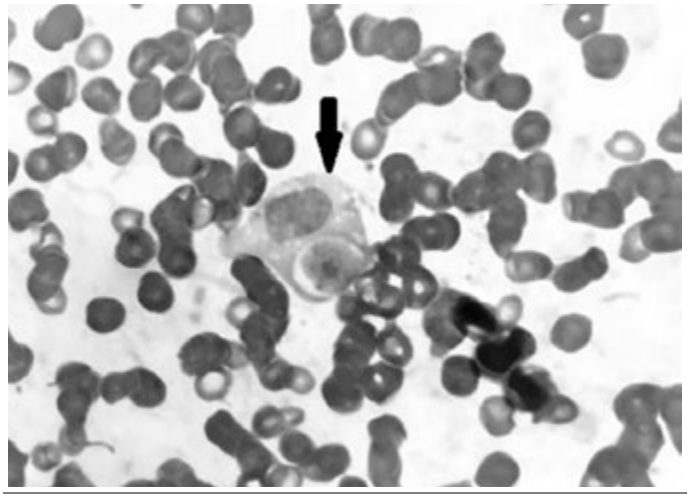


Figure 1.

Summary/Conclusion: In our case EBV seems to be the responsible factor for both hemophagocytosis and lymphomatoid granulomatosis. Although the phagocytic activity of monocytes is well known it was occasionally find in the peripheral blood smear, in this case the interesting fact is that phagocytic activity is observed in the peripheral smear and bone marrow examination.

PB2066

UNUSUAL CLINICAL PRESENTATION OF CANDIDA TROPICALIS INFECTION IN A AML NEUTROPENIC PATIENT

F. Cuellar-Ambrosi[1,*], M. Monsalve[1], I.M. Trompa[2], G. Gaviria Cardona[1], I.F. Vega-Gonzalez[3], O.A. Osorio[4]

[1]Hematology, [2]Infectology, Clinica Leon XIIII, [3]Radiology, [4]Nuclear Medicine, Clinica Las Americas, Medellin, Colombia

Background: Invasive fungal infections (IFIs) cause considerable morbidity and mortality in patients with hematological neoplasia. Physicians frequently rely on a constellation of clinical signs, imaging, cultures, histopathology to establish a diagnosis and a suitable follow-up.

Aims: We presente a 20 y-o boy with AML who developed severe systemic *candida tropicalis* infection during induction chemotherapy neutropenia period and detected by cultures and PET CT.

Methods: This report is from the Hematology and HSCT Service of the Clinica Leon XIII - IPS Universidad de Antioquia in Medellin - Colombia. An unusually descriptive case of IFI by *candida tropicalis* in order to highlight some diagnostic clues and follow-up support.

Results: A 20 y-o young man presented with AML-M2, CD3,CD117, CD34CD45d,HLADR,CD15, all (+), MPOd 100%. PML-RARA (-). Current 7+3 protocol was started. Standard prophylaxis with acyclovir and fluconazole was done. At day +15 neutropenic fever appeared and some days after hemocultures yielded *candida tropicalis.* Concomitantly, disseminated absceded subcutaneous skin nodules were observed. In cultures from these abscess *candida tropicalis* grew again. PET CT revealed disseminated nodular lesions, *photo.* Amphotericine deoxyclate and high dose fluconazole were started. On the other hand, the patient reached leukemia complete remission. One month later his clinical condition was stable with nodular skin lesions clinically resolved, except the persistent abscess in the pericardium as shown on PET CT. Successful surgical drain of the persistent pericardial abscess was done. HiDAC consolidations were followed without candida reactivation.

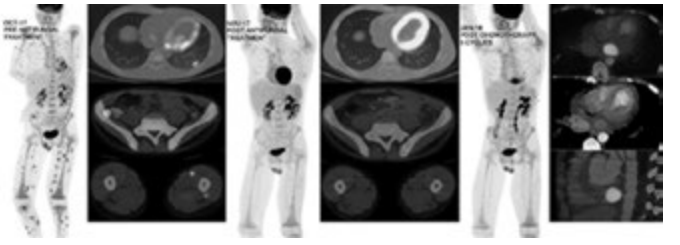


Figure 1.

Summary/Conclusion: IFIs are common during treatment of hematological malignancies. Diagnostic methods some times are inconclusive to evaluate their clinical extension and for monitoring evolution. We present here a young man with chemotherapy induced neutropenia who developed disseminated *candida tropicalis* infection with unusual definite extension and the monitoring of response by serial PET CT.

PB2067

PRIMARY ANTIFUNGAL PROPHYLAXIS WITH MICAFUNGIN IN HEMATOLOGICAL MALIGNANCIES: A SINGLE CENTER EXPERIENCE

M. Porrazzo*, M. Piedimonte, A. Campagna, G. Galassi, S. Pelliccia, E. Conte, A. Ferrari, A. Tafuri

Hematology, Sapienza, Rome, Rome, Italy

Background: Micafungin is a clinically important echinocandin antifungal drug, recently approved as prophylaxis treatment in patients undergoing allogeneic transplantation and in those undergoing prolonged neutropenia (>10 days). The use and the role of micafungin however prophylaxis is still questionable in term of comparison with azole compounds and on the potential selective pressure of micafungin toward resistant fungi. Furthermore, the frequency of breakthrough invasive fungal diseases (IFI) during echinocandin therapy is unclear.

Aims: To evaluate the frequency of breakthrough IFI in a single center experience of hematological malignancies treated with primary antifungal prophylaxis by micafungin.

Methods: To evaluate retrospectively the use of micafungin in 11 adult hematological patients treated at our institution and characterized by high and intermediate risk of infections for development of IFI according to the latest NCCN Guidelines (table 1).

Table 1. Patients characteristics.

Median age, years (range)	60 (33-74)
Female: Male	1:10
Patients at high risk for IFD n. (%) - Induction/consolidation chemotherapy for acute leukemia - Severe Aplastic Anemia - AML during other treatments	6/11 (54.5%) 3/11 (27%) 1/11 (9%) 2/11 (18%)
Patients at intermediate risk for IFD n. (%) - ASCT with severe mucositis	5/11 (45%)
History of previous infections - Bacterial - Viral - Fungal	3/11 (27%) 2/3 (67%) 1/3 (33%) 0/3
CIRS SCORE - < 6 - > 6	9/11 (82%) 2/11 (18%)
Days of neutropenia at micafungin discontinuation, median (interquartile range)	9 (6-13)

Results: All patients received prophylactic micafungin at 50 mg/day during neutropenia until neutrophils recovery or breakthrough IFI. The median of the days of neutropenia at micafungin discontinuation was nine. Febrile neutropenic patients with suspected IFI underwent an infective work up including serum galactomannan and/or bronchoalveolar lavages (BAL), CT scan and multiple samplings of blood cultures. IFI occurred in three patients affected by high risk of IFI, with two proven IFI, Aspergillus and Mucor

species, respectively (table 2). All patients had a grade IV neutropenia and the IFI occurred respectively after 30, 24 and 11 days of neutropenia from the first administration of micafungin. We observed two lung infections, with sepsis in a patient affected by severe aplastic anaemia and a CNS involvement in an AML patient, respectively. The third patients with AML secondary to myelodysplastic syndrome developed a probable liver fungal infection revealed by CT scan. All patients were treated empirically with liposomal amphotericin B followed by voriconazole: two died of IFI after 25 and 23 days, the third died because of refractory leukemia. No IFI were documented in the five patients at intermediate risk receiving micafungin prophylaxis, while the other three high-risk patients developed a fungal infection several months after micafungin discontinuation.
Summary/Conclusion: In our experience, micafungin was active as profilaxis treatment in intermediate risk patients. Whereas a feeble approach has been shown in patients with high risk of IFI. We suggest therefore evaluating in a larger series of patients this approach to clarify which patients may take advantage of micafungin prophylaxis

Iron metabolism, deficiency and overload

PB2068

SOLUBLE TRANSFERRIN RECEPTOR LEVEL AS PREDICTOR OF IRON OVERLOAD IN PATIENTS WITH B-THALASSEMIA AND SICKLE CELL DISEASE

A. El-Beshlawy[1,*], A. Abdel-Salam[1], M. El-Tagui[1], M. Farhan[2], M. Ramadan[3], W. Mouthanna Saeed Kassim[4], M. El-Ghamrawy[1]

[1]Pediatric Hematology & BMT Unit, [2]Clinical Pathology Department, [3]Faculty of Medicine, Cairo University, Cairo, Egypt, [4]Pediatrics, Faculty of Medicine, Cairo University, Cairo, Egypt

Background: Soluble transferrin receptor (sTfR) is one of the main regulators of cellular iron homeostasis, and is an emerging diagnostic tool of iron status.
Aims: To investigate the diagnostic value of sTfR as compared to serum ferritin (SF) in assessment of iron overload expressed as liver iron concentration (LIC) measured by magnetic resonance imaging (MRI) in patients with β-thalassemia major (BTM) and sickle cell disease (SCD).
Methods: Eighty transfusion dependent patients (64 BTM and 16 SCD) with a mean age of 10.8±4.5 years were recruited. Steady state SF and sTfR levels were assessed quantitatively by enzyme linked immunoassay (ELISA). LIC values, within 6 months' duration, as identified by quantitative MRI of hepatic iron stores as a signal intensity ratio method based on T1 and T2* contrast imaging without gadolinium were retrieved. We categorized iron overload status in our patients into 2 subgroups: group A; 52 patients with LIC >7mg/g dw, and group B; 28 patients with LIC <7mg/g dw. Informed consent was obtained from patients' legal guardians before enrollment. The study protocol was approved by Cairo University Research Ethics Committee.
Results: Mean sTfR, SF and LIC of studied group (n=80) were 121 nmol/L, 2478 ng/ml and 18.6 mg/g dw respectively. BTM and SCD patients had comparable mean sTfR and SF (p>0.05 for both) but LIC was significantly higher in BTM group (p<0.001). Group A had significantly higher median sTfR and SF compared to group B (p=0.026 and 0.003 respectively). However, sTfR did not correlate with SF or LIC (p=0.273, 0.725 respectively). A positive correlation was evident between SF and LIC (r=0.49, p=0.001). Both sTfR and SF were good predictors of iron overload at certain cutoff levels; ROC curve of sTfR and SF at cutoff values of 110.4 nmol/L and 1220 ng/ml respectively demonstrated a sensitivity of 90.6%, specificity 62.5% for both (p 0.05). Using *Cohen's kappa testing*, agreement between serum sTfR at a level of 110.4 nmol/L and LIC was evident (p=0.001); but this significance was lost at sTfR values ≥133.3 nmol/L. Significant agreement between SF and LIC were evident at both values of 1220 ng/ml and 1591.5ng/mL and higher (p=0.001 and 0.014 respectively).
Summary/Conclusion: sTfR was a valuable quantitative assay of iron overload in children with BTM and SCD. Both sTfR and SF could accurately predict hepatic iron overload among our patients at certain cutoff values. However, the overall accuracy and agreement of sTfR with LIC was lost at high serum values.

PB2069

THE INCREASING FERRITIN SERUM LEVEL AND OVERALL SURVIVAL IN PATIENTS WITH MALIGNANT LYMPHOMAS

T. Babaeva*, T. Pospelova, O. Seregina

Novosibirsk State Medical University, Novosibirsk, Russian Federation

Background: It is known, that extracellular ferritin takes part in mechanisms of neoplasm progression in solid cancer, and can stimulate proliferation of malignant cells lines. Similarly, the ferritin serum level correlates with tumor mass volume and tumor activity in patients with hematological malignancies, such as malignant lymphoma and acute leukemia. On the other hand, the normalization of ferritin serum level in remission has proved the clinical value of ferritin for initial assessment and therapy response evaluation, especially in patients with malignant lymphomas.
Aims: Was to evaluate the increasing ferritin serum level in newly diagnosed patients with malignant lymphomas.
Methods: A total of 98 patients with malignant lymphoma, including 72 patients with non-Hodgkin's lymphoma (73,5%) and 26 patients with Hodgkin's lymphoma have been examined in our study. 84,7% of the patients with non-Hodgkin's lymphomas have advanced staged of disease (III-IV). All patients have received 4-6 courses of chemotherapy. The ferritin serum level was detected by Enzyme-linked Immunosorbent Assay (ELISA).

Results: the overall survival was evaluated in two groups of newly diagnosed patients: in first group of patients (n=43) the ferritin serum level was <350ng/ml, in another group of patients (n=55) the ferritin serum level was >350 ng/ml. The median survival in patients with ferritin level >350ng/ml was lower (40 months; p=0,004) than in patients with normal level of ferritin at onset of disease - in this group of patients the median survival has not been reached. The initial level of hemoglobin at the onset of disease was used as the reference marker of poor prognosis. The overall survival (OS) in the group of patients with normal level of hemoglobin (>120g/L) has not reached the median of survival, whereas patients with anemia (hemoglobin 120 g/L) has tended to deteriorate the overall survival (OS) - the median of survival was 40 months (p=0,007). Besides that, the low level of hemoglobin, correlates with significant increasing the mortality risk (Odds ratio (OR)-6,333; confidence interval (CI) – 95%; 2,152-18,638; p 0,05). Patients with high ferritin serum level at the onset of disease also has poorer results of survival and higher risk of mortality (OR 8,122; CI-95%, 1,764-37,396; p 0,05) by comparison with the group of newly diagnosed patients with ferritin serum level 350ng/ml.
Summary/Conclusion: These findings allow to conclude the patients with hyperferritinemia at the onset of disease in the group of patients with poor prognosis and lower overall survival. The high ferritin serum level in patients without prior blood transfusion should consider as a significant marker of poor prognosis. The analysis of ferrokinetics in patients with lymphomas before starting the chemotherapy make it possible to use preventive approach for combining groups with poor prognosis and elimination of negative factors.

PB2070

DIFFERENTIATION OF IRON DEFICIENCY ANEMIA AND THALESSEMIA TRAIT IN CHILDREN WITH MICROCYTIC HYPOCHROMIC ANEMIA

G. Aydogan*, S. Keskin, N. Ozdemir, F. Akici, Z. Salcioglu, C. Bayram, E. Pasli, E. Yilmaz, A. Aycicek, T.N. Tahtakesen, G. Ersoy

Pediatric Hematology, Kanuni Sultan Suleyman Education And Research Hospital, Istanbul, Turkey

Background: Microcytic anemia is defined as anemia with a low mean corpuscular volume (MCV) for age, race, and sex. The most common causes of microcytic anemia in children are iron deficiency and thalassemia. Iron deficiency and thalassemia are characterized by decreased hemoglobin production, due to insufficient availability of heme or globin, respectively and both cause hypochromic microcytic anemia. Iron deficiency is frequently seen in parts of the world where nutrition is inadequate and the socioecomic status is low. Thalassemias are common in the Mediterranean region. Thalassemia trait may be identified as an incidental finding. Differentiation and detection of coexistence is essential for genetic counselling and to set a treatment plan on the basis of etiology.
Aims: The aim of the study was to characterise the frequency of iron deficiency anemia (IDA) and thalassemia trait among children who presented with microcytic anemia in our pediatric hematology clinic.
Methods: Data were available for 200 children (6 months-18 years) attending the outpatient pediatric hematology clinic between August 2013-July 2014. Screening for iron deficiency was done by analysing serum ferritin, iron and iron binding capasity. Haemoglobin variants were diagnosed by HPLC or capillary electrophoresis and molecular methods.
Results: Of the 200 enrolled, 93 were female (46.5%) and 107 were male (53.5%). Fifty-three children were <2 years of age (26.5%), 52 (26%) between 2-4 years, 56 (28%) between 5-11 years and 39 (19.5%) were >12 years old. One-hundred-fiftyfour had IDA (77%), 27 had thalassemia trait (13.5%), and in 11 both conditions co-existed. Eight of the thalassemia trait patients were found to have an alpha-thallesemia gene mutation, in 3 of these there was also IDA. RBC, MCV, Mentzer index (MI), serum iron, total iron binding capacity, ferritin were significantly different between IDA and thalassemia trait patients (p<0.001) however RDW was not different between the 2 groups (p>0.05). Sensitivity and specifity of MI for detection of thalassemia trait 100% and 69.4% respectively. The positive and negative predictive values of MI in diagnosing thalassemia trait were 36.6 and 100%. In patients with co-existing IDA and thalassemia trait; MI had a sensitivity of 90.9%, specifity of 69.4%, pozitive predictive value of 17.5% and a negative predictor value of 99.7%.
Summary/Conclusion: The differentiation between βTT and IDA, requires Hb A2 estimation by Hb electrophoresis, examination of a peripheral blood film, serum ferritin, iron, TIBC, and transferrin saturation. The requirement of simple distinguishing parameters between IDA and thalassemia trait in a child presenting with hypochromic microcytic is needed as several studies have shown the effect of coexistence of IDA on HbA2 synthesis resulting in confusing levels of HbA2 in thalessemia. Hemoglobin electrophoresis is also not helpful in patients with alpha- thalassemia trait.

PB2071

PARENTERAL IRON THERAPY IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE: AUDIT ON A SINGLE CENTRE CLINICAL PRACTICE

S. Lovato*

Postgraduate, London North West University Healthcare NHS Trust, London, United Kingdom

Background: Iron deficiency is common in Inflammatory Bowel Diseases (IBD), unfortunately oral iron supplements are often poorly tolerated and parenteral iron is used instead. National and international guidelines establish the best practice regarding diagnosis of iron deficiency, indication for parenteral iron therapy and follow up recommendations. Regular audits are an effective tool to ensure the guidelines are followed and to improve local practice.
Aims: The aim of the study was to compare the parenteral iron therapy practice in Ealing hospital with the national and international standards. We looked at the blood test used to diagnose iron deficiency: haemoglobin, ferritin and transferrin saturation if ferritin normal or high. We also evaluated the appropriateness of parenteral iron therapy according to the guidelines; first line parenteral therapy is indicated for haemoglobin levels lower than 100g/l, IV iron is used as second line in patients not responsive or intolerant to oral iron. Follow up should be performed at least four weeks after the infusion and within three monthsand should include haemoglobin and ferritin; in case of iron deficiency the patient should be prescribed further iron therapy.
Methods: Parenteral iron prescriptions for patients with IBD were identified. The hospital informatics system was used to retrieve clinical data and blood test results. Twelve months data were taken in account.
Results: We identified 19 prescriptions for 15 patients. One patient, who had 3 prescriptions, had blood test done with an unusual pattern, she was on iron infusion maintenance every four months and she had follow up blood tests done two weeks after the infusion, too early for follow up and too old for diagnosis of iron deficiency at the time of the iron infusion. In the remaining 16 cases iron deficiency was confirmed in the months before by FBC and ferritin; transferrin saturation was performed in all 4 cases with normal ferritin. Of the 19 prescription 15 were definitely appropriate; in 4 cases appropriateness was not evaluable (3 due to lack of recent blood tests, in 1 case haemoglobin was >100g/L, too high to justify IV iron as first line therapy, and there was no clinical information on previous oral iron therapy). Follow up was done in 18 cases but timing was variable. Only in 8 cases follow up was arranged within 3 months as suggested by the guidelines, in 5 cases the follow up was arranged earlier and in the remaining 5 later. In 4 cases the follow up was not acted upon, the patients were still iron deficient but no further iron therapy was prescribed.
Summary/Conclusion: The results were discussed with the IBD team to create an action plan to improve patients' care. Diagnosis was good and appropriateness wad acceptable, the only patient who had an abnormal pattern of blood tests will be contacted to modify how therapy is given and monitored. Follow up practice was the main issue, this was due to lack of dedicated specialist nurse who could organise day unit appointment and follow up. The clinician seeing the patient and prescribing parenteral iron will take responsibility of ensuring that iron deficiency is confirmed with recent blood tests and appropriate follow up, either with an appointment in the IBD clinic or through the General Practitioner, is organised. Once applied these changes the audit should be repeated in one year to ensure the changes improved the compliance with international guidelines.

PB2072

WHICH IS THE ETIOLOGY OF BREATH HOLDING SPELL? CARDIAC CONTRACTION OR IRON DEFICIENCY ANEMIA

S. Akarsu[1,*], T. Kasar[2]

[1]University of Firat, Faculty of Medicine, Pediatric Haematology, Elazig, [2]University of Ordu, Faculty of Medicine, Pediatric Cardiology, Ordu, Turkey

Background: Breath-holding spell (BHS) is a prevalent, nonepileptic, recurrent, potentially frightening early childhood disease with varying clinical manifestations. In addition to underlying genetic predisposition, dysregulation of central nervous system (CNS) is responsible for BHS. Autonomic dysfunction which leads to cardiac arrest, and cerebral anoxia, and increased

vagal tonus play roles in its pathogenesis. It may accompany iron deficiency anemia (IDA). In cases with BHS, increase in corrected QT (QTc), and QTc dispersion (QTcd) which are electrocardiographic (ECG) reflection of regional differences in myocardial repolarization have been demonstrated.
Aims: In our study, we determined whether BHS develops as a result of dysrhytmia secondary to abnormal ventricular depolarization inherent to the disease, or as an outcome of IDA.
Methods: Study groups were formulated prospectively. BHS-IDA group consisted of cyanotic children aged between 9 and 60 months. The cases were evaluated in 3 groups as follows: Group 1 (Group BHS-IDA, n: 12), Group 2 (Group IDA without BHS, n: 34), Group 3 (Healthy control group, n: 26). ECGs were evaluated by the same pediatric cardiologist at one session. The difference between the longest, and the shortest QTc intervals was accepted as QTcd. Cases in all three groups were compared as for QTc, and QTcd values. Statistically analysis was performed using Student's *t*, and Mann-Whitney *U* tests, and $p<0.05$ was accepted as the level of statistical significance.
Results: QTc intervals were 412.5±19.1 ms in Groups 1, 394.4±27.1 ms in Group 2, and 395.8±20.2 ms in Group 3. QTcd intervals in children diagnosed as BHS-IDA 12.0±6.5 ms, IDA 16.4±10.5 ms, and healthy control group 27.6±9.0 ms were as indicated ($p>0.05$).
Summary/Conclusion: IDA may affect cardiac, and nervous system which play an important role in changing autonomic nervous system balance. It induces hypoxia which leads to dysrhytmia. However in cases with BHS, ventricular repolarization which develops as a result of autonomic dysregulation, and increased vagal stimulation may differ.

PB2073

AN OLD CANDY IN A NEW WRAPPER! NUTRITIONAL DEFICIENCY IN SAMPLES OBTAINED FOR HAEMOGLOBINOPATHY SCREENING

A. Memon[1,*], I. siddiqui[2], N. ali[1]

[1]Department of Pathology & Laboratory Medicine, [2]Aga Khan University Hospital, karachi, Pakistan

Background: Anemia is a major health concern globally, yet devastating for the developing countries. Iron deficiency considers contributing 70% of total nutritional deficiencies and 30% are said to be due to vitamin B12/ folate and deficiency of other micronutrients. Iron deficiency and megaloblastic anemia cause variations in haemoglobin A_2 levels which may lead to misdiagnosis. It has a financial and psychological implication to that person. True prevalence of iron or vitamin B12 / folate deficiency is uncertain in our population.
Aims: This study aim to determine iron and vitamin B12 / folate deficiency in samples received for haemoglobinopathy screening.
Methods: Retrospective review of first 200 chromatograms and peripheral blood film of samples received for haemoglobinopathy screen by HPLC (High performance liquid chromatography) during the February 2016 at Section of Haematology, Department of Pathology & Laboratory Medicine, Aga Khan University Hospital Karachi. Cases with haemoglobinopathies and with alternative diagnosis like malaria, leukemia and pancytopenia were excluded from this study.
Results: Total 200 consecutive chromatograms and peripheral blood films of samples were reviewed retrospectively. Out of these 200 cases 47% cases, (n=94) showed nutritional deficiency with female to male ratio of 2:1 and mean age of 13 years. Among these 47% cases, 44.5% cases (n=89) showed iron deficiency with 5% suspicion of suppressed haemoglobin A_2 levels. Vitamin B12/ folate deficiency was found in 2.5% (n=5) of cases with raised haemoglobin A_2 levels.
Summary/Conclusion: Nutritional deficiency was found in 47% cases in current study. This is an ongoing study, further data will be included while completion of a study period. Authors recommend treating an underlying nutritional deficiency prior to haemoglobinopathy screen.

PB2074

AUTOIMMUNE IRON DEFICIENCY ANEMIA, IS IT REAL? CASE REPORT

N. Vaynyunskaya*

Pediatric hematology, Saint-Petersburg State Children Hospital [1], Saint-Petersburg, Russian Federation

Background: Revalence of iron deficiency (IDA) are staggering: 2 billion people, *i.e.* more than 30% of the world's population suffer from that. IDA is one of the most studied pathological condition. It is "the most grateful anemia" and after normalization of diet, adequate ferrotherapy and treatment of the digestive tract relapses should not happens. The most difficult is to understand causes of recurrence IDA in previously examined and treated patients with no obvious reason.
Aims: Our aim is to describe the clinical case of recurrent iron-deficiency anemia presumably autoimmune etiology in 17 year old girl.
Methods: Determination of specific antibodies to human-ferritin by the method of detection of lupus anticoagulant in patients with prolonged APTT. Ferritin of the patient was 0,008ng/ml.The ferrinin of the control sample was 171ng/ml. After mixing in equal proportions plasma of the patient and control, incubation for two hours ,the level of ferritin in the control sample decreased from 171 to 51ng/ ml. We do not have in Russia Anti-Ferritin antibody.
Results: The patient had history of 5 years long IDA. All possible examinations were made, there was no bleeding, malnutrition or hereditary IDA. At the time of admission her blood test was - HB 47g/l, MCV 54,Ret-Hb-14%. As we have found "anti ferritin" antibody by our original method it was chosen intravenous immunoglobulin(IVIG) at a dose of 2mg/kg for treatment. Simultaneously with the treatment IVIG our patient received iron supplementation. The treatment was successful. On day 14, the HB level increased to 106g/l. One month after the treatment HB was 126g/l, erythrocytes-4,3 x10/12/l, MCV 83, 346 MCHC, MCH 28, Ret-Hb -29%, ferritin 146ng/ml.
Summary/Conclusion: There are a lot of immune disease in our century. We can not forget about it when we treat patients with reccurent IDA. Sometimes anti ferritin antibodies may be very useful. And if there are no anti ferritin antibodies in your countries we offer to make the incubation with control as we did.

Myelodysplastic syndromes – Biology & Translational Research

PB2075

HLA-G GENE AND PROTEIN EXPRESSION IN PATIENTS WITH PRIMARY MYELODYSPLASTIC SYNDROME

S. Giannouli[1,*], A. Nezos[2], E. Zintzaras[3], A. Papageorgiou[4], K. Kampas[4], I. Stergiou[4], M. Voulgarelis[4]

[12nd Department of Internal Medicine, [2]Department of Physiology, National and Kapodistrian University of Athens, Athens, [3]Department of Biomathematics, University of Thessaly School of Medicine, Larissa, [4]Department of Pathophysiology, National and Kapodistrian University of Athens, Athens, Greece

Background: In myelodysplastic syndromes (MDS) deregulation of immune effectors pathogenetically drives abnormal haemopoiesis and enhanced leukemic propensity. Human leukocyte antigen G (HLA-G) is a nonclassical MHC class I antigen, regularly not expressed in normal tissues except in trophoblasts from early gestation placentas and other immune-privileged tissues. Evidence for HLA-G silencing by a DNA methylation process has been reported. Interestingly, the use of demethylating agents such as 5-azacytidine further demonstrated that the repression of HLA-G gene activity in cultured various cell lines is reversed by demethylating treatment. HLA-G primary transcript generates seven alternative mRNAs that encode membrane-bound HLA-G1, -G2, -G3, -G4 and soluble HLA-G5,-G6 and –G7 isoforms. HLA-G has a direct inhibitory effect on the cytolytic activity of NK cells, cytotoxic T lymphocytes and is implicated in the induction of Foxp3-regulatory T cells. Therefore, HLA-G possesses immune tolerogenic activity with potential implications in antitumor immune responses.
Aims: In order to investigate potential implication of HLA-G in immune deregulation underlying MDS pathogenesis and evolution we studied HLA-G gene and protein expression in CD34 cells of patients with primary MDS.

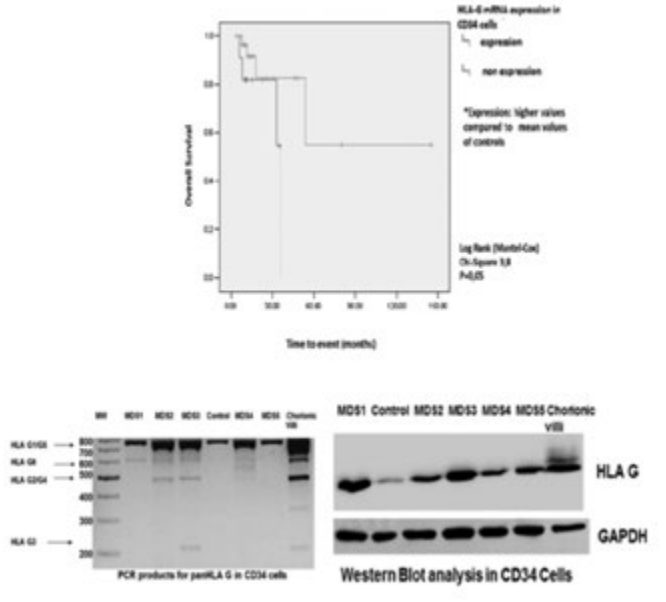


Figure 1.

Methods: Real Time PCR for HLA-G mRNA expression was performed in CD34 bone marrow (BM) cells derived from 35 primary untreated MDS patients of all subtypes, 7 patients with high-grade Non Hodgkin Lymphoma without BM involvement, as well as first trimester trophoblasts from 2 donors served as controls. CD34 BM HLA-G protein levels were evaluated by Western blot in 10 study participants and plasma HLA-G protein levels were evaluated by ELISA in 22 MDS samples and 17 apparently healthy age/sex matched controls. Canonical variate analysis (CVA) was used to discriminate MDS subtypes using a set of several well established laboratory parameters, as well as the HLA-G expression in CD34+ cells. The Kaplan–Meier method was used for calculation of survival probabilities and the Log-rank test for comparison of survival curves between expression levels of HLA-G. Cox regression was used for Overall Survival (OS).
Results: Increased HLA-G mRNA and protein expression was observed in CD34 cells from MDS patients compared to controls (p=0.04 and p=0.0095, respectively). Plasma HLA-G levels were significantly higher in MDS patients compared to controls (p=0.0008). A distinct pattern of expression of various HLA-G mRNA isoforms was noted between MDS patients and controls. HLA G1/G5 was the commonest isoform expressed in CD34 cells, but other less common isoforms namely HLA-G2/-G4,-G3, and –G6 were also expressed. Interestingly, the CVA revealed that the major parameters that play a role in discrimination of the three major MDS subtypes (RCUD, RCMD and RAEBs), were not only the expected parameters of WPSS and percentage of blasts, but also the HLA-G mRNA expression in CD34 cells. OS curves of MDS with HLA-G mRNA overexpression differed significantly during follow-up, compared to MDS patients without expression (p=0.05).
Summary/Conclusion: Given the immune inhibitory properties of the HLA-G molecule, its increased expression in MDS CD34 cells may implicate the mechanisms of resistance or escape to immune surveillance contributing to MDS evolution and prognosis. Moreover, in the era of demethylating treatment the finding of HLA-G expression by MDS CD34 cells is of specific clinical interest.

PB2076

GENE EXPRESSION AND EPIGENETIC PROFILING SEPARATES CASES WITH DIC(1;7)(Q10;P10) FROM T-MDS/AML

A.G. Lema Fernandez[1,*], B. Crescenzi[1], V. Pierini[1], V. Di Battista[1], G. Barba[1], F. Pellanera[1], D. Di Giacomo[1], R. Piazza[2], E. Adelman[3], M.E. Figueroa[4], C. Mecucci[1]

[1]Department of Medicine, University of Perugia, Perugia, [2]School of Medicine and Surgery, University of Milano Bicocca, Milano, Italy, [3]Department of Pathology, University of Michigan School of Medicine, Ann Arbor, [4]Department of Human Genetics, University of Miami Miller School of Medicine, Miami, United States

Background: The dic(1;7)(q10;p10) is a centromeric-centromeric DNA juxtaposition, usually found in Myelodysplastic Syndromes (MDS) and Acute Myeloid Leukemia (AML) arising after chemo/radio-therapy. Due to the absence of genes within the epigenetically defined centromeric region, no specific molecular targets have been identified yet.
Aims: The aim of this study was to characterized biological features of MDS/AML with dic (1;7) by investigating their genetic and epigenetic landscape.
Methods: Three series of 5 cases each were selected for the study: 1) MDS/AML with dic(1;7); 2) t-MDS/AML; 3) non-neoplastic cytopenias. DNA and RNA were extracted from unsorted bone marrow cells. Genomic characterization of dic(1;7) included karyotype, Fluorescent *In situ* Hybridization (FISH), Single Nucleotide Polymorphism Array (SNPa), Whole Exome Sequencing (WES), and RNA sequencing. Global epigenetic approach used Enhanced Reduced Representation Bisulfite Sequencing (mERRBS). All amplified libraries were sequenced on Illumina HiSeq2500 using manufacturer's recommended protocol and were aligned against hg19.
Results: In all dic(1;7) cases breakpoints fell within α-sat DNA at chromosome 1 and 7, resulting in a 1q trisomy and 7q monosomy. SNPa excluded copy neutral loss of heterozygosity or cryptic copy number alterations, confirming dic(1;7) as a cytogenetically primary abnormality. WES did not identify a common mutational background in dic(1;7) series. Two patients harbored 4 somatic mutations never described in MDS/AML (*EB1*; *GGPS1*; *CCDC8*; *PSMF1*). Gene expression profile (FDR ≤0.1, $\log_2$FC ≥I1I) provided us with a specific downregulation signature differentiating dic(1;7) from both controls [4860 differentially expressed genes (DEGs): 889 up and 3971 down] and t-MDS/AML [4317 DEGs: 482 up and 3835 down]. To characterize the biological differences, we analyzed DEGs within functional pathways, which identified downregulation of ATP-binding cassette transporters and upregulation of p53 signaling only in dicentrics (FDR ≤0.05). We next investigated the gene dosage effect of 1q trisomy and 7q monosomy. In keeping with 7q monosomy, 95% of DEGs on 7q were downregulated in dic(1;7). Surprisingly, the gene dosage effect of 1q trisomy contributed only partially, with more than 50% of 1q DEGs being downregulated in dicentrics. In exploring the basis for the dic(1;7)-related 1q downregulation, the DNA methylation profile identified hypermethylation at 1q in dicentrics in keeping with downregulation signature despite trisomy. Global epigenome analysis, which capture around 3.2M of CpGs, was able to separate dic(1;7) and t-MDS cases from each other and from controls. Moreover, mERRBS showed a specific dic(1;7) hypermethylation pattern in non-promoter regions, particularly at intronic enhancers. We used PET Module tool to identify putative enhancer-target genes, which hypo-expression gave account of around 30% of the downregulated signature. Furthermore, Hypergeometric Optimization of Motif EnRichment analysis showed that hypermethylated enhancers were specifically enriched for the Krüppel-like factor protein family of transcription factors.
Summary/Conclusion: In conclusion our results first showed that the cytogenetically unbalanced dic(1;7) in MDS/AML is the hallmark of a specific

gene expression profile originated by epigenetic events, mostly enhancer hypermethylation. Biological features of dic(1;7), particularly its rare occurrence in complex karyotypes and the presence of p53 up-regulation, are separating MDS/AML with dic(1;7) from t-MDS/AML.

PB2077

CIRCULATING INFLAMMA-MIRNAS IN MYELODYSPLASTIC SYNDROMES

D. Mattiucci[1], E. Rossi[1], V. Mari[1], M. Mariani[1], T. Martini[1], V. Santini[2], E. Masala[2], S. Mancini[3], A. Olivieri[1], A. Poloni[1,*]

[1]Scienze Cliniche e Molecolari, Università Politecnica delle Marche, Ancona, [2]University of Florence, Firenze, [3]Università Politecnica delle Marche, Ancona, Italy

Background: Myelodysplastic Syndromes (MDS) are a heterogeneous group of clonal stem cell disorders affecting mainly the elderly and characterized by ineffective haemopoiesis, peripheral blood cytopenias and risk of developing acute myeloid leukemia. MicroRNAs (miRNAs) are small non coding single strand RNAs. miRNAs act inhibiting messenger RNAs (mRNAs) translation and decreasing their stability through a complementary base pairing and, on the basis of this regulatory role, abnormal miRNA levels are related to several pathological states. The presence of miRNAs in body fluids may represent a gold mine of noninvasive biomarkers in cancer. In particular, miRNAs in serum are linked with the presence of hematologic malignancies. In MDS, immune system dysregulation may represent a primary pathophysiologic abnormality and a key driver of the pathological evolution of MDS and it looks like chronic inflammation role is essential in MDS pathogenesis and progression: there is growing evidence implicating inflammation-related changes, inhibitory cytokines and increased intramedullary apoptosis as contributors to ineffective hematopoiesis.
Aims: In this study, we evaluated the expression of 15 miRNA which have been demonstrated to be dysregulated during ageing and in inflammatory conditions: miR-17; miR-9; miR-22; miR-152; miR-335; miR-19b; miR-20a; miR-34a; miR-146a; miR-181a; miR-9; miR-21; miR-126; miR-29a; miR-155.
Methods: Total RNA was isolated from the serum of 60 High Risk (HR) and Low Risk (LR) MDS patients and 30 donors and a quantitative analysis of the circulating miRNA was performed by Real Time PCR.
Results: Among 15 miRNA studied, the levels of miR-9, miR-17, miR-22, miR-34a, miR-152, and miR-335 were not detectable both in patients and donors. MiR-126, miR29a, miR20a, miR181a, 19b and miR21 show a statistically significant differential expression in both HR and LR patients with respect to controls. MiR-146a and miR-126 were altered only in LR-MDS (IPSS and R-IPSS) patients.
Summary/Conclusion: In this work it has been possible to evaluate inflamma-miRNAs' levels in MDS. In particular, these data could have a considerable significance in LR MDS, in which inflamma-miRNAs are aberrantly expressed respect to controls and could be the key regulators in the pathogenesis of ineffective erythropoiesis and anemia and also in myeloproliferation and oncogenic transformation.

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TREG LEVELS IN BONE MARROW OF LOW RISK MDS PATIENTS CORRELATE WITH PREFERENTIAL EXPANSION OF CYTOTOXIC T CELLS IN BONE MARROW AND A MORE FAVOURABLE PROGNOSIS

S. Leone[1,*], V. Rubino[2], A. Giovazzino[3], A.T. Palatucci[4], G. Cerciello[1], F. Pane[1], G. Ruggiero[3], F. Alfinito[1], G. Terrazzano[2]

[1]Dipartimento ad Attività Integrata di Ematologia, Oncologia, Anatomia Patologica e Diagnostica per Immagini, AOU Federico II, Naples, [2]Dipartimento di Scienze, Università della Basilicata, Potenza, [3]Dipartimento di Scienze Mediche Traslazionali, Università degli studi di Napoli Federico II, [4] Istituto per l'Endocrinologia e l'Oncologia Sperimentale, Consiglio Nazionale delle Ricerche (IEOS-CNR), Naples, Italy

Background: Several data have been showing that immune-dependent mechanisms might be relevant for the selection, expansion and dominance of dysplastic clone/s in a subgroup of Myelodysplastic (MDS) patients. To date, valuable criteria to identify patients in which a deranged immune response might account for the selection/shaping of the dysplastic precursor/s are still lacking. Immune response is a microsite phenomenon, thus the analysis of the immune effectors in the Bone Marrow (BM) offers a unique possibility to specifically focus the mechanisms underlying the immune-mediated processes likely involved in the selection/expansion of dysplastic clone/s in MDS.
Aims: We previously described that specific alterations of immune profile, as represented by low Treg levels and high expression of CD54 on CD8 effectors in BM, allow the identification of a subgroup of MDS patients in which an immune-mediated pathogenesis of the disease might be inferred. This study aims to confirm and extend these data trying to correlate tolerance control derangement in BM with pathological expansion of T cell effectors in Low Risk MDS patients. Moreover, we address the evaluation of Treg level as a valuable prognostic marker in Low Risk MDS.
Methods: To investigate the occurrence of antigen-dependent clonal expansion of CD4 and CD8 T lymphocytes, we analysed, by flow cytometry, TCR Vb repertoire in BM as compared with peripheral blood in 26 Low Risk patients and healthy donors. To evaluate the prognostic role of BM Treg at diagnosis, we have investigated the occurrence of leukemia evolution and overall survival in our cohort of patients in a minimal 36 month follow up.
Results: Preliminary data confirm that Treg, a key element for cell-mediated tolerance control, show a clustered distribution in BM; moreover, their quantitative defect correlates with the recruitment and the activation of T cell cytotoxic effectors in BM. The analysis of preferential CD4 and CD8 T cell expansion in BM, respect to peripheral blood, reveals the presence of oligoclonal BM expansions of CD8 and CD4 T lymphocytes. Notably, CD8 BM expansions are significantly related to low Treg level (<2% of BM lymphocytes). Moreover, our data indicate that the subgroup of patients with low BM Treg level, at diagnosis, shows significant lower leukemia evolution and death in a minimum 36 month follow up.
Summary/Conclusion: These data suggest that BM Treg level may represent a valuable criterion to identify the subgroup of Low Risk MDS patients in which immune-mediated mechanisms are relevant for MDS pathogenesis. A more homogeneous grouping of Low Risk patients will improve clinical management of the disease, hopefully allowing a more effective employment of innovative immune-modulating strategies in MDS.

PB2079

THE ANALYSIS OF EXPRESSION OF FOXP3 MOLECULE'S ISOFORMS BY REGULATORY CELLS IN PERIPHERAL BLOOD IN MYELODYSPLASTIC SYNDROME PATIENTS

G. Dudina[1,*], A. Mitin[2], M. Litvina[2], A. Donetskova[2]

[1]Hematology, Moscow Clinical Scientific Center (MCSC), [2]NRC Institute of Immunology, Moscow, Russian Federation

Background: Assuming that the immune system plays an active role in pathogenesis of myelodysplastic syndrome (MDS), it is expected that certain immune responses, namely, the number of regulatory T-cells (Treg cells), can be used as prognostic criteria. Participation of Tregs in pathogenesis of MDS can partly explain the link between MDS and autoimmune disorders, as well as tumor transformation.
Aims: Estimate the number of regulatory T-cells and level of expression of FOXP3 molecule isoforms differing by the exon-2 in peripheral blood of patients with MDS at early and late stages.
Methods: The evaluation was performed on peripheral blood mononuclear cells (PBMC). Various fluorochrome-labeled monoclonal antibodies (eBioscience) were used. As a negative control for establishing of gates for $FOXP3^+$ cells, we used a non-expressing FOXP3 molecules subpopulation of CD3-negative cells.
Results: 55 patients with MDS (WHO 2008) were examined. Patients with low and intermediate-1 risk of IPSS were included in the early-stage MDS group (E-MDS), and intermediate-2 and high-risk IPSS patients were included into the late stage MDS group (L-MDS). The obtained values for E-MDS and L-MDS groups did not differ significantly. When compared with the age control group, the absolute number of leukocytes, lymphocytes, and $CD4^+$ T-cells was found to be decreased, which is typical for MDS. In both groups, more than two-fold decrease in the absolute number of Tregs was registered. Treg decrease was proportional to the degree of leukopenia and somewhat more pronounced than decrease level of the number of lymphocytes and all $CD4^+$ T-cells, which is due to decrease of the proportion of Tregs among $CD4^+$ T-cells. The decrease of Treg percentage was statistically significant in the E-MDS group only, which suggests a higher probability of autoimmune disorders in this group. A significant decrease of the number of Tregs was shown, both expressing the complete FOXP3 molecule (FOXP3-FL Treg) and expressing only the FOXP3 molecule without exon-2 (FOXP3D2 Treg). Decrease of the number of FOXP3D Treg in peripheral blood was more pronounced than the decrease of FOXP3-FL Treg, which is due to a significant increase of the relative proportion of the latter. Presumably, the Treg functional activity in MDS is attenuated due to decrease of the proportion of FOXP3D2 Treg, known to be the most active suppressor of the immune response.
Summary/Conclusion: The data obtained shows a functionally intermediate role of Treg in MDS compared to inflammatory (autoimmune) diseases and

the norm, and its opposite role in malignant neoplasms. The basic mechanisms of MDS pathogenesis, including Treg function, need further study, which may lead to subdivision of different diseases with a distinctive clinical picture and outcome.

PB2080

VARIATION IN THE LEVEL OF AKT1 IN LYSATES OF BONE MARROW CELLS IN MYELODYSPLASTIC SYNDROMES

N. Klimkovich[1,*], O. Krasko[2], T. Kozarezova[1], M. Tishkevich[3]

[1]Department of pediatric oncology and hematology, Byelorussian Medical Academy of Post-Graduate Education, [2]Laboratory of Bioinformatics, United Institute of Informatics Problems of the National Academy of Sciences, [3]Institute of Bioorganic Chemistry of the National Academy of Sciences, Minsk, Belarus

Background: Oncoprotein kinase Akt (proteinkinase B) plays an important role in the regulation of the cell cycle, apoptosis, glucose metabolism and angiogenesis. There is an opinion that the quantitative level of the Akt kinase phosphoform can be considered as an independent prognostic marker for some types of human malignant neoplasms. Studies of the activated Akt protein are mainly produced in solid tumors. Such studies are few in hematology, but the prognostic and therapeutic prospects make them relevant.
Aims: Study of the level of Akt1 in lysates of bone marrow cells in patients with MDS and an estimation of its prognostic value.
Methods: The level of the activated form of the anti-apoptotic protein Akt1 was analyzed in74 patients with *de novo* myelodysplastic syndromes (MDS) and 11 with acute myeloid leukemia (AML) as a comparison group. Akt1 in the lysates of bone marrow cells was determined by the EIU the Total-Akt1 DuoSet IC kit (R&D Systems, Inc. USA). The measurements were carried out on an iMark microplate reader (BioRad, Japan). Statistical analysis was made with statistical tools R version 3.1.3.
Results: A high level of the activated form of Akt1 in the lysates of bone marrow cells was established for AML (1428 (103 ... 2319) pg/ml) and for MDS (528.25 (225.9 ... 1788.3) pg/ml) in relation to the control (165.95 (104.5 ... 236.1) pg / ml). In RA, 5q-syndrome and RCMD there was low content of Akt1 compared to RAEB variant: 286.95 (225.9 ... 1788.3) pg/ml for RA and 5q-syndrome, 526.7 (235.8 ... 1765.8) pg/ml with RCMD, 714.3 (235.8 ... 1775.3) pg/ml for RAEB. Analysis of the content of Akt1, depending on the cytogenetic status of bone marrow cells in patients with MDS, showed that the maximum concentration of Akt1 (726.8 (289.6 ... 1316.7) pg/ml) corresponds to multiple karyotype anomalies. The minimum concentration of Akt1 was registered in isolated del 5q31 (286.25 (225.9 ... 289.5) pg/ml). In groups with normal karyotype and isolated chromosomal abnormalities the content of Akt1 in the lysates of bone marrow cells did not differ. Expression of CD95 and FLT3 (CD135) by bone marrow cells has a nonlinear dependence on the level of Akt1. At the Akt1 level in bone marrow cell lysates over 500 pg/ml, the CD95 expression fast falls below 20% and FLT3 (CD135) expression increases by more than 40%. Death of patients with Akt1 content in bone marrow cell lysates more than 500 pg/ml was recorded in 82.5% of cases, and in patients with a concentration of Akt1 less than 500 pg/ml, 44.1% of cases ($p<0.001$). Transformation to acute leukemia was more frequent with an Akt1 content of more than 500 pg/ml (62.5%) compared to lower values of this parameter (41.2%), $p=0.067$. The timing of the onset of MDS transformation into leukemia is statistically significant: 71 (6 ... 340) weeks with Akt1 less than 500 pg/ml and 23.5 (3 ... 133) weeks with Akt1 greater than 500 pg/ml ($p<0.001$).
Summary/Conclusion: An increase in the concentration of the activated form of the Akt1 protein in bone marrow cell lysates corresponds to unfavorable variants of MDS that have a high risk in the IPSS system. The level of Akt1 in bone marrow cell lysates is more than 500 pg/ml and is the threshold for blocking apoptosis and the activity of clonal proliferation. The increased level of the activated form of Akt (more than 500 pg/ml) in the lysates of bone marrow cells can be considered as an independent prognostic marker of the progression of MDS.

PB2081

HIF-1A EXPRESSION IS ASSOCIATED WITH PRIMARY RESISTANCE TO AZACITIDINE IN MDS PATIENTS – DATA FROM THE HELLENIC MDS STUDY GROUP

V. Bakou[1], A. Spathis[2], F. Kontsioti[1], A. Bouhla[1], G. Stavroulaki[1], I. Glezou[1], K. Godopoulos[1], S. Papageorgiou[1], E. Bazani[1], D. Vasilatou[1], N. Viniou[3], A. Galanopoulos[3], K. Zafeiropoulou[4], I. Kotsianidis[5], A. Symeonidis[4], G. Dimitriadis[1], V. Pappa[1,*]

[1]Second Department of Internal Medicine and Research Institute, [2]Department of Cytopathology, Attikon University General Hospital, [3]The Hellenic MDS study group, Athens, [4]The Hellenic MDS study group, Patra, [5]The Hellenic MDS study group, Alexandroupoli, Greece

Background: Hif-1α expression has been previously associated with an adverse prognosis in patients with solid tumors and some hematological malignancies. Moreover, it was recently correlated with poor overall survival and disease progression in MDS. Hif-1α is also known to regulate the expression of human equilibrative nucleoside transporters (ENTs) and ribonucleotide reductase (RR), both of which are involved in the metabolism of hypomethylating agents, such as azacitidine. AZA is the standard of care for patients with high-risk myelodysplastic syndromes (MDS) and AML patients not candidate for treatment with intensive chemotherapy. Nevertheless, approximately 40% of patients fail to respond, whereas even responders will inevitably relapse.
Aims: This study aims at investigating the expression level and clinical significance of Hif-1α in both MDS AZA responders and non-responders, before and after treatment.
Methods: Bone marrow samples from 39 *de novo* MDS patients, 5 CMML patients and 12 AML [43 M/13 F, median age 75 (52-89)] and 10 healthy donors were collected. MDS patients were classified according to WHO as RCMD (4/39), RAEB I (11/39) and RAEB II (24/39) and according to IPSS as low (3/39), intermediate-1 (7/39), intermediate-2 (13/39) and high (16/39). All patients received AZA treatment at the dose of 75mg/m^2 x7 days SC. Low and inter-1 MDS patients received azacitidine as off label therapy. BM samples from 29 MDS [RCMD (1/29), RAEB I (9/29), RAEB II (19/29)], 2 CMML and 2 AML of the aforementioned patients were also collected 6 months after AZA therapy [26 M/7 F, median age 75 (52-89)]. After BM mononuclear cells' isolation (Ficoll-paque method), RNA extraction (TRIzol method) and cDNA preparation (Superscript II reverse transcriptase), Hif-1α expression was estimated by Rt-PCR TaqMan gene expression assay. Relative gene expression was calculated by comparative threshold cycle (ΔΔCt) method. β-actin was used as a housekeeping gene. Statistical analyses were performed through Kruskal Wallis, Mann-Whitney U and Wilcoxon signed rank tests.
Results: Out of the 56 patients used in our study, 32 responded to azacitidine-treatment (including CR, PR and HI) while 24 failed to respond. AZA responders presented with a statistically significantly increased $2^{-\Delta\Delta Ct}$ ratio of Hif-1α/β-Actin median expression compared to both control samples and non responders (1.377 *vs* 0.698 and 0.73, $p=0.004$ and 0.008, respectively). AZA non-responders presented no statistical significance compared to control samples. Hb levels were not significantly different between R and NR patients' groups. Moreover, an increase in Hif-1α expression was observed comparing Inter-I and Inter-II IPSS risk groups (0.730 *vs* 1.672, $p=0.037$) in all MDS patients examined, while a positive correlation between Hif-1α expression and IPSS-R risk scores (CC=0.371, $p=0.024$) was also found. Finally, the $2^{-\Delta\Delta Ct}$ ratio of Hif-1α/β-Actin median expression after 6-months of therapy presented a decreasing trend for both responders [from 1.377 to 1.269 (7.85% decrease)] and non-responders [from 0.730 to 0.598 (18% decrease)].
Summary/Conclusion: Our data indicate that patients with increased pretreatment Hif-1α expression seem to better respond to AZA therapy. Hif-1α expression gradually increases across IPSS and IPSS-R subgroups, suggesting an association of Hif-1α with a more aggressive disease. Moreover, AZA treatment seems to downregulate Hif-1α expression in both responders and non responders, indicating a potential role of hypoxia signaling in azacitidine resistance.

PB2082

TARGET NEXT GENERATION SEQUENCING FOR SOMATIC MUTATION SCREENING IN PATIENTS WITH A LOWER-RISK MDS TREATED WITH THE IRON-CHELATOR DEFERASIROX

E. Fabiani[1,*], C. Calabrese[2], P. Niscola[3], E. Balleari[4], A. Molteni[5], C. Finelli[6], G. Falconi[1], S. Fenu[7], L. Fianchi[8], M. Criscuolo[8], C. Gurnari[1], F. Buccisano[1], L. Maurillo[1], F. Lo Coco[1,9], D. Cilloni[2], M.T. Voso[1]

[1]Department of Biomedicine and Prevention, University Tor Vergata, Rome, [2]Department of Clinical and Biological Sciences, University of Turin, Orbassano, [3]Department of Hematology, S. Eugenio Hospital, Rome, [4]Department of Haematology and Oncology,, IRCCS AOU San Martino, Genova, [5]Hematology, ASST Grande Ospedale Metropolitano Niguarda, Milan, [6]Department of Hematology,, S. Orsola-Malpighi Hospital, Bologna, [7]Department of Hematology, San Giovanni-Addolorata Hospital, [8]Hematology Institute, Università Cattolica del Sacro Cuore, [9]Laboratory of Onco-Hematology, Santa Lucia Foundation, Rome, Italy

Background: Somatic mutations are one of the major factors driving the

pathogenesis of myelodysplastic syndromes (MDS) and are closely associated with clinical phenotype. The iron-chelator deferasirox (DFX) has been shown to induce hematologic response (HR) in about 10% of patients with MDS, with a yet unknown mechanism.

Aims: Our aim was to evaluate the role of somatic mutations in critical genes as predictors of hematologic response to DFX.

Methods: Sixty diagnostic bone marrow samples from 19 patients responsive and 41 resistant to DFX were tested in this study. Thirty genes known to be frequently mutated in haematological malignancies were screened for somatic mutations in 15 patients (7 responsive and 8 resistant) using NGS based on the commercial Myeloid Solution by SOPHiA GENETICS (SOPHiA GENETICS, Saint-Sulpice, Switzerland). The resulting captured libraries were further processed on a HiSeq® sequencing platform (Illumina, San Diego, California). Generated FASTQ sequencing files were then uploaded to SOPHiA DDM® platform (SOPHiA GENETICS, Saint-Sulpice, Switzerland). An additional cohort of 45 patients was screened for mutations in the hot-spot regions of spliceosome machinery enzymes (SF3B1, SRSF2, U2AF1) and epigenetic regulators (IDH1, IDH2 and DNMT3A), using Sanger sequencing.

Results: Using NGS, we identified 53 mutations with a variant allele frequency (VAF) ≥1%, with at least 1 mutation in 14 of 15 patients (93.3%). The median number of mutations per patient was 3.53 (range, 0-6). As reported in figure 1a, the most commonly mutated genes were: ASXL1 in 9 of 15 pts (60%) and RUNX1, DNMT3A, SF3B1 and TET2 in 4 of 15 pts (27%). In this analysis, none of the single gene was predictive of HR to Deferasirox treatment, while mutation frequency in general was lower in responders, as compared to resistant patients (mean 2.4 *vs* mean 4.5 mutations/patient, respectively; p=0.0232). When extending the mutational screening to further 45 patients by Sanger sequencing, we identified SF3B1 as the most commonly mutated gene. Although the cumulative frequency of SF3B1 mutations in the entire cohort of patients (23/60 pts, 38.3%) appeared to be higher in resistant as compared to responsive patients (17/41, 41.5%, *vs* 6/19, 31.6%), the difference did not reach statistical significance.

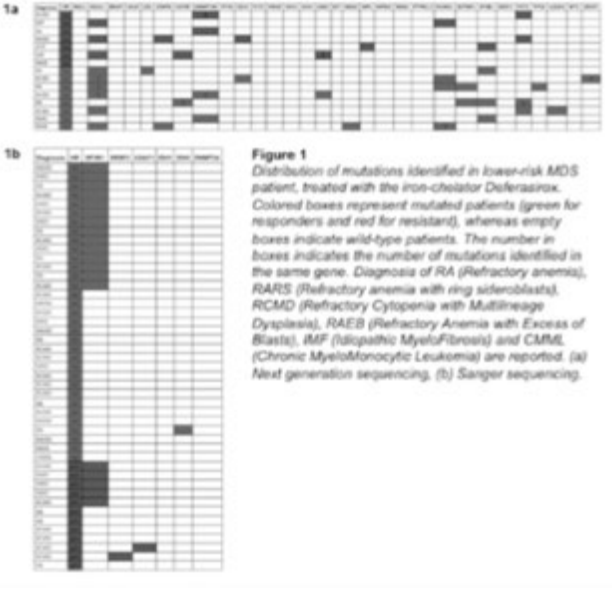

Figure 1
Distribution of mutations identified in lower-risk MDS patient, treated with the iron-chelator Deferasirox. Colored boxes represent mutated patients (green for responders and red for resistant), whereas empty boxes indicate wild-type patients. The number in boxes indicates the number of mutations identified in the same gene. Diagnosis of RA (Refractory anemia), RARS (Refractory anemia with ring sideroblasts), RCMD (Refractory Cytopenia with Multilineage Dysplasia), RAEB (Refractory Anemia with Excess of Blasts), IMF (Idiopathic MyeloFibrosis) and CMML (Chronic MyeloMonocytic Leukemia) are reported. (a) Next generation sequencing, (b) Sanger sequencing.

Figure 1.

Summary/Conclusion: Our preliminary data show that mutational screening performed by NGS of lower-risk MDS may have a role in predicting hematologic response to Deferasirox treatment, whereas the sole presence of SF3B1 mutations is not associated to hematologic response.

PB2083

SOMATIC MUTATIONS IN PATIENTS WITH CLONAL CYTOPENIA OF UNDETERMINED SIGNIFICANCE, MYELODYSPLASTIC SYNDROME AND RELATED MYELOID NEOPLASMS: A PRELIMINARY STUDY WITH A NORTHEASTERN POPULATION IN BRAZIL

L. Oliveira[1,2,*], R. Rahimy[2], V. Lisboa[3], V. Maffili[2], I. Saldanha[2], S. da Paz[4], S. Teles[4], C. Pereira[5], M. Gonçalves[2], K. Coelho[2], M.A. Salvino[1,6], D. Zanette[2]

[1]Hematology, São Rafael Hospital Monte Tabor Bahia, [2]LIGHT - Laboratory of Investigation of Genetics and Translational Hematology, Gonçalo Muniz Institute - Oswaldo Cruz Foundation, [3]STS - Hematology Center, [4]Gonçalo Muniz Institute - Oswaldo Cruz Foundation, [5]CBTC - Center for Biotechnology and Cell Therapy, São Rafael Hospital Monte Tabor Bahia, [6]Hematology, Federal of Bahia University, Salvador, Brazil

Background: The importance of the identification of somatic mutations in myeloid neoplasms has increased dramatically in the last ten years. The study of these mutations has improved the understanding of bone marrow (BM) clonal expansion that occurs in these malignancies. Mutations in genes participating in the mechanism of splicing and epigenetics are found in patients with clonal cytopenia of undetermined significance (CCUS), myelodysplastic syndrome (MDS), myelodysplastic/myeloproliferative neoplasm (MDS/MPN) and acute myeloid leukemia with myelodysplasia-related changes (AML-MRC). The evaluation of somatic mutations profiles in the Brazilian population has clinical relevance due to the great ethnic differences found in our population as compared to the North American and European populations, where the vast majority of these studies were conducted.

Aims: Evaluation of the frequency of somatic mutations in genes: *SF3B1, TET2, U2AF1, IDH1/2, tp53* in patients from the city of Salvador, Bahia, Brazil, with diagnoses of CCUS, MDS, MDS/MPN and AML-MRC.

Methods: In this observational study, 50 untreated patients ≥18 years were analyzed. Blood counts, cytogenetic and BM analysis were performed. DNA was isolated from BM and PCR analyzes was performed. All patients provided informed consent.

Results: From the 50 patients analyzed, 24 were diagnosed with cytopenias (48%), 18 with MDS (36%), 5 with MDS/MPN (10%) and 3 with AML (6%). Overall, 29 patients were female (58%) and 21 were male (42%). The mean age was 60.5 years (range 22-84). Mutated genes were found in 19 patients (38%). Ten patients showed mutations in *U2AF1* gene (20%), 6 (12%) in *TET2*, one (2%) in *SF3B1*, one (2%) in *IDH2* and one (2%) in *TP53* mutated. The coexisting mutations were found in four patients, as follows: *IDH2* + *SF3B1*, *SF3B1* + *TET2*, *U2AF1* + *IDH2* and *U2AF1* + *TET2*. Eleven patients (45,8%) presented mutations in the cytopenias group. From these, 7 cases showed mutations in *U2AF1* and the remaining four cases had mutations in *SF3B1*, *TET2*, *IDH2* and *TP53*. All the coexisting mutations found were in this group. In the MDS group, *TET2* mutations were found in four (38,9%) patients and *U2AF1* mutations were found in 16,7% (n=3). No mutations were observed in the AML group and one patient (20%) presented mutation in *TET2* gene in the group MDS/MPN (p=0,66). From the 27 patients <60 years of age, 9 (40,9%) showed mutations: four had *U2AF1* mutated, two patients presented mutation in *TET2* and three presented mutations in *SF3B1*, *IDH2* and *TP53*. From the 27 patients >60 years of age, 10 (37%) presented mutations: 6 in *U2AF1* and four in *TET2* (p=0,66). All of the patients with coexisting mutations were <60y. Mutations were not differentially associated with sex. Of 21 male patients, 9 (42,9%) had one or more mutations. Of the 29 female patients, 10 (34,5%) had mutations (p=0,55). The majority of patients analyzed had <2% blasts in the BM, hemoglobin levels ≥10g/dL, neutrophils ≥800/μL and platelets ≥100,000/μL (Figure 1).

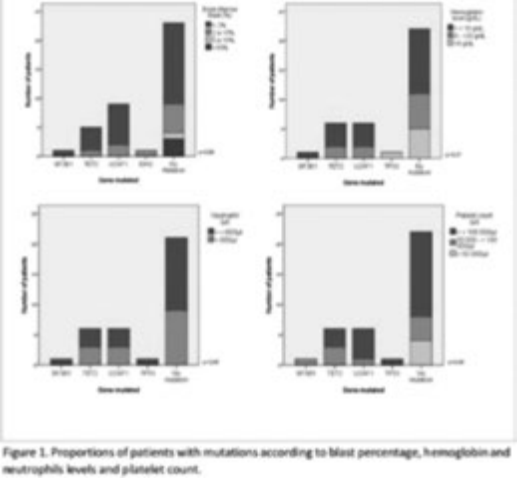

Figure 1.

Summary/Conclusion: Differently from literature data, the main gene mutated was *U2AF1*, found in 20% of cases here, as opposed to ~ 8%

found in other studies. The cytopenias group presented the highest number of cases with mutations. Moreover, the number of coexisting mutations was higher in this group and the same were found only in patients <60y. This is a preliminary study, that is under development to increase the number of enrolled individuals and might be added to other Brazilian studies, aiming to define the mutation profile of myeloid neoplasms in Brazil.

PB2084

THE HYPERMETHYLATION PROFILE OF APOPTOTIC GENES IN MYELODYSPLASTIC SYNDROMES

F. Zaker[1,*], N. Nasiri[1], N. Amirizadeh[2], M. Razavi[3]

[1]Lab haematology, Iran University Of Medical Sciences, [2]Higher education institution, iranian blood transfusion service, [3]Department hematology oncology, Iran University Of Medical Sciences, tehran, Iran, Islamic Republic Of

Background: MDS is a clonal disorder which patients have pancytopenia, anemia and transfusion dependent. This disorder progress to AML in 1:3 of cases and resistant to chemotherapy. Several molecular mechanisms involved in pathogenesis of MDS including hypermethylation of selected genes in different manner like apoptosis, cell cycle, and tumor suppressor.
Aims: In this work we studied methylation and epigenetic of several genes including FOXO3, CHK2, HRK and PTEN involved in apoptosis and relationship to biological characteristic and karyotype and subgroup of MDS and IPSS.
Methods: We studied 54 patients enrolled in Shariati Hospital, Firozgar hospital and other therapuetic center in Tehran. PB or BM were collected and DNA and RNA extracted. DNA was bisulfitted, methylated and measured by MS-HRM. cDNA synthesized from RNA and expression of genes studied by REAL TIME PCR. SPSS 16 software was used to analyse the data.
Results: The most frequency of methylation occurred in CHK2 gene which has been seen in all subtypes of MDS including RAEB1, 2. The advanced stage of MDS showed the most hypermethylation status compare to early PCR stage. The most hypermethylation of gene occurred in high and very high subgroup of IPSS-R. Real time showed downregulation of selected genes which were in parallel to hypermethylation pattern.
Summary/Conclusion: The data showed hypermethylation of all selected genes inviolve in apoptosis could explain the mechanisms and pathogenesis in MDS in partly. This hypermethylation was parallel to decease in expression of selected genes.

Myelodysplastic syndromes – Clinical

PB2085

THE ROLE OF FLT3-ITD MUTATION ON DE NOVO MDS AMONG CHINESE POPULATION

F. Xu[1,2], R. Han[2], J. Deng[3], Q. Qiu[2], H. Shen[2], S. Chen[2], D. Wu[2], S. Xue[2,*]

[1]Department of Medical Oncology, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, [2]The First Affiliated Hospital of Soochow University,Jiangsu Institute of Hematology, [3]Suzhou Center for Disease Control and Prevention, Suzhou, China

Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of myeloid neoplasms characterized by aberrant myeloid differentiation, ineffective hematopoiesis and potency of secondary acute myeloid leukemia (sAML) progression. The underlying mechanism of this transformation has long been the hit topic among hematologists. Besides, with the nature of heterogeneity seen in MDS, scoring systems were introduced to evaluate the prognosis of patients with MDS, such as IPSS-R, yet leaving molecular patterns out of them. Meanwhile, the role of FLT3 gene in AML has been well documented and put into practice. Nevertheless, with a rather low incidence of mutation in MDS group (0.6%>6%), the role of FLT3 mutation has not been revealed fully in real world MDS.
Aims: The present study was performed to investigate: the mutation patterns of FLT3 in MDS among Chinese patients; the impact of FLT3 on prognosis of Chinese MDS patients; and the association between FLT3 mutations and secondary AML transformation.
Methods: 311 *de novo* MDS patients diagnosed between 2010 and 2016 under the 2016 WHO criteria were enrolled, with written informed consent. Studies were approved by the ethics committee of the First Affiliated Hospital of Soochow University (FAHSU) in accordance to the declaration of Helsinki protocol. FLT3 testing was applied only at initial diagnosis and at the first time of AML progression. Hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated using Cox proportional hazards models, which were adjusted for possible confounding factors.
Results: The mutation patterns of FLT3-ITD seen in our patients were similar with their AML counterparts. With basic clinical characteristics well balanced, leukemia transformation rate was significantly different between FLT3-ITD and wild type groups, with 42.9% (3/7) and 10.2% (31/304) of each. Two groups also showed diverse prognosis. The progression free survival (PFS) was 43(16-310) days and 365(8-2208) days in ITD positive and negative group, respectively(P<0.001). And the overall survival (OS) was 218(16-334) days and 414(8-2208) days (P<0.001). In later univariate and multivariate analysis, five factors were found having independent impact on OS: bone marrow blast percentage, WBC count, cytogenetics subtype, treatment and FLT3-ITD mutation status. Here the treatment was graded into three parts, transplantation, chemotherapy or demethylation agents and supportive care only.
Summary/Conclusion: When observed at MDS stage, patients harboring FLT3-ITD mutations had higher AML-transformation rate, quicker disease progression and shorter survival than wild type patients among Chinese population, laying in accordance with data from previous studies.

PB2086

Abstract withdrawn.

PB2087

DIAGNOSTIC POTENTIAL OF ALDEHYDE DEHYDROGENASES ISOENZYMES IN MDS AND AML

A.C. Gonçalves[1,2], B. Macedo[3], A. Barbosa Ribeiro[1,2,4], R. Alves[1,2], J. Jorge[1,2], G. Marques[5], L. Jorge[5], E. Cortesão[1,2,4], A.B. Sarmento Ribeiro[1,2,4,6,*]

[1]Coimbra Institute for Clinical and Biomedical Research (iCBR) - Group of Environment Genetics and Oncobiology (CIMAGO), FMUC, [2]Laboratory of Oncobiology and Hematology and University Clinic of Hematology, Faculty of Medicine, University of Coimbra (FMUC), Coimbra, [3]Chemistry Department, Aveiro University, Aveiro, [4]Clinical Hematology Department, [5]Clinical Pathology Department, Centro Hospitalar e Universitário de Coimbra (CHUC), [6]Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra, Portugal

Background: Aldehyde dehydrogenases (ALDH) are critical to the protection against toxic aldehydes and have been associated with multiple diseases,

namely with cancer. Myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) are two myeloid neoplasias with a complex multistep development involving abnormal differentiation, cellular proliferation, and apoptosis. Since ALDHs are involved in some of these biological processes, the deregulation of these enzymes may influence MDS and AML development.

Aims: This study aimed to evaluate the gene expression levels of ALDHs isoenzymes in patients with MDS and AML in order to verify their potential as a biomarker for the diagnosis and/or prognosis of these diseases.

Methods: To this end, we analyzed the gene expression levels of *ALDH1A1*, *ALDH1A2*, *ALDH1A3*, *ALDH1B1*, *ALDH1L1*, *ALDH1L2*, *ALDH2*, *ALDH3A1*, *ALDH3A2*, *ALDH3B1*, *ALDH3B2*, *ALDH4A1*, *ALDH5A1*, *ALDH7A1*, *ALDH16A1*, and *ALDH18A1*. The ALDH expression levels were analyzed using RT-PCR and the differentially expressed genes were quantified by qPCR. This study enrolled 34 MDS patients [median age of 72 years (ranging from 49 to 89 years) and 59% males], 20 AML [median age of 56 years (ranging from 26 to 92 years) and 45% males], and 34 healthy controls [median age of 70 years (ranging from 32 to 88 years) and 59% males]. According to the 2016 WHO classification, MDS group included: 4 MDS with single lineage dysplasia (MDS-SLD), 15 MDS with multilineage dysplasia (MDS-MD), 7 myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN), 4 MDS with excess blasts (MDS-EB), 3 MDS with ring sideroblasts (MDS-RS), and 1 MDS associated with isolated del(5q). According to WPSS, 5 MDS patients have very low risk, 9 low risk, 6 intermediate risk, 2 high risk, 1 very high risk (4.4%), and 11 patients were unclassified. The AML group included 7 AML with minimal differentiation (AML-MD), 5 acute myelomonocytic leukemia, 4 acute promyelocytic leukemia with PML-RARA, and 4 AML with myelodysplasia-related changes (AML-MRD). The statistical analysis was carried out by uni- and multivariate tests. A value of $p<0.05$ was considered significant.

Results: The results indicate that *ALDH3A2*, *ALDH3B1*, *ALDH4A1*, and *ALDH18A1* had a differential expression among the study groups and were posteriorly quantified by real-time PCR. MDS and AML patients showed higher median expression levels of *ALDH3A2* [MDS: 1.93 interquartile range (IR) 1.28; $p<0.001$; AML: 1.51 IR 0.99; $p=0.008$)] and *ALDH4A1* (MDS: 0.18 IR 0.47; $p=0.011$; AML: 0.16 IR 0.78; $p=0.012$) in comparison with controls (*ALDH3A2*: 0.4624 IR 1.53 *ALDH4A1*: 0.0388 IR 0.12). The expression of *ALDH3B1* was higher in MDS patients (1.64 IR 1.39) than in AML patients (0.45 IR 0.47; $p<0.001$) and controls (0.35 IR 0.51; $p<0.001$). Moreover, this isoenzyme was significantly higher in MDS-SLD and in very low-risk patients (WPSS) comparatively with the others subgroups. Additionally, patients with MDS and AML with myelodysplasia-related changes (AML-MRC) did not express ALDH18A1. ROC curve analysis showed that *ALDH3A2* ($p=0.001$), *ALDH3B1* ($p<0.001$), and *ALDH4A1* ($p=0.012$) were able to discriminate MDS patients from controls. Moreover, *ALDH3A2* was the only isoform with diagnostic value for AML patients.

Summary/Conclusion: ALDH isoforms have differential expression patterns in MDS and AML patients when compared to controls and with each other, and can be good diagnostic biomarkers of these diseases. However, further studies are needed to prove the potential of these enzymes as diagnostic/prognostic biomarkers.

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PB2088

RISK-MITIGATION PLANS TO REDUCE INCIDENCE OF URINARY ADVERSE EVENTS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS) TREATED WITH ORAL RIGOSERTIB (RIGO) IN COMBINATION WITH AZACITIDINE (AZA)

S.C. Navada[1,*], G. Garcia-Manero[2], M.N. Rajeh[3], J.M. Shammo[4], E.L. Atallah[5], E.A. Griffiths[6], S.K. Khaled[7], P.S. Zbyszewski[8], M.E. Petrone[8], S.M. Fruchtman[8], L.R. Silverman[1]

[1]Mount Sinai Medical Center, New York, [2]University of Texas MD Anderson Cancer Center, Houston, [3]Saint Louis University, St. Louis, [4]Rush University Medical Center, Chicago, [5]Froedtert Hospital & the Medical College of Wisconsin, Milwaukee, [6]Roswell Park Cancer Institute, Buffalo, [7]City of Hope, Duarte, [8]Onconova Therapeutics, Inc., Newtown, United States

Background: MDS are a rare group of blood cancers that occur as a result of disordered development of blood cells within bone marrow. MDS occur most commonly in older adults, with a median age at dx of ≥65 yrs. Previous studies have shown oral RIGO at a dose of 560mg BID in Low-Risk MDS (LR-MDS) patients (pts) demonstrate a transfusion independence rate (IWG2006) of 44% (Raza *et al*., Blood 2017). Oral RIGO in combo with AZA in pts with MDS is being studied (Navada S, Blood 2016). In monotherapy & combination trials, oral RIGO has been associated with urinary adverse events (UAEs) of interest, shown to be dose & administration scheme dependent (Garcia-Manero G, Blood 2016).

Aims: Reported are initial results of a dose exploration study in MDS pts focusing on impact of risk-mitigation plans in reducing incidence of UAEs including hematuria.

Methods: Pts w MDS or leukemia (N=168) were given oral RIGO monotherapy in doses escalating from 70mg-1680mg 2x daily for either 14 consecutive days per 21-day cycle (intermittent schedule) or for 21 consecutive days per 21-day cycle (continuous schedule). In Part 1 of a trial of oral RIGO with AZA 75mg/m^2/d SC or IV (N=54) pts were administered RIGO 2x daily on Day 1-21 of a 28-day cycle in escalating cohorts, max dose of 560mg qAM & 280mg qPM (total 840mg) & AZA, administered for 7days/month starting on Day 8. In an ongoing Part 2 Study, oral RIGO at a total dose of 1120mg in 2 cohorts 560mg BID or 840mg/280mg is administered on Days 1-21 of a 28-day cycle in MDS pts with AZA; applying risk-mitigation plans to reduce UAEs: 1. Second RIGO dose must be taken at 3 PM (±1 hour) at least 2 hours after lunch to avoid a nocturnal bladder dwell time (Maniar M, *et al.* ASCO 2018 Sub for Pub); 2. Oral hydration of at least two liters of fluid per day is encouraged; 3. Mandatory bladder emptying prior to bedtime; 4. Urine pH approx. 2 hours after AM dose. Sodium bicarbonate suggested 650 TID if pH tests <7.5.

Results: The most frequent treatment emergent AEs observed in safety-evaluable pts in MDS studies w oral RIGO monotherapy (N = 168) were urinary. Higher frequency was identified for pts treated with continuous RIGO dosing *versus* intermittent dosing. Due to UAEs, continuous 560mg BID dosing is no longer being studied. Incidences of renal and urinary disorders have been identified with single agent AZA. In a trial of RIGO (total dose of 840mg) & AZA, incidence of UAEs, including hematuria, was 74%, w Gr ≥3 UAEs of 29%. In 37 pts studied w oral RIGO 1120mg & AZA, implementation of risk-mitigating plans to reduce UAEs, UAEs were 30%; Gr ≥3 5% have been seen to date (Table 1).

Table 1.

Table: UAEs Comparison Between RIGO Mono & Combo Therapy*

Patients on Monotherapy**	168
Patients with Urinary AEs	108 (64%)
Patients with hematuria	37 (22%)
Grade 1/2	35 (21%)
Grade ≥3	6 (3.5%)
Patients on Part I RIGO 840mg & AZA	42
Patients with Urinary AEs	31 (74%)
Patients with hematuria	20 (48%)
Grade 1/2	17 (40%)
Grade ≥3	5 (12%)
Patients on Part 2 RIGO (1120mg) & AZA with risk mitigation plan	37
Patients with Urinary AEs	11 (30%)
Patients with hematuria	4 (11%)
Grade 1/2	4 (11%)
Grade ≥3	0 (0%)

* preliminary analysis of platelet count to be presented
** AEs were Graded per National Cancer Institute's Common Toxicity Criteria ver4.0

Summary/Conclusion: Dose optimization & risk mitigation plans to reduce UAEs associated with oral RIGO in combination with AZA have resulted, to date, in a decrease in frequency of UAEs. This study is ongoing. Reduction of AEs permits pts to continue treatment to optimize benefit. Reduction in incidence of UAEs enables continued study of oral RIGO in LR-MDS based on promising transfusion independence rate previously reported (Raza *et al*., Blood 2017).

PB2089

THE HIDDEN IMPACT OF PATIENT GENERAL CONDITION ON THE OVERALL SURVIVAL OF MYELODYSPLASTIC SYNDROMES AND RELATED DISEASES (CHRONIC MYELOMONOCYTIC LEUKEMIA AND ACUTE MYELOID LEUKEMIA)

P. González Carmona[1,*], V. Martínez-Robles[1], S. Cerdá[1], J. Sánchez-del-Real[1], S. Jacoubi[1], F. Rondón[1], N. Bonache[2], M.L. Villalobos[1], J.A. Rodríguez-García[1], F. Ramos[1]

[1]Hematology, [2]Internal Medicine, Hospital Universitario de León, León, Spain

Background: Patient clinical situation at diagnosis (Dx) of MDS is a function of general condition (GC) *before Dx* and the impact of the new disease.

ECOG performance status probably depends on both of them, and consequently may be highly dependent on the very moment that it is measured. GC at baseline can be measured by Lee Index (LIx) for Older Adults (Lee SJ *et al.*, 2006), a 12-item validated scale that has been shown to predict 4-year mortality among community-dwelling adults in USA and also to predict overall survival (OS) in a prospective and molecularly annotated cohort of Spanish MDS patients (Ramos F *et al.*, 2017). To our knowledge, LIx has not been applied yet to MDS-related conditions (CMML and AML).
Aims: To analyze whether patient GC before Dx can be considered an independent determinant of OS in a heterogenous population of Pts diagnosed with MDS and related diseases.
Methods: We have reviewed the clinical charts of 132 pts (84 M, 48 F), median age 79y (IQR 73-82y), diagnosed in our center with MDS (n=88), CMML (17) or AML (27), whose GC had been evaluated by means of the LIx. The study was approved by our Institutional Review Board. All Pts were Dx and classified according to WHO 2008 and stratified prognostically (Px) according to disease-specific criteria (IPSS-R in MDS, CPSS in CMML and MRC/LRF scoring system in AML). ECOG was 0 in 23 Pts (17.4%), 1-2 in 93 (70.4%), 3-4 in 15 (11.4%) and NA in 1 (0.75%). Twenty-two Pts (16.7%) had received therapy known to prolong OS, while the rest had received only supportive care. After a median follow-up of 38.1 months (IQR 37.7-71.6), 70 Pts (53.0%) had died, 45 (34.1%) were alive, and 17 (12.9%) were lost to follow-up. The proportion of Pts that received disease-modifying therapy was similar across the Dx and Px groups. OS was evaluated by Log-rank tests (for trend, as appropriate) and Cox regression models.
Results: OS was longest for CMML Pts (median NR), intermediate for MDS (53.4 months, IQR 29.1-77.7) and shortest for AML (8.5, IQR 0-19.1), p<0.001 (Log-rank). As expected, it was also progressively shorter as the disease-specific Px categories were worse (p<0.001, Log-rank for trend). ECOG categories were strongly associated with both LIx and Px categories (Chi-Square for trend 10.84, p=0.001 and 7.94, p=0.006, respectively), but not to the Dx category (p=0.55). LIx score ranged 0-19 points (median 8, IQR 6-10). Fifty-three Pts (40.2%) were included in the Q1 (score 0-5), 45 Pts (34.1%) in Q2 (6-9), 25 (18.9%) in Q3 (10-13) and 9 (6.8%) in Q4 (score 14+). As expected, LIx categories were associated neither with the Dx nor with the Px categories (p=0.15 and p=0.36, respectively). At first sight, we observed a non-significant univariate trend towards a shorter OS as the LIx was higher: Q1 68.2 months (IQR 25.6-110.9), Q2+Q3 45.2 (IQR 14.9-75.4) and Q4 16.2 (IQR 0-41.8); p=0.211, Log-rank for trend. Interestingly, multivariate models disclosed that LIx score was an independent determinant of OS in this heterogeneous population, whether the type of treatment is included in the model (p=0.019) or not (p=0.034).
Summary/Conclusion: Patient general condition, as evaluated multidimensionally by the Lee Index for Older Adults, is an independent determinant of OS in patients with MDS, CMML and AML. This statement is now being evaluated in a larger and prospective patient cohort.

PB2090

SOLUBLE ADHESION MOLECULE LEVELS PREDICT RESPONSE TO AZACYTIDINE IN PATIENTS WITH HIGH RISK MYELO DYSPLASTIC SYNDROME – RESULTS OF A PIVOTAL TRIAL

T. Kupsa[1,2], P. Belohlavkova[2], L. Jebavy[1,2], P. Zak[2], J.M. Horacek[1,2,*]

[1]Department of Military Internal Medicine and Military Hygiene, University of Defence, Faculty of Military Health Sciences, [24]th department of Internal Medicine, Hematology, University Hospital and Charles University, Faculty of Medicine, Hradec Kralove, Hradec Kralove, Czech Republic

Background: Since introduction of azacytidine therapy for high risk myelodysplastic syndromes (MDS) several attempts have been made to define predicitive and prognostic factors in this patient cohort. Cytokines and adhesion molecules have been studied as markers of immune system activation in many diseases including MDS and acute myeloid leukemia (AML). Further knowledge gained from baseline cytokine levels assessment may help to improve treatment outcomes.
Aims: The aim of this study is to evaluate baseline levels of selected cytokines and soluble adhesion molecules and their relationship to azacytidine therapy response.
Methods: Baseline serum levels of 19 MDS patients, age 70.7±3.5 years, median 72 years, were collected prior to azacytidine therapy in the period 2015-2017. These patients were not candidates for intensive chemotherapy or allogeneic stem cell transplantation. All patients were treated with azacytidine 75mg/m^2 per day for 7 consecutive working days. Dose reduction was applied when necessary. Two subgroups were identified according to response to therapy within 6 cycles. Age, IPSS, normal karyotype, complex karyotype and bone marrow blast cell infiltration over 15% were variables included into analysis. We evaluated serum levels of the following 17 analytes: interleukins (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10), Epidermal Growth Factor (EGF), Interferon-γ, Monocyte Chemotactic Protein-1 (MCP-1), Tumor Necrosis Factor-α (TNF-α), Vascular Endothelial Growth Factor (VEGF), E-selectin (E-SEL), P-selectin (P-SEL), L-selectin (L-SEL), Intercellular Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Molecule-1 (VCAM-1). All biomarkers were measured by biochip array technology on Evidence Investigator analyzer (Randox). Statistical analysis was performed in STATISTICA 2.0.
Results: Based on response to azacytidine therapy, the subgroups of responders (n=9, 47%) and non-responders (n=10, 53%) were identified. CR was achieved in 3 cases (16%). The subgroups have not differed in age, IPSS, bone marrow blast cell infiltration or frequency of normal karyotype or complex karyotype. The responders to azacytidine had lower levels of ICAM-1 (229.08±37.85 *vs* 290.97±45.3, *P*=0.0363), E-SEL (6.16±1.85 *vs* 16.38±9.89, *P*=0.0431) and L-SEL (759.84±206.74 *vs* 1166.16±359.83, *P*=0.0383).
Summary/Conclusion: The results indicate that high risk MDS patients can be further stratified according to cancer microenvironment, which is a sine qua non for development of new treatment approaches. We plan to analyse these cytokines and soluble adhesion molecules as possible prognostic markers in the future.
The work was supported by a long-term organisation development plan 1011 (FMHS).

PB2091

MIR-16 IS ASSOCIATED WITH VEGF UPREGULATION IN HIGH-RISK MYELODYSPLASTIC SYNDROMES

Y. Nie[1,*], Y. Yu[2], S. Wang[3], Y. Jia[1], B. Xiong[2], X. Zuo[2]

[1]sino-us diagnostics, Tianjin, [2]Department of Hematology, Zhongnan Hospital of Wuhan University, Wuhan, [3]Institute of Hematology & Blood Disease Hospital, Tianjin, China

Background: Myelodysplastic syndromes (MDS) is a heterogeneous group of hematological disorders characterized by impaired hematopoiesis with a high risk of leukemic transformation. Recently, overexpression of Vascular endothelial growth factor (VEGF), a major angiogenic factor, has been found in MDS and showed different expression status in different risk of MDS.
Aims: Our aim is to investigate the possible role of miR-15a and miR-16 on regulation of VEGF expression and their effects on angiogenesis in different risk of MDS.

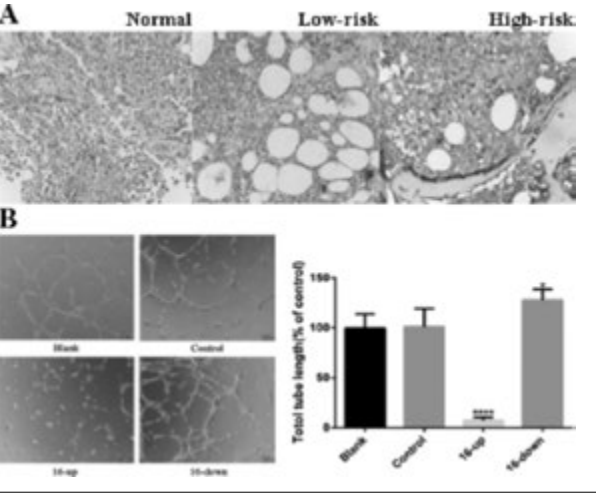


Figure 1.

Methods: ELISA, immunohistochemistry assay and immunofluorescence were used for detection of VEGF secretion in peripheral blood of MDS patients, protein expression in biopsies of MDS patients and several leukemia and MDS cell lines, repectively. qRT-PCR was performed to detect the expression of miR-15a and miR-16 in MDS bone marrow samples and cell lines. A human MDS SKM-1 cell line was transfected with pre-miR-16, anti-miR-16 and their respective negative control. ELISA and western blotting were performed to test the effect of miR-16 on the secretion and protein expression of VEGF. The luciferase reporter assays was conducted to confirm VEGF is a potential target of miR-16. The migration and tube formation of human umbilical vein endothelial cells were performed in miR-16 transfected SKM-1 condition medium (CM).

Results: Levels of miRNA-16 were significantly decreased in high-risk MDS patients as well as SKM-1 cell line, while the expression levels of VEGF were upregulated. Transfection of miR-16 in SKM-1 cells resulted in reduced secretion and protein expression of VEGF. The migration and tube formation of human umbilical vein endothelial cells were decreased in miR-16 transfected SKM-1 CM compared to CM from SKM-1 cells transfected with vector control. Direct binding of miR-16 to the 3' untranslated region of VEGF was confirmed by luciferase reporter assay.
Summary/Conclusion: These data suggest that miR-16 may play a role in disease progression of MDS partially by modulation of angiongenesis through targeting VEGF and that miR-16 might serve as a novel therapeutic target in MDS.

PB2092

ACCESS TO DIAGNOSTIC AND THERAPEUTIC TOOLS FOR MYELODYSPLASTIC SYNDROMES IN GENERAL PRACTICE: SURVEY AMONG LATIN-AMERICAN HEMATOLOGISTS

R. Crisp[1,2,3,*], G. Vidal-Senmache[3,4], J. Navarro Cabrera[3,4], F. Huaman-Garaicoa[3,5], B. Maldonado[3,5], M.S. Undurraga Sutton[3,6], X. Valladares[3,6], J. Reyes[3,7], V. Abello[3,7], M. Pimentel[3,8], A. Ayala[3,8], S. Grille[3,9], C. Guillermo[3,9], J. Choque[3,10], V. Salinas Viedma[3,11], M. Royg[3,11], M. Iastrebner[2,3], C. Belli[2,3]

[1]Clinical Hematology, Hospital Nacional Alejandro Posadas, [2]Sociedad Argentina de Hematología, Buenos Aires, Argentina, [3]Grupo Latinoamericano de Sindromes Mielodisplasicos (GLAM), Montevideo, Uruguay, [4]Sociedad Peruana de Hematología, Lima, Peru, [5]Sociedad Ecuatoriana de Hematología, Guayaquil, Ecuador, [6]Sociedad Chilena de Hematología y Hemoterapia, Santiago de Chile, Chile, [7]Asociación Colombiana de Hematología y Oncología, Bogota, Colombia, [8]Sociedad Dominicana de Hematología, Santo Domingo, Dominican Republic, [9]Sociedad de Hematología de Uruguay, Montevideo, Uruguay, [10]Sociedad Boliviana de Hematología y Hemoterapia, La Paz, Bolivia, Plurinational State Of, [11]Sociedad Paraguaya de Hematología, Asuncion, Paraguay

Background: MDS refers to a heterogeneous group of closely related clonal hematopoietic disorders. Among the diagnostic and prognostic tools, pathology, cytogenetics, immunophenotyping and, more recently, point mutations have been shown to identify patients at different risk for survival. Standard treatment options include supportive care, disease-modifying agents and allogeneic hematopoietic stem cell transplantation (HSCT).
Aims: To assess the real-world hematologist practice patterns within Latin-America (LA).
Methods: A printed 23-question survey consisting mainly of multiple-choice questions covering demographics of responders, diagnosis, therapy, evaluation of response, and cause of stopping treatments was collected since August 2015.
Results: Among the 458 respondents: 234 were from Argentine, 8 Bolivia, 47 Chile, 17 Colombia, 16 Dominican Republic, 36 Ecuador, 10 Paraguay, 64 Peru and 26 Uruguay. The response rate was 22-90% depending on their respective Hematology Societies. The majority of responders practiced hematology with a similar distribution within a 5-years range, except for Paraguay (<10: 70%) and Ecuador (>10: 92%). The practice setting was highly heterogeneous: public (0-90%), private/health insurance institution (0-59%) and combined (10-75%). The age-target practice was mostly split between adult and pediatric, however, restricted to adult patients in Paraguay/Uruguay, and mixed in 49% - Peru. Morphological description is a common practice. Additional tests include the histological examination of the BM (87-100%), being mostly done at the institution of attendance (except for Bolivia/Chile). The cytogenetic analysis (53-100%) and immunephenotyping by flow cytometry are heterogeneous (42% - 100%), being mostly referred. Physicians from Chile, Colombia, Ecuador and Peru selected to introduce MDS diagnosis as a pre-leukemia, cancer or neoplasia while others avoided these words. The IPSS and IPSS-R are preferred by most hematologists, however, age becomes the main factor for therapeutic decisions. Regarding therapies, most responders indicated transfusions of blood components and erythropoietin +/- other growth factor without differences. Chelation therapy ranged between 0% Bolivia and 100% Colombia/Ecuador. Azacytidine has been highly indicated in 89% Argentine and in 100% Colombia/Ecuador/Dominican Republic. The average for 5-aza-2'-deoxicytidine was lower (77% Colombia followed by 60% Argentine). Lenalidomide was less indicated in 23% Chile and 22% Uruguay. HSCT indication ranged among 33-94% and was not available in Paraguay/Bolivia/Dominican Republic. The access to clinical trials is significantly limited (8-0%). The suspension of treatment was mainly related to lack of response while chelation-therapy to side effects.
Summary/Conclusion: This is the first study that explores the real-world clinical practice for MDS in LA showing a heterogeneous access to complementary diagnostic tools, management and treatment in the surveyed countries.

PB2093

HEALTH IMPACT OF THE 5-AZACYTIDINE ADMINISTRATION IN THE DOMICILIARY CARE IN PATIENTS WITH MYELODYSPLASTIC SYNDROME

C. Alarcón-Payer[1,*], J.M. Puerta Puerta[2], A. Jiménez Morales[1], F. Hernández Mohedo[2], J. Badiola González[2], M.E. Clavero Sánchez[2], Z. Mesa Morales[2], P.A. González Sierra[2], E. López Fernández[2], R. Ríos Tamayo[2], A. Romero Aguilar[2], M. Jurado Chacón[2]

[1]Servicio de Farmacia, Hospital Universitario Virgen de las Nieves, [2]UGC Intercentro Hematología y Hemoterapia, Hospitales HUVN-PTS, Granada, Spain

Background: Most patients with intermediate-2 and high-risk myelodysplastic syndrome (MDS) have a median age of 75 years and 25% of them are diagnosed beyond 80 years of age. Therefore, many of them may have great difficulty to travel to the hospital during the 7 days of duration of each cycle of 5-azacytidine treatment.
Aims: To analyse the health impact of the administration of 5-azacytidine in the domiciliary care and to evaluate the therapeutic adherence.
Methods: 2-year prospective observational study on 20 MDS patients with a median of age of 75 years, with difficulty to travel to the day hospital to received treatment with 5-azacytidine during 7 days. The drug was prepared in the hospital pharmacy service, using the water reconstitution method for refrigerated injections and kept in refrigerator (2-8 ºC), resulting in both chemically and physically stable solutions for 22 hours. Once the inclusion of the patient in the study was confirmed by the haematologist, the prescribed treatment regime was informed to the pharmacy service and to the nurse to organize medication regime in the domiciliary care. The variables considered in this study were: beginning of treatment with 5-azacytidine, treatment duration, level of satisfaction of patients, treatment adherence and detected side effects.
Results: 20 MDS patients received treatment with 5-azacytidine in domiciliary care during a mean of 15 months of treatment. 70% of the patients showed great difficulty to travel to the day hospital because they required an accompanying person and 30% did not possess supporting infrastructure. 100% of the patients were highly satisfied with the service, therapeutic adherence improved in 95% and it was detected side effects in 15% of them (neutropenia, anaemia and gastrointestinal reactions).
Summary/Conclusion: The administration of 5-azacytidine in domiciliary care in MDS older patients with difficulty to travel to the day hospital has allowed to bring support to these patients, improving the day hospital logistics, increasing the satisfaction of the patients and its adherence to the treatment and offering a better quality healthcare.

PB2094

STUDY ON QUALITY OF THE TRANSFUSION PROCESS IN OUTPATIENT CARE IN A TERTIARY HOSPITAL

B. Cidoncha Morcillo[1,*], L.J. Garcia Frade[2], E. Fernández Fernández[2], E. Bonis Izquierdo[2], A. Campano Garcia[2], N. Andres Hernandez[2]

[1]Hosp. Univ Del Rio Hortega, Valladolid, Spain, [2]Hematology and Hemotherapy, Hosp. Univ Del Rio Hortega, Valladolid, Spain

Background: The evidences in transfusional matter, are realized in patients admitted, reason why the established criteria so much for patients in the Units of Hospitalization as in the Day Hospital, are not the same There is currently enough evidence to affirm that the transfusion of blood products can be carried out effectively, safely and in accordance with the American Blood Bank Association guidelines in the HDD, which entails, among other benefits, a significant reduction in the number of revenues. We have no knowledge of publications of works with this content by hospital centers equivalent to ours in population area and / or number of beds in our country. Therefore, our study is focused on the care activity, developed mainly in the Day Hospital, in relation to the transnfusional activity.
Aims: The main one is the quantification of the percentage of transfusion episodes of packed red blood cells (CH) according to the criteria of the AABB.
Methods: Retrospective, open and unicentric study. The period 2012-2013 is compared with 2015-2016. The inclusion criteria cover all patients over 14 years of age, who were transfused in the HURH Day Hospital, regardless of the medical service prescribing the transfusion.The lists of patients and number of CH transfused were obtained from the Blood Bank management program (DELPHIN).The pretransfusional haemoglobins were obtained from the MODULAB laboratory program.The diagnoses of the patients

were extracted from the hospital records (SICLINICA).The data was collected anonymously in an Access ® database designed to preserve confidentiality.The statistical analysis has been performed using Stata program (StataCorp, Texas, USA).
Results: From 2012 to 2013, 203 patients were transfused in HDD. Among them, 41.38% were treated in haematology service. The pretransfusional haemoglobin was 7.7g/dl, with a range of 7.0-8.3g/dl. 319 transfusion episodes (63.55% of the total) were considered correctly indicated. Haematology service had the highest percentage of transfusions indicated, 230 (72.59%). The difference in adecuated indications between Haematology and the rest of services was 24.4%, which is statistically significant (p<0.001). From 2015 to 2016, 270 patients were transfused in HDD. Of those, 35% belonged to Haematology service. The pretransfusional haemoglobin was 7.9g/dl, with a range of 7.2-8.4g/dl. A total of 373 transfusion episodes (54.61% of the total) were considered correctly indicated. Haematology service had the highest percentage of transfusions indicated, 233 (61.97%). The difference in indications between Haematology and the rest of services was 16.4%, which statistically significant (p<0.001).

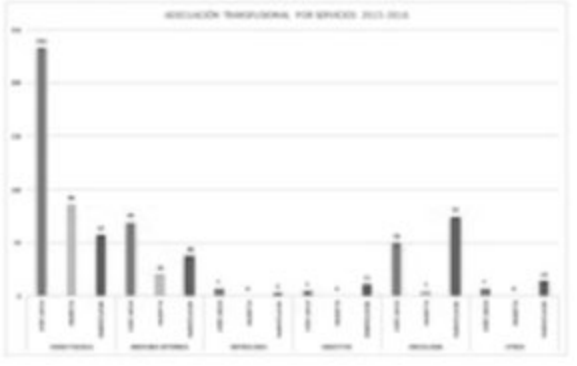


Figure 1.

Summary/Conclusion: The increase in transfusion in our center was 25% from the periods of 2012-13 to 2015-16. This finding is related to the increasing complexity of care and the chronification of several pathologies. The figures of pretransfusional Hb and infusion times were within the current recommendations, however, between 5.1%-6.4% of transfusions were performed without recent analysis. The comparison of adequate and inadequate transfusion, between haematology and the other services was statistically significant in the two periods of time studied (p<0.001), which can be considered reasonable given the greater involvement of haematology in the transfusion process.

PB2095

CHARACTERISTICS AND SURVIVAL OF DIABETIC AND NON-DIABETIC PATIENTS WITH MYELODYSPLATIC SYNDROMES

Y. Moshe[1,2,*], E. Joffe[2,3], G. Uri[4,5], M. Broitman[1], K. Filanovsky[6,7], G. Man-El[3], H. Oster[2,8], P. Raanani[2,3], I. Avivi[1,2], M. Mittelman[1,2,8]

[1]Hematology, Tel-Aviv Sourasky Medical Center, [2]Sackler Faculty of medicine, Tel Aviv University, Tel Aviv, [3]Hematology, Rabin Medical Center, Petah Tikva, [4]Hematology, Soroka Medical Center, [5]Faculty of Medicine, Beer Sheba and Ben-Gurion University, Beer Sheba, [6]Hematology, Kaplan Medical Center, Rehovot, [7]Hadassah Medical School, Hebrew University, Jerusalem, [8]Medicine, Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel

Background: Diabetes mellitus (DM) is a common co-morbidity in patients with myelodysplastic syndromes (MDS). In the general population, DM is associated with a shorter life expectancy, greater risk for infection, and anemia even in the absence of severe renal disease. It would be reasonable to assume that these issues would have a considerable impact on the management and outcomes of patients with MDS and DM.
Aims: We aimed to determine differences in the clinical chracteristics and disease course of diabetic *vs* non-diabetic MDS patients.
Methods: We retrospectively analyzed the characteristics and survival of MDS patients from four medical centers in Israel. We compared disease parameters at presentation, co-morbidities and overall survival between diabetic MDS patients and non-diabetic MDS patients.
Results: We reviewed records of 700 patients diagnosed with MDS between 2004 and 2016. Of these, 185 MDS patients (26.4%) had DM prior to MDS. The median age (75 *vs* 76.5, p=0.322) and gender distribution (43.3% *vs* 40.5% females, p=0.581) were similar for MDS patients with and without DM, respectively. Diabetic patients had a higher median body mass index (27.7 *vs* 26.7, p=0.02). MDS patients with DM had more co-morbidities than non-diabetic patients: cardiovascular disease (32.9% *vs* 22.6%, p=0.018), chronic kidney disease (37.8% *vs* 19.2% p<0.001), and hypertension (89.6% *vs* 67.7% p<0.001). COPD and history of previous chemotherapy were not significantly different between the two groups. Among patients with sufficient data to assign an IPSS-R risk category (n=259) diabetic patients had a trend towards lower risk disease (IPSS-R very low/low in 76% of diabetics *vs* 66% non-diabetic, p=0.09). Survival data were available for 481 patients. Median follow-up time was 74 months (95% CI 64-87 months). We observed no significant difference in overall survival curves or median overall survival between diabetic and non-diabetic MDS patients (**Figure 1**) (median OS 67.7 months 95% CI 57-78.3 months *vs* 62.9 months 95% CI 48-77.8 months, p=0.778).

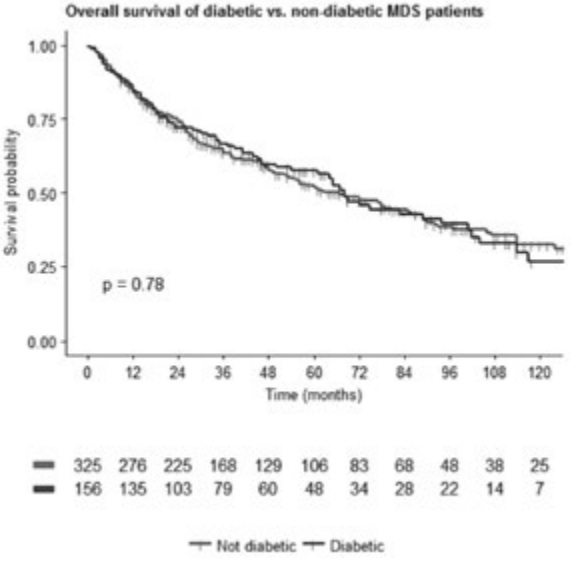


Figure 1.

Summary/Conclusion: MDS patients with DM have more co-morbidities than MDS patients who are non-diabetic. In contrast to expectations, in our series, diabetic and non-diabetic patients with MDS had similar overall survival. Additional studies are needed to confirm this finding, and to elucidate whether this is due to specific biological differences between diabetic and non-diabetic MDS patients or whether this is due to closer medical follow-up of diabetic patients, leading to diagnosis of MDS in earlier stages.

PB2096

CHRONIC MYELOMONOCYTIC LEUKEMIA AND AUTOIMMUNE DISORDERS

G.A. Pinto Solano*, M. Calbacho, J. López

Hematology, Hospital Ramon Y Cajal, Madrid, Spain

Background: Multiple autoimmune conditions have been described in the context of chronic myelomonocytic leukemia (CMML), but it is not clear if they could have an impact on clinical evolution.
Aims: We describe the subgroup of CMML related to autoimmune diseases (AD) and the main clinical features, compared to the patients who have other types of CMML.
Methods: We have studied 38 patients over 14 years (2003-2016), and compared the subgroup with autoimmune *versus* non-autoimmune disorders, in order to establish differences in some clinical parameters and the free progression survival (PFS) defined as the time from diagnosis to transformation to acute myeloid leukemia.
Results: We found a prevalence of AD of 23.6%, with the following autoimmune conditions: primary immune thrombocytopenia (22.2%), psoriasis (22.2%), adult-onset Still's disease (11.1%), giant cell arteritis (11.1%), ankylosing spondylitis (11.1%), rheumatic polymyalgia (11.1%) and unspecified arthropathy (11.1%). The main clinical features for both groups are described in table 1. The mean follow-up was 32.5 months. PFS at 2 years was 76.5% *versus* 47.2% in the AD group compared to non-AD group (p: 0.536), respectively.
Summary/Conclusion: The patients with CMML and AD represent a special subgroup that could have a better prognosis, as occur in other hematological

malignancies. This group of AD tend to have a normal karyotype and dysplastic subtype. More studies could be necessary to confirm the features and clinical evolution of this association.

Table 1.

Clinical features	Autoimmune disease (N=9)	Non-autoimmune Disease (N=29)
Mean age (years)	69.4	71.2
Gender (male/female %)	77/23	62/38
Normal karyotype (% patients)	77	51
CPSS (low or int-1/ int-2 or high %)	44/56	58/42
FAB subtype (dysplastic/proliferative)	33/67	51/49
WHO subtype (0/1/2 %)	75/6.8/17.2	78/11/11

PB2097

DEPLETION OF PROGENITORS B CELLS IN CHRONIC MYELOMONOCYTIC LEUKEMIA

G.A. Pinto Solano*, M. Calbacho, J. Lopez

[1]Hematology, Hospital Ramon y Cajal, Madrid, Spain

Background: Depletion of progenitors B cells (PBC) has been described as a frequent event in myelodysplastic syndromes and aplastic anemia, but we do not know if this event occurs in other related conditions as chronic myelomonocytic leukemia (CMML), and if it has any potential diagnostic or prognostic significance.
Aims: Describe the prevalence of depletion of PBC at bone marrow of CMML at diagnosis, and correlate if exists difference in the progression-free survival (PFS) compared to patients without this event.
Methods: We retrospectively reviewed the bone marrow flow cytometry of CMML patients at diagnosis during the period of 2008-2016. Depletion of PBC (CD34+CD19+CD10+) was defined as a value less than 0.05% of the total nucleated cell at the bone marrow. PFS was defined as the time from diagnosis to progression to acute myeloid leukemia.

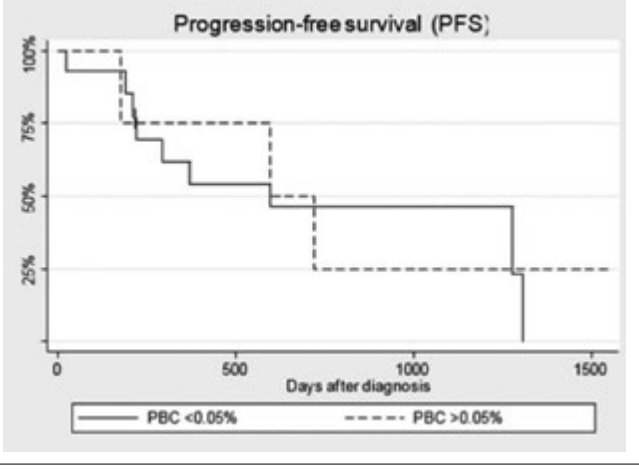


Figure 1.

Results: The studied group had a median age of 73.1 years, with a distribution by sex (male/female) of 61% and 49% respectively, and a mean follow-up of 24.2 months. According to WHO subtype (0, 1 and 2), the distribution was 66.7%, 11.1% and 22.2%, respectively. We found that 78% of the patients had depletion of PBC. The depleted patients had the CPSS prognostic scale (low/int-1/int-2/high) of 23%, 15.3%, 46.1% and 15.3%, and the non-depleted of 20%, 20%, 60%, 0%, respectively. By FAB subtype the proportion of dysplastic *versus* proliferative disease was 46% and. 54% in depleted patients, and 60% and 40% in non-depleted. The PFS to 2 years was 50% in non-depleted patients *versus* 46% in depleted patients (p: 0.816, see figure 1).

Summary/Conclusion: Based in our data, it is possible to consider that the depletion of PBC as a frequent event in CMML, and there is not a prognostic correlation with statistical significance in terms of PFS to 2 years in depleted or non-depleted patients. The depletion of PBC occurred without specific tendency in all subgroups by CPSS and FAB classification. Due to small sample size, further studies are necessary to clarify the relevance of this event.

PB2098

Abstract withdrawn.

PB2099

ASIA-INCLUSIVE CLINICAL DEVELOPMENT OF PEVONEDISTAT IN COMBINATION WITH AZACITIDINE IN HIGHER RISK MYELODYSPLASTIC SYNDROMES, CHRONIC MYELO MONOCYTIC LEUKEMIA, OR LOW-BLAST ACUTE MYELOID LEUKEMIA

X. Zhou[1,*], S. Friedlander[1], F. Sedarati[1], S. Kuroda[2], Z. Hua[1], Y. Yamamoto[3], D.V. Faller[1], T. Takubo[2], S. Sugizaki[2], K. Nakai[2], E. Bedell[1], K. Venkatakrishnan[1]

[1]Millennium Pharmaceuticals Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, MA, United States, [2]Takeda Pharmaceutical Company Limited, Tokyo, [3]Takeda Pharmaceutical Company Limited, Osaka, Japan

Background: Pevonedistat (PEV), a first-in-class NEDD8-activating enzyme inhibitor, combined with azacitidine (AZA) has demonstrated clinical activity in Western patients (pts) with acute myeloid leukemia (AML). PEV is currently in global clinical development for higher risk myelodysplastic syndromes (HR MDS), chronic myelomonocytic leukemia (CMML), and low-blast AML in a global phase 3 pivotal trial PANTHER (P3001).
Aims: To enable timely enrollment of Study P3001 for pts in East Asia, we proposed to apply multi-regional clinical trial (MRCT) principles of the ICH E17 draft guidelines informed by ICH E5 principles from an exploratory point of view to define the dose and minimum required number of East Asian pts in the pooled East Asian region.
Methods: Disease epidemiology, cytogenetic and mutational profiles of MDS, CMML, and AML among Caucasian and East Asian populations, treatment landscape, and efficacy of AZA were investigated. PEV pharmacokinetics (PK) and safety data in East Asian pts were collected in Pevonedistat-1012 (P1012), a phase 1 dose-finding study of PEV in combination with AZA conducted in Japan, South Korea, and Taiwan. Informed consent was provided for Study P1012. Population PK analyses were conducted to compare PEV exposures between East Asian and Western pts. Statistical considerations were provided to estimate the probabilities of consistency in efficacy between East Asian pts and the overall study population to be enrolled in the P3001 phase 3 study.
Results: According to epidemiological data reported by US, EU, Japan, and South Korea, the incidence of MDS and AML are comparable across these regions. Comparison of the molecular alterations in AML, MDS, and CMML across Caucasian and East Asian populations as well as the frequencies of these alterations suggested that the cytogenetic and mutational landscape is generally similar across these ethnicities. Literature research indicated that AZA shows comparable efficacy (response rate and hematologic improvement) in pts from Japan and South Korea. Preliminary PEV PK data from 18 pts (including 8 from Japan, 7 from Taiwan, and 3 from South Korea in Study P1012) indicated similar PEV PK profiles in East Asian (37%) and Western pts (geometric mean dose-normalized AUC_{48h} of 1167 [CV of 37%] and 1065 h*ng/mL [CV of 32%], respectively). In addition, systemic PEV exposures were consistent across the major East Asian races (ie, Japanese, Korean, Chinese). The safety profile of PEV alone or in combination with AZA in East Asian pts was generally comparable to that observed in Western pts. Based on the assumption that the treatment effects in Japanese/East Asian pts are similar to those in the overall population, with a total of 30 East Asian pts, the estimated probabilities of achieving consistent efficacy outcomes (measured by event-free survival and overall survival) between East Asian pts and the overall 450 pts in the global pivotal trial P3001 are >80%.
Summary/Conclusion: Intrinsic and extrinsic factors relevant to disease (HR MDS, CMML, and low-blast AML) and PEV were inferred to be similar across the planned countries of enrollment in the pooled East Asian region (Japan, South Korea) and between East Asian and Western regions. Clinical efficacy and safety data from the P3001 phase 3 study are therefore applicable to inform benefit-risk assessment for the East Asian population and are additionally justified to be pooled across pts enrolled for purposes of assessing consistency in the benefit-risk profile in East Asian populations.

PB2100

DEVELOPING A CORE OUTCOME SET FOR MYELODYSPLASTIC SYNDROMES – THE MDS-RIGHT HEALTH PROFESSIONAL DELPHI SURVEY

I. Stojkov[1,*], A. Conrads-Frank[1], U. Rochau[1], K. Koinig[2], M. Arvandi[1], A. Symeonidis[3], S. Guillermo[4], P. Fenaux[5], D. Bowen[6], M. Mittelmann[7], H. Garelius[8], U. Germing[9], C. van Marrewijk[10], J. Cermak[11], E. Hellström-Lindberg[12], A. Smith[13], T. de Witte[10], F. Efficace[14], U. Siebert[15], R. Stauder[2]

[1]Institute of Public Health, Medical Decision Making and Health Technology Assessment, Department of Public Health, Health Services Research and Health Technology Assessment, UMIT - University for Health Sciences, Medical Informatics and Technology, Hall in Tirol, [2]Department of Internal Medicine V (Hematology and Oncology), Innsbruck Medical University, Innsbruck, Austria, [3]Department of Medicine, Div. Hematology, University of Patras Medical School, Patras, Greece, [4]Department of Hematology, Hospital Universitario y Politécnico La Fe, Valencia, Spain, [5]Service d'Hématologie, Hôpital Saint-Louis, Assistance Publique des Hôpitaux de Paris (AP-HP) and Université Paris [7], Paris, France, [6]St. James's Institute of Oncology, Leeds Teaching Hospitals, Leeds, United Kingdom, [7]Department of Medicine A, Tel Aviv Sourasky (Ichilov) Medical Center and Sackler Medical Faculty, Tel Aviv University, Tel Aviv, Israel, [8]Department of Medicine, Section of Hematology and Coagulation, Sahlgrenska University Hospital, Göteborg, Sweden, [9]Department of Hematology, Oncology and Clinical Immunology, Universitätsklinik Düsseldorf, Düsseldorf, Germany, [10]Department of Tumor Immunology - Nijmegen Center for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands, [11]Department of Clinical Hematology, Inst. Of Hematology & Blood Transfusion, Praha, Czech Republic, [12]Department of Medicine, Div. Hematology, Karolinska Institutet, Stockholm, Sweden, [13]Epidemiology and Cancer Statistics Group, Department of Health Sciences, University of York, York, United Kingdom, [14]Health Outcomes Research Unit, Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA), Rome, Italy, [15]Institute of Public Health, Medical Decision Making and Health Technology Assessment, Department of Public Health, Health Services Research and HTA, UMIT - University for Health Sciences, Medical Informatics and Technology, Hall i.T., Austria/, Center for Health Decision Science, Department of Health Policy and Management, Harvard Chan School of Public Health, Boston, MA, United States/, Institute for Technology Assessment and Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States

Background: Myelodysplastic syndromes (MDS) represent a complex group of myeloid clonal disorders. Decision making in MDS is complicated by advanced age of MDS-patients and a high prevalence of comorbidities. Moreover, development of treatment strategies is complicated by the inconsistent outcome reporting in MDS studies and by the resulting lack of comparability.
Aims: The aims of this research were (1) to provide an overview of accepted and implemented outcomes in studies in MDS patients, (2) to identify the most important outcomes for the treatment of MDS from the perspective of health care practitioners (HCPs) and (3) to initiate the development of an unified MDS-specific core outcome set (COS).
Methods: To identify potential MDS core outcomes we performed a comprehensive systematic literature review in the ClinicalTrials.gov database and four clinical trial registries (ISRCTN, ICTRP, EU-CTR and NCI). We included studies evaluating MDS patient-relevant outcomes registered between 2012 and 2016 and excluded studies focusing solely on pharmacokinetics, pharmacodynamics or molecular research. Data regarding the registration year, funding, intervention, study population characteristics, primary and secondary outcomes were extracted using a predefined data extraction form and summarized in a comprehensive evidence table. All studies were screened by at least two independent reviewers. After summarizing the data, researchers together with MDS clinical experts actively participated in the discussion and derived a consensus on the potential MDS core outcomes. The selection process included a three-round online Delphi survey among HCPs from 17 different countries as part of the MDS-RIGHT project. Each outcome was ranked using a scale from 1 to 9. Following recommended criteria, highly important outcomes were defined as those ranked 7-9 by at least 70% of participants and ranked 1-3 by not more than 15%. Outcomes were excluded if ranked 1-3 by at least 70% of the participants and 7-9 by not more than 15%. All outcomes proceeded to the next round and were presented together with the ratings of the previous round. The final, third round was intended for deriving consensus and defining the outcomes specifically for MDS.
Results: From 425 (observational and interventional) included studies, we extracted 1,341 patient and/or clinically relevant outcomes that were condensed into 26 potential MDS core outcomes. 56 responses (24% response rate) obtained in the first round resulted in 15 outcomes ranked as highly important, and one additional outcome, suggested by two participants. None of the outcomes could be excluded. 38 responses (17%) were analyzed in the following round, where six outcomes were ranked highly important. No outcomes fulfilled the exclusion criteria, leading to the overall selection of six MDS core outcomes (quality of life, treatment-related mortality, overall survival, performance status, safety, hematological improvements). Final consensus on the remaining outcomes and definition of all included outcomes is ongoing.
Summary/Conclusion: Our study succeeded in identifying six outcomes that experts agreed on being included in the MDS-COS. The selected outcomes show the experts' awareness of the importance of patient-reported outcomes in MDS. MDS-specific definition of the MDS core outcomes and inclusion of perspectives of different stakeholders, namely of patients and health-care providers are currently ongoing.

PB2101

THE PREDICTIVE VALUE OF FERRITIN, FOLIC ACID AND VITAMIN B12 AS BIOMARKERS FOR THE DEVELOPMENT OF LEUKEMIA IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES. A PILOT STUDY

D.L. Lighezan[1,*], S.O. Cismas[1], C. Sorica[1], O. Potre[1], B. Timar[2], H. Ionita[1], I. Ionita[1]

[1]Hematology, [2]Diabetes, Nutrition and Metabolic Diseases, University of Medicine "Victor Babes" Timisoara, Timisoara, Romania

Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of hematologic disorders defined by ineffective hematopoiesis, manifesting in peripheral cytopenias. The progression of these clonal hematopoietic disorders is highly unpredictable and leukemic transformation is an often occurring hematological feature. No accurate prediction model has been so far established, due to the fact that no efficient prognostic markers have been identified.
Aims: The aim of this study was to evaluate whether the serum levels of ferritin, folic acid and vitamin B12 can be regarded as biomarkers of acute leukemic transformation in patients with MDS.
Methods: In this single-center, retrospective, registry-based study we included 128 patients diagnosed with primary MDS between 2012 and 2017. Data regarding serum levels of ferritin, folic acid, vitamin B12 were collected at the moment of diagnosis and during follow-ups. The initial medullary blast count and disease progression were also noted. The evaluated patients with MDS were divided into two groups: the patients with stable disease and those with leukemic progression.
Results: Out of the one hundred and twenty eight patients, 38.3% (n=49) suffered disease progression and ultimately acute leukemia transformation, with a gender ratio of 61% male to 39% female. At the moment of diagnosis mean ferritin levels were lower in patients with stable disease 409.8 ng/ml *vs* 504.5 ng/ml in patients with leukemic progression; whereas the opposite was observed for mean B12 and folate levels. The mean B12 levels were higher in stable disease patients 479.5 pg/ml vs, 455.2 pg/ml and the mean folate levels were 9.0 ng/ml *vs* 8.0 ng/ml in patients with leukemic progression. The blast count at diagnosis was significantly higher in patients with disease progression into acute leukemia (8.8 *vs* 4.3) with a p <0.001. Follow-up data revealed a significantly elevated Δferritin (ferritin last evaluation - ferritin diagnosis) in patients with subsequent acute leukemia transformation (1072.3ng/ml *vs* 680.4ng/ml), p=0.006, highlighting a continuous trend of increase. An elevation of Δfolate levels (3.7 ng/ml *vs* 4.7 ng/ml) and a decrease of ΔVitamin B12 levels (251.9 pg/ml *vs* 213.4 pg/ml), without statistical significance, were also observed.
Summary/Conclusion: Despite the small sample size which may lead to possible type 2 statistical errors, the results of this study may be regarded as triggers for further research. Our results are pointing to a possible use of ferritin as a predictor for disease progression in patients with MDS. In the same time, the results are pointing to a lack of association between serum vitamin B12 or folic acid and the disease progression.

PB2102

A SINGLE CENTRE EXPERIENCE OF RESPONSE RATES TO AZACITIDINE FOR MDS, AML AND CMML IN RELATION TO CYTOGENETICS RISK AND PERFORMANCE STATUS

V. Murthy*, N.D.G. Kapuge, E. Shereef, J. Suhr, C. Kartsios, E. Xenou, J. Ewing, E. Nikolousis

Haematology, Heart Of England NHS Foundation Trust, Birmingham, United Kingdom

Background: The advent of targeted therapy has increased the repertoire of therapeutic options. In particular the methyl transferase inhibitor 5 Azacytidine, that targets epigenetic changes in MDS and AML.The myelodysplas-

tic syndromes (MDS) are an acquired form of clonal stem cell disorders that manifest heterogeneously but are unified clinically by progressive bone marrow failure, susceptibility to life threatening infections, and a risk of transforming to leukemia.We report a single centre experience of using Azacitidine in the licensed NICE indications, comparing with the published data.
Aims: Aim of our study was to assess azacitidine response rates in different cytogenetic risk groups and varying performance status.
Methods: We retrospectively reviewed patients with MDS and AML who received Azacitidine at the Heart of England NHS Foundation Trust from Apr 2012- January 2018. Patient's demographic data, disease characteristics, treatment, outcome and follow up data were obtained and all patients were included irrespective of the number of the cycles.
Results: 57 patients were included in the analysis. Median age of treatment was 73.9 years. Median number of cycles was 6 (range from 1 to 43). There were 68% of MDS patients, 22.8% of AML and 8.8% of CMML2 patients in the cohort. Varying degree of performance stage was noted including ECOG 0,1 and 2 at 40.4%, 43.9% and 7.7% respectively. 42.1% had high risk cytogenetics depending upon the IPSS-R risk based classification. 19.3% patients had complex karyotype (3/>3). Median Hb was 9g/dl, Neutrophil count was $0.7x10^9$/l and platelet count was $41x10^9$/l in this population. Median bone marrow blast percentage was 10%. Responding patients showed reduced transfusion requirement. 10, 26 and 25 patient had not had any transfusions in first second and third quarters correspondently. Causes of death were mainly disease progression and sepsis. 42.2% died due to disease progression and 35.1% died due to sepsis. Overall, median progression free survival (PFS) was 19 monts while median overall survival (OS) was 12 months. Overall survival was not affected by age, Bone marrow median blast percentage and median neutrophil count, however, survival was affected by presenting peripheral blast percentage, IPSS-R, Haemoglobin and median platelet count that seemed to be statistically significant (P<0.05). There is no statistically significant PFS or OS for patients who had varying performance stage (p=0.78 and p0.65 respectively). Nevertheless, there is significance difference noted in the group of who had high risk cytogenetic *versus* other cytogenetic risks with PFS of 8 months and 15 months respectively (p=0.017), OS of 15 and 29 months respectively (p=0.09).

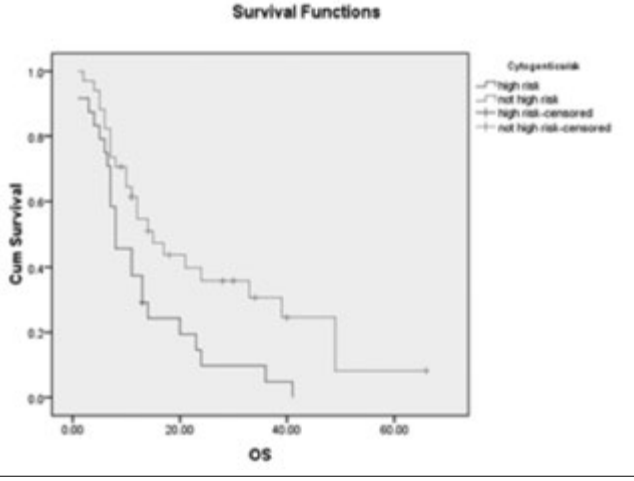


Figure 1.

Summary/Conclusion: Azacidine therapy has benefited for the patients who had advanced age AML CMML and MDS irrespective of their performance stage at ECOG 0-2 and patient who has non high risk cytogenetic based on IPSS-R risk categorization. However, the group of patients with high risk cytogenetic and poor performance considered historically very poor survival had considerable PFS and OS benefitting treatment rather than best supportive care.

PB2103

UNUSUAL FAVOURABLE CLINICAL COURSE IN A PATIENT WITH LOW-RISK MYELODYSPLASTIC SYNDROME WITH DEL (5Q) AND ACQUIRED TP53 MUTATION: CLINICAL, BIOLOGICAL AND MOLECULAR SEQUENTIAL STUDY

C. Finelli[1,*], E. Ottaviani[1], M. Follo[2], N. Testoni[1], M. Raffini[1], M.T. Bochicchio[1], S. Parisi[1], S. Mongiorgi[2], L. Cocco[2], M. Cavo[1]

[1]Ematologia, Policlinico S.Orsola-Malpighi, [2]Biomedical Sciences-Human Anatomy, University of Bologna, Bologna, Italy

Background: Lenalidomide (LEN) is the approved first line treatment for lower risk myelodysplastic syndromes (MDS) with del (5q) and transfusion-dependent anemia. However, the acquisition of a TP53 mutation in these patients is considered a high-risk factor, predictive of a poor clinical outcome, due to a high risk of evolution in acute myeloid leukemia (AML).
Aims: To present the clinical, biological and molecular data, sequentially assessed, of a patient with low-risk MDS with del(5q, who acquired a TP53 mutation during the course of the disease, but, nevertheless showed a particularly favourable clinical course.
Methods: The patient at the time of the first diagnosis, on November 1996, underwent a complete diagnostic work-up, with complete blood counts, bone marrow aspiration and cytogenetic study. Subsequently, starting from July 2009, when she started LEN, she also underwent biological and molecular studies, performed sequentially throughout the subsequent course, until now. Mutation analysis of TP53 exons 5-9 was performed on mononuclear cells by PCR and direct bidirectional Sanger sequencing. Each chromatogram was compared with wild-type sequence (GenBank; NM_001126114). Gene expression analyses were performed on mononuclear cells at diagnosis and during treatment by a TaqMan-based Real-Time approach (Follo, PNAS 2009).
Results: At diagnosis (November 1996) the patient, a 55-year-old female, showed transfusion-dependent anemia. Refractory anemia (according to FAB) with the presence of del (5q)(q13;q33) in 8/9 metaphases was diagnosed. IPSS risk was low, and IPSS-R risk was intermediate. The patient, refractory to erythropoietin, continuously received red cell transfusions, and also iron chelation, (subcutaneous deferoxamine from March 1998, and subsequently oral deferasirox, starting on July 2003), until July 2009, when LEN was started. At that time no mutation of TP53 was detected. The patient discontinued transfusions after 2 months of LEN, after 3 months the Hb level reached 12.2 g /dL, and after 6 months no del(5q) metaphases were detectable, while 3 metaphases with + 8 were observed. Complete cytogenetic remission was achieved after 12 months, and persisted after 24 months, on July 2011, when LEN was discontinued. Cytogenetic remission was maintained for another 6 months, and hematologic remission, in the absence of treatment, lasted for 55 months, until February 2016, when the patient showed hematologic relapse, with transfusion need, but without excess of marrow blasts or high-risk cytogenetic alterations. LEN was restarted on April 2016, and a quick second hematologic response was observed after 2 cycles (Hb 11.1 g/dL), with a partial cytogenetic response (14/30 normal metaphases) on November 2017, after 11 cycles. The patient is still continuing LEN, still maintaining complete hematologic remission. Starting from February 2015, a TP53 mutation was detected for the first time, and was confirmed on November 2015 and November 2017: a missense variant in exon 5 C135Y (c.404G>A), intron variant in intron 6-7 (rs1825895). As to biological studies, the patient showed an increased expression of Beta-Globin mRNA during response to LEN, that was associated with an induction of PI-PLCgamma1, therefore hinting at an activation of erythroid differentiation.
Summary/Conclusion: This case shows that the detection of a TP53 mutation is not necessarily associated with a poor clinical outcome in all cases, and that probably not all types of TP53 mutations might have a negative prognostic impact.

PB2104

NEXT GENERATION SEQUENCING IN PREDICTING OUTCOME IN MDS- DAWN OF NEW ERA IN PAKISTAN

N. Anwar[1,*], A. Arshad[1], S. Shahid[2], S. Ahmed[2], N. Fatima[3], T. Shamsi[1]

[1]Hematology, [2]Genomics, [3]Research and development, National Institute of blood diseases and bone marrow transplant karachi Pakistan, Karachi, Pakistan

Background: Myelodysplastic syndromes (MDS) are a heterogenous group of hematologic malignancies characterized by clonal expansion of bone marrow myeloid cells with impaired differentiation. Studies utilizing next-generation sequencing (NGS) have identified a core set of recurrently mutated genes in the majority of patients with AML and MDS. The identification of recurrent mutations in MDS has led to incipient insights into the pathophysiology of this disorder including those with mundane cytogenetics. DNA-level mutations in several of these genes including ASXL1, ETV6, EZH2, RUNX1 and TP53 in MDS contribute to disease pathogenesis, outcome and currently is an active area of research.
Aims: Our institution is first in the country to perform next generation sequencing analysis. This study is a beginning to the insight of genomics in this disorder with respect to racial difference as much limited has been done

on regional level with none reported nationally. With this aim the study was done to assess mutation analysis in MDS and correlate the data with the clinical outcome of the patient.
Methods: A total of 20 MDS diagnosed patients by bone marrow biopsy and cytogenetics were included. Informed consent and detailed history was taken from all patients. The next generation sequencing analysis for a panel of 54 genes including tumor suppressor and oncogenic hotspots associated with myeloid malignancies (AML, MDS, MPN, CMML and JMML) was performed using DNA samples.
Results: A total of 20 MDS patients (14 males and 6 females) meeting the criteria of WHO classification were included. The mean age of the patients was 42 years. Most common presenting complain was weakness in 13 (65%) patients followed by fever and lethargy in 6 (30%). The mean IPSS was 01. Cytogenetic analysis revealed 13(65%) patients with abnormal karyotype while 7(35%) with normal karyotype. Using next generation sequence analysis, 10 (50%) patients had mutations including 2 novel mutations {RUNX1 p.lle428Thr(het) and GATA2 p.Thr358Pro} and 8 reported mutations including p.Pro384Leu (het) RunX1,p. Pro75Leu CDKN2A,Tet-2 c5162 T>G mutation,p.Gln1039Ter (het) ASXL1,p.Gly12Ser, NRAS and DNMT3A NPM. Patients who carried mutations received blood transfusion, lenalidomide, hematinics and azacitidine for treatment. Out of these, 03 patients were transformed into acute myeloid leukemia and received chemotherapy. Mutations in MDS are associated with changes in patient outcomes and it was found statistically significant in our cohort. Numbers of expiry in patients identified with mutations were comparably higher than those who did not carry any mutation (P-value=0.017).
Summary/Conclusion: Sequencing studies suggest that multiple mutations are required for MDS initiation and progression to acute myeloid leukemia (AML). In addition to providing prognostic information, these gene mutations can be used to monitor patient disease burden through the use of ultrasensitive detection techniques like NGS. The past several years have yielded many incipient insights, but the consummate genetic landscape of MDS is not yet kenned. Additional studies will be required to understand the prognostic implications of these mutations for treatment, disease progression and survival.

PB2105

ASSEMENT OF TRANSFUSION FREQUENCY AS A MORTALITY INDICATOR IN MYELODYSPLASTIC SYNDROMES. A SINGLE CENTER DATA

S. Cismas[1,*], D. Lighezan[1], C. Sorica-Romanescu[1], M. Delamarian[2], O. Potre[1], I. Ionita[1]

[1]Hematology, University of Medicine "Victor Babes" Timisoara, [2]Hematology, Municipal Emergency Hospital Timisoara, Timisoara, Romania

Background: Myelodysplastic syndromes (MDS) are a heterogenous set of clonal stem cell neoplastic disorders defined by ineffective hematopoiesis which translates to peripheral cytopenia with one or more lineages being affected. According to WHO, MDS is classified into six distinct entities based on morphologic, quantative and qualitative evaluation of both peripheral blood and bone marrow. Flow cytometry immunophenotyping, bone marrow biopsy, cytogenetics and molecular biology offer further information relating to prognosis and treatment options. An independent prognostic factor in MDS is transfusion dependency. Higher mortality rates have, especially in low-risk patients, been related to higher transfusion frequency and consequential iron overload.
Aims: The aim of this study is to evaluate the prognostic value of transfusion requirement, anaemia severity at moment of diagnosis, chelation therapy and to estimate mortality as it relates to these factors.
Methods: We analysed data retrospectively from one hundred twenty eight patients with myelodysplastic syndrome diagnosed between January 2012 and February 2018 and we evaluated transfusion requirement, admission frequency, anaemia severity at diagnosis, chelation therapy and overall survival.
Results: Mortality rate was high, at 67% (n=86); for these patients, haemoglobin at diagnosis was 8.7, transfusion requirement median was 18, with an average of 10 admissions per patient, as opposed to a haemoglobin at diagnosis of 9.3, transfusion requirement of 19 and a number of admission of 15 for the 33% (n=42) of patients that survived. In cases where chelation therapy was needed, mortality was related to the number of transfusions administered with a median of 30 in the higher morality group and a median of 7 transfusions for the lower mortality group.
Summary/Conclusion: In conclusion, a lower haemoglobin level at diagnosis resulted in a higher mortality rate, the same as with patients with a higher transfusion requirement and post transfusion iron overload that required chelation therapy. This retrospective data supports the use of transfusion frequency as an negative prognostic indicator.

PB2106

AZACITIDINE CAN INDUCE LIFE-THREATENING SKIN LESIONS

C. Vo[1,*], A. Thoresen[1], I. Dybedal[2]

[1]Hematology, Innlandet Hospital, Gjoevik, [2]Hematology, Oslo University Hospital, Rikshospitalet, Oslo, Norway

Background: Azacitidine (AZA) was the first drug demonstrating survival benefit over conventional care in patients with high risk MDS, including AML with 20-30% blasts. It has been the standard of care in patients not eligible for allogeneic stem cell transplantation and is lately also used in elderly patients with AML. The medication is usually well tolerated. We will report two patients with cutaneous side effects of AZA, which turned into a life-threatening systemic affection in one of them.
Aims: By these cases, we want to demonstrate that AZA can cause life-threatening skin lesions. High-dose steroids can dramatically improve a nearly fatal clinical situation.
Methods: The two patients included in this report have both given written informed consents to this publication. We describe two patients who have been treated at a local and a university hospital. **Patient 1** was a 79-year-old woman with MDS-EB-2 treated with AZA 100 mg/m^2 subcutaneously (sc) days 1-5. She was admitted to hospital on day 5 because of dyspnea, coughing and high fever. Chest X-rays showed a consolidation. She was treated with broad-spectrum antibiotics. After 7 days she developed darkly red and swollen skin lesions on her right hand. This rapidly changed into necrotic tissues as demonstrated at the picture. In addition, 3 more necrotic skin lesions were observed, also affecting her other hand. Surgery was performed several times. She became critical ill and circulatory instable with low blood pressure. It was considered unlikely that she would survive. Since no bacteria were found and AZA can give necrotizing fasciitis, acute febrile dermatosis and lung infiltrations, we as a last try, started with high-dose steroids. Within a short time, her condition was dramatically improved. **Patient 2** was a 72-year-old man with relapse of AML. He was treated with AZA 100mg/m^2 sc day 1-5. Before cycle 4, he was admitted to hospital because of neutropenic fever and received empirically broad-spectrum antimicrobials. Feeling better after 4 days he started AZA cycle 4.The following day he rapidly developed darkly red and swollen skin lesions on both hands and forearms in addition to high fever. The similarity between his skin affection and the findings in patient 1, made us consider AZA-induced side effects likely. We chose to treat him with high-dose steroids. Already the next day his condition had improved.
Results: For patient 1 laboratory values revealed neutrophils 42.3x10^9/L. Microbiological samples from skin and blood were all negative. Biopsy of the skin lesions showed neutrophilic dermatosis as in Sweet syndrome.
For patient 2 a skin biopsy showed subacute inflammation with no signs of vasculitis or infection, but findings comparable with drug-induced changes.

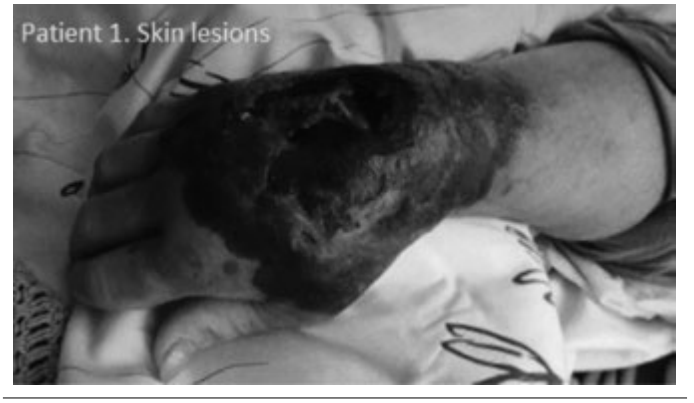


Figure 1.

Summary/Conclusion: AZA-induced Sweet syndrome appears to be the cause of the progressing, necrotic skin lesions with the fulminant systemic affection and circulatory collapse in patient 1. High-dose steroids dramatically improved her situation. A similar development may have been prevented by introducing steroids to patient 2.

PB2107

CLINICAL IMPLICATION OF SERUM FERRITIN LEVEL AT DIAGNOSIS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

S. Osanai, M. Shiseki*, K. Yoshinaga, N. Mori, J. Tanaka

Department of Hematology, Tokyo Women's Medical University, Tokyo, Japan

Background: Iron overload due to an ineffective erythropoiesis and/or red cell transfusion is often observed in myelodysplastic syndrome (MDS) patients, resulting in damage of target organs including heart, liver, and pancreas. Several reports showed clinical impacts of iron overload in MDS.
Aims: In the present study, we retrospectively analyzed relationship between serum ferritin level at diagnosis and clinical features in MDS patients to evaluate clinical implication of serum ferritin level.
Methods: This retrospective observational study was conducted according to the Declaration of Helsinki, and approved by institutional ethics committee (No. 4301). We reviewed medical records and laboratory data base of the patients who were diagnosed as MDS between January 2003 and December 2016. Patients in whom serum ferritin level at the time of MDS diagnosis were available, were included this study. Patients with therapy-related MDS, with history of transfusion, any specific therapy for MDS were excluded from the study.
Results: A total of 98 patients (59 males and 39females, median age, 71 years, range, 20-91 years) were included into this study. Median follow-up period was 22.5 months (mean 39.0, range 0.4-165.7 months). We analyzed relationship between serum ferritin level and clinical parameters and laboratory data to evaluate clinical implication of serum ferritin level. Inverse correlation was observed between serum ferritin level and hemoglobin concentration (r=-0.4022, P<0.0001). Positive correlation was observed between serum ferritin level and ring-sideroblast percentage in bone marrow (r=0.4163, P=0.0002). No significant difference in serum ferritin level was observed among MDS subtypes, IPSS and IPSS-R risk categories. The Cox proportional hazards model was employed to evaluate prognostic significance of serum ferritin level, demonstrating that high serum ferritin level (more than 500 ng/ml) was associated with worse OS (hazard ratio=3.04, 95% CI, 1.58-5.70, P=0.0012). Patients were classified into two groups according to serum ferritin level, high (500ng/ml or more) ('H'), and low (less than 500 ng/ml) ('L') groups. Kaplan-Meier plots indicated that 'H' group showed shorter OS than 'L' group (log-rank test, P=0.0014). Estimated OS rates at 2 years, 5 years, and 10 years from diagnosis were 48.8%, 42.1%, and 12.0% in 'H' group, and 82.4%, 66.5%, and 50.4% in 'L' group, respectively. Subgroup analysis demonstrated that prognostic significance of serum ferritin level was obviously observed in patients with lower IPSS (Low and Int-1), but not in those with higher IPSS (High and Int-2). Among patients with lower IPSS, Kaplan-Meier plots demonstrated significant worse OS in 'H' group compared with 'L' group (log-rank test, P=0.0003); estimated 2-year, 5-year and 10-year OS rates were 44.0%, 30.8%, and 0% in 'H' group, and 73.2%, 62.5%, and 47.1% in 'L' group, respectively. In contrast, among patients with higher IPSS, no difference in OS was observed between 'H' and 'L' groups (log-rank test, P=0.9224).
Summary/Conclusion: Our present results showed correlation between serum ferritin level at diagnosis and degree of anemia, suggesting serum ferritin level reflects dyserythropoiesis. High serum ferritin level at diagnosis was associated with worse survival in low risk MDS patients, indicating clinical implication of serum ferritin level as a prognostic marker.

PB2108

IS MDS REALLY TREATABLE? EXPERIENCE FROM PAKISTAN

N. Anwar[1,*], A. Arshad[1], S. Sharif[2], N. Fatima[2], T. Shamsi[1]

[1]Hematology, [2]Research and development, National Institute of blood diseases and bone marrow transplant karachi Pakistan, Karachi, Pakistan

Background: Myelodysplastic syndromes (MDS) represent a diverse group of hematopoietic stem cell disorders characterizing ineffective hematopoiesis, morphological dysplasia and leukemic transformation. Although the international prognostic scoring system (IPSS) represents gold standard for risk assessment, it is still difficult to predict survival and median time to leukemic transformation in the majority. MDS being considered a relatively uncommon disorder in our region having no national registry for evaluation of disease burden is presumed to be an incurable disease with majority of patients conventionally being treated by only blood product support. Most of the patients are unable to avail standard treatment and those who are treated with recommended options are presumed to have hopeless outcomes. Hence, the study was planned to evaluate the fate of this disorder in our region with respect to various given treatment options.
Aims: The aim of the study was to assess disease outcome in the region with best available treatment option given and establish local data on it.
Methods: It was an analytical cross sectional study conducted at National Institute of Blood Diseases and Bone Marrow Transplantation, Karachi Pakistan from June 2010 to January 2018. Baseline investigations done including complete blood counts, bone marrow biopsy and cytogenetics. Clinical parameters of all patients were recorded. Patients were classified according to world health organization (WHO) 2008 classification and IPSS was calculated. Approval from the institutional ethics committee was obtained prior to the study. Differences in clinical measurements were evaluated using SPSS version 23. Moreover overall survival (OS) was also observed.
Results: A total of 186 patients were included in the study. The median age was 55 (range 3-90) years. There were 139 males and 47 females. In our patients the most common presenting complaint was loss of appetite 110(59%) followed by weakness and fever in 76(41%). The mean hemoglobin (Hb%) was 7.94±2.17g/dl, total leucocyte count (WBC) 7.51±13.3 × 10^9/l, platelet count 81±93.7 × 10^9/l. Cytogenetic data was available for 104 patients which revealed normal karyotype in 58 (56%) and abnormal karyotype in 46 (44%). The mean IPSS of the patients was 01. Out of total, supportive treatment was offered to (n=70,38%), hematinics (vitamin B12 and folic acid) (n=45,24%), recombinant erythropoietin (n=25,14%), lenalidomide (n=19,10%), thalidomide (n=11,06%), hypomethylating agents in (n=06,03%), steroids (n=06,03%) and growth factors (GCSF) in (n=04,02%). Death of 124 patients occurred during the study period. Cause of death was ascertained in 84 patients. Septicemia was the most common cause of death and found in (n=27, 32%) followed by shortness of breath (n=26, 31%), severe anemia (n=21, 25%) and intracranial bleeding in (n=10, 12%). The overall survival was 33% analyzed by using Kaplan meier curve. Risk of progression to AML was found in 06 patients with median time of 15 months.
Summary/Conclusion: Although MDS is considered as an uncommon disease yet the numbers of patients are increasing in Pakistan. There is a need to develop disease registry in our region to understand and approximate better measures which will help to treat patients with definitive treatment. Moreover, further studies with large sample size are needed to convince state's health department to finance treatment and research in the context so that better treatment options are made available.

PB2109

EVALUATION OF THE EFFICACY OF LENALIDOMIDE IN PATIENTS WITH HIGH-RISK MYELODYSPLASTIC SYNDROME (MDS) WHO ARE PARTIALLY RESISTANT TO HYPOMETHYLATING THERAPY

G. Dudina*

Hematology, Moscow Scientific&Clinical Center, Moscow, Russian Federation

Background: In Russia, there are no approved drugs for MDS patients who retain blood transfusion dependence despite the antineoplastic effect observed after hypomethylating therapy. The median survival rates in this cohort are significantly lower than those in the complete response group. The risk of relapse is significantly higher in these patients.
Aims: To assess the possibility of increasing the median survival rate and reducing blood transfusion dependence in patients partially resistant to hypomethylating therapy with azacitidine by administering second-line lenalidomide therapy.
Methods: 9 high-risk MDS patients (6 women, 3 men) were enrolled in the study. Cytogenetic analysis of bone marrow revealed no del(5q). The median age was 67 (52 to 84). Before enrollment patients received 4 to 12 cycles of azacitidine at a dose of 75 mg/m^2. All patients remained transfusion-dependent. The mean number of blast cells in the bone marrow was 2.8±0.3% (0.8±0.04%-5.2±0.2%). Lenalidomide was administered at a dose of 10 mg per day for 21 days. In cases when bone marrow relapse had been documented or when no decrease in the degree of blood transfusion dependence was observed for 3 cycles, the drug was discontinued. The average period from diagnosis to beginning lenalidomide therapy was 22 months. The median duration of lenalidomide therapy was 81 days (range: 42 to 168 days). The follow-up period was 18 months.
Results: 4 (44%) patients responded with a decrease in blood transfusion dependence. Three patients had complete response, one demonstrated a 50% reduction in need for transfusions (intervals between transfusions were increased up to 2 months, dose of packed RBCs reduced from 5–6 to 2–3 per month). All patients responded during the first three cycles of therapy. Five (56%) patients fully retained a need for blood transfusions. As for side effects, episodes of grade 3 or 4 neutropenia (neutrophil counts below 1,000 in 1 μl) occurred in 50% of patients during lenalidomide therapy. However, neutropenia was detected during Cycle 1 in one patient among those with a complete response, while in the non-responded group, 4 (80%) patients had neutropenia during 2-3 cycles of treatment. The treatment was discontinued after cycle 2 in 1 patient due to disease progression into acute leukemia. Four patients stopped taking the drug after cycle 3 due to a lack of effects. Blood transfusion therapy was continued for these patients. Within six months, all four patients underwent disease progression to acute myeloblastic leukemia. The mean duration of the response, characterized

by blood transfusion independence, was 8 months. The recurrence of blood transfusion dependence in all patients was accompanied by disease progression, which required third-line therapy with low-dose cytarabine at 15 mg/m2 for 14 days at 21- to 38-day intervals. A partial response was achieved in 5 (55%) patients. In terms of overall survival, in the general group, 7 (77%) patients were alive for 12 months. 3 patients (33%) were alive for 18 months.
Summary/Conclusion: Use of lenalidomide in MDS patients with a high risk of progression to acute leukemia with persistent blood transfusion dependence after hypomethylation therapy is clearly justified. The effect observed in the form of cessation/reduction of transfusion dependancy and prolongation of disease-free and overall survival, with satisfactory drug tolerance. This treatment can be recommended in elderly patients with a high comorbidity index when allogeneic stem cell transplantation cannot be performed.

PB2110

CHRONIC MYELOMONOCYTIC LEUKAEMIA A RETROSPECTIVE STUDY FROM A SINGLE CENTRE IN ROMANIA

N.A. Dobrete[1], C. Ghita[1], D. Coriu[1,2], A. Tatic[1,2,*]

[1]Hematology, Fundeni Clinical Institute, [2]Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Background: Chronic myelomonocytic leukaemia (CMML) represents a clonal hematopoietic disorder with myelodysplastic and myeloproliferative features. Some CMML cases are similar to a myelodysplastic syndrome with persistent blood monocytosis (MDS-CMML). Other cases present with leucocytosis, neutrophilia, persistent peripheral blood monocytosis and splenomegaly and are similar to a myeloproliferative syndrome (MPN-CMML). According to the 2016 WHO classification CMML is classified into 3 groups based on the peripheral/ bone marrow blast count: CMML-0, CMML-1, CMML-2.
Aims: The aim of this study was to analyse the features and treatment response in the CMML patients in our centre.
Methods: Between 2011- 2017, in our clinic, 38 patients met the criteria for CMML and were reclassified according to the 2016 WHO classification for the purpose of this retrospective study. The median monitoring time was 18 months.
Results: There were 25 (65.79%) men and 13 (34.21%) women, with a sex ratio ♂/♀= 1.9. Women presented a younger median age at diagnosis 62 years (47, 67) *vs* men 68 years (62, 75), (p=0,025), with an overall median age of 66 years (59, 75). The majority of patients were over 60 years old at diagnosis (73.68%). Half the patients presented multiple cardiovascular comorbidities at diagnosis. In this lot, 16 patients (42.1%) were categorized as MPN-CMML and 22 patients as MDS-CMML (57.9%). According to the WHO 2016 classification 13 patients (34.21%) were CMML-0, 11 patients (28.95%) CMML-1 and 14 patients (36.84%) CMML-2. There was a statistically significant difference between the median value for leukocytes (p<0,001), neutrophils (p<0,001) and monocytes (p<0,001) for MDS-CMML and MPL-CMML, but not regarding the median value for haemoglobin, platelets, bone marrow blast and LDH level. Between the 3 WHO groups no statistically significant difference was observed for the median value for leucocytes, neutrophils, monocytes platelets and haemoglobin. The difference between the median LDH levels for the three groups was statistically significant (p=0.038). The transformation rate to AML for this lot was 34.2%, with a median time to progression of 17 months and a survival time of 2.2 months afterwards. No difference regarding the transformation rate for the 2 groups (MDS-CMML, MPN-CMML) respectively the 3 groups (CMML-0, CMML-1, CMML-2) was observed. The median survival time for CMML-0 was 32 months, for CMML-1 25 months and for CMML-2 18 months with an overall median survival time of 32 months. Regarding the treatment, 8 patients (21.05%) received hypomethylating agents, 9 patients (23.68%) received Hydroxycarbamide and 2 patients (10.52%) received intensive chemotherapy including allogeneic stem cell transplant (1 patient ongoing at 47 months after allogeneic stem cell transplant, while another died 3 months after the same procedure). No statistically significant difference was found between these treatment groups for our study.
Summary/Conclusion: We conclude that CMML is a heterogeneous disease, mostly affecting the elderly with a reserved outcome. The only curative treatment with an impact on survival is allogeneic stem cell transplantation.

PB2111

NEUTROPHIL TO LYMPHOCYTE RATIO AND PLATELET COUNT AS PROGNOSTIC INDEX IN MYELODYSPLASTIC SYNDROME

I. Mandac Rogulj*, K. Mišura, N. Popović, B. Ačamović Stipinović·

A. Beganović, S. Ostojić Kolonić

Clinical Hospital Merkur, Zagreb, Zagreb, Croatia

Background: Inflammatory and immune response in tumor microenvironment is important in the pathophysiology of tumor progression, and has been studied so far in many solid tumors and haematological malignancies. In tumor microenvironment, neutrophils secrete different cytokines that stimulate tumor growth, while lymphocytes represent immune defense against tumor cells and mediate cytotoxic cell death. Absolute neutrophil to lymphocyte ratio (NLR) was previously analyzed as prognostic parameter in solid tumors and haematological disorders. Thrombocytopenia is a known hematologic parameter of poor survival in patients with myelodysplastic syndrome (MDS). In this work, we tried to determine the prognostic significance of NLR and platelet count in MDS patient group.
Aims: In the retrospective cohort of 58 untreated MDS patients, we analyzed clinical and laboratory data of MDS patients and the prognostic significance of the NLR and platelet count in correlation with well known prognostic indices (IPSS, IPSSR).
Methods: The study included 58 patients (24 women, 34 males), median age 73 years. Patients with IPSS <1.5 had significantly higher platelet count compared to those with IPSS>1.5 (138 *vs* 58, p<0.001), as well as in the R-IPSS group <4.5 *versus* those in the group with R-IPSS>4.5 (122 *vs* 68, p<0.01).
Results: No statistically significant difference in NLR (p<0.087 for IPSS or p<0.112 for R-IPSS) was found. The number of platelets showed a negative correlation with IPSS (rho=-0.500, p<0.001) and R-IPSS (rho=-0.456, p<0.001). Analyzing other parameters, statistically significant correlation of IPSS with LDH (p<0.008) and CRP (p<0.01) was observed. In the IPSS group <1.5 there was a significantly lower LDH level compared to patients with IPSS>1.5 (189 *vs* 233) as well as for CRP (3.25 *vs* 11.05) The same observation was for R-IPSS (for LDH 189.5 *vs* 227.5, p<0.01, for CRP 2.55 *vs* 12.1, p<0.002).
Summary/Conclusion: In a small number of our patients, we have shown that platelet count, LDH and CRP, have negative correlation with IPSS and R-IPSS prognostic index. NLR was not statistically significant, but this should be analyzed on a larger number of MDS patients.

PB2112

FLOW CYTOMETRIC CHARACTERIZATION OF THE CD4 AND CD8 T CELL IN MDS – IS IT CLINICALLY RELEVANT?

A. Arshad*, N. Anwar, M. Saud, S. Munzir, T. Shamsi

Hematology, National Institute of blood diseases and bone marrow transplatation, Karachi, Pakistan

Background: Myelodysplastic syndromes MDS is a hematopoietic disorder of clonal pluripotent stem cells, bone marrow failure, varying degree of pancytopenia and a propensity for conversion to Acute Myeloid Leukemia. The myeloid lineage is mostly involved but there is ongoing immune responses occurs in MDS involving B cell with or without T cell involvement. Studies suggests an increasing evidence of an autoimmune process in pathogenesis of the bone marrow failure that accompanies MDS. Analysis of CD4/CD8 subsets analysis shows abnormal expansion in MDS and could predict response to treatment.
Aims: The current has been done to evaluate the intimacy of t cells in the pathogenesis of MDS.
Methods: The study was done at National Institute of Blood Diseases and Bone Marrow Transplantation, Karachi Pakistan during the period of June 2017 to January 2018.A total of 12 MDS including (7 males and 5 females) confirmed patients meeting criteria of WHO classification were included.24 aged and sex matched were also part of study. Approval for the study was obtained from the Institutional Review Board of NIBD. Written informed consent of each participant was also obtained. However, the illiterate participants had the consent forms read and interpreted to them in their native languages and with their oral consents, literate family representatives of the illiterate participants signed the consent forms on behalf of each of them.In this cross-sectional study, using sterile disposable needles and syringes, 5 ml of blood was aseptically collected from each participant by venipuncture of the cubital vein. Samples were each placed in BD vacutainers containing anticoagulant, and conveyed to the Flow Cytometry and Hematology Departments.CBC was done utilizing XN-1000.CD3,CD4 and CD8 T cells were enumerated using BD FACScount flow cytometer (BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions.
Results: A total of 12 MDS patients with 24 aged and gender matched controls were included. The mean age of the patients was 43yrs ±18.4. The common presenting complain was weakness, fever in 8(66.6%) patient followed by abdominal pain and gums bleeding in 4(33.3%) patients. The mean Hb of patients was 8.71±2.05(g/dl), TLC 4.39±2.03*10^9/l and platelets count

132±168*10⁹/l with ipss of o.8 while in control group Hb 14.24±1.29(g/dl), TLC 7.18±1.87(*10⁹/l) and platelets counts were 231±68(*10⁹/l). In patient CD4 counts were 693±426 and elevated CD8 were 620±313.8 while in control group CD4 counts were 858±355 and CD8 counts 537±208.
Summary/Conclusion: MDS is although a disease of bone marrow failure but there is an strong evidence that autoimmune processes are involment in the pathogenesis of bone marrow failure mds..Further studies with large sample size are needed to determine whether CD4/CD8 T cells analysis could be useful in predicting responders to immunosuppressive treatment.

PB2113

CHILDHOOD SECONDARY MYELODYSPLASTIC SYNDROME CASE REPORT OF A RARE DIAGNOSIS

J. Medeiros[1,*], I. Oliveira[2], A. Ferrão[3], M.J. Palaré[3], A. Morais[3]

[1]Serviço de Hematologia e Transplantação de Medula, Centro Hospitalar Lisboa Norte, Hospital Santa Maria, [2]Serviço de Pediatria, Centro Hospitalar Setúbal, E.P.E., Hospital São Bernardo, [3]Unidade de Hematologia, Departamento de Pediatria, Centro Hospitalar Lisboa Norte, Hospital Santa Maria, Lisboa, Portugal

Background: Myelodysplastic syndrome (MDS) in children is rare and accounts for less than 5% of hematopoietic malignancies in childhood. Most are secondary to cytotoxic therapy, inherited bone marrow failure disorders or acquired severe aplastic anemia. Abnormalities in chromosome 7 are seen both in *de novo* and therapy-related MDS.
Aims: We aim to present a case of a child with a rare diagnosis of secondary MDS.
Methods: Clinical case presentation.
Results: A 4 year-old female child from Angola presented with pancytopenia in 2014. She was first treated with quinine and clindamycin due to an ongoing malaria endemic outbreak, although the diagnosis was not confirmed, and later with ceftriaxone, cloxacillin, meropenem and fluconazole for persistent fever and high inflammatory levels. In July 2014 she was transferred to Namibia, where she was diagnosed with aplastic anemia (AA) and treated with 2 cycles of cyclosporine and antithymocyte globulin (ATG), and subsequently becoming transfusion independent until April 2017. At that point, due to pancytopenia, a new bone marrow aspirate was performed, showing dysgranulopoiesis, dyserythropoiesis and dysplasia. She was then sent to Portugal for diagnostic and treatment purposes. Upon arrival she presented with normal growth and development. The complete blood count showed severe anemia (hemoglobin 4.5g/dL), mild neutropenia (1290 neutrophils/µL) and moderate thrombocytopenia (83000 platelets/µL). Infectious disease were excluded, including malaria, tuberculosis and viral serologic tests. The initial bone marrow aspirate showed less than 10% dysplastic erythroid cells and 5% myeloblasts. Since MDS was not confirmed, a second bone marrow aspirate was obtained, which revealed significant dyserythropoiesis and dysmegakaryopoiesis, as well as monosomy 7 in FISH analysis, compatible with a diagnosis of MDS. She is currently scheduled to undergo allogeneic hematopoietic stem cell transplantation.
Summary/Conclusion: We present a case of a patient with a probable diagnosis of acquired aplastic anemia and secondary MDS. We can only speculate about the underlying etiology of the AA. Quinine can be associate with the development of aplastic anemia. Patients treated with immunosuppressive therapy have an increased risk of developing clonal hematopoietic disorders, with MDS being one of the most frequent, although there's no conclusive evidence that cyclosporine and ATG increase the risk for secondary MDS. The underlying dyspoiesis of aplastic anemia is the most acceptable reason for MDS in the current literature. Monosomy 7 is the most frequent cytogenetic alteration in children with MDS and is associated with a poor outcome, making allogeneic hematopoietic stem cell transplantation the treatment of choice.

PB2114

THE 5 - AZACITIDINE THERAPY IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA

H. Ionita[1,*], I. Ionita[1], M. Delamarian[2], D. Calamar[1], M. Ionita[3], M. Ifrose[2], I. Pascu[2], C. Ionita[4], M. Samfireag[2]

[1]Hematology, University of Medicine and Pharmacy Victor Babes Timisoara, [2]Hematology, Clinical County Hospital, [3]Internal Medicine, [4]Surgery, University of Medicine and Pharmacy Victor Babes Timisoara, Timisoara, Romania

Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of neoplastic disorders, with variable clinical course, originated from a clonal disorder of hematopoietic cell. MDS affects most frequently old individuals, who usually have an increased prevalence of comorbidities. In intermediate-2 and high risk myelodysplastic syndromes (MDS), chronic myelomonocitic leukemia (CMML) with 10-29% marrow blasts, and acute myeloid leukemia (20% to 30% marrow blasts) 5-azicitidine induces hematologic responses and prolongs overall survival.
Aims: We retrospectively evaluated the efficacy and tolerability of azacitidine in patients with MDS, CMML and AML in our department of hematology.
Methods: From 2012-2017, 30 patients affected by int-2 and high risk MDS, CMML or AML with marrow blasts <30%, who were not candidates to aggressive therapy, have been treated with azacitidine at a dosage of 75 mg/m2/d subcutaneously 7 days, every 28 days. The diagnosis was established according to the 2008 WHO criteria, IPSS and WPSS prognostic scores were calculated. The WHO diagnoses were: 20 MDS with IPPS risk int-2 or high, 8 AML with blasts 20-30% and 2 CMML. The median age was 71 (range 45-78), male to female ratio was 0.8 and the median number of cycles received was 7 (range 1-20).
Results: The overall response rate (OPR) was 19/30 patients (59%). According to International Working Group (IWG) 2006 criteria, we detecte complete remission (CR) in eight patients (27%) after a median of 6 cycles (range 6-8), hematologic improvement with bone marrow complete remission in four patients (13%) after 8 and 13 cycles of therapy, hematologic improvement in 9 patients (30%) after 6 cycles (range 4-8), stable disease (SD) in 6 patients (20%), and progressive disease (PD) in 3 patients (10%) after 6 cycles (range 4-8). Median duration of response was 14 months (range 6-28); median overall survival, for all patients treated, from the beginning of azacitidine was 15.2 months (range 8-35). No differeces in response rate could be appreciated according to age, bone marrow fibrosis and transfusion requirements. Among the non-responding patients, five (17%) recquired several admisions to hospital because of infectious or hemorrhagic complications.
Summary/Conclusion: Besides a high rate of response (ORR 59%, CR 27%), azacitidine was well tolerated. In particular the low rate of serious adverse events and of hospital admissions despite severe cytopenias, improved quality of life and reduced utilization of standard medical resources. Azacitidine confirmed to be an active therapy in patients not candidate to high intensity treatment for age and/or comorbidities.

Myeloma and other monoclonal gammopathies – Biology & Translational Research

PB2115

Abstract withdrawn.

PB2116

Abstract withdrawn.

PB2117

DETECTION OF MYD88 L265P MUTATION BY DIGITAL PCR IN PERIPHERAL BLOOD SAMPLES FROM IGM MONOCLONAL GAMMOPATHY PATIENTS

N. Garcia-Gisbert[1,*], E. Abella[2], C. Fernandez-Rodriguez[1,3], A. Ferrer[3], M.D.C. Vela[3], L. Camacho[1,3], X. Calvo[3], S. Montesdeoca[3], L. Arenillas[3], E. Gimeno[2], C. Besses[1,2], A. Salar[1,2], B. Bellosillo[1,3]

[1]Grup de Recerca Clínica Aplicada a Neoplàsies Hematològiques, IMIM, [2]Hematology Department, [3]Pathology Department, Hospital del Mar-IMIM, Barcelona, Spain

Background: Assessment of *MYD88* p.L265P mutation has been implemented in clinical routine as a diagnostic tool in IgM monoclonal gammopathy. Although bone marrow sample is usually available at diagnosis, the study of peripheral blood is useful for patient follow-up or in absence of bone marrow sample. However, the proportion of cells with the mutation in peripheral blood may be very low, which makes it difficult to detect them, leading to a false negative result. A strategy to overcome this situation is to enrich the target population and to use high sensitivity techniques. Enrichment of the sample in mutated cells can be done by depletion of CD3-positive lymphocytes (as the expression of CD19 is usually weak in the B-cell clonal population). Digital PCR is a technology that allows the detection and quantification of mutated DNA, even when it is present in a low proportion of total DNA.
Aims: To analyze the mutational status of *MYD88* p.L265P in peripheral blood samples by real-time allele-specific PCR (AS-qPCR) and digital PCR in a series of patients with IgM monoclonal gammopathy, as well as to determine the utility of the enrichment of the CD3-negative population for the detection of this mutation.
Methods: 41 peripheral blood samples were collected from 36 patients with IgM monoclonal gammopathy (28 with Waldenström macroglobulinemia (WM) and 8 with IgM monoclonal gammopathy of undetermined significance (IgM MGUS)). Depletion of CD3-positive lymphocytes was performed and DNA was obtained from the fraction of total mononuclear cells and from the CD3-negative subfraction. The *MYD88* p.L265P mutation was analyzed by AS-qPCR (7500Fast, Applied Biosystems) and digital PCR (QuantStudio 3D, Applied Biosystems).

Table 1.

Table 1. Number of positive samples for the *MYD88* p.L265P mutation according to the cell population studied and the technique used.

	AS-qPCR	digital PCR
PBMC	22/41 (53.7%)	31/41 (75.6%)
CD3- PBMC fraction	24/41 (58.5%)	33/41 (80.5%)

PBMC: peripheral blood mononuclear cells.

Results: When the total mononuclear cell fraction was analyzed, *MYD88* p.L265P was detected in 22 from the 41 samples studied (53.65%) using AS-qPCR and in 31/41 samples (75.6%) by digital PCR. The analysis of the CD3-negative fraction allowed the detection of the *MYD88* p.L265P in 24/41 samples (58.5%) by AS-qPCR and in 33/41 (80.5%) by digital PCR. These results are summarized in Table 1. In the CD3-negative fraction, the average mutational load was of 1.72% (range: 0.05%>16.75%), which was significantly higher than the observed in the total mononuclear cell population: mean 1.13% (range: 0.05% >11.21%), p=0.004. The negative cases by AS-qPCR corresponded to cases with low mutational load (<0.25%). No significant differences were observed in the allelic load between WM and IgM MGUS cases. As a whole, *MYD88* p.L265P was detected in 29 from the 36 cases studied, 6/8 (75%) IgM MGUS and 23/28 (82%) WM patients. This later percentage rose up to 91% (21/23) when only untreated WM patients were considered.
Summary/Conclusion: In patients with MW and IgM MGUS, the application of high sensitivity techniques such as digital PCR and, to a lesser extent, the enrichment of the sample in tumoral cells by T-cell depletion, improve the detection of *MYD88* p.L265P mutation.

PB2118

CIRCULATING TUMOR DNA ANALYSIS OF KRAS, NRAS AND BRAF MUTATIONS USING MASS SPECTROMETRY IN MULTIPLE MYELOMA PATIENTS

L.-T. Hsiao[1,2,*], T.-J. Chiou[1,3], J.-P. Gau[1,2]

[1]School of Medicine, National Yang-Ming University, [2]Division of Hematology, Department of Medicine, [3]Division of Transfusion Medicine, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, Republic of China

Background: Multiple myeloma (MM) is an incurable malignancy of plasma cells and the incidence of Taiwan MM has markedly increased in recent decades. There were frequent mutations in KRAS (particularly in previously treated patients), NRAS, BRAF, FAM46C, TP53, and DIS3 genes.
Aims: The study is to examine the prevalence of hotspot gene mutations in Taiwan MM patients by using the DNA from MM bone marrow samples, and further develop so-called liquid biopsy and to detect several hotspot gene mutations in patients' plasma DNA.
Methods: BM DNA was extracted from 122 MM patients at diagnosis or relapse. In addition, cell-free DNA (ccfDNA) was collected from 24 of these patients at the comparable time of BM collected. Mass Spectrometry was used to simultaneously detect a panel of assay including 26 types of KRAS, 26 types of NRAS and 6 types of BRAF mutations.
Results: We found that total 45 (36.89% of 122) and 50 (40.98% of 122) patients had mutations in KRAS and NRAS genes in the BM, respectively. KRAS_A59T, NRAS_G12S and NRAS_A146T were the most frequent 3 mutations. For 24 ccfDNA, RAS/BRAF mutations were detected in 70.83% (17 of 24) of patients, including 41.67% (10/24), 41.67% (10/24), 4.17% (1/24) patients. The same mutations detected in the plasma of 6 patients could be also seen in the bone marrow. When compared the detection of RAS/BRAF mutations in plasma and bone marrow, 12 patients (50% of 24) had RAS/BRAF mutations detected in both plasma and bone marrow, and 3 patients (12.5% of 24) did not mutations in both specimens. Four patients (16.67%) had mutations only detected in BM, and 5 patients (20.83% of 24) had mutations only detected in the plasma. When RAS/BRAF mutations was detected in the plasma of MM patients by MassArray, the sensitivity and specificity of RAS/BRAF mutations in the bone marrow was 75% and 37.5%, respectively. The positive predictive value and negative predictive value were 70.59% and 42.86%, respectively.
Summary/Conclusion: Our study found a comparable mutation rate of RAS/BRAF gene in Taiwan MM patients, when comparing those in recent reports of Western countries. We further used Mass Spectrometry to detect circulating tumor DNA, and one fifth of patients (5 of 24, 20.83%) had mutations only detected in the plasma.

PB2119

MOGROL REPRESENTS A NOVEL ANTI-MYELOMA AGENT VIA ERK AND NF-KB INHIBITION

S. Mu*, L. Ai, Y. Hu

Hematology, Wuhan Union Hospital, Wuhan, China

Background: Multiple myeloma (MM) is the second most prevalent hematologic malignancy after non-Hodgkin's lymphoma. Drug resistance and relapse limit MM to be an incurable disease. Mogrosides are reported to represent a novel class of bioactive plant compounds with anti-cancer effects, which are digested to mogrol *in vivo.*
Aims: Here, we sought to verify the anti-myeloma effect of mogrol both *in vitro* and *in vivo*, and investigate the pharmacological mechanism to provide new therapeutic strategy for MM.
Methods: Inhibitory rates under different concentrations of mogrol between 0 and 50umol/L were measured via cell count kit-8 (CCK-8) to ascertain

the maximal inhibitory concentration (IC50). Peripheral blood mononuclear cells (PBMCs) from health donors was used to make sure the safety of mogrol. Next, 2 myeloma cell lines (ARH77 and U266) were exposed to mogrol at different concentrations of 0, 0.5×IC50 and IC50, and cell viability, apoptosis and cell cycle distribution were measured to verify the anticancer effects of mogrol on MM cells. Moreover, myeloma xenografted BALB/c nude mice model was established to validate the pharmacological effects of mogrol *in vivo*. The Raf/MEK/extracellular regulated kinase (ERK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways were investigated through detecting related proteins expression by western blot.

Results: CCK-8 test showed that mogrol significant inhibited MM cell proliferation in a time- and dose-dependent manner. However, viability of PBMCs was not influenced by mogrol at any concentrations. And the inhibitory rate on MM cells changed from 7.73% to 78.9% at concentrations between 0.62 and 50umol/L, thus IC50 of mogrol on myeloma cells was 26.52±0.21umol/L. In addition, we also found that mogrol not only decreased MM cell viability, increased the total apoptosis rate, and caused G1/S arrest *in vitro*, but also reduced tumor volume and slowed down tumor growth *in vivo*, with an average size of tumors of 228.20±11.02mm^3 *vs* 422.90±50.07mm^3 after 4 weeks (p<0.05). Accordingly, western blot results displayed the dysregulation of relative proteins expression, such as up-regulated Bax and Cleaved caspase-3, and down-regulated Bcl-2. Moreover, ERK phosphorylation was inhibited in MM cells treated with mogrol, and NF-κB was also decreased in the mogrol-treated MM cells. Legends: A. Inhibitory effect of mogrol on ARH-77 cell proliferation after 24h. B. Apoptotic effects of mogrol on ARH-77 cells as determined by flow cytometry. C. Mogrol induced G1/S phase arrest in ARH-77 cells. D. Mogrol inhibited tumor growth in the myeloma xenografted BALB/c nude mice model. E. Effects of mogrol on the expression of p-NF-κB, p-ERK1/2, Bcl-2, Bax, and Cleaved caspase-3.

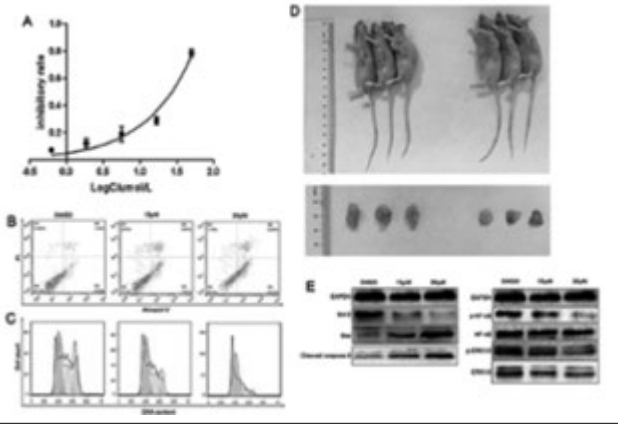


Figure 1.

Summary/Conclusion: Our results showed that mogrol not only inhibited MM cells growth *in vitro*, but also hampered MM development and progression *in vivo*. Besides, we found that ERK and NF-κB pathways was inhibited in the MM cells treated with mogrol. These findings suggested that mogrol, a novel ERK inhibiting agents, might grow to be a safe and promising anti-myeloma drug.

PB2120

CIRCULATING EXOSOMAL LONG NON-CODING RNA PRINS FIRST FINDINGS IN MONOCLONAL GAMMOPATHIES

D. Vrábel[1,*], L. Sedlaříková[1], B. Bollová[1], L. Radová[2], L. Brožová[3], J. Jarkovský[3], M. Almáši[4], M. Penka[4], P. Kuglík[5], V. Sandecká[6], M. Štork[6], Z. Adam[6], L. Pour[6], M. Krejčí[6], S. Ševčíková[1,4]

[1]Babak Myeloma Group, Department of Pathological Physiology, Faculty of Medicine, [2]Central European Institute of Technology, [3]Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, [4]Department of Clinical Hematology, University Hospital Brno, [5]Department of Experimental Biology, Faculty of Science, Masaryk University, [6]Department of Internal Medicine, Hematology and Oncology, University Hospital Brno, Brno, Czech Republic

Background: Monoclonal gammopathies (MG) are heterogeneous diseases with focal lesions in the bone marrow (BM); therefore, analysis of biopsy specimen obtained from a single biopsy site in the BM may not always contain information about all pathological clones. In the case of MM, these subclones directly influence survival of patients and may not be present in the sampled BM. The so-called liquid biopsies (biopsies of peripheral blood) represent a real promise for these diseases since circulating molecules that are detectable in peripheral blood (PB) mirror the complex heterogeneity of MG and can serve as potential diagnostic, prognostic and predictive markers. So far, others and we showed that these molecules include cell-free DNA and non-coding RNA (ncRNA), especially microRNA (miRNA) and long non-coding RNA (lncRNA). LncRNA expression is tissue-specific and implicated in diverse biological functions, including physiological and pathological processes, including tumorigenesis. At the same time, it is known that these molecules circulate in body fluids but their potential as MG diagnostic markers has not been clarified yet.

Aims: We investigated lncRNA expression profiles in exosomal fraction of peripheral blood serum of newly diagnosed multiple myeloma (MM) patients, monoclonal gammopathy of undetermined significance (MGUS) patients in comparison to healthy donors (HD) to find out if circulating lncRNA may be used as diagnostic markers of MG.

Methods: We performed expression profiling of 84 lncRNA by a commercial lncRNA PCR Array, followed by validation of chosen lncRNA by quantitative real-time PCR on a cohort of 50 MM, 49 MGUS patients and 30 healthy donors.

Results: Our analysis revealed dysregulation of exosomal lncRNA PRINS in MM *vs* HD with sensitivity of 80.8% and specificity of 76.9%; for exosomal PRINS in MGUS *vs* HD, sensitivity was 83.3% and specificity 80.8%. Overall, expression of exosomal lncRNA PRINS distinguished MM and MGUS patients from HD with sensitivity of 84.9% and specificity of 83.3%.

Summary/Conclusion: Our study suggests a possible diagnostic role for exosomal lncRNA PRINS in monoclonal gammopathies patients.

PB2121

A ROLE OF LIGHT AS POTENTIAL BIOMARKER FOR PROGRESSION OF SMOLDERING TO SYMPTOMATIC MULTIPLE MYELOMA

R. Rizzi[1,*], B. Giacomina[2], A. Mestice[1], G. Storlino[3], S. Bortolotti[3], P. Sindaco[1], R. De Robertis[1], P. Curci[1], S.C. Colucci[2], G. Specchia[1], M. Grano[3]

[1]Hematology Section, Dept. of Emergencies and Organ Transplantation, Bari University Medical School, [2]Department of Basic and Medical Sciences, Neurosciences and Sense Organs, Section of Human Anatomy and Histology, University of Bari, [3]Section of Human Anatomy and Histology, Dept. of Emergencies and Organ Transplantation, Bari University Medical School, Bari, Italy

Background: In 2014 the definition of smoldering multiple myeloma (sMM) was updated by the International Myeloma Working Group (IMWG). Thus, at present, the detection of SLIM CRAB can be considered for treatment need. In addition, several clinical and biological predictors of progression to symptomatic MM can be used to discriminate sMM patients on the basis of their different degree of risk of progression. Beside to the validated criteria included in Mayo Clinic or Spanish models, several other potential biomarkers have been each time proposed. We previously demonstrated that LIGHT/TNFSF14, a TNF superfamily member, is over-expressed on CD14+ monocytes, CD8+ T-cells and neutrophils of patients with MM lytic bone disease.

Aims: Given lytic bone disease as the most frequent MM-defining event in symptomatic MM patients, here we aimed to test LIGHT as potential biomarker for progression of sMM to symptomatic MM.

Methods: PB samples were obtained from 58 patients (30 M/28 F, 63±10 years) newly diagnosed as having symptomatic MM with related-bone disease, 60 with sMM (36/24, M/F, 54±20 years) and 50 healthy controls. Patients and controls were age and sex matched. Patients were diagnosed as having symptomatic MM or sMM based on IMWG criteria. By means of multiparameter flow cytometry (MFC), we evaluated LIGHT expression on circulating CD14+-monocytes, CD16+-neutrophils, CD4+- and CD8+-T cells. The results were compared to those obtained in healthy controls.

Results: The levels of LIGHT measured on CD14+ monocytes of symptomatic MM, sMM patients, compared to those of healthy controls gave the following results: 47.1%±9.5% (range 30-90%), 14.75%±20% (range 0-88%) *vs* 2.1%±1.2 (range 0-4%), p<0.01. In addition, we found that in sMM patients, LIGHT levels positively correlated with high involved/uninvolved serum free light chain (FLC) ratio r=0.613 p<0.001.

Summary/Conclusion: Several clinical and biological biomarkers have recently been proposed to stratify sMM patients on their risk of progression to symptomatic disease. Based on the positive correlation between LIGHT expression levels on circulating monocytes, and involved/uninvolved serum FLC ratio, we could argue that LIGHT may be further assessed as a potential biological precursor of lytic bone-disease development in sMM patients. Reliable clinical and biological biomarkers for risk stratification of sMM

can allow to plan optimized follow-up as well as to identify further subgroups of patients, who could benefit from an early treatment. The high levels of LIGHT expressed by circulating monocytes and their correlation with FLC ratio, suggest a possible role of this cytokine as biological predictor of sMM progression to symptomatic MM.

PB2122

DIFFERENTIAL MIRNOME EXPRESSION PROFILE IN NON TUMOR LYMPHOMONOCYTES OF THE PERIPHERAL COMPARTMENT OF MGUS SUBJECTS

O. Bianco[1,*], A. Allegra[1], M. Mania[2], S. Campo[2], V. Innao[1], M. Scuruchi[3], D. Vaddinelli[1], A. Avenoso[2], A. D'Ascola[3], G.M. Campo[3], A.G. Allegra[1], C. Musolino[1]

[1]Division of hematology, Department of Patologia Umana dell'adulto e dell'età evolutiva, [2]Department of Biomedical and Dental Sciences and Morphofunctional Images, [3]Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy

Background: Monoclonal gammopathy of undetermined significance (MGUS) is a pre-malignant abnormality of plasma cells, with increased serum levels of immunoglobulins. Patients with MGUS may evolve to multiple myeloma (MM) through a multistep process, including also deregulation of gene expression.
Aims: We aimed to demonstrate that MGUS patients display a deregulation of miRNA gene expression (short non-coding RNAs, regulating gene expression at the translational level) in peripheral lymphomonocytes.
Methods: Utilizing reverse transcription quantitative polymerase chain reaction we independently measured MiRNome expression profile in healthy and MGUS subjects.
Results: We obtained a specific MGUS microRNAs signature. Furthermore, six different pathways involved in important cellular processes of lymphomonocytes were analysed using *in silico* analysis, in order to identify dysregulated miRNAs in MGUS compared to controls. IL6 pathway, intrinsic and extrinsic apoptosis pathways, G1/S transition of the cell cycle, AKT-dependent pathway and PI3K pathway were influenced by 141 dysregulated miRNAs, making lymphomonocytes anti-apoptotic, non-proliferative, metabolically altered and inhibiting activation of angiogenic and immune responses.
Summary/Conclusion: This study demonstrates that MGUS patients have a different microRNA profile in peripheral blood cells in comparison with healthy donors, and that these microRNAs may play a role in the immune response and in anti-apoptotic processes.

PB2123

PDCD1, PDL1 AND CTLA4 POLYMORPHISMS ASSOCIATED WITH THE SUSCEPTIBILITY TO AND CLINICAL FEATURES OF MULTIPLE MYELOMA

T. Kasamatsu[1,*], M. Awata[1], R. Ishihara[1], M. Ohata[1], Y. Kanai[1], Y. Murakami[1], K. Murata[1], S. Watanabe[1], N. Takahashi[1], N. Gotoh[1], M. Takizawa[2], A. Yokohama[3], N. Tsukamoto[4], H. Handa[2], T. Saitoh[1], H. Murakami[1]

[1]Department of Laboratory Sciences, [2]Department of Medicine and Clinical Science, Gunma University, [3]Blood Transfusion Service, [4]Oncology Center, Gunma University Hospital, Gunma, Japan

Background: Programmed cell death-1 (PD-1), PD-1 ligand 1 (PD-L1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) plays an important role in immune checkpoint pathways. Single nucleotide polymorphisms (SNPs) of *PDCD1*, *PDL1* and *CTLA4* have been reported to be associated with susceptibility to some cancers. However, the potential association between SNPs in these immune checkpoint genes and risk of multiple myeloma (MM) remain controversial and obscure.
Aims: The aims of this study were to clarify the influence of *PDCD1*, *PDL1* and *CTLA4* SNPs on the risk of developing MM and its clinical features.
Methods: We extracted the genomic DNA from 125 MM patients and 211 healthy controls, and determined 3 *PDCD1* SNPs (rs36084323, rs41386349, rs2227982), 2 *PDL1* SNPs (rs2297136, rs4143815) and 4 *CTLA4* (rs733618, rs1157131, rs231775, rs3087243) by using the PCR-restriction fragment length polymorphism or the TaqMan allelic discrimination real-time PCR method. Haplotypes were statistically inferred from *PDCD1*, *PDL1* and *CTLA4* genotype data using Haploview version 4.2 (www.broad.mit.edu/mpg/haploview). All statistical analyses were performed with the IBM SPSS software package ver. 24 (IBM, Armonk, NY, USA). This study was approved by the Institutional Review Board of Gunma University Hospital (Approval #160007).
Results: The patients with MM had a significantly higher frequency of the *PDCD1* GCC/GCC haplotype (rs36084323 high-expression type/rs41386349 high-expression type/rs2227982) compared with healthy controls (10.4% *vs* 4.3%, OR=2.61, p=0.028). However, no statistically significant differences were observed in the genotype and the haplotype of *PDL1* and *CTLA4* SNPs between MM patients and healthy controls. In MM patients, the *PDCD1* rs41386349 CT & TT genotypes (low-expression type) was associated with significantly higher frequency of patients with plasmacytoma at diagnosis than the CC genotype (high-expression type) (30.4% *vs* 14.5%, p=0.039). The *PDCD1* rs2227982 CC genotype was associated with significantly higher frequency of patients with bone lesion than the CT & TT genotypes (86.0% *vs* 68.3%, p=0.033). Moreover, the *PDL1* rs2297136 TT & TC genotypes (high expression type) was associated with significantly lower albumin level than the CC genotype (low expression type) (mean±SD 3.84±0.65 *vs* 4.36±0.54, p=0.038). The *CTLA4* rs733618 AG & GG genotypes (low-expression type) was associated with significantly higher frequency of patients with plasmacytoma at diagnosis than the AA genotype (high-expression type) (27.0% *vs* 9.8%, p=0.029). In addition, the *CTLA4* rs1157131 GG genotype had a higher frequency of patients with ISS stage III than the AA & AG genotypes (43.8% *vs* 26.2%, p=0.040). However, we observed no significant difference in OS of MM patients among *PDCD1*, *PDL1* and *CTLA4* SNPs.
Summary/Conclusion: Our findings indicate that *PDCD1* polymorphisms may contribute to susceptibility of MM. The polymorphisms in immune checkpoint gene is not associated with the prognosis of MM but *PDCD1*, *PDL1* and *CTLA4* SNPs may be associated with extra-medullary disease and severity of MM.

PB2124

NOVEL THERAPEUTICS FOR MULTIPLE MYELOMA TESTED IN A 3D BONE MARROW MODEL

M. Braham[1,*], A. Deshantri[2], M. Minnema[3], Z. Sebestyen[3,4], T. Straetemans[3,4], J. Kuball[3,4], C. Öner[1], R. Schiffelers[2], M. Fens[2,5], J. Alblas[1]

[1]Department of Orthopaedics, [2]Department of Clinical Chemistry and Hematology, [3]Department of Hematology, [4]Laboratory of Translational Immunology, University Medical Center Utrecht, [5]Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, Netherlands

Background: Multiple myeloma is an incurable malignancy of plasma cells that develops in the bone marrow (BM). 2D cell line cultures are commonly used to assess the therapeutic potential of experimental anticancer treatments against myeloma *in vitro*. These 2D cultures do not mimic the dependency of primary myeloma cells on the surrounding BM, nor the BM itself. Since myeloma progression and resistance to therapy are dependent on the BM microenvironment, these essential characteristics need to be taken into account when assessing the therapeutic potential of experimental anticancer interventions.
Aims: Our first aim was to analyze the feasibility of testing novel therapeutics in a previously developed 3D BM myeloma model. For this, both a cellular immunotherapy (αβT cells engineered to express a defined γδTCR; TEGs) and a nanomedicine delivery system (VLA4 targeted liposomal drug delivery) were tested. Secondly, the effect of the therapies on both the cultured myeloma cells and the surrounding BM was analyzed.
Methods: The 3D BM myeloma model components are mesenchymal stromal cells, endothelial progenitor cells and (primary) myeloma cells co-cultured in hydrogel. The 3D BM myeloma model allows non-invasive time-lapse imaging, following the (primary) myeloma cells in the culture system before and after treatment. After 7 or 14 days of pre-culture (allowing myeloma outgrowth), the novel therapeutics were added. The delivery, migration and effect of the therapy were followed for 48 hours. The targeted VLA4 liposomes were loaded with doxorubicin and compared to standard doxorubicin treatment.
Results: The TEGs, when added to the 3D BM myeloma model, were capable of migrating through the 3D model, exerting a killing response towards the primary myeloma cells in 6 out of 8 donor samples after both 24 and 48 hours. This was not observed when adding non-functional control T cells. The supporting stromal microenvironment was unaffected in all conditions after 48 hours. The VLA4 targeted liposomes of different sizes (70 nm, 100 nm and 200 nm) were injected in the 3D BM myeloma model. Only the 70 nm and 100 nm liposomes were capable of passively diffusing through the 3D model. Cellular uptake of the liposomes was observed throughout the model, displaying a concentration gradient after injection. When comparing standard doxorubicin therapy to liposomal doxorubicin

therapy, similar killing effects towards myeloma cells were observed. However, the targeted liposomal drug therapy induced significantly less unwanted stromal and endothelial cell death compared to the standard doxorubicin therapy.

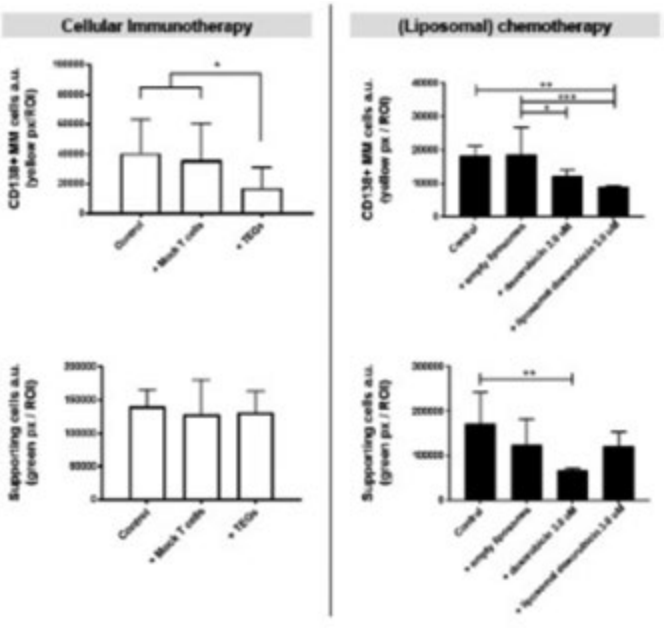


Figure 1.

Summary/Conclusion: The previously developed 3D BM myeloma model allows the *in vitro* study of both cellular immunotherapy and a nanomedicine delivery system on (primary) myeloma cells. Equally important, this model allows testing within an engineered BM environment, analyzing treatment effects on the BM environment as well. The model can thus be used to study cellular targeting throughout the model and general effectiveness of the added therapy by analysis of both on- and off-target effects.

PB2125

FEATURES OF STROMAL BONE MARROW MICROENVIRONMENT IN PATIENTS WITH MULTIPLE MYELOMA WITH INEFFECTIVE TRANSPLANTATION OF PERIPHERAL HEMATOPOIETIC STEM CELLS

N. Semenova*, I. Kostroma, S. Gritsaev, S. Bessmeltsev, V. Rugal

Russian research institute hematology and transfusiology, Saint-Petersburg, Russian Federation

Background: High-dose chemotherapy and subsequent Autologous hematopoietic stem cell transplantation (AutoHSCT) are widely used at the stage of remission consolidation in patients with multiple myeloma (MM). AutoHSCT is not equally effective for all patients. The ineffective AutoHSCT group includes patients undergone unsuccessful mobilization and ones with early relapse after AutoHSCT. AutoHSCT success depends on many factors, including the patient's age, general status and the status of comorbidity, the intensity and duration of prior therapy, etc. No less important is the state of the niche of HSC, which has been subjected to toxic effects of aggressive treatment, as well as the success of homing and fixing poured HSC in niches, which is largely determined by the morphological and functional characteristics of the main stromal components of the niche.
Aims: To investigate the morphological and functional characteristics of the main stromal components of the niche HSC in patients with multiple myeloma and inefficient AutoHSCT.
Methods: 12 trepanobiopsy of bone marrow from patients diagnosed with progressive MM (5 men and 7 women) were used in the study. The age of the patients ranged from 52 to 68 (median 57 years). Inefficient mobilization of HSC was noted for 7 patients and early relapse (*i.e.* within 12 months after AutoHSCT) for five. The study applied histological, immunohistochemical (IHC) and morphometric methods (VideoTest®).
Results: Assessment of the amount of infiltration of the bone marrow plasma cells is one of the main criteria for diagnosis of MM. Histological examination of trepanobiopsies of patients with MM showed non-uniform cellular infiltration of myeloma cells in the bone marrow. All types of bone marrow infiltration were identified in the group studied: focal (2 cases), interstitial 5-10% lesions (4 cases), interstitial 20-30% lesions (4 cases), diffuse (2 cases). Bone marrow damage did not exceed 10% for half of the cases (6 patients). We carried out a histomorphometric analysis of niche-forming structures of bone marrow microenvironment: vessels, endosteal cells, proteins of extracellular and intratrabecular matrix (collagen I, III, IV). Following trends were revealed during analysis. An increase of microcirculation vessels number especially in 8 patients It comprises 15.2±2.8% and 9.1±1.2% for experimental and control (bone marrow donors) respectively($p<0.05$). A marked increase in cell per unit length of bone trabecular was observed for endost of 2.7±0.5 compared with 1.4±0.2 in the control ($p<0.05$). Pronounced expression of CD56 tumor cells in subendosteal area was revealed in case of damage to the bone marrow exceeded 30%. Osteolysis was observed by the type of smooth resorption. Increased expression of collagen type IV was observed around all blood vessels of microcirculation, venules, arterioles. A typical feature was the appearance of focal network of reticulin fiber in subendosteal and perivascular spaces. Despite the treatment, microenvironment of the bone marrow retained the features of the tumor-associated environment.
Summary/Conclusion: Discovered key features of stromal elements of the bone marrow niche HSC evidence of serious violation of their morphological status, which is directly reflected in the results AutoHSCT. Studies of the stromal component of bone marrow can give clinicians additional information for successful training of patients with MM to AutoHSCT.

PB2126

MYELOMA CELL DEATH INDUCED BY ZEBULARINE LEADS TO CMYC DOWNREGULATION AND IS DECREASED BY CYTIDINE DEAMINASE OVEREXPRESSION

P. Krzeminski[1,*], R. García-Sanz[2], N. Gutiérrez[2]

[1]Centro de Investigacion del Cancer, [2]Hematologia, Hospital Universitario. IBSAL, IBMCC (USAL-CSIC), Salamanca, Spain

Background: A significant progress has been made in the treatment strategies for multiple myeloma (MM) over the last decade. However, the genetic complexity is hindering the cure of the disease. Epigenetic therapies have shown clinical efficacy in several hematological malignancies. The most investigated drugs modulating DNA methylation are 5-azacitidine and decitabine, which are approved by FDA for treatment of myelodysplastic syndromes. Although both agents have been investigated in MM, their therapeutic benefit is rather marginal. Other compounds like zebularine, which is considered a second generation DNA methylation inhibitor, has not been well explored in MM. In contrast to 5-azacitidine and decitabine, zebularine is stable, less toxic and inhibits not only DNA methylation but also cytidine deaminase (CDA), an enzyme responsible for a resistance to nucleoside analogs.
Aims: To investigate the cytotoxic effect of zebularine on MM and to elucidate it potential mechanisms of action.
Methods: The KMS12BM, KMS12PE, MM1s/r, OPM2, H929, RPMI and U266 human MM cell lines were obtained from ATCC, and JJN3 was obtained from DSMZ. Zebularine was bought from Santa Cruz Biotech and decitabine was obtained from Sigma Aldrich. The following antibodies were purchased and used as recommended by the manufacturer: GAPDH and cMyc (Abcam), DNMT3a, DNMT3b, E2F1 and PSME1 (Santa Cruz Biotech), pH2Ax was bought from (Novius). Total DNA methylation was estimated by ELISA kit and Anexin/PI kit was used to analyze apoptosis by FacsScalibur.
Results: DNA methylation evaluated by ELISA showed that zebularine treatment induced DNA demethylation in a panel of 6 MM cell lines and this effect was similar to demethylation induced by decitabine. DNA demethylation was associated with DNMT3a/b decrease and was accompanied by the reduction in cell viability tested by MTT and Anexin V experiments in 8 out of 9 MM cell lines. Interestingly, zebularine reduced cell viability more than decitabine in H929, KMS12BM, MM1s and RPMI cells. The cytotoxic effect of zebularine was associated with the induction of DNA damage and the decrease of cMyc protein in 4 cell lines, regardless of *TP53* mutation status. Moreover, zebularine potentiated the effect of bortezomib by decreasing PSME1 protein involved in bortezomib resistance. Surprisingly, overexpression of CDA enzyme that can be inhibited by zebularine, reduced cytotoxic effect of the compound and abrogated DNA damage and cMyc protein decrease.
Summary/Conclusion: Zebularine induces cytotoxicity in MM cell lines resistant to decitabine and sensitizes myeloma cells to bortezomib treatment. The anti-myeloma activity of zebularine is associated with the decrease of cMyc protein.

PB2127

PODOCYTURIA IN MULTIPLE MYELOMA PATIENTS HAS A TENDENCY TO INCREASE WİTH TREATMENT

T. Kuzu[1], S. Paydaş[2], E. Gurkan[3,*], S. Paydas[4], A.H. Uğuz[5]

[1]Dept of Internal Medicine, [2]Dept of Nephrology, [3]Dept of Hematology, [4]Dept of Oncology, [5]Dept of Pathology, Çukurova University Medical School, Adana, Turkey

Background: Multiple myeloma (MM) is a clonal B-cell disease of proliferating plasma cells. Renal disease is a common complication of MM that occurs in 20-25% of newly diagnosed patients. Cast nephropathy, amyloid light chain amyloidosis and monoclonal Ig deposition disease are the most common causes of chronic kidney disease in MM patients. Podocytes are unique cells with complex interdigitating foot processes that cover the outer surface of the glomerular basement membrane and thereby form an important layer of the glomerular filtration barrier. Podocyte injury is the hallmark of many glomerular diseases.
Aims: We have performed this study in order to investigate whether podocyte injury contributes to the pathogenesis of renal disease in MM patients and tried to detect correlations with treatment outcomes.
Methods: A total of 27 patients with newly diagnosed multiple myeloma and 20 healthy subjects were enrolled in the study and assessed concerning urinary podocytes and podocyte-associated molecules at bbaseline and end of the 6th month. Seven of the patients were lost due to death before the end of the study period. All the patients received bortezomib-based treatment. Determination of urine protein and creatinine (Cr) concentrations and quantitative analyses of Podocyn-mRNA (Pod), nephrin mRNA (Nep), and VEGF-A mRNA expression were performed using RT-PCR in pelleted urine samples. Levels of mRNA expression of podocyte proteins were also corrected by urine Cr concentration., cDNA was produced and PCR was processed. Podocytes were identified by PCR tagging nephrin, podocyn and VEGF-A which are biomarkers of podocyte, was detected.
Results: Proteinuria was found in 74% and renal failure in 33% of the cases at diagnosis. Median proteinuria levels were significantly higher compared to controls (725 mg *vs* 45 mg, (P<0.001). Proteinuria significantly decreased at the end of sixth months of treatment (P=0.002). Podocyturia monitored by Pod-mRNA, VEGF-A mRNA and Nep-mRNA expression was unremarkable compared to healthy controls in newly diagnosed MM patients. However, although was insignificant, there was a tendency towards an increase in urinary podocyte proteins concomittantly with a significant increase in urinary podocyte proteins, Pod-mRNA:/Cr, Nep mRNA/Cr and VEGF-A mRNA/ Cr ratios after treatment as compared with the pretreatment levels (P=0.001; P:0.039 and P=0.001 respectively).
Summary/Conclusion: Podocyturia as a marker of glomerular injury is unremarkable in MM patients at diagnosis. However, increase in podocyturia and related proteins during treatment might indicate glomerular injury as a consequence of therapeutic drugs especially bortezomib which is known as an nonnephrotoxic agent except causing thrombotic angiopathy. Further studies with more patients and longer observation period are needed in order to understand the extent of glomerular injury in MM patients while on treatment with different and new agents.

PB2128

HUMAN MONOCLONAL IGG DERIVED FROM PATIENTS WITH MULTIPLE MYELOMA ARE ABLE TO PENETRATE LIVING NEOPLASTIC CELLS AND EXHIBIT INTRACELLULAR BIOLOGICAL FUNCTIONS

I. Sarrigeorgiou[1], E. Terpos[2,*], T. Stivarou[1], P. Chalas[1], E. Kastritis[2], M.A. Dimopoulos[2], P. Lymberi[1]

[1]Immunology Laboratory, Department of Immunology, Hellenic Pasteur Institute, [2]Department of Clinical Therapeutics, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece

Background: Our group has previously described that high frequency of serum monoclonal immunoglobulins (M-Igs) of G and A class (M-IgG, M-IgA) from patients with Multiple Myeloma (MM) exhibit polyreactivity, which is a common feature with Natural Antibodies (NAbs) (*Abs present in the serum of healthy subjects, able to recognize self- and non self- antigens*). This and other findings support the view that in MM, certain clones producing M-Igs originate from NAb-producing clones occurring under normal conditions. Antibodies able to penetrate living cells (CPAbs) have been detected and well characterized in patients with systemic lupus erythematosus and in mouse models; recognize DNA and accumulate in the nucleus. Their penetration ability seems to be directly related to their polyreactivity. Our laboratory has recently described the existence of Cell-Penetrating Antibodies (CPAbs) in healthy conditions, derived either from BALB/c mice (monoclonal CPAbs), or from intravenous immunoglobulin (IVIg) (polyclonal human IgG). The development of human monoclonal IgG-CPAbs is of major importance in drug delivery and in cancer therapy.
Aims: The aim of the present study was to investigate the cell-penetrating ability of M-IgG exhibiting NAb–like activity, as well as their intracellular biological functions in neoplastic cells.
Methods: Seventy-one sera of patients with IgG-MM (IgG M-peak >5 g/l: 42 IgGκ/29 IgGλ) were studied by in-house ELISAs against a panel of self and non-self antigens (actin, tubulin, myosin, carbonic anhydrase, thyroglobulin, DNA and the hapten trinitrophenyl (TNP). We selected and purified five polyreactive IgG and one non-polyreactive IgG (negative control) by protein-G affinity chromatography. We then established the optimum conditions in terms of concentration, temperature and time-course in order to examine their ability to: 1) penetrate FcγR$^+$ (Raji & MDA-MB-231) and FcγR$^-$ (NIH-3T3 & HeLa) cells by immunofluorescence, 2) induce apoptosis on the aforementioned cells by flow cytometry (FACS), and 3) hydrolyze plasmic and genomic DNA.
Results: The five purified M-IgGs tested (3 IgGκ and 2 IgGλ) were able to penetrate all cell types, either FcγR$^+$ or FcγR$^-$, at 37^{o}C in a dose- and time-dependent mode of entry, while three of them were able to also penetrate cells at 4^{o}C (energy-independent mode of entry); optimum conditions of cell-penetration: 300 μg/ml, 2h-incubation. All M-IgGs: 1) were polyreactive, exhibiting a unique polyreactivity profile with the antigens of the panel used, 2) accumulated in the cytoplasm, 3) induced apoptosis especially in MDA-MB-231 cells, and 4) were able to hydrolyze plasmid DNA isoforms, while one of them also hydrolyzed genomic DNA.
Summary/Conclusion: We demonstrate herein that the sera of MM-patients with M-IgG represent an excellent source of human monoclonal IgGs exhibiting cell-penetrating ability and intracellular functionality. These human monoclonals can be exploited as a potential therapeutic tool in several disorders including malignant diseases, using them either *per se*, or as carriers for intracellular drug delivery, or even both.

PB2129

HIGH-RISK YOUNG MULTIPLE MYELOMA PATIENTS TREATED UP-FRONT WITH LENALIDOMIDE: IMPACT OF KIR 3DL1-EDU AND KIR HAPLOTYPES ON CLINICAL OUTCOME

F. Puglisi[1,*], A. Romano[1], C. Conticello[1], S. Toscano[2], M.P. Azzaro[2], M. Parisi[1], V. Calafiore[1], E. Martino[1], G. Sapienza[1], S. Leotta[1], G. Milone[1], G. Sortino[2], F. Di Raimondo[1]

[1]Division of Hematology and Bone Marrow Transplantation Units – Policlinico Hospital, [2]Tissue Typing Laboratory - Division of Hematology and Bone Marrow Transplantation Units – Policlinico Hospital, University of Catania, Catania, Italy

Background: Natural killer (NKs) cells provide rapid responses to viral-infected and malignant cells, including Multiple Myeloma (MM) cells. The balance among inhibitory and activating signals, delivered by multiple interactions between ligands (NKRL) on target cells and NK receptors (KIR), determines the posture of the NK cell response to either one of target cell elimination or tolerance. Despite novel therapeutic options, 15% to 20% of newly-diagnosed MM patients experience early death and rapid relapse following or even during initial induction therapy, the majority of them carrying high-risk cytogenetics (HRC) features, such as del (17p), del (1p), t(4;14) or t(14;16), as detected by FISH.
Aims: The aim of this work was to study the influence of the differential expression of activating or inhibitory KIR and the inhibitory HLA class I ligands on clinical outcome of HRY-MM patients treated up-front with regimens followed or not by lenalidomide maintenance.
Methods: KIR expression, KIR haplotype AA/Bx, and their HLA ligands were determined in 31 healthy subjects (controls) and 69 MM patients using the Genotyping SSP and SSO kit. In 50 HRY-MM patients (median age 54.1 years, 30 males), carrying at diagnosis del (17p), t(4;14) or t(14;16) and who were randomly assigned to lenalidomide maintenance until progression or observation after one autologous stem cell transplantation, we evaluated the role of KIR expression, KIR haplotype AA/Bx, and their HLA ligands on progression free survival (PFS).
Results: We observed that presence of KIR3DL1-edu was significantly more prevalent among controls as compared to MM patients (76,7% *vs* 43,5, 9%, p<0,0023). After median follow-up of 45 months, HRY-MM-patients carrying on KIR3DL1-edu had longer PFS than those without it (53.8 *versus* 41.5 months, p=0.08), without significant impact on overall survival (OS). In MM patients the AA and Bx genotype frequencies were 24,6% (AA) and 74,4% (B/x) with an A:B ratio of 1:3.05. Among the healthy controls, the AA and B/x genotype frequencies were 33% and 67% with an A:B ratio of 1:2. There was no significant difference in KIR haplotype AA/Bx frequencies in MM *versus* healthy subjects, however, in HRY-MM-patients, B/x haplotype was associated to longer PFS (56.4 *versus* 27.8 months, p=0.06). In

multivariate analysis, only complete remission achievement after induction and consolidation treatment were predictors of outcome, independently from KIR and haplotype genetics.
Summary/Conclusion: Our data show that 3DL1-edu and B/x haplotype are associated to clinical outcome in HRY-MM-patients receiving a lenalidomide based approach, implying a role of NK cytotoxicity against MM residual cells. These data will be confirmed in larger prospective series.

PB2130

THE INVOLVEMENT OF MESANGIOGENIC PROGENITOR CELLS IN MULTIPLE MYELOMA PATHOGENESIS

C.P. Schifone[1,*], S. Pacini[1], M. Montali[1], S. Barachini[1], F.M. Panvini[2], G. Buda[1], F. Mazziotta[1], F. Martini[1], M. Petrini[1]

[1]Hematology, University of Pisa, [2]Scuola Superiore Sant'Anna, Pisa, Italy

Background: Mesangiogenic Progenitor Cells (MPCs) are a bone marrow (BM) cell population isolated in humans able to differentiate into mesenchymal stromal cells (MSCs) and retaining an angiogenic potential. These two differentiation fates are mutually-exclusive, MPC-derived MSCs are not able to take part in angiogenic process but they can differentiate into adipocytes, chondroblasts or osteoblasts. The MPC differentiation toward the mesengenic lineage has been demonstrated to activate the non-canonical Wnt pathway, not involved during angiogenesis. More specifically, the MSCs differentiation takes place toward two hierarchical steps: a first differentiation into "early MSCs" (also called P1-MSCs) with the activation of Wnt-5/calmodulin pathway, then a terminal differentiation into "late MSCs" (also called P2-MSCs) independent from this pathway. Indeed, it has been demonstrated that Calmidazolium Chloride (CLMDZ), a potent calmodulin inhibitor, blocks the MPC mesengenic differentiation acting on the early phase. Previous studies, conducted on BM samples of non-hematological patients, demonstrated that the MPC angiogenic differentiation is, instead, inhibited by Bortezomib. For these peculiar characteristics, MPCs can be thought to be involved in the pathogenesis and progression of Multiple Myeloma (MM). The same *in vitro* experiments performed on BM samples from newly diagnosed MM patients, surprisingly showed that both mesengenic and angiogenic differentiations were impaired by Bortezomib while CLMDZ did not affect any differentiation. This data suggests that possibly MPCs would be restricted to an angiogenic fate, losing the mesengenic potential in the pathological setting.
Aims: To evaluate a possible involvement of MPCs in MM pathogenesis, we assessed the angiogenic potential of P1-MSCs applying sprouting tests.
Methods: After written consent, BM samples were obtained from 11 newly diagnosed MM patients. We isolated MPCs, as previously described, from each sample and performed mesengenic differentiation applying a specific medium for MSC expansion. After six days of culture, P1-MSCs were detached and two 3D-spheroids were produced by the hanging drop method. The spheroids were then plated on Matrigel thick gel and cultured in EGM-2 endothelial growth medium for one week. Sprouting distance was then measured by image analysis software and the mean values obtained from three different observers were recorded and analyzed by t-test.
Results: Six of eleven patients have a mean sprouting value of 41.2±16.1μm while the other five have a mean value of 206.7±15.2μm, the statistical analysis demonstrates a significative difference ($p<0.01$) between these two groups (figure 1). Surprisingly, higher sprouting values have been detected into patients with a more aggressive stage of disease and reporting osteolytic lesions, instead, a reduced sprouting has been detected in patients without osteolytic lesions or affected by smoldering MM.

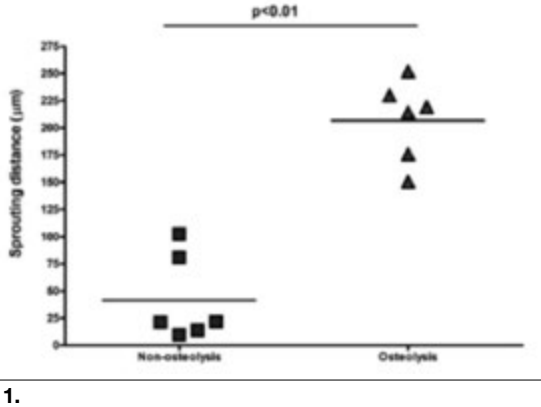


Figure 1.

Summary/Conclusion: Multiple Myeloma as a BM niche disease, affects the microenvironment. Particularly, our results suggest that disease in advanced stage (characterized by osteolytic lesions) probably forces MPCs toward an angiogenic differentiation regardless of the culture conditions. Thus, MPCs could be involved in the transition from a sub-clinical and "non-vascular" stage of disease to the advanced stages. Moreover, this MPCs restriction to the angiogenic fate could correlate *in vivo* with a reduced mesengenesis which cooperates to osteolytic lesions.

PB2131

MYELOID AND LYMPHOID DENDRITIC CELLS IN PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE - POSSIBLE LINK WITH DISEASE PROGRESSION

E. Grywalska[1,*], M. Pasiarski[2], A. Stelmach-Goldys[3], B. Sosnowska-Pasiarska[4], P. Steckiewicz[2], A. Kowalska[1], K. Gosik[1], B. Garus[2], S. Gozdz[5], J. Rolinski[1]

[1]Department of Clinical Immunology and Immunotherapy, Medical University of Lublin, Lublin, [2]Department of Hematology, Holycross Cancer Center, [3]Department of Hematology, Holycross Cancer Center, [4]Department of Oncocardiology, [5]Department of Clinical Oncology, Holycross Cancer Center, Kielce, Poland

Background: Monoclonal gammopathy of undetermined significance (MGUS) is a premalignant, clonal plasma cell disorder, characterized by the presence of a monoclonal (M) protein, <10% clonal plasma cells in the bone marrow, and absence of multiple myeloma or related lymphoplasmacytic malignancies. MGUS is present in 3% of the general population ≥50 years old. Dendritic cells (DCs) are a heterogeneous population of leukocytes defined as professional antigen presenting cells playing a key role in anticancer immunity.
Aims: The purpose of this study was to evaluate subpopulations of myeloid and lymphoid DCs in the peripheral blood (PB) and bone marrow (BM) of patients with MGUS in correlation with known prognostic factors.
Methods: The study involved 40 patients diagnosed with MGUS and 20 individuals belonging to the control group. The mean percentage of myeloid and lymphoid DCs was determined using flow cytometry.
Results: In the present study, we demonstrated a significant reduction in the percentages of both myeloid and lymphoid DCs in MGUS patients, more pronounced in those with the worse prognosis as determined by the high levels of M protein and low concentration of hemoglobin. Accordingly, a marked decrease in the proportions of both myeloid and lymphoid DCs in the BM of patients with MGUS in comparison with healthy controls was also found. The frequencies of both myeloid and lymphoid DCs correlated negatively with the percentages of plasmocytes in the BM.
Summary/Conclusion: Our results suggest that the degree of DC subpopulations deficit could be related to the MGUS progression, which in consequence may contribute to the MGUS-related impairment of the immune responses. This work was supported by research grants no. DS460 of the Medical University of Lublin and no. UMO-2016/21/B/NZ6/02279 of the Polish National Science Centre.

PB2132

QUANTIFICATION AND CHARACTERIZATION OF BONE MARROW MESENCHYMAL STROMAL CELLS IN MONOCLONAL GAMMOPATHIES

R. Leite[1], J. Barradas[2,*], B. Paiva[3], I. Silva[4], P. Rocha[2], H. Silva[2], C. Geraldes[5], A. Paiva[1,4]

[1]College of Health Technology of Coimbra, Coimbra, [2]Clinical Hematology Department, Centro Hospitalar Tondela-Viseu, Viseu, Portugal, [3]Center for Applied Medical Research University of Navarra, Navarra, Spain, [4]Flow Cytometry Unit – Clinical Pathology Service, [5]Clinical Hematology Department, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

Background: Monoclonal Gammopathies (MG) are plasma cell (PC) dyscrasias caused by clonal accumulation of PC in the bone marrow (BM). The BM microenvironment plays a crucial role in disease development. BM Mesenchymal Stromal Cells (MSC) are able to interact with clonal PC contributing for a microenvironment suitable for disease progression.
Aims: To evaluate the relationship between PC dyscrasias progression with the frequency and phenotype of BM MSC.
Methods: Clinical process consultation and BM aspirates were proceded in patients diagnosed with MGUS (n=32), smoldering Multiple Myeloma (sMM) (n=5) and MM (n=24), and in a control group (n=10) of normal BM samples. Multidimensional flow cytometry was used to identify, quan-

tify and characterize BM MSC (based on the expression of CD13, CD24, CD29, CD49e, CD73, CD90, CD105, CD106 and CD271).
Results: After frequency normalization, by removing clonal PCs from all BM nucleated cells, a significantly increased in the frequency of MSC (0,137%) was observed in symptomatic MM patients when compared with sMM (0,027%), MGUS (0,027%) and normal BM (0,034%). We also found that the higher frequency of BM MSC was associated with lower hemoglobin concentrations, higher frequency of BM PC and higher ISS staging. In MM patients, a higher MSC frequency was observed in the cases whereas clonal PC didn t express CD56. Moreover, a lower expression of CD73 and a higher of CD90 by MSC, was observed through disease progression, from MGUS to MM.
Summary/Conclusion: The higher frequency of MSC and the altered phenotype observed in BM samples from patients with PC dyscrasias, could play an important role in MM pathogenesis.

PB2133

ANALYZING BONE AND EXTRAMEDULLARY PLASMACYTOMA SUBSTRATES IN PATIENTS WITH MULTIPLE MYELOMA

M. Firsova*, L. Mendeleeva, A. Kovrigina, N. Deineko, M. Solovev, V. Savchenko

National Research Center for Hematology, Moscow, Russian Federation

Background: At the time of the diagnosis of multiple myeloma (MM) the incidence of plasmacytomas varies from 3,5 to 18%. We defined plasmacytomas as bone (plasma cell tumor extending from the intramedullary spaces of the bone marrow through the cortical bone) and extramedullary (spread to organs distant from bone). The presence of extramedullary plasmacytoma at the time of the diagnosis of MM has a negative impact on the disease prognosis, while bone plasmacytomas in patients with MM are treatable. The pathogenesis of extramedullary disease has not been thoroughly analyzed, thought cell adhesion molecules and chemokine receptors are assumed to participate in this process. Study of tumor plasmacytoma substrate is great interest due to the rarity of this pathology.
Aims: Analyzing CD56, CD166, CXCR4, Ki-67 and c-MYC expression in the tumor substrate of bone and extramedullary plasmacytomas in patients with MM.

Table 1.

Marker	MM patients with:	Expression values (%)		p
		M	± m	
CD56	bone plasmacytoma (n=14)	54.29	10.18	0.165
	extramedullary plasmacytoma (n=7)	28.57	14.86	
CD166	bone plasmacytoma (n=14)	36.29	7.61	**0.044**
	extramedullary plasmacytoma (n=7)	9.57	8.46	
CXCR4	bone plasmacytoma (n=14)	45.50	9.35	0.413
	extramedullary plasmacytoma (n=7)	31.71	13.91	
Ki-67	bone plasmacytoma (n=14)	25.36	5.63	**0.004**
	extramedullary plasmacytoma (n=7)	61.43	10.50	
c-MYC	bone plasmacytoma (n=14)	33.29	8.37	0.748
	extramedullary plasmacytoma (n=7)	37.86	10.57	

Table 1. Markers expression values of bone and extramedullary plasmacytomas in patients with MM

Methods: From October 2013 to April 2017 21 patients with newly diagnosed MM (10 males and 11 females) 23-77 years old (mean value: 52 y.o.) were included in the study. The disease was diagnosed in accordance to IMWG criteria (2014). 14 patients were diagnosed with bone plasmacytoma associated with skeleton bones (vertebrae, ribs, skull and pelvis) and 7 patients were diagnosed with extramedullary plasmacytoma distant from bone (liver, pancreas, stomach, soft tissues, muscles and skin). In all cases a tumour biopsy was taken which confirmed the presence of plasma cell infiltration. Paraffin block slices from tumour biopsy material were used to perform an immunohistochemistry analysis with an antibody panel to CD56, Ki67, CXCR4, CD166, c-MYC. Marker expression level was analyzed with Leica DM4000B microscope by viewing 10 fields of view at 400-fold magnification. Marker expression assessment was carried out by means of semi-quantitative method. The percentage of cells expressing the protein in question against the total number of tumor substrate cells was calculated. To assess statistical differences between the mean values we applied Student's *t*-test (including preliminary assessment using Levene's test for equality of variances).
Results: Analyzing mean values of marker expression in bone and extramedullary plasmacytomas resulted in revealing significant differences in CD166 and i-67 (Table 1), while no significant differences between expressions of other markers in question were found. A higher Ki-67 index (p=0.004) was reported for extramedullary plasmacytoma cells, comparing to that of bone plasmacytoma cells, with their mean values of 61.43±10.50% and 25.36±5.63% respectively. The expression of cell adhesion molecule CD166 in bone plasmacytoma cells was significantly higher than that of extramedullary plasmacytoma cells (36.29±7.61% and 9.57±8.46% respectively, p=0.044).
Summary/Conclusion: Aggressive course of MM complicated with extramedullary plasmacytoma may be caused by significantly higher proliferative activity of extramedullary plasmacytoma cells, comparing to that of bone plasmacytoma cells. Low expression of adhesion molecule CD166 in extramedullary plasmacytoma cells may be the reason behind weakening bond between myeloma cell and bone marrow microenvironment, which leads to the occurrence of hematogenic dissemination of plasma cells in various organs and tissues. In the same time, high expression of CD166 in bone plasmacytoma cells may point to this marker's participation in modelling osteogenesis and forming osteodestructions.

PB2134

ASSOCIATION OF HLA-SPECIFICITIES OF CLASSES I, II WITH IMMUNOCHEMICAL VARIANTS OF MULTIPLE MYELOMA

E. Poponina[1,*], E. Butina[1], G. Zaytseva[1], N. Minaeva[2], I. Paramonov[3]

[1]Immunohematology, [2]Hematology, [3]Director, Federal State Institute of Science "Kirov research Institute of Hematology and Blood Transfusion of the Federal Medical and Biological Agency of Russia", Kirov, Russian Federation

Background: The main histocompatibility complex plays an important biological role, participating in the regulation of the immune response. There is some information in the literature about the relationship of specific HLA-specificities to various diseases, including oncohematological ones. In multiple myeloma (MM) the malignant clone develops from B-lymphocyte precursor cells, retaining the ability to produce pathological monoclonal immunoglobulins (the most common immunochemical variants are IgG, IgA) or light chains of immunoglobulins (the Bence-Jones variant). It is of interest to investigate the dependence of the onset of MM and its immunochemical variants on the specificity of the genes of the HLA system.
Aims: Identify the dependence of the occurrence of MM variants on the characteristics of patients HLA-genotypes.
Methods: The results of typing the HLA-genes of loci A, B, DRB1, DQA1, DQB1 by the SSP method were analyzed in 134 MM patients: 78 (58.2%) with variant G, 26 (19.4%) with A, 26 (19,4%) - with Bence-Jones. The frequency of occurrence of HLA-genes in patients with various forms of MM was compared with the frequency of these genes in healthy residents of the region – donors of blood components.
Results: It was found that in the general group of patients with MM this alleles were revealed significantly more often than in a healthy population: A*19 (21.6% *vs* 13.1% in healthy persons, p<0.05, RR=2.0), B*16 (13.4% *vs* 7.7% in healthy subjects, p<0.05, RR=2.0) and the DRB1*09 (10.9% *vs* 1.5%, p<0.01; RR=8.4). The HLA-B*05 gene, on the contrary, was less common than in healthy individuals (5.2% *vs* 11.6%, p<0.05, RR=0.3), which indicates its preventive effect. A detailed analysis of the distribution of HLA-genes revealed specific genes for each immunochemical variant of MM. The HLA-A*19 gene (23.1% *vs* 13.1% in healthy p<0.05, RR=2.1) and B*16 (17.9% *vs* 7, 7%, p<0.01, RR=2.5) predisposed to the immunochemical variant with IgG secretion. In the variant with the secretion of pathological IgA, the specificity of HLA-A*03 was detected 3 times less frequently than in healthy individuals (11.6% *vs* 33.7%, p<0.05, RR=0.3). For Bence-Jones myeloma, there was an increase in the occurrence of HLA-A*10 and B*18, often heritarily linked (HLA-A*10 - 38.5% *vs* 16.3%, p<0.01, RR=3.7; HLA-B*18-30.8% *vs* 11.3%, p<0.01, RR=3.9). The DRB1 * 09 gene was detected in 1.5% of a healthy population, while in the variant with IgA secretion - in 4.8%, IgG - in 7.5% of patients (the differences did not reach statistical significance). In patients with the Bence-Jones variant, the DRB1 * 09 frequency was 28.0% (p<0.001, RR=22.7).
Summary/Conclusion: To the development of MM predispose the genes A*19, B *16, DRB1*09; to the variant G - A*19, B*16; to the light chain variant - A*10, B*18, DRB*09. Protective to the development of MM is the gene B*05, to the development of variant IgA - A*03.

PB2135

RECONSIDERING JAK2 IN AN INTER-TUMOUR HETEROGENEITY OF MULTIPLE MYELOMA

S. Sommaluan[1], B. Rerkamnuaychoke[1], T. Puavilai[2], S. Chuncharunee[2], T. Chareonsirisuthigul[1], P. Pornsarayuth[1], T. Siriboonpiputtana[1,*]

[1]Pathology, [2]Medicine, Mahidol University, Bangkok, Thailand

Background: Multiple myeloma is a hematological malignancy characterized by abnormal accumulation of clonal plasma cells in the bone marrow. Beside the complexity and heterogeneity of the disease, we and others attempt to molecularly identify the biomarkers of multiple myeloma aiming for early detection, risk-assessment, monitoring, and importantly, the potential therapeutic markers.
Aims: We molecularly identify the biomarkers of multiple myeloma aiming for early detection, risk-assessment, monitoring, and for the development of potential therapeutic markers.
Methods: We combined our routinely genetic testings including the conventional cytogenetic analysis, iFISH for CD138 enriched samples, MLPA analysis, and a pan-screening array CGH to identify genetic alterations in 35 newly diagnosed myeloma patients.
Results: While several recurrent genetic alterations in multiple myeloma such as translocation involving immunoglobulin gene rearrangements, Del(17p), Del(13q), and Amp(1q) were observed in similar frequency as compared to previous publications, the amplification of short arm of chromosome 9 (JAK2) by MLPA analysis was predominantly observed (10/35; 29%) in our tested samples. Consistency results were observed by using array CGH technique. Moreover, our preliminary data in the transcriptional analysis of multiple myeloma patients using expression microarray was able to identify the dysregulation of JAK2 in all multiple myeloma subgroups.
Summary/Conclusion: Our findings further highlight the inter-tumour heterogeneity of multiple myeloma between populations and the alteration of JAK2 may play a role as a key driving mutation in multiple myeloma which mainly positive in our tested population.

PB2136

TARGETING SIGLEC-7: A NOVEL IMMUNOTHERAPEUTIC APPROACH TO POTENTIATE THE CYTOTOXIC FUNCTIONS OF NATURAL KILLER CELLS AGAINST MULTIPLE MYELOMA

J. Daly*, S. Sarkar, A. Natoni, J. Hu, S. Chauhan, R. Henderson, T. Duggan, L. Kirkham, C. McEllistrim, M. O'Dwyer

Medicine, National University of Ireland, Galway, Galway, Ireland

Background: Multiple Myeloma (MM) is a malignant plasma cell disorder, accounting for 10% of all haematological malignancies. The immune-suppressive functions of sialylated glycans, abundant on the surface of myeloma cells, has not previously been addressed. We hypothesize that hypersialylation of MM enables evasion of Natural Killer (NK) cells within the bone-marrow niche. Disrupting the interactions between these sialylated glycans and their cognate sialic acid-binding lectin (Siglec) receptors on NK cells could lead to the development of novel immunotherapeutic strategies for treating MM.
Aims: Determine the expression of Siglec-7 ligands on a panel of MM cell lines and primary MM cells and determine the expression of Siglec-7 on primary bone marrow-derived NK cells. Determine the effect de-sialylating MM cell lines has on their sensitivity to NK-mediated cytotoxicity using both a sialidase and sialyltransferase inhibitor and the effect blocking Siglec-7/Ligand interactions has on NK-mediated cytotoxicty.
Methods: Siglec-7 ligand expression was measured by staining with a recombinant Siglec-7 Fc chimera (1.25µg/ml, R&D systems) and flow cytometry was used to determine Siglec-7 ligand positive cells. Siglec-7 expression was measured using an anti-Siglec-7 antibody (Miltenyi) and flow cytometry.
In order to desialylate MM both the sialidase Neuraminidase (Sigma-Aldrich) and sialyltransferase inhibitor P-3Fax-Neu5Ac (Merck) were used. Cells were either treated with 1X neuraminidase for 45mins prior to co-culture, or with 200µM 3Fax for 5 days. To block Siglec-7 interacting with its concomitant ligands, RPMI8226 myeloma cells were treated with recombinant Siglec-7 Fc chimera (10µg/ml, R&D systems) for 30mins prior to co-culture with KHYG-1 NK cells.
Results: We observed that Siglec-7 ligands (Siglec-7L) are highly expressed across a panel of MM cell lines. Furthermore, Siglec-7L expression was also observed on CD38+/CD138+ primary MM cells isolated from BM aspirates of newly diagnosed and relapsed MM patients (N=3,N=2 respectively). Immunophenotyping analysis revealed that the BM-derived NK cells of MM patients have significant cell surface expression of the cognate Siglec-7 receptor (Siglec-7R)(82±25%, n=5). Cytotoxicity assays and flow cytometry analysis revealed that abolishing sialylated glycans from the cell surface of MM cells using a sialyltransferase inhibitor results in a 1.3-1.6 fold increase ($p<0.05$) in NK cell induced cell death in MM cell lines RPMI8226 and H929, but not MM1S. (Fig.1, n=3). Furthermore, to validate our observations we pre-treated RPMI8226 cells with 10µg/ml of recombinant Siglec-7 Fc chimera to block Siglec-7 ligands on the cell surface. We observed statistically significant enhancements of NK cytotoxicity against the Siglec-7 Fc chimera treated RPMI 8226 *versus* an isotype Fc control and at all E:T ratios (P=0.003 1:1, P=0.001 2.5:1, P=0.03 5:1).

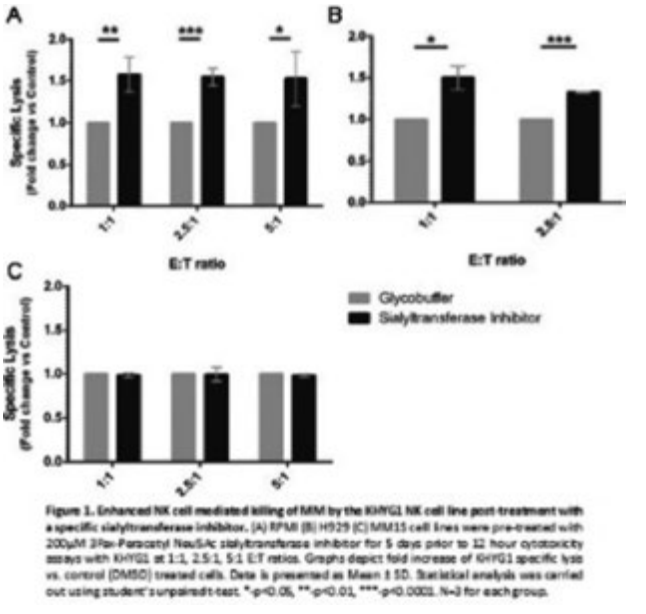


Figure 1. Enhanced NK cell mediated killing of MM by the KHYG1 NK cell line post-treatment with a specific sialyltransferase inhibitor. (A) RPMI (B) H929 (C) MM1S cell lines were pre-treated with 200µM 3Fax-Peracetyl Neu5Ac sialyltransferase inhibitor for 5 days prior to 32 hour cytotoxicity assays with KHYG1 at 1:1, 2.5:1, 5:1 E:T ratios. Graphs depict fold increase of KHYG1 specific lysis vs. control (DMSO) treated cells. Data is presented as Mean ± SD. Statistical analysis was carried out using student's unpaired t-test. *-p<0.05, **-p<0.01, ***-p<0.0001. N=3 for each group.

Figure 1.

Summary/Conclusion: We observed that Siglec-7 and it's cognate ligands are abundantly expressed on the surface of NK and MM cell lines respectively. Additionally, Siglec-7 is significantly expressed on primary NK cells circulating within the BM of MM patients. Functional assays revealed that Siglec-7L and Siglec-7R receptor interactions have significant immune suppressive effects on the cytotoxicity of NK cells towards MM cell lines. Thus, we can leverage the findings of this study to develop NK cell based cellular therapies to target Siglec-7 ligand- receptor interactions in MM.

PB2137

BONE MARROW MESENCHYMAL STROMAL CELLS FROM HEALTHY DONORS SECRETE ANTI-ANGIOGENIC EXTRACELLULAR VESICLES AS WELL AS SOLUBLE FACTORS HAVING GROWTH INHIBITORY EFFECT IN MULTIPLE MYELOMA

T. Umezu[1,*], S. Imanishi[2], N. Gladkova[3], K. Ohyashiki[1], J. Ohyashiki[4]

[1]Department of Hematology, [2]Institute of Medical Science, Tokyo Medical University, Shinjyuku, [3]Kintaro Cells Power Corrporation, Shinagawa, [4]Department of Advanced Cellar Therapy, Tokyo Medical University, Shinjyuku, Japan

Background: The bone marrow mesenchymal stromal cells (BM-MSCs) from healthy donor is one of the most promising sources of cell therapy. We have previously shown that extracellular vesicles (EVs) secreted from BM-MSCs had anti-angiogenic effect rather than direct growth inhibition in multiple myeloma (MM) (Umezu *et al.* Blood Adv 2017).
Aims: To elucidate the molecular mechanism of BM-MSCs administration in cancer patients, we investigated both EVs and soluble factor mediated effect on multiple myeloma cells using a freshly obtained BM-MSC specimens.
Methods: BMSCs from Russian healthy volunterrs (KNT_D_170714, KNT_L_170714, KNT_80_170825 and KNT_86_170825: KNT cells) are kindly provided by Kintaro cells power Co. (Tokyo, Japan). Fresh bone marrow specimens were collected at Federal Research and Clinical Center of Federal Biomedical Agency of Russia (FRCC FMBA, Moskva, Russia) after obtaining approval of institutional ethical committee. Spectral karyotyping (SKY), immunophnotyping was done to validate KNT cells. In addition, KNT cells was evaluated after induction using MSC differentiation media, according to the manufacuture's protocol (PromoCell). To analyze the factors secreted by the KNT cells, 90% confluent, passage 2-4 KNT cells in T25 tissue culture flask, were transferred to a serum-free AIM-V

medium (Invitrogen) during 48 h. The EVs were isolated from conditioned medium (CM) using a Exoquick-TC (SBI). The size of EVs was confirmed using a NanoSight LM10 (Malvren). CM was fractionated using 3-kDa, 10-kDa and 50-kDa MW cut-off filter units (Millipore). To determine the effect of secretion from KNT cells, we used a MM cell line, RPMI8226. Endothelial tube formation assay was used for validating function of MM cell-endothelial cell (HUVECs) communication.
Results: We first validated KNT cells as follows. The KNT cells had a fibroblast-like morphology in culture regardless of donor's age. Immunophenotyping and karyotyping showed a normal karyotypes. The cells were positive for all MSC markers (CD90, CD73, CD105), but negative for hematopoietic markers (CD34 and CD45). When cultured in appropriate culture conditions KNT cells showed adipogenic or osteogenic potential. The nanoparticle size distribution of EVs secreted from KNT cells (KNT-EVs) were approximately 50 nm. We found that the donor's age involved the properties of KNT-EVs, We found that KNT-EVs derived from young healthy donors significantly reduced tube formation of HUVECs co-cultured with a MM cell line (RPMI8226), but did not affect the survival of RPMI8226 *in vitro*. We also found KNT cell-derived CM (KNT-CM) derived from young healthy donors markedly inhibited the growth of RPMI8226 as well as the tube formation of HUVECs. Especially the MM cell growth inhibitory effect was remarkable in the CM fractionated by 50-kDa MW cut-off filter.
Summary/Conclusion: In the current study, we used freshly obtained BM-MSCs (KNT cells) instead of commercially avairable BM-MSCs, such as widely used BM-MSCs from LONZA co. Our results indicates that the young healthy donor-derived KNT cell secretome including EVs and other soluble factors have the ability to inhibit MM-induced angiogenesis. In contrast, growth inhibitory effect was only seen at the molecules up to a MW of 50-kDa in KNT-CM. These findings indicated the limitation of EV-based therapy, however, analysis of EVs and fractionated CM may shed light on the complex molecular mechanism of BM-MSC-based therapy.

PB2138

IMPACT OF THE EXPRESSION OF LRP GENE ON THE SENSITIVITY OF TUMOR PLASMA CELLS TO BORTEZOMIB *IN VIVO* AND *IN VITRO*

Y. Chernykh[1,*], A. Golenkov[1], E. Rybalkina[2], S. Shushanov[2], T. Mitina[1], E. Kataeva[1], E. Trifonova[1], E. Klinushkina[1], S. Zakharov[1], L. Vysotskaya[1], K. Belousov[1]

[1]Clinical haematology, Moscow Regional Research and Clinical Institute, [2]N.N.Blokhin Russian Cancer Research Center, Moscow, Russian Federation

Background: The impact of the expression of Lung Resistance Protein (LRP) gene on the development of drug resistance to the first generation proteasome inhibitor Bortezomib is currently under active study.
Aims: To study the impact of LRP gene expression on the sensitivity of tumor plasma cells of bone marrow aspirate of patients with stage III multiple myeloma (MM) and tumor plasma cells of human MM cell lines to Bortezomib.
Methods: The LRP gene expression was assessed in bone marrow mononuclear cell fraction containing plasmocytes of 15 patients with newly diagnosed multiple myeloma Durie-Salmon stage III and in the human MM cell lines RPMI8226 and IM9. The assessment of LRP gene expression was measured by reverse transcription polymerase chain reaction test. The sensitivity of tumor plasmocytes to Bortezomib-action *in vivo* was assessed by the percentage reduction in the absolute level of paraprotein after 6 courses of Bortezomib-containing treatment, as well as in the overall survival (OS) index. OS were analyzed by the Kaplan-Meier method using the Cox-Mantel criterion. Differences were considered statistically significant at $p < 0.05$. The expression of the LRP gene in MM cell lines was examined before and after prolonged cells culture on a medium with Bortezomib.
Results: The LRP gene expression was found in 10 of 15 patients (67%) before start of cytostatic therapy. The intensity of gene expression was different. Were show 2 subgroups of patients: a subgroup with high LRP gene expression and a subgroup with low intensity of gene expression. After 6 cycles of induction with Bortezomib, there was not a significant decrease of paraprotein levels in the subgroups. Overall survival was negatively associated with high LRP gene expression only (median of overall survival in patients with high LRP gene expression was 17 months and in those with low expression –62 months, $p<0.05$). *in vitro* IM9-cells with low LRP expression were more sensitive to Bortezomib- action than RPMI8226-cells with high LRP expression (IC50 were 0.8±0.3x10-8 M and 2.7±0.6x10-8 M respectively). Cultivation of the cell lines on the Bortezomib containing medium for 90 days was leads to increase of IC50 of Bortezomib, as well as to increase of LRP expression in both human MM cell lines.
Summary/Conclusion: The high LRP gene expression can impact on the development of tumor plasma cell`s resistance to Bortezomib.

PB2139

EFFECT OF AUTOLOGOUS STEM CELLS TRANSPLANTATION OF PATIENTS WITH MULTIPLE MYELOMA ON THE CALORIMETRIC MARKERS OF THE SERUM PROTEOME. CORRELATION WITH THE IMMUNOLOGICAL MARKERS

L. Gartcheva[1,*], A. Danailova[2], S. Todinova[2], K. Dimitrova[1], V. Petkova[1], S. Krumova[2], M.G. Guenova[1], S. Taneva[2], B. Spassov[3]

[1]Laboratory haematopathology and immunology, University National Specialised Hospital for Active Treatment of Haematological Diseases, [2]Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, [3]University National Specialised Hospital for Active Treatment of Haematological Diseases, Sofia, Bulgaria

Background: The thermodynamic stability of biofluids is currently extensively studied by means of differential scanning calorimetry (DSC), a biophysical technique that measures thermally induced conformational transitions of biomolecules in solution.
Aims: With the aim to develop a novel strategy for monitoring of patients with multiple myeloma (MM) that underwent autologous stem cells transplantation (ASCT) we have explored the potential of differential scanning calorimetry (DSC).
Methods: Blood sera were derived from 11 patients, defined as eligible for ASCT – 8 secretory, 2 non secretory(NS) and 1 with Free Light Chain (FLC) MM before and at 1; 2; 3; 6; 9; 12;16 months after the ASCT. In addition, 21 age matched control samples were studied. The quantification of immunoglobulin heavy/light chains (HLC) and the ratio FLC (rFLC) was performed by "Hevylite immunoassay" and "Freelite" R test, with SPAPLUS (Binding Site, UK) analyzer. Blood sera were heated with constant scanning rate (1 °C/min) in the range 20–95 °C by means of highly sensitive DASM 4 (Privalov, BioPribor)-built-in calorimeter. Origin software package was used to evaluate the calorimetric parameters: transition temperature (T_m) and excess heat capacity (C_p^{ex}) of the successive thermal transitions; temperature of the major peak (T_m^{mp}) and weighted average center of the thermogram, T_{FM} ($T_{FM}=^{T1}\int_{T2} T\, C_p^{ex}(T)\, dT / C_p^{ex}(T)\, dT$), where T_1 and T_2 are the initial and final temperatures of the thermogram. We applied linear correlation in the thermogram's shape (Pearson's correlation coefficient) and non-parametric statistical test.
Results: Before and after ASCT however the M protein concentration, rFLC and HLC varied in very large intervals. The calorimetric profiles and the thermodynamic parameters before the ASCT derived from the thermograms deviated strongly from the control thermogram. The excess heat capacity at and above 70 °C was clearly larger in the MM cases than in the control samples. The T_m^{mp} shifts from 61.9 °C in the control samples to 72.6–76. 3 °C and the T_{FM}, shifts from 64 to 68.0–74.5 °C, for all MM samples before ASCT. These changes in the C_p^{ex} and the denaturation temperatures of the individual transitions resulted in greatly reduced values of the shape similarity parameter, r, (0.09–0.9) as compared to those typical for healthy thermograms (0.97). Before the ASCT the Cp^{HSA} value is ca. 1.5–2.0 times, lower than that of the control. For NS MM and FLC MM cases before ASCT, Cp^{HSA} is lower by 1.5–2.3 times than the control, r was 0.74–0.90 and T_m^{mp} was 62–64 °C. After ASCT there was a trend for strong reduction in the M protein content and the HLC for all secretory cases. There was also an inverse correlation between the changes in the M protein level and in the thermogram's shape similarity parameter, r. The decrease in M protein concentration was also related to strong reduction in T_m^{mp} from 78 °C to 62 °C as well as in T_{FM}, but less pronounced – from 76 °C to 68 °C; those correlations are less strong than that between the M protein level and r. The calorimetric scans recorded for the two NS MM cases both before and after ASCT deviated strongly from those of healthy controls.
Summary/Conclusion: We established that the change in the paraprotein level and thus the patient's clinical status is clearly reflected in the serum thermogram. Hence, DSC can be used as complementary bioanalytical tool for the monitoring of MM remission/progression in a fast and cheap way.

PB2140

STUDY OF MULTIPLE MYELOMA CHROMOSOMAL ABNORMALITIES BY MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION: CORRELATIONS WITH FLUORESCENCE IN SITU HYBRIDIZATION

F. Carbone[1,*], F. Nozza[2], G. Vona[2], A. Dell'Acqua[1], S. Oronzo[3], C. Mannarella[4], A. Ciancio[4], B. Daraia[4], G. Pietrantuono[5], I. Attolico[6], R. Nuccorini[6], S. Pascale[6], P. Musto[7], M. Pizzuti[6], A. Fragasso[4]

[1]Hematology Unit, Madonna delle Grazie Hospital (Laboratory "Muscas"), Matera, [2]"Carla Alagia" Laboratory of Clinical Research and Advanced Diagnostics, IRCCS-CROB, Rionero in Vulture, [3]Genetics, [4]Hematology Unit, Madonna delle Grazie

Hospital, Matera, [5]Department of Onco-Hematology, IRCCS-CROB, Rionero in Vulture, [6]Hematology Unit, San Carlo Hospital, Potenza, [7]Scientific Direction, IRCCS- CROB, Rionero in Vulture, Italy

Background: Chromosomal abnormalities play an important role in prognostic stratification of Multiple Myeloma (MM). Current technology used to evaluate such genetic abnormalities is Fluorescence *In Situ* Hybridization (FISH). Nevertheless, it is difficult for FISH to screen all lesions simultaneously, due to high costs and technique limitations. Multiplex Ligation-dependent Probe Amplification (MLPA) is a polymerase chain reaction that permits the amplification of multiple targets by a single reaction. This technique is useful to detect Copy Number Variations (CNVs), but it is unable to find out balanced translocations. Furthermore, MLPA analysis detects abnormalities only if they are present in 30-40% of pathological cells.
Aims: In this study we evaluated the MLPA technique in the identification of chromosomal abnormalities occurring in MM patients, comparing the results with those obtained by FISH.
Methods: Cohort study included 24 MM patients (male to female ratio 15/9, median age 69 years, range 53-85 years). Bone marrow samples were taken in line with good medical practice. After evaluating the presence and percentage of plasma cells with optical microscopy, a sample part of bone marrow was used for the FISH assay. MLPA analysis (SALSA MLPA P425-B1 MM probemix, MRC-Holland, Amsterdam, Netherlands) was conducted on DNA extracted from marrow aspirates with commercially kits. PCs were purified from MM samples using CD138 microbeads and magnet-assisted cell sorting (MiltenyiBiotec).We enriched 7/24 (29%) samples. The PCR products were initially analyzed using ABI 3130 Genetic analyzer (Applied Biosystems), later using SeqStudio Genetic Analyzer (Applied Biosystems).
Results: FISH analysis detected genetic alterations in 20 of 24 patients tested for assay: 13q deletion was the most frequently genetic aberration (9/20) followed by 17p deletion (3/20) and 1q amplification (3/20). Other genetic alterations included 1p deletion in 1patient and a deletion of Y chromosome in another one. Out of these 20 patients, 16 were positive for IGH rearrangements. Overall, out of 24 patients, 12 cases were positive for CNVs. We could identify genetic alteration in 10/24 multiple samples by using MLPA. The most frequently genetic aberrations included 13q deletion (6/10) followed by 1q amplification (4/10). There was 1 positive case for chromosome 1p deletion, while the chromosome 17p deletion was positive in 1 patient. Interestingly, we also found 2 patients with chromosome 5q amplification, 2 with 15q amplification, 1 with 12p deletion and 1 with Y chromosome deletion. Overall, concordant results were detected in 18/24 (75%) cases. The discordant results were 6/24 (25%). Out of these 6 cases, MLPA analysis were conducted on DNA isolated from CD138-enriched plasma cells in 2 patients, while MLPA tests were made on DNA isolated from whole bone marrow mononuclear cells in 4 patients.
Summary/Conclusion: In this study there was a consistent concordance between FISH and MLPA for detection of CNVs. However, some discrepancies emerged, which were probably attributable to the non-isolation of CD138+ cells in some samples. MLPA can't detect balanced aberrations, but identified some additional cytogenetic abnormalities that are not usually investigated by a standard FISH approach; therefore, taken together, MLPA and FISH represent complementary techniques, both useful to find cytogenetic aberrations in MM patients. The study is still recruiting new patients.

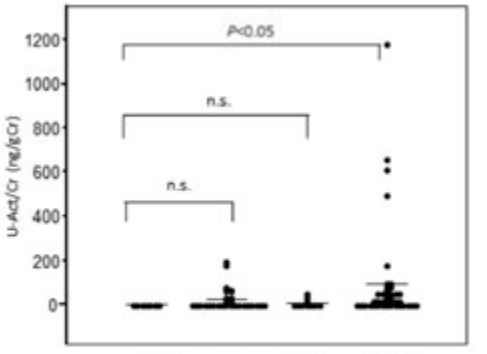
PB2141

ACTIVIN A: A NOVEL URINARY BIOMARKER REFLECTING EARLY RENAL IMPAIRMENT IN MULTIPLE MYELOMA

H. Iriuchishima[1,*], A. Maeshima[2], S. Takahashi[2], T. Ishizaki[1], A. Yokohama[3], N. Tsukamoto[4], T. Saitoh[5], H. Murakami[5], H. Handa[1]

[1]Hematology, [2]Nephrology and Rheumatology, [3]Blood Transfusion, [4]Oncology Center, [5]Laboratory Sciences, Gunma University, Maebashi city, Gunma, Japan

Background: Renal impairment (RI) is a common complication of multiple myeloma (MM), which significantly affects not only treatment efficacy but also patient mortality. The conventional parameters defining RI in MM (the level of serum creatinine (Cr) >2.00 mg/dl or Ccr <40 ml/min) are not sensitive, resulting in the delay of diagnosis and treatment of RI. Recently, it is reported that monoclonal gammopathy of renal significance (MGRS), which represents all renal disorders caused by a monoclonal immunoglobulin secreted by a B-cell or plasma cell clone, is associated with high morbidity. Therefore, sensitive reliable parameters for early diagnosis of RI in MM are required. Activin A, a multifunctional cytokine belonging to the TGF-β superfamily, has been reported to be involved in kidney development, tubular repair after injury and fibrotic process of the kidney. However, it is unclear whether activin A plays a role in renal damages in MM.
Aims: To clarify the involvement of activin A in renal damages in MM, we examined whether activin A can be detectable in the urine from patients with newly diagnosed MM (NDMM), asymptomatic (smoldering) (SMM), and MGUS including MGRS.



	control	MGUS	SMM	NDMM
U-Act/Cr (ng/gCr)	0	25.0	6.61	97.3

Figure 1.

Methods: We investigated the data of patients with NDMM (n=41), SMM (n=10), and MGUS (n=28) diagnosed in our department between October 2012 and September 2017. Along with common blood and urine chemistry determinations, serum and urinary activin A were measured by ELISA. In some cases, renal histological evaluation was performed using renal biopsy samples. This study was approved by the local institutional review board, and written informed consent was obtained from all patients.
Results: Urinary activin A, which was undetectable in healthy controls (HC), significantly increased in NDMM (97.3±36.0 ng/gCr, p<0.05 *vs* HC), but not in MGUS (25.0±9.4 ng/gCr) and SMM (6.61±3.9 ng/gCr). There were no significant differences in serum activin A level among these subgroups. Urinary activin A level was significantly reduced after initial treatment in NDMM (73.9 *vs* 12.0 ng/gCr, $p<0.01$). Improvement rate of urinary activin A from baseline after treatment was much higher than that of serum Cr or eGFR (84%, 30%, 12%, respectively). There was a significant correlation of urinary activin A with urinary protein level (p<0.001), but not with eGFR, serum Cr, and N-acetyl-glucosaminidase. Interestingly, there was no significant correlation between serum and urinary activin A level. Immunohistochemical analysis revealed that activin A, which was absent in normal kidney, was present at the tubulointerstitial area of the kidneys from patients with MGRS.
Summary/Conclusion: These data suggest that urinary activin A is a novel urinary biomarker detecting renal damages in MM. Measurement of urinary activin A might be an useful tool to perform early diagnosis of RI or to predict the severity of renal damages in MM.

PB2142

RAMAN SPECTROSCOPY IS ABLE TO DISCRIMINATE BETWEEN CD138-/CD138+ PLASMA CELLS OF MULTIPLE MYELOMA PATIENTS

A. Allegra[1,*], O. Bianco[1], E. Fazio[2], M. Bonsignore[2], F. Neri[2], G. Ferlazzo[3], S. Trusso[4], D. Franco[2], L.M. De Plano[5], M.G. Rizzo[5], S. Crea[5], S.P.P. Guglielmino[5], C. Musolino[1]

[1]Division of Hematology, Department of Patologia Umana dell'Adulto e dell'Età Evolutiva, [2]Dep. of Mathematical and Computational Sciences, Physical Science and Earth Science, [3]Laboratory of Immunology and Biotherapy, Department of Human Pathology, University of Messina, [4] Institute of Chemical-Physical Processes (IPCF)-CNR, CNR-Messina, [5]Department of Chemical Sciences, Biological, Pharmaceutical and Environmental, University of Messina, Messina, Italy

Background: Multiple myeloma is a B cell neoplastic disorder characterized by clonal proliferation of malignant plasma cells in the bone marrow. CD138 is a marker of plasma cells and MM cells. The expression of CD138 highly correlates with that of VEGFR3. However, a low CD138 expression is frequently observed in MM patients and several studies have reported how a decreased CD138 expression can affect the prognosis and the treatment response.

Aims: We proposed Micro-Raman spectroscopy as an easily accurate and non invasive method to discriminate plasma cell immune-phenotype.

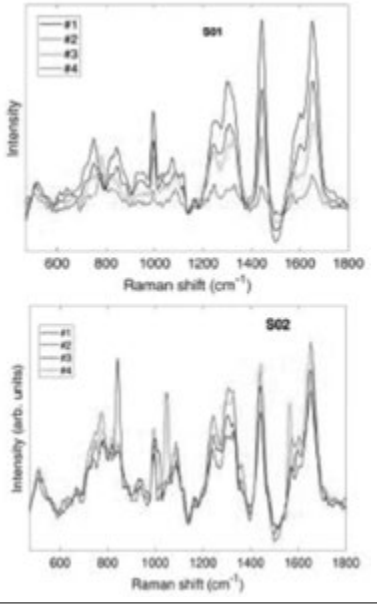


Figure 1.

Methods: two distinct cell subtypes for antigen expression pattern: i) subtype 2 CD45-/CD56-/CD38+ (namely, S01); ii) subtype 3 CD45+/CD38+/CD138+ (namely, S02) separated by the conventional flow cytometry approach and then fixed on CaF2 substrate, were analyzed carrying out Raman mapping analyses. Raman contributions were observed in selected spectral regions where markers of specific functional groups, useful to characterize the cell state, are present. Hence, comparing Raman spectra, the investigated cell subtypes can be distinguished, analyzing several spectra for each sample. So, intracellular variability, repeatability and sensitivity of the analyses for each sample was evaluated.
Results: - Regarding S01cells: the spectra acquired in the #3 and #4 points are characterized by DNA and PO-P vibrational modes in the 637-665 and 776-827 cm-1, respectively. In addition, RNA features at 725 cm-1 and in the 1560-1580 cm-1 regions are more evident. C-C or C-O vibrational modes, ascribed to membrane phospholipids, are envisaged in the 1075-1116 cm-1 region. Regarding S02 cells: The main spectral differences respect to S01 cells are referred: 1) to the nucleic acids contributions (DNA/RNA) and 2) to the protein features (1255-1670 cm-1 region) more pronounced and defined in the S02 cells in comparison to the S01 ones. Furthermore, cells S01 show an high internal variability respect to S02, suggesting a high metabolic activity.
Summary/Conclusion: Plasma cells are a eterogenous population in Multiple Myeloma patients. A different rate of CD138+ cells is able to modify the prognosis. Raman Spectroscopy could increase our knowledge of the structure and problably the function of these cells contribuiting to a better awereness of the mechanisms of Multiple Myeloma progression.

PB2143

PDGFR-B AND CXCR4 IN MULTIPLE MYELOMA DISEASE STAGES: PRELIMINARY RESULTS

L. Correia[1,*], M.M. Tenreiro[1], M. Silveira[2], M.A. Brito[1], R. Malhó[3]

[1]DBBH, FFUL, [2]IPOFG, [3]FCUL, Lisboa, Portugal

Background: Multiple myeloma (MM) is characterized by the clonal expansion of plasma cells (PCs) in the bone marrow (BM) that leads to bone destruction, anaemia and renal failure. The evolution from the asymptomatic stage of monoclonal gammopathy of undetermined significance (MGUS) to MM and the progression of the disease itself are related with cellular and molecular alterations in the BM microenvironment, including the development of the vasculature. Many factors are involved in the mobilization of endothelial progenitors, including C-X-C motif chemokine ligand 12 (CXCL12) that stimulates the motility of cells and its receptors, namely the C-X-C motif chemokine receptor 4 (CXCR4). Another factor is platelet-derived growth factor receptor (PDGFR)-β, a marker of pericytes, mural cells recruited to stabilize newly formed blood vessels. Despite the increasing knowledge about the disease, there is still no effective cure and the standard survival is up to 4 years. Therefore a better understanding of the disease evolution at cellular and molecular levels is needed.
Aims: To evaluate the changes in PDGFR-β and CXCR4 positive cells, and their relation with PCs, in the bone marrow, along the several disease stages.
Methods: We performed a longitudinal study, using BM smears archived at the Hematology Service of Instituto Português de Oncologia Dr. Francisco Gentil, Lisbon. BM smears from patients evolving from MGUS to MM and from MM to remission (MMr) were used (n=3). Smears were May-Gr nwald-Giemsa stained for PCs analysis, and were subjected to immunofluorescence analysis of PDGFR-β and CXCR4.
Results: As expected, the evolution of MGUS to MM was characterized by a significant increase in PCs levels, from 5.8% to 23.4% ($p<0.01$), and by a decrease from 39.0 to 2.9% from MM to MMr ($p<0.001$). Analysis of PDGFR-β+ cells revealed no significant difference from MGUS to MM but a significant increase from 14.5% in MM to 39.0% in MMr ($p<0.001$). The results for CXCR4 showed that the patients who evolved from MGUS to MM with PCs>20% in MM, presented a decrease in CXCR4+ cells from 55.1% to 27% ($p<0.05$), whereas the group of patients with less severe disease (PCs ≤20% in MM) showed no statistical significance. To establish if the levels of PDGFR-β and CXCR4 were correlated with those of plasma cells, we analysed the results obtained in MM patients as fold change from those of the same patients in MGUS and determined the correlation coefficients between PCs, and PDGFR-β or CXCR4. PCs were 4-fold higher in MM than in MGUS, whereas the levels of PDGFR-β+ and CXCR4+ cells were nearly 0.8-fold of those of MGUS. Analysis of the correlation coefficients revealed that PCs were negatively correlated with CXCR4+ cells ($r=-0.825$, $p<0.05$). In contrast, no significant correlation was observed concerning PDGFR-β+ cells, or between CXCR4+ and PDGFR-β+ cells, probably due to the limited study population.
Summary/Conclusion: Although preliminary, these results point to variations in PDGFR-β+ and CXCR4+ cells along different stages of the disease that deserve to be further studied in larger groups of patients. Moreover, they indicate that CXCR4 and PDGFR-β are involved in the BM microenvironement alterations along MM disease stages.

PB2144

INTRODUCTION OF A EUROPEAN QUALITY ASSESSMENT SCHEME FOR GENETIC TESTING IN MYELOMA: PROGRESS AND UPDATE

P. Talley[1,*], A. Chantry[2], A. Cox[2], K. Rack[3], R. Hastings[3]

[1]Haematological Malignancy Diagnostic Service (HMDS), St James's University Hospital, Leeds, [2]Department of Oncology and Metabolism, University of Sheffield, Sheffield, [3]GenQA, John Radcliffe Hospital, Oxford, United Kingdom

Background: Recent guidelines have highlighted the importance of genetic analysis in plasma cell myeloma (PCM) patients. GenQA (Genomics Quality Assessment) are a branch of UKNEQAS (UK National External Quality Assessment Schemes) and offer external quality assessment (EQA) schemes covering a range of haematological disease. In 2014, a pilot EQA scheme for PCM was launched. The scheme was aimed at all laboratories offering genetic testing for PCM patients, and included a questionnaire assessing the current status of PCM genetic testing. As well as providing a method to evaluate and assess laboratories analytical and interpretative skill and ability, the scheme aimed to provide an education component with the idea of creating a more equitable and consistent diagnostic genetic service. We present an update on the progress of the EQA scheme to date.
Aims: To introduce an EQA scheme for PCM to evaluate analytical and interpretative ability. To assess the status of PCM genetic testing at scheme introduction. To influence equity and consistency of genetic testing through educational components of scheme participation and production of best practice guidelines for genetic laboratories.
Methods: The pilot GenQA scheme involved analysis of two known abnormal cases; the first case involved analysis of online FISH images, the second was presented as fixed patient cells for internal FISH processing and analysis. For each case, the participating laboratory produced a report that mirrored their standard reports. A panel of four experts, as well as the GenQA scheme organiser/deputy, assessed the analytical ability and the interpretative components presented in the report. This methodology has been followed in subsequent years. A questionnaire interrogating the laboratories referral patterns, sample numbers, turnaround times, techniques employed and gene regions examined was also issued in the inaugural year.
Results: At scheme introduction, 39 laboratories participated; of those, 33 produced satisfactory reports. Participation rates have shown an overall increase and plateau over the period of four years, 56 laboratories in year 2, 65 in year 3 and 61 in year 4. The number of poor performance laboratories demonstrated a decrease over the first three years with an upward

spike in year 4; 15.4%, 10.7%, 3.1% and 14.8% over 2014, 2015, 2016 and 2017 respectively. This peak in poor performance was due to a change in testing strategy employed by some laboratories, with penalities being applied to the use of specific probes in an inappropriate context. Case 2, the wet sample, results in more poor performance, but also involves increased assessment of the testing strategy, technical and analytical ability of laboratories. The inaugural scheme survey, provided evidence for the *ad hoc* nature of genetic testing in PCM, although the majority of laboratories did carry out the essential tests.
Summary/Conclusion: In summary, we present the successful implementation of an EQA for genetic diagnosis in myeloma, which is now entering its fifth year. We have seen an increase and plateau of participants over this period, and a positive effect of the educational component provided by the scheme. Although the essential tests are being provided by the majority of laboratories, an inequitable service is provided. This highlights the need, not only for the educational component of the EQA scheme, but for the production of best practice guidelines (BPG) in this area. BPG are currently in progress, to address this shortfall.

PB2145

Abstract withdrawn.

PB2146

MOLECULAR CYTOGENETIC ANALYSIS IN MULTIPLE MYELOMA/ PLASMA CELL LEUKEMIA PATIENTS WITH CIRCULATING PLASMA CELLS

J. Balcárková*, M. Mlynárčiková, J. Minařík, T. Pika, P. Krhovská, H. Urbánková, T. Papajík

Hemato-oncology, University hospital Olomouc, Czech republic, Olomouc, Czech Republic

Background: The common feature shared by primary plasma cell leukemia (pPCL) and multiple myeloma (MM) with circulating plasma cells (CPCs) is the expansion of plasma cells independent of bone marrow (BM). The presence of CPCs is considered as a marker of highly proliferative disease and an adverse independent prognostic factor. The diagnosis of pPCL is based upon absolute number (≥2x10^9/L) and/or the percentage (≥20%) of CPCs in peripheral blood (PB). Cytogenetic studies showed that CPCs have a variety of genetic abnormalities. Majority of cytogenetic abnormalities in patients with CPCs are non-hyperdiploid with higher frequency of major IgH translocations, *i.e.* t(11;14)(q13;q32), t(4;14)(p16;q32) and t(14;16) (q32;q23). The most common abnormalities are deletion of 13q/ monosomy 13, deletion of 17p and abnormalities of chromosome 1 (1q21 gain/ deletion 1p21).
Aims: The aims of the present study were to analyze cytogenetic changes in a cohort of 7 patients (3 with pPCL and 4 MM with CPCs).
Methods: We used array comparative genomic hybdridization (arrayCGH) and FICTION method (fluorescence immunophenotyping and interphase fluorescence *in situ* hybridization) with commercially available probes specific for RB1/c15, IgH, 1q21/1p32, TP53/c17, to assess clonal evolution in BM and CPCs and to evaluate the prognostic importance.
Results: We detected translocation of *IgH* gene in 4 out of 7 patients- t(11;14) in 3 patients, t(4;14) in one patient. In one patient we delineated der(4)t(4;7)(p16;q?)ins(4;7)(p16;q?) including FGFR3/MMSET. Gain of 1q21 was found in 5/7 patients. Deletion of RB1 gene was detected only in 2/7 patients and TP53 deletion in one patient. Abnormalities identified in BM were also found in CPCs. ArrayCGH found common deletions of 1p, 6q, 13q, 16q and gain of 1q, chromosome X abnormalities (monosomy/Xq gain). Chromotripsis of chromosome 8 and hyperdiploidy with trisomies of chromosomes 3, 5, 7, 9, 11, 15 and 19 was found in 2 other patients.
Summary/Conclusion: In summary, genetic aberrations were recorded in all 7 analyzed cases with PCL and MM with CPCs. We found numerical changes as well as structural aberrations similarly as in MM patients. Our findings underline that genetic abnormalities are more frequent in PCL/MM with CPCs than in MM patients and they tend to be more complex.
This work was supported by grant IGA_LF_2018_004.

PB2147

ALTERED MICRORNA EXPRESSION PROFILE IN THE PERIPHERAL LYMPHOMONOCYTES OF MULTIPLE MYELOMA PATIENTS WITH BISPHOSPHONATE-INDUCED OSTEONECROSIS OF THE JAW

V. Innao[1,*], A. Allegra[1], G. Oteri[2], M. Mania[2], A. D'Ascola[2], O. Bianco[1], A. Avenoso[2], D. Vaddinelli[1], A.G. Allegra[1], S. Campo[2], C. Musolino[1]

[1]Division of Hematology, Department of Patologia Umana dell'Adulto e dell'Età Evolutiva, [2]Department of Biomedical, Dental Science and Morphological and Functional Images, University of Messina, Messina, Italy

Background: Bisphosphonates are formidable inhibitors of osteoclast-mediated bone resorption employed for therapy of multiple myeloma (MM) subjects with osteolytic lesions. Osteonecrosis of the jaw (ONJ) is an uncommon drug-induced adverse event of these agents. MicroRNAs (miRNAs) are a group of small, noncoding RNAs nucleotides, which are essential post-transcriptional controllers of gene expression. They have a central role in the normal bone development.
Aims: The goal of our study was to investigate 18 miRNAs, whose targets were previously validated and described in MM subjects without ONJ, in peripheral lymphomonocytes of MM subjects with bisphosphonate-induced ONJ.
Methods: Utilizing reverse transcription quantitative polymerase chain reaction we evaluated miRNAs in five healthy subjects and in five MM patients with ONJ.
Results: Our experimental data revealed that a diverse miRNA signature for ONJ subjects emerged respect to control subjects. Using the filter for *in silico* analysis, among 18 miRNAs we recognized 14 dysregulated miRNAs. All of these miRNAs were significantly over-expressed in patients *vs* controls (MIR-16-1, MIR-21, MIR-23A, MIR-28, MIR-101-1, MIR-124-1, MIR-129, MIR-139, MIR-145, MIR-149, MIR-202, MIR-221, MIR-424, MIR-520). Among them, six were strongly up-regulated (4-fold up-regulated and more). These miRNAs target numerous pathways and genes implicated in calcium ion binding, bone resorption, mineralization of bone matrix, differentiation and maintenance of bone tissue.
Summary/Conclusion: A modified microRNA expression profile after zoledronate therapy could participate to the onset of ONJ. Targeting these miRNAs could provide a new treatment for the prevention or treatment of ONJ.

PB2148

RELATIONSHIP OF MOLECULAR PECULIARITIES WITH CYTOGENETIC ABERRATIONS IN MULTIPLE MYELOMA

E. Nazarova*, M. Sarpova, N. Minaeva, J. Suhorukova, I. Paramonov, E. Zotina, I. Dokshina, V. Shardakov

Kirov Research Institute of Hematology and Blood Transfusion, Kirov, Russian Federation

Background: Multiple myeloma (MM) is characterized by pronounced genomic heterogeneity due to a complex combination of numerical and structural changes in chromosomes that play a key role in oncogenesis. In studies of malignant plasma cells using fluorescence *in situ* hybridization (FISH) and flow cytometry, karyotype abnormalities are detected in more than 90% of MM patients, frequency and severity of which correlates with the stage of the disease, the prognosis and response to therapy. Genetic mutations also play an important role in the pathogenesis of MM.
Aims: To study the relationship between the mutational status of a number of immune response genes and cytogenetic aberrations in MM patients.
Methods: 39 patients with MM aged from 33 to 75 years (median - 57 years) in the onset of the disease were evaluated, 14 men (35.9%), 25 women (64.1%). FISH-method of the bone marrow cells interphase nuclei is performed with Kreatech FISH Probe DNA probes. Genotyping of 20 polymorphic locus of the 13 immune response genes of genomic DNA from peripheral blood leukocytes was carried out by polymerase chain reaction with allele-specific primers (single nucleotide polymorphism - SNP).
Results: The patients were divided into two groups. The first group included 24 (61.5%) patients with MM who had no chromosomal abnormalities in the FISH analysis. The second group consisted of 15 (38.5%) patients with different cytogenetic aberrations in the form of monosomy 13, trisomy 4, 11, 13, 14, 17, 20 chromosomes, amplification of 1q21, del DLEU1 (13q14), del IGH (14q32), del TP53 (q13;q32), t(4;14) (p16;q32) and t(14;16) (q32;q23). When comparing the obtained cytogenetic data with the results of SNP genotyping, it was found that the presence of the mutant allele G in the haplotypes (CG+GG) of the interleukin-6 (*IL6*) gene at the locus-174 by four times (p=0.04), and the presence of the «wild» type G allele in the homozygous state at the mutation point -915 of the transforming growth factor-β (*TGF-β*) gene, it reduces the detection rate of cytogenetic aberrations in MM by 33 times (p=0.02). The conducted studies confirmed the generally recognized molecular heterogeneity of MM. The genes *IL6* and *TGF-β* are localized in 7 and 19 chromosomes, respectively. Aberrations in these chromosomes are detected in MM with hyperdiploid karyotype, which has a favorable prognosis. Proteins synthesized by these genes play a particular role in MM. IL-6 is one of the growth and survival factors of malignant plasma cells, inhibiting their apoptosis. In addition, IL-6 is able

to interact with other factors involved in the pathogenesis of MM, such as adhesion molecules, tumor suppressor genes and oncogenes. The transforming growth factor-β (TGF-β) refers to a transcription factors family that are involved in cell proliferation, differentiation, and apoptosis. In MM, TGF-β can act as a suppressor and a tumor growth promoter.
Summary/Conclusion: Cytogenetic studies in MM are used to predict the course of the disease. The detection of genetic characteristics of malignant disorders creates prerequisites for the development of targeted therapy. Given the important role of IL-6 and TGF-β in this disease, it is advisable to use the mutation status of the *IL6* and *TGF-β* genes to predict the course of MM and personalized treatment. Thus, the complex determination of chromosomal changes, the spectrum of mutations and the evolution of subclones in MM requires further research in this direction.

PB2149

EPIDEMIOLOGY OF MULTIPLE MYELOMA IN A LARGE METROPOLITAN AREA OF RUSSIA (NOVOSIBIRSK)

N. Skvortsova*, T. Pospelova, I. Kovynev

Novosibirsk State Medical University, Novosibirsk, Russian Federation

Background: Morbidity and prevalence of MM in some regions of Russia have not been studied enough and are characterized by a significant spread of indicators reflecting the large territory of the country, the heterogeneity of its geographical and climatic conditions, migration processes and the level of diagnostic capabilities of each region. Novosibirsk is the largest commercial, business, cultural, industrial, transport and scientific center of Western Siberia and the third city of Russia with a population of 2 million 84.4 thousand people. The obtained data will allow to reveal the peculiarities of MM epidemiology in this region of Russia and will help in the development and implementation of regional and national programs for the provision of timely and qualified medical care to patients with this disease.
Aims: Analysis of the dynamics of the main epidemiological indicators of MM in a large metropolitan area of Russia (Novosibirsk).
Methods: The study included 335 patients with newly diagnosed MM who were observed at the City Hematology Center in Novosibirsk between 2006 and 2016. The median age of the patients was 67 years (range from 30 to 89 years). The dynamics of intensive morbidity, prevalence, lethality and survival of MM patients in Novosibirsk for the analyzed period was retrospectively studied. Statistical processing of data was carried out using the statistical software STATISTIKA (version 7.0) and SPSS (version 23.0). Overall survival was calculated using the Kaplan-Meier method. Reliability of differences in survival in the study groups was calculated using a log-rank test, the differences were considered reliable at $p<0.05$.
Results: The average intensive morbidity of MM in Novosibirsk over the past 10 years has increased 1.6 times and amounted to 2.4 cases per 100 000 population per year, which corresponds to the incidence in Russia as a whole and lower than in Europe and The United States. The prevalence of MM for the period under review increased from 2.8 to 13.8 per 100,000 people per year, indicating an increase in the number of newly diagnosed patients with MM and prolonging their life span. Among women, MM morbidity and prevalence is statistically significantly higher than among men, which is associated with better survival rates for women and fewer males in the region according to regional statistics. The annual mortality of MM patients decreased from 28.3 to 8.2 with a negative linear trend throughout the analyzed period, which is most likely due to the high efficacy of bortezomib and lenalidomide-containing regimens introduced since 2006. The median overall survival (OS) of patients with MM for the study period was 70 months. In patients younger than 65 years, the median OS was 79.1 months, in the 65-75 year-old group, 54.3 months, and in patients over the age of 75, 36.1 months, respectively.
Summary/Conclusion: Thus, the epidemiological data obtained by us indicate an increase in the incidence of MM during the analyzed period. An increase in the prevalence of MM along with a decrease in mortality indicates an increase in the life of patients due to the rather high efficiency of modern chemotherapy programs based on new medicinal agents. The overall survival of MM patients in different age groups corresponds to international literature data.

PB2150

THE MOLECULAR SIGNATURES OF HIGH RISK MULTIPLE MYELOMA IN THAI POPULATION

P. Kamseng[1,*], T. Siriboonpiputtana[1], T. Puavilai[2], K. Paisooksantivatana[1], T. Chareonsirisuthigul[1], S. Chuncharunee[2], B. Rerkamnuaychoke[1]

[1]Department of Pathology, [2]Department of Medicine, Faculty of Medicine Ramathibodi hospital, Mahidol university, Bangkok, Thailand

Background: Multiple myeloma (MM) is recognized as a hematological malignancy characterized by the accumulation of abnormal plasma cell clones in bone marrow. MM is a heterogeneous disease in which clinical representation, cell morphology, immunophenotype, prognosis, disease progression as well as treatment outcome, harbors specific genetic alterations. Advanced genomic techniques including microarray have been currently used to study the heterogeneity/complexity of the disease which could help further identify biomarkers as well as develop the new effective therapy.
Aims: In this work, we aimed to primarily explore the transcriptional signature of different subgroups of myeloma patients in Thai population.
Methods: We performed gene expression profile analysis using microarray to analyze a total of seventeen CD138-enriched samples from newly diagnosed MM patients. The risk-assessment was based on the Mayo Stratification of Myeloma and Risk-Adapted Therapy. Finally, gene set enrichment analysis (GSEA) was performed by using Hallmark gene set version 4.0 MSigDB collections as a gene set database.
Results: We could categorize individual patients in to different subgroups as following: 2 patients with high-risk (11.8%), 7 patients with intermediate-risk (41.2%), and 8 patients with standard-risk (47.1%). Markedly, 2 patients with high-risk group shared both del(13q) and del(17p). Additionally, we found that several genes are differentially expressed in different subgroups of multiple myeloma. Moreover, GSEA analysis revealed that TNFA signaling via NFKB, TGF beta signaling, and KRAS signaling are differentially expressed in high-risk group when compared with the standard-group (FDR<25% and nominal p-value <0.05).
Summary/Conclusion: We provide the preliminary data of a transcriptional signature in different subgroups of MM in Thai patients. The results from GSEA reveal and highlight several molecular pathways that are critical for the development of high-risk MM phenotype in Thai population.

PB2151

THE NOVEL TRANSLOCATION OF T(1;21) IN MULTIPLE MYELOMA WITH COMPLEX KARYOTYPE

M. Okay*, R. Ciftciler, E. aladag, H. Goker

Hematology, Hacettepe University, Ankara, Turkey

Background: Multiple myelomas (MM) is characterized as the neoplastic proliferation of plasma cells producing a monoclonal immunoglobulin. Multiple myeloma is a heterogeneous disease. The prognosis of patients with MM is dependent on four factors: staging, patient factors, disease biology and response to therapy. The cytogenetic anomalies of patients have prognostic significance.
Aims: The cytogenetic anomalies of patients have prognostic significance. Accurate identification of high-risk and low-risk cytogenetic abnormalities plays a crucial role in predicting the response or resistance to the treatment. The aim of this paper is to report complex karyotype with novel translocation leading to a fatal clinical course in a patient with MM.

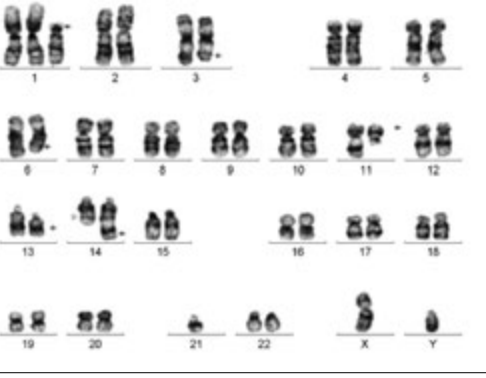


Figure 1.

Methods: We report the case of a 48-year-old man presenting with black stool, bicytopenia, high creatinine level and hypercalcemia. The bone marrow aspiration of the patient showed diffuse neoplastic plasma cell infiltration and complex karyotype was detected in all of 20 metaphases on cytogenetic analyses. The karyotype of the patient was 46,XY,dic(1;21)(p11;p11), del(3)(q25;q29), del(6)(q24;q26), t(11;14)(q13;q32), del(13)(q14q21). The anomaly of t (1; 21) (p11; p11), which we detected in this

case and which has not been detected between these breaking points in any malignancies before, was detected in a case with MM for the first time.
Results: The anomaly of t (1; 21) (p11; p11), which we detected in this case and which has not been detected between these breaking points in any malignancies before, was detected in a case with MM for the first time. Although the prognostic impact of this unique anomaly may be unclear. Regarding the very aggressive clinical presentation of the MM, cytogenetic abnormalities could be linked to poor prognosis.
Summary/Conclusion: Multiple myeloma is a heterogeneous disease. The cytogenetic anomalies of patients have prognostic significance. We report the case of complex karyotype was detected in all of 20 metaphases on cytogenetic analyses. The karyotype of the patient was 46,XY,dic(1;21)(p11;p11), del(3) (q25;q29), del(6) (q24;q26), t(11;14) (q13;q32), del(13) (q14q21). The anomaly of t (1; 21) (p11; p11), which we detected in this case and which has not been detected between these breaking points in any malignancies before, was detected in a case with MM for the first time.

Myeloma and other monoclonal gammopathies – Clinical

PB2152

Abstract withdrawn.

PB2153

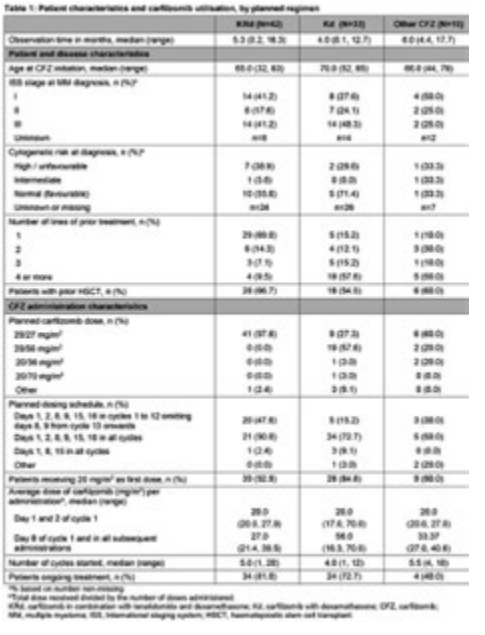
REAL-WORLD EVIDENCE OF THE USE OF CARFILZOMIB AMONG PATIENTS WITH RELAPSED MULTIPLE MYELOMA IN EUROPE: AN INTERIM ANALYSIS FROM A PROSPECTIVE OBSERVATIONAL STUDY

E. Terpos[1,*], J. Caers[2], M. Sohne[3], F. Suzan[4], M. Schoonen[5], S. Wetten[5], C. Turkington[6], M. Ladetto[7], D. Lechner[8]

[1]Department of Clinical Therapeutics, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece, [2]Department of Hematology, CHU de Liège, Liège, Belgium, [3]St. Antonius Hospital, Nieuwegein, Netherlands, [4]Amgen (Europe) GmbH, Zug, Switzerland, [5]Centre for Observational Research, Amgen Ltd., [6]Global Biostatistical Science, Amgen Ltd., Uxbridge, United Kingdom, [7]Hematology Division, Azienda Ospedaliera Santi Antonio e Biagio e Cesare Arrigo, Alessandria, Italy, [8]Department of Hematology and Medical Oncology, Ordensklinikum Linz Elisabethinen, Linz, Austria

Background: Carfilzomib (CFZ) is a next-generation proteasome inhibitor currently approved in combination with lenalidomide and dexamethasone (KRd) or with dexamethasone alone (Kd) for treatment (Tx) of adult multiple myeloma (MM) patients (pts) with at least one prior Tx in the European Union (EU). Access to CFZ is ongoing throughout the EU. Real-world evidence is crucial to understand how CFZ based regimens are used in practice and in relation to EU prescribing information (PI).
Aims: To describe CFZ utilisation in routine clinical practice as well as the pt population, safety profile, response to Tx and selected healthcare resource utilisation. Results from a first planned interim analysis are reported.
Methods: This prospective cohort study (NCT03091127) recruited adults who at the time of CFZ initiation had experienced a relapse, received ≥1 prior line of MM Tx and ≥1 dose of CFZ in a combination regimen in routine clinical practice. Medical history and pt characteristics prior to CFZ initiation are collected as well as further data during pt observation until 30 days after final CFZ administration or until 18 months after initiation, whichever is earlier. All adverse events of grade 3 or above (Gr3+, including serious adverse events [SAE]) are collected.

Table 1.



Results: First pt was enrolled on 14th March 2017. As of 30 October 2017, 85 pts have been included from 4 countries participating so far: Austria

(22), Belgium (23), Greece (26), and the Netherlands (14). The reported regimens planned to be prescribed were KRd (49%), Kd (39%), and other CFZ regimen combinations (12%) which included triplets with cyclophosphamide (n=5), pomalidomide (n=3), methylprednisolone (n=1), and elotuzumab (n=1). Overall, 34.1% of pts reported a history of hypertension, 15.3% of cardiac disorders, 15.3% of diabetes and 5.9% of pulmonary embolism prior to CFZ. On average, pts with planned KRd were younger than pts with planned Kd and 69% of KRd pts had received 1 prior line of Tx compared with 15% of Kd pts. For nearly all KRd pts (97.6%) and over half of Kd pts (57.6%), the planned CFZ dose was per EU PI, 20/27 mg/m^2 and 20/56 mg/m^2 respectively. For all remaining Kd pts physicians planned a dose lower than the recommended biweekly dose of 112 mg/m^2. For nearly half of KRd pts (47.6%) and around three quarters of Kd pts (72.7%), the planned administration schedule was per EU PI. Regardless of regimen, most pts received the starting dose, 20 mg/m^2. The average dose for administrations from day 8 of cycle 1 onwards was largely in line with the EU PI. After a median observation time of 4.6 months, 81% and 73% of KRd and Kd pts, respectively, are still ongoing CFZ Tx. Treatment-emergent adverse events Gr3+ were reported in 26.2% of KRd (16.7% SAEs), 21.2% of Kd (18.2% SAEs) and 60% of other combinations (10.0% SAEs) and one fatal event of cardio-respiratory arrest occurred in the Kd group. The most frequent events were neutropenia (n=4), pneumonia (n=4), anaemia (n=3), decreased platelet count (n=3) and sepsis (n=2) and one pt experienced cardiac failure. Only 3 pts discontinued CFZ Tx due to an AE.

Summary/Conclusion: These first results suggest that KRd may be preferably used as second-line Tx and Kd may be deferred to later Tx lines, however, KRd was generally available before Kd in participating countries. The planned CFZ dose reduction among Kd pts may reflect a real-life approach to manage frail and heavily pretreated pts. Further observation of increasing number of pts in routine practice with further follow up is ongoing to assess longer-term pt management and response.

PB2154

ASSESSMENT OF PNEUMOCOCCAL VACCINATION RESPONSE IN MULTIPLE MYELOMA

M. Ellis[1,2,*], N. Runnegar[1], P. Hogan[1], P. Mollee[1,2]

[1]Princess Alexandra Hospital, [2]University of Queensland, Brisbane, Australia

Background: Patients with myeloma are at increased risk of infective complications and current international guidelines recommend the use of immunization against S. pneumoniae, H. influenzae and influenza A and B. Prior studies assessing response to influenza vaccine have demonstrated poor rates of seroconversion. Modern myeloma therapy is based around combinations of immunosuppressive proteasome inhibitors (PI) and immunomodulatory (IMiD) agents.

Aims: We sought to assess the rate of seroconversion in patients with myeloma receiving different treatment regimens.

Methods: This study was conducted as a pilot study. Patients had serological assessment for the pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F and 23F prior to vaccination with the 23-valent purified polysaccharide vaccine Prevenar 23 using an ELISA platform. Repeat serology was taken approximately six weeks following vaccine administration. Patients were stratified in to three groups based on whether they were newly diagnosed (vaccinated prior to any therapy), or currently on (or within three months of ceasing) either PI or IMiD based therapy. An appropriate response was defined as a four-fold increase in antibody concentrations or an absolute value increase of >1.3 ug/mL. An optimal response was defined as an appropriate response in >75% of tested serotypes (>5 of the 7 tested serotypes).

Results: A total of 63 patients were vaccinated and had baseline serology, 51 follow-up serology samples were available for analysis. Median age was 67 (range 43-89), and 42 (67%) patients were male. Median ages did not differ between treatment groups. There was a significant difference between weekly dexamethasone dose (49mg *vs* 17.5mg *vs* 30.7mg, p<0.001), prior lines of therapy (0 *vs* 1.9 *vs* 0.89, p=0.002), paraprotein size (32 *vs* 5.5 *vs* 14.6g/L p<0.001) and level of residual gamma globulins (3 *vs* 5.4 *vs* 4.1g/L, p=0.003). Baseline pre-vaccination anti-Pneumococcal IgG antibody geometric mean concentrations (GMCs) were 0.12, 0.21, 0.16, 0.47, 0.20, 0.34 and 0.20 respectively for serotypes 4, 6B, 9V, 14, 18C, 19F and 23F. Post vaccination GMCs were 0.34, 0.61, 0.56, 2.25, 1.26, 1.05 and 0.55. One-way ANOVA demonstrated significant increases in antibody concentration (P<0.001) however with differences only seen with serotypes 14 and 18. 41 (80%) patients responded to at least one serotype. Median number of seroconverted serotypes was 3 (IQR=4). 18 (35%) had an optimal response to vaccination. There was one case of documented Pneumococcal infection following vaccination. Two-way ANOVA demonstrated a difference in response between serotypes (p<0.001) but not treatment group (p=0.36).

Summary/Conclusion: Baseline levels of pneumococcal immunity in this population are low. Although most patients are capable of seroconverting to at least one serotype, the rates of protective post-vaccination antibody concentrations remain low. Choice of therapy does not appear to influence seroconversion rates however this may be confounded by other clinical and disease related factors.

PB2155

POMALIDOMIDE PLUS DEXAMETHASONE (PD) IN THE TREATMENT OF ASIAN PATIENTS WITH RELAPSED/REFRACTORY MYELOMA (RRMM) WHO ARE PREVIOUSLY REFRACTORY TO LENALIDOMIDE-A TRIAL BY THE ASIAN MYELOMA NETWORK (AMN)

W. Chng[1,*], K. Kim[2], S.Y. Huang[3], C.-S. Chim[4], N. Takezako[5], H. Asaoku[6], H. Kimura[7], H. Kosugi[8], J. Sakamoto[9], S. Gopalakrishnan[10], Y. Wei[11], S.L. Lee[11], J. Lee[12], S.S. Yoon[13], J.-S. Kim[14], C.-K. Min[15], J.-H. Lee[16], B. Durie[17]

[1]National University Cancer Institute, Singapore, Singapore, Singapore, [2]Sungkyunkwan University, Samsung Medical Center, Seoul, Korea, Republic Of, [3]National Taiwan University, Taipei, Taiwan, Republic of China, [4]University of Hong Kong, Hong Kong, Hong Kong, [55]National Hospital Organization Disaster Medical Center, Tokyo, [66]Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital, Hiroshima, [7]Northern Fukushima Medical Center, Fukushima, [8]Ogaki Municipal Hospital, Tokyo, [9]Tokai Central Hospital, Toki, Japan, [10]Singapore General Hospital, [11]Singapore Clinical Research Institute, Singapore, Singapore, [12]Cheonnam National University Hwasun Hospital, [13]Seoul National University Hospital, [14]Yonsei University College of Medicine, Severance Hospital,, [15]Seoul St Mary's Hospital, Seoul, [16]Gachon University Gil Medical Center, Incheon, Korea, Republic Of, [17]Cedars-Sinai Medical Center, Los Angeles, United States

Background: Pomalidomide is a 2nd generation immunomodulatory drug that has been approved for the treatment of patients who progress after prior treatment with proteasome inhibitor and lenalidomide. Experience with pomalidomide in Asian patients is very limited to date.

Aims: We aim to study the efficacy and toxicity of Pd in Asian patients with relapse or refractory myeloma and if the additional of cyclophosphamide can elevate response in suboptimal responders to Pd.

Methods: We conducted a prospective Phase 2 trial of pomalidomide (4mg daily for 21 days every 4 weeks) plus dexamethasone (40mg once weekly) in myeloma patients who have relapsed after prior bortezomib and are refractory to lenalidomide Asia (NCT02158702). If there was less than a minimal response after three cycles of Pd, including progression within three cycles, cyclophosphamide 300mg/m^2 (day 1, 8, 15) can be added. This report presents data available up till the data cut-off date of 17 Jan 2018.

Results: One hundred and thirty patients have available base line information and safety data. At the cut-off date, 51% of the patients have either progressed or died. Cohort has typical disease characteristics of relapse MM. Median prior line of treatment is 3. 81% of patients experienced adverse events (AEs) of any grade (27% of episodes grade 3 or higher), with 67% of these episodes related to the study drugs. 71.5% of patients experienced serious AEs (SAEs) of any grade (72% of episodes grade 3 or higher), with 32% of these episodes related to the study drugs. Almost all grade 3 or higher events were related to cytopenias and infections. Only 1 patient experienced each of the following AEs: grade 3 peripheral neuropathy, venous thromboembolism or grade 3 renal impairment. 45 patients have died, 22 from disease progression, 10 from sepsis or pneumonia, and 4 from cardiac events.The median follow-up of the whole group is 9.2 months. The median progression free survival (PFS) (N=119) was 10.2 months. Those treated with only Pd (n=81) have a median PFS of 9.2 months. Patients on Pcd (n= 38) had a median PFS of 11.37 months. Achievement of a partial response (PR) or better was significantly associated with improved PFS. There was no significant difference in PFS by age, number of prior lines of treatment, or the presence of high-risk genetics. Overall median OS was 18.4 months. For those treated with Pd, the median OS was 18.4 months whereas it is not yet reached for those on Pcd. One hundred and two patients have data for response assessment. Fifty-three (52%) achieved a ≥PR response with 1 achieving CR and 1 stringent CR. The median duration of response was 11.7 months for those who had achieved a ≥PR. If minimal response was included, the response rates would be 87%. For those on Pd (N=64), 58% achieve ≥PR. Thirty-eight patients had cyclophosphamide added due to suboptimal response after three cycles of Pd, 16 (42%) of these achieve ≥PR. In these patients the median duration of response is 13.5 months.

Summary/Conclusion: This is the first prospective report of the efficacy and safety of Pd in Asian patients with RRMM. Our results compare favorably with previously published data from the US and Europe. In patients with suboptimal response, the addition of cyclophosphamide upgraded responses,

rendering survivals comparable to those Pd-responsive patients. A randomized Phase 3 study conducted by the AMN comparing Pcd to Pd has commenced enrolment recently.

PB2156

EVALUATION OF PULMONARY FUNCTION TESTS IN PATIENTS WITH MYELOMA REVEALS THAT PULMONARY ABNORMALITIES ARE COMMON AND ARE INDEPENDENTLY ASSOCIATED WITH WORSE OUTCOME

E. Kastritis*, D. Fotiou, G. Trakada, M. Gavriatopoulou, I. Panagiotidis, D.C. Ziogas, E. Eleutherakis-Papaiakovou, M. Roussou, M. Migkou, N. Kanellias, I. Ntanasis-Stathopoulos, L. Velentza, A. Kallianos, E. Terpos, M.A. Dimopoulos

Department of Clinical Therapeutics, National and Kapodistrian University of Athens, Athens, Greece

Background: Pre-existing pulmonary disease may affect the treatment choices, toxicity and the survival of patients with Multiple Myeloma (MM). However, data on the prognostic value of Pulmonary Function Tests (PFTs) in myeloma patients' outcome, at the time of initial assessment of newly-diagnosed patients, are scarce.
Aims: To evaluate the incidence and prognostics importance of lung function abnormalities in patients with symptomatic myeloma
Methods: We prospectively performed PFTs in 121 consecutive newly-diagnosed MM patients, before initiation of treatment and we evaluated possible associations of baseline lung function with their outcomes.

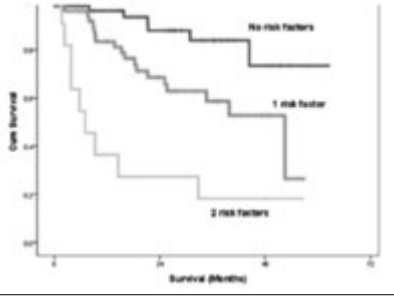


Figure 1.

Results: Pulmonary function evaluation with PFTs revealed that 54 patients (44.63%) had either obstructive or restrictive pulmonary function defects, even among patients that did not report a history of lung disease. The survival was significantly worse in patients with obstructive pulmonary defect (median OS: 32.8 months) *vs* those with restrictive (median OS: 52.5 months) or normal lung function (median not reached, 3-years survival 76%) (p=0.013). In the univariate analysis specific indices of lung function that were associated with survival included Forced Vital Capacity (FVC)(lt)(p=0.012), FVC(%)(p=0.006), Forced Expiratory Volume in 1 sec (FEV1)(lt)(p=0.018), FEV1(%)(p=0.013), Peak Expiratory Flow (PEF)(lt/min)(p=0.008), PEF(%)(p=0.005), carbon monoxide diffusion capacity corrected for hemoglobin (DL_{CO})(p=0.012), maximal expiratory (Pe)(kPa)(p=0.032) and Pe(%)(p=0.024) and inspiratory pressures (Pi)(kPa)(p=0.023) and Pi(%)(p=0.027). Other baseline factors associated with survival included ISS stage (p=0.008), hypercalcemia (p=0.064), and the presence of high risk cytogenetics (any of t(4;14), t(14;16) or del17p) (p=0.004). Abnormal PFTs were associated with early mortality (<1 year from initiation of therapy). Low PEF was strongly associated with early death (p<0.001); other indices included FVC (p=0.001), FEV1 (p=0.001) and DL_{CO} (p=0.005). Abnormal breathing pattern was also associated with early death, especially obstructive pattern (HR:8, 95%CI 2.1-30, p<0.001) and less restrictive (HR:2.2, 95%CI 0.9-9.7, p=0.068), compared to normal pattern. We identified that PEF <65% of predicted (33 months *vs* not reached at 3 years, HR:2.8, 95%CI 1.47-5.5, p=0.001) and a DL_{CO} <65% (median OS of 33 months *vs* not reached, HR:2.54, 95%CI 1.3-5.1, p=0.005) were associated with worse survival. There was a strong association of the two indices (p<0.001): 21% of patients had both, 19% only PEF <65%, 6% only DL_{CO} <65% and 53% none of the two. Multivariate analysis indicated that R-ISS-3 and the presence of either or both PEF(%)<65% and DL_{CO}<65% of predicted were the strongest prognostic factors for survival. Thus, we formulated a prognostic score encompassing myeloma-related and myeloma-independent factors that discriminates 3 groups with different survival: 3 year survival was 85% and 59% for patients with none or either of the risk factors and 18% (median survival of 7 months) if they had both (p<0.001) (see figure). Importantly, the prognostic significance of this score was independent of the age of the patients.
Summary/Conclusion: We conclude that PEF and DL_{CO} could be useful in the initial assessment of newly-diagnosed MM patients as significant predictors of survival. Respiratory screening should be included in the routine initial evaluation of myeloma patients, despite the presence or absence of respiratory symptoms or abnormal clinical respiratory examination.

PB2157

EARLY PARAPROTEIN REDUCTION AND RESPONSE TO FIRST-LINE TREATMENT IN MULTIPLE MYELOMA

M. Binder*, S.V. Rajkumar, M.Q. Lacy, M.A. Gertz, F.K. Buadi, A. Dispenzieri, Y.L. Hwa, A. Fonder, M. Hobbs, S.R. Hayman, S.R. Zeldenrust, J.A. Lust, S.J. Russell, N. Leung, P. Kapoor, R.S. Go, W.I. Gonsalves, T. Kourelis, R. Warsame, R.A. Kyle, S.K. Kumar

Division of Hematology, Mayo Clinic, Rochester, United States

Background: The introduction of novel agents including immunomodulators and proteasome inhibitors has led to an improvement in response rates and survival outcomes in patients with newly diagnosed multiple myeloma. Despite these therapeutic advances a subset of patients do not respond to first-line treatment and biomarkers are needed to identify those at risk.
Aims: To evaluate whether early paraprotein reassessments can predict response to first-line treatment and identify patients at risk for treatment failure.
Methods: We studied 419 patients with newly diagnosed multiple myeloma who were treated in routine clinical practice (n=225, training cohort) or on prospective clinical trial protocols (n=194, validation cohort) between 12/2003 and 12/2015 at Mayo Clinic. All patients had serum M-spike and free light chain (FLC) measurements performed before and after the first treatment cycle. Response to first-line treatment was evaluated using the International Myeloma Working Group Uniform Response Criteria. The biomarkers of interest were the relative decrease in M-spike and absolute FLC difference (ΔFLC). The measurements were standardized to a 28-day interval (divided by the number of days between reassessments and multiplied by 28). Three milestones were defined: Achieving neither a 25% reduction in M-spike nor a 25% reduction in ΔFLC within one treatment cycle (M0), achieving a 25% or greater reduction in at least one of the two biomarkers (M1), and achieving a 25% or greater reduction in both biomarkers (M2). Receiver operator characteristics (ROC) analysis was performed to determine the performance characteristics of these milestones in regards to predicting the best response to first-line treatment.

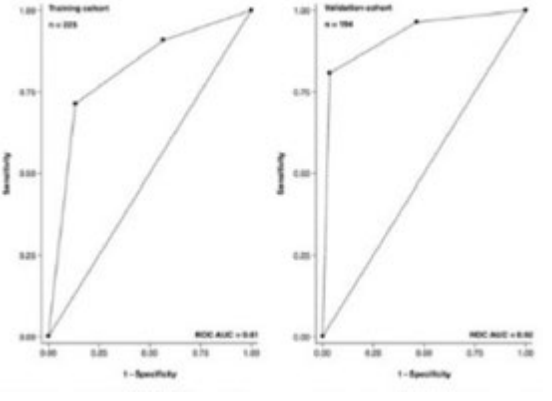


Figure 1.

Results: The median age at diagnosis in the training and the validation cohort was 66 (32-94) and 66 years (38-85), respectively. One hundred forty-nine (61%) and 103 patients (53%) were male, respectively. The three most common regimens in the training cohort were lenalidomide + dexamethasone, bortezomib + cyclophosphamide + dexamethasone, and bortezomib + lenalidomide + dexamethasone. The three most common regimens in the validation cohort were carfilzomib + thalidomide + cyclophosphamide, lenalidomide + cyclophosphamide + dexamethasone, and ixazomib + cyclophosphamide + dexamethasone. One hundred ninety-five patients in the training (87%) and 166 patients in the validation cohort (86%) achieved a partial response or better to first-line treatment (PR+). One hundred thirty-nine of the 143 patients (97%) in the training cohort and 134 of the 135 patients (99%) in validation cohort who reached M2

achieved PR+ later on. Thirty-eight of the 51 patients (75%) and 26 of the 38 patients (68%) who reached M1 achieved PR+ later on. Eighteen of the 31 patients (58%) and 6 of the 21 patients (29%) who reached M0 achieved PR+ later on. The ROC curves for the milestones in regards to achieving PR+ are shown in **Figure 1**. Reaching M2 was fairly sensitive (71%, 95% CI 64-78 and 81%, 95% CI 74-86) and highly specific (87%, 95% CI 69-96 and 96%, 95% CI 82-100) for achieving PR+, translating into a high positive predictive value (97%, 95% CI 93-99 and 99%, 95% CI 96-100).
Summary/Conclusion: Patients with newly diagnosed multiple myeloma treated in routine clinical practice and on clinical trial protocols were highly likely to respond to treatment if they experienced at least a 25% decrease in serum M-spike and ΔFLC during the first treatment cycle. Biomarker reassessment after the first treatment cycle may help to identify patients at risk for treatment failure early on.

PB2158

CHARACTERIZATION AND TREATMENT OUTCOME OF IGE MULTIPLE MYELOMA - A CASE SERIES

A. Jurczyszyn[1,*], J.J. Castillo[2], D.H. Vesole[3], J. Liu[4], I. Avivi[5], A. Waszczuk-Gajda[6], E. Lech-Maranda[7,8], M. Gentile[9], G. Mikala[10], T. Guerrero-Garcia[11], A. Suska[1], M.A. Gertz[12]

[1]Department of Hematology, Jagiellonian University Medical College, Cracow, Poland, [2]Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, [3]John Theurer Cancer Center, Hackensack University Medical Center, Hackensack, [4]Rutgers New Jersey Medical School, Newark, NJ, United States, [5]Tel Aviv Medical Center, Tel Aviv, Israel, [6]Department of Hematology, Oncology and Internal Diseases, Warsaw Medical University, [7]Institute of Hematology and Transfusion Medicine, [8]Centre of Postgraduate Medical Education, Warsaw, Poland, [9]Hematology Unit, Department of Onco-Hematology, A.O. of Cosenza, Cosenza, Italy, [10]Department of Hematology and Stem Cell Transplantation, South-Pest Central Hospital, Natl. Inst. Hematol. Infectol., Budapest, Hungary, [11]Dana-Farber Cancer Institute at St. Elisabeth`s, Boston, MA, [12]Division of Hematology, Mayo Clinic, Rochester, MN, United States

Background: IgE multiple myeloma (MM) is an uncommon hematologic malignancy, which represents <0.01% of all diagnosed MM cases. In the current literature, there are only several dozen cases described. The clinical features and treatment outcomes of IgE MM patients have not been compared to other MM isotypes.
Aims: To describe the clinical characteristics and outcomes of patients with IgE MM.
Methods: We completed a retrospective chart review using a standardized protocol from 50 clinical centers specializing in MM management and treatment. We describe the clinical presentation, diagnostic results, management and treatment outcomes of patients with IgE MM. Clinical and pathological features are presented using descriptive statistics. Response was assessed using IMWG criteria. Overall survival was estimated using the Kaplan-Meier method.

Table 1.

Patients' characteristics at diagnosis

Characteristic		n/n$_{miss}$	
Age	median (range), years	18/0	60 (35 - 85)
Gender	male, n (%)	18/0	9 (50%)
Serum IgE	median (range), IU/ml	10/8	2 158 (810 – 50 000)
Hemoglobin	median (range), g/dl	16/2	11 (8 - 14)
Glomerular filtration rate	median (range), ml/min	9/9	45 (9 – 99)
Serum calcium	median (range), g/dl	15/1	10 (9 – 12)
Albumin	median (range), g/dl	16/2	3.6 (2.6 – 4.9)
β2 microglobulin	median (range), g/dl	12/6	3.2 (1.7 – 17.8)
ISS I	n (%)	10/8	8 (80%)
ISS II			1 (10%)
ISS III			1 (10%)
Bone lesions present	n (%)	12/6	10 (83%)

n/n$_{miss}$ – number of patients with data available/missing

Results: We report data on 18 patients with IgE MM diagnosed between 1982 and 2016 from 9 centers from Hungary, Italy, Israel, Poland and the United States. The table summarizes patient characteristics at diagnosis. The most common symptoms reported were available in 14 patients, and included bone pain (n=12, 86%), constitutional symptoms (n=5, 36%), anemia (n=3, 21%), renal failure and paresthesia (n=1 each). Flow cytometry data were available in only 6 patients. CD38 and CD138 expression was positive while CD20 expression was negative in all patients. CD56 expression was positive in 3 patients (50%). Cytogenetic testing was available in 10 patients: 2 (20%) with t(11;14), 2 (20%) with del13q, 2 (20%) with del17p, and 1 (10%) with t(4;14). First line treatment included the following: 6 (38%) received an immunomodulatory drug, 10 (63%) received a proteasome inhibitor, 11 (69%) received conventional chemotherapy, and 7 (44%) subsequently underwent autologous stem cell transplantation. Response to first line treatment was available in 10 patients: 4 (40%) complete response, 3 (30%) very good partial response and 3 (30%) partial response, for an overall response rate of 100%. At a median follow-up of 8.1 years, the 5-year and 10-year overall survival (OS) rates were 63% (95% CI: 35 - 82%) and 54% (95% CI: 26 - 76%), respectively. The median OS was not reached. The cause of death was known in 3 patients and included disease progression, amyloidosis and secondary plasma cell leukemia.
Summary/Conclusion: The present case series is the largest reported to date of this rare type of MM. The clinical, pathological features and responses of patients with IgE MM appear similar to other isotypes. The OS time was longer than previously reported in this small series. However, it should be noted that most previously published cases came pre-date the availability of novel agents. Most of the patients reported in this study were exposed to novel agents, which could have impacted on the longer survival observed here.

PB2159

GOOD OUTCOMES USING ACTIVE TREATMENT WITH NOVEL AGENTS IN ELDERLY MYELOMA PATIENTS

N. Ediriwickrama*, T. Oyebamiji, S. Munisamy, S. Bowcock

Haematology Department, King's College Hospital, London, United Kingdom

Background: Despite recent improved survival with the use of novel agents in myeloma, elderly patients have benefited less than younger patients. The median survival for patients age >75 in previous studies is 24-27 months and patients who were untreated survived only 2 months median. Our unit pursues an active policy of offering anti-myeloma treatment with novel agents to all patients regardless of age, performance status (PS) and comorbidity, provided some reversibility is likely. Treatment doses are reduced for those age >75 for the first 1-2 cycles and dosage subsequently escalated if tolerated. We audited the outcome.
Aims: This study aims at evaluating the outcome of the elderly myeloma cohort who were mainly treated with novel agents
Methods: All patients age ≥75 years with symptomatic myeloma presenting to a general hospital from January 2011-June 2017 were included. The following were collected: baseline demographics, ECOG PS, age-adjusted Charlson comorbidity index (aCCI), International Staging System (ISS). Data were analysed using SPSS 20 software and Kaplan-Meier survival curves plotted.

Table 1.

Key baseline characteristics for all 52 patients (above) Responses to treatment for 50 treated patients (below)				
Age median=79 (range 75-99) (N=52)	ISS stage II=31% ISS stage III=42% (N=45)		PS ≥2=56% PS 3+4=23% (N=45)	aCCI ≥5=64% (N=50)
Response rate to treatment in all patients who started 1st and 2nd line therapies				
	1st line treatment (n=50)	Overall response rate to 1st line (CR+VGPR+PR)	2nd line treatment (n=30)	Overall response rate to 2nd line (CR+VGPR+PR)
Complete response (CR)	9(19%)	72%	2(8%)	58%
Very good partial response (VGPR)	9(19%)		2(8%)	
Partial response (PR)	16(34%)		10(42%)	
Stable disease (SD)	8(17%)		8(33%)	
Progressive disease (PD)	5(11%)		2(8%)	
Died before response assessment	3		6	

Results: 52 patients were found age 75-99 (median 79 years) PS ≥2=56%, aCCI 3-8 (median 5), ISS 3=42%. 50 (96%) patients received therapy and only 2 patients were not treated (1 declined, 1 severe dementia). Of the 50 patients who received first line therapy, 49 were treated with novel agents (bortezomib based=36(72%), thalidomide based=12(24%), lenalidomide based=1(2%)) and 1(2%) with dexamethasone only. 72% achieved a partial response (PR) or better. 8 patients died during treatment (toxicity 1, disease

progression 4, unrelated causes 3). 30 patients received second line treatment and the mean time to commence second line treatment (from the commencement of first line treatment) was 16.3 months. All patients were treated with novel agents (43% proteasome inhibitor based, 30% thalidomide based, 26% lenalidomide based). 13 patients underwent third line treatment or more. At the data cut-off date 24/11/2017 62% (32) patients were still alive. The median overall survival (OS) for the cohort was 45.4 months (95% CI 37-57). Of the 20 patients who died during the study period, the majority (16, 80%) died of disease progression while only 2 died due to treatment related toxicity (the other 2 patients died of unrelated causes). The 3 patients with advanced dementia fared poorly and did not complete treatment. Mild dementia (6) was manageable with support.
Summary/Conclusion: Our OS for this group aged ≥75 is better than that reported from the Mayo clinic at 27 months. They reported a similar study from 1999-2008 where only 39% patients received novel agents. Our more recent cohort was treated almost exclusively with novel agents and this may have contributed to better OS. Our patients were a poor prognosis group in view of their age, PS, ISS and aCCI. Treatment was therefore dose reduced from the start and this may have contributed to the small number of toxic deaths (2) and the overall tolerability of treatment with few discontinuations. Despite the dose reductions, patients gained useful responses and OS was encouraging. Although significant numbers of patients had poor prognostic factors (PS, aCCI, ISS stage) in this group, we have shown that the majority of patients tolerated treatment and had a positive response. We observed more than double the OS in our cohort compared to those published in the literature (45.4 months *vs* 22 months). In conclusion, an active treatment strategy regardless of age and comorbidity can benefit elderly patients.

PB2160

MONTELUKAST COMBINED WITH THE LIPID LOWERING DRUG GEMFIBROZIL REVERSES RESISTANCE TO COMBINATION CHEMOTHERAPY IN PATIENTS WITH MULTIPLE MYELOMA RESULTS OF PROSPECTIVE PHASE 1/2 CLINICAL TRIAL

S. O'Mara[1,2,3,*]

[1]New England Northwest Haematology, [2]Tamara Hospital, [3]Haematology, Laverty Pathology, Tamworth, Australia

Background: The asthma drug Montelukast has been shown to have many inhibitory physiological effects at an *in vitro* level that could be used to inhibit malignant processes. There is also no known maximal tolerated dose of this drug. It was thus proposed that that montelukast could be repurposed for use in malignancy. A solitary patient who had rapidly progressive lambda light chain myeloma while on Thalidomide based combination chemotherapy was treated with Montelukast in addition to Bortezomib based chemotherapy. This patient achieved a strict Complete response measured at 12 weeks despite the dire prognosis. A small phase 1 /2 longnitudinal cross over clinical trial was conducted in relapsed and refractory multiple myeloma patients to determine whether this benefit was due to montelukast and to establish whether the treatment is safe.
Aims: To determine whether montelukast combined with bortezomib based therapy is safe.To determine whether montelukast would reverse resistance to chemotherapy in high risk relapsed/refractory/resistant multiple myeloma patients.
Methods: Subjects had previously received a median of five treatments including stem cell transplant. Three of the patients had lambda light chain myeloma. The patients would have been excluded from clinical trials based on refractoriness. Patients were given CyBorD chemotherapy on a 4 week cycle. They received a minimum of two cycles. Paraproteins were measured at the end of each four week cycle. If there had been an increase in the paraprotein from the previous treatment cycle montelukast and gemfibrozil was added to their drug regimen. Five of the six patients had enough measurements and were eligible for review. The paraprotein levels were again measured following each 4 week cycle following montelukast administration. The patients were seen weekly to determine toxicity. The response in paraprotein was compared using a paired t-test as each patient acted as their own control.
Results: The patients had a median of five previous forms of therapy for myeloma including stem cell transplant. The increase in paraprotein on CyBorD was statically significant prior to the addition of montelukast. Three patients received two cycles of montelukast/ CyBorD. Two patients received a solitary cycle. There was no toxicity up to a dose level of montelukast 60mg a day, Gemfibrozil 600mg per day. Addition of these two agents lead to an improvement in the response to combination chemotherapy in all five patients. p<0.01 Student's T test. Four of the five patients achieved a partial response including a VGPR following the addition of montelukast and gemfibrozil. The other had a partial response in bone marrow alone. The five patients are alive at a median of 15 months post treatment despite having highly advanced and refractory disease. Median progression free survival has not been reached and is over 8 months.
Summary/Conclusion: The addition of montelukast and gemfibrozil to standard chemotherapy leads to deeper responses in multiple myeloma patients resistant/refractory to chemotherapy including patients that have progressed following stem cell transplant. The treatment appears extremely effective, is safe and has few to no side effects.

PB2161

AUTOLOGOUS TRANSPLANTATION WITH REDUCED PRE-TRANSPLANT CONDITIONING IN PATIENTS WITH MULTIPLE MYELOMA AT A HIGHER AGE - TOXICITY VERSUS EFFICACY

A. Jungova[1,*], E. Havlova[2], T. Zabranska[1], D. Lysak[1], P. Jindra[1]

[1]Hemato-oncology department, [2]Institute of Biochemistry and Hematolog, Charles University Hospital Pilsen, Pilsen, Czech Republic

Background: Multiple myeloma (MM) is a relatively usual hemato-oncological disease the incidence of which has been increasing. Despite a significant progress in drug development, autologous transplantation (Tx) of peripheral hematopoietic stem cells remains the fundamental pillar of the treatment. The standard administration consists in a dose of melphalan 200mg/m^2, and this regimen is preferably used with younger patients due to the toxicity risk of the preparation regimen. The dose of melphalan can be reduced to 100-180mg/m^2 in older patients depending on the overall patient's condition.
Aims: The objective of our work was to compare the length of hospitalization, event. infection and GIT complications and also the influence on PFS and OS in a group divided by age.
Methods: Retrospective analysis in 179 patients with MM transplanted consecutively in the Department of Hematology and Oncology, University Hospital in Pilsen, between 2013-2017. The data evaluation was performed after the first Tx. In all patients, melphalan was only administered as a part of conditioning.

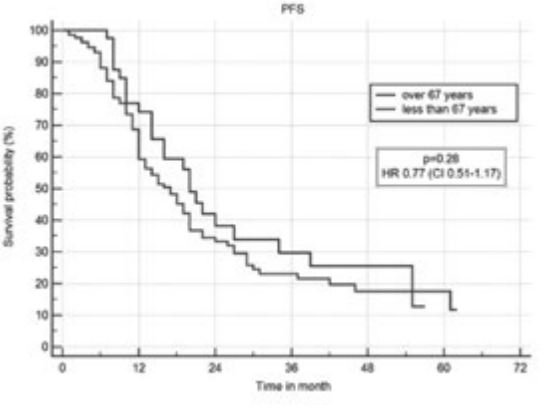


Figure 1.

Results: The group of 179 patients was divided by age. In the group of patients >67 years of age, there were a total of 47 patients with median age 70 (67-75), while the group of patients <67 years of age at the time of Tx (B) was represented by 133 patients with median age 59 (41-66). Besides age, there was no statistical difference between the basic parameters. In group A, the average dose of melphalan was 154mg/m^2, in group B, it was 188mg/m^2. The total hospitalization time showed median of 15 days (14-28) in group A and 16 days (13-63) in group B. The engraftment was the same in both groups. Gastrointestinal toxicity was completely comparable in both groups, the median showed grade I (0-IV), in group A, there was no more serious complication than grade III, in group B there was grade III+IV just in 2%. Infective complications in group A were relatively rare, the median was grade 0 (0-II) with no occurrence of more serious infective complications, in group B, just 1% of very serious infections (gr. III+IV), median of grade I (0-IV). Our analysis did not even show any apparent influence of the lower dose of melphalan on PFS or OS. Median PFS in group A was 20 months and 15 months in group B (p=0.28, HR 0.77, CI 0.51-1.17). Median OS in group A was not reached and in group B, it was 40 months (p=0.23, HR 0.66, CI 0.36-1.22).

Summary/Conclusion: Autologous transplantation is an integral part of the treatment in patients having the diagnosis of multiple myeloma. So far, it has been standardly used in younger patients, however, our data show that even older patients may be transplant eligible and that it is possible to get the same results as in younger patients including PFS and OS while the toxicity risk remains the same. Take message home: Autologous Tx with a reduced dose in patients up to 70 years of age is more convenient than regimens based on 'new drugs'.

PB2162

THE PROSPECTIVE INTERSECTORAL NATIONAL COHORT STUDY MYRIAM TO STUDY CHARACTERISTICS, TREATMENT AND OUTCOME OF PATIENTS WITH MULTIPLE MYELOMA IN GERMANY

W. Knauf[1,*], M. Engelhardt[2], C. Losem[3], J. Lipke[4], C. Kasper[5], L. Houet[6], C. Vannier[6], M. Jänicke[6], N. Marschner[7], T. Dechow[8], H. Einsele[9]

[1]Centrum für Hämatologie und Onkologie Bethanien, Frankfurt a.M., [2]Universitätsklinikum Freiburg, Freiburg i. Br., [3]MVZ für Onkologie und Hämatologie im Rhein-Kreis Neuss, Neuss, [4]Gemeinschaftspraxis für Hämatologie und Onkologie, Dortmund, [5]Onkologische Schwerpunktpraxis Hof - Medizinisches Versorgungszentrum, Hof, [6]iOMEDICO, [7]Praxis für interdisziplinäre Onkologie & Hämatologie, Freiburg i. Br., [8]Gemeinschaftspraxis für Hämatologie und Onkologie, Ravensburg, [9]Universitätsklinikum Würzburg, Würzburg, Germany

Background: In Germany, multiple myeloma (MM) represents the third most common hematologic malignancy and is still considered incurable with standard treatment regimens. Therapeutic options have markedly increased over the last decade and a number of new treatments that showed promising results in clinical trials may be approved in the near future. However, few so called "real world" data is available regarding the implementation of these new treatments into routine care and their impact on the prognosis of patients with MM treated outside of clinical trials.
Aims: The purpose of MYRIAM is to create a prospective, intersectoral, national, longitudinal, multicenter cohort study to document patient and disease characteristics, treatments, course of disease, including clinical and patient-reported outcomes and consent to perform translational research.
Methods: Between 2017 and 2021 about 2.000 patients with MM giving informed consent at the start of their first or second systemic treatment will be prospectively recruited in 150 different sites, including university hospitals, community hospitals and outpatient clinics (office-based practices) and will be followed until death or for a maximum of 5 years. Data will be collected in electronic case report forms with implemented completeness and plausibility checks, regularly examined by data managers and randomly monitored. Patient-reported outcomes will be assessed at the time of recruitment, every 3 months for the first 24 months and every 6 months thereafter, for a maximum of 5 years altogether using the EORTC-QLQ-C30+MY20 and the Brief Pain Inventory. Patients will be asked to give informed consent for future translational research of their unused tumor samples. Annual interim analysis will be performed. The study was approved by local ethics committees and is registered under clinicaltrials.gov (identifier: NCT03308474).
Results: The first patient was recruited in September 2017. At the time of abstract submission (February 2018), 138 patients had been recruited in 43 sites, and a further 63 sites had already agreed to participate. Data cut for the first planned interim analysis will be April 30th 2018. First results on patient and disease characteristics and initial treatments will be presented.
Summary/Conclusion: MYRIAM will for the first time present prospective, intersectoral, longitudinal data on patient characteristics, treatment and outcome of MM patients across all health care sectors in Germany. Data will shed light to the current state of care outside of clinical trails, allow identification of unmet medical needs that can be used to create recommendations to improve care, and will facilitate generation of hypothesis for clinical trials to improve current best practice.

PB2163

URINARY RETINAL BINDING PROTEIN IN UNTREATED SYSTEMIC IMMUNOGLOBULIN LIGHT CHAIN AMYLOIDOSIS AT BASELINE

T. Rezk[1,*], J. Gilbertson[1], S. Packer[2], R. Salota[3], H. Lachmann[1], S. Walsh[4], P. Hawkins[1], J. Gillmore[1]

[1]UCL Division of Medicine, National Amyloidosis Centre, London, [2]BBI Solutions, BBI, Kent, [3]Epsom and St Helier University Hospitals, Biochemistry Departement, Carshalton, [4]UCL Division of Medicine, Centre for Nephrology, London, United Kingdom

Background: Renal involvement causing progressive proteinuric chronic kidney disease is present in 70% of patients with systemic AL amyloidosis at diagnosis. Patient and renal survival are dependent upon both successful free light chain suppression and degree of renal dysfunction at the time of diagnosis[1]. Urinary excretion of retinal binding protein (RBP), an indicator of renal tubular injury, has been used in detecting early renal involvement in multiple myeloma[2,3] Free light chains (FLC) are known to be toxic to the proximal tubule which contributes to a range of pathologies including cast nephropathy and Fanconi's syndrome.[4] Furthermore, treatment with systemic chemotherapy (often proteasome inhibitor based) to suppress FLC production can be associated with acute kidney injury (AKI).
Aims: We hypothesized that a significant proportion of patients with newly diagnosed renal AL amyloidosis have proximal tubular dysfunction and sought to determine whether this may predict renal outcomes.
Methods: All patients (n=158) who attended the National Amyloidosis Centre (NAC) from September 2016 to October 2017 and were enrolled into 'ALchemy', the centre's prospective observational study for newly diagnosed patients with AL amyloidosis, underwent measurement of urinary RBP (uRBP) excretion in conjunction with routine clinical, biochemical and scintigraphy assessments.
Results: Median age was 69 yrs (44-90) with median serum creatinine of 89 umol/L and eGFR of 67 ml/min/1.73m^2. Median urinary protein creatinine ratio (uPCR) was 311 mg/mmol. Median uRBP was 285µg/L. There was a significant correlation between uRBP and serum creatinine (Pearson correlation p<0.0001, R 0.6440) and uRBP and uPCR (Pearson correlation p<0.0001, R 0.5247) with a weaker correlation with uACR (Pearson correlation p<0.0001, R 0.3039). Despite this however, a markedly elevated uRBP/Creatinine (>1000mg/mmol) was detected in 35 patients including 9 who had an eGFR >30ml/min/1.7m^2, and 8 who had uPCR <300mg/mmol and ACR <50mg/mmol. There was a strong correlation between altered fractional excretion of both phosphate and urate with uRBP (Pearson correlation, p<0.0001, R 0.6181).
Summary/Conclusion: Although there is a significant correlation between low molecular weight protein excretion indicating proximal tubular dysfunction with both serum creatinine and uPCR, there is a cohort of patients with both preserved renal excretory function and absence of significant proteinuria who have elevated uRBP excretion,. The association with fractional excretion of both phosphate and urate demonstrate that elevated uRBP excretion in patients with newly diagnosed untreated renal AL amyloidosis specifically indicates proximal tubular dysfunction. The analysis of whether uRBP predicts renal outcomes is underway and may be of relevance to other renal pathological lesions.

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PB2164

FIRST-LINE THERAPY WITH BENDAMUSTINE/PREDNISONE/BORTEZOMIB (BPV) FOR NON-TRANSPLANT ELIGIBLE SYMPTOMATIC MULTIPLE MYELOMA PATIENTS

W. Knauf[1,*], G. Dingeldein[2], R. Schlag[3], M. Welslau[4], C. Kunz[5], T. Moehler[6], C. Habermehl[5], H. Goldschmidt[7], M.S. Raab[7], T.G. BPV[1]

[1]Onkologische Gemeinschaftspraxis, Frankfurt, [2]Onkologische Praxis, Darmstadt, [3]Hämatologisch-onkologische Schwerpunktpraxis, Würzburg, [4]Onkologische Praxis, Aschaffenburg, [5]Dept. of Biostatistics, German Cancer Research Center, Heidelberg, [6]Medical Science, IQVIA, Neu-Isenburg, [7]Dept. of Medicine V (Hem/Onc), University Hospital, Heidelberg, Germany

Background: Elderly patients with multiple myeloma (MM) frequently are ineligible for intensive therapies including autologous stem cell transplantation and require an individualized therapy.
Aims: This study investigated efficacy and safety of the 3-drug-combination bendamustine/prednisone/bortezomib (BPV regimen) as first-line therapy for elderly patients with multiple myeloma (MM).

Methods: Elderly patients with symptomatic MM not eligible for intensive therapy and autologous stem cell transplantation were enrolled in this phase IIb study for first-line treatment with the bendamustine/prednisone/bortezomib regimen. All 46 patients were included in the safety and intention-to-treat (ITT) analysis for the secondary objectives. 34 patients were eligible for the primary efficacy analysis of overall response rate (ORR) defined as CR and PR in the per protocol (PP) population, which consisted of all patients who completed at least 3 cycles of BPV therapy and were evaluable for response. For evidence of superiority compared to historical MPV (melphalan, prednisone, bortezomib) data we postulated the lower confidence boundary for the primary endpoint of ORR to be at least 67%. The study was conducted as a multicenter, single-arm, open-label clinical trial. The treatment regimen consisted of bendamustine 90mg/m^2 iv: d1, 2; prednisone 60mg/m^2 po: d1-4; bortezomib 1.3mg/m^2 iv: cycle 1 [d1-d42]): d1, 4, 8, 11, 22, 25, 29, 32; cycle 2-9 [d1-28]: d1, 8, 11, 22. Treatment was scheduled for 9 cycles with 42 d for cycle 1 and 28 d for cycle 2-9.
Results: From November 2014 to October 2016, 46 patients were included into the trial. Patients had the following key baseline characteristics: median age 76 years, female: 61%, median glomerulofiltration rate (GFR) of 64ml/min. The ORR was 76.5% with a lower 95% confidence bound of 62.7%. The clinical benefit rate (CBR) including MR was 91.2%. 19 patients with renal impairment at baseline had a median GFR of 42.6 ml/min [range 12.9-49.7]. A renal response (defined as improvement of renal function by IMWG criteria, Dimopoulos *et al.* J Clin Oncol 2010) was observed in 11 pts.. 6 pts. achieved a complete recovery of the renal function. The BPV regimen was well tolerated. 33 of 46 pts. (71.7%) experienced AEs of CTC grade 3 and 4. The most common grade 3/4 AEs were neutropenia (26%), infections (26%), and thrombocytopenia (19.5%). Pneumonia was documented in 4 patients. Cardiac grade 3 and 4 complications were atrial fibrillation (3 events) and hypertension (2 events). No new safety signals for the study drugs were observed. 46% of patients developed at least 1 SAE.
Summary/Conclusion: BPV may serve as a well tolerated first-line regimen for transplant ineligible elderly MM patients with an encouraging ORR of 76%. BPV can be also considered, if a fast renal response is required in patients with myeloma induced renal impairment. However, the study did not provide statistical evidence of superiority compared to historical MPV response data.

PB2165

LENALIDOMIDE MAINTENANCE CHEMOTHERAPY; AN ANALYSIS OF REAL WORLD SURVIVAL DATA IN MULTIPLE MYELOMA PATIENTS TREATED WITH AUTOLOGOUS STEM CELL TRANSPLANT AND BORTEZOMIB-BASED INDUCTION

H. Cherniawsky[1,*], I. Sandhu[2], M. Chu[2], J. Hewitt[2], I. Ismail[2], A. Belch[2], L. Pilarski[2], T. Nikitina[2], C. Venner[2]

[1]Internal Medicine, University of Alberta, [2]Cross Cancer Institute, Edmonton, Canada

Background: Multiple myeloma (MM) is an incurable plasma cell malignancy. The primary goal of therapy is to obtain a deep and durable response to improve disease control and survival. In the post-transplant setting, large phase 3 trials suggest daily low-dose lenalidomide has a positive impact on progression free survival (PFS) and overall survival (OS).
Aims: We sought to evaluate the impact of lenalidomide maintenance in a real world setting in patients treated with bortezomib based induction chemotherapy and autologous stem cell transplant (ASCT) at our centre.
Methods: We evaluated all patients with MM from the Cross Cancer Institute who received frontline bortezomib-based induction chemotherapy and ASCT between December 2004 and January 2016 to ensure a minimum of 2 years follow-up. Patients were analyzed as receiving or not receiving maintenance based on intention-to-treat. Maintenance therapy involved lenalidomide monotherapy or in combination with bortezomib, an option at our center for patients with high risk cytogenetics. OS was measured from treatment initiation to death or last follow-up. PFS was measured from treatment initiation to relapse, death or last follow-up. Maximal response to treatment was assessed according to the International Myeloma Working Group criteria with an additional endpoint of near complete response (nCR) where CR was not confirmed by immunofixation or bone marrow biopsy.
Results: 207 patients were included. 131 received lenalidomide based maintenance and 76 did not. Median ISS score was 2 in both groups (p=0.14). A mean of 28 cycles of lenalidomide (0.5-89) was given. 18% patients discontinued therapy prior to relapse. In the maintenance arm, 96% obtained a VGPR or greater compared to 76% in the no maintenance group (p<0.01). The median follow-up is 70 months in the non-maintenance cohort and 47 months in the maintenance cohort likely due to more recent adoption of lenalidomide use and therefore less time for follow-up in this cohort. The estimated 4-year OS was 87.1% in the maintenance group and 70.7% in the non-maintenance group (p<0.01, figure 1a). To date 105 patients have relapsed (47 in the maintenance and 58 in the non-maintenance cohort). The estimated 4-year PFS was 61.5% in the maintenance group and 34.6% in the non-maintenance group (p<0.01, figure 1b). The incidence of thromboembolism (TE) (3.1% *vs* 1.3% (p=0.43)) and second primary malignancies (SPMs) (2.3% *vs* 6.6% (p=0.12)) were low in the maintenance and non-maintenance groups respectively. 37.4% of patients in the maintenance group required dose adjustments. When first adopted, lenalidomide maintenance was given in a 28/28 day schedule and was pursued in 92 patients. Of these, 74.0% received all cycles at the intended dose. The current standard is a 21/28 day dosing schedule and was used in 38 patients. Of these, 77.6% received all cycles at the intended dose. In all, 18.3% of patients discontinued medication for reasons other than relapse.

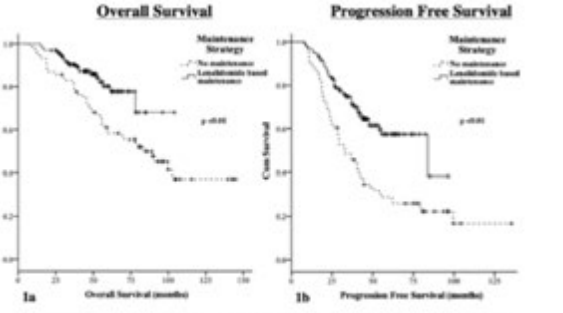


Figure 1. OS (1a), PFS (1b) in patients treated with and without lenalidomide based maintenance following bortezomib based induction and autologous stem cell transplant.

Figure 1.

Summary/Conclusion: Our data illustrates the positive impact of lenalidomide on depth of response, PFS and OS. Treatment was well tolerated with the majority of patients receiving the intended dose regimen. Few patients discontinued treatment prior to relapse. The rates of TEs and SPMs and consistent with previous reports. This data supports the ongoing use of lenalidomide maintenance in myeloma patients post-ASCT as a standard of care.

PB2166

CARFILZOMIB AND DEXAMETHASONE VERSUS 8 CYCLES OF BORTEZOMIB AND DEXAMETHASONE: AN INDIRECT COMPARISON AND EXPLORATORY ANALYSIS OF THE EFFICACY AND SAFETY OF THE RANDOMIZED, PHASE 3 ENDEAVOR TRIAL

K. Weisel[1,*], I. Majer[2], L. DeCosta[3], A. Oriol[4], H. Goldschmidt[5], H. Ludwig[6], M. Campioni[2], Z. Szabo[7], M. Dimopoulos[8]

[1]Medical Clinic II, Department of. Hematology, Oncology, Immunology, Rheumatology and Pulmonology, Tuebingen, Germany, [2]Global Health Economics, Amgen (Europe) GmbH, Zug, Switzerland, [3]Global Biostatistical Science, Amgen Ltd., Uxbridge, United Kingdom, [4]Institut Català d'Oncologia, Institut Josep Carreras, Hospital Germans Trias i Pujol, Barcelona, Spain, [5]Department of Internal Medicine V, University Hospital Heidelberg, and National Center for Tumor Diseases (NCT), Heidelberg, Germany, [6]Wilhelminen Cancer Research Institute, Wilhelminenspital, Vienna, Austria, [7]Amgen (Europe) GmbH, Zug, Switzerland, [8]School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

Background: In patients with relapsed and/or refractory multiple myeloma, carfilzomib and dexamethasone (Kd56) was superior to bortezomib and dexamethasone (Vd) in the randomized, phase 3 ENDEAVOR trial for progression-free survival (PFS) (hazard ratio [HR] 0.53; 95% confidence interval [CI] 0.44–0.65; P<0.0001). In ENDEAVOR, both Kd56 and Vd were planned to be administered until disease progression, while in other clinical trials, e.g., the randomized, phase 3 CASTOR trial comparing Vd with or without daratumumab, Vd was administered for up to 8 cycles (6 months) according to its EU label.
Aims: To estimate how efficacy and safety results for Kd56 *vs* Vd would have differed if Vd had been given for up to 8 cycles only.
Methods: For the efficacy analysis, published PFS data from the ENDEAVOR (Dimopoulos *et al.*, *Lancet Oncol* 2017) and CASTOR (Spencer *et al.*, ASH 2017) trials were used in three steps. First, ENDEAVOR Vd patients were matched to average characteristics of CASTOR Vd patients using matching-adjusted indirect comparison methodology. Then, a piecewise Cox regression model was fitted to the matched ENDEAVOR Vd data and

re-constructed virtual patient-level CASTOR Vd data that allowed assessing the increased PFS risk due to stopping Vd treatment after 8 cycles *vs* continuing Vd treatment beyond 8 cycles till progression. Finally, the PFS HR for Kd56 *vs* Vd from ENDEAVOR was adjusted according to the increased PFS risk for the period beyond 8 cycles. The presented modeling approach has been previously accepted by the National Institute for Health and Care Excellence in the UK (Technology Appraisal Guidance TA457, 2017). For the safety analyses, using most recent data on file, only adverse events (AEs) that occurred during the first 8 cycles plus 30 days of follow-up after the last dose were considered for patients in the Vd arm of the ENDEAVOR trial. Besides the incidence of treatment-emergent adverse events (TEAEs) of grade 3 or more (Gr3+) for Vd 8 cycles, Kd56/Vd 8 cycles exposure-adjusted risk ratios were estimated for Gr3+ TEAEs.

Results: During the first 8 cycles, the risk reduction in PFS for Kd56 *vs* Vd 8 cycles was equal to that estimated for Kd56 *vs* Vd in ENDEAVOR. For Vd patients, the increase in PFS risk associated with stopping Vd beyond 8 cycles was estimated to be 32% (HR: 1.32, 95% CI 0.89-1.96, P=0.17). This corresponds to a 60% risk reduction in PFS for Kd56 *vs* Vd (HR: 0.40; 95% CI 0.26-0.63; P<0.0001) beyond 8 cycles (Figure). The frequency of Gr3+ TEAEs was 81.9% for Kd56, 71.1% for Vd, and was estimated to be 64.5% for Vd 8 cycles. Incidence of Gr3+ peripheral neuropathy was 2.4%, 9.6%, and 8.6% for Kd56, Vd, and Vd 8 cycles, respectively. The exposure-adjusted risk ratios of Kd56 *vs* Vd 8 cycles were estimated to be 0.61 (95% CI 0.53-0.71) for all Gr3+ TEAEs and 0.08 (95% CI 0.04-0.17) for Gr3+ peripheral neuropathy.

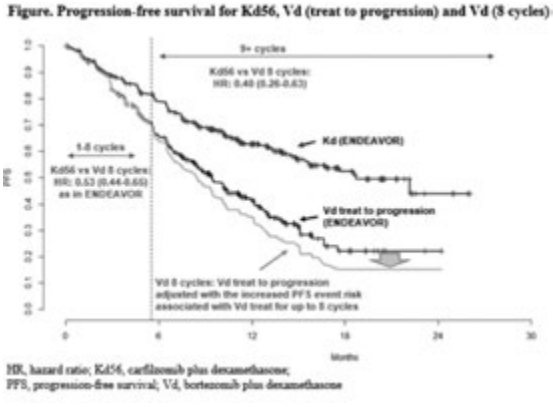


Figure 1.

Summary/Conclusion: Results from the matched-efficacy analysis indicated that the duration of Vd treatment has a significant impact. If Vd had been given for 8 cycles, the relative decrease in PFS risk with Kd56 would have been larger than that observed in ENDEAVOR where Vd treatment was continued beyond 8 cycles till progression. The approach used in this study adjusts for differences in trial design such as Vd treatment duration, allows to reduce bias, and is a robust methodology. In contrast, indirect treatment comparisons including network meta-analyses that do not adjust for differences in trial design and/or patient populations are susceptible to greater bias.

PB2167

MYC REARRANGEMENT IN COHORT OF NEWLY DIAGNOSED SLOVENIAN MM PATIENTS

H. Podgornik*, N. Zima, J. Nussdorfer, H. Čibej, A. Doplihar Kebe, S. Zver

Department of Haematology, University Medical Centre, Ljubljana, Slovenia

Background: *MYC* rearrangements (*MYC*r) are late progression events in multiple myeloma (MM). Reported frequency differs considerably among studies ranging from 15% and up to 50% of MM patients. Increased *c-MYC* expression was found to be involved in MM progression, is associated with it's aggressive nature, short remissions and overall survival.

Aims: We determined the frequency and type of *MYCr* in newly diagnosed MM patients in Slovenija and analysed correlation with concomitant cytogenetic aberrations as well as with some clinical parameters.

Methods: 69 patients were included, aged 45 to 93 (median 68) years. Observation time was 0-8.3 years with (median 1.1 year). *MYC* was analysed in all but one patient before the induction MM treatment. *MYC* rearrangement was determined on CD-138 isolated plasma cells by FISH which was also used to detect other MM specific cytogenetic abnormalities: del(13q), del(17p), *IGH* rearrangements, amp(1q), del(1p), and hyperdiploidy. Poseidon DNA probe MYC TC with established cut-off value 2.5% was used not only to detect *MYC*r but trough the co-localization patterns of signals also to distinguish different breakpoints in *MYC* gene. Differences between categorical variables were determined by Fisher's exact test using Medcalc software.

Results: *MYCr* was found in 17% (12/69) of patients. The clone size ranged from 5 - 99% of plasma cells. We observed all three breakpoints: distal and proximal to *MYC* gene and in the *MYC* gene. Dominant breakage variant in 7/12 pts was distal. The later was commonly observed as a sole subtype as well as a prevailing clone. Besides typical signals also proximal and distal breakage accompanied by deletion of the corresponding chromosomal region was observed. Considering other routinely determined chromosomal rearrangements, *MYC* was more frequently rearranged in patients with del(13q) (P=0.004), amp(1q) (P=0.035), and hyperdiploidy (P=0.012). *MYCr* was found in 2/12 patients as a sole abnormality. MYCr was accompanied with secondary cytogenetic changes In 9/10 patients and only in 1/10 patients with primary change (hyperdiploidy). The difference between groups was significant (P=0.0011). When we focused on cytogenetic risk groups 25% (7/28) of patients in high risk group was MYC positive, while only 19% (4/21) and 6% (1/17) of patients were positive in standard and low risk group respectively. Difference among groups was however, not statistically significant. For 52 patients clinical data were available. We couldn't find difference between *MYC* positive and negative groups for ISS score and due to the short observation time neither for non-responsiveness to induction MM treatment and survival.

Summary/Conclusion: The observed frequency of *MYCr* in our cohort of almost exclusively newly diagnosed MM patients is consistent with previous literature reports. We observed strong correlation between *MYCr* and secondary chromosomal aberrations as well as with overall high risk cytogenetic group. This confirms not only that *MYCr* itself is a secondary genetic event but also the need for its early recognition and more effective/specific therapeutic approaches in *MYCr* patients. The short observation time is a limitation of the current analysis but will enable us to obtain valuable data in the future work. Namely, majority of MM patients were treated with the same therapeutic regimen.

PB2168

SUPERVISED AND HOME-BASED EXERCISE IN PATIENTS NEWLY DIAGNOSED WITH MULTIPLE MYELOMA – A RANDOMIZED CONTROLLED FEASIBILITY STUDY

R.F. Larsen[1,*], M. Jarden[2], L.R. Minet[3], U.C. Frølund[4], N. Abildgaard[5]

[1]Department of physiotherapy and occupational theray, Zealand University Hospital, Roskilde, [2]Department of Hematology Finsen Center, Copenhagen University Hospital Rigshospitalet, Copenhagen, [3]Department of Rehabilitation, Odense University Hospital, Odense, [4]Department of Hematology, Zealand University Hospital, Roskilde, [5]Department of Hematology, Odense University Hospital, Odense, Denmark

Background: Exercise is considered to be feasible, safe and beneficial for patients with haematological cancer. However, patients with Multiple Myeloma (MM) are not only underrepresented in exercise studies (3 RCT, 1 pilot), the studies also primarily include younger patients with MM prior to, undergoing and after autologous stem cell transplantation. Due to the bone disease in MM, exercise can be challenging. Our ongoing RCT examines the effect of an individualized, early initiated exercise intervention (EI) in newly diagnosed patients with MM, irrespective of age.

Aims: The aim of the present study is to report the interim analysis of feasibility and safety of the EI.

Methods: This is a two-center RCT with blinded outcome assessors. Informed consent is obtained. Exclusion criteria include radiology assessed risk of fractures according to scoring system. Baseline tests are carried out within 4 days after starting anti-myeloma treatment, followed by randomization to control group (CG) or intervention group (IG). Randomization is stratified according to planned treatment (high dose chemotherapy with autologous stem cell transplantation or non-intensive treatment), WHO performance status (0-1 or ≥2) and study site. The EI starts within one week and is a 10-week supervised and home-based exercise program comprising aerobic and strengthening exercises and physical activity in accordance with international guidelines. Both groups (CG and IG) receive written information about physical activity and transfer techniques (usual care). Main objectives are muscle strength (knee extensor strength and grip strength), physical function (6 minutes walk test and 30 seconds sit to stand test) and physical activity (monitored by use of ActivPal 3 micro). Feasibility outcome measures were eligibility, acceptance and drop-out rates. Further, adherence, tolerability and safety (adverse events (AEs)) of the supervised exercise sessions (SES) were obtained.

Results: Of 49 patients screened, 40 (82%) were eligible for inclusion; 30 (75%) accepted participation and were randomized (IG n=17, CG n=13). The participants (n=30) age in years (median (range)) was 69 (38-90). Of the 17 IG participants, 14 (82%) started intervention. Withdrawals prior to start of EI (n=3) was caused by no surplus energy (n=1), wish for treatment closer to home (n=1), and sudden impairment (n=1). Two withdrew during the EI period due to stroke (n=1) and because exercise was found too hard (n=1). One CG participant dropped out after receiving usual care because of no surplus energy. In the total period from baseline to end of the intervention 12 participants (71%) completed the intervention, and in the total period five IG participants dropped out (29%). Of those starting EI (n=14) the adherence to SES was 94%, and the tolerability was 98%; two patients discontinued one SES each due to non-serious AEs (symptoms of pain, and dyspnea and dizziness). No serious AEs were reported, and importantly, no patients had pathological fractures during the EI.
Summary/Conclusion: Early initiated exercise in newly diagnosed patients with MM, regardless of age and intensity of anti-myeloma treatment, is feasible, safe and tolerable. Early initiated exercise may be important in preventing physical decline during treatment for MM.

PB2169

DETECTION OF ASYMPTOMATIC FEMORAL HEAD AVASCULAR NECROSIS ON ROUTINE WHOLE BODY MRI IN PATIENTS WITH MULTIPLE MYELOMA PROVIDES OPPORTUNITIES FOR JOINT PRESERVATION

P. Sriskandarajah[1,2,*], N. Ahmed[3], C. Burd[3], V. Shah[2,4], A. Riddell[3], K. Boyd[2], M. Kaiser[4], C. Messiou[3]

[1]Cancer Therapeutics, Institute of Cancer Research, [2]Haematology, [3]Radiology, Royal Marsden Hospital, [4]Molecular Pathology, Institute of Cancer Research, Sutton, United Kingdom

Background: Whole body MRI is emerging as the most sensitive imaging technique for patients with multiple myeloma (MM) and as such is recommended by the IMWG for all patients with an apparent diagnosis of asymptomatic myeloma. In the UK whole body MRI is first line imaging for all patients with a suspected diagnosis of myeloma, follow up, response assessments and restaging at relapse. MRI is also the most sensitive imaging test for detection of avascular necrosis (AVN) of the femoral heads, a complication of corticosteroid use in MM patients.
Aims: To assess the prevalence and outcome of MM patients with features of AVN on whole body MRI.
Methods: We conducted a retrospective analysis of all whole body MRIs between1st January 2010 to 1st May 2017 for MM patients at the Royal Marsden Hospital. All scans were reported by consultant radiologists with a special interest in myeloma for whom review for skeletal complications of myeloma is routine practice. These radiological reports were searched for the following terms "AVN" and "avascular necrosis". Images of the patients with AVN were interrogated and correlating clinical information documented.
Results: Out of 650 whole body MRI scans performed in 226 patients, 15 patients (6.6%)(10 male, 5 female, median age 66.5 years [range 31-74]) were identified to have typical MRI features of AVN which included subchondral serpiginous lines and oedema. 2/15 had femoral head collapse and 4/15 had bilateral AVN. 10/15 patients had active disease according to IMWG criteria and 8/15 were being treated with steroid-combination regimens. Indications for scanning included disease assessment post-induction therapy (6/15), biochemical progression (5/15) and bony pains at other sites (1/15). Only 3/15 (20%) patients had reported hip/pelvic pain at the time of AVN detection. Patients with AVN had received a median cumulative dexamethasone dose of 540mg (range 80-5040mg), while 3/15 patients had had prior femoral radiotherapy. None of the patients had known diabetes. All patients were receiving bisphosphonates on a monthly to 2-monthly basis. During surveillance, 8/15 patients had scans demonstrating progressive AVN, including worsening femoral head oedema and increasing effusions. Of these, 4/8 reported worsening symptoms in the affected hip(s) and subsequently required surgical intervention with total hip replacements. All other patients were managed conservatively and are continuing to be reviewed by the orthopaedic team.
Summary/Conclusion: In keeping with previous published data which has shown a 9% incidence of AVN, the incidence of AVN in MM patients imaged with routine whole body MRI was low (6.6%). However we have shown that asymptomatic AVN can be detected early on whole body MRI before femoral head collapse. Early orthopaedic referral provides an opportunity for interventions to preserve the joint before femoral head collapse and the requirement for total hip replacement. Additionally, when early signs of AVN are identified, rapid de-escalation of steroid dosing should be considered once the patient's myeloma is well controlled. We recommend that whole body MRI scans in patients with MM should always be interrogated for early signs of AVN.

PB2170

ASSOCIATION OF CLINICAL RESPONSE WITH SURVIVAL AND QUALITY OF LIFE (QOL) AFTER CARFILZOMIB AND DEXAMETHASONE (KD56) TREATMENT IN THE PHASE 3 ENDEAVOR TRIAL

D. Siegel[1,*], H. Ludwig[2], P. Moreau[3], M.-V. Mateos[4], R. Niesvizky[5], W.J. Chng[6], K. Mezzi[7], Z. Yang[7], S. Panjabi[7], M. Dimopoulos[8]

[1]John Theurer Cancer Center, Hackensack University Medical Center, Hackensack, United States, [2]Wilhelminen Cancer Research Institute, Wilhelminenspital, Vienna, Austria, [3]University Hospital Hotel-Dieu, Nantes, France, [4]Hematology Service, University Hospital Salamanca, Salamanca, Spain, [5]Center for Myeloma, New York Presbyterian Hospital-Weill Cornell Medical Center, New York, United States, [6]National University of Singapore, Singapore, Singapore, [7]Amgen Inc., Thousand Oaks, United States, [8]University Athens School of Medicine, Athens, Greece

Background: ENDEAVOR demonstrated superior response rates, depth of response, PFS, and OS with Kd56 *vs* Vd (bortezomib-dexamethasone).
Aims: Here, we assessed post-hoc the association between response and clinical outcomes/QoL.
Methods: PFS and OS were assessed for responders (≥partial response [PR]) *vs* non-responders (<PR; a 2-mo Simon-Makuch landmark analysis was conducted; Kaplan-Meier [KM] estimates conditioned at the landmark) and by response status (KM estimates; medians not adjusted for responder bias). QoL was assessed by the EORTC Global Health Status/QoL (GHS/QoL) subscale with higher scores indicating better QoL. Changes from baseline in GHS/QoL scores by response status were explored using mixed models for repeated measures. PFS and OS were assessed for responders (≥partial response [PR]) *vs* non-responders (<PR; a 2-mo Simon-Makuchlandmark analysis was conducted; Kaplan-Meier [KM] estimates conditioned at the landmark) and by response status (KM estimates;medians not adjusted for responder bias). QoL was assessed by the EORTC Global Health Status/QoL (GHS/QoL) subscale withhigher scores indicating better QoL. Changes from baseline in GHS/QoL scores by response status were explored using mixed modelsfor repeated measures.
Results: In the Kd56 arm, 77% of patients (pts) had a response: 13% ≥complete response (CR), 42% very good partial response (VGPR), and 22% PR. In pts at risk at the 2-mo landmark, median PFS beyond the landmark was not estimable (NE; 95% CI: 20.2, NE) in responders *vs* 10.9 mos (95% CI: 7.6, NE) in non-responders. Median OS beyond the landmark was 49.3 mos (95% CI: 45.6, NE) in responders *vs* 25.8 mos (95% CI: 19.8, 32.9) in non-responders. Simon-Makuch analysis indicated a significant difference in PFS and OS between responders and non-responders ($p<0.001$). For ≥CR, VGPR, or PR, median PFS (95% CI) was NE (NE, NE), 22.2 (18.7, NE), and 10.3 (8.8, 14.9) mos, respectively. Median OS (95% CI) was NE (NE, NE) for ≥CR, 51.3 (NE, NE) for VGPR, and 42.5 (33.6, 47.6) mos for PR. Kd56 responders had improved GHS/QoL scores *vs* Kd56 non-responders (difference in mean change [diff]: 7.22 [95% CI: −0.68, 15.12]). Among Kd56 responders, changes were similar by response depth (diff for ≥CR *vs* VGPR: 0.35; ≥CR *vs* PR: 1.59).
Summary/Conclusion: In the Kd56 arm, 77% of pts had a response and OS beyond the 2-mo landmark improved by 24 mos *vs* non-responders. Responders also had better GHS/QoL scores *vs* non-responders.

PB2171

EFFECT OF COMPOUND KUSHEN INJECTION ON IMMUNE FUNCTION FOR PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA UNDERGOING CHEMOTHERAPY

H. Tu[1,*], L. Zhang[2], L. Ding[1], Y. Jin[1], F. Zhou[1,3]

[1]Department of Hematology, Zhongnan Hospital of Wuhan University, Wuhan, [2]Department of Traditional Chinese medicine, Inspection test center of Xiaogan Food and Drug, Xiaogan, [3]Department of Hematology, Second Affiliated Hospital, Medical School of Xi'an Jiaotong University, Xi'an, China

Background: Therapy integrating traditional Chinese and Western medicine has been the most distinctive method for treating malignant tumors in China. As a representative of traditional Chinese medicine (TCM), compound Kushen injection (CKI, also known as Yanshu injection) is extracted from the Kushen (*Radix sophorae flavescentis*) and Baituling (*Rhizoma smilacis glabrae*) herbs, using modern standardized Good Manufacturing Processes (GMP). There have been numerous clinical reports demonstrating the anti-

cancer effect of CKI, including the use of CKI to treat gastric cancer, liver cancer, lung cancer, breast cancer, ovarian cancer, colorectal cancer and additional cancer types.However, the literature contains little experimental data involving CKI in MM, and the underlying complex mechanisms of its anti-cancer effects are not fully understood.
Aims: The aim of the present study was to assess the clinical effectiveness and safety of compound Kushen injection (CKI) plus (vincristine, doxorubicin and dexamethasone) VAD regimen chemotherapy for the treatment of newly diagnosed multiple myeloma (MM).
Methods: A total of 142 newly-diagnosed MM patients were randomly assigned to the treatment group (n=72), and were treated with CKI plus chemotherapy, or the control group (chemotherapy only; n=70). After four treatment cycles, the treatment effect was evaluated including, overall response rate (ORR), quality of life (QoL), lymph cellular immunity function, secretion of cytokines and toxicity reactions.
Results: Before treatment, the peripheral blood $CD3^+CD4^+$, $CD3^+CD8^+$, NK cells and cytokine (IL-2, IFN-γ) levels of MM patients were significantly decreased compared with health volunteers (p<0.05). In addition, TNF-α levels were significantly increased in MM patients (p<0.05) compared with healthy volunteers. Post-treatment, patients in the treatment group showed significantly higher levels of $CD3^+CD4^+$, $CD3^+CD8^+$, NK cells, IL-2 and IFN-γ than the control group (p <0.05). Furthermore, TNF-α levels were significantly lower in the treatment group compared with the control group (p<0.05). ORR and QoL improvement rate in the treatment group was significantly higher than the control (87.5% *vs* 71.4%, p<0. 05; 65.3% *vs* 54.3%, p<0. 05, respectively), as was bone pain relief 3 months later for chemotherapy (p<0.05). In addition, the control group had more incidences of hematologic and nonhematologic toxicity compared with the treatment group (p<0.05).
Summary/Conclusion: In conclusion, CKI plus standard chemotherapy appears to have enhanced short-term efficacy and lower toxicity compared with standard chemotherapy alone, which may depend on increasing the patient's immunological function and improving the QoL.

PB2172

ANALYTICAL CRITICALITIES ASSOCIATED TO DIFFERENT METHODS FOR SERUM FREE LIGHT CHAIN DETECTION: IMPACT ON THE INTERNATIONAL MYELOMA WORKING GROUP (IMWG) CRITERIA DEFINING SYMPTOMATIC MULTIPLE MYELOMA

K. Weisel[1,*], H. Salwender[2], M. Ums[3], L. Kanz[3], R. Klein[3], A. Peter[1]

[1]Hematology and Oncology, University Hospital of Tuebingen, Tuebingen, [2]Asklepios Clinic, Hamburg, [3]University Hospital of Tuebingen, Tuebingen, Germany

Background: Current criteria differentiating between monoclonal gammopathy of unknown significance (MGUS), smoldering multiple myeloma (SMM) and multiple myeloma (MM) are included in the IMWG guidelines published in 2003. Recently in 2014, the IMWG updated the criteria and revised the definition of multiple myeloma by adding a serum free light chain ratio (sFLC) of >100 as a definition for multiple myeloma requiring treatment. At present, two commercial assays for sFLC quantification are available: The TBS Freelite assay and the Siemens N Latex FLC assay. The first is based on polyclonal antibodies directed against a variety of FLC epitopes. It may be run on a wide range of nephelometers as well as turbidimeters. The second method uses a probe mixture of monoclonal antibodies and runs exclusively on Siemens nephelometers.
Aims: Here, we present a prospective monocentric trial evaluating sFLC measurement and calculated kappa/lambda ratio with the N Latex FLC and the Freelite assays in patients with MGUS, SMM and MM either newly diagnosed (NDMM), or relapsed and refractory (RRMM).
Methods: Between Apr. and Aug. 2016, 40 patients were included into the clinical trial. Patients underwent routine clinical analysis for sFLC. The trial included 5 patients with MGUS and 35 patients with MM. 8 had SMM, 33 NDMM and 2 RRMM. The involved light chain was kappa in 23 and lambda in 17 cases. sFLC analysis was performed at study entry and for a maximum of 6 analyses during follow-up. All samples were analyzed for sFLC on a single Siemens BN II analyzer with Freelite reagents (The Binding Site) and with N Latex reagents (Siemens Healthcare Diagnostics). Clinical diagnoses matching submitted serum samples were retrieved from the patients' medical file. For method comparison Passing Bablok correlation analysis after log/log transformation was performed.
Results: Based on their established reference ranges, sFLC results were classified in different groups to perform an interrater agreement analysis. Values for concordance between the N Latex FLC and the Freelite test for kappa FLC, lambda FLC and kappa/lambda ratio in the total patient population were 0.85, 0.73 and 0.93 showing a very good concordance above 0.81 for FLC kappa and FLC ratio and still a good concordance for FLC lambda. In the regression analysis, the Pearson correlation coefficient (r) showed an overall good correlation with r=0.977 for kappa, r=0.900 for lambda and r=0.975 for kappa/lambda ratio, respectively.In 35 patients with NDMM either smoldering or symptomatic, 17 (49%) patients showed sFLC ratio >100 and involved FLC >100mg/L in the Freelite test. 7/17 of those identified patients had a sFLC ratio >100 in the N Latex FLC assay plus one further patient. Applying a cutoff of 50 for N Latex FLC ratio identified 4 additional patients.
Summary/Conclusion: sFLC analysis requires continuous awareness of analytical limitations. Concordance between the Freelite and N Latex FLC assays show overall good concordance, however in single cases large differences are observed especially in measurement of lambda FLC. As the sFLC ratio >100 is implemented in the IMWG criteria defining MM disease requiring treatment physicians should be aware that this criterion might be solely rely on the method or the analyzer used for determination of sFLC values. This might lead to discrepant decisions in a proportion of NDMM patients. Therefore, IMWG guidelines for treatment of NDMM including patients solely showing sFLC ratio >100 should be carefully revised.

PB2173

ELIGIBILITY CRITERIA FOR CLINICAL TRIALS AND COMORBIDITY AS PREDICTORS OF CHEMOTHERAPY DOSE ADJUSTMENT IN PATIENTS WITH MULTIPLE MYELOMA

E. Pérez Persona*, A. Unamunzaga, I. Oiartzabal Ormategui, A. Vega Gonzalez de Viñaspre, R. Diez Angulo, A. Santamaría Lopez, L. Cuevas Palomares, C. de Miguel Sanchez, D. Robles de Castro, A. Pereda Vicandi, M. Gabilondo Jalon, J.M. Guinea de Castro

Hematology, Hospital Universitario de Araba-Sección Txagorritxu, Vitoria, Spain

Background: Approval of new standards of treatment in cancer is usually preceded by phase III clinical trials to demonstrate whether or not a product offers a treatment benefit to a specific population, providing information on safety and efficacy data. There is important selection bias on clinical trials. Strict eligibility criteria could compromise extrapolation of results to the general population.
Aims: Asses if comorbidity by Cumulative Illness rating score for geriatrics (CIRS) score or the possibility of been eligible to participate in clinical trials is associated with dose drug reduction for unsuitable-transplant patients with newly diagnosed multiple myeloma in real setting
Methods: CIRS score, and dose adjustment was assessed retrospectively for transplant-ineligible multiple myeloma patients diagnosed at Hospital Universitario de Araba (Spain). Eligibility criteria of 5 clinical trials: 3 commercial (VISTA, FIRST, ALCIONE) and 2 academic (GEM05MAS65 and GEM10MAS65) were verified for all the patients.
Results: From feb/10 to dec/17, 143 multiple myeloma patients were diagnosed in Hospital Universitario de Araba. 64% of them (92 patients) were transplant-ineligible. Median age was 77 years old, higher than published trials (median age VISTA: 71, FIRST: 73, ALCIONE: 71, GEM05: 73, GEM10: 75). CIRS score identify different prognostic groups in overall survival: CIRS <4: 52.3 months; CIRS 4 to 8: 57 months and CIRS>8: 13.5 months (p: 0.017). There was a high proportion of patients ineligible for participating in clinical trials: VISTA: 75% FIRST: 70%; ALCIONE 75%; GEM05: 75%; GEM10: 77%. 72% of the patients were treated with bortezomib, melphalan and prednisone scheme, and 28% with bortezomib/dexamethasone +/- ciclophosphamide. In general, tolerance to treatment was good, no deaths attributed to treatment were observed. 47.3% of the patients need to reduce any of the drugs prescribed, and 11% withdrawn treatment due to toxicity. By drugs, melphalan need to be reduce or withdrawn in 57% of patients, bortezomib in 29%, and corticoids in 18% of the patients. Interestingly, we could not observe correlation between the CIRS score and the possibility of been eligible for any of the selected clinical trials. Neither CIRS score nor eligibility for clinical trials was associated with the possibility of reduce of withdrawn chemotherapy.
Summary/Conclusion: Clinical trials are excessively restrictive. There is no apparent correlation between the possibility of been eligible for participating in clinical trials, comorbidities and chemotherapy s adjustment. Eligibility criteria of clinical phase 3 trials compromised extrapolation of security and efficacy of results to real setting patients.

PB2174

A COMPARATIVE ANALYSIS OF FREE LIGHT CHAIN CONCENTRATIONS IN SERUM AND CEREBROSPINAL FLUID FOR PATIENTS WITH MULTIPLE MYELOMA COMPLICATED BY MYELORADICULOPATHY

?. Трифонова [1,*], ?. Голенков [1], ?. Митина [1], ?. Москалец [1], ?. Когарко [2], ?. Белоусов [1], ?. Катаева [1], ?. Кедров [1]

[1]ГБУЗ МО МОНИКИ им. М.Ф. Владимир, [2]Институт химической физики им. Н.Н. Семенова, Москва, Russian Federation

Background: To formulate the concept of intrathecal tumor growth in patients with multiple myeloma (MM), complicated by myeloradiculopathy based on model concentration gradients of free light chains in serum (sFLC) and the free light chain in cerebrospinal fluid (FLC CSF).
Aims: To formulate the concept of intrathecal tumor growth in patients with multiple myeloma (MM), complicated by myeloradiculopathy based on model concentration gradients of free light chains in serum (sFLC) and the free light chain in cerebrospinal fluid (FLC CSF).
Methods: 12 patients with MM complicated by myeloradiculopathy were included in this study. Paraprotein (PIg) G was determined in 6 patients (κ- at 3; λ - in 3), PIg M/κ – in 1 patient, an isolated BJ disease - in 5 patients (λ-5). A newly diagnosed MM was present in 5 patients, a resistant MM - in 4 patients; 3 patients were receiving maintenance treatment during the study. All patients received chemotherapy treatment, including bortezomib, melphalan (cyclophosphamide) and prednisone (dexamethasone), lenalidomide administered when tumor resistance occurred. Concentrations of sFLCand FLC CSF were measured by ELISA method on a spectrophotometer (Kenstar, USA).
Results: The average concentrations of κ- sFLC was 15,9±4 mg/l, which was greater than this value for healthy individuals (7,3 mg/l). Distribution of values for concentrations reached 6,7±2 and 28,9±7 mg/l. The average value of κ- FLC CSF was 0,03±0,01 mg/l with a distribution of 0,02±0,05 and 0,05±0,02 mg/l. The average concentration of λ- sFLC was 54,3±16,4 mg/l (values for healthy individuals - 12.7 mg/l), with distribution of 12,6 and 98,7 mg/l. Concentration of λ -FLC CSF was 0.5±0.18 mg/l with a distribution of 0.17 and 0.83 mg/l. To determine the concentration gradient for sFLC and FLC CSF the concentrations of κ- and λ – FLC were determined in serum and in cerebrospinal fluid. The ratios for FLC concentration in serum *vs* FLC concentration in cerebrospinal fluid were calculated for κ-FLC and for λ – FLC, which was 1,7 and 1,6 respectively. Comparison of this model with the performance of each patient showed that 6 out of 12 results were below the 1.7 or 1.6, indicating an intrathecal growth. Tumor cytosis in cerebrospinal fluid was determined in one case.
Summary/Conclusion: Results of this study could serve as indicators for intrathecal chemotherapy for MM complicated by tumor myeloradiculopathy.

PB2175

THE CLINICAL SIGNIFICANCE OF THE IMMUNOPHENOTYPIC PROFILE OF TUMOR PLASMA CELLS IN THE BONE MARROW AND PERIPHERAL BLOOD IN MULTIPLE MYELOMA COMPLICATED BY PLASMACYTOMAS

K. Belousov [1,*], ?. Митина [1], ?. Чуксина [1], ?. Голенков [1], ?. Трифонова [1], ?. Катаева [1], ?. Черных [1], ?. Высоцкая [1], ?. Захаров [1], ?. Клинушкина [1], ?. Митин [2], ?. Караулов [3]

[1]ГБУЗ МО МОНИКИ им. М.Ф. Владимир, [2]ФГБУ «ГНЦ институт иммунологии» ФМБА России, [3]Первый Московский государственный медицинский университет имени И. М. Сеченова, Москва, Russian Federation

Background: the study of the immunophenotype of tumor plasma cells of the bone marrow (PC BM) and circulating tumor cells (CTC MM) can reveal new mechanisms of metastasis and generalization of the disease in multiple myeloma (MM) complicated by plasmacytomas.
Aims: the study of the immunophenotypic profile of bone marrow tumor cells and circulating tumor cells in patients with multiple myeloma complicated by plasmacytomas, depending on the clinical course of the disease.
Methods: 18 patients with MM (9 women, 9 men), from 41 to 85 years (median 59 years) were examined. All patients had 3 stages of the disease according to Salmon-Durie, and plasmacytomas of different localization. The average level of plasma cells in CM according to the myelogram is 19.3% (0.4-69.0%). The first detected MM was in 9 patients, 9 previously received therapy (VCP, VMP, RVP). In 13 (72.2%) patients there were bone plasmocytomas, in 5 (27.8%) - extramedullary plasmacytomas. In 14 (77.8%) patients, the plasmacytomas were more than 7 cm in diameter (bulky disease). Differences between PC KM and COCMM in patients with primary and pre-treated, with solitary bone and extramedullary plasmacytomas, in patients with plasmacytomas more than 7 cm were assessed.
Results: in primary and treated patients a significantly higher level of CD138 + on PC KM is determined in comparison with COC MM. In both groups, the detectability of CD138 cells was significantly higher at CIC MM. There was no significant difference in the detection of CD56, CD11c, CD117, CD81, CD33, CD27, CD28, CD19, CD20, CD79b in both groups. Patients with bone plasmacytomas have a higher detectability of CD138 + on PC KM compared to COCMM. Detectability of CD138- was higher at COC MM. In the group of patients with extramedullary plasmacytomas, there was no significant difference in the detection of CD138 + and CD138- between CM of PC and COC of MM, there were no other antigens. When comparing the detectability of plasmocyte antigens *versus* the size of the plasmacytoma - more than 7 and less than 7 cm in both groups, a significant predominance of CD138 + cells in the CM KM and a higher detection of CD138 cells at COC MM were revealed.
Summary/Conclusion: immunophenotypically CTCs of MM are less mature cells compared to PC KM, losing the antigen CD138 + and the connection with the bone marrow stroma. These results were noted in both primary and treated patients, as well as when comparing patients with plasmacytomas more than 7 cm and less than 7 cm. In this connection, the loss of surface CD138 + by the plasma cell can be regarded as one of the mechanisms of metastasis and progression of MM. In the group of extramedullary plasmocytes, there were no significant differences between PC BM and CTC MM, which allows us to conclude that the immunophenotypic identity of these cells is medullar. However, these data require further study due to the small sample of patients in this group.

PB2176

THE OCCURRENCE AND ECONOMIC BURDEN OF TREATMENT INDUCED PERIPHERAL NEUROPATHY IN PATIENTS WITH MULTIPLE MYELOMA IN SWEDEN

H. Nahi[1,*], G. Walinder[1], F. Gatta[2], H. Beydogan[3], A. Levine[3], C. Lourdudoss[3], I. Majer[2], S. Gonzalez-McQuire[2], M. Hansson[4,5]

[1]Department of Medicine, Huddinge, Karolinska University Hospital, Stockholm, Sweden, [2]Amgen (Europe) GmbH, Zug, Switzerland, [3]IQVIA, Solna, Stockholm, [4]Wallenberg Center for Molecular Medicine, Lund University, [5]Hematology clinic, Skåne University Hospital, Lund, Sweden

Background: Approval of novel regimens for multiple myeloma (MM) in Europe have improved the efficacy and safety of treating patients with MM. Peripheral neuropathy (PN) is both a MM complication and a toxicity that can occur from some anti-myeloma treatments. Treatment-induced peripheral neuropathy (TIPN) impairs quality of life of patients; however, the economic burden of TIPN is not well known.
Aims: The primary aim of the study was to understand the treatment patterns in patients with MM. This analysis focused on secondary and exploratory objectives assessing the occurrence and economic burden of TIPN in a real-world setting in Sweden.
Methods: This retrospective cohort study used data from electronic medical records (EMR) from three large haematology clinics in Stockholm, Sweden that were linked to national health registries. Eligible patients were those with a diagnosis of MM (ICD-10:CD90.0) in the Swedish Cancer Registry between 2006–2015 and had initiated MM treatment during the same period. Follow-up was until last EMR visit, death or study end (March 2017). For this analysis, patients who had no record of PN or TIPN for the past two years before the initiation of the analysed treatment line were included. Diagnosis of TIPN was retrieved from the National Patient Register (ICD-10:G62.0) and EMR case notes. An index matching method with replacement was used to match patients with TIPN and those without for baseline characteristics, MM treatment that induced TIPN and line of therapy. For patients with TIPN, the time from treatment initiation to the TIPN diagnosis date was estimated (TIPN lag days). For patients without TIPN, a pseudo TIPN diagnosis date was set according to the TIPN lag days of the related case, after the initiation of the matched treatment. Cost calculations were based on 2015 DRG tariffs. Statistical analyses were descriptive in nature.
Results: In total, 1298 MM patients were identified from EMRs; of which 550 were eligible for the overall study. Of these patients, 385 met this analysis criterion (135 received stem cell transplantation [SCT] and 250 did not [non-SCT]). Overall, 26% of patients were diagnosed with TIPN. At first line (1L), most patients with TIPN had received a bortezomib-based regimen (81%) while at second line (2L), most patients with TIPN had received a lenalidomide-based regimen (55%) (Table 1). Median TIPN lag days was similar in both SCT and non-SCT patients (4.3 and 4.4 months respectively, for 1L bortezomib-based treatment and 1.3 months each, for 2L lenalidomide-based treatment). Matched patients (n=73) were well balanced on all matching variables. Median follow-up from TIPN date and pseudo date was 1.7 and 1.8 years, respectively. Overall, patients with TIPN had increased healthcare resource utilisation (HRU) compared with those without TIPN: mean hospital inpatient visits (3.5 *vs* 2.3 visits), median total length of stay (7.2 *vs* 1.2 days) and mean hospital outpatient visits (17.8 *vs* 12.6 visits). Similarly, patients with TIPN showed significant increased total cost of HRU per patient-year than those without TIPN (mean €52,353 *vs* €31,194, respectively), mainly driven by outpatient prescription drugs costs (mean €27,399 *vs* €18,808).

Table 1.

Table 1. Number of MM patients experiencing occurrence of TIPN by MM treatment and line of therapy

Treatment regimens	Number of MM patients experiencing TIPN occurrrence 1L (n = 57)	2L (n = 31)	3L (n = 12)	4L (n = 2)
Bortezomib-based[a]	46 (81%)	11 (35%)	5 (42%)	0 (0%)
Bortezomib + thalidomide-based[b]	2 (3%)	1 (3%)	0 (0%)	1 (50%)
Lenalidomide-based[c]	4 (7%)	17 (55%)	7 (58%)	1 (50%)
Thalidomide-based[d]	5 (9%)	2 (6%)	0 (0%)	0 (0%)

1L, first line; 2L, second line; 3L, third line; 4L, fourth line; MM, multiple myeloma; TIPN, treatment-induced peripheral neuropathy

[a]Total number of bortezomib-treated patients included in this analysis: 259 in 1L; 66 in 2L; 30 in 3L; 7 in 4L
[b]Total number of bortezomib + thalidomide-treated patients included in this analysis: 10 in 1L; 9 in 2L; 2 in 3L; 3 in 4L
[c]Total number of lenalidomide-treated patients included in this analysis: 46 in 1L; 102 in 2L; 35 in 3L; 7 in 4L
[d]Total number of thalidomide-treated patients included in this analysis: 67 in 1L; 21 in 2L; 11 in 3L; 1 in 4L

Summary/Conclusion: Findings showed that most patients with TIPN had received bortezomib-based treatments. Patients with TIPN had increased HRU and ~70% higher cost per patient-year *versus* those without TIPN. There is a need for effective MM treatments which reduce the occurrence of TIPN in order to ensure benefit to patients and decrease healthcare expenditure.

PB2177

OUTCOMES OF TREATMENT OF MULTIPLE MYELOMA IN PATIENTS REQUIRING RENAL REPLACEMENT THERAPY: "REAL WORLD" SINGLE CENTRE EXPERIENCE OVER AN 11 YEAR PERIOD

A. Ratnayake[1,*], M. Srikantharajah[2], D. Makanjuola[1], S. Stern[2]

[1]Renal, [2]Haematology, Epsom & St Helier University Hospital NHS Trust, Carshalton, United Kingdom

Background: Renal impairment is a recognised complication of multiple myeloma (MM), ranging from mild chronic kidney disease to end-stage renal failure. The incidence of renal impairment at diagnosis of myeloma is between 20-50%; of these, approximately 10% present with severe acute kidney injury (AKI) requiring dialysis. Treatment regimens including both the proteasome inhibitor Bortezomib and Dexamethasone are now most commonly used as first line therapy in such patients. These regimens have improved the overall prognosis of these patients, where successful therapy can also result in partial or full recovery of renal function. However, these treatments also cause side effects such as peripheral neuropathy, infection and cytopenias.
Aims: We reviewed patients managed at our centre to determine whether the treatment of myeloma patients requiring renal replacement therapy (RRT) was associated with a high incidence of the above side effects, and to look for an improvement in outcomes over an 11 year period.
Methods: Patients diagnosed with MM with associated renal impairment between January 2007- December 2017 were identified. Only patients who required RRT were included in the analysis. Patient data including demographics, type of myeloma, chemotherapy regimens, associated complications, response to treatment and survival rates were obtained from electronic patient records.
Results: 29 patients were identified who met the above inclusion criteria. Median age was 64 years (range 45-86). 59% patients were male; 72% were Caucasian. Median follow up time was 33 months (range 8-96 months). 65% (N=19) patients were treated with first line chemotherapy regimens which included Bortezomib; of these 84% showed a complete (21%) or partial (63%) response to therapy. 1 year survival was 89% (N=19), 5 year survival was 71% (N=14). Complications of therapy included thrombosis (11%), neuropathy (53%), and infection requiring hospital admission (42%). 37% of patients to-date recovered enough renal function to come off RRT. 35% (N=10) patients did not have Bortezomib as part of initial chemotherapy regimen. 50% of these patients responded to therapy; all with partial response, none with a complete response. 1 year survival was 90% (N=10), 5 year survival was 50% (N=10). Thrombosis (20%) and infection requiring hospital admission (20%) were also recognised complications. None of these patients were recorded to have experienced neuropathy. Only 10% of patients recovered renal function sufficiently to stop RRT.
Summary/Conclusion: Advances in therapies for MM have improved prognosis and quality of life for patients. The time frame of our study coincides with the introduction in this centre of Bortezomib as first-line treatment for MM in patients with renal impairment in 2010. In our cohort of patients who required RRT, Bortezomib treatment resulted in an increased likelihood of response to first line therapy, sufficient improvement of renal function to cease RRT, and improved overall survival. However, our cohort also demonstrates a larger side effect profile with Bortezomib-containing regimens, notably peripheral neuropathy and infection, thereby increasing overall morbidity for some of these patients.

PB2178

PROGNOSTIC ROLE OF NEUTROPHIL-LYMPHOCYTE RATIO IN PATIENTS WITH MULTIPLE MYELOMA

A. Luchinin[1,*], N. Minaeva[1], S. Semochkin[2]

[1]Kirov Scientific Research Institute of Hematology and Blood Transfusion of the Medical-Biological Agency, Kirov, [2]Pirogov Russian National Research Medical University, Moscow, Russian Federation

Background: The neutrophil-lymphocyte ratio (NLR) has been recently identified for multiple myeloma (MM) as prognostic factor. There are several trials and one meta-analysis confirm it. The NLR is neutrophil count (cells/μL) divided by lymphocyte count (cells/μL) in common blood count.
Aims: In our research we studied the NLR prognostic significance for overall survival (OS) and for distribution by Durie-Salmon stages of patients with MM. We checked hypothesis that NLR can serve as a negative prognostic immune biomarker in newly diagnosed MM.
Methods: Our study included 314 patients with newly diagnosed MM at the Kirov Research Institute of Hematology and Blood Transfusion from 1990–2015. All patients fulfilled the IMWG criteria for symptomatic MM. The correlation of NLR with various parameters was assessed with ANOVA corrected by Turkey HSD test for multiple comparisons. For assessment categorical parameters we used Kruskal-Wallis rank test. The Cox proportional hazards model, Wilcoxon and log-rank tests for comparison survival curves was used to evaluate NLR at diagnosis as a prognostic factor for OS. Statistical analysis was performed using R language (v 3.4.3). A p-value of <0.05 was considered statistically significant.
Results: In our group, the median age was 64 years old (range: 29–90) and 190 patients (60%) were female. Overall, 179 patients (57%) had IgG type, 74 (23%) had IgA type, 43 (14%) had light chain disease and 15 (5%) had non-secretory MM. The MM type of 3 (1%) patients was unknown. The median NLR at diagnosis was 1.6. The cutoff point of NLR 2.6 that yielded the greatest differential to segregate cohorts, was based on the analyzed at different cutoff points from the long-rank test. At the time of this study, 256 (82%) patients had died. The median of OS in all group was 35 months, 5-year OS – 30%. We found that patients with stage IIB and IIIB by Durie-Salmon had more superior average NLR level *versus* patients with stage IA, IIA and IIIA (ANOVA test, $p<0.0001$). During multiple comparative analysis patients with A and B subclasses differed among themselves by NLR level (Turkey HSD test, $p<0.05$). In this regard we compare medians in this group because they did not have normal distribution. The median of NLR level in patients in B stages by Durie-Salmon was higher than among patients with A stages (2.45 *versus* 1.45, Kruskal-Wallis test, $p<0.0001$). Also, patients with baseline NLR 2.6 had shorter median OS (28 months *versus* 40 months, respectively; HR=1.38, 95% CI: 1.05–1.8, p-value=0.023; Wilcoxon test, p=0.008).
Summary/Conclusion: The MM patients with higher NLR (≥2.6) are more likely to have poorer prognosis by OS than those with lower NLR. Also, in patients with B stages (IIB or IIIB) by Durie-Salmon with high creatinine level (≥2mg/dL) the average NRO level is higher than those, who don't have myeloma nephropathy.

PB2179

BLEEDING AND THROMBOTIC COMPLICATIONS IN PLASMA CELL DISORDERS IN THE "NOVEL AGENTS ERA": RISK FACTORS, OUTCOME ,PROGRESSION AND STRATEGIES OF PREVENTION A REAL-LIFE MONOCENTRIC RETROSPECTIVE ANALYSIS

L. Torti[1,*], A.M. Morelli[1], F. Bacci[2], P. Di Bartolomeo[1]

[1]Clinical Hematology, Department of Hematology, Transfusion Medicine and Biotechnology, "Spirito Santo" Civic Hospital, Pescara, [2]Section of Hematopathology, Department of Hematology and Oncological Sciences, S.Orsola Malpighi Hospital, Bologna, Italy

Background: "Thrombosis is often reported in cancer" Trousseau wrote in 1865. Multiple myeloma(MM) is associated with venous-thromboembolism. Moreover bleeding-complications are possible in plasma-cell-disorders and are cause of early mortality. Data available in this setting are rare.
Aims: We'd like to share our experience regarding 138 patients with these events retrospectively analysed.
Methods: We reviewed 20 bleeding and 118 thrombotic events consecutively diagnosed and managed between 1999 to 2018 in the median-time of 18 years, median follow up 12,5 years (10,2-19).

Results: We reported 4 bleeding events in amyloidosis patients:3 periorbital hemorrhages related to X-factor-deficiency and one intestinal-bleeding. We described 15 hemorragic events in MM patients under treatment: one of them was lifethreatening (an extensive brain hemorrhage)and 4 gastrointestinal-bleeding. 11 events were minor mucocutaneous-haemorrhages. Among risk-factors we have high monoclonal-component at diagnosis (13). A 49-year old female died during induction-therapy of a fatal brain hemorrhage. On CT an extended intracranial hemorrhage developed in right parietal region (Fig.2c-e). Platelet and coagulation-exams were normal. Hypothesized mechanisms are an acquired platelet disfunction o an amyloidotic deposition of fibrils. We had 5 events after osteomedullary-biopsies:we recommend a good post-procedure-compression. Within thrombotic manifestations we have 69-events in MGUS(59 venous, 10 arterial), 29 events in MM (22 venous,7 arterial) and 20 events in SMM patients (18 venous, 2 arterial). Thrombophilic screening was negative.Venous thromboses described were at diagnosis not at relapse(75/99). We had one case of massive lethal pulmonary embolism. We have described 7 thrombotic manifestations in MM-patients at the time of diagnosis before the start of therapy, highlighting how the disease is associated with a thrombogenic condition. Among risk factors of disease we have ISS score (III 39, II 7 and I 3), elevated serum-FLC(40/49). Therapy-consisted-of dexamethasone(29), chemotherapy(9), lenalidomide(8), KRD(2),doxorubicin(3), thalidomide(5), pomalidomide(2 patients). We chose low molecular heparin for prophylaxis. Among MGUS patients 48 evolved with median TTP of 56 months(14-90). We compared TTP with an homogeneous group of 48 patients (69 months,51-110) matched by age and risk-profile, with evidence of a shorter progression-time in patients with MGUS and thrombosis (Fig1a, Longrank-test-95% IC 2, 9-9, HR5). M-protein level is a risk factor. Moreover in our study 14 SMM-patients evolved with medium time of 34 months (11-53). We have compared TTP with an age and risk-profile-matched-SMM-patients-group (14-patients), median-time-69-months,range-29-75(Fig1d). L-Rtest, 95%CI, HR3,4), showing a shorter time for SMM-series with thrombosis. Crosstalk between microenvironment and clone can facilitate progression. Finally we compared OS and PFS among our 44 MM patients under therapy and thrombosis and a similar group regarding age and ISS score,demonstrating how outcome and PFS are worse in patients with thrombosis (Fig 1b-c). For first series median OS is of 36 months(20-60)and PFS of 18,5 months (range 7-29) and for second-series without thrombosis median OS of 69 months (35-110) and PFS of 31 months (6-39), L-R test, 95%CI, HR 4,7 for OS and HR4,2 PFS).

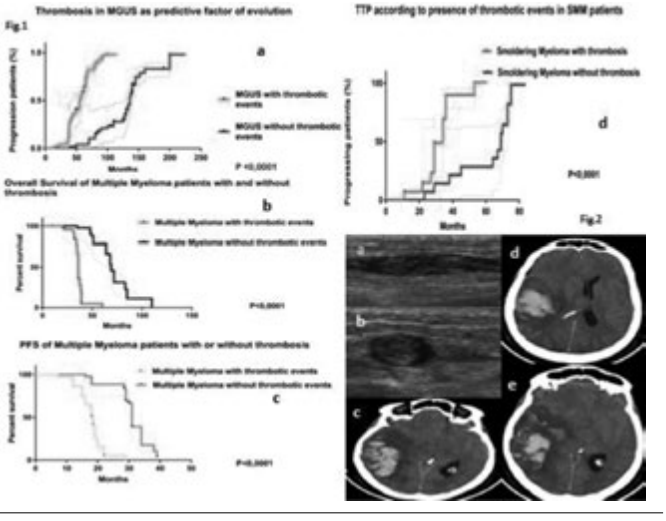


Figure 1.

Summary/Conclusion: Our study is limited:heterogeneity of population and no information about cytogenetics. Cancer protrombogenicity may promote angiogenesis, metastasis and poorer prognosis. Prospective studies are needed to verify our conclusions and to investigate exact pathogenesis.

PB2180

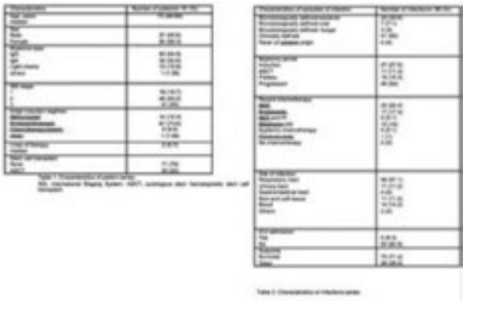
MULTIPLE MYELOMA AND INFECTIONS IN THE "NOVEL AGENTS ERA": A REAL-LIFE RETROSPECTIVE UNICENTRIC EXPERIENCE

M.S. Infante*, I. Gonzalez, C. Muñoz, M.A. Foncillas, C. Heras, K. Marin, J.A. Hernandez-Rivas

Hospital Infanta Leonor Madrid, madrid, Spain

Background: Multiple myeloma (MM) is a clonal plasma cell malignancy that is increasing in prevalence as the population ages and the survival improves. Infection is a leading cause of morbidity and mortality, contributing to nearly 50% of early deaths (Augustson *et al.*, 2005). Infection risk is increased due to patient-, disease- and treatment-related factors. New treatment options in the last decades have improve survival in MM patients but their effect on the risk of infections remains to be established yet.
Aims: To describe retrospectively the development of infections requiring hospitalization in 91 MM patients diagnosed in our institution between 2008 and 2017.
Methods: Median age 73 years (46-89), 54 females. There were 51 patients with 1 or 2 infections and 9 patients with >3 infections. 31 patients had no infection. Characteristics of the studied patients are detailed in Table 1.
Results: 98 episodes of infection were identified. Of these, 43 (43.8%) were microbiologically identified, 51 (52%) clinically defined and 4 (4%) were fever of unknown origin. Of the 43 microbiologically defined infections, 77% (33) were bacterial, 7% (3) fungal and 16.2% (7) viral. Of the 33 bacterial infections, 18 (54.5%) were caused by gram negative and 15 (45.4%) gram positive microorganisms. *Escherichia Coli* was the most frequently isolated organism (21%). Vaccine-preventable encapsulated bacteria *Str.pneumoniae* was isolated in 18% of all bacterial infections. The 7 viral infections were due to influenza virus (57%), respiratory syncytial virus (28%) and herpes simplex reactivation (14%).There were 3 fungal infections: 2 candidemias and 1 *Pneumocystis Jiroveci* pneumonia. Respiratory tract (57,1%) and blood (14.2%) were the most common sites of infection. Bacterial infections occurred more frequently in relapse phases (84%), during neutropenia (53%) or in hypogammaglobulinemic patients (62%).All the patients with viral-infections had previous PIs (Proteosome Inhibitors) based therapies. All the patients with fungal-infections had previous IMiDs (Immunomodulatory drugs) therapies and were receiving HD (high dose) steroids.Infections occurred more frequently at disease progression (50%) and during induction (27.5%) and the majority of patients were older than 65 (74%). 11/16 infections occurring during plateau were of low severity and had good outcome.Most recent therapies administered before infection development were IMiDs (22,4%) and Bortezomib (17.3%). Admission to the intensive care unit (ICU) was required in 8.3% infection episodes. 28 infections resulted in death (28.5%): 22/28 occurring during disease progression, 2/28 during induction (1 patient with herpetic encephalitis and 1 multiorganic failure MOF), 1 in ASCT and 3 deaths during plateau (1 heart failure in VGPR, 1 MOF after prolonged hospitalization for toxicity in PR, 1 pneumococcal pneumonia in CR). Overall Survival (OS) was 63 months. More details of infections are shown in table 2.

Table 1.



Summary/Conclusion: Our study identifies a high risk patient group for infections: older age, late disease, advanced ISS, multiple treatments, HD corticoids treatments. Infections represent a significant-comorbidity factor especially during induction and in refractory/relapsed-patients, with 28.5% infection related mortality in our population. Immunoglobulin replacement therapy, vaccination for encapsulated bacteria or antibiotic-prophylaxis may have a role in older patients with high ISS stage. Trials are needed to target new approaches for prevention, early detection and treatment of infection in MM patients.

PB2181

OUTCOME OF RELAPSED OR REFRACTORY MULTIPLE MYELOMA PATIENTS TREATED WITH PCCD (POMALIDOMIDE - CYCLOPHOSPHAMIDE-CLARITHROMYCIN - LOW DOSE DEXAMETHASONE) IN THE REAL-LIFE SETTING

R. Ríos-Tamayo[1,*], A. Martín[2], D. Sánchez-Rodríguez[3], M.E. Clavero[1],

A.B. Rivera[1], T. Rodríguez[4], M.J. Olivares[4], R. Sánchez[5], C. Alarcón[6], A. Jiménez[6], J. Badiola[7], E. Morente[7], P. Garrido[7], J. Sainz[7], M. Jurado[7]

[1]Monoclonal Gammopathies Unit, University Hospital Virgen de las Nieves, [2]Clinical Trials Unit, [3]Monoclonal Gammopathies Unit, FIBAO, [4]Immunology, [5]Nuclear Medicine, [6]Pharmacy, [7]Hematology, University Hospital Virgen de las Nieves, Granada, Spain

Background: Multiple myeloma (MM) is a complex and heterogeneous malignancy. Despite the widespread use of new agents and the use of less toxic and more efficient regimens, the treatment of relapsed and/or refractory MM (RRMM) is challenging, with poor outcome in most patients. Pomalidomide and low-dose Dexamethasone (Pd) is considered a standard of care in RRMM. To further improve its results, Pd has been associated with Cyclophosphamide (PCd)(Baz RC *et al.* 2016; Chari A *et al.*2016) achieving an impressive 64.7% and 67% overall response rate (ORR) respectively. Many other combinations have been used (Ríos-Tamayo R *et al.* 2017) without showing clear advantage over PCd. However, to our knowledge, the baseline addition of clarithromycin (c) to PCd (PCcd) has not been previously explored.
Aims: To assess in a preliminary way if the addition of c to PCd add clinical value to the triplet
Methods: Patients included in our MM population-based registry fulfilling RRMM status according with IMWG criteria, were prospectively treated with P 4 mg/day/21 days, C 50 mg/day/21 days, c 500mmg/12h/continous and d 40 mg/weekly, in cycles of 28 days. We estimated efficacy in terms of overall response rate (ORR), progression free survival (PFS) and overall survival (OS), as well as the main grade 3-4 toxicity.
Results: Since 2015, 24 RRMM patients have been treated with P-based regimens, and 19 of them received complete (not scaled) PCcd, 9 men and 10 women, median age at diagnosis 63 years (48-81). The ISS was I 47%, II 32% and III 21%. 5 patients (26.3%) had light chain MM. PCcd was used as third line of therapy (LT) in 8 patients (42%), fourth LT in 3 (16%), fifth in 4 (21%) and subsequent LT in 3 (21%). Median of cycles administered was 5 (1-25). 12/17 patients achieved at least PR, with 70.5% ORR (11.8% CR). Median PFS and OS were 6.7 and 11 months, respectively. 4 patients have died (3 with progressive disease, 1 renal failure) whereas 11 remain ongoing in follow-up. No patient died due to infection. 5 patients (26.3%) are long-term responders, receiving 12 or more cycles. Neutropenia (grade 3-4) was documented in 57.9%. Infection (grade 3-4) was present in 26.3%. Thrombocytopenia (grade 3-4) was shown in 21.1%.
Summary/Conclusion: PCcd seems to be a convenient and optimized way to use P in real-life RRMM patients. This is an all-oral four-drug regimen with a remarkable ORR and a manageable safety profile. If PCcd is able to improve the outcome of PCd must be confirmed in a clinical trial.

PB2182

SURVIVAL OF RELAPSED MYELOMA PATIENTS WITH A SECOND AUTOGRAFT: LOCAL EXPERIENCE

C. Hendry[1,*], R. Soutar[2]

[1]University of Glasgow, [2]Haematology, Beatson Oncology Centre, Glasgow, United Kingdom

Background: In the UK five year overall survival in myeloma is estimated to be 47%, with 10 year overall survival (32.5%) ranking 6th lowest of the 20 most common cancers. The Beatson Oncology Centre serves the west of Scotland, encompassing some of the most deprived areas in Europe. Male life expectancy in Glasgow city is 73.4 years, while the average for Scotland is 77.1. Female life expectancy is 81.1 years in Glasgow (78.7 in Scotland). For comparison male and female life expectancy is 7 years greater in Sweden than Glasgow (male life expectancy 80.56 years and female life expectancy 84.09 years). Poor life expectancy is reflected in cancer survival rated.
Aims: In light of this, we were interested to see how this impacted on the overall survival of a group of unselected patients who were treated for relapsed multiple myeloma with a second autologous stem cell transplant (ASCT) at the Beatson Oncology Centre, Glasgow. Second autologous stem cell transplantation is 'standard of care' for our myeloma patients.
Methods: Patients were identified from data registration for EBMT. Electronic case records were retrospectively analysed for data retrieval for patients who underwent a second autograft between 1 January 2005 and 31 December 2015. Survival analysis was carried out by *IBM SPSS Analytics.*
Results: Of the 40 patients analysed 65% were male, 35% female. The average age was 59 (range 50-71). 57.5% of patients were diagnosed with IgG disease, 20% light chain, 15% IgA, 2.5% IgE, 2.5% non-secretory and 2.5% unrecorded. The mean average time from diagnosis until first transplant was 12 months (range 5-61 months, median =8). Average hospital stay for the first transplant was 22.1 days (range 11-54, median =18). The average time until time to next treatment (TNT) from the first ASCT was 45.5 months (range 6-199, median =35.5). The mean average time until next second transplant was 55.65 months (range 20-208, median =46.5). Average hospital stay for the second transplant was 21.2 days (range 14-53, median =18). The average time until next treatment from second transplant was 23.1 months (median =18, range 2-51). To date, 25% of patients have not required treatment post-transplant. Patients who did require treatment were generally retreated with a Lenalidomide based regimen (n=30) as per national Scottish guidance. 13 (32.5%) patients have died since their second treatment, the majority of progressive myeloma. Median overall survival post second transplant is 80.3%.

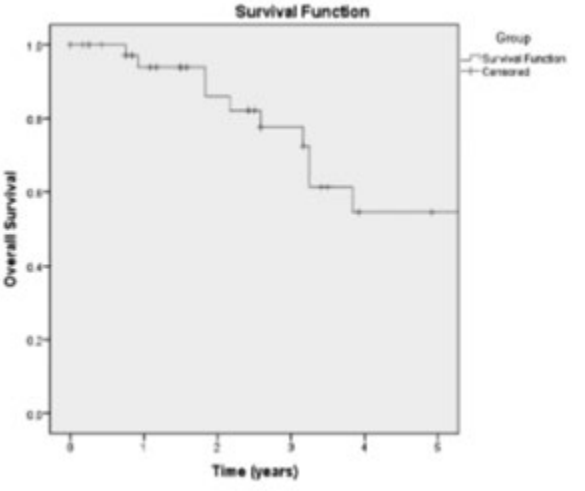


Figure 1.

Summary/Conclusion: The 'NCRI Myeloma X Relapse' trial demonstrated the benefit of a second autologous stem cell transplant with three year overall survival as 80.3%. In our cohort, three year survival compared favourably at 81.9% (95% Confidence Interval [CI] 64.9-97.5). Projected five year survival is calculated to 59.4% (CI 42.8-47.5). Our data confirms the good outcome associated with a second autologous transplant in a non-trial unselected population despite background adverse life expectancy. It is therefore likely that our data can be extrapolated to our European populations with poor survival demographics.

PB2183

NORWEGIAN REAL-LIFE EXPERIENCE OF PREAPPROVAL CARFILZOMIB THROUGH COMPASSIONATE USE NAMED PATIENT PROGRAM (NPP) FOR RELAPSING/REFRACTORY MYELOMA (RRMM)

I. Haarstad[1,*], A. Lysén[2], F. Schjesvold[2]

[1]Faculty of Medicine at University og Oslo, [2]Oslo Myeloma Center, Oslo, Norway

Background: The proteasome inhibitor carfilzomib was recently approved for treatment of RRMM. In the period before approval, drugs can be provided to critically ill patients with few treatment options, through expanded access programs.
Aims: This retrospective study of patients who received carfilzomib before marketing, was designed to describe the patients' characteristics and their corresponding treatment responses. An additional intention was to use the real-life experience to highlight a possible benefit of making new drugs available before marketing approval.
Methods: 33 Norwegian patients received carfilzomib through an NPP in the period from March 2015 to January 2016, for RRMM within 10 different Norwegian hospitals. The patient journals were printed and sent from the respective hospitals to Oslo Myeloma Center. Baseline values and treatment responses was assessed and entered into our myeloma database. Statistical analysis was done to determine response rates and time to event outcomes.
Results: The participants had received a median of 6 (1-11) previous lines of therapy, frequently including bortezomib (100%) and thalidomide and/or lenalidomide (97%), which was refractory in respectively 63,5% and 65,6% of the exposed. The most frequently used regimen was carfilzomib and dexamethasone (Kd). Response rates showed a median overall response rate (ORR) of 25% and a clinical benefit rate (CBR) of 44,4%. The median progression free survival (PFS) was 3.0 months, with a median overall survival

(OS) of 7.0 months. Patients who achieved at least partial response (PR) showed a significantly longer progression free survival with a median PFS of 8,5 months, and patients who received lenalidomide in addition to carfilzomib (KRd) showed a significantly better response with an ORR of 55,6%. Among the patients refractory to bortezomib, ORR was 10,7% and CBR 28,6%; among the patients refractory to thalidomide/lenalidomide ORR was 14,8% and CBR 29%. The patients refractory to both bortezomib and thalidomide/lenalidomide demonstrated ORR of 10,5% and CBR of 31,6%. Adverse events occurred in 90,6% of the patients, with 59,4% experiencing one or more cytopenias, 25% fatigue, 21,9% dyspnea, 18,8% cardiovascular complication and 18,8% kidney failure. 3/32 (9,4%) of patients discontinued treatment owing to adverse events. 2/33 died before ending carfilzomib, and 25/33 (75%) was dead by the time of the end of study. Carfilzomib was not considered as a direct cause of death in any patient.
Summary/Conclusion: MM is incurable in most cases, and the response is inversely correlated with the number of treatment lines. The majority of our real-life patients had advanced MM and where heavily treated prior to carfilzomib. Still a significant part of the patients benefited from the treatment in terms of prolonged PFS (3 months) and OS (7 months), with significantly increased PFS of 8,5 months, among those who experienced PR or better. A superior response was shown by those treated with lenalidomid in addition to carfilzomib (KRd). The results confirms the advantage of access to medicines prior to marketing authorization.

PB2184

PHASE III (IKEMA) STUDY DESIGN: ISATUXIMAB PLUS CARFILZOMIB AND DEXAMETHASONE (KD) VS KD IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM)

P. Moreau[1], M.-A. Dimopoulos[2], K. Yong[3], J. Mikhael[4,5], M.-L. Risse[6], G. Asset[7], T. Martin[8,*]

[1]University Hospital, Nantes, France, [2]National and Kapodistrian University of Athens, Athens, Greece, [3]University College London, London, United Kingdom, [4]Mayo Clinic, Phoenix, [5]International Myeloma Foundation, Los Angeles, United States, [6]Sanofi, Vitry-Alfortville, [7]Sanofi, Chilly-Mazarin, France, [8]University of California, San Francisco, United States

Background: Multiple myeloma remains an incurable disease requiring multiple lines of therapy, in which carfilzomib-based treatments are a standard of care. Isatuximab (ISA), an anti-CD38 monoclonal antibody with antitumor and immunomodulatory activities in preclinical models of MM, has shown clinical activity as monotherapy and in combination with current standard of care in patients with RRMM.
Aims: This Phase III, prospective, randomized, open-label study (NCT03275285; IKEMA) will evaluate the clinical benefit of ISA in combination with Kd *vs* Kd alone in patients with RRMM.
Methods: Patients with RRMM who have received 1–3 prior lines of therapy will be enrolled. Patients who have received prior carfilzomib therapy, have primary refractory disease, or have free light chain measurable disease only will be excluded. Approximately 300 patients will be randomized according to 2 stratification factors (prior lines of therapy: 1 *vs* >1; revised International Staging System [R-ISS] criteria at baseline: I or II *vs* III *vs* unknown) in a 3:2 ratio to receive either ISA (10 mg/kg weekly in Cycle 1 then once every 2 weeks thereafter) in combination with Kd (carfilzomib [56 mg/m^2 on Days 1, 2, 8, 9, 15, and 16; 20 mg/m^2 on Days 1 and 2 of Cycle 1] and dexamethasone [20 mg on Days 1, 2, 8, 9, 15, 16, 22, and 23]) or Kd in 28-day cycles until disease progression (PD), patient choice, or unacceptable toxicity. Patients will provide written, informed consent before participation in the trial. The primary endpoint is progression-free survival (PFS), defined as the time from the date of randomization to the date of first documentation of PD (according to International Myeloma Working Group Criteria) or death. Comparison between arms will be conducted through a log-rank test procedure stratified by number of previous lines of therapy and R-ISS. Key secondary endpoints include overall response rate, rate of very good partial response or greater, minimal residual disease negativity rate, complete response rate, and overall survival. Other secondary endpoints include safety, duration of response, time to progression, PFS2 (defined as the time from randomization to the time of second PD or death), pharmacokinetics, and quality of life. Safety evaluations will include adverse events and laboratory parameters, vital signs, and physical examination.
Results: The IKEMA study is currently enrolling patients; recruitment is planned in 16 countries worldwide.
Summary/Conclusion: This Phase III, randomized, multicenter study will provide an evaluation of the efficacy and safety of ISA added to Kd standard of care in patients with RRMM.
Funding: Sanofi

PB2185

PHASE III (IMROZ) STUDY DESIGN: ISATUXIMAB PLUS BORTEZOMIB, LENALIDOMIDE, AND DEXAMETHASONE (VRD) VERSUS VRD IN TRANSPLANT-INELIGIBLE PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

T. Facon[1,*], H. Goldschmidt[2], M. Cavo[3], T. Martin[4], G. Paux[5], C. Oprea[5], R. Orlowski[6]

[1]Hôpital Claude Huriez, Lille, France, [2]University of Heidelberg, Heidelberg, Germany, [3]University of Bologna, Bologna, Italy, [4]University of California, San Francisco, United States, [5]Sanofi, Vitry-sur-Seine, France, [6]University Texas MD Anderson Cancer Center, Houston, United States

Background: Patients with transplant-ineligible, newly diagnosed multiple myeloma (NDMM) require therapies which prolong survival and improve quality of life. The combination of bortezomib (V), lenalidomide (R), and dexamethasone (d) significantly improves progression-free (PFS) and overall survival compared with Rd in NDMM, and has an acceptable safety profile. Combining VRd with a monoclonal antibody (mAb) may further improve efficacy. Isatuximab (ISA) is an anti-CD38 mAb that demonstrates antitumor and immunomodulatory activities with strong potentiation when combined with V and R in MM xenograft models.
Aims: This Phase III, randomized, open-label, multicenter study (NCT03319667; IMROZ), is being conducted to evaluate the clinical benefit of ISA plus VRd *versus* VRd alone in the treatment of adult patients with transplant-ineligible NDMM.

Table 1.

Table. Dosing schedule during the induction and continuous phases

Induction phase: Cycles 1–4 (6-week cycles)																			
Day	1	2	4	5	8	9	11	12	14	15	21	22	23	25	26	29	30	32	33	42
ISA (10 mg/kg)	x				x[a]					x		x[a]				x				
Bortezomib (1.3 mg/m^2)	x		x		x		x					x		x		x		x		
Lenalidomide (25 mg)	Days 1–14											Days 22–35								
Dexamethasone (20 mg)	x[b]	x	x[b]	x	x[b]	x	x[b]	x		x[b]		x[b]	x	x[b]	x	x[b]	x	x[b]	x	
Continuous phase: Cycle 4 onwards (4-week cycles)																				
ISA (10 mg/kg)	x									x										
Lenalidomide (25 mg)	Days 1–21																			
Dexamethasone (20 mg)	x				x					x		x								

[a]: Cycle 1 only; [b]: Patients ≥75 years receive dexamethasone only on these days

Methods: All enrolled patients will provide written informed consent. Approximately 440 patients with symptomatic MM (International Myeloma Working Group [IMWG] criteria) will be randomly assigned, according to Revised International Staging System criteria (I or II *vs* III *vs* unknown) and age (<70 *vs* ≥70 years), in a 3:2 ratio to ISA 10 mg/kg plus VRd or VRd alone for four 6-week cycles (induction phase) (Table). After Cycle 4, patients will receive Rd with or without ISA in 4-week cycles until disease progression, unacceptable adverse events (AEs), or patient decision to discontinue (Table) (continuous phase). ISA will be reduced to monthly dosing from Cycle 18. Patients in VRd arm who progress during the continuous phase could be eligible for cross-over to ISA plus Rd. The primary endpoint is PFS, which is defined as the time from randomization to the date of disease progression (assessed by a blinded independent review committee according to IMWG criteria) or death, and will be compared between the two arms with a 1-sided stratified log-rank test. Key secondary endpoints will include rate of very good partial response or better, minimal residual disease negativity rate, and complete response rate. Safety evaluations include AEs, laboratory parameters, vital signs, and physical examination.
Results: The IMROZ study is currently enrolling patients; recruitment is planned in approximately 100 sites worldwide, including Japan and China.
Summary/Conclusion: This Phase III, randomized, multicenter study will provide an evaluation of the efficacy and safety of ISA plus VRd in patients with transplant-ineligible NDMM.
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PB2186

A MULTI-CENTER RETROSPECTIVE STUDY OF BONE DESTRUCTION ASSOCIATED WITH 419 NEWLY DIAGNOSED MULTIPLE MYELOMA

Y. Jin[1,*], H. Liu[2], L. Ding[3], X. Tong[4], H. Tu[3], P. Liu[3], H. Shen[5], F. Zhou[1]

[1]Department of Hematology, Zhongnan Hospital of Wuhan University, Wuhan,

[2]Department of Hematology, Second Affiliated Hospital, Medical School of Xi'an Jiaotong University, Xi'an, [3]Department of Hematology, Zhongnan Hospital, Wuhan University, [4]Department of Hematology, hongnan Hospital, Wuhan University, Wuhan, [5]Department of Hematology, Zhongnan Hospital of Wuhan University, Wuhan, China

Background: Multiple Myeloma (MM) patients with bone destruction is difficult to restore, so deeply explore the factors affected the bone destruction associated with MM will be important in clinic. This study retrospectively analyzed 419 cases with multiple myeloma.
Aims: In this study, the factors correlated with bone destruction and factors affecting survival and prognosis of myeloma bone destruction were analyzed by a retrospective study.
Methods: Comparison between categorical variables was made by the chi-square analysis with pearson test. The parametric t test with independent samples was used to compare the mean of variables between without bone destruction and with bone destruction patients. Correlation was analyzed using bivariate correlation analysis by pearson test (r). The multivariate analysis that factors associated with bone destruction were determined by binary logistic regression model with forward stepwise. The survival analysis was evaluated according to the Kaplan-Meier method with the two-sided log-rank test
Results: Multiple linear regression analysis showed that those MM patients with higher concentration of Ca^{2+} in serum, higher positive rate of CD138 immuno-phenotype and advanced in stage with 13q34 deletion in cytogenetics would more prone to bone destruction, while total bile acid (TBA) and Kappa chain isotope negatively correlated with bone destruction in MM patients. The Kaplan-Meier analysis indicated that Ca^{2+}, serum β2-microglobulin (β2-MG), hemoglobin (HGB), creatinine (CREA), uric acid (UA) and age correlated with the survival of bone destruction in MM patients. Cox regression analysis further showed that the independent prognostic factors of β2-MG and CREA had higher risk for early mortality in bone destruction patients. Moreover, a novel index based on β2-MG and globulin (GLB) to white blood cell (WBC) ratio were associated with high-risk and predicted poor survival of bone destruction patients, which will be a novel index predicts prognosis of myeloma patients using routine examination method instead of bone marrow aspiration and has an important significance for clinical guide.
Summary/Conclusion: In a conclusion, for MM patients, those with higher concentration of Ca^{2+} in serum, higher positive rate of CD138 immunophenotype and advanced in Stage with 13q34 deletion in cytogenetics would more prone to bone destruction. Hypercalcaemia, elevated serum β2-MG, anemia, renal insufficiency, elevated UA and advanced in years were correlated with poor survival and high risk in bone destruction of multiple myeloma. Combined β2-MG, GLB and WBC significantly predicts prognosis of bone destruction patients, which will be an important significance for clinical guide.

PB2187

MONTELUKAST AND GEMFIBROZIL WHEN ADDED TO BORTEZOMIB BASED THERAPY INDUCES RAPID RESPONSE IN PATIENTS WITH REFRACTORY AND HIGH RISK DISEASE

S. O'Mara[1,2,3,*]

[1]Tamara Hospital, [2]Haematology, Laverty pathology, [3]New England Northwest Haematology, Tamworth, Australia

Background: The CyBorD drug regimen when administered to patients with multiple myeloma results in a complete response rate of 40% in previously untreated multiple myeloma and a complete response rate of 8% in relapsed or refractory myeloma. It has been found that Montelukast and Gemfibrozil when used in poor prognosis multiple myeloma deepens the response and reverses resistance to Bortezomib based treatment (MOM trial). The response was demonstrable within 1 cycle of CyBorD (28 days) and there was no additional toxicity. The question arises as to whether these agents when used up front earlier in the disease process leads to deeper and more rapid response.
Aims: To determine whether montelukast and gemfibrozil when added to bortezomib chemotherapy from the first cycle leads to an increase in complete responses over expected responses and whether the time taken to reach response is faster and deeper.
Methods: Three patients with relapsed/refractory Multiple Myeloma and two patients with high risk *de novo* disease have been given Montelukast 30mg orally twice daily with Gemfibrozil 600mg orally twice daily in combination with CyBorD regimen. Patient 1 and 2 had progressive disease while on CTD treatment including a patient with multiple soft tissue plasmacytomas and 17p deletion. The third patient had dialysis dependent renal failure for a period of 3 months prior to receiving treatment. The Fourth patient had recurrent nephrotic syndrome on the basis of AL Amyloid previously treated 6 years earlier with CTD. The fifth patient had Multiple Myeloma with suspected cardiac amyloid causing cardiac failure. The expected number of complete responders in this cohort was 1/5 and an expected ORR of 60%.
Results: 4/5 patients achieved a complete haematological resonse in paraprotein with a mean time to complete response of less than 35 days. 80% CR. 1/5 patient has achieved a haematological partial response. ORR 100% Expected 2= 40% chi^2 =14. df =2 p<0.01. Treatment is continuing and it is anticipated that all four patients in complete remission will undergo bone marrow biopsy and minimal residual disease assessment at the completion of four cycles of treatment. The soft tissue plasmacytomas resolved in 10 days, The symptoms signs of nephrotic syndrome improved markedly in 10 days. The renal failure appears to be improving (lower creatinine, more urine output) in 3 weeks. One patient was also found to have stage II co-existing breast cancer which partially responded to non breast cancer chemotherapy. There has been a partial response with post mastectomy histology to be reported at the congress. Patient 1 has been in complete remission for a period of two years.
Summary/Conclusion: The addition of Montelukast to standard CyBorD chemotherapy in high risk patients leads to an extremely rapid and much deeper response in most patients treated with CyBorD chemotherapy. There is little toxicity. The ORR is 100% with a haematological CR rate of of 80%. The rate of sCR will be published following further bone marrow studies when all four cycles of CyBorD are completed.

PB2188

EFFICACY, SAFETY AND TOLERABILITY OF CARFILZOMIB LENALIDOMIDE-DEXAMETHASONE (KRD) REGIMEN IN RR/MM

V. Calafiore*, E. Martino, M. Parisi, G. Sapienza, V. Del Fabro, S. Leotta, A. Romano, C. Conticello, F. Di Raimondo

Division of Hematology, Azienda Policlinico-OVE, University of Catania. Via Santa Sofia n.[78], [95100], Catania, Italy, CATANIA, Italy

Background: Carfilzomib, Lenalidomide and Dexamethazone (KRd) is currently utilized in relapsed/refractory multiple myeloma (RRMM) patients (pts) that have experienced almost one line of treatment. Improved progression free survival and overall survival was detected in this setting of pts treated with this combination in clinical trials. Although the onset of new cardiovascular events (CV) or worsening of pre-existing CV events have been described in less than 10% of pts, the regimen has an accettable safety and tolerability. Since its approval in Italy, data on real life KRd use are still lacking.
Aims: We conducted an analysis of RRMM pts treated with KRd out of clinical trials to provide further insights on efficacy, tolerability and safety.
Methods: From November 2016 up to now 35 RRMM pts were exposed to KRd. Median number of prior therapies was 2 (range 1-6); 16 (45%) was classified as high risk pts (r-ISS) and 17 (48%) were previously autotransplanted.
Results: We report results of the first 12-months safety interim analysis on KRD treatment of RRMM. Data cut-off was January 2018. 35 pts with a median age of 65 years (range 50-79) were evaluated. All the pts received almost 2 cycles, 1 pts 16 cycles, 2 pts 15 cycles, 3 pts 14 cycles, 2 pts 13 cycles, 2 pts 12 cycles, 3 pts 11 cycles, 2 pts 10 and 9 cycles, 5 pts 8 cycles, 2 pts 7, 6, 5 and 4, 2 pts only 3 cycles, 3 pts only 2 cycles, At least 5 KRD treatment cycles were given to 28 (80%) pts. All pts had a response to treatment at the first/second cycle. At the end of the second cycle 26 (74%) pts obtained a response. Overall response rate with KRD was nearly 80% among the 28 pts that have completed 5 cycles.The majority of the pts(n=26, 96%) responded after at least 1 cycle of chemotherapy. Partial response were observed in 6 pts(21%). Rate of very good PR was 32% (9 pts); 3 pts(10%) achieved complete response (CR), 1 patient minimal response (MR), 6 pts obtained a stable disease, 6 pts obtained a stable response and then progressed. To identify pts that could obtain the most advantage by KRd treatment, we distinguished pts that have completed almost three cycles in two groups, based on previous exposure or not to lenalidomide (Table1). The 13 pts previously treated with lenalidomide (group A), compared to 15 pts not exposed to lenalidomide (group B), showed a worst profile: in the group A there was a higher% of refractory pts(25% *vs* 11%, p=0.0007) who received more prior treatments, despite the duration of the last treatment was similar in both groups. The regimen was well tolerated. with grade 3-4 haematological and non-haematological AEs on 10 (35%) and 15 (53%) pts respectively. Grade 3-4 non- haematological AEs occurred in 53% of patients, the most common being pneumonia/fever (3/2, 10/7%), hypertension (8, 28%) hiperglicemia (1, 3%), and cardiac failure (2, 7%). Cardiac

AEs were 35% (10 pts). Pts with ≥1 CV risk factor at enrolment had a an increased risk of developing a CV AE during treatment as compared to pts with no CV risk factors.1 pt died of infection (not treatment-related). In pts who developed serious AE, KRD dose reduction (2, 7%) and discontinuation (3, 10%) were applied. Onset of CV events significantly increased the rate of dose reductions and treatment discontinuation. CV AEs may significantly impact treatment compliance and survival.

Table 1.

	Previous exposure to lenalidomide (n = 13) Group A	No previous exposure to lenalidomide (n = 13) Group B	p-value
Median age, years (range)	65 (49-77)	66 (51-78)	0.2
Males, n (%)	10 (77%)	8 (66%)	0.2
Paraproteins (isotype), n (%)			
Immunoglobulin G	10	7	0.13
Immunoglobulin A	1	3	0.6
Light chain only	2	3	0.39
Cytogenetics			
del13q	2	2	>0.99
t4,14 or t 11,14	3	4	>0.99
del17p	1	1	>0.99
normal	7	8	>0.99
Stage ISS			
I	7	6	0.7
II	2	4	0.65
III	4	5	>0.99
Prior treatments			
Single autologous SCT	4 (14%)	1 (3.5%)	0.15
Double autologous SCT	4 (14%)	7 (25%)	0.46
Median of prior treatments	5	2	0.46
Status disease			
Relapse	6 (21%)	12 (43%)	0.0007
Refractory	7 (25%)	3 (11%)	
PFS from the last treatment			
< 6 months	5 (18%)	5 (18%)	0.12
6-12 months	4 (14%)	6 (21%)	0.11
> 12 months	4 (14%)	4 (14%)	0.12
Response after two cycles			
At least PR	5 (18%)	11 (39%)	0.0005
Less than PR	8 (29%)	4 (14%)	
Best response			
At least PR	6 (22%)	12 (43%)	0.0004
Less than PR	7 (25%)	3 (10%)	

Summary/Conclusion: Rate of VGPR obtained with KRd combination was high with an overall response of about 80%. Safety profile was acceptable with a 35% of CV events. Additional analyses are needed to evaluate the impact of these patterns on efficacy outcomes.

PB2189

AUTOLOGOUS STEM CELL TRANSPLANT IN PATIENTS WITH MULTIPLE MYELOMA AND RENAL FAILURE

L. Foster[1,*], S. Rassool[1], Y. Li[1], F. Dignan[2], D. Osborne[3], J. Norman[2], C. Barnes[4], R. Brown[5], C. Lobetti-Bodoni[2], S. Roberts[6], C. Gregory[7], R. Beezley[2], M. Waller[2], S. Choudhuri[3], H. Osman[7], E. Abu-Rashed[2], S. Raj[5], R. Thomas-Dewing[4], M. Rowlands[3], K. Brice[2], J. Sudhir[2], E. Tholouli[2], A. Rocci[2,8]

[1]Medical School, The University of Manchester, [2]Haematology, Manchester Royal Infirmary, Manchester University NHS Foundation Trust, [3]Haematology, Royal Oldham Hospital, The Pennine Acute Hospitals NHS Trust, [4]Haematology, Salford Royal Hospital, Salford Royal NHS Foundation Trust, [5]Haematology, Trafford General Hospital, Manchester University NHS Foundation Trust, [6]Haematology, Royal Bolton Hospital, Bolton NHS Foundation Trust, [7]Haematology, Royal Albert Edward Infirmary, Wrightington Wigan and Leigh NHS Foundation Trust, [8]Faculty of Biology, Medicine and Health, Division of Cancer Science, The University of Manchester, Manchester, United Kingdom

Background: Different degrees of renal impairment are commonly observed in patients with multiple myeloma (MM) due to direct or indirect damage to the kidneys by plasma cells or light chains. Most of the patients do partially recover kidney function, but for those with permanent kidney impairment the treatment options can be limited, particularly in regard to the autologous stem cell transplant (ASCT).
Aims: To retrospectively review the outcome of a cohort of patients with varying degrees of renal failure, treated with ASCT.
Methods: A representative group of patients with MM, treated in the Greater Manchester area and referred to Manchester Royal Infirmary for ASCT between 1/1/2010 and 31/12/2016 have been included in this study. They have been randomly selected from over 300 patients treated with ASCT. Patients received Melphalan 200mg/m^2 or 140mg/m^2 if creatinine clearance (CrCl) <30 ml/min or serum creatinine >200µmol/L. Time To Next Treatment (TTNT) and Overall Survival (OS) curves have been compared using log-rank test.
Results: A cohort of 158 patients was analysed. At the time of ASCT, 71 patients had CrCl ≥90ml/min (normal), 51 patients had CrCl of 60-89 ml/min (Chronic Kidney Disease (CKD) 2), 22 patients had CrCl of 30-59 ml/min (CKD 3) and 14 patients had CrCl of ≤30ml/min (CKD4, including 3 patients on dialysis). As expected, induction treatment differed according to CrCl: 70% patients with CKD1+2 received a Thalidomide-containing induction compared to 44% of those with CKD3+4, whilst a Bortezomib-based regimen was used in 26% *vs* 55%, respectively. The response after induction was similar: in the patients with CrCl ≥60 the CR was 32% (38% in patients with CrCl <60), VGPR was 48% (vs 49%), PR 18% (vs 13%), MR 1% (vs 0%) and PD 1% (vs 0%). Three months after ASCT, both groups' responses improved: CR 43% in patients with CrCl ≥60 (vs 62%), VGPR 47% (vs 25%), PR 9% (vs 10%) and 1 patient had PD in the CrCl<60 group. These translated into a 5-year TTNT of 65.6% for the patients with CrCl ≥60 *vs* 50.6% for patients with CrCl <60 (HR 0.526, CI 0.255-1.086, p=0.0824). The 5-year OS was the same at 81.6% for both groups (HR 0.848, CI 0.365-1.969, p=0.701). The toxicity profile in the 2 groups was largely similar: in patients with CKD1+2 the median days of hospitalisation for ASCT was 19, compared to 20 for patients with CKD3 and 23 for those with CKD4, and the median number of days with CRP >50mg/L was 5 and 7, respectively. Median time to engraftment showed no differences between the CKD1+2 and CKD3+4: Absolute Neutrophil Count >1x10^9/L in 13 *vs* 12.5 days, platelets >100x10^9/L in 19 *vs* 22 days and haemoglobin >10g/L after 28 *vs* 29 days, respectively. Rates of readmission to hospital were lower in the CKD1+2 group at 11.5% compared to 19.4% in CKD 3+4. Moreover, median days of readmission per patient were lower in CKD1+2 groups: 6.7 *vs* 10. Of note, sequential eGFR measurements in patients with CKD3+4 showed an improvement in kidney function over time: in patients with CKD 3 (and CKD 4) the median eGFR at diagnosis was 35 (and 12.5), pre-ASCT was 46 (and 20), 3 months after ASCT was 45.5 (and 20.5) and 12 months after ASCT was 42.0 (and 25).
Summary/Conclusion: Patients with MM and kidney impairment seem to benefit from ASCT to a similar level to patients with normal kidney function in terms of quality of response and long term survival. Patients with poorer kidney function show greater improvement in eGFR following ASCT. A higher rate of readmission has been observed in patients with kidney impairment, suggesting the need for closer follow-up in this particular population.

PB2190

KRD TREATMENT OF RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS: A REAL LIFE EXPERIENCE

G. Barilà[1,2,*], V. Meneghini[1,3], A. Bonalumi[1,3], A. Furlan[1,4], F. Patriarca[1,5], F. Colasante[1,5], F. Scognamiglio[1,6], R. Sartori[1,7], M. Rupolo[1,8], M. Tinelli[1,9], N. Pescosta[1,10], C. Minotto[1,11], A. Liço[1,2], T. Berno[1,2], G. Semenzato[1,2], R. Zambello[1,2]

[1]Multiple Myeloma Triveneto Network, Triveneto, [2]Department of Medicine, Hematology and Clinical Immunology section, Padua University School of Medicine, Padua, [3]Department of Medicine, Section of Hematology, University Hospital of Verona, Verona, [4]Hematology Unit, Santa Maria di Ca' Foncello Hospital, Treviso, [5]Clinica Ematologica, Azienda sanitaria Universitaria Integrata, DAMe, Università di Udine, Udine, [6]Department of Hematology, San Bortolo Hospital, Vicenza, [7]Department of Medicine, Section of Hematology, Hospital of Castelfranco Veneto, Castelfranco Veneto, [8]Department of High Dose and Cellular therapy, C.R.O. Aviano hospital, Aviano, [9]U.O.C. Internal Medicine, San Bonifacio hospital, San Bonifacio, [10]Ematologia e Centro TMO, Ospedale Centrale Bolzano, Bolzano, [11] UOC di Oncologia ed Ematologia Oncologica, Mirano hospital, Mirano, Italy

Background: Multiple Myeloma (MM) is a hematologic neoplasm characterized by the proliferation of malignant clonal plasmacells in the bone marrow. The outcome of MM patients has significantly improved in the last years with the introduction of proteasome inhibitors (PI) and immunomodulatory (IMIDs) drugs as bortezomib and lenalidomide respectively. Besides, triplet regimens containing IMIDs with PI or monoclonal antibodies represent nowadays the backbone treatment for relapsed patients treatment. In detail, in the ASPIRE trial, the combination of second generation PI carfilzomib with lenalidomide and dexamethasone (KRD) led to a unprecedented results in term of high quality responses and progression free survival (PFS) with respect to lenalidomide-dexamethasone standard treatment. These results led to the approval in 2016 in Italy of KRD treatment for relapsed MM patients.
Aims: Here we report a multicentric real life experience of treatment with KRD in relapsed MM patients on behalf of the Gruppo Mieloma Triveneto.
Methods: A cohort of 80 patients affected by relapsed MM patients according to IMWG criteria was treated with KRD, in 10 hematological unit of Triveneto, Italy. Clinical characteristics, including response to treatment and toxicities, were collected.
Results: Median patient's age was 62 years (42-76) with 32/80 patients (40%) with >65 years. Patients previously received a median of 2 lines of

therapy (1-7), with 25/80 (31%) underwent to at least 3 regimens. Most patients (54/80, 68%) were treated due to clinical relapse, with 9/54 (17%) developing extramedullary disease, while only 26 patients (32%) received KRD due to biochemical relapse. With a median number of 5 cycles (1-17) of KRD received, the overall response rate (ORR) was 76% (61/80), including 40% of high quality response (21% Very Good Partial Response [VGPR] and 19% Complete Response [CR]). ORR and high quality response rate were higher although not significantly different among patients who previously received 1-2 lines of therapy towards patients who received >2 regimens (80% *vs* 65%, p=0.16 and 53% *vs* 35%, p=0.15). Eight patients (10%) during KRD treatment underwent to peripheral blood stem cells (PBSC) apheresis, 7 using high dose cyclophosphamide, and one using lenograstim with plerixafor. Almost all patients (7/8, 88%) completed PBSC collection, with a mean 7.39x10^6/Kg PBSC harvested. Besides, 7 patients already received stem cell transplantation, 5 using autologous PBSC and 2 using allogenic PBSC. Hematological toxicities were mild with neutropenia present in 27% of patients, thrombocytopenia in 26% and anemia in 11%; grade 3 toxicities were even lower (11%, 9% and 4% respectively). Non hematological toxicities were mostly infective, with 22 patients (28%) involved (with 8% of grade 3 events). Cardiovascular events including hypertension and heart failure were mild, with 8% of any grade events and only 5% grade 3 events. With a median follow up of 5 months, median PFS not reached; moreover, median PFS of patients who received at least 2 lines of therapy is already significantly higher than in patients who received >2 lines of therapy (17 months *vs* undefined, p=0.03).

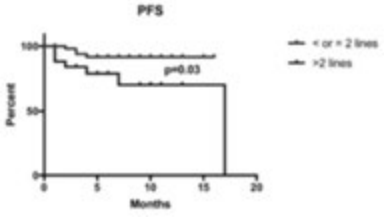

Figure 1.

Summary/Conclusion: In our experience, KRD treatment is feasible and effective in a non selected cohort of relapsed MM patients. The safety profile was acceptable, with main toxicities being represented by hematological and infective events, while cardiovascular side effects were fewer and less serious. Although our results need to be confirmed with a longer follow up, we point to the worst outcome in term of PFS of heavily treated patients.

PB2191

ORAL CYCLOPHOSPHAMIDE'S ADDITION IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS WITH BIOCHEMICAL PROGRESSION DURING LENALIDOMIDE-DEXAMETHASONE TREATMENT

L. Cesini[1,*], A. Siniscalchi[2], S. Grammatico[1], A. Andriani[3], A. Fiorini[4], L. De Rosa[5], T. Za[6], A. Rago[7], T. Caravita[2], M.T. Petrucci[1]

[1]Department of Cellular Biotechnologies and Haematology, Sapienza University of Rome, [2]Institute of Haematology, S. Eugenio Hospital, [3]Department of Haematology, Nuovo Regina Margherita Hospital, Rome, [4]Department of Hematology, Belcolle Hospital, Viterbo, [5]Department of Haematology, S. Camillo Hospital, [6]Institute of Haematology, Catholic University, Rome, [7]Department of Cellular Biotechnologies and Haematology, Sapienza University of Rome, Polo Pontino, Latina, Italy

Background: Multiple Myeloma (MM) remains an incurable disease despite recent advances in therapy and supportive care. Retreatment for relapsed-refractory multiple myeloma (RRMM) represents one of the therapeutic challenge as well as the change of family drugs. Alkylating drugs have been the standard of care for MM patients (pts) for many years, with melphalan and prednisone (MP) being considered the standard approach for pts not eligible for autologous transplantation. Lenalidomide combined with dexamethasone (Rd) is one of the current standards for treatment of RRMM. However the majority of pts become resistant during the treatment.
Aims: We evaluated the addiction of cyclophosphamide (CRd) in RRMM pts who experienced biochemical relapse during Rd treatment, to slow down the progression in active relapse.
Methods: This analyses included 31 pts with RRMM treated with Rd who received cyclophosphamide at biochemical relapse. Data were collected in 7 Italian Centers of the Multiple Myeloma GIMEMA-Latium Region Working Group. The median age of pts was 70 years (range: 50-86); 15 males and 16 females. The median line of previous treatments was 1 (range: 1-4). Median time from diagnosis to the first CRd cycle was 32.4 months (range: 8.5-187). The CRd regimen was continued in responding pts or in stable disease (SD) until disease progression (PD).
Results: All pts received Rd with a median of 15 cycles (range: 1-52). Responses to Rd were observed in 18 pts (58%), particularly 12 partial responses (PR), 3 very good partial responses (VGPR) and 3 complete rsponses (CR). Three pts maintained a SD. Grade 3/4 side effects were neutropenia (19%), anemia (9.7%), muscle cramps (9.7%), polyneuropathy (9.7%), diarrhea (6.5%), infections (6.5%), esophagitis (3.2%) and rash (3.2%). One patient experienced pulmonary embolism (3.2%). All 31 pts developed a biochemical relapse during Rd treatment, with a median time of 18.5 months (range: 1-49) and received cyclophosphamide without lenalidomide dose modification. The median number of CRd cycles administered was 8 (range: 1-35) and the median time to response after CRd was 2.5 months. A response was observed in 9 (29%) pts, particularly 3 VGPR and 6 PR. Ten patients obtained a SD and 12 a PD. Among the responding pts, 3 are still in therapy with CRd. No correlation between Rd and CRd responses was observed. After a median observation time of 40 months, the median OS from the diagnosis of MM was 78.8 months, while the median OS from the beginning of CRd was 17.7 months. No significant difference in OS was observed between pts treated with 1 or >1 prior antimyeloma regimen (OS 22.9 months *vs* 13.5 months, p=0.65). The age at diagnosis (≥ or <70 years) was not predictive of outcome, with a median OS of 17.7 and 13.8 months for patients ≥ of 70 or <70 years, respectively (p=0.94). In addition median time to progression (TTP) was 7.6 months (range: 1-33) and the median PFS from the beginning of CRd was 13.1 months. No additional adverse events were observed by adding cyclophosphamide to Rd. After a median follow up of 11 months (range: 1.3-50.9), there were no treatment-related deaths, but 17 pts (55%) died for progressive myeloma, with a median time of 9.1 months (range: 1-38).
Summary/Conclusion: We confirm that Rd is an effective and well tolerated regimen for RRMM pts, inducing a high response rate. However, the addition of oral cyclophosphamide delays the progression in few pts, who present a biochemical relapse during Rd treatment.

PB2192

EARLY RELAPSE AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION IS A MAJOR PREDICTIVE FACTOR OF LOWER OVERALL SURVIVAL IN MULTIPLE MYELOMA PATIENTS

A. Roque[1,2,*], M.C. Afonso[2], E. Cortesão[1,2], A.I. Espadana[2], A.B. Sarmento-Ribeiro[1,2], C. Geraldes[1,2], M.L. Ribeiro[2]

[1]Faculty of Medicine, University of Coimbra, [2]Clinical Hematology Department, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

Background: The new generation drugs and consolidation of response with high-dose chemotherapy and autologous hematopoietic stem cell transplantation (aHSCT) have revolutionized the prognosis of multiple myeloma (MM) patients (pts). However, MM remains a not curable disease and ultimately all pts will relapse, with different studies indicating 16-20% of relapse in the first year after aHSCT. Nevertheless, the relevance of early relapse after aHSCT in prognosis remains unclarified in the era of more and more effective and diversified treatments.
Aims: We aimed to evaluate the characteristics of pts that early relapse after aHSCT as well as its impact in overall survival (OS).
Methods: We analyzed 207 consecutive aHSCT performed in MM pts in our centre between January/2007 and December/2016. Pts were divided into two groups: R1 (relapse ≤12 months from aHSCT) and R2 (either relapsed >12 months after aHSCT or are disease free at the last follow up). The assessment of response was based on the International MM Working Group consensus criteria (2016).
Results: The median age of the cohort was 59 (27-70) years, 59.4% were male, which was similar between the two groups (p=NS). Thirty-two pts (15.5%) relapsed in the first year post-aHSCT (R1), while 175 (84.5%) relapsed after 1 year or maintain response at the time of the last follow-up (R2). Considering the pts with follow up over 3 years in group R2 (n=127), 30 (23.6%) relapsed between 12-36 months post-aHSCT, 27 (21.3%) relapsed after 3 years and 70 (55.1%) did not relapse after a median follow up of 54.6 months. Clinical and laboratorial characteristics such as International Staging System score, bone involvement, extramedullary disease and cytogenetic alterations were similar between the two groups. The percentage of pts treated with IMIDs was higher in R1 group (34.4 *vs* 16.6%; p=0.02), while the number of pts treated with proteasome inhibitor, the number of previous therapeutic lines, and the time between diagnosis and aHSCT were similar (p=NS). The percentage of patients with complete response (CR) criteria before aHSCT was similar between R1 e R2 (p=NS). However, there was a significant difference when we considered the achieve-

ment of CR 100 days after aHSCT, that was higher in group R2 (9.7 *vs* 13.8%; p<0.001). The early relapse group presented lower OS after diagnosis (36.3 months *vs* not reached [NR]; *Hazard ratio* [HR]=0.18; p<0.001) as well as after aHSCT (15.4 months *vs* NR; HR=0.14; p<0.001). Considering the group of pts that relapsed during the follow-up (n=89), the OS after relapse was significantly higher in those who relapse after the first year (11.3 *vs* 79.9 months; HR=0.43; p=0.015).
Summary/Conclusion: This study corroborates the concept that early relapse after aHSCT is a major negative prognostic factor, in the era of novel agents, with the sustainability of response being a very important variable. No clinical or analytical variables analyzed seemed to influence relapse timing.
Relapse at the first year correlated inversely with the depth of response at day 100 after aHSCT, in a more relevant way compared with the previous response. These results, conjoining with the absence of correlation between the time diagnosis-aHSCT and the time of relapse, suggest that aHSCT should not be delayed in order to obtain a prior deeper response.

PB2193

Abstract withdrawn.

PB2194

Abstract withdrawn.

PB2195

LIGHT-CHAIN DEPOSITION DISEASE: DIAGNOSIS AND TREATMENT CHALLENGES. A SINGLE CENTER EXPERIENCE

S. Badelita[1,*], A. Jercan[1], R. Irimia[1], M. Popescu[1], D. Vasilache[1], C. Pantoc[2], D. Coriu[3]

[1]Hematology and Bone Marrow Transplant Department, Fundeni Clinical Institute, [2]Nephrology, Carol Davila, [3]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

Background: Light-chain deposition disease is characterized by deposition of nonamyloid monoclonal light chains in multiple organs. It is a rare disease caused by overproduction of kappa chains and very rarely lambda chains by a single clone of plasma cells. LCDD can occur in any organ but kidneys are always involved as a rapidly progressive glomerulonephritis or acute tubulointerstitial nephritis. A mandatory step in LCDD diagnosis is identifying the monoclonal pathological light chain in serum or urine. LCDD should be distinguished from other monoclonal proteins associated diseases like cryoglobulinemia, myeloma cast nephropathy, amyloidosis and Fanconi syndrome.
Aims: Large multicenter series of patients are needed to develop an optimal standardized treatment strategy.
Methods: We present a single center experience with twelve cases of LCDD diagnosed betweed 2015 and 2017. We assessed the patients with the same panel of investigations used in the case of amyloidosis, including serum electrophoresis and immunofixation, free light chain assay, proteinuria, fibroscan, echocardiogram, electromyography and whole body-low dose CT. Renal biopsy, bone marrow aspirate and biopsy, abdominal fat biopsy and salivary gland biopsy were performed on every patient. All diagnosis were confirmed following renal biopsy (electronic microscopy and immunofluorescence) that presented glomeruli with dense linear subendothelial deposits in capillary loops; patchy dense deposits ; mezangiosclerosis areas; multiple endothelial detachments; disorganised tubular basal membrane with spidery, linear dense deposits.In our group of patients all abdominal fat biopsies were Congo red stain negative under polarized light.
Results: Eleven of them had kappa LCDD and one presented lambda LCDD. All the patients had a bone marrow plasmacytic infiltrate of under 10%. None of them had osteolytic bone lesions. Three patients had hepatic involvement (Fibroscan >8,6 kPa) and one patient presented restrictive cardiomiopathy.Two patients had Sicca Syndrome. Electromyography showed sensitive neuropathy in one patient, chronic motor-sensitive axonal polyneuropathy in one patient and chronic demyelinating polyneuropathy in one patient. Renal involvement was present in six patients as stage 3 CKD, two had CKD stage 4 and the other four stage 5 CKD. Four patients required hemodyalisis initiation. Three patients presented with nephrotic syndrome and seven patients had subnephrotic range proteinuria. Three had microscopic haematuria. Nine patients presented with secondary hypertension.
Ten patients received Bortezomib-based therapy and two patients received Melphalan based therapy. Two of them underwent autologous bone marrow transplant without complications and without additional renal function reduction. After the completion of the treatment, in 2018 all twelve patients are alive with controlled disease. None of them required therapy resumption. Four patients had CR/VGPR and three of them showed renal function improvement. ASCT was performed even in advanced CKD stages.
Summary/Conclusion: LCDD should be considered in patients with MGUS and CKD. Bortezomib based therapy followed by ASCT showed promising results.

PB2196

EFFECTIVENESS OF BENDAMUSTINE IN RELAPSED OR REFRACTORY AL AMYLOIDOSIS

N. Forgeard[1,*], S. Harel[1], A. Talbot[1], B. Royer[1], I. Madelaine[2], M. Vignon[1], M. Baron[1], J. Verine[3], J.-P. Fermand[1], B. Arnulf[1]

[1]Immuno-hematology, [2]Pharmacy & Chemotherapy preparation Unit, [3]Anatomopathology, Hôpital St-Louis, Paris, France

Background: Treatment of AL amyloidosis (non IgM) currently aims at suppressing amyloidogen free light chains by targeting the underlying pathological plasmocyte clone. First line therapy relies on melphalan or bortezomib-cyclophosphamide with dexamethasone. However, there is no standardized treatment for relapsed or refractory patients. Bendamustine is an alkylating agent already used in multiple myeloma, with low cardiac, nephrologic or neurologic toxicities, and has already been studied in two series (one prospective and the other retrospective) without available data concerning organ response.
Aims: Our aim was to assess efficacy and tolerance of bendamustine as a salvage therapy in relapsed of refractory non IgM AL amyloidosis.
Methods: We retrospectively studied the data of 22 patients who received bendamustine for relapsed or refractory amyloidosis (IgA, IgG, kappa or lambda free light chain), treated in our center between 2012 and 2016. Patients had to receive at least 3 courses to be included. IgM amyloidosis were excluded. Bendamustine was administered in 28 days cycles, in association with high dose steroids. Associated treatments were determined by physicians on a case-by-case basis. Immunochemical and organ responses were assessed according to the First Roundtable on Clinical Research in Immunoglobulin Light-Chain Amyloidosis guidelines.
Results: The 22 patients (13 men, 9 women, median age 58 years) in this study had previously received a median number of 2 lines of therapy (melphalan 59%, bortezomib 86%, ASCT 9%). Organ involvement prior to therapy was as follows: heart 64%, renal 68%, liver 23%, GI tract 23%, neurologic 45%. Five patients (23%) had associated smoldering multiple myeloma. All patients received associated treatment with high dose steroids, 5 (23%) received bortezomib and 2 (9%) received thalidomide. Twelve patients (55%) achieved haematologic response, with complete response in 1 case (5%) and VGPR in 4 cases (18%). Among patients with hematologic response, 5/12 (42%) experienced organ response. The median overall survival was 31 months. Among patients who received ulterior treatment, the median time to next treatment was 5.2 months. Severe adverse events (grade 3 or 4) occurred in 6 patients (27%), among whom 5 experienced febrile neutropenias. One patient previously treated with melphalan, cyclophosphamide and lenalidomide developed secondary AML with complex caryotype, 3 years after 8 courses of bendamustine, of fatal evolution.
Summary/Conclusion: These results concur with those previously reported, and confirm bendamustine's efficacy from an immunochemical point of view, resulting in organ response in 42% of cases. Immediate tolerance is good, but the potential delayed occurrence of secondary myeloid hemopathies should be taken into account, as is the case with all alkylating agents.

PB2197

FACTORS ASSOCIATED WITH RENAL FAILURE AND RENAL FUNCTION RECOVERY IN MULTIPLE MYELOMA

A. Senturk Yikilmaz[1,*], S.M. Bakanay[1], S. Akinci[2], M. Gündüz[2], S. Küçükyurt Kaya[1], S. Doğan[1], I. Dilek[1]

[1]Department of Hematology, Yıldırım Beyazıt University, [2]Department of Hematology, Atatürk Education and Research Hospital, Ankara, Turkey

Background: Renal failure is a common initial presentation of multiple myeloma (MM) and causes significant morbidity and mortality.
Aims: This study aimed to investigate the factors associated with renal failure and renal recovery in MM.
Methods: The clinical features and prognostic factors were retrospectively analyzed for 169 newly diagnosed MM patients at single center. Statistical analyzes were performed using chi-square test using SPSS version 16.0 (SPSS

Inc., Chicago, IL, USA). A value of less than 0.05 was considered significant.The patients with renal failure (Cre ≥2mg/dl) at diagnosis were compared with the cases with normal renal function (Cre<2 mg/dl). Patients with renal failure at diagnosis were further analyzed with regards to renal function recovery after 2 and 4 cycles of anti-myeloma treatment.
Results: The median age of the patient population was 64.8 (39-88) years with male/female 98/71. Forty (23.7%) patients presented with renal failure and 19 patients required hemodialysis. According to the International Scoring System, 43(25.4%) patients had low (I), 46(27.2%) patients had intermediate (II), and 80(47.3%) patients had high (III) ISS score. There were no significant difference between the patients presenting with normal renal function (RN) and the patients presenting with renal failure (RF) in terms of age, gender, bone involvement, the ratio of first line bortezomib containing regimen. Compared with the RN group significantly more patients in RF group had light chain myeloma (p=0.028), high ISS (p=0.003), elevated serum lactate dehydrogenase value (p=0.001), initial hypercalcemia (p=0.001) and anemia (p=0.002). Response rates (≥partial response (PR)) after 2 cycles of anti-myeloma treatment and referral to high dose therapy and autologous transplantation were similar in both groups. However, in the RN group, the ratio of patients achieving ≥PR after 4 cycles of treatment was higher than RF group (p=0.019). Sixteen (42%) of the forty patients with renal failure at the time of initial diagnosis fully recovered after first line anti-myeloma treatment. Patients whose renal functions recovered (RFc) were compared with the patients whose renal functions did not recover (RFnc). There was no statistically significant difference between the RFc and RFnc groups in terms of age, gender, ISS, serum lactate dehydrogenase level, hypercalcemia, anemia, bone involvement and initial requirement for hemodialysis. Response (≥PR) to 2 cycles of anti-myeloma treatment was similar in each group. On the other hand, having light chain myeloma (p=0.012) and lambda light chain (p=0.002) had negative impact on renal function recovery. Fourteen of the 27 patients (51.9%) who received bortezomib containing first line treatment had renal function recovery. Renal function recovery was significantly associated with the use of bortezomib in first line therapy (p=0.004).
Summary/Conclusion: Initial presentation with renal failure is a poor prognostic feature and has negative impact on response to anti-myeloma treatment. On the other hand, renal function recovery is not always parallel to myeloma response. Along with sufficient supportive therapy, having a non-light chain myeloma and prompt initiaion of bortezomib based first line treatment seem to be best indicators of renal function recovery.

PB2198

DEGREE OF VULNERABILITY IN PATIENTS WITH MULTIPLE MYELOMA: A COMPARATIVE STUDY BETWEEN THE APPLICATION OF CLINICAL JUDGMENT AND THE SCALE OF ASSESSMENT (GA SCALE)

E. Abella*, F. García-Pallarols, R. Ben Lashen, A. Senín, L. Martinez-Serra, S. Montesdeoca, I. Párraga, E. Torres, D. Román, M. Ferraro, A. Angona, C. Besses

Hematology Department, Hospital del Mar/IMIM, Barcelona, Spain

Background: The International Myeloma Working Group (IMWG) has defined a frailty score (GA scale) based on comorbidity index, Charlson score and Katz basic activities of daily living scale for elderly myeloma patients. This score has demonstrated to be more sensitive for geriatric assessment and aids in choosing the most appropriate treatment for each patient. Although GA scale calculation is defined as easy, fast and effective, it is sometimes difficult, due to time constraints, to perform it during the pre-treatment visit. For this reason, the therapeutic decision is still often based on clinical judgment.
Aims: To compute the concordance between clinical judgment and GA scale. In discordant cases, to investigate whether the therapeutic decision has been adequate, assessing toxicity, tolerance and treatment discontinuation.
Methods: From 2015 to 2017, 47 evaluations in 43 patients were performed. The clinical judgment was established during the clinical visit considering age, ECOG, family support and cognitive evaluation and patients were classified as Fit, Unfit and Frail. The GA scale was calculated by the case manager before or during the first cycle of treatment except for 3 patients who did not finally receive treatment. Discordant cases were revised according to the clinical follow up. We defined as undervalued and overvalued, patients with a clinical judgment lower or greater than GA scale respectively.
Results: Twenty-five evaluations were done in patients between 65-75 years and 22 in patients >75 years. Discordant results have been found in 21/47 cases (44%) with a low concordance (Kappa= 0,338). Eleven cases were undervalued and 10 cases were overvalued. The treatment was discontinued in 3/11 (27%) cases of the undervalued group and 2/10 (20%) cases of the overvalued group. In both groups, toxicity was the main cause of treatment discontinuation. There were no treatment related deaths. There were no significant differences in number of discrepancies between age groups (p=0.96).
Summary/Conclusion: A low concordance between clinical judgment and GA scale was observed (Kappa=0.338).For discordant cases, discrepancies that led to treatment discontinuation were observed in more than 20% of the cases (20% in overvalued group and 27% in the undervalued group). Both the low concordance observed and the amount of significant discrepancies demonstrate once again that the application of the GA scale in elderly patients is more sensitive than the clinical assessment to establish the most appropriate treatment.

PB2199

IMPACT OF IMPROVEMENT IN RENAL FUNCTION AND WAITING TIME BEFORE INDUCTION THERAPY ON SURVIVAL IN NEWLY DIAGNOSED MULTIPLE MYELOMA

N. Wohner*, G. Varga, P. Farkas, L. Horvath, G. Szombath, E. Horvath, A. Balogh, A. Masszi, J. Varkonyi, T. Masszi

3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary

Background: Multiple myeloma (MM) is the second most common haematological malignancy among adults in Hungary. Renal impairment is frequent in MM patients and has fundamental impact on overall survival (OS).
Aims: Our research question was how long the waiting time of patients is from the detection of renal impairment (GFR <40 mL/min) until the start of induction therapy and how this waiting time influences OS. We also studied if reversibility/irreversibility of renal failure has an impact on OS.
Methods: We analyzed the medical history of 212 MM patients at Semmelweis University 3rd Department of Internal Medicine, Budapest, Hungary from 2007 to 2017. We studied if renal impairment (GFR <40 mL/min) was reversible (GFR >40 mL/min) or irreversible, as well as how long the patients waited from the diagnosis of renal disease until the first induction therapy for MM. We also compared ISS and FISH data and determined how all these factors influence the OS.
Results: One-third of the patients (74/212) had renal impairment at diagnosis, and in half of these cases (36/74) the renal function was reversible. Before 2012 the renal function was not reparable in 80% of patients, however after 2012 the ratio decreased to 53%, most probably due to the introduction of new generation drugs. OS was significantly lower in patients with irreversible renal failure compared to those without renal impairment (15 *vs* 55 months, p=0.013), however we did not find similar significant difference in patients whose renal impairment was reversible (55 *vs* 47 months, p=0.32). Importantly, the waiting time of those whose renal failure became irreversible was significantly longer compared to reversibly affected patients (7 weeks *vs* 2.5 weeks, p<0.0001).
Summary/Conclusion: Our results point out the importance of speed in the diagnosis of MM. Any delay in the diagnosis and start of the induction therapy worsens the outcome of patients with MM and renal failure. Despite the positive tendency that we detected in the last few years we still have to improve the interdisciplinary cooperation in order to diagnose and treat MM patients as soon as possible.

PB2200

Abstract withdrawn.

PB2201

COMPARING EFFICACY OF INITIAL INDUCTION IN NEWLY DIAGNOSED MULTIPLE MYELOMA. A VALUABLE INFORMATION IN POPULATION-BASED REGISTRIES

R. Ríos-Tamayo[1,*], M.J. Sánchez Pérez[2], D. Sánchez-Rodríguez[3], A. Martín García[4], Y.-L. Chang-Chan[2], S. Sequera[5], J.L. García de Veas Silva[6], J. Sainz Pérez[7], A.B. Rivera Ginés[1], M.E. Clavero Sánchez[1], C. Alarcón Payer[6], M. Jurado Chacón[7]

[1]Monoclonal Gammopathies Unit, University Hospital Virgen de las Nieves, [2]Granada Cancer Registry, Andalusian School of Public Health, [3]Monoclonal Gammopathies Unit, FIBAO, [4]Clinical Trials Unit, [5]Pharmacy, [6]Immunology, [7]Hematology, University Hospital Virgen de las Nieves, Granada, Spain

Background: Prognosis in multiple myeloma (MM) depends on specific patient and disease characteristics, staging and response. Strong evidence supports the quality and duration of the response, in accordance with IMWG criteria, as a key prognostic factor. Therefore, a careful monthly monitoring of response is mandatory, as well as a global evaluation after

each phase of the treatment. Outside clinical trials (ct), heterogeneity remains in relation to response evaluation of real-life patients. Even in the setting of population-based MM registries, the response is rarely reported.
Aims: Assessing the evolution of the response to old and current induction regimens.
Methods: The Granada MM Registry is the second largest single-institution population-based registry (Ríos-Tamayo R *et al.*2015), working since 1985. Evaluation of the response was prospectively recorded since 2012, and retrospectively during the previous period. IMWG criteria have been used, including imaging (PET/CT) and flow minimal residual disease (MRD)(Kumar S *et al.*2016). All consecutive newly diagnosed MM (NDMM) patients with residence in our reference area, which were considered fit to receive the standard induction regimen at the moment of diagnosis, were included. Overall response rate (ORR) and complete response (CR) rate have been pointed out. When information was not available, the response was informed as unknown/not valuable.

Table 1.

REGIMEN	n	ORR %	CR %	MRD NEG%	UNKNOWN	CLINICAL TRIAL
MP	25	20	4	-	44	N
VAD	74	50.1	4.1	-	33.8	N
VCMPVBAD	6	66.6	33.3	-	16.7	N
VD	58	58.6	19	3.5	22.4	N
VMP	53	52.9	18.9	-	35.8	N
VCD	97	79.3	24.7	2.1	8.3	N
VRD	20	95	70	35	5	Y(18)
VTD	3	100	33.3	-	0	N
PAD	4	75	25	-	25	N
RD	9	66.7	55.6	22.2	22.2	Y(3)
RDC	4	100	25	25	0	Y
KRD	6	100	66.7	33.3	0	Y

Results: 359 NDMM patients have been evaluated in relation to 12 regimens, including two or more drugs: melphalan-prednisone (MP), vincristine-adriamycin-dexamethasone (VAD), V-BCNU-M-cyclophosphamide-P/V-BCNU-A-D (VBMCP/VBAD), bortezomib-D (VD), VMP, VCD, V-lenalidomide-D (VRD), V-thalidomide-D (VTD), bortezomib-A-D (PAD), RD, R-D-clarithromycin (RDC), carfilzomib-R-D (KRD). Table 1 shows the number (n) of patients in each combination, ORR and CR rates. If the regimen has been used in the context of a ct, it has been indicated.
Summary/Conclusion: The information about response should be prospectively recorded in MM population-based registries. The retrospective evaluation of response in old regimens is inconclusive due to a high proportion of unknown response. The new triplets, particularly VRD and KRD, whose approval in real-life is still pending, are highly effective as induction. Triplets are more effective than doublets. However, selected patients treated with doublets can achieve CR with MRD negativity. The heterogeneity in response highlights the need to implement a risk-adapted, individualized, and patient-centered therapy.

PB2202

EFFICACY OF NEW DRUGS PRIOR TO SINGLE OR TANDEM AUTOLOGOUS STEM CELL TRANSPLANTATION IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS. A SINGLE CENTER EXPERIENCE

S. Grammatico[1,*], E. Lisi[1], F. Vozella[1], L. Cesini[1], A. Piciocchi[2], S. Capria[1], R. Foà[1], M.T. Petrucci[1]

[1]Department of Cellular Biotechnologies and Hematology, Policlinico Umberto I, [2]GIMEMA Foundation Data Center, Roma, Italy

Background: In the last decade numerous studies have shown that proteasome inhibitors and immunomodulatory drugs lead to notable advancements over conventional chemotherapy in multiple myeloma patients. Indeed, a 3-drug regimen with these novel agents is the best induction treatment for young newly diagnosed multiple myeloma (YNDMM) patients before autologous stem cell transplantation (ASCT).
Aims: To compare the data reported in the literature with the "real-life" clinical practice at a single Center. We report our experience over the last 25 years comparing the efficacy of old drugs *vs* novel agents. In addition, the responses obtained with a single *vs* double ASCT in this cohort of patients were also evaluated.
Methods: We retrospectively analyzed 258 YNDMM patients treated at our Center between October 1988 and November 2014. All patients received old or novel agents as induction treatment, followed by stem cell mobilization with high-dose cyclophosphamide plus filgrastim and conditioning chemotherapy with melphalan at 200 mg/m^2. Tandem ASCT was performed sequentially 3-6 months after the first transplant. The primary endpoint was overall survival (OS) and the secondary end point progression-free survival (PFS).
Results: Between October 1988 and October 2008, 173/258 patients received as induction treatment old drugs, *i.e.* VAD-vincristine, doxorubicin and dexamethasone (n=167) or MP- melphalan and prednisone (n=6), while, between February 2005 and November 2013, 85/258 patients were treated with novel agents, *i.e.* bortezomib-based (n=67) or IMiD-based regimens (n=18). All 258 patients received high-dose melphalan and a single (n=153) or tandem (n=105) ASCT. Patients treated with old drugs obtained a CR/nCR/VGPR in 17.9% of cases after induction, in 28.9% after a single ASCT and in 34.5% after a tandem ASCT. Patients treated with new drugs obtained a CR/nCR/VGPR in 42.3% of cases after induction, in 42.3% after a single ASCT and in 54% after a tandem ASCT. The contribution of the second ASCT was statistically significant for patients treated with both old and new drugs (p=0.005 *vs* p=0.001).Patients in CR/nCR/VGPR after induction showed a better OS and PFS at 10 years (62.1% *vs* 40.7%; p=0.0632 and 36.2% *vs* 17.2%; p=0.0685).Patients treated with new drugs had a slightly better OS than patients treated with old drugs (66.1% *vs* 51.5%, p=0.26) and a significantly better PFS (55% *vs* 25.3%, p=0.0047). Among our 258 patients, 144 presented a first relapse. They had been treated as follows:1) 51 patients (35.4%) received old agents as first and second line therapy; 2) 79 patients old agents for first line therapy and novel agents for second line therapy (54.9%); 3) 2 patients novel agents for first line therapy and old agents for second line therapy (1.4%); 4) 12 patients novel agents both as first and second line treatment (8.3%). Our analysis focused on groups 1 and 2. OS and PFS at 10 years were better for patients of group 2 (20.4 *vs* 2.4%; p <0.0001 and 10% *vs* 2.3%; p=0.02). PFS2 (the interval from treatment start at the onset to the second relapse) at 10 years was also significantly better for group 2 (25.7% *vs* 9.2%; p=0.0002).
Summary/Conclusion: Our real-life experience confirms that novel agents have replaced the old drugs as induction treatment prior to ASCT in YNDMM patients. Moreover, according to our results, tandem ASCT still maintains an important role even in the era of novel agents. Novel agents have also a significant impact in the subsequent lines of treatments.

PB2203

BENDAMUSTINE-BORTEZOMIB-DEXAMETHASONE (BVD) IN HEAVILY PRETREATED MULTIPLE MYELOMA

C. Cerchione[1,*], L. Catalano[1], D. Nappi[1], A.E. Pareto[1], S. Basile[1], L. Marano[1], I. Peluso[1], L. Simeone[1], O. Vitagliano[1], S. Palmieri[2], S. Rocco[2], G. Monaco[2], A. Romano[3], F. Pane[1], F. Ferrara[2]

[1]Hematology - Department of Clinical Medicine and Surgery, University Federico II, Naples, [2]Hematology, A.O.R.N. Cardarelli, Napoli, [3]Department of General Surgery and Medical-Surgical Specialties, Haematology Section, University of Catania, Catania, Italy

Background: Bendamustine is a bifunctional alkylating agent, with low toxicity, proved to be effective in relapsed, refractory and in new diagnosed Multiple Myeloma (MM).
Aims: It has been evaluated efficacy and tolerance of Bendamustine, in combination with bortezomib-dexamethasone (BVD) in patients with relapsed and refractory MM (rrMM), whose prognosis is particularly severe. A regional retrospective real-life analysis of patients with rrMM who had been treated with BVD as salvage therapy has been performed.
Methods: 56 patients (31 M/25 F), with rrMM, median age at diagnosis 57.3 years (r. 36-82), median age at start of treatment 61.8 years (r.37-83) treated with several lines of treatments (median 6, r. 2-11), every refractory to all the drugs previously received (also Bortezomib), received BVD (Bendamustine 90 mg/sqm days 1,2; Bortezomib 1.3 mg/sqm days 1,4,8,11, Dexamethasone 20 mg days 1,2,4,5,8,9,11,12, Pegfilgrastim day +4) every 28 days, until progression. ISS was equally distributed, and cytogenetic was evaluable in 12 patients, and in particular one del13q and one t(11;14). All the patients had previously been treated with schedule containing bortezomib and IMIDs, and 30% had also received radiotherapy. 67% of them had undergone at least to a single auSCT. All patients were relapsed and refractory to last therapies received before BVD.
Results: Bendamustine was well tolerated, with grade 3 transfusion-dependent anemia in 41% of patients, and 37% grade 3 neutropenia (no ospedalization was required, no septic shocks were observed). No severe extrahematologic toxicity was observed, only grade 1 gastrointestinal side effect (nausea), treated by common antiemetic drugs. According to IMWG, after a median follow-up of 14 months (r.2-36), ORR was 64% (36/56: 4 CR, 7 VGPR, 16 PR, 9 MR) with 8 PD and 12 patients in SD, which can be considered as an impressive result in this subset of rrMM patients. In particular, for 11 patients, BVD was, after having achieved at least a PR, a bridge to second auSCT, and for two patients a bridge to alloSCT. Three patients have shown a notable PR after failure of novel agents (*i.e.* Carfilzomib and Pomalidomide). Median time to response was 1.2 months (r.1-3), median OS from diagnosis was 62.7 months (range 6-151), median OS from start of Bendamustine was 9.8 months (range 2-36).

Summary/Conclusion: The triplet Bendamustine-Bortezomib-Dexamethasone has shown significant efficacy in a particularly severe setting of patients, relapsed and refractory to all available therapeutic resources, and, in particular cases, it could be considered as a bridge to a second autologous or allogenic SCT.

PB2204

EFFICACY OF RETREATMENT WITH IMMUNOMODULATORY DRUGS AND PROTEASOME INHIBITORS FOLLOWING DARATUMUMAB MONOTHERAPY IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA PATIENTS

R. Oostvogels*, M. Jak, R. Raijmakers, R. Mous, M. Minnema

Hematology, University Medical Center Utrecht, Utrecht, Netherlands

Background: Patients with relapsed and/or refractory multiple myeloma represent a major clinical challenge as data on optimal treatment regimens in this group are limited and trials of new drugs are often difficult to compare. Responses are generally limited and short-lived and their prognosis remains unfavorable.
Aims: In a single-center retrospective observational study we analysed the efficacy of retreatment with immunomodulatory agents (IMiDs) and/or proteasome inhibitors (PIs) after treatment with daratumumab monotherapy in patients with relapsed and/or refractory multiple myeloma (RRMM).
Methods: In total 55 patients were treated with daratumumab monotherapy between 2010 and 2017. From this group 29 (53%) IMiD-refractory patients were retreated with an IMiD after daratumumab treatment and 6 (11%) PI-refractory patients were retreated with a PI-based regimen.
Results: From the IMiD-refractory patients 20/29 (69%) showed an improved response (defined as stable disease or better) upon IMiD retreatment compared to their previous IMiD-response before daratumumab treatment. In the PI-refractory group 5/6 patients (83%) had a superior response to PI retreatment compared to the pre-daratumumab PI-response. In many patients, variable regimens and/or doses were used, precluding an exact comparison between pre- and post-daratumumab. However, in 3 lenalidomide-refractory patients retreatment with the same lenalidomide dose in a similar schedule resulted in improvement of response compared to the pre-daratumumab treatment. The immunomodulatory effects of daratumumab treatment leading to an altered balance between immunosuppressive cell subsets and effector T cells may play a role in the observed high response rates in previously refractory patients. Furthermore, the excellent tolerability of daratumumab treatment may enable patients to recover from prior lines of treatment and receive full dosing of subsequent therapies.
Summary/Conclusion: In conclusion, a high proportion of RRMM patients benefitted from retreatment with IMiDs and PIs after daratumumab treatment. These retreatment options should therefore be explored in relapsed refractory patients progressing on daratumumab monotherapy.

PB2205

THE EFFECT OF SURGICAL INTERVENTION IN THE TREATMENT OF SOLITARY PLASMACYTOMA OF THE BONE

M.T. Koning[1,*], I. Noordhoek[2], P.A. von dem Borne[1], L.A. Daniëls[3], W. Pondaag[4], A.H. Cleven[5], H. Veelken[1], S. Dijkstra[6]

[1]Department of Hematology, [2]Department of Surgery, [3]Department of Radiotherapy, [4]Department of Neurosurgery, [5]Department of Pathology, [6]Department of Orthopedic Surgery, Leiden University Medical Center, Leiden, Netherlands

Background: Solitary plasmacytomas of the bone (SPB) are malignant, intramedullary, monoclonal plasma cell expansions in absence of systemic myelomatous disease. Left untreated, many SPB progress to systemic multiple myeloma (MM). Adequate and timely treatment is vital, since SPB represent a window of curative opportunity that is lost with progression to MM. The current gold standard for SPB treatment is radiotherapy (RT). However, a recent population study (Thumallapally *et al*., 2017), suggested that additional surgical treatment improves patient survival.
Aims: We reviewed the literature and performed a meta-analysis to assess the use and effect of surgery in SPB.
Methods: We searched PubMed, EMBASE and Web of Science for papers describing cases of surgically treated SPB. We included original reports from clinical practise written in Dutch or English from 1990 or later describing ≥1 case of surgically treated SPB without evidence of systemic myelomatous disease at the time of diagnosis. Individually described cases for which intervention and follow-up data were available, were analysed in Kaplan-Meier plots.

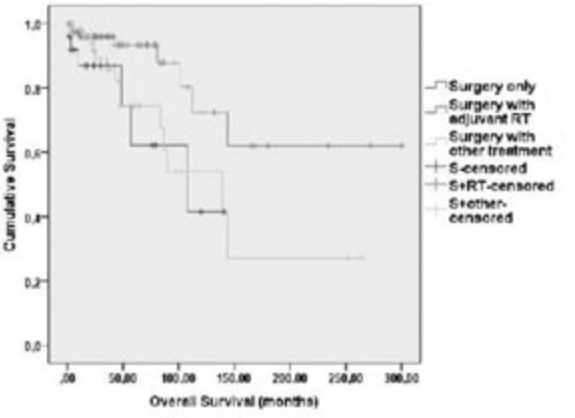

Figure 1.

Results: From 709 results, we included 113 suitable papers describing 700 SPB cases in total (skull and facial bones: 98; spine: 300; chest wall: 98; pelvis: 69; extremities: 86; unspecified: 49). Intriguingly, only 4 authors stated that their surgical intervention was driven by consideration for the patient's prognosis. More commonly, surgical intervention was performed due to initial misdiagnosis of the lesion or the need for acute intervention. In the skull, many authors performed craniotomies expecting to find meningiomas, spinal SPB was mostly operated upon for decompression and stabilisation and SPB of the extremities often presented as a pathological fracture with a need for stabilisation with osteosynthetic material. 195 cases were described in adequate detail to be included in a survival analysis. The male to female ratio was 2,2:1 and median age at diagnosis 53 years (range: 5-82), in line with previously published population data. Skull SPB cases were disproportionally often female (p=0,016). No other sex or age preference was observed between SPB localisations. Kaplan-Meier survival analysis showed better overall survival for combined treatment with surgery and RT (n=79) over surgery alone (n=25)(p=0,020) or surgery in combination with other treatment (n=53)(p=0,021). Progression-free survival was not significantly different between treatments. Our cohort had too few cases treated by RT alone to yield significant results.
Summary/Conclusion: Although surgical intervention with adjuvant RT was shown to confer the best survival in population studies, the choice for surgical intervention is rarely made for the benefit of long-term patient survival. Our findings warrant consideration of additional surgical excision to the treatment plan of patients otherwise treated by RT alone. Moreover, SPB patients who undergo surgical intervention for stabilisation, central nervous system decompression, diagnostic excision etc., may clearly benefit from adjuvant RT. Comprising only case reports, case series and retrospective cohorts, the papers included in this study are at a high risk for selection bias. Therefore we recommend validating these results in a prospective clinical trial. Additionally, these outcomes may be compared with patients treated by RT alone, and the research question may be extended to solitary extramedullary plasmacytomas and plasmacytomas in the context of MM.

PB2206

UNINVOLVED IGA SUPPRESSION PREDICTS EVOLUTION TO SYMPTOMATIC MYELOMA IN CONTEMPORARY DIAGNOSED SMM; PRELIMINARY RESULTS

P. Papaioannou[1], A. Gkioka[1], K. Tsalimalma[2], A. Koudouna[1,*], S. Kotsanti[1], K. Bitsani[1], D. Maltezas[1], T. Iliakis[1], M. Dimou[1], ?. Kafassi[2], P. Sfikakis[1], P. Panayiotidis[1], M. Kyrtsonis[1]

[1]Hematology Section - [1]st Department Of Propaedeutic Internal Medicine, [2]Immunology Laboratory, Laikon Hospital Athens, Athens, Greece

Background: Smoldering Myeloma (SMM) is an asymptomatic form of Multiple Myeloma (MM) that might have a course as indolent as monoclonal gammopathy of undetermined significance (MGUS), especially after the first 5 years. Some patients however may insidiously rapidly become symptomatic and as follow-up visits are less frequents in SMM, end organ damage may occur. To avoid such events, three biomarkers (FLCR>100, ≥1 osteolysis and ≥60% plasma cell bone marrow infiltration) were recently established to discriminate asymptomatic MM patients at increased risk of quick evolution, characterizing them, when present, as symptomatic and in need of immediate treatment (ref.1). In spite of the usage of the new IMWG definition criteria, there are still patients evolving relatively early and the actual notion of High risk SMM is quite subjective among scientific groups.

The Ig heavy chain can be accurately determined with the Heavy/Light Chain (HLC) 'Hevylite' method that measures separately HLC-IgA, -G, -M kappa or lambda, thus allowing exact quantification of the amount of pure monoclonal fraction but also the degree of suppression of uninvolved polyclonal Igs, both being reflected by the corresponding ratios (HLCR). HLCR values in MM (ref.2) and of the uninvolved isotype-specific heavy / light chain (HLC-pair suppression) in MGUS (ref.3) were shown to constitute independent risk factors for progression.
Aims: The aim of this study was to investigate weather involved and uninvolved HLCs values or their ratio can identify accurately SMM patients at risk to progress to symptomatic MM.
Methods: We studied 59 patients with SMM from whom 20 were men and 39 women, with median age 65 (31-84), immunoglobulin type consisted IgG in 51 patients (86,4%) and IgA in 8 patients (13,6%). None of the patients FLCR>100, ≥1 osteolysis or ≥60% plasma cell bone marrow infiltration. Patients were regularly followed (each 3 months) since SMM diagnosis and for a long period (median 98 months). "HevyLite" assay measurements were performed by nephelometry according to the manufacturer's instructions in frozen sera sample drawn at the time of diagnosis. Statistical analysis was performed by standard methods using the SPSS v24.0. software.
Results: HLCR measurements failed to add prognostic information; the same was true for uninvolved IgG and IgM values. However the summated uninvolved IgA kappa plus IgA lambda (SUAKL) values in IgG SMM and uninvolved isotype-specific IgA alone in IgA patients were significantly prognostic of evolution to MM (p<0,01). Time to evolution was 30,5 months in the 12 patients that progressed; their median SUAKL was 0,95g/L *versus* 1,8 g/L in the rest (p<0,01).
Summary/Conclusion: SUAKL will probably prove to be useful as a marker of high-risk SMM; its value should indeed be confirmed in a larger series

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PB2207

COMPARISON OF TWO MOBILIZATION REGIMENS FOR NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

E. Mariggiò[1,*], L. Lombardi[1], F. Vozella[1], F. Fazio[1], A. Piciocchi[2], R. Foà[1], M.T. Petrucci [1]

[1]Hematology, Sapienza University of Rome, [2]Gimema Data Center, Rome, Italy

Background: Induction therapy with novel agents followed by autologous stem cell transplantation (ASTC) is the worldwide gold standard for frontline treatment of younger patients with multiple myeloma (MM). Notwithstanding, a variable proportion of these patients fail to mobilize CD34+ peripheral blood stem cells (PBSC) at all or to collect an adequate number for a safe ASTC or sufficient for tandem or savage procedures ("poor mobilizer").
Aims: The use of lenalidomide and hematological toxicity developed during induction were taken into account as possible factors associated with poor mobilization. The use of Plerixafor with G-CSF for PBSC mobilization significantly improves the chances of a successful mobilization.
Methods: We report the unicentric experience of the Department of Cellular Biotechnologies and Hematology, "Sapienza" University, Rome in 48 patients (pts) with newly diagnosed MM treated as induction therapy with Bortezomib, Thalidomide, dexamethasone (VTd; 33 pts 68,7%) or Carfilzomib, Lenalidomide-dexamethasone (KRd; 15 pts 31,3%). The median age was 55,4 years (range 48-61), 28 men (58,3%) and 20 women (41,7%). In all cases the mobilizing regimen was cyclophosphamide (3g/m^2) associated to G-CSF (10mcg/Kg). International Staging System (ISS) was I-II-III in 25 (52,1%), 19 (39,6%) and 4 (8,3%) patients respectively. After induction, 7 pts (14,6%) achieved complete response (CR), 27 pts (56,3%) achieved a very good partial response (VGPR), and 14 (29,2%) partial response (PR). Filgrastim was used as G-CSF in 38 pts (79,2%) and Lenograstim in 10 pts (20,8%). The use of Plerixafor was necessary in 7 cases (46,7%) for patients treated with KRD induction regimen, in 3 cases (9%) for VTD.
Results: The use of Plerixafor (yes or not) has been compared with the following variables: sex, age, ISS, type of G-CSF, Induction Regimen, type of monoclonal component, time between mobilization date and therapy end. In univariate analysis type of induction regimen was the only statistically significant factor (KRd 7 cases used Plerixafor *vs* 3 VTd); p=0.003. CD34+ cell median final collection (x10^6 /Kg) was 8,60x10^6 /Kg (range 4.40-17) for KRd and 10,38x10^6 /Kg (range 1.49-18.8) for VTd respectively; the difference is statistically significant, p=0,047.
Summary/Conclusion: Our data though revealing a possible negative effect of lenalidomide-based regimens on PBSC mobilization used also with carfilzomib association. Lenalidomide is myelosuppressive and alters the stromal milieu thereby suppressing stem cell mobilization. Plerixafor with G-CSF has been shown to improve successful stem cell mobilization rates in patients receiving Lenalidmide based induction therapy.

PB2208

IMPACT OF NOVEL AGENTS ON AGGRESSIVE RELAPSE OF MULTIPLE MYELOMA: A SINGLE CENTER EXPERIENCE

E. Martino*, V. Calafiore, M.S. Parisi, V. Del Fabro, G. Sapienza, A. Romano, C. Conticello, F. Di Raimondo

Division of Hematology, A.O. "Policlinico-Vittorio Emanuele", University of Catania, Via Citelli [6], [95124] Catania, Italy, Division of Hematology, A.O. "Policlinico-Vittorio Emanuele", University of Catania, Via Citelli [6], [95124] Catania, Italy, Catania, Italy

Background: Therapeutic advances, notably the development of regimens that incorporate immune-modulatory drugs (lenalidomide and pomalidomide) and proteasome inhibitors (*e.g.* bortezomib and carfilzomib), have improved survival outcome in multiple myeloma (MM). However, MM remains largely incurable and patients invariable develop relapsed or refractory (R/R) disease, a new unmet clinical challenge for hematologists. Relapse can be characterized according to disease aggressiveness and the presence of clinical symptoms. Aggressive disease relapse can occur at biochemical level, due to rapid and relevant increase of monoclonal component or LDH, or at clinical level it can be defined by the presence of extramedullary disease, acute renal injury or progression to secondary plasma cell leukemia.
Aims: In this retrospective study we evaluated 76 patients who relapsed after a front-line regimen containing lenalidomide or bortezomib to identify the rate of biochemical and clinical aggressive relapses and clinical outcome upon treatment with second generation novel agents (pomalidomide and carfilzomib).
Methods: From July 2014 we evaluated 76 R/R patients (41 males and 35 females, median age 62 years, range 45-78). Median number of previous line was 3 (1-6), half of them (43/76) underwent ASCT. Pd consisted of pomalidomide 4 mg daily given orally on Days 1–21 of each 28-day cycle and dexamethasone 40 mg weekly. KRd consisted of Carfilzomib 20mg/m^2 IV on days 1 and 2 of the first cycle, then 27mg/m^2 on days 8, 9, 15 16 of the first cycle and days 1, 2, 8, 9, 15 and 16 of the subsequent cycles, Dexamethasone 20 mg on days 1, 2, 8, 9, 15, 16 and lenalidomide 25 mg daily given orally on days 1-21 of each 28-day cycle.
Results: 51 patients were both bortezomib and/or lenalidomide refractory and received Pd according to Italian Health System. 7 of them further progressed and received KRd, the remain 25 received KRd as second and third line therapy. 53 patients had a non aggressive relapse, while the remain 23 experienced an aggressive relapse of disease. 43 patients experiencing relapse after ASCT, 44% had asymptomatic serological relapse or progression, 66% experienced symptomatic relapse (with 38% high risk cytogenetic detected by FISH) and one-third experienced relapse featuring extramedullary disease, plasma cell leukemia or severe renal failure. In the remaining 33 patients not eligible to ASCT, 78% had not aggressive relapse. As expected median PFS obtained in Pd patients was higher for NA group (6 *vs* 4 months). Even if the majority of KRd patients are still under treatment, the OR was 90% with greater PFS in NA than in A group (8 *vs* 6 months).
Summary/Conclusion: In our community setting data, heavily pretreated patients achieved improvement of outcome obtaining a median PFS >4 months, using KRd and Pd as salvage therapies. We found that an aggressive relapse was more frequent in young patients and that earlier treatment at biochemical asymptomatic relapse is associated to better outcome.

PB2209

REAL WORLD DATA OF AGE RELATED CYTOGENETIC ABNORMALITY DISTRIBUTION IN MULTIPLE MYELOMA PATIENTS AT DIAGNOSIS: SINGLE CENTRE EXPERIENCE FROM THE UNITED KINGDOM

J. Tam*, R. Krishna, K. Marshall, L. Cresswell, C. Morrow, L. Barton, G. Asagba, M. Garg

Haematology, University Hospitals of Leicester, Leicester, United Kingdom

Background: Cytogenetic study/ Fluorescence *in situ* Hybridization (FISH) has become an essential tool in assessing prognosis and survival in patients newly diagnosed with multiple myeloma. Cytogenetic abnormalities have various implications in terms of treatment response. It would be useful to

identify patients with a higher incidence of high risk cytogenetic abnormalities in order to target further research in the vulnerable population.
Aims: To review distribution of cytogenetics abnormalities by FISH testing in different age groups of newly diagnosed multiple myeloma patients seen in the University Hospitals of Leicester NHS Trust between 2015-2018.
Methods: Data was collected from hospital computer-based patient records and the database of the cytogenetics department. All newly diagnosed patients with multiple myeloma who underwent a bone marrow biopsy were included in this analysis.. The bone marrow aspirates were screened morphologically. If there were ≥10% of plasma cells, the sample was referred for cytogenetics testing. The FISH panels consist of t (4;14), t (14;16), t (14;20), Del 17p, 1q amplification, and deletion 1p.

Table 1.

Abnormalities		Age group		
Type	No.	<45	45 to 65	>65
t (4;14)	4	0	1	3
t (14;16)	1	0	0	1
t (14; 20)	0	0	0	0
Del 17p	8	0	2	6
Amp1q	30	0	6	24
Del 1p	0	0	0	0
Not analysed	39	2	7	30
No ab detected	59	0	14	45
>1 abnormalities	16	0	2	14
Subtotal		*2*	*32*	*123*
Total	**157**		**157**	

Results: 157 patients (92 male and 65 female) with newly diagnosed multiple myeloma underwent bone marrow biopsy over the three-year period. 39/157 were not analysed as either the plasma cells on the screening sample was insufficient or the number of cells cultured were inadequate for analysis. 59/157 did not have any FISH abnormality. 59/ 157 patients were tested positive for FISH abnormalities, out of which 16/59 had more than one FISH abnormality. The results were categorised under three age groups (<45 years, 45-65 years and >65 years). None of the patients under 45 had an abnormal FISH result while 11/32 patients between the ages of 45-65 and 48 /123 in the >65 year-old age group were identified with FISH abnormalities. The most common FISH anomalies were amplification 1q followed by del 17P in both the intermediate age group and the older age population.
Summary/Conclusion: In our study it was observed that there was an increasing frequency of high risk mutations with increasing age. There is also a significantly higher proportion of del 1q seen in this UK cohort, this being a well known poor prognostic indicator. Cancer research UK data shows poorer patient survival ,shorter time to progression and poorer outcomes in this age group. More research needs to be targeted at the elderly population which may not be suited for more intensive therapies, to overcome high risk cytogenetics; but may benefit from targeted therapies given as maintenance treatment which may prevent progression and ensure better quality of life.

PB2210

Abstract withdrawn.

PB2211

HIGH IMPLEMENTATION ADHERENCE TO LENALIDOMIDE IN MULTIPLE MYELOMA

I. Amitai[1,*], H. Magen[2,3], A. Rozenthal[1], S. De Geest[4,5], E. Tousset[6], I. Vaxman[1,2], P. Raanani[1,2], A. Nagler[3], A. Leader[1,2]

[1]Institute of Hematology, Davidoff Cancer Center, Rabin Medical Center, Petah-Tikva, [2]Sackler School of Medicine, Tel Aviv University, Tel Aviv, [3]Division of Hematology, Chaim Sheba Medical Center, Tel Hashomer, Israel, [4]Institute of Nursing Science, Department Public Health, University of Basel, Basel, Switzerland, [5]Department Public Health and Primary Care, Academic Centre of Nursing and Midwifery, KU, Leuven, [6]AARDEX Group, Visé, Belgium

IA and HM contributed equally; AL and AN contributed equally.

Background: Lenalidomide (LEN) is an important backbone of regimens in the multiple myeloma (MM) treatment arsenal. Non-adherence (NA) to anticancer therapy occurs in approximately 30% of cancer patients and is generally associated with adverse outcomes. A recent study used pharmacy refill data to show LEN implementation NA in 14.5% of MM patients (defined as medication possession ratio <80%), but ON-OFF cycling with LEN can affect the reliability of such data. Electronic methods of measuring adherence provide richer sampling and better reliability. There is no prior prospective longitudinal data on LEN adherence, and specifically no reports on electronically measured (EM) LEN implementation adherence.
Aims: (1) To determine the period prevalence of EM implementation adherence to LEN; (2) To delineate patterns of ON-OFF cycling.

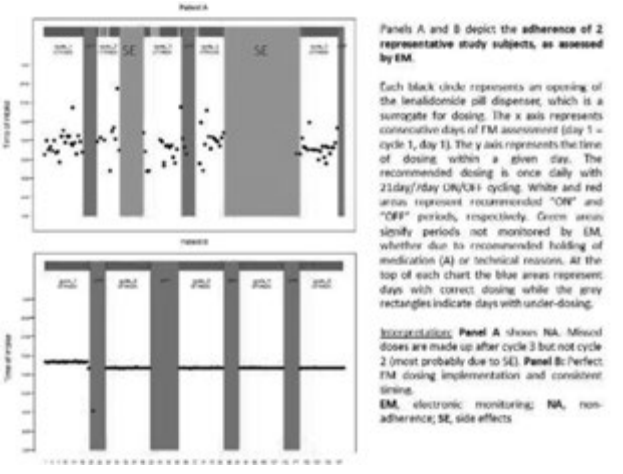


Figure 1.

Methods: Methods: We report on an ad-hoc interim analysis of a prospective observational pilot cohort study of LEN-naïve adult patients receiving LEN-based regimens for treatment of MM. Patients completing follow-up by Feb 1, 2018 were included in this analysis. Convenience sampling was used to enroll 37 patients cared for in the hematology outpatient clinic (all from 1 tertiary center), 34 of whom were included in this analysis (3 excluded due to technical defects or inability to use the EM device). *Sample characteristics* were as follows: median age, 64.5 years [interquartile range (IQR) 58-74]; 35% female; median no. of medications, 5 [IQR 3-6]; median ISS, 2 [IQR 2-3]; median prior disease duration, 22.5 months [IQR 6.5-56]; LEN regimen: 71% LEN-dexamethasone; line of treatment: 1st=6%, 2nd=73%, 3rd=21%. Implementation adherence to once daily LEN in 21day/7day (ON/OFF) cycles was monitored using EM (MEMS®, AARDEX) from study index (*i.e.* LEN cycle1, day1) until the end of cycle 5. This provided data on daily dosing (Figure 1). Adherence was expressed as the percentage of days with LEN taken as prescribed during ON cycling, independent of dosing timing. Descriptive statistics were used to show the median [IQR] for continuous variables and percentages for categorical data.
Results: Median LEN implementation adherence was 98.9% (IQR 93.1-100%; min 51.7%), while the mean was 93.8% (*i.e.*% of days with correct dosing during ON cycling). Representative cases of suboptimal and perfect adherers are shown in Figure 1. The median duration of follow-up was 132 days (IQR 76-138), while the median no. of cycles was 4.5 (IQR 2-5). The reasons for completing less than 5 LEN cycles with EM (n=17) were as follows: treatment change (n=7) mostly due to side effects (n=4); withdrawal of consent (n=5); death (n=1); others (n=4). 41% of patients had no adjustment in ON cycle length, while 53% had ≥1 shortened cycles. 80% (24/30) of evaluable patients achieved partial response or more at 6 months.
Summary/Conclusion: LEN implementation adherence was remarkably high yet showed large variability in this pilot study. Most patients had adjustments in cycle length, emphasizing the limitations of adherence analyses assuming fixed 21/7day cycling and the importance of rich sampling (e.g. EM). Importantly, a quarter of patients had correct dosing on less than 93% of days, which may be suboptimal. These patients may be candidates for adherence enhancing interventions. Thus, future research should focus on strategies for identifying this small subgroup of non-adherers.

PB2212

LONG TERM DISEASE CONTROL WITH POMALIDOMIDE AND DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS: A REAL LIFE EXPERIENCE

M.S. Parisi*, V. Calafiore, E. Martino, S. Leotta, M.C. Pirosa, G. Sapienza, V. Del Fabro, A. Romano, C. Conticello, F. Di Raimondo

Division of Hematology, A.O. "Policlinico-Vittorio Emanuele", University of Catania, Via Citelli [6], [95124] Catania, Italy, Division of Hematology, A.O. "Policlinico-Vittorio Emanuele", University of Catania, Via Citelli [6], [95124] Catania, Italy, Catania, Italy

Background: Pomalidomide is currently utilized in relapsed/refractory multiple myeloma (RRMM) patients that have experienced bortezomib and

lenalidomide treatment. In this setting the drug has shown in clinical trials improved survival and good tolerability.Since its approval in Italy, data on real life are still poor.
Aims: We conducted an analysis of RR MM patients treated with pomalidomide and dexamethasone (PomaD) in real life, previously enrolled in an interventional (STRATUS, MM-010) or currently enrolled in an observational study (MM-015) to provide further insights on this salvage therapy.
Methods: Between January 2016 and July 2017, 41 RR MM patients were treated with pomalidomide 4 mg daily po on days 1–21 of each 28-day cycle and dexamethasone 40 mg weekly (≤75 years) and 20 mg weekly (>75 years). Among patients 14 were enrolled in MM-015 study (an observational study) whereas additional 15 come from MM-010 study (a single-arm phase 3b study).
Results: We describe a total of 56 patients (median age 68 years, range 42-78). Median number of prior therapies was 4 (range 2-7). 29 (51%) patients had pre-existing severe anemia, 5 (9%) thrombocytopenia, 14 (25%) had renal impairment, 10 (18%) extramedullary myeloma;14 (25%) was high risk patients (r-ISS), 7 (12%) previously allo-transplanted. Median number of 8 (range 1-21) PomaD cycles were given. Overall treatment duration mean was 7.7 months. A half of the patients responded after at least one cycle. After a median follow-up of 12 months, median PFS and OS for patients were 6,7 and 9,9 months, respectively. At enrollment, 29 (51%) of patients were anemic, 15 (27%) neutropenic. The regimen was well tolerated with grade 3-4 haematological and non-haematological adverse events in 10 (18%) and 27 (48%) patients respectively. Grade 3-4 non-haematological AEs occurred in 48% of patients [most common: fatigue (7, 12,5%), pneumonia (6, 11%), diarrhea (3, 5%), glucose metabolism alteration (3, 5%), thromboembolism (2, 3,5%), diffuse erythema (2, 3,5%), hyponatremia (2, 3,5%), atrial flutter (1, 2%), acute renal failure (1, 2%)]. In case of serious AE, pomalidomide dose reduction (7, 12,5%) and discontinuation (11, 20%) were applied. All patients responded to treatment at the I/II cycle. Mostly PR and SD were observed. After a median time of 8 months (range 2-21) all patients relapsed. Data on 6-Months control of disease are favorable and related to Durie and Salmon stage. Almost 50% of patients obtained a control of disease with clinical benefit lasting 6 months. Moreover, patients with Stage I-IIA at PomaD-beginning had better probabilities of obtaining control of disease lasting almost 6 months. An unexpected data was the comparison between PFS obtained with the treatment before pomalidomide (previous treatment PFS, pPFS) and the PFS obtained with PomaD treatment (poPFS). 17 patients (30%) obtained a PFS longer than the precedent one. Among these patients, 6 were from STRATUS protocol and treatment was stopped at biochemical progression (25% increase in protein M) according to protocol indication. Difference between poPFS and pPFS was not statistically significant (log-rank p value 0.5).
Summary/Conclusion: Real life PomaD is well tolerated in RR MM patients prolonging PFS and OS with acceptable toxicity. Notably PFS could be superior to that obtained with precedent treatment and treatment may induce almost 6 months disease control in most patients intensively pretreated.

PB2213

THE DETERMINATION OF FACTORS AFFECTING ON THE PROGRESSION-FREE SURVIVAL OF MULTIPLE MYELOMA PATIENTS

A. Garifullin[1,*], S. Voloshin[1], A. Kuvshinov[1], A. Kuzyaeva[1], A. Schmidt[1], Z. Chubukina[1], V. Shuvaev[1], E. Krasnikov[2], S. Bessmeltsev[1]

[1]Russian Research Institute of Hematology and Transfusiology, [2]City Hospital N 40, Saint-Petersburg, Russian Federation

Background: The progression-free and overall survival are key indicators that reflect the effective therapy in patients with hematological disease. The use of different prognostic models based on various factors and their combinations allows to determine the optimal strategy for treating patients, including patients with multiple myeloma (MM) and improve survival. There are quantitative factors and qualitative signs which are determined before and after the induction therapy, and after autologous stem cells transplantation (AutoSCT), if this option was available. However, the advantage of each prognostic parameter should be revised when using new drugs and their combinations.
Aims: To define the factors influencing on progression-free survival (PFS) by means of one-factor and multifactor analyses.
Methods: We analyzed 72 patients with MM (median age 59 years, male/female – 1.25:1). The induction therapy with Bortezomib-based regimens (VD, CVD, VMP, PAD) was used in 48/72 (66.7%) patients, Immunomodulator-based regimens (Thal+D, RD, VRD, PomD) – in 20/72 (27.8%), chemotherapy – in 4/72 (5.5%). Autologous stem cell transplantation (ASCT) is carried out 48 (66.7%) patients. We used the sex, age, the variant induction antimyeloma therapy, response after treatment, MFC MRD status, PET-CT status, tumor load, AutoSCT, variant of maintenance therapy as prognostic factors. The MFC MRD status and PET-CT status were evaluated after 4-6 cycles of induction therapy and after ASCT, if it (AutoSCT) was performed. For definition of MRD we used 5-color flow cytometry with CD38, CD138, CD45, CD19, CD20, CD27, CD56 and CD117 antibodies. The MFC MRD– ($<10^{-4}$) response was stated at identification less than 0.01% of clonal plasma cells and MFC MRD– ($<10^{-5}$) – less than 0.001%. The PET-CT was done in 30 patients only. The PET-CT– status was established if specific accumulation of 18-FDG was absent.
Results: The general regression models (one-factor analysis) show reliable influence of complete response (CR) (p=.0004) and tumor load in bone marrow (p=.000006) on PFS. The multifactor analysis among these parameters shows the CR above the non-CR (VGPR, PR, SD), MFC MRD– status (MRD $<10^{-4}$) above MFC MRD+ (p<0.05). Simultaneous use of factors (achievement of CR and MRD $<10^{-4}$) show reliable impact on PFS (p=.0006). The MFC MRD– ($<10^{-4}$) was reached in 25% (18/72), The MFC MRD– ($<10^{-5}$) – in 9.7% (7/72). The PFS median in MFC MRD+ ($>10^{-4}$) group was 23 months, in the MFC MRD– ($<10^{-4}$) was 65 months (p=.0004). The reliable MFC MRD– ($<10^{-5}$) above MFC MRD– ($<10^{-4}$) and MFC MRD+ ($>10^{-5}$) was not achieved (p>.05). The PFS median in MFC MRD– ($<10^{-5}$) group was 67 months, in the MFC MRD+ ($>10^{-5}$) was 47 months (p>.05). It is probably caused that small number of patients with MFC MRD– ($<10^{-5}$) and short period of observation. The sex, age, variant of induction antimyeloma, PET-CT status, AutoSCT and variant of maintenance therapy were not impact on PFS (p>.05).

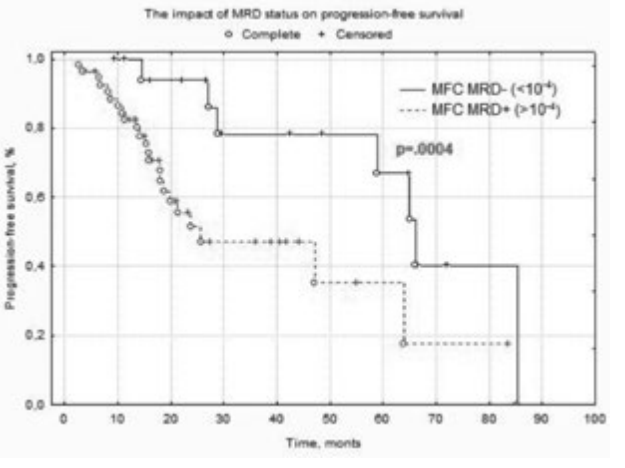


Figure 1.

Summary/Conclusion: The presence of MFC MRD is unfavorable prognostic factor. Achievement CR and MRD– ($<10^{-4}$) status are favorably influence on duration of PFS in MM patients.

PB2214

PHASE 2 STUDY FOR CARFILZOMIB PLUS ELOTUZUMAB PLUS DEXAMETHASONE FOR MYELOMA PATIENTS RELAPSED AFTER 1-3 PRIOR TREATMENT LINES

R. Silvennoinen[1,2,3,*], H. Nahi[4], P. Anttila[1], P. Koskenvesa[1], J. Lievonen[1], S. Luoma[1], A. Räsänen[5], V. Varmavuo[5], A. Sikiö[6], M. Nurmi[6], M. Säily[7], M. Sankelo[8], E. Jantunen[2,9,10], T.-T. Pelliniemi[11], S. Ilveskero[12], M.M. Majumder[13], C. Heckman[13]

[1]Comprehensive Cancer Center Hematology, Helsinki University Hospital, Helsinki, [2]Medicine, Kuopio University Hospital, Kuopio, [3]University of Helsinki, Helsinki, Finland, [4]Medicine, Karolinska Institutet Huddinge, Stockholm, Sweden, [5]Medicine, Kymenlaakso Central Hospital, Kotka, [6]Medicine, Central Finland Central Hospital, Jyväskylä, [7]Hematology, Oulu University Hospital, Oulu, [8]Hematology, Tampere University Hospital, Tampere, [9]Clinical Medicine, University of Eastern Finland, Kuopio, [10]Medicine, North Carelia Central Hospital, Joensuu, [11]Fimlab Laboratories Ltd, Tampere, [12]Clinical Chemistry and Hematology, HUSLAB Helsinki University Hospital, [13]Institute for Molecular Medicine Finland (FIMM), Helsinki Institute of Life Science, University of Helsinki, Helsinki, Finland

Background: The median progression free survival (PFS) and overall survival (OS) of multiple myeloma (MM) patients have prolonged due to novel agents combined with ASCT. However, the vast majority of patients will

progress after a median of 2-3 years with median OS of 7-8 years. Thus, the possible benefit of new combinations and dosing of available agents has to be investigated. The first proteasome inhibitor (PI), bortezomib (B), combined with elotuzumab and dexamethasone (d) showed superiority to Bd resulting in PFS of 9.7 *vs.* 6.9 months, respectively, without excessive toxicity (Jakubowiak *et al.* Blood 2016;127:2833-40[1]).
Aims: The aim of this study is to investigate the safety, feasibility and initial efficacy of a second generation PI carfilzomib plus a SLAMF7 antibody elotuzumab plus dexamethasone (KEd) in relapsed MM patients.
Methods: Forty patients with relapsed MM after 1-3 prior lines will be included in this phase 2 study after written informed consent. The primary endpoint is overall response rate. In patients achieving at least very good partial remission (VGPR) the quality of response and bone marrow lymphocyte subgroups will be assessed with high-sensitivity multicolour flow cytometry according to the 8-colour EuroFlow protocol. Carfilzomib (K) is given once weekly 20mg/m^2 on D1C1 and thereafter 70mg/m^2 in 28 day cycles on days 1, 8 and 15 in cycles 1-8 and on days 1 and 15 thereafter combined with weekly elotuzumab (E) 10 mg/kg on days 1, 8 and 15 in cycles 1-2, thereafter on days 1 and 15; dexamethasone (d) on days 1, 8, 15 and 22 on cycles 1-8, thereafter on days 1 and 15. Treatment will continue until progression or excessive toxicity. Carfilzomib dose was 20/56mg/m^2 for the first two cycles for the first five patients to evaluate the safety. Follow-up samples will be stored for later response analysis of elotuzumab interference on IgG-kappa paraprotein. Additionally, patient samples collected prior to treatment will be comprehensively profiled by whole exome and RNA sequencing and evaluated for *ex vivo* response to the agents. Furthermore, the previously described[1] association of FcγRIIIa polymorphism with response will be measured. Together, the study addresses clinical response, *ex vivo-in vivo* translation, identifies molecular biomarkers for the KEd combination and facilitates precision guided clinical trials for relapsed refractory MM.
Results: By the end of Feb 2018 eight patients have been enrolled. After a median of four cycles one patient is in VGPR, four patients are in PR, one in MR, and two that are too early to assess. One patient had grade 2 infusion reaction after premedication. One patient developed autoimmune hemolytic anemia (AIHA) possibly related to elotuzumab and recovered with steroids. She continued on Kd without reappearance of AIHA, while elotuzumab was permanently discontinued. A grade 3 adverse event with asymptomatic elevation of liver transaminase was detected in another patient who recovered with dose reduction of dexamethasone to 12mg and carfilzomib to 56mg/m^2 without any change in elotuzumab dose.
Summary/Conclusion: To the best of our knowledge this is the first study evaluating the carfilzomib plus elotuzumab plus dexamethasone combination in relapsed MM with comprehensive molecular annotations. Preliminary results of KEd treatment with weekly dosing of 70mg/m^2 resulted with at least PR response in 5/6 patients. We noticed an unexpected severe adverse event of AIHA in one study patient. AIHA should be excluded if unexpected anemia will appear during elotuzumab treatment.

PB2215

THE TREATMENT WITH PEGYLATED LIPOSOMAL DOXORUBICIN, CYCLOPHOSPHAMIDE AND DEXAMETHASINE (CED) FOR RELAPSED AND REFRACTORY MULTIPLE MYELOMA

M. Ionita[1], I. Ionita[2], F. Ghilezan[3], D. Calamar[2], V. Todorescu[2], C. Ionita[4], D. Oros[2], C. Sorica[2], H. Ionita[2,*]

[1]Internal Medicine, [2]Hematology, University of Medicine and Pharmacy Victor Babes Timisoara, [3]Hematology, Clinical County Hospital, [4]Surgery, University of Medicine and Pharmacy Victor Babes Timisoara, Timisoara, Romania

Background: Multiple myeloma is a malignant plasma cell disorder. It is the second most frequent haematological malignancy and characterized by malignant plasma infiltration or the bone marrow and is associated with an increased level of monoclonal protein in the blood and/or urine. Patients affected by multiple myeloma become chemo-resistant : hence, one of the main topics in clinical research is the quest for therapeutic alternatives to overcome refractory disease.
Aims: Since 2012, we have treated patients affected by Multiple Myeloma, relapsed and refractory to most of the therapeutic options available, with a chemotherapy based on a combination of pegylated liposomal doxorubicin, cyclophosphamide and dexamethasone (CED), at monthly doses respectively of 35mg/m^2 on day 1800mg/m^2 on day 1 and 20mg/ m^2 on days 1-4.
Methods: Twenty (20) patients (11men, 9 women), with a median age of 62 years, (range: 40-74) affected by advanced, relapsed and progressive multiple myeloma, whose median number of previous treatments was 3 lines (range 2-5) were treated with monthly CED courses (median number of courses: 3; rage: 3-10).
Results: Three patients (15%) had a complete remission (CR), in 11 patients (55%) there was a partial response (PR); the disease remained stable in two patients (10%) and four patients (20%) did not benefit from CED treatment. Median response duration was 9 months (range: 4-25). The toxicity profile of CED was satisfactory: hematological toxicity (WHO grade 2) was observed in 6 patients (30%).There was 2 patients with gastrointestinal disturbances, one patient with infections, an episode of acute renal failure in one patient. One toxic death due to sepsis was noted.
Summary/Conclusion: These results can encourage further studies as they have been obtained in patient with severely advanced disease stage, previously not exposed to anthracyclines. The preliminary data point to a significant results in 50% of patients with particularly advanced disease.

PB2216

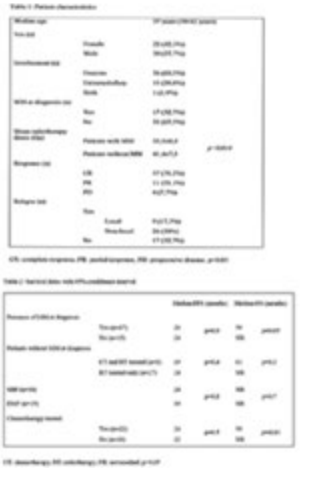
CLINICAL FEATURES AND OUTCOMES OF SOLITARY PLASMACYTOMAS: SINGLE-CENTER EXPERIENCE FROM TURKEY

Y. Ulusoy[1,*], E. Arslan Davulcu[1], F. Sahin[1], N. Soyer[1], E.S. Kamer[2], N. Ozsan[3], M. Hekimgil[3], G. Saydam[1]

[1]Hematology, [2]Radiation Oncology, [3]Pathology, Ege University Hospital, Izmir, Turkey

Background: Solitary plasmacytoma (SP) is a plasma cell dyscrasia that presents as a mass lesion consists of plasma cells that produces monoclonal immunoglobulins without any systemic involvement. SP accounts for approximately 5% of all plasma cell dyscrasias and mostly occurs in osseous tissue, however one third of patients have extramedullary plasmacytoma.
Aims: In this paper, we analyzed our patients and aimed to reveal patient characteristics and outcomes.
Methods: A total of 52 patients who were referred to our clinics of hematology and radiation oncology with the diagnosis of SP from January 2006 to March 2017, were evaluated retrospectively. Age, sex, treatment procedures, radiotherapy doses, involved tissue datas, relapse and response status, death times were recorded from hospital registries.

Table 1.



Results: Median age at the diagnosis was 57 years (30-82 years), 57,7% of them (n=30) were male. Seventeen patients were diagnosed as concurrent multiple myeloma (MM) and 15 of those treated with radiotherapy and chemotherapy both. Patients without MM at the diagnosis (n=35) were irradiated and followed periodically after completion of radiotherapy, 8 of those were treated with chemotherapy adjacent to radiotherapy due to widespread disease. Solitary bone plasmacytoma (SBP) was the most common diagnosis with a rate of 69,2% (n=36), one patient was diagnosed with SBP and extramedullary plasmacytoma (EMP) at the same time. Patients those whom had concurrent MM was irradiated palliatively with a dose of 20 to 45 Gray (Gy), curative radiotherapy was performed to all other patients at a dose of 45 to 54 Gy. Median duration of follow-up for patients was 45 months (0-144 months). Local recurrence defined as relapse from the affected site at the diagnosis was detected in 9 patients (17,3%), all relapsing patients (67,3%, n=35) were received mean 38,2±9,0 Gy dose of irradiation while patients with no recurrence were treated with mean 42,2±5,8 Gy dose of irradiation during diagnosis. Local recurrence was not related with the dose of radiotherapy (p=0,5). Patients responded well to all therapies, partial and complete response were achieved in 92,3% of patients (n=48). During

follow-up MM was diagnosed in 7 of all 35 patients without MM at the time of diagnosis, five of them had SBP. Median progression time to MM for those patients was 29 months (14-104 months). Median overall survival (OS) was not achieved, progression free survival (PFS) was median 26 months. The presence of MM at the diagnosis was not a risk factor for progressive disease (p=0,9). Median OS for patients with MM at the diagnosis was 59 months and was not reached in patients without MM. There were no significant PFS and OS differences between SBP and EMP groups (p=0,8, p=0,7, respectively). The outcomes of patients without concurrent MM treated with chemo-radiotherapy and treated with radiotherapy alone were not different also (Table 2).
Summary/Conclusion: This registry-based study represents our experience on SP and provides the data showing that radiotherapy as a single regimen results in good outcomes. Due to limited number of patient, it is difficult to hypothesize that radiotherapy is the golden standard, but radiotherapy alone to involved area seems to be adequate and safe treatment option. Our findings is consistent with literature, however there is no prospective or large patient group of retrospective analysis in the literature.

PB2217

BORTEZOMIB-CYCLOPHOSPHAMIDE-DEXAMETHASONE INDUCTION THERAPY IS COMPARABLE TO BORTEZOMIB-THALIDOMIDE-DEX AMETHASONE IN NEWLY DIAGNOSED MYELOMA: A SINGLE CENTRE EXPERIENCE

F. Al-Kaisi*, D. Smith, F. Wadelin, E. Das-Gupta, S. Henshaw, S. Adams, C. Williams

Clinical Haematology, Nottingham University Hospitals, Nottingham, United Kingdom

Background: Bortezomib-based triplet therapy is the current standard of care for induction treatment in myeloma prior to autologous stem cell transplant (ASCT) in combination with dexamethasone and an IMiD such as thalidomide (VTD) or an alkylator such as cyclophosphamide (VCD). Induction therapy aims to achieve the deepest remission whilst limiting treatment toxicity. Peripheral neuropathy secondary to bortezomib and thalidomide can result in dose reduction or treatment discontinuation and potentially poorer response. Substituting thalidomide for cyclophosphamide may limit neuropathy but could theoretically reduce efficacy due to losing the synergistic effect between bortezomib and thalidomide.
Aims: To compare the response to treatment, incidence of neuropathy and rate of bortezomib and thalidomide dose reduction or discontinuation in patients who received VTD *vs* VCD induction for newly diagnosed myeloma prior to ASCT.
Methods: Patients were treated in 2 consecutive cohorts. Response rates were assessed prior to ASCT using IMWG criteria.

Table 1.

	VTD (n=39)	VCD (n=30)
Median age	62	63
Cytogenetics		
Standard Risk	16	14
High Risk	4	5
Unknown	19	11
Median Bortezomib dose	20.8 (mg/m²)	24 (mg/m²)
Median bortezomib cycles	4	5
PFS (1 year)	87%	76.5%
Response Rate		
ORR	86.8 %	96.5 %
≥ VGPR	24 (61.5 %)	16 (53.3%)
PR	9 (23%)	12 (40%)
SD	1 (2.56%)	0
PD	4 (10.25%)	1 (3.3%)
Unknown	2 (5.1%)	1 (3.3%)
Peripheral Neuropathy	14 (35.8%)	13 (43.3%)
Grade 1	5 (12.8%)	2 (6.6%)
Grade 1 plus pain	3 (7.6%)	3 (10%)
Grade 2	3 (7.69)	7 (23.3%)
Grade 3	2 (5.12%)	1 (3.3%)

Results: Between 2014 and 2017, 39 patients were treated with VTD and subsequently, a further 30 were treated with VCD. Median age at diagnosis was 62 years and 63 years and the median performance status was 1 and 0, respectively. ORR was 86.8% with VTD and 96.5% with VCD. The rate of VGPR or better was higher in the VTD group (61.5% *vs* 53%). 4 patients (10.25%) in the VTD group and 1 patient (3.3%) in the VCD group progressed during treatment, with 3 of 4 in the VTD group having high-risk cytogenetics. More patients treated with VTD proceeded to ASCT (84.6% *vs* 70%). Median follow up was 16 months. The 1-year PFS was 87% for VTD *vs* 76.5% for VCD. Comparing VTD to VCD, the median total cumulative bortezomib dose was 20.8mg/m^2 *vs* 24 mg/m^2 and the median number of cycles given was 4 and 5 respectively. 14 patients (35.9%) with VTD developed neuropathy of any grade compared to 13 (43.3%) with VCD leading to dose reduction in 20.5% and 43.3% respectively. Median cumulative bortezomib dose prior to the first bortezomib dose reduction was 12.45mg/m^2 with VTD and 16.1mg/m^2 with VCD. The rate of bortezomib discontinuation due to neuropathy (all grades) was 10% in each group. 15 patients (38.5%) treated with VTD had thalidomide dose reduction and 13 (33.3%) discontinued it after a median of 4 cycles. Median bortezomib dose per patient before discontinuation was 10.63mg/m^2 for VTD and 19.43mg/m^2 for VCD.
Summary/Conclusion: ORR was higher in patients treated with VCD, with 96.5% achieving remission. Patients treated with VTD required earlier dose reductions of bortezomib and received a lower median dose. Whilst patients treated with VTD achieved a higher rate of VGPR or better, VCD still resulted in an impressive 53% VGPR or above. VGPR rate for VCD is higher than that seen in some previous studies and comparable to that in the IFM2013-04 trial although our results were achieved using a lower dose of cyclophosphamide (500mg weekly). 1-year PFS was higher in the VTD group but more patients in this arm proceeded to ASCT so the PFS data may not accurately reflect the efficacy of induction therapy. Interestingly the rate of neuropathy was higher with VCD. This may reflect the need for more active dose reduction of bortezomib in the VTD group, discontinuation of thalidomide in around one-third of VTD patients and the higher cumulative bortezomib dose in VCD patients. Our findings suggest VCD is effective, delivers a higher bortezomib dose and is a viable alternative to VTD for induction therapy in myeloma.

PB2218

RETROSPECTIVE AUDIT OF BISPHOSPHONATE USE IN MULTIPLE MYELOMA PATIENTS IN TWO DISTRICT GENERAL HOSPITALS AND FRACTURE CONSENSUS

Z. Saleem[1,*], R. Allam[2], T. Hui[2], A. Bebb[2], A. Danga[1], T. Sugai[1]

[1]Haematology, Hillingdon Hospital, [2]Haematology, west middlesex hospital, London, United Kingdom

Background: Bisphosphonates are specific inhibitors of osteoclastic activity and form a cornerstone in the treatment of Patients with multiple myeloma to reduce the incidence of Skeletal related events. They are shown to be effective in reducing the incidence of Pathological fractures and pain.Skletal related events in multiple myeloma patients are associated with adverse outcomes in the form of increased morbidity, more hospital admissions and poor quality of life.We conducted a retrospective audit of bisphosphonates use in multiple myeloma patients in two district general hospitals against the UK NICE guidelines. NICE published guidelines in February, 2016 regarding management of Multiple Myeloma Patients, recommending the use of Intravenous bisphosphonates in all multiple myeloma patients soon after diagnosis
Aims: Our Primary objectives were: 1: To assess our compliance with the NICE guideline, 2: To assess the efficacy of I/V Bisphosphonates in reducing the incidence of Pathological fractures in multiple myeloma patients. Our secondary objective was to assess the incidence of osteonecrosis of Jaw in patients receiving I/V Bisphosphonates.
Methods: We collected the data for newly diagnosed multiple myeloma patients between January, 2010 to December, 2017 at the Hillingdon and west Middlesex hospiatals from the clinic letters, medical notes and radiology reports.
Results: There were 110 patient diagnosed with multiuple myeloma during this period. 102 (92.7%) of the patients received I/V bisphosphonates, out of these 62 pateints received Zolendronic acid and 40 patients received Pamidronate.Only 8(7.2%) Patients did not receive Bisphosphonates, 2 of these patients were intolerant of the treatment, for the rest of the 6 patients no reason was identified from the medical notes. Mean Duration of treatment was 24 months(range 12-36 months), 56(50%) of the patients had pathological fractures at the time of Diagnoses. Only 16(14%) of the patients receiving bisphosphonates were found to have a new pathological fracture. On the other hand the incidence of pathological fracture was much higher in patients who did not receive any bisphosphonate, 3 (37.5%) patients not recieving bisphosphonates were found to have a new pathological fracture. None of the patients treated with I/V bisphosphonates were found to have osteonecrosis of jaw.
Summary/Conclusion: Although our data is relatively small but clearly demonstrates, our compliance with NICE guidelines regarding the use of

I/V Bisphosphonates in multiple myeloma patients, reduction in the incidence of Pathological fractures in multiple myeloma patients receiving bisphonates(14% *vs* 37.5% in treated and untreated groups respectively), none of the patients were identified to have developed osteonecrosis of jaw with I/V bisphonate treatment, (International consensus is 1 in 1000 patient being treated with I/V Bisphosphonates will develop osteonecrosis of jaw).

PB2219

PROGNOSTIC SIGNIFICANCE OF THE CHROMOSOME 1 ABNORMALITIES IN PATIENTS WITH MULTIPLE MYELOMA

J. Bila[1,*], V. Djurasinovic[1], A. Sretenovic[2], J. Jelicic[2], M. Dencic Fekete[2], M. Perunicic Jovanovic[2], D. Antic[1], B. Mihaljevic[1]

[1]Clinic of Hematology, Clinical Center of Serbia, Medical Faculty, University of Belgrade, [2]Clinic of Hematology, Clinical Center of Serbia, Belgrade, Serbia

Background: For the past two decades, several chromosomal abnormalities (CA) of high-risk significance on the course of multiple myeloma (MM) were defined, aiming to establish different prognostic groups.
Aims: The aim of this study was to analyze the prognostic significance of the chromosome 1 molecular abnormalities in MM patients.
Methods: A total of 104 newly diagnosed MM transplant-ineligible (median age 68 years, range 65-80 years; 56 male/48 female), were analyzed in the study, with following distribution: IgG myeloma had 68 patients (65.4%), IgA 17 (16.3%), IgD 1 patients (1%), light chains 16 (15.4%), and non-secretory 2 (1.9%). According to the clinical stage (CS, Durie&Salmon), advanced III CS was found in 82 patients (78.8%), II in 14 (13.5%), and symptomatic I CS in 8 (7.7%) patients. The ISS score 1 had 30 (28.8%) patients, 40 (38.5%) ISS 2, and 34 patients (32.7%) had ISS 3. Renal impairment existed in 29 patients (27.9%). According to the Revised ISS core (R-ISS), the distribution was as follows: RSS I 43 patients (41.3%), II 37 (35.6%), and III 24 patients (23.1%). Applying interphase fluorescent in-situ hybridization (iFISH) with probe 1p32/1q21 (CDKN2C/CKS1B), chromosome 1 abnomalities were identified in 54 (59.3%) patients: del1p32 in 16 patients (29.6%); and +1q21 in 38 patients (70.4%). Thalidomide based combinations were applied in 86 patients (82.7%), while bortezomib based combinations were applied in 18 patients (17.3%) with high-risk features.
Results: The overall treatment response (CR/VGPR/PR, IMWG criteria) with chr1 abnormalities, was achieved in 32 patients (51.9%): in 10/32 patients (31.3%) with del1p32; and 22/32 patients with +1q21 (68.7%). The median follow up of analyzed group was 22 months (range 6-100 months). Patients with chromosome 1 abnormalities had shorter PFS, still without statistical significance (Breslow 2.499; p=0.114), and statistically significant shortness of OS (Breslow 5.344; p=0.016). However, statistical analysis did not confirm the prognostic impact of +1q21 (PFS: Breslow 2.123, p=0.145; OS: Breslow 1.833, p=0.176), or del1p32 (PFS: Breslow 0.366, p=0.545; OS: Breslow 0.505, p=0.477). In addition, Cox regression analysis indicated R-ISS3 as the most important prognostic parameter that influenced OS (95% CI, 1.760-7.1429; p=0.006).
Summary/Conclusion: Molecular abnormalities of the chromosome 1 are of the negative prognostic significance in the MM patients. Still, it seems that R-ISS score 3 is of major impact on the course of disease with subsequent implications on the treatment approach.

PB2220

PROGNOSTIC SIGNIFICANCE OF THE REVISED INTERNATIONAL STAGING SYSTEM IN TRANSPLANT-ELIGIBLE PATIENTS WITH MULTIPLE MYELOMA

J. Bila[1,*], M. Todorovic Balint[1], D. Antic[1], V. Djurasinovic[1], A. Sretenovic[2], J. Jelicic[2], M. Dencic Fekete[2], M. Perunicic Jovanovic[2], B. Mihaljevic[1]

[1]Clinic of Hematology, Clinical Center of Serbia, Medical Faculty, University of Belgrade, [2]Clinic of Hematology, Clinical Center of Serbia, Belgrade, Serbia

Background: In attempt to define different prognostic groups, with a final goal to personalize optimal approach in treatment of patients with multiple myeloma (MM), Revised International Staging System (R-ISS) was established.
Aims: The aim of study was to analyze the prognostic significance of the R-ISS score in MM patients eligible for autologous stem cell transplantation (ASCT).
Methods: A total of 112 newly diagnosed MM patients (median age 54 years, range 22-65years; 63 male/49 female), were analyzed in the study, with following distribution: IgG myeloma had 72 patients (64.2%), IgA 19 (17.0%), IgD 3 patients (2.7%), light chains 14 (12.5%), and non-secretory 4 (3.6%), and. According to the clinical stage (CS, Durie&Salmon), advanced III CS was found in 87 patients (77.7%), II in 15 (13.4%), and symptomatic I CS in 10 (8.9%) patients. The ISS score 1 had 56 (50.0%) patients, 21 (18.7%) ISS 2, and 35 patients (31.3%) had ISS 3. Renal impairment existed in 14 patients (12.5%). According to the R-ISS score, the distribution was as follows: RSS I 58 patients (51.8%), II 39 (34.8%), and III 15 patients (13.4%). Patients were treated with induction therapy based on triple combinations with thalidomide and/or bortezomib, followed with high-doses of Melphalan (HDT, 200mg/m^2), and supported with ASCT.
Results: The overall treatment response (CR/VGPR/PR, IMWG criteria), analyzed on +100. day after HDT+ASCT, was achieved in 108 patients (96.4%). According to the R-ISS score, overall treatment response was achieved in all of 58 patients with R-ISS I; in 38/39 (97.4%) with R-ISS II; and in 32/35 (91.4%) with R-ISS III. The median follow up of analyzed group was 52 months (range 12-143 months). The R-ISS was highly statistically relevant regarding both EFS (Log Rank=13.729, p=0.001) and OS (Log Rank=10.486, p= 0.001). Cox regression analysis confirmed that R-ISS was the most important prognostic parameter that influenced OS. (95% CI, 1.056-7.120; p=0.038).
Summary/Conclusion: The R-ISS score is highly significant prognostic factor in transplant eligible myeloma patients. It represents currently most sensitive prognostic tool in multiple myeloma with consequent implications to personalized treatment approach.

PB2221

EFFECT OF BISPHOSPHONATE AND ANTI-MYELOMA THERAPY ON BONE TURNOVER MARKERS IN MULTIPLE MYELOMA

A. Jandial[1,*], S.H. J.[1], K. Mishra[1], S. Bhadada[2], A. Sood[3], D. Lad[1], R. Aggarwal[4], M. Prakash[5], G. Prakash[1], V. Suri[1], A. Khadwal[1], S. Kumari[1], N. Varma[6], P. Malhotra[1]

[1]Department of Internal Medicine, [2]Department of Endocrinology, [3]Department of Nuclear Medicine, [4]Department of Immunopathology, [5]Department of Radiodiagnosis, [6]Department of Hematology, PGIMER, Chandigarh, India

Background: Bone involvement is one of the defining features of symptomatic multiple myeloma (MM). There is little information on changes in bone mineral metabolism that occur with bisphosphonates and anti-myeloma medication.
Aims: Assessment of bone mineral metabolism in MM patients at diagnosis and three months after treatment with antimyeloma drugs and bisphosphonates.
Methods: Newly-diagnosed MM patients were prospectively enrolled from January 2017 to December 2017. Besides standard myeloma work-up (including skeletal survey), baseline evaluation included estimation of serum bone turnover markers [carboxy-terminal cross-linking telopeptide of type I collagen (CTX), aminoterminal propeptide of type 1 procollagen (P1NP), and Osteocalcin (OC)], DEXA scan, and Tc99 bone scintigraphy. Patients were treated as per institutional protocol with antimyeloma drugs and monthly bisphosphonates. Skeletal survey and bone turnover markers were re-assessed after 3 months.
Results: Twenty-four newly-diagnosed MM patients were enrolled. Median age was 55 years (range 35 – 76 years); 79.16% were males. Majority had bone pains (83.3%) and anemia (83.3%); renal failure (45.8%) and hypercalcemia (45.8%) were not uncommon. IgG subtype was most common (52%), followed by IgA (21%) and light chain myeloma (16%). Majority (83.3%) had ISS stage III disease; mean value of β-2 microglobulin was 17.81 (± 25.16) mg/mL. 17 patients (70.83%) had multiple lytic lesions and seven (29.16%) fracture at baseline (pathological 4, traumatic 3). On DEXA scan, 10 patients (41.67%) had osteopenia and three (12.5%) had osteoporosis. Bone scintigraphy revealed meaningful uptake at baseline in 19 patients (79.16%); mainly in axial skeleton. All bone markers [CTX, P1NP, and OC] showed a graded but statistically insignificant correlation with the extent of bone involvement on baseline skeletal survey, P >0.05. Baseline CTX levels in patients with pathological fractures were significantly higher than those without fracture (P=0.041). Baseline β-2 microglobulin significantly correlated with CTX (r=0.44) and P1NP (r=0.43) levels; OC showed no correlation with β-2 microglobulin. After 3 months, significant decline was observed only in CTX levels [0.46 (±0.84) *v* 1.16 (±1.19), P=0.001]; minimal upsurge was observed in P1NP and OC levels (P >0.05). Fall in CTX levels among patients receiving VTD [bortezomib, thalidomide plus dexamethasone; n=5] was significantly greater than those receiving VCD [bortezomib, cyclophosphamide plus dexamethasone; n=19], P=0.012. Decline in CTX among patients exclusively treated with zoledronate [n=17] was significantly larger than those who received initial ibandronate (due to renal failure) followed by zoledronate [n=7], P=0.017. After 3 months, overall response rate was 75% [CR 16.7%, VGPR 50%, PR 33.3%].

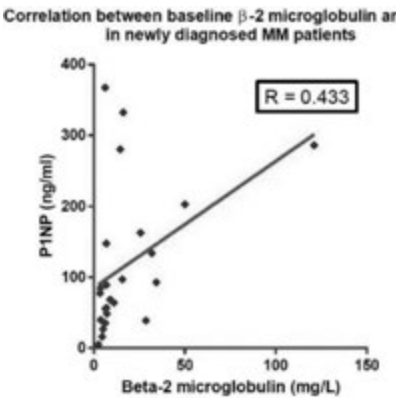


Figure 1.

Summary/Conclusion: We observed that bone turnover markers significantly correlated with myeloma disease burden (β-2 microglobulin). Their relationship with the severity of skeletal involvement was graded albeit inconsequential. Bisphosphonates and anti-myeloma medications considerably reduced CTX (bone resorption marker) but had trivial impact on P1NP and OC (bone formation markers). Predominance of ISS stage III patients, small study cohort and mitigation by concomitant unapparent factors (e.g., senile osteoporosis) shall be the possible explanations for these results. Larger prospective studies with longer follow up shall be required to interpret dynamics of bone turnover markers in myeloma.

PB2222

Abstract withdrawn.

PB2223

CARFILZOMIB-LENALIDOMIDE-DEXAMETHASONE IN THE MANAGEMENT OF LENALIDOMIDE-REFRACTORY MULTIPLE MYELOMA

C. Cerchione[1,*], K. Ferrara[1], I. Peluso[1], M. Di Perna[1], I. Zacheo[1], D. Nappi[1], A. E. Pareto[1], A. Romano[2], F. Pane[1], L. Catalano[1]

[1]Hematology - Department of Clinical Medicine and Surgery, University Federico II, Naples, Napoli, [2]Department of General Surgery and Medical-Surgical Specialties, Haematology Section, University of Catania, Catania, Italy

Background: Carfilzomib is an epoxyketone proteasome inhibitor of second generation, proved to be effective and safe in relapsed and refractory Multiple Myeloma (rrMM), in combination with dexamethasone or lenalidomide and dexamethasone.
Aims: In this retrospective observational trial, it has been evaluated efficacy and safety of carfilzomib, in combination with lenalidomide-dexamethasone (KRD) as salvage regimen in patients with rrMM, refractory to lenalidomide, whose prognosis is particularly severe.
Methods: 27 patients (16 M/11 F), with rrMM, median age at diagnosis 63 years (r. 47-79), median age at start of treatment 66 years (r. 53-83) treated with several lines of treatments (median 3, r. 2-11), underwent to KRD regimen (ASPIRE trial schedule) for a median treatment cycles of 4 (r 1-12). ISS was equally distributed, and cytogenetic was evaluable in 8 patients, and in particular one del13q14 1q gain, one del13q14 and one t(11;14). All patients had previously been treated with bortezomib and IMIDs, and were refractory to this agents. 59% (16/27) of them had undergone at least to a single autologous SCT.
Results: According to IMWG criteria, after a median follow-up of 3 months (r.1-13), ORR was 66,7% (14/21: 8 VGPR, 6 PR) with 3 progressive diseases (PD) and 2 patients in stable disease (SD): this can be considered as an impressive result in this subset of rrMM patients, refractory to lenalidomide. In particular, for 1 patient, KRD was, after having achieved at least a PR, a bridge to second autologous SCT. Median time to response was 2 months (r.1-4), median OS from diagnosis was 51 months (r. 9-170), median OS from start of Carfilzomib was 3 months (r. 1-13). Carfilzomib was well tolerated, with grade 2 anemia in 33% (9/27) of patients, successfully managed by ESAs, without necessity of blood transfusions; 18% (5/27) grade 3-4 neutropenia (pegfilgrastim in primary prophylaxis was given, no ospedalization was required, no septic shocks were observed); 29% (8/27) grade 2, 18% (5/27) grade 3 and 7% (2/27) grade 4 thrombocytopenia, without hemorrhagic events and necessity of transfusions. Concerning severe extra-hematologic toxicity, it was observed pneumonia in 44% (12/27) of patients, treated by common antibiotic drugs; hypertension (grade 2-3) in 33% (9/27) of patients; arrhythmias in 7% (2/27) of patients; dyspnea in 11% (3/27) of patients; fatigue in 29% (8/27) of patients. All patients were carefully monitored by expert cardiologists of our deparment.
Summary/Conclusion: KRD has shown significant efficacy in a particularly severe setting of patients, relapsed and refractory to all available therapeutic resources, also lenalidomide, and, in particular cases, it could be considered as a bridge to a second autologous or allogenic SCT.

PB2224

FREQUENCY AND CAUSES OF NOT RECEIVING SUCCESSIVE LINES OF TREATMENT IN REFRACTORY MULTIPLE MYELOMA: A CROSS-SECTIONAL STUDY

A. Senín*, F. García-Pallarols, R. Ben Lashen, L. Martínez-Serra, S. Montesdeoca, D. Román, M. Ferraro, I. Párraga, C. Besses, E. Abella

Hematology Department, Hospital del Mar-IMIM, Barcelona, Spain

Background: The introduction of novel agents during the last decade has improved the clinical outcome in patients with recurrent/refractory multiple myeloma (R/R MM), which has allowed to increase the number of lines and the types of treatment received. Nevertheless, most studies are performed within clinical trials and there are few data on sequential treatments in real-world clinical practice, where treatments are not completed.
Aims: To study the frequency and reasons for not receiving next line of treatment in a real-life cohort of patients with R/R MM.
Methods: Main clinical-biological characteristics of 108 patients diagnosed of MM between 2010 and 2016 in a tertiary centre were collected. Patients were categorized according to the last line of treatment received, including palliative treatment with cyclophosphamide as a line.The reasons for not receiving a subsequent treatment in each line were defined as: response (not needing further therapy), treatment-related toxicity and death (due to toxicity, disease progression or other causes).Overall survival and time to next treatment curves were calculated by kaplan meier method. A competitive risk analysis was carried out to estimate the projected proportion of patients receiving second and third lines considering death, toxicity disqualifying for further therapy or loss of follow-up as competitive events.

Table 1.

Table 1. Reasons for not receiving a next line of treatment

Patients who do not receive a next line	1st line n=35	2nd line n=29	3rd line n=21	4th line n=13	5th line n=10
Response	11 (31)	4 (14)	2 (8)	0	0
On going	7 (20)	10 (35)	7 (33)	5 (38)	5 (50)
Exitus due to progression of MM	5 (14)	5 (17)	8 (40)	5 (41)	4 (40)
Exitus due to treatment-related complications	4 (11.5)	5 (17)	3 (14)	2 (16)	1 (10)
Exitus due to others Causes	4 (11.5)	2 (7)	0	0	0
Exitus due to another neoplasia	3 (9)	3 (10)	0	0	0
Toxicity by treatment	1 (3)	0	1 (5)	1 (5)	0

Results: The reasons for not receiving a next line of treatment, at the time of the analysis, are summarized in Table1.Thirty-five out of 108 patients (32%) received only a first line. Thirty-one percent of them (11/35) remained in response without requiring rescue treatment. Fourteen percent (5/35) discontinued treatment due to death in the context of progression and 11.5% (4/35) due to toxicity-related death. Twenty percent (7/35) died due to causes not related to MM, as other neoplasms (9%; 3/35). Only 3% of patients could not receive further treatment due to serious toxicities that did not result in death.Twenty-seven percent of patients (n=29) received only treatment up to the second line. The discontinuation due to death by progression and death by toxicity was 17% (5/29), in both cases.Twenty percent of patients (n=21) received treatment only up to the third line. Death by progression was the most frequent cause of not receiving successive lines (40%; 8/21), whereas severe toxicity and death by treatment-related com-

plications were the reason in 5% and 14% of patients, respectively.Only 12 patients received treatment up to fourth line, 7 up to fifth line and 1 up to sixth line. The cause of not continuing treatment was mainly death by progression (41% and 40% in fourth and fifth lines, respectively). No responses were observed in these lines.The median overall survival of the series was 25 months. Median of time to next treatment were 16, 15 and 13 months in first, second and third line, respectively. The projected probability of receiving a second line of treatment at 60 months was 78% whereas probability of receiving third line at 60 months was 53% by competitive-risk analysis (68% of the 78% remaining).
Summary/Conclusion: -In our series, 78% of patients were estimated, by competitive risk analysis, to receive a second line of therapy at 60 months. Patients do not access a second line mainly due to death. While the main reason for discontinuation in the first line was not needing further therapy, the main cause in successive lines was death by progression (25% of the global series) increasing its frequency according to the number of lines received.

PB2225

AUTOLOGOUS STEM CELL TRANSPLANTATION IN NEWLY DIAGNOSED ELDERLY MULTIPLE MYELOMA PATIENTS: A SINGLE CENTRE EXPERIENCE

T. Berno*, M. Riva, L. Ammirati, A. Visentin, C. Gurrieri, A. Branca, L. Checuz, A. Lico, G. Barilà, M. Leoncin, S. Imbergamo, F. Piazza, L. Trentin, G. Semenzato, R. Zambello

Department of Medicine, Hematology and Clinical Immunology Unit, University Hospital, Padua, Italy

Background: Multiple myeloma (MM) is a plasma cell malignancy that affects older adults with a median age at diagnosis of 70 years. Autologous stem cell transplantation (ASCT) is the standard of care for young newly diagnosed MM patients. The safety and efficacy of High Dose Therapy (HDT) as upfront treatment in elderly patients remain still uncertain, because elderly age is frequently associated with increased comorbidities and suspected increased treatment-related toxicity.
Aims: In our centre we analyzed the outcome and toxicities in a homogeneous cohort of newly diagnosed elderly MM patients treated with HDT approach.
Methods: We retrospectively evaluated 24 newly diagnosed elderly MM patients, according to International Myeloma Working Group (IMWG). All of them were fit/low-risk according to revised Myeloma Comorbidity Index (R-MCI). Median age at diagnosis was 66 years (range 65-68 years), and at transplantation 66.5 years (range 65-69). The revised International Staging System (R-ISS) stage was: I in 12 patients (50%), II in 8 cases (33%), III in 3 patients (12.5%) and unknown in 1 patient (4%). FISH analysis were performed at diagnosis in all patients, and we found standard-risk in 11 patients (46%), intermediate-risk in 4 cases (16%), high-risk in 3 cases (12.5%) and not assessable in 6 patients. Immunoparesis occurred in 19 patients (80%) at diagnosis. The induction therapy was bortezomib-based in association with dexamethasone (VD) with or without thalidomide (VTD). The mobilization regimen included cyclophosphamide followed by G-CSF. The conditioning regimen consisted of melphalan 140 mg/mq or 200 mg/mq given over two days.
Results: As induction therapy 10 patients (41.5%) received VD, 11 patients (46%) VDT and 3 patients received bortezomib-based therapy plus infusional chemotherapy regimens (12.5%). Melphalan 140 mg/mq conditioning regimen was administered in 14 patients (60%) and 200 mg/mq in 10 patients (40%). The overall response rate (ORR) to induction therapy (≥PR) was 91.6% including 5 CR (21%), 10 VGPR (41.5%), 7 PR (29%) and 2 SD (8.5%). The ORR after ASCT increased to 96%: 5 CR (21%), 14 VGPR (58.5%), 4 PR (16.5%) with 1 death. Immunoglobulin recovery 1 year after ASCT occurred in 15 patients (62.3%) out of 19 with immunoparesis at diagnosis. Median time to neutrophil and platelet engraftment was 10 days (range 6-18 days). No significant difference in toxicity was found among the two groups (MEL140 *vs* MEL200). The non-hematological toxicities after ASCT (G3-G4) included infections in 12 patients (50%) and gastrointestinal disorders in 10 cases (41.5%). In 1 patient we observed cardiac toxicity (G2). The day-100 post ASCT treatment-related mortality (TRM) was 4% (1 patients died after conditioning regimen). During follow up we reported secondary malignancies in 2 cases (8%) and one therapy-related myeloid neoplasm (t-MDS). The mean PFS was 30 months in MEL140 group *vs* 53 months in MEL200 (p=0.11), no significant difference in OS was observed (p=0.3).
Summary/Conclusion: Our results suggest that ASCT is a safe and well-tolerated procedure in our small cohort of elderly and fit patients. We detected among the two regimens (MEL140 and MEL200) a similar profile of toxicities. The dose of Melphalan (MEL200) seems to impact on the duration of response, with a statistically significant trend in PFS. We confirm that an accurate assessment of elderly patients performance status and comorbidities at diagnosis, can help to develop risk-adapted treatment strategies to improve outcome, in elderly MM patients.

PB2226

INCORPORATION OF TECHNOLOGY IN OUR MULTIPLE MYELOMA CONSULTATION: A YEAR OF EXPERIENCE

J.M. Sánchez-Raga*, A. Ortuño-Cabrero, A. Pérez-Montaña, N. Beltran, T. Ros, B. López-Andrade, M.D.C. Ballester-Ruiz, L. Lo Riso, L. Bento, L. García-Maño, M.A. Duran, A. Sampol-Mayol

Hospital Universitari Son Espases, Palma, Spain

Background: Multiple myeloma is a chronic pathology diagnosed usually in elderly patients. It often presents with systemic involvement (bone lesions, renal failure, anemia, infections) secondary to the pathology itself or as a treatment side effect. Patients often come to consult or the emergency department spotaneously for problems arising from this pathology. Thanks to the collaboration of Celgene we have developed a link project in our center, whereby a case management hematologist through a telematic medical consultation (telephone and via email), detects side effects of treatment, prescribes complementary tests or modifies adjuvant treatment and derives early to other specialists in the cases that it is necessary. With this, diagnostic and therapeutic delays have been avoided in patients affected by Multiple Myeloma, improving their quality of life and strengthening the doctor-patient relationship.
Aims: We summarize the activity of the first year of implementation of the telematic consultation and the benefits obtained from it.
Methods: In March 2017, the project was initiated and presented personally to each patient with multiple myeloma. In our center we have 104 patients in follow-up in this period of time, of which 80% have been included in the project for the time being.
Results: 51 patients have attended at least one telematic consultation (median 2, range 1-21). Tables 1 and 2 summarize the type of activity attended. We have save more than 135 spontaneous visits of the patients to our consultations or emergencies. We have also reduced the time until resolution of the reason for consultation. More detail information will be provided in the congress. 51 patients have performed at least one telematic consultation (median 2, range 1-21). Tables 1 and 2 summarize the type of activity attended in the consultation. With this consultation we have saved 137 spontaneous visits of the patient to consultations or emergencies. We have also reduced waiting times until resolution of the reason for consultation. More detailed information will be provided in the congres.

Table 1.

REASONS FOR CONSULTATION	Number of consultations	(%)
Medical consultation	75	36.8%
Claim citations / tests	51	25%
Information	31	15.2%
Referral specialists	20	9.8%
Prescription of medicines	16	7.8%

REASONS FOR MEDICAL CONSULTATION	Number of consultations
Digestive: Nausea, diarrhea, constipation	21
Respiratory: Respiratory infection, dyspnea	15
Pain	13
Cardio-vascular	9
Psychological:Anxiety, depression	6
Skin	5
Urological	3
Others	2

Summary/Conclusion: The telematic consultation can save hospital visits, reduce attention time, and improve the monitoring and quality of life of our patients. In an increasingly technological society, the incorporation of mobile phone, email and other tools will be more common in our clinical practice.

PB2227

TREATMENT WITH IXAZOMIB IN HEAVILY PRETREATED PATIENTS WITH MULTIPLE MYELOMA IN SLOVENIA: REAL LIFE DATA

A. Čebokli, N. Zima, S. Zver*

Hematology, University clinical center Ljubljana, Ljubljana, Slovenia

Background: Based on the TOURMALINE-MM1 trial, Ixazomib (Ixa) is a novel oral proteasome inhibitor (PI), which in combination with lenalidomide and dexamethasone (IxaRD) prolongs progression free survival and increases the overall response rate over RD alone.
Aims: To evaluate the efficacy and safety of IxaRd in the real-world clinical practice in Slovenia.
Methods: We retrospectivelly analysed data on heavily pretreated patients with RRMM, treated with IxaRD regimen from July 2016 to January 2018, that were included in the compassionate ixazomib use Named-Patient Program in Slovenia. We compared our data with significantly less pretreated patients in Tourmaline MM1 study. Primary end point was overall response rate (ORR). Secondary endpoints included duration of treatment, number of patients experiencing adverse effects (AE) and treatment discontinuation reasons.
Results: We treated 47 patients; Female/Male ratio was 30%/70%, median age at enrollment was 68 (46-82), median age at diagnosis 62, ISS I or II 59% and ISS III 41%. They were previously treated with median 5 (1-11) lines of therapy. 94% of patients were previously treated with bortezomib based regimens, 11% were primarily refractory. 69% of patients received lenalidomide based regimen (24% were refractory), and 13% received pomalidomide. At diagnosis, cytogenetics was not assessed in 47% (22/47) of patients: 15% (7/47) had high risk cytogenetics, 23% (11/47) intermediate risk and 15% (7/47) standard risk. Translocation (4;14) and gaining of (1q21) were determined as intermediate risk. 45 patients (96%) were treated with the IxaRD combination, 5 of those (10%) have continued without lenalidomide due to side effects. Two patients (4%) started treatment with ixazomib-dexamethasone regimen. Median number of cycles administered was 4,5 and 12 patients are still receiving treatment. Response rate was assesed according to IMWG criteria. ORR in these patients was 33%; 5 patients (10%) achieved CR, 5 (10%) VGPR, 6 (13%) PR. In addition we observed minimal response or at least stable disease in 17% of patients. In 27% of patients disease progressed. In 23% of patients we were not able to evaluate the response rate due to the short period of treatment. In 5 patients refractory to bortezomib, 1 VGPR and 1PR were observed. There were no AE reported amog 62% of patients. 6% of patients had polyneuropathy, 16% had cytopenias, 2% dizziness, 4% infection, 2% generalized skin rash, and 2% acute renal impairment, leading to treatment discontinuation in 8 patients.
Summary/Conclusion: The dataset from this real life study describes results of IxaRD regimen in our heavily pretreated and high risk patients. It also emphasises the difference between real life data and clinical trials. Namely our patients received many more lines of therapy and had more often unfavorable disease markers. The overal response rate, including MR and SD patients, was 50%, which is significantly less than the ORR published in Tourmaline study (78%). Clinical benefit rate was much lower in patients treated beyond 5th treatment line, including bortezomib and lenalidomide refractory patietns. We noticed that resistance to bortezomib doesn't always mean, that the patient is ixazomib resistant too; 40% of bortezomib refractory pts responded to ixazomib. It would be interesting to find out how it is with cross resistance interactions between karfilzomib and ixazomib and vice versa. During the observational period there were no ixazomib related deaths. IxaRd appears to be a reasonablly safe and effective treatment option.

PB2228

SERVICE DELIVERY OF APPROVED DARATUMUMAB TREATMENT FOR RELAPSED MYELOMA; CHALLENGES OF NORMAL IMMUNOGLOBULIN AND UNAFFECTED FREE LIGHT CHAIN SUPPRESSION, ESPECIALLY WHEN ASCT IS INCLUDED

R. Powles[1,*], L. Little[1], J. Sheldon[2], R. Wheeler[2]

[1]Myeloma Unit, Cancer Centre London, [2]Immunology, St Georges Hospital, London, United Kingdom

Background: The anti-CD38+ monoclonal antibody Daratumumab (dara) is established as part of the treatment pathway for MM, along with autologous stem cell transplantation (ASCT) (IMWG 2017). Patients who have previously received, or are receiving Daratumumab have altered cellular inmmunological status (ASH 2017 abstract 3148).
Aims: To review suppression of normal serum immunoglogulins in dara treated patients, and possible increased infection risk (EBMT 2018 Powles *et al.* Abstract B232), and whether this impinges on the safety of ASCT. To determine if in ASCT patients, this altered immune status, results in poor stem cell harvesting, delayed engraftment, infections, possible autologous GVHD, and dara infusion- related reactions when re-challenged post ASCT. We also look to see if suppression of normal Free Light Chain (FLC) levels occurs on dara, which may effect interpretation of FLC ratio.
Methods: In this study 26 patients with progressive MM (1-13 prior lines of therapy) aged 65y(36-83); IgG: 13, IgA: 8, BJ: 4, NS 1, were treated with dara plus/minus velcade or revlimid at our institution; 21 with minimum follow-up 6 months. Three patients underwent ASCT.
Results: Of 26 patients with progressive MM at baseline, 24 responded (MRD-ve 35%, VGPR 35%, PR 19%, SD 4%, PD 8%) **Immunoglogulin suppression;** There was profound suppression of normal unaffected Ig levels at 6 months in 25/26pts inc those in CR; *e.g.* 7 patients with IgG myeloma on dara monotherapy ≥6 months; at 1 month normal IgA was reduced by 30-86% (m 80%); IgM by 25-86% (m 66%). this pattern occurred in non-IgG patients. **Infections**; there were 95 infection events seen including 27% URTIs (grade 3) & 1 grade 4 influenza associated death. **Unaffected FLC Suppression;** in 24 (of 26) patients unaffected FLCs dropped at 1 month (2 were stable), *e.g.* in 7 IgGk patients λ light chains had dropped from 1.3mg/L to 0.7; 6.3 to 4.9; 13.6 to 5.3; 5.9 to 1.2; 17.1 to 7.9, 10 to 6.6; 9.7 to 7.0 at 1 month. This was compared with a rise in unaffected λ light chains in 6 (of 7) of these patients 1 month into a prior (non-dara) MM therapy. **ASCTs** Three patients received planned ASCTs; two had PBSCs harvested following dara treatment. **Pt1.** F 72, IgAλ. 13 previous lines (9 yrs) treatment, 6 cycles dara pre ASCT; 10 post. ANC engraftment; 12 days. Inpatient stay (los): 14 days. Pre ASCT: MRD+ve (PP14g/L) post ASCT: MRD-ve. **Pt 2.** F 49, IgGk. 4 previous lines (9 yrs) treatment. 7 cycles Dara pre ASCT; 10 post. ANC engraftment 13 days. los: 15 days. MDR+ve status (BMB IP+iv) pre SCT. post MRD-ve. **Pt 3.** M 63 IgAλ /AL. 2 previous lines (2yrs) treatment. 7 cycles Dara pre ASCT; 10 post. ANC engraftment: 13 days. los: 22 days. MRD-ve status pre SCT; post MRD-ve. All three had serum dara levels at time of ASCT.
Summary/Conclusion: We describe profound normal immunoglogulin suppression with associated infection for patients on dara, with or without serum dara levels present. Three ASCT patients engrafted and were discharged from hospital promptly. They mobilized standard stem cell yields, had no evidence of autologous GVHD, unexpected infections, or an IRR with their next infusion of dara. The effect of dara in suppressing the unaffected FLC requires consideration when monitoring FLC ratios in these patients.

PB2229

MONOCLONAL GAMMOPATHY AND TRANSTHYRETIN AMYLOIDOSIS

A. Alarcon Tomas[1,*], C. Salas[2], I. Zegrí[3], P. García-Pavia[3], J. Vázquez Cobos[4], P. Massó[5], I. Krsnik[1]

[1]Hematología, [2]Pathology, [3]Cardiology, Hospital Universitario Puerta de Hierro, [4]Hematología, Hospital Reina Sofía, [5]Hematología, CNIC, Madrid, Spain

Background: Amyloidosis is a rare disease characterized by tissue deposition of insoluble fibrils created by the aggregation of misfolded proteins. Cardiac involvement is common and associated with an increased risk of morbidity and mortality. The most frequent types of amyloidosis are light-chain (AL) and mutant or wild-type transthyretin (TTR). AL amyloidosis caused by the deposition of misfolded kappa or lambda light chains in patients with an underlying plasma cell malignancy. Differential diagnosis between AL and TTR cardiac amyloidosis is mandatory because prognosis and therapy are different, but it is not always easy as non-invasive techniques are not specific.
Aims: We report three patients with a monoclonal gammopathy and wild-type TTR amyloidosis.
Methods: Case reports, Patient 1. A 66-year old men was sent for haematological evaluation after an ischemic stroke. He was otherwise asymptomatic. An echocardiogram showed a concentric left ventricular hypertrophy suggesting cardiac amyloidosis. Blood tests showed normal counts, a monoclonal component and an increase in free light chains (FLC). A cardiac biopsy showed Congo Red positive deposits. Immunohystochemistry (IHC) was not diagnostic. Mass spectrometry (MS) confirmed the diagnosis of TTR amyloidosis. Fifty months from diagnosis, he remains asymptomatic with stable FLC and NT-proBNP. Patient 2. A 87 year old men was admitted to our Hospital with a two-month history of cough, edemas, and dyspnea. He had anemia, thrombopenia, a biclonal component and an increase in FLC. A cardiac biopsy with IHC and MS confirmed the diagnosis of TTR amyloidosis. He was begun on cyclophosphamide, prednisone and thalidomide with improvement of the blood counts and decrease of the M-spike. Thirteen months from diagnosis, he remains alive with cardiological support. Patient 3. A 75-year-old male was evaluated at our centre with a three-

month history of edemas and shortness of breath. A diagnosis of monoclonal gammopathy of unknown significance (MGUS) had been made elsewhere nine years before. Echocardiography showed a severe concentric left ventricular hypertrophy. A cardiac biopsy with IHC and MS confirmed the diagnosis of TTR amyloidosis. Ten months from diagnosis he remains stable with diuretic therapy. MGUS remains also stable.
Results: Our three patients are elderly males with a MGUS and a positive SC. Although IHC studies showed TTR deposition in the heart, a faint positive staining with anti-TTR can be seen in AL specimens. In the three cases, MS confirmed the presence of TTR and ruled out deposition of light chains.

Table 1.

	Patient 1	Patient 2	Patient 3
Age	66	87	75
Sex	Male	Male	Male
TTR gene Wild Type (WT)/Mutant	WT	WT	WT
MS (cardiac biopsy)	TTR	TTR	TTR
IHC	TTR expression +++, Amiloid A -, Kappa+, Lambda +	TTR expression +++, Amiloid A -, Kappa-, Lambda -	TTR expression ++, Amiloid A -, Kappa+, Lambda +
99mTechnetium-DPD scintigraphy	Score 2	Score 3	Score 3
Echo: LVEF/IVS	55%/21mm	50%/15mm	60%/18mm
NYHA stage	1	2	2
NT-proBNP, pg/ml	483	1557	3867
M-spike (g/dl)	IgG λ (1.2)	Biclonal λ (2.1)	IgG λ (1.3)
Proteinuria	Negative	Negative	Negative
% plasma cells in BM	35%	9%	1%
FLC κ/λ(mg/l)	14.9/147.5	35.2/272.8	31.3/23.9
Plasma Cell disorder	Asymptomatic MM	MM	MGUS

Table 1: clinical data and biochemical data, imaging techniques and biopsy results

Summary/Conclusion: Most cardiac amyloidosis are either due to TTR or light chain deposition. AL cardiac amyloidosis has a worse prognosis and the underlying neoplastic clone can be treated with anti-myeloma drugs. Differential diagnosis between AL and TTR amyloidosis may be complicated. Firstly, wild-type TTR amyloidosis may be more prevalent among elderly patients than previously thought. Secondly, many elderly patients have monoclonal gammopathies so the presence of a MGUS does not mean that amyloidosis is AL. Echocardiography or MRI of the heart cannot differentiate between the two types. Scintigraphy (SC) with 99Tc is always positive in patients with cardiac TTR-amyloidosis but up to 10% of AL cases may show a positive score. Immuno-electronic microscopy is not available in most centres. Differential diagnosis between cardiac AL and TTR amyloidosis is mandatory because subtyping of the amyloid has significant implications in the management of patients and prognosis. MS might be essential.

PB2230

DOES ASYMPTOMATIC D-DIMER ELEVATION PREDICT THROMBOSIS IN PATIENTS ON IMMUNOMODULATORY DRUGS IN MULTIPLE MYELOMA: REAL-LIFE DATA

M.S. Aydın[1,*], S.C. Yazgan[2], E. Karakulak[1], R. Çiftçiler[1], E. Kelkitli[3], M. Turgut[3], H. Goker[1], H. Demiroglu[1], O. Ozcebe[1], I. Haznedaroglu[1], S. Aksu[1], F. Tekin[1]

[1]Hematology, [2]Internal Medicine, Hacettepe University Faculty of Medicine, Ankara, [3]Hematology, [19] Mayıs University, Samsun, Turkey

Background: Immunomodulatory drugs (IMiDs) bring the risk of venous thromboembolism (VTE) in patients with Multiple Myeloma (MM). Prophylactic measures such as asetylsalicylic acid (ASA), low-molecular heparins (LMWH) or warfarin usage are routine but there are no well-defined criteria in which patients which measure will be taken. Risk assessment models with disease-related, treatment-related and patient-related risk factors help in decision process. Routine D-dimer monitoring in asymptomatic patients on IMiDs may help early diagnosis of VTE.
Aims: To evaluate if routine D-dimer monitoring on IMiD usage will detect asymptomatic VTE.
Methods: Retrospective data from a total of 55 MM patients on IMiD-containing regimens diagnosed between 01.01.2001 and 01.06.2017 were analyzed. D-dimer values and radiologically or pathologically diagnosed thromboses events were recorded. Univariate analyses were done by Chi-square test for categorical and by t-test for numerical variables, respectively.
Results: Median age of diagnosis was 58 (range, 33-79 years) and median age was significantly high in thrombosis group (n=8) with respect to patients without thrombosis (n=47) (p=0.040). Thromboprophylaxis regimens were recorded in 40 patients; 19 patients with ASA and 21 patients with LMWH. Of these, 50 patients were monitored with plasma D-dimer levels during IMiD usage. 23 patients (46%) showed an elevation of D-dimer (≥1 mg/L), and there were 32 patients in whom D-dimer was <1 mg/L or was not monitored. There were 8 thromboses events (3 deep venous thromboses and 5 pulmonary venous embolisms). All thromboses events were symptomatic. Of 11 patients with asymptomatic D-dimer elevation, all were investigated radiologically for possible thrombosis and none had thromboses. There was no median D-dimer value difference between symptomatically and asymptomatically D-dimer elevated patient groups (p=0.178). There was no thromboprophylaxis regimen choice difference between thrombosis and no-thrombosis groups (p=0.664).
Summary/Conclusion: VTE events may occur despite thromboprophylaxis in IMiD using patients. In this study, symptoms suggesting thrombosis seem more important with respect to D-dimer elevation in diagnosing VTE. But there is no enough data for foregoing serial plasma D-dimer monitoring because early intervention may help to prevent symptomatic or lethal VTE events.

PB2231

RENAL SALVAGE WITH BORTEZOMIB/CYCLOPHOSPHAMIDE/DEXAMETHASONE INDUCTION THERAPY IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS WITH RENAL INSUFFICIENCY: A SINGLE-CENTER STUDY FROM NORTH INDIA

U. Nath*, R. Chetia, J. Nanda, A. Baveja

Medical Oncology Haematology, All India Institute of Medical Sciences (AIIMS), Rishikesh, Rishikesh, India

Background: Renal impairment is a common and potentially life-threatening complication of multiple myeloma. Among newly diagnosed myeloma patients, 20-50% have acute kidney injury at the time of diagnosis. The commonest form of myeloma-related acute kidney injury is light chain cast nephropathy. Bortezomib/Cyclophosphamide/Dexamethasone (CyBorD) regimen is the preferred initial induction therapy in newly diagnosed myeloma patients with acute renal insufficiency.
Aims: Our study reports the renal salvage response to CyBorD induction therapy in newly diagnosed multiple myeloma patients with renal insufficiency in a tertiary hematology center in north India.
Methods: The study included 34 newly diagnosed patients of multiple myeloma with renal insufficiency (RI) between March 2016 & January 2018. RI was defined as either serum creatinine >2 mg/dL or creatinine clearance (CrCl) <40 mL/min related to myeloma.[1] Baseline tests of renal function included serum creatinine & estimation of CrCl, serum electrolyes, calcium, phosphorus & uric acid. The investigations done for myeloma diagnosis included bone marrow aspiration+biopsy, serum protein electrophoresis, serum immunofixation & free light chain assay, β2 microglobulin & X-ray skeletal survey. Renal biopsy could be performed in two patients only. Renal insufficiency was aggressively managed with intravenous hydration (3 liters/m^2/day, unless oliguric with volume overload) and intravenous dexamethasone. Induction therapy with CyBorD was initiated immediately after confirmation of myeloma diagnosis (21 days' cycle: Bortezomib 1.3 mg/m^2 intravenously on days 1,4,8,11; Cyclophosphamide 300 mg/m^2 orally on days 1,8,15; and Dexamethasone 40 mg/day orally on days 1,4,8 & 11 of each cycle). Hemodialysis was performed in patients with oliguric renal failure, volume overload, or severe hyperphosphatemia unresponsive to treatment. Plasmapheresis was not performed in any of the patients. Zoledronate was deferred till normalization of renal function. Renal response to anti-myeloma therapy was defined as per International Myeloma Working Group (IMWG) criteria [Complete renal response: CrCl >60 mL/min; Partial renal response: CrCl 30-59 mL/min].[1]
Results: The median patient age was 61 years (22-76 years). Majority (72%) of the patients had kappa light chain paraprotein (either kappa light chain myeloma or IgG/IgA kappa myeloma). Median serum creatinine & calcium levels at presentation were 3.1 mg/dl (2.1-11 mg/dl) & 11.2 mg/dl (8.1-15.4 mg/dl) respectively. Four patients (12%) required hemodialysis initially as per indication. Complete renal response was achieved in 94% (32/34) patients, including those who required dialysis. Two patients discontinued treatment & were lost to follow up. The median time from initiation of treatment to achievement of renal response was days (range 3 - 35 days).
Summary/Conclusion: Myeloma-related acute renal insufficiency is a medical emergency. Aggressive supportive treatment and prompt initiation of Bortezomib-based anti-myeloma therapy are the cornerstones of management. Hemodialysis is indicated in a minority of patients but can be life-saving. Bortezomib/Cyclophosphamide/dexamethasone induction regimen is associated with excellent renal salvage response in myeloma-related acute renal insufficiency.

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PB2232

CLINICAL EFFICACY AND SAFETY OF LENALIDOMIDE IN NHS GREATER GLASGOW & CLYDE

L. Doherty[1,*], R. Soutar[1], K. MacLure[2]

[1]Haematology, Beatson West of Scotland Cancer Centre, Glasgow, [2]Robert Gordon University, Aberdeen, United Kingdom

Background: Lenalidomide in combination with dexamethasone is accepted for use in Scotland as 1st line therapy in transplant ineligible patients who are unsuitable for thalidomide treatment, 2nd line in patients who are unsuitable for thalidomide treatment and unrestricted use as 3rd line treatment.
Aims: This retrospective Real World Data (RWD) study examined the clinical efficacy and safety of lenalidomide in the treatment of multiple myeloma patients in NHS Greater Glasgow and Clyde (GG&C), with an approximate population of 1.2million.
Methods: All multiple myeloma patients treated in GG&C with lenalidomide from 01/01/2013 to 01/01/2016 were eligible for inclusion. Exclusion criteria were any patients with myeloma associated amyloidosis, those treated as part of a novel combination therapy or as part of maintenance therapy post-transplant. 131 patients met the criteria. Data were censored for 01/01/18. Patient characteristics including disease stage, ECOG status and renal function were determined. Clinical efficacy was evaluated using International Myeloma Working Group criteria for Time to Progression (TTP) and Progression Free Survival (PFS). Overall survival data were immature. Adverse effects and reasons for discontinuation were assessed.
Results: The overall median TTP was 90 weeks compared to the TTP of 58 weeks in the Pooled Pivotal Study[1] (PPS) of the MM-009 and MM-010 trials. PFS was 71 weeks, compared to 48 weeks in the PPS. The improved PFS/TTP was despite patients being older and having poorer performance status and renal function to the PPS. The results compare favourably to Spanish[2], Korean[3], Dutch[4] and Polish[5] RWD studies (see table). TTP was found to be significantly higher than PFS. The TTP metric creates a less robust endpoint as it censors deaths that have not been positively attributed to disease progression. This challenges the applicability of TTP in myeloma patients, a primarily elderly group, who have higher mortality rates irrespective of their underlying malignancy. In total, 22% (n=29) of patients either discontinued lenalidomide due to adverse effects (n=18) or died (not attributable to progression) while on lenalidomide (n=11). Although generally higher, rates of discontinuation due to adverse effects and deaths were comparable to those in published literature.

Table 1.

	GG&C	PPS	Spanish RWD	Korean RWD	Dutch RWD	Polish RWD
Median Age	71	63	66.5	62	61	60.5
ECOG Status 0 1 2 3 4	25% 31% 41% 4% 0	44% 44% 11% 0 0	NA	76.4 (0-1) 17.2 (2-4)	NA	Only 0-2 included
Median Previous Therapies	2 (0-5) 1 = 2.2% 2 = 15.2% 3≥ = 82.4%	1 = 18.4% 2 ≥ =81.6%	3	3.5	3.5	1-2 Lines =31% 3-5 Lines =56% ≥ 6 Lines =13%
Previous ASCT	45%	58%	35%	67%	66%	60%
Renal Impairment CrCl = Creatinine Clearance	CrCl>90 = 21% CrCl60-89 =21% CrCl30-59 =44% CrCl15-29 =12% CrCl<15 =2%	Patients with Creatinine > 221 μmol/L were excluded	Creatinine > 177 μmol/L = 12.6%	CrCl< 30 =9%	Cr Cl<50= 7%	Creatinine > 177 μmol/l = 7.5%
TTP weeks	90	58	56	35	39	87
PFS weeks	71	48	NA	NA	43	NA

Summary/Conclusion: This is the first study of lenalidomide use in unselected patients with a median age over 70 years and better reflects those treated in actual clinical practice. Despite the older age, poorer ECOG status and renal function, TTP/PFS were better than those seen in the PPS/RWD and confirms lenalidomide's efficacy in an elderly and pre-treated patient group.

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PB2233

MINIMAL RESIDUAL DISEASE NEGATIVITY AFTER MOLECULARLY TARGETED VENETOCLAX THERAPY OF SECONDARY PLASMA CELL LEUKEMIA WITH TRANSLOCATION T(11;14)

G. Mikala*, A. Cegledi, O. Csacsovszki, M. Peto, Z. Szemlaky, M. Udvardy, S. Tasnady, A. Kozma, P. Remenyi, I. Valyi-Nagy

Hematology and Stem Cell Transplantation, South-Pest Central Hospital, National Inst. Hematology & Infectology, Budapest, Hungary

Background: Secondary plasma cell leukemia presenting as a myeloma relapse after multiple prior lines of therapy is a condition with dismal prognosis and a usual survival of only a few weeks. Recently, it has been shown that multiple myeloma with translocation t(11;14) may be exquisitely sensitive to the bcl-2 inhibitor venetoclax.
Aims: Here we report the highly successful therapeutic result of a myeloma patient diagnosed 11 years ago who received 8 prior lines of therapy that included all available therapeutic agents (including thalidomide, lenalidomide, bortezomib, carfilzomib, bendamustine, daratumumab and twice high-dose melphalan with autologous stem cell transplantation). The patient presented at our institution with hypercalcaemia (3.01 mmol/l), cytopenias, ECOG 3 physical condition due to profuse bone pain. Blood smear indicated plasma cell leukemia, flow cytometry analysis confirmed plasma cell leukemia with circulating plasma cells at the level of 14 G/l.
Methods: We are reporting on a clinical case documented with clinical laboratory, FISH and flow cytometry analysis.
Results: Since the patient was known to have broad resistance to available myeloma therapies, however had translocation t(11;14) since diagnosis, after initial zoledronic acid infusion, we elected to administer bortezomib-dexamethason-bendamustine combination with the addition of clarithromycine 500 mg BID and 400 mg daily dose of venetoclax orally. On day 3, the circulatory plasma cells disappeared and grade II tumor lysis syndrome developed (LDH max at 3107 U/l, with manageable hyperuricaemia, hyperkalemia, and hyperphosphatemia). The overall clinical state of the patient was steadily improving, he currently continues to receive venetoclax in monotherapy at the dose level of 400 mg daily. As CYP3A4 inhibition, initially clarithromycine was administered, but after 1 month due to GI symptoms, clarithromycine was switched to fluconazole 100 mg daily that was well tolerated. After 5 month of venetoclax 400 mg OD (only) in this pharmacologically enhanced monotherapy, the patient is ECOG 0, his M-protein is detectable only by immunofixation, free light chains and their ratio are normal, blood counts are in the normal range. Bone marrow aspiration indicated pathological plasma cells below the level of detection of available flow cytometry.
Summary/Conclusion: Our case indicates that in secondary plasma cell leukaemia refractory to available prior therapies and exhibiting translocation t(11;14), venetoclax may be a potentially effective salvage therapy. Additionally, pharmacological enhancement of 400 mg daily dose of venetoclax - with CYP3A4 inhibitors clarithromycine or azole antifungal agents may provide a cost effective alternative to the generally recommended 1200 mg daily dose of this expensive novel medication.

PB2234

SALVAGE THERAPY WITH POMALIDOMIDE-BASED REGIMEN IN RELAPSED/REFRACTORY MYELOMA. EFFICACY, AND SAFETY RESULTS

M. Sirvent Auzmendi[1,*], S. Herraez Rodriguez[2], E. Perez Persona[3], N. Caminos Altuna[1], I. Urreta Barallobre[4], E. Perez Santaolalla[1], I. Ceberio Echechipia[1], M. Araiz Ramirez[1]

[1]Hospital Universitario Donostia, San Sebastian, [2]Hospital Basurto, Bilbao, [3]Hospital Txagorritxu, Vitoria, [4]Epydemiology, San Sebastian, Spain

Background: The combination of pomalidomide and low-dose dexamethasone has proved to be effective and safe in patients with end-stage relapsed/refractory multiple myeloma (RRMM), otherwise characterized by a very poor outcome. MM remains an incurable disease with unavoidable relapses. However, with the new therapeutic approaches available to date, prolonged survival of patients has been demostrated. Pomalidomide is a distinct immunomodulatory drug with potent antimyeloma activity, and has demonstrated efficacy even in patients that are refractory to Lenalidomide. In order to increase response rate, commonly it is used in combination.
Aims: We analize retrospectively, in a real-worl clinical practice setting, the efficacy, and safety of a pomalidomide-based regimens in patients with RRMM, treated in 3 centers. We evaluated the efficacy either when given with dexamethasone in 23 patients or in combination with oral cyclophsphamide +/- claritromycin in 25 patients. We evaluated the impact of adverse prognostic factors of cytogenetics and refractoriness to previous lenalidomide.
Methods: This multicenter estudy included fourty eight patients treated with Pomalidomide-based regimen for RRMM, between 2013-2017.
Results: Overall response rate (ORR) was 55.4% including CR 4.4%, VGPR in 24.4%, and PR in 26.6% patients. 13.3% showed stable disease, and 31% progression. There was no significant difference in patients who received pomalidomide in combination *vs* alone with dexamethasone, in terms of ORR (66.6% *vs* 71.43%, p 0.75) and PFS (mean 7.2 *vs* 8.5 months, p 0.55). If the treatment was given early (at second, third or four line) there was a trend towars improved PFS (mean 8.6 *vs* 5.8 months at ≥5 lines), but was not statistically significant (p 0.24). Lenalidomide refractory patients present disease progression in 38.24% and ORR in 50%. In patients whithout refractoriness, all of them had response (p0.043). The mean PFS was 10 months in the non-refractory *vs* 5 month in the refractory group (p 0.11).There was no significant difference in ORR and mean PFS was 8.4 *vs* 7.1 months in standar *vs* high risk citogenetic groups (p0.62). The most common adverse events were infections (19.1%), cytopenias (10.6%) and inestability (6.4%). 12 patients (25%) need dose reduction and 5 (10.4%) had to discontinued because of AEs.
Summary/Conclusion: Pomalidomide-based treatment is effective, even in heavily treated RRMM, and adverse citogenetic group. In our study there was no benefit from using pomalidomide in combination with oral cyclophosphamide +/- claritromycin, but this should be confirmed in further studies. Lenalidomide refractory patients had shorter progressión free survival.

PB2235

RISK FACTORS FOR EARLY MORTALITY IN MULTIPLE MYELOMA WITH DIFFERENT SURVIVAL TIME CUT-OFF POINTS: REAL-LIFE DATA

M.S. Aydın[1,*], E. Karakulak[1], R. Çiftçiler[1], H. Demiroğlu[1], H. Goker[1], M. Turgut[2], E. Kelkitli[2], N. Sayinalp[1], Y. Buyukasik[1], F. Tekin[1]

[1]Hematology, Hacettepe University Faculty of Medicine, Ankara, [2]Hematology, 19 Mayıs University, Samsun, Turkey

Background: Overall survival in Multiple Myeloma (MM) improved in last decades with the introduction of newer agents and broader use of autologous hematopoietic stem cell transplantation. Besides this, early mortality (EM) is still a concern in newly diagnosed MM. Data for EM comes from large prospective trials of selected MM populations and different time cut-off points are used in different studies.
Aims: To evaluate most common causes of EM in MM, risk factors for EM and to flash on do's and don'ts in high risk cases.
Methods: Retrospective data from 37 newly diagnosed MM patients between 01.01.2003-30.10.2017 and died within 12 months of diagnosis and as control group 52 newly diagnosed MM patients who lived longer than 12 months were analyzed. Four different cut-off time points for early mortality was defined as 1 month (EM1), 3 months (EM3), 6 months (EM6) and 12 months (EM12). Univariate analyses were done by Chi-square test for categorical and by t-test for numerical variables, respectively. Multivariate analyses were done by Cox regression analysis.
Results: Median age was 59 (range, 45-82 years) and was not different from cases lived longer than 12 months (p=0,822). Males and females were 56.8% and 43.2% respectively. Disease characteristics and comorbidities are summarized in Figure 1. Most common cause of mortality was acute renal injury (86.5%). Median survival was 3.8 months for EM12. Multivariate analysis showed anemia (HR: 4.20, p: 0.003), and hypercalcemia (HR: 2.44, p: 0.012) as independent risk factors for EM12; anemia (HR: 3.79, p: 0,015) and hypercalcemia (HR: 2.39, p: 0.029) for EM6, anemia (HR: 4.69, p: 0.041) for EM3. Renal failure was also more frequent in EM12 (p=0,200). Congestive heart failure was associated with increased mortality in EM12 (HR: 3.10, p: 0.005) and EM6 (HR: 3.46, p: 0.005). Bortezomib based induction regimens were less frequently associated with mortality in EM12 (HR: 0.23, p<0.001), EM6 (HR: 0.22, p: 0,003) and EM3 (HR: 0.14, p: 0.01) in multivariate analysis. Types of paraprotein, extramedullary disease, adjusted Charlson Comorbidity Score were not statistically different between early mortality groups at any cut-off point of survival times. Response to induction regimen was not included in analysis due to missing data.

Table 1.

Figure 1. Disease characteristics and comorbidities

	Cases survived less than 12 months (n=37) (%)	Cases survived more than 12 months (n=52) (%)	p
Disease characteristics			
ISS III	14 (56.0)	22 (45.8)	p=0.465
Anemia	27 (72.9)	24 (48.0)	p=0.001
Bone morbidity	29 (93.0)	37 (78.7)	p=0.110
Hypercalcemia	16 (50.0)	11 (21.5)	p=0.009
Renal failure	14 (43.8)	15 (29.0)	p=0.238
Common Comorbidities			
Chronic renal disease	8 (21.6)	18 (34.6)	p=0.239
Congestive heart failure	8 (21.6)	2 (3.8)	p=0.015
Liver disease	4 (10.8)	0 (0)	p=0.027
Coronary artery disease	4 (10.8)	10 (19.2)	p=0.380
Chronic lung disease	3 (8.1)	2 (3.8)	p=0.645

Summary/Conclusion: Our study was first to detect anemia as a risk factor for EM in MM. Congestive heart failure was a risk factor as a comorbidity. Renal failure was also a risk factor for EM although not significant but there are studies showing renal failure as a risk factor for EM. These high risk patients should be treated with newer, less toxic agents.

PB2236

SPLENIC INVOLVEMENT IN AL AMYLOIDOSIS: A CAUSE FOR PANCYTOSIS

A. Jercan[1,*], S.N. Badelita[1], C. Dobrea[1], M. Popescu[1], D. Vasilache[1], S. Iacob[2], D. Coriu[1]

[1]Hematology and Bone Marrow Transplantation, [2]Gastroenerology, Fundeni Clinical Institute, Bucharest, Romania

Background: Light Chain Amyloidosis (AL) is a systemic disease which causes extracellular deposition of amyloid fibrils composed of immunoglobulin light chains secreted by monoclonal plasma cells. Splenic involvement is defined by functional hyposplenism identified by the presence of Howell-Jolly bodies in the red cells on peripheral blood smear in patients without splenectomy. Prevalence of hyposplenism in AL patients is 24%. Hyposplenism was identified in patients with AL and FX deficit. Splenomegaly is not a useful sign for the diagnosis of functional hyposplenism in amyloidosis. Hyposplenic patients had a worse survival compared to normosplenic amyloidosis patients, probably due to the extensive body burden of amyloid.
Aims: The aim was to study the presence of hyposplenism and it's association with pancytosis in light chain amyloidosis.
Methods: We studied the patients diagnosed with AL in the Hematology Department of Fundeni Clinical Institute, Bucharest, Romania, between 2007 and 2017. Functional hyposplenism was identified by the presence of Howell-Jolly bodies in the red cells on the peripheral blood smear. We evaluated CBC, age, sex, AL type, FX level and involved organs.
Results: In the last 10 years, there were diagnosed 124 patients with AL, 40 patients (32%) had Howell-Jolly bodies in the red cells and 13 patients (10%) had leukocytosis +/- thrombocytosis. Median age of patients with functional hyposplenism was 62 years, M:F ratio was 1:1 and 38% were κ

AL and 62% λ AL. The association of leukocytosis and presence of Howell-Jolly bodies in the red cells on peripheral blood smear was present in 6 patients, of which 4 had cardiac and kidney involvement and 4 had liver and peripheral nervous system involvement. Hepatomegaly was seen in 4 of the 6 patients. The average FX level was 69% (min. 30% and max 110%), but without clinically significant bleeding, except amyloid vasculitis.
Case presentation. A 47 years old male diagnosed in 2014 with pancytosis (WBC 14 310/μL; PLT 906 000/μL, HB 19.4 g/dl; Ht 56.4%) and hepatomegaly. Bone marrow aspirate and biopsy were performed and he was diagnosed, in another Hematology Department, with chronic myeloproliferative disease for which he received 4 phlebotomies and hydroxyurea for 6 months. In October 2014 he is diagnosed with nephrotic syndrome and has a renal biopsy that shows amyloid deposits (kappa light chain). In November 2014 he was admitted to our Clinic and was diagnosed with kappa light chain amyloidosis with systemic involvement: kidney (nephrotic syndrome), liver (massive hepatomegaly, Stiffness of 75 KPa on Fibroscan), splenic (pancytosis, Howell-Jolly bodies in the red cells, splenomegaly). We started treatment with CyBorD (Cyclophosphamide, Bortezomib and Dexamethasone), but the patient wasn't compliant and was lost from treatment and follow up for about 1 year. In this period, he developed severe kidney disease and started hemodialysis. He came back to our Clinic in November 2015 and received 7 cycles of CyBorD. Involved free light chain decreased from 519 mg/l to 44 mg/l, there was an impressive improvement in Fibroscan stiffness (75->13.8 KPa) and maintained a slight leukocytosis and thrombocytosis. He is without treatment since June 2016, in excellent clinical condition, normal blood count, but he is still on hemodialysis.
Summary/Conclusion: Amyloidosis with splenic involvement and secondary hyposplenism can be the cause of pancytosis and so it should be considered, especially if Howell-Jolly bodies in the red cells are present on the peripheral blood smear.

PB2237

MAINTENANCE TREATMENT WITH THALIDOMIDE AFTER FRONTLINE BORTEZOMIB-BASED REGIMENS IN TRANSPLANTATION INELIGIBLE PATIENTS WITH MYELOMA MULTIPLE (MM). EXPERIENCE OF REAL LIFE

D. De Miguel Llorente[1,*], A. Gil[2], H. Guillen[3], A. Vazquez[2], N. Golbano[4], A. Pérez[5], D. Subira[4], J. Arbeteta[4], S. Herrero[6], D. Morales[7]

[1]Hematologia, Hospital Universitario de Guadalajara, [2]H. U. Guadalajara, [3]H.U. Guadalajara, [4]H.U.G, [5]H.U.G., Guadalajara, [6]H.U.G, Gyadalajara, [7]HUG, Guadalajara, Spain

Background: Phase IIIB UPFRONT trial was designed to compare three frontline bortezomib-based regimens in transplantation ineligible patients with Myeloma Multiple (MM) (Niesvizky R, *et al.* JCO 2015;33:3921-3929. Median progression free survival (PFS) with VD, VTD and VMP was 14.7, 15.4 and 17.3 months, respectively; median overall survival was 49.8, 51.5 and 53.1 months. Nowadays, lenalidomide continuous frontline therapy in elderly MM patients has showed better results in PSF and OS in this group of patients.
Aims: The aim of our review was to evaluate the efficacy and clinical outcome of initial bortezomib-based therapies and the maintenance treatment with thalidomide in elderly MM patients.

Table 1.

	PFS (months, median)	OS (months, median)
VMP	44.5	69.5
VD	25	35
VCD	31	31

Methods: we report a total number of 30 elderly MM patients (age median 80 years old, range 66-89; 19 females) since January 2008 to December 2017. 18 patients were treated in frontline with Bortezomib and Dexamethasone (VD), 8 patients with Bortezomib. Melphalan and prednisone (VMP) and 5 patients with Bortezomib. Cyclophosphamide and dexamethasone (VCD). The patients after completed the planned cycles of Bortezomib (median 7 cycles, range 3-14), received maintenance therapy with oral thalidomide (50mg/d) until disease progression or toxicity. Response rate, progression-free survival (PFS) and overall survival (OS) were the outcome measures.
Results: Nowadays, 6 patients are ongoing with thalidomide therapy. 11 (36.6%) patients had to stopped the thalidomide due to progression of disease. Only one patient (3,3%) had discontinued thalidomide therapy by tolerability. In overall group, we reported a PFS of 33,5 median months, and 35 median months of OS. In the table 1, we described the results of PFS and OS, in the patient's different subgroup.
Summary/Conclusion: 1) Thalidomide maintenance offer an advantage in PFS and OS in all bortezomib containing regimens (VTD; VD; VCD) in transplantation ineligible patients with MM; 2) Thalidomide maintenance was feasible without producing cumulative toxicity. 3) The continuous treatment with thalidomide (low dosage, 50mg/d) is efficacious, tolerable and low cost, and it should be taken into consideration.

PB2238

EFFICACY AND SAFETY OF TREATMENT WITH BORTEZOMIB, CYCLOPHOSPHAMIDE, DEXAMETHASONE (VCD) VERSUS BORTEZOMIB, DEXAMETHASONE (VD) REGIMENS IN NEWLY DIAGNOSED PATIENTS WITH MULTIPLE MYELOMA (NDMM)

M. Liangming*

Hematology, Shan Xi Da Yi Hospital, Taiyuan, China

Background: Bortezomib (BTZ) represents an important progress in the treatment of multiple myeloma MM. BTZ combined with other agents is becoming a standard care.The combination of BTZ with dexamethasone previously shown the superior to VAD.
Aims: To observe the efficacy and safety of bortezomib cyclophosphamide dexamethasone VCD) versus bortezomib dexamethasone (VD) regimens in treatment of patients with newly diagnosed multiple myeloma (NDMM).
Methods: To retrospective analysis of the 73 patients with NDMM from January 2013 to January 2016 in Department of Hematology Dayi Hospital.they were divided into VCD arm and VD arm, The outcomes of two different regimens were analyzed, including response and adverse events
Results: He overall response rate was 80.5% (33/41) with VCD arm and 78.1% (25/32) with VD arm,with no statistically significant difierences (P=0.804). However,complete response was 36.6% (15/41) with VCD arm and 15.6% (5/32) with VD arm,and differences were statistically significant (P=0.046), The median progression free survival (PFS) was 27 and 24 months in VCD arm and VD arm, the median overall survival (OS) was 35 and 33 months in VCD arm and VD arm,with no statistically significant difierences (P>0.05). Peripheral neuritis (PN), thrombocytopenia,diarrhea and constipation are the most common adverse events in two groups. There was no statistically significant difference between the two groups (P>0.05). Most adverse events were in grade 1 and 2.
Summary/Conclusion: Both VCD and VD regimens are effective induction chemotherapy choices for NDMM. VCD is preferable to VD terms of achieving complete remission.

PB2239

THE AUTOPHAGY INHIBITOR HYDROXYCHLOROQUINE ADDED TO THALIDOMIDE BASED THERAPY FOR MULTIPLE MYELOMA DEEPENS RESPONSE AND PROLONGS REMISSION IN PATIENTS PREVIOUSLY TREATED WITH THALIDOMIDE

S. O'Mara[1,2,3,*]

[1]Haematology, Laverty pathology, [2]Tamara Hospital, [3]New England Northwest Haematology, Tamworth, Australia

Background: Autophagy inhibitors are being tested in clinical trials for the treatment of malignancy. In multiple myeloma the autophagy inhibitor plaquenil has been tested with bortezomib *in vitro* with mixed results as well as a clinical trial with disappointing outcomes. Recently the antibiotic clarithromycin has been shown to be an autophagy inhibitor and has also been shown to synergistically increase the response to thalidomide in vitro. There have been previous single cohort studies using thalidomide/ clarithromycin combinations in multiple myeloma. At the time of that study, it was not known that clarithromycin was an autophagy inhibitor. Clarithromycin when trialled with Bortezomib in a clinical trial lead to unacceptable gastrointestinal toxicity without clear benefit. Does the addition of an autophagy inhibitor Hydroxychloroquine when added to an IMID such as thalidomide lead to an improvement over thalidomide alone as suggested by the *in vitro* studies. A longnitudinal crossover study was undertaken on the effect of adding hydroxychloroquine to patients who had previously been given thalidomide therapy previously. It would be expected

that patients would have an inferior response and duration of response the second time they were given thalidomide.
Aims: To determine whether the autophagy inhibitor Hydroxychloroquine when added to thalidomide based therapy improves the response to thalidomide based treatment.
Methods: Three patients received the CTD regimen for a period of 6 months. Thalidomide 100mg a day, Cyclophosphamide 100mg a day orally and Dexamethasone 20mg orally once weekly. One had a partial response 5g/L lasting 16 months from 2014. The second patient had a VGPR 2g/L lasting 17 months 2014. The third patient had no response to either thalidomide or lenalidamide in 2008 at 14g/L. She subsequently underwent bortezomib based therapy and stem cell transplant. All three relapsed with bone pain and increasing paraprotein level >20% from plateau level. The patients again received CTD at the same dose and duration. Hydroxychloroquine (plaquenil) was added at 200mg twice a day. This dose is a dose used to treat common rheumatological diseases and is safe even in combination with other agents. The absolute response in paraprotein level was documented and the duration of response has been measured every three months.
Results: All patients symptoms of bone pain have resolved. Two patients have achieved a complete remission of 0 g/L paraprotein measured at the end of 6 months therapy with CTD and Hydroxychloroquine. The third patient achieved a Partial remission at 3g/l a 70% reduction. Two patients response duration has already been maintained for a longer period than in their first remission with CTD and the third continues. There has been no grade two or higher toxicity.
Summary/Conclusion: Hydroxychlroquine when used with thalidomide based therapy is a cheap non toxic addition to myeloma therapy that appears to deepen the response in all patients tested. It has also increased the response duration in 2 treated patient with the others not progressing. Although the clinical trials have focused on adding autophagy inhibitors to poteasome inhibitors, it appears that they may be more useful when used with IMIDS.

PB2240

CT AND MRI MAY RESULT IN OVERDIAGNOSIS OF SKELETAL DISEASE IN MULTIPLE MYELOMA

A.E.A. Dahm[1,*], V. Dalen[2], A.-S. Vegsgaard Olsen[2], C.-P. Jerome[3], J.-T. Geitung[3]

[1]Haematology, Akershus University Hospital, Lørenskog, [2]University of Oslo, Oslo, [3]Radiology, Akershus University Hospital, Lørenskog, Norway

Background: Investigations for skeletal disease is essential in the diagnostic work-up of multiple myeloma. According to international guidelines, low dose CT, MRI or PET-CT are the preferred choices of modality.
Aims: To compare the ability of MRI and CT to detect of skeletal disease in multiple myeloma. To investigate the inter-observer agreement between two radiologists in the interpretation of the images.
Methods: A retrospective, comparative study of multiple myeloma patients that that had taken MRI and CT within four months between 2007 and 2015. We included only patients where the entire vertebral column was portrayed and ended up with 12 patients. The radiologists looked at the images independently blinded for the colleague's' previous descriptions. The images were assessed for malignant fracture, osteoporotic fracture, malignant infiltration and osteoporosis. Later they sat down together, to make up a gold standard, using all pictures and information available to them.
Results: Radiologist 1 diagnosed 20 malignant fractures in the vertebral column on CT, and 26 on MRI, while Radiologist 2 diagnosed 12 fractures on CT and 22 on MRI. The gold standard, however, showed 10 fractures. The two radiologists agreed that 1 patient had no malignant fractures on CT, while the gold standard showed that 6 of 12 patients did not have malignant fractures. The radiologists had a total of 24 different diagnostic assessments of malignant fractures on CT and 18 on MRI. Radiologist 1 diagnosed 9 osteoporotic fractures on CT and 23 on MRI, and Radiologist 2 diagnosed 9 fractures on CT and 24 on MRI. The gold standard showed 14 osteoporotic fractures. The sensitivity for radiologist 1 to discover malignant fractures on CT was 0.83, specificity 0.5. For radiologist 2, the sensitivity for malignant fracture on CT was 0.5 and the specificity 0.67. On MRI, the sensitivity of Radiologist 1 for detecting malignant fractures was 0.83, specificity 0.17. Radiologist 2 had sensitivity of 1 on MRI, but specificity of 0.17. The inter-observer agreement for detection of malignant fractures showed a Cohen's kappa of 0.42.
Summary/Conclusion: Both radiologists diagnosed malignant and osteoporotic fractures when the gold standard showed that there was none. MRI was not better than CT. Furthermore, the agreement between the radiologists was unsatisfactory. Our study shows that the current guidelines for skeleton imaging in multiple myeloma may result in overdiagnosis of skeletal disease.

PB2241

BORTEZOMIB INDUCTION AND MAINTENANCE THERAPY IN MULTIPLE MYELOMA PATIENTS INELIGIBLE FOR TRANSPLANTATION

K. Milcheva[1,*], D. Todorieva[2], I. Kindekov[3], E. Naseva[4], B. Spasov[1]

[1]Clinic of Hematology, National Specialised Hospital for Active Treatment of Haematological Diseases, Sofia, [2]Clinic of Hematology, University Hospital "St.Stransky", Pleven, [3]Clinic of Hematology, [4]Department of Health Economics, Medical University - Sofia, Sofia, Bulgaria

Background: The current report represents the interim results of clinical observation, estimating the efficiency and safety of Bortezomib based induction and maintenance therapy in transplant ineligible patients.
Aims: The aim of the current investigation includes assessment of progression free survival (PFS), treatment response rates, overall survival (OS) and adverse events.
Methods: The analysis includes 20 patients, 9 male and 11 female, who received treatment in the hematology clinics of the Military Medical Academy and UMPHAT "G.Stranski" -Pleven. Inclusion criteria: patients with multiple myeloma, who received Bortezomib-based first line treatment and Bortezomib maintenance for two years or till progression/toxicity; transplants ineligible; Exclusion criteria: polyneuropathy Grade >=2 or heavy comorbidity. Patients were assessed by laboratory exanimations, including FBC, biochemistry, β2-microglobulin, and serum and urine electrophoresis with immune fixation. Patients' diagnose, stage and response were evaluated according to the IMWC 2010 recommendations. Bortezomib-induced hematology and non-hematology toxicity was graded according to the CTCAE v.4.03 criteria. Methods of descriptive statistics were applied. The survival rate was assessed by the Kaplan-Meyer method. The survival in the individual groups was compared with the Log Rank test (Mantel-Cox) and the follow up time - by Kaplan-Meyer's inverted method. Results were accepted significant at $p < 0.05$.
Results: After 27 months median follow up, the OS had not yet been reached, the PFS was 24 months. At 32 month, 61, 1% of patients were still alive. The two year survival was 76%. The majority of the patients were stage II-III ISS- 8 (80,0%) and Ig G type - 11(55%). After a median follow-up time of 24 months, the PFS was 24 months. The OS has not yet been reached. At 32-month, 61,1%of patients were still alive. The two-year survival was 76%. Median time to first response was 4 month (2-8), with predominant response rate (PR) in 75% of the patients. Median time to best response was 2 months (2-31), with predominant response rate CR in 45% of the patients. During the follow-up, 9 patients experienced loss of response. At the last visit, 11 patients continue their treatment. In the course of treatment, the safety of the drug was also monitored. In 20% (n=4) of patients, reversible Bortezomib-related toxicity was recorded and only one patient required dose reduction due to painful neuropathy. There were no serious CTCAE Grade 3-4 events, death or discontinuation of therapy due to Bortezomib.
Summary/Conclusion: Despite the small number of patients, the obtained results are comparable to those from the UPFRONT Phase 3 trial. Bortezomib (Velcade®) -based therapy, including induction and maintenance phase, in patients with multiple myeloma, unsuitable for transplantation, is an effective and safe therapeutic strategy.

PB2242

INCIDENCE AND PREVALENCE OF MULTIPLE MYELOMA IN A LARGE NATIONWIDE HEALTH PLAN IN ISRAEL

C. Weil[1,*], S. Gelerstein[2], G. Chodick[3], S. Sharman Moser[1], N. Barit Ben-David[2], V. Shalev[3], D. Meytes[4]

[1]Epidemiology & Database Research, Maccabi Healthcare Services, Tel Aviv, [2]Janssen Israel, J-C Health Care Ltd., Kibbutz Shefayim, [3]Epidemiology and Database Research & Sackler Faculty of Medicine, Maccabi Healthcare Services & Tel Aviv University, [4]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Background: Multiple myeloma (MM) is the second most common hematologic malignancy.
Aims: This study aims to describe the real-world incidence and prevalence of MM in Israel.
Methods: A retrospective database study was conducted in Maccabi Healthcare Services (MHS), a 2-million-member health payer-provider in Israel. MM was defined by cross-linking ICD-9 diagnoses with dispensed MM treatments and free light chain assays. MM prevalence (31/12/2016) and incidence (2012-2016) rates were calculated and age-adjusted to the WHO World standard population. Prevalent patients were characterized in terms of socio-demographic characteristics, comorbidities, and medication use.
Results: The crude and age-standardized prevalence of MM was 3.2 and

2.6 per 10,000 population, respectively. The mean (± SD) age was 68.1±11.0 years (55% male). The prevalence of cardiovascular disease, diabetes, hypertension, chronic kidney disease (eGFR <60) and osteoporosis were 32%, 25%, 59%, 59%, and 38%, respectively. Overall, 88.5% ever had a record of dispensed MM treatment. Among patients treated in 2016 (N=427), their last purchase consisted of 43% first line, 30% second line, 18% third line, and 9% fourth line or more. The crude and age-standardized annual incidence rate was 5.1 and 4.7 per 100,000 population, respectively. The mean age at diagnosis was 68.5±11.4 (58% male). Adjusting to the Israeli population, there were an estimated 485 new cases of MM in Israel in 2016.
Summary/Conclusion: These results indicate a substantial burden of MM in Israel, in line with international estimates. This study serves as the basis for further research on MM patient outcomes.

PB2243

BORTEZOMIB, LENALIDOMIDE AND DEXAMETHASONE IN THE MANAGEMENT OF RELAPSED AND REFRACTORY MULTIPLE MYELOMA: A REAL-LIFE EXPERIENCE

C. Cerchione[1,*], A.E. Pareto[1], I. Zacheo[1], M. Di Perna[1], D. Nappi[1], I. Peluso[1], K. Ferrara[1], A. Romano[2], F. Pane[1], L. Catalano[1]

[1]Hematology - Department of Clinical Medicine and Surgery, University Federico II, Naples, Napoli, [2]Department of General Surgery and Medical-Surgical Specialties, Haematology Section, University of Catania, Catania, Italy

Background: Bortezomib, Lenalidomide and Dexamethasone is one of the best option for frontline treatment, approved in USA but not available in Italy. However, it can show interesting results also in relapsed and refractory patients, thanks to the synergistic effect of these agents.
Aims: In this retrospective observational study, it has been evaluated the safety and efficacy of the combination of bortezomib plus lenalidomide plus dexamethasone (VRD) in patients with relapsed and refractory Multiple Myeloma (rrMM).
Methods: 29 patients (19 M, 10 F), with rrMM, median age 64 years (range 38-79), were treated with the VRD regimen (Bortezomib 1.3 mg/sqm days 1,4,8,11; dexamethasone 20 mg days 1, 2, 4, 5, 8, 9, 11, 12 and oral lenalidomide 25 mg daily on days 1-21), with a median of 6 cycles (range 1-21). Patients had previously received 3 median (range 1-6) lines of therapy. 83% (24/29) of them had undergone to autologous SCT.
Results: According to IMWG, ORR was 79.3% (23/29: 6 CR, 5 VGPR, 7 PR, 5 SD). Median time to response was 3 months (range 1-6), median OS from diagnosis was 56 months (range 12-221). Bortezomib-lenalidomide-dexamethasone was well tolerated, with grade 1-2 anemia in 5 patients, successfully managed with ESAs, and, thanks to the way of administration, also compliance is good. Peripheral neuropathy was seen in 48% (14/29) patients.
Summary/Conclusion: Bortezomib-lenalidomide-dexamethasone triplet, thanks to a notable proved synergistic mechanism of action between bortezomib and lenalidomide, had shown significant efficacy in severe setting of heavily pretreated patients, relapsed and refractory to bortezomib and lenalidomide.

PB2244

OUTCOME OF PATIENTS WITH MULTIPLE MYELOMA AND RENAL FAILURE IN ERA OF NOVEL AGENTS- EXPERIENCE OF A TERTIARY CARE CENTRE FROM NORTHERN INDIA

N. Gupta*, F. Naim, A. Garg, K. Purohit, M. Mulam

Clinical Hematology and bone marrow/stem cell department, Sir Ganga Ram Hospital, New Delhi, India

Background: Renal involvement in myeloma is a serious complication which occurs in 20-30% of patients. In 10% of patients, it is severe enough to require dialysis. Historically outcome to patients with myeloma and renal failure have been poor with median survival of less than 1 year. Prognosis depends upon the reversibility of renal function with a survival similar to other patients in whom creatinine returns to normal. With the advent of novel agents like bortezomib and thalidomide, which can be safely given in these patients, remission rates and renal recovery rates have significantly improved.
Aims: To study the outcomes in patients of multiple myeloma presenting with renal failure treated with bortezomib based triple drug regimens treated at our centre.
Methods: This is a retrospective study in which 20 patients treated at the Sir Ganga Ram hospital, New Delhi, India with diagnosis of MM and renal dysfunction and at least six months of follow up were enrolled from January 2015 to June 2017. Demographic data, baseline serum creatinine, hemoglobin, serum calcium level, M protein type and level, bone marrow plasma cells, need for dialysis were recorded. Bortezomib plus Thalidomide plus dexamethasone (BTD) and Cyclophosphamide plus Bortezomib plus dexamethasone (CyBorD) were the two regimens employed in non-random fashion on treating physician's discretion.
Results: The mean age of the patients was 53.9 years. The median baseline serum creatinine was 5.05 mg/dL (2.79–15.39). Anemia (hemoglobin <10 g/dL) was noted in 70% and hypercalcemia (calcium >11mg/dL) in 40% of patients. Immunofixation revealed myeloma to be Ig G in 10/20 (50%), Ig A in 7/20 (35%) and light chain only myeloma in 3/20 (15%) patients. BTD and CyBorD were used in 12 (60%) and 8 (40%) of patients respectively with high dose dexamethasone (40mg/day for 4 days) given in first cycle to all patients. Hemodialysis was performed in 10 patients (50%) and two of them underwent high cut off (HCO) dialysis. Serum free light chain levels returned to normal after 4 sessions of 8 hours each of HCO dialysis. Serum creatinine came to normal level in all (80%) patients except four patients after 4 cycles of chemotherapy. Two (10%) of them remained dialysis dependant. Six patients (30%), who achieved a very good partial response (VGPR) after 4 cycles of bortezomib based chemotherapy but then suffered early relapse and died of progressive disease. The median progression free survival has not reached after 18 months of follow up (range 6-32 months). Six patients (30%) underwent autologous stem cell transplantation and continue to be complete remission. Median progression free survival in patients who underwent transplant (not reached after 25 months of follow up) *vs* non- transplanted group is (10.5 months).
Summary/Conclusion: In our patients with the incorporation of novel agents including bortezomib and thalidomide, outcome of patients with myeloma with renal failure has improved. Moreover, autologous transplant can further improve outcome in carefully selected patients. Longer follow up is needed to determine exact benefit of these agents in improving overall survival of these patients.

PB2245

YOUNG ADULTS WITH MULTIPLE MYELOMA – UNVEILING A DIFFERENT SPECTRUM IN A RESOURCE CONSTRAINED SETTING

S. Mirgh*, M. Mahapatra, T. Seth, R. Saxena, H.P. Pati, S. Tyagi, M. Aggarwal, R. Dhawan, R. Halder, S. Kothari, R. Chauhan

Hematology, All India Institute of Medical Sciences, New Delhi, New Delhi, India

Background: Traditionally, Multiple Myeloma (MM) has been considered a disease of the elderly with peak incidence at 60-70 years of age. However, there exists a certain subset of extremely young patients. Data on young adults with MM is scarce. We present the disease characteristics for this unique age group.
Aims: 1. To understand the frequency of patients with young myeloma (<=40 years), their clinical profile and response to therapy. 2. To compare with older population in terms of clinical profile.
Methods: Retrospective single-centre study conducted at a single tertiary care centre from New Delhi, North India. Records of all young patients (18-40 years) with MM managed in our department in the last 6 years (January 2012 – December 2017) were reviewed.
Results: A total of 112 evaluable patients were included in the study. Interestingly, the frequency of young MM patients was 27.6% (31/112), 3 of whom were less than 30 years of age at diagnosis. Unlike elderly, females almost equated the males in this age group (male:female : 1.06:1). Main presenting manifestations were back pain (64.5%), fatigue (58%) and referral for renal dysfunction (19.3%). Other presenting features included plasmacytomas (13%), weight loss (6%), bleeding manifestations (3%) and cord compression (3%). Hypercalcemia, renal dysfunction, anemia and lytic lesions were present in 19.3%, 35.4%, 71% and 77.4% patients respectively. Of these, one-fourth patients (25.8%) underwent autologous stem-cell transplant. Baseline beta-2-microglobulin levels were available in 23 patients. 82.6% patients were in high risk group as per ISS – 3 (International Staging System) staging. Almost one-third cases (n=10) (32.2%) were relapsed-refractory. FISH panel was done in 8 patients, 2 (25%) of whom revealed 13q deletion.
Summary/Conclusion: Our study shows a trend towards gender equality in young patients with MM, a higher prevalence of high-risk (ISS-3) disease, higher prevalence of appendicular skeleton lesions and good response to therapy.

PB2246

NON-MYELOMA KIDNEY DISEASE AND MANAGEMENT ALGORITHM IN MYELOMA PATIENTS

K. Westwood[1,*], S. Natarajan[1], S. Elhanash[1], I. Ali[2], S. Watt[1]

[1]Haematology, [2]Renal Medicine, Manchester Foundation NHS Trust, Manchester, United Kingdom

Background: In multiple myeloma, the kidney is a major organ that can be affected during the disease process resulting in 40% of patients developing renal impairment. In an ageing population, there are multiple comorbidities that can have a negative impact on renal function and hence non-paraprotein related disease has previously been described in up to 25% of myeloma patients. Declining renal function in myeloma results in difficulties giving optimum chemotherapy and worse outcomes for patients.
Aims: To optimise management of renal impairment from none myeloma causes.
Methods: We reviewed an unselected group of 34 patients, median age 75 (53-87), attending our unit with multiple myeloma and graded their renal function using a combination of glomerular filtration rate (GFR) and Albumin Creatinine Ratio (ACR).
Results: Findings show that 54.8% of the patients have a mild to moderate decrease in renal function and 32.3% show a moderate to severe decrease. 58% of patients had an ACR outside of the normal range (<3mg/ml). See Table. This leaves only 12.9% of patients had no markers of chronic kidney disease (CKD). We identified 12% of the patients are pre-diabetic (HBA1c 42-47 mmol/mol) and 5.9% of patients are known to be diabetic, but had HBA1C greater than 58 mmol/mol. 24% of patients were identified as having a systolic blood pressure ≥140 mmHg.
Summary/Conclusion: In patients who have myeloma at least partial remission we follow our algorithm summarised below for monitoring and intervention to preserve renal function, 1. Clinical testing of Blood pressure, HBA1C, ACR, EGFR at least 12 monthly; 2. Patients with stage 3b renal failure are referred for a renal opinion; 3. If the patient is diabetic and poorly controlled despite GP management we refer to endocrinology for intervention; 4. If they are pre-diabetic or newly diagnoses diabetic then we refer to GP for intervention; 5. We ask GPs to review for patients with a systolic BP over 140 or diastolic over 90. Initially with ambulatory BP monitoring and pharmaceutical intervention if confirmed; 6. If ACR >30 we advise an ACE inhibitor at a dose tolerated by the patient; 7. We consider addition of a statin if eGFR <60 due to increased cardiovascular risk. The full algorithm defines the frequency of CKD, diabetic and blood pressure monitoring needed for specific patient groups within the multiple myeloma population. In conclusion, the aim of our patient review and algorithm is to identify, target and manage all possible reversible causes of CKD in the multiple myeloma cohort. We believe this is a cheap and effective intervention to improve quality of life and may have an impact in the overall survival in multiple myeloma by preserving renal function. We intend to monitor our patient cohort longitudinally, but further studies would be useful to prove the effectiveness of these interventions in patients with multiple myeloma.

PB2247

THE MYELOMA CANADA RESEARCH NETWORK CANADIAN MULTIPLE MYELOMA DATABASE (MCRN CMM-DB): MULTI INSTITUTIONAL SHARING OF CLINICAL DATA FOR RESEARCH

C.P. Venner[1,*], E. Masih-Kahn[2], T. Truaon[3], V. Jimenez-Zepeda[4], A. McCurdy[5], M. Sebag[6], K. Song[7], T. Reiman[8], R. LeBlanc[9], J. Stakiw[10], M. Louzada[11], R. Kotb[12], D. White[13], M. Aslam[14], A. Del Col[15], S. Tavoularis[15], E. Gul[16], D. Reece[2]

[1]Medical Oncology, Cross Cancer Institute, University of Alberta, Edmonton, [2]Medical Oncology and Hematology, [3]Health Informatics, Princess Margaret Cancer Centre, Toronto, [4]Tom Baker Cancer Centre, Calgary, [5]The Ottawa Hospital, Ottawa, [6]McGill University, Montreal, [7]The Leukemia and Bone Marrow Transplant Program of British Columbia, Vancouver General Hospital, Vancouver, [8]Horizon Health Network, Saint John, [9]The Maisonneuve-Rosemont Hospital Research Centre, Montreal, [10]Saskatchewan Cancer Agency, Saskatoon, [11]London Regional Cancer Centre, London, [12]Cancer Care Manitoba, Winnipeg, [13]Nova Scotia Health Authority, Halifax, [14]Allan Blair Cancer Centre, Regina, [15]Myeloma Canada, Montreal, [16]Myeloma Canada Research Network, Vaughn, Canada

Background: We have seen dramatic improvements in survival for patients living with multiple myeloma (MM). To better understand the current national landscape for patients with MM the Myeloma Canada Research Network (MCRN) developed a centralized Canadian Multiple Myeloma Database (MCRN CMM-DB) aimed at collecting all disease specific outcomes-based endpoints.
Aims: The goals of the MCRN CMM-DB are to: 1) benchmark current outcomes of MM patients treated with available treatment strategies in Canada; 2) identify regional therapeutic differences across the country; 3) inform future care strategies with evolving funded novel treatment approaches and 4) better inform future trial initiatives and national translational programs.
Methods: Sites with recognized expertise in treating MM across Canada were invited to participate in data collection. Capitalizing on existing local disease specific databases, endpoints were identified to determine the final data dictionary and mandatory data elements used in the MCRN CMM-DB. Given broad representation across multiple regions and institutions a governance structure was developed, which included Operations and Steering Committees. This was essential to ensure the development of a representative data collection platform, data quality and integrity, protection of patients' privacy, financial sustainability, guide data access, prioritize data usage and disseminate findings to the broader MM community nationally and internationally. The aim is to enroll all newly diagnosed patients from participating sites over the next 10 years. Where available, legacy data on previously diagnosed patients going back to 2007 will also be uploaded to represent the patient experience through the novel agent era.
Results: To date, 13 sites representing the major MM treating and academic centres across Canada have committed to the initiative. After user acceptance testing was completed the final MCRN-MM-DB was built according to agreed specifications approved by the organizing and steering committees. Using the eCancerCare platform a user-friendly web-based interface was developed to facilitate the entry of anonymized data to a secure centralized data repository. In parallel to the system build, each participating site obtained research ethics approval consistent with local requirements. Institutions signed a data sharing agreement with MCRN to facilitate ease of data transfer across jurisdictions. Access to the MCRN-MM-DB was deployed to sites with ethics approval, a signed data sharing agreement and with designated data coordinators to consent patients and enter data. Six sites that had existing data registries obtained REB approval to migrate legacy data. The first site was activated in July 2017. Thirteen sites representing the provinces of Ontario, Quebec, Alberta, Nova Scotia, Manitoba, Saskatchewan, British Columbia and New Brunswick are now contributing data. 20 users across sites have received formal training and were provided with Case Report Form Completion Guidelines. A total of 50 records from different sites have been audited centrally. Prospective data on 102 patients have been entered. The legacy dataset is estimated at 4000 patients. An aggregate summary of pooled data available in the MCRN-MM-DB will be presented at the meeting.
Summary/Conclusion: We anticipate that the MCRN CMM-DB will be one of the largest, most comprehensive nation-wide disease specific repositories. It will provide locally and nationally relevant benchmarks for current outcomes and better inform future clinical and academic ventures.

PB2248

BULKY EXTRAMEDULLARY SOFT-TISSUE PLASMACYTOMAS IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS: A SINGLE CENTER EXPERIENCE FROM NORTH INDIA

U. Nath*, R. Chetia, J. Nanda, A. Baveja

Medical Oncology Haematology, All India Institute of Medical Sciences (AIIMS), Rishikesh, Rishikesh, India

Background: Multiple myeloma (MM) is a clonal plasma cell neoplasm typically confined to the bone marrow and skeleton. In addition, a number of patients develop extramedullary disease, which may sometimes present as the leading clinical manifestation. The incidence of extramedullary plasmacytomas has been reported in the western literature as 7% - 18% at the time of multiple myeloma diagnosis and up to 20% at myeloma relapse. However, there is very little data regarding the same in the Indian scenario.
Aims: The present abstract reports the high incidence of bulky extramedullary soft-tissue plasmacytomas in newly diagnosed multiple myeloma patients hailing from the western parts of Uttar Pradesh and adjoining Haridwar district of Uttarakhand, who attended AIIMS Rishikesh for diagnosis & treatment.

Table 1.

Sl	Age/Sex	Diagnosis	Plasmacytoma site	Treatment & outcome
1	68 y / M	IgA Kappa MM	Bulky retroperitoneal mass; Multiple left cervical & supraclavicular lymphadenopathy (Biopsy+IHC: Plasmacytoma)	CyBorD x 2 cycles: No response. Bortezomib+ Bendamustine+ Dexa x 4 cycles: CR
2	62 y / F	Kappa Light Chain MM	Plasmacytoma involving left lung & adjacent thoracic wall (10.8 x 7.9 x 5.6 cm)	CyBorD x 4 cycles. Partial response.
3	60 y / M	Kappa Light Chain MM	Soft-tissue plasmacytomas on chest wall. Sphenoid bone plasmacytoma, adjacent to sphenoid sinus (24x13mm). Right Facial nerve palsy	Refractory to CyBorD. RVD started. Lost to follow up.
4	75 y / M	IgG Lambda MM	Multiple, large soft-tissue plasmacytomas in paraspinal region & on scapula	CyBorD x 3 cycle: Progressive disease. Lost to follow up.
5	39 y / F	Non-secretory MM	Presented with Left lumbar region mass & paraplegia. Biopsy: plasmacytoma. (25 x 14 cm) with intra-abdominal & intra-spinal extension	Bortezomib+ PegLiposomal Doxorubicin + Dexa x 2 cycles. Radiation (40Gy).
6	47 y / M	IgG Kappa MM, del17p13	D3-D4 paraspinal soft-tissue plasmacytoma with intraspinal extension. Large soft-tissue plasmacytoma in sacral region	Bortezomib + Lenalidomide + Dexa (RVD) started - February 2018.

Methods: The study included 32 newly diagnosed patients of multiple myeloma between March 2016 & December 2017. The investigations done for confirmation of myeloma diagnosis included bone marrow aspiration+biopsy, serum protein electrophoresis+immunofixation, serum

free light chain assay, and X-ray skeletal survey. MRI was done for imaging of the soft-tissue masses. Diagnosis of extramedullary soft-tissue plasmacytoma was confirmed by core biopsy/excision biopsy & immunohistochemistry (IHC) in all cases. PET-CT imaging facilities were not available at the institution. All the six patients were treated with Bortezomib-containing triple-drug regimens. Local radiation therapy was administered for compressive symptoms.
Results: Six patients (18.7%) out of total 32 consecutive newly diagnosed myeloma patients had one or more extramedullary soft-tissue plasmacytomas at presentation. The median age of these patients was 61 years (range 39-75 years). The patient characteristics & treatment outcomes are given in Table 1.
Summary/Conclusion: The frequent finding of bulky extramedullary soft-tissue plasmacytomas in newly diagnosed multiple myeloma patients in the western districts of Uttar Pradesh & adjacent Uttarakhand state in India could be the manifestation of unique myeloma disease biology. Possible association of aggressive plasmacytomas with kappa light chain paraprotein also needs to be explored in larger number of patients.

PB2249

POMALIDOMIDE-DEXAMETHASONE IN THE MANAGEMENT OF HEAVILY PRETREATED MULTIPLE MYELOMA

C. Cerchione[1,*], D. Nappi[1], A.E. Pareto[1], I. Migliaccio[1], I. Zacheo[1], M. Di Perna[1], I. Peluso[1], K. Ferrara[1], A. Romano[2], F. Pane[1], L. Catalano[1]

[1]Hematology - Department of Clinical Medicine and Surgery, University Federico II, Naples, Napoli, [2]Department of General Surgery and Medical-Surgical Specialties, Haematology Section, University of Catania, Catania, Italy

Background: Pomalidomide is a new generation IMID, with a very good compliance, thanks to oral administration, which can be used also in heavily pretreated patients, in a domestic setting.
Aims: In this retrospective observational trial, It has been evaluated efficacy and tolerance of pomalidomide plus dexamethasone (PD) as salvage regimen in heavily pretreated patients with relapsed and refractory MM (rrMM), whose prognosis is particularly severe.
Methods: 26 patients (14 M/12 F), with rrMM, median age at diagnosis 69 years (r. 52-84), and median age at start of treatment 73 years (r.56-87) treated with several lines of treatments (median 6, r. 2-9), every refractory to all the drugs previously received (also Bortezomib, Thalidomide and Lenalidomide), received PD (Pomalidomide 4 mg for 21 days, dexamethasone 40 mg days 1,8,15,22, pegfilgrastim day +8) every 28 days, until progression. ISS was equally distributed, and cytogenetic was evaluable in 12 patients, and in particular three del13q and one t(11;14) were present. All the patients had previously been treated with schedule containing bortezomib and IMIDs. 57% (15/26) of them had undergone at least to a single ASCT. All patients were relapsed and refractory to last therapies received before PD.
Results: Pomalidomide was well tolerated, with grade 3 anemia in 46% (12/26) of patients, 34% (9/26) grade 3 neutropenia (pegfilgrastim in primary prophylaxis was given, no hospitalization was required, no septic shocks were observed), 23% (6/26) grade 3-4 thrombocytopenia without hemorrhagic events and transfusion-dependence. No severe extra-hematologic toxicity was observed. According to IMWG, ORR1 (≥PR) was 42% (11/26: 2 CR, 3 VGPR, 6 PR), but, considering that we are evaluating a cohort of heavily pretreated patients without any other alternative treatment, with really poor prognosis, another parameter should be considered, ORR2 (≥SD), considering stable disease as a successful result in progressive MM. ORR2 was 73% (19/26: 2 CR, 3 VGPR, 6 PR, 8 SD). These can be considered as impressive result in this subset of patients. Oral treatment gives a really good compliance, in frail and unfit patients, and response, when present, is always really fast (median time to response: 2 months (r.1-6)), median OS from diagnosis was 87 months (range 21-228), median OS from start of pomalidomide was 8 months (range 1-14).
Summary/Conclusion: Pomalidomide-dexamethasone has shown significant efficacy and a very good compliance, thanks to oral administration, in a particularly severe setting of heavily pretreated patients, relapsed and refractory to all available therapeutic resources.

PB2250

PROGNOSTIC SIGNIFICANCE OF HEAVY/LIGHT CHAIN RATIO IN INTACT IMMUNOGLOBULIN MULTIPLE MYELOMA PATIENTS PRELIMINARY RESULTS

I. Kakkas[1,*], I. Konstantellos[2], S. Delimbasi[2], K. Papageorgiou[1], N. Harhalakis[2], A. Tsirogianni[1]

[1]Immunology and Histocompatibility, [2]Hematology and Lymphoma, Evangelismos Hospital, Athens, Greece

Background: During 2009, Bradwell *et al.* presented a new technique for intact Immunoglobulin Multiple Myeloma (IIMM) and Waldenstrom's Macroglobulinemia (WM) monitoring. They developed and validated a method for the separate quantification of the kappa and lambda bounded amounts of circulating IgG, IgA and IgM (Heavy/Light Chain-HLC assay). This was achieved by developing antisera with specificity for unique epitopes present at the junction between the heavy and light chains constant regions of each immunoglobulin molecule. This assay allows the quantification of the absolute value of the involved IgGκ, IgGλ, IgAκ, IgAλ, IgMκ and IgMλ along with their deriving ratios (IgGκ/IgGλ etc, Heavy/Light Chain ratio, HLC ratio). According to the literature, these measurements have been proven sensitive and specific for the monitoring of patients with IIMM. Additionally, the prognostic significance of HLC measurements for symptomatic IIMM patients (before treatment initiation) has been investigated. According to the results of two relatively recent studies (Bradwell 2013, Ludwig 2013), extreme low or high HLC ratios (<0.01 or >200) were associated with decreased overall survival of symptomatic IIMM patients.
Aims: The investigation for existence of any prognostic significance of HLC measurements for symptomatic IIMM patients (before treatment initiation) diagnosed and treated in our Hospital's Hematology and Lymphoma Department.
Methods: Forty-one newly diagnosed symptomatic IIMM patients were studied. Twenty-five of them were men and 16 women. Their median age was 68 years (range: 43-83). The isotype of paraprotein was in 31 cases IgG and in 10 cases IgA. Twenty-four patients were ISS stage I, 13 stage II and four stage III. Patients median follow-up was 16 months (range: 6-24). HLC ratio was determined in all patients before treatment initiation. HLC measurements were performed by using the Hevylite™ assays (The Binding Site Group Ltd, UK) on a SPA PLUS turbidometer.
Results: Statistical analysis was done by using the x^2 test. At the time of last evaluation, 36 patients were alive. Five patients had died due to disease progression and their median survival was seven months (range: 2-14). Extreme HLC ratios (<0.01 or >200) emerged in 14 patients (7/31 IgG and 7/10 IgA, $p<0.05$). Two out of five deceased patients were IgG and three IgA. Also, four out of the five deceased patients had extreme HLC ratios ($p<0.05$). It is noted that all three IgA deceased patients emerged extreme HLC ratios ($p<0.01$).
Summary/Conclusion: Despite the limited number of patients in our study, it is clear from the above-mentioned that there is a statistically significant correlation between IgA isotype of paraprotein and HLC ratio extreme values (<0.01 or >200). Also, there is a statistically significant correlation between mortality and HLC ratio extreme values, especially for IgA patients.

PB2251

RESPONSE TO INDUCTION THERAPY IN MULTIPLE MYELOMA PATIENTS; A SINGLE CENTRE EXPERIENCE

M. Borhany[1,*], M. Afzal[2], A. Hayee[2], U. Zaidi[2], T. Shamsi[2]

[1]Haematology and Blood Bank, [2]National Institute of Blood Disease and Bone Marrow Transplantation, Karachi, Pakistan

Background: Multiple myeloma is a clonal B-cell disorder which is characterized by proliferation and accumulation of plasma cells in the bone marrow. The disease accounts for 13% of all hematologic malignancies.
Aims: To determine clinical presentation and compare response to induction treatment of multiple myeloma (MM) in newly diagnosed patients registered with the National Institute of Blood Disease (NIBD), Karachi.
Methods: Prospective, observational study was done from January 2013 to June 17 at our hospital in newly diagnosed MM patients and treatment was followed in study patients. Remission was documented after 4 cycles of treatment according to the standard response criteria of International Myeloma working group (IMWG).
Results: Eighty seven MM patients were included in the study. There were 52 males and 35 females. The median age was 57.26 (ranged from 40-65 years). The male to female ratio was 1.4:1. The main presenting complains included backache in 57% (n=50), pain in the legs 46% (n=40) and generalized weakness 63% (n=55). Anemia with hemoglobin level <10 g/dl was found in 71% (n=62) patients. The mean hemoglobin value was 8.9±2.1g/dl. Renal impairment was present in 49.4% (n=43) patients while 29% (n=25) patients had hypercalcemia. Patients were staged according to International staging system; 49% of the patients (n=43) were in stage II, 30% (n=26) patients in stage I and 21% (n=18) in stage III disease. About 48% (n=42) patients received thalidomide/melphalan/dexa (TMD), out of which 43%

(n=18) achieved complete remission (CR) after 4 cycles while 57% (n=24) had partial response (PR). Thirty patients (34%) received lenalidomide/melphalan/dexa (LMD) of which 77% (n=23) achieved CR while 23% (n=7) patients had PR. Fifteen patients received lenalidomide/bortezomib/dexa (LBD), out of which 93% achieved CR. Chi square was applied to observe the association in treatment response and it was found statistically significant that treatment B and C had higher percentage of CR compared to treatment A (p-value = >0.05). However no significant results were found when treatment B and C was compared (p-value = <0.05). Twenty eight partial responders of treatment A and B received LBD, out of which 90% achieved remission. Overall survival at 3 years was 59%. Management related toxicities included weakness, constipation, diarrhea, nausea, vomiting and peripheral neuropathy.

Summary/Conclusion: Treatment with lenalidomide/melphalan/dexa and bortezomib combination was superior to the thalidomide group. Moreover, bortezomib addition results in complete remission in non-responders at induction and was well tolerated.

PB2252

ADDRESSING UNMET MEDICAL NEEDS IN MAINTENANCE TREATMENT FOR NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM): CURRENT TREATMENT LANDSCAPE AND EMERGING THERAPEUTIC OPTIONS

W.J. Chng[1,*], M. Beksac[2], R. Hajek[3], K. Weisel[4], M.-V. Mateos[5], A. Spencer[6], S. Iida[7], D. de Farias[8], R. Labotka[9], T. Skacel[9], A. Palumbo[9], S. Lonial[10], S. Kumar[11], M. Dimopoulos[12]

[1]Department of Hematology-Oncology, National University Cancer Institute, Singapore, Singapore, [2]Department of Hematology, Ankara University, Ankara, Turkey, [3]Department of Hematooncology, University Hospital Ostrava, Ostrava, Czech Republic, [4]Department of Hematology, University of Tuebingen, Tuebingen, Germany, [5]Hospital Universitario de Salamanca, Instituto Biosanitario de Salamanca (IBSAL), Salamanca, Spain, [6]Australian Centre for Blood Diseases, Monash University, Malignant Haematology & Stem Cell Transplantation, Alfred Hospital and Department of Clinical Haematology, Monash University, Melbourne, Australia, [7]Department of Hematology & Oncology, Nagoya City University, Nagoya, Japan, [8]Hospital das Clinicas, Universidade Federal de Goiás (UFG), Goiânia Goiás, Brazil, [9]Millennium Pharmaceuticals Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, MA, [10]Department of Hematology and Medical Oncology, Winship Cancer Institute, Emory University, Atlanta, GA, [11]Division of Hematology, Mayo Clinic, Rochester, MN, United States, [12]Department of Clinical Therapeutics, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece

Background: In NDMM, following response to primary therapy, maintenance therapy prolongs progression-free survival (PFS) and overall survival (OS) (McCarthy *et al*, J Clin Oncol 2017; Ludwig *et al*, Blood 2012). However, although multiple drugs have been investigated, maintenance is not yet an established treatment option worldwide; lenalidomide is the only approved agent, and only as post-autologous stem cell transplant (ASCT) maintenance.

Aims: We reviewed published/ongoing phase 3 trials of current and emerging maintenance treatment options to evaluate the treatment landscape and identify unmet medical needs and how these needs might be addressed.

Methods: A focused literature review and a search of clinical trial registries were conducted to identify publications and ongoing phase 3 studies of maintenance treatment approaches in NDMM.

Results: Maintenance with lenalidomide, bortezomib, or thalidomide has been well-studied and offers differential benefit, leaving unmet needs in some populations. Lenalidomide maintenance post-ASCT prolongs PFS and OS *versus* placebo/observation (McCarthy *et al*, J Clin Oncol 2017) but increases risk of second primary malignancies and has a less pronounced benefit in some subgroups, including patients with high-risk cytogenetics and those with high ISS stage. Lenalidomide has shown improved PFS, but not OS, in the non-ASCT setting (Palumbo *et al*, N Engl J Med 2012). Thalidomide maintenance demonstrates a significant risk reduction for PFS, but not in patients with high-risk cytogenetics (Ludwig *et al*, Blood 2012), and with notable toxicity. Although both lenalidomide and thalidomide have shown OS benefits, these benefits are not consistent across all individual studies. Bortezomib has activity post-ASCT, including in patients with high-risk cytogenetics (Sonneveld *et al*, J Clin Oncol 2012), and in the non-ASCT setting (Palumbo *et al*, J Clin Oncol 2010; Mateos *et al*, Lancet Oncol 2010), but has not been studied in a placebo-controlled setting. Long-term use of bortezomib and thalidomide may be limited by short- and long-term toxicity, such as peripheral neuropathy, as well as by the treatment burden associated with repeated parenteral administration. Although these agents are being used for maintenance, several unmet medical needs still remain, including the need for a therapy that can be dosed for an extended time without cumulative or late-onset toxicity or the emergence of resistant clones at relapse. Further, there is a need for a maintenance therapy offering extended benefit in all patients, including subgroups with high-risk disease (advanced stage, high tumor burden, high-risk cytogenetics, comorbidities). Ideally, maintenance should deepen patient responses instead of just sustaining an existing response. There are several ongoing phase 3 studies of ixazomib, carfilzomib, and daratumumab as maintenance (Table) that may address some of the unmet needs. Long-term treatment with regimens containing these agents results in deepening responses and improved outcomes, including in multiple high-risk patient subgroups. Oral ixazomib potentially offers a convenient and feasible approach to long-term proteasome inhibitor therapy, with a manageable toxicity profile.

Table 1.

Table. Ongoing phase 3 maintenance trials

Agent	Study	Design	NCT number
Ixazomib	TOURMALINE-MM3 NCT02181413	Ixazomib vs placebo in post-ASCT NDMM	NCT02181413
	TOURMALINE-MM4 NCT02312258	Ixazomib vs placebo in post-induction, non-ASCT NDMM	NCT02312258
	GEM2014MAIN NCT02406144	Ixazomib-lenalidomide vs lenalidomide in post-ASCT NDMM	NCT02406144
Carfilzomib	University of Chicago NCT02659293	KRd vs lenalidomide in post-ASCT NDMM	NCT02659293
Daratumumab	Cassiopeia NCT02541383	Daratumumab vs observation post-ASCT	NCT02541383

Summary/Conclusion: Current maintenance therapies for NDMM have resulted in improved long-term outcomes but are associated with some limitations. Emerging therapies may offer a feasible approach due to manageable long-term toxicity profiles and convenience, especially oral agents.

PB2253

POSSIBLE FACTORS OF PROGRESSION IN MONOCLONAL GAMMOPATHY IN PATIENTS OF GOMEL REGION IN BELARUS

Z. Kozich[1,*], V. Martinkov[2], Z. Pugacheva[3], L. Smirnova[4]

[1]Hematology, [2]Molecular genetics, [3]Laboratory diagnostics, The Republican Research Center for Radiation Medicine and Human Ecology, Gomel, [4]Hematology, BelMAPO, Minsk, Belarus

Background: Monoclonal gammopathy of undetermined significance (MGUS) is a group of diseases with the proliferation of cells of lymphoid or plasmocyte nature <10% secreting various pathological immunoglobulins in blood serum and / or urine <30 g/l with no foci of lysis, kidney damage, hypercalcemia and risk of progression of 1% in a year

Aims: to detect possible risks of progression of monoclonal gammopathy in patients of Gomel region.

Methods: The material for the study was samples of whole venous blood and bone marrow from 28 patients with pathological paraprotein who underwent examination and treatment during the period 2014-2017 in the SI "RRCRM&HE" in Gomel (Belarus). The diagnosis was confirmed by the presence of pathological immunoglobulin in the blood and / or urine and tumor immunophenotype of plasma or lymphoid cells of bone marrow. Antigen expression was determined by flow cytometry. The results were estimated at the time of determination of the pathological protein. The number of clonal plasma cells in the bone marrow was 4.6% (1.2-15%) on an average. Monoclonal gammopathy was more common in women (62.9% were female). The median age was 64 years (46-80 years).

Results: In our study pathological paroprotein was represented by IgG (50.0%), IgA (17.9%), IgM (7.1%), Bens-Jones protein (14.3%) and absence of secretion of Ig (10.7%%). Monoclonal gammapathy with the presence of IgG and Bence-Jones protein progressed to multiple myeloma during the first year of follow-up in 9 (32.1%) patients. In one patient, the disease was transformed into Waldenstrom's macroglobulinemia within three years. During this period of time, a significant increase in CD20 expression was detected proportionally to the increase in the M-protein index. Two patients from this group showed a significant increase in CD117 expres-

sion (p=0.052) along with a high level of pathological M-protein. A high risk of progression was associated with the M-protein with IgM secretion. Excess of lactate dehydrogenase (LDH) indices was also significant (p=0.05) in patients with a high risk of progression, changes in B2-microglobulin (possibly due to a small percentage of plasma cells in the bone marrow) were not detected.
Summary/Conclusion: Monoclonal gammopathy of undetermined significance (MGUS) is a premalignant proliferation of B-lymphocytes with a high risk of progression to multiple myeloma, Waldenstrom's macroglobulinemia. Despite a very short observation period, during our study we detected a significant increase in CD117 and CD20 expression in patients who progressed to multiple myeloma and Waldenstrom's macroglobulinemia, which may be an unfavorable prognostic factor. Perhaps this will help to identify patients at high risk for progression initially, which is important in determining the time of the start of specific therapy.

PB2254

DARATUMUMAB-IXAZOMIB-DEXAMETHASONE: A NEW THERAPEUTIC REGIMEN FOR RELAPSED MULTIPLE MYELOMA?

S. Martín-Batista[1,*], M. Moreno-Vega[1], P. Hernández-Hernández[1], A. Martín-Martín[2], P. Machado-Machado[2], S. Lakwhani[2], C. De Bonis-Braun[2], B. González-González[2], M.J. Rodriguez-Salazar[2], B. Soria-Santabárbara[2], S. Iraheta-Reyes[2], T. Martín-Santos[2], M.R. Arcas-Vega[2], K. Mayani[2], J.M. Raya[2], M.T. Hernández[2]

[1]Hematology, [2]Universitary Hospital of Canary Islands, La Laguna. Canarias, Spain

Background: Patients with relapsed and refractory multiple myeloma (MM) have poor prognosis. Life expectancy is increasing because of a rising number of agents with various mechanisms of action. However, MM remains incurable because of the emergence of resistant clones. Among these drugs, Daratumumab (DARA) is the first-in-class human monoclonal antibody against CD38 cells showing an overall response rate of 36% and a median overall survival (OS) of 17 months in monotherapy, now it can be used in combination with lenalidomide and dexamethasone, or bortezomib and dexamethasone, for the treatment of patients who have received at least one prior therapy. Ixazomib is a boron-containing selective proteasome inhibitor that demonstrated antimyeloma activity with excellent safety profile. Ixazomib is a proteasome inhibitor approved in combination with lenalidomide and dexamethasone for the treatment of patients who received at least one prior therapy.
Aims: We report two cases of relapsed MM treated with DARA-Ixazomib-Dexamethasone as rescue treatment, to our knowledge, the first study reporting this combination.
Methods: Description of two case reports about patients treated on our Unit.

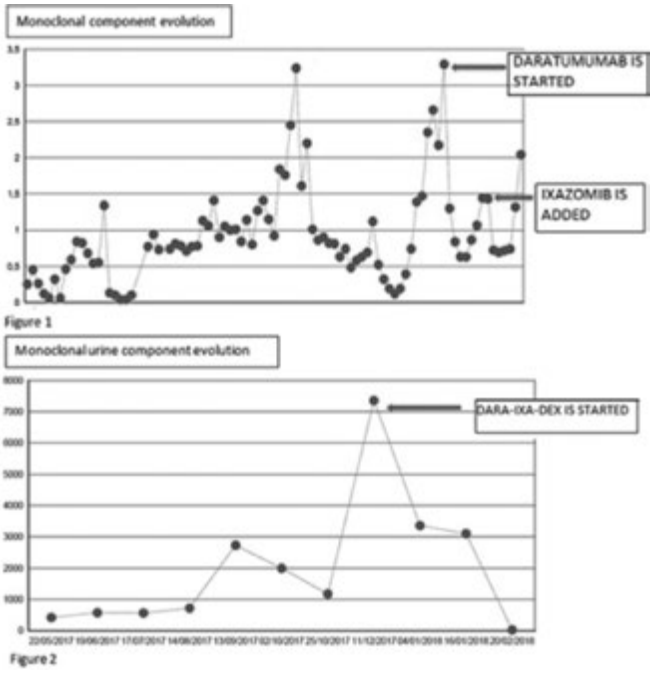


Figure 1.

Results: A 67-years-old woman with a known diagnosis of IgA lambda MM presented with relapsed MM. She first presented in 1999, was started on VBMCP/VBAD followed by auto transplant (ASCT), achieving a complete response (CR) but relapsed five years later, so she received bortezomib achieving a second CR. In 2007, a second relapsed was detected and she experimented a large sequence of treatment-relapsed consisting of VMP, Lenalidomide-dexamethasone, Bendamustine and Elotuzumab-thalidomide-dexamethasone. While the 13th cycle, she suffered a severe lung infection and was admitted in the critical care unit. Fortunately, she recovered well and was started on Carfilzomib. Next year, the disease progressed so we restarted Carfilzomib adding Dexamethasone (Kd), achieving a VGPR. Later, the disease progressed so the treatment was restarted but MM was refractory; in February 2017 a treatment based on DARA-bortezomib was initiated, but we stopped bortezomib because of severe neuropathy. After an optimal response, it got worse and Ixazomib was added; the disease evolution was optimal (Figure 1). After 12 cycles IgA levels increased slowly. At present, MM is in progression. Our second case is a 60-years-old woman whose kappa light chain MM was detected in 2016, and was started on VTD achieving a good response, because of many complications, we stopped the treatment. After few months, she progressed so we started bortezomib-cyclofosphamide-dexamethasone (VCD) without response, so Rd was initiated. It was interrupted due to vertebral fractures. Promptly, she was started on Kd, but she debuted with a sepsis. After recovering, she received dexamethasone-cyclophosphamide-etoposide-cisplatin, but after one cycle she was diagnosed with a spinal cord compression. In December 2017, a regimen consisting of DARA 16mg/kg/week -Ixazomib 4 mg/week -dexamethasone was initiated. As striking decrease of the protein M in urine was observed (Figure 2), but a pancreatic plasmacytoma was detected. An ASCT was made and currently, after two months, MM is in a VGPR.
Summary/Conclusion: Daratumumab has shown benefit in patients who have progressed, and whose combination with other drugs just started to investigate. We combined this drug with Ixazomib in an attempt to control the disease, the response was good. However, optimization of the regimen could prolong response duration and improve patients' outcomes.

Myeloproliferative neoplasms – Biology & Translational Research

PB2255

HIGHER SCLEROSTIN/SOST EXPRESSION IS ASSOCIATED WITH LOWER PERCENTAGE OF CIRCULATORY BLASTS AND BETTER PROGNOSIS IN PATIENTS WITH MYELOFIBROSIS

M. Lucijanic[1,*], A. Livun[2], K.M. Tupek[3], T. Stoos-Veic[4], G. Aralica[5], V. Pejsa[1], R. Kusec[1]

[1]Hematology Department, [2]Clinical Department of Laboratory Diagnostics, University Hospital Dubrava, [3]Clinical Department of Laboratory Diagnostics, Clinical Hospital Dubrava, [4]Cytology and Cytometry Department, [5]Pathology Department, University Hospital Dubrava, Zagreb, Croatia

Background: Sclerostin, a product of *SOST* gene, is a main negative regulator of bone metabolism which exerts its properties through inhibition of WNT signaling pathway in osteoblasts. WNT signaling pathway is implicated in pathogenesis of Philadelphia chromosome negative myeloproliferative neoplasms (Ph- MPNs). These diseases are characterized by remodelling of bone marrow (BM) stroma and development of BM fibrosis/osteosclerosis during course of the disease.
Aims: We aimed to investigate Sclerostin/*SOST* expression in BM tissue of patients with primary (PMF) and secondary myelofibrosis (SMF) and to assess its clinical correlations.
Methods: Using immunohistochemistry (IHC) and real-time-polymerase-chain-reaction (RT-PCR) we investigated Sclerostin/*SOST* expression in totally 66 diseased (51 PMF, 15 SMF) and 18 control BM samples. Sclerostin expression measured by IHC was expressed as a percentage of positive cells. *SOST* expression measured by RT-PCR was normalized to *Abl* and expressed as a ΔCT value. Samples were collected retrospectively in period from 2006 to 2016. Correlations with clinical parameters were made. The Mann Whitney U test, the Spearman rank correlation, the log-rank test and the Cox regression analysis were used. The ROC curve analysis was used to define optimal cut-off values for survival. *P* values <0.05 were considered to be statistically significant.

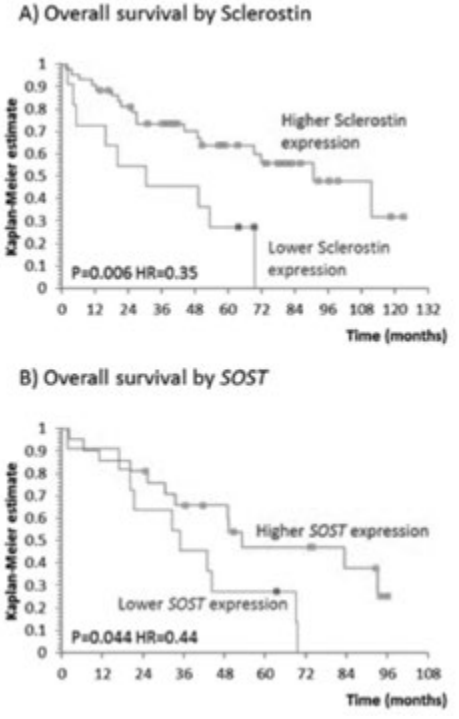


Figure 1.

Results: Median age of patients was 67 years, 59.2% were males. Median follow up of our cohort was 74 months. Sclerostin/*SOST* expression did not significantly differ between healthy and diseased patients, nor between PMF and SMF. However, higher Sclerostin expression in myelofibrosis patients was significantly correlated with lower percentage of circulatory blasts (Rho -0.28, P=0.042) and transfusion dependency (P=0.049). Higher *SOST* expression in diseased patients was similarly significantly correlated to lower percentage of circulatory blasts (Rho -0.44, P=0.042), but also higher platelets (Rho 0.4, P=0.031) and smaller spleen size (Rho -0.6, P=0.001). We found no significant association of Sclerostin/SOST expression with driver mutations or degree of bone marrow fibrosis. Patients with higher Sclerostin expression measured by IHC (HR=0.35, P=0.006) and *SOST* expression measured by RT-PCR (HR=0.44, P=0.044) had better overall survival than patients presenting with lower Sclerostin/*SOST* expression as shown in a Figure. This association remained significant for *SOST* (HR=0.21, P=0.025) after adjusting for age, gender and circulatory blasts (HR=1.06, P=0.002).
Summary/Conclusion: Sclerostin expression might affect stem cell mobilization. Patients with higher Sclerostin/*SOST* expression experienced improved survival, effect which might be prognostically independent of reduction in circulatory blasts and which is probably mediated through canonical WNT inhibition. Our findings emphasize the role of bone metabolism regulating cytokines, such as Sclerostin, in pathogenesis of Ph- MPNs and suggest that WNT inhibition might be an interesting therapeutic approach in myelofibrosis patients.

PB2256

HOW RUXOLITINIB IMPACTS ON DRIVER AND ADDITIVE MUTATIONS IN PATIENTS WITH MYELOFIBROSIS

S. galimberti[1,*], S. Balducci[2], F. Guerrini[2], S. Grassi[3], D. Cattaneo[4], A. Iurlo[5], P. Vigneri[6], M. Petrini[2], G.A. Palumbo[7], C. Baratè[8]

[1]clinical and experimental medicine, university of Pisa, [2]hematology, university of Pisa, pisa, [3]GENOMEC, university of Siena, siena, [4]policlinico ca' granda, hematology, [5]IRCCS ca' granda, hematology, Milano, [6]oncology, university of Catania, [7]hematology university of Catania, catania, [8]hematology pisa, pisa, Italy

Background: Introduction of ruxolitinib in the clinical practice has changed the outcome of patients with myelofibrosis (MF), offering longer survivals and improvement of the quality of life. Nevertheless, about 50% of patients loss clinical response, and some authors ascribed this phenomenon to driver and non-driver mutations: Patel *et al.* (Blood 2015) reported that having more than 3 mutations well correlated with shorter time to discontinuation and shorter overall survival (OS), but other investigators in the COMFORT-II trial reported that non-driver mutations did not correlate with response or survival (Guglielmelli, Blood 2014).
Aims: in order to further investigate if ruxolitinib could play any role in changing the mutational landscape in MF patients, we assessed the 3 driver and 8 non-driver mutations in 36 MF patients at diagnosis; 19 were tested also after 12 months of ruxolitinib, and compared with 4 cases receiving hydroxyurea.
Methods: JAK2, CALR, and MPL mutations were screened by qualitative/quantitative PCR. For the non-driver mutations, we designed a PCR plate with pre-spotted primers able to amplify ASXL1, EZH2, DNMT3A, IDH1, IDH2, SRSF2, TET2, TP53, for total 38 hot-spot sites (*Custom qBiomarker Somatic Mutation PCR Array®* - Qiagen, Italy). These genes were chosen because already included in the high molecular risk subgroup (*ASXL1*, *EZH2*, *SRSF2*, *IDH1/2*) or for their prognostic negative role in myeloid hematological neoplasias (DNMT3A, TP53).
Results: JAK2 was mutated in 70% of cases, CALR in 20%, whereas 10% were triple-negative. The median OS was significantly longer for primary MF (160 months) *vs* post-ET (80 months) or post-PV MF (35 months)(p=0.03), and for CALR- *vs* JAK2-mutated patients. At the last follow-up, 4 patients (11%) progressed to AML, and 12 (33%) died. The non-driver mutations were found at diagnosis in 33% of cases receiving ruxolitinib and in one/4 patients treated with hydroxyurea. Considering both driver and non-driver mutations, 24 cases (67%) were mutated, with 16 carrying one mutation, and 10 two mutations. The most frequently detected mutations belonged to the methylation pathway (DNMT3A, IDH, TET2=75%), followed by TP53 (17%), SRSF2 (8%), ASXL1 (8%), and EZH2 (8%). During treatment, JAK2 VAF remained stable, whereas non-driver mutations changed in 13 cases: 9 acquired a new mutation, while 4 lost the previously detected mutations. Acquisition of DNMT3A mutation was found in 5 patients, of IDH2 in one, and of TP53 in another one. None of the CALR-mutated cases carried non-driver mutations. In the 4 cases treated with hydroxyurea, during treatment one acquired TP53 and another one DNMT3A mutation. On the other hand, 4 cases lost mutations previously present at diagnosis (TP53, IDH2, ASXL1, DNMT3A) in the group of ruxolitinib, but nobody in the group of hydroxyurea. Presence/absence of non-driver mutations, their number (>1), the molecular subgroup (methylation, splicing, chromatin) did not significantly condition OS.
Summary/Conclusion: In this work, even if on a small series of patients, we showed that during ruxolitinib about the half of cases develops non-driver mutations, a percentage overlapping to that observed in cases receiving

hydroxyurea. Interestingly, ruxolitinib allowed disappearance of mutations in one third of cases. Acquisition of new mutations or the type of non-driver mutations did not correlate with higher rate of death or ruxolitinib failure.

PB2257

TUMOR-SPECIFIC METHYLATION OF THE CANDIDATE TUMOR SUPPRESSOR GENE HLS5 IS AN EPIGENETIC BIOMARKER FOR ACUTE MYELOID LEUKEMIA(AML) AND MYELOPROLIFERATIVE NEOPLASM(MPN)

J. Huang*, X. Ye

Hematology, the Fourth Affiliated Hospital, College of Medicine ,Zhejiang University, Yiwu, China

Background: Aberrant promoter methylation is an epigenetic mechanism for silencing tumor suppressor genes (TSG), and is also a biomarker for early cancer diagnosis and prognosis prediction. HLS5 belongs to the RBCC (Ring-finger, B-box, coiled-coil) family, a family of tumor suppressive genes including Pm, Tif1-α,Herf and Rfp, it has been reported to regulate erythroid differentiation, to be activated during the conversion of J2E erythroleukemic cells to monocytoid cells, and assumed to be a tumor supresor.However, the role of HLS5 in acute myeloid leukemia and myeloproliferative neoplasms has never been explored.
Aims: the role of HLS5 in acute myeloid leukemia and myeloproliferative neoplasms.
Methods: Bone marrow of 35 *de novo* AML-M2, 20 *de novo* AML-M5 and 68 JAK2/V617F mutation positive MPN patients were collected, the mononucleated cells separated, RNA extracted and the HLS5 expression level detected using real-time quantitative PCR and MSP. SPSS 16.0 used for data analysis.
Results: We identified HLS5 as a functional tumor suppressor gene frequently methylated in multiple myeloid neoplasms. We further uncovered HLS5 as one of the up regulated genes in myeloid neoplasm cell lines after pharmacologic demethylation with 5 aza 2' deoxycytidine (Aza). Methylation of HLS5 was detected in most acute myeloid leukemia cell lines, including KG1, Kasumi-1, U937, THP, HL60 cell lines and JAK2/V617F mutation positive cells. Aza treatment led to HLS5 promoter demethylation and transcriptional reactivation in silenced cell lines, indicating a methylation mediated silencing. Aberrant methylation was further detected in 40% (14/35) AML-M2, 60% (12/20) AML-M5 and 60-80% of various types of JAK2/V617F mutation positive MPN, but not in any normal PBMC sample, and is thus tumor specific. Moreover, HLS5 methylation was detected in 3/10 (30%) serum samples from MF patients.
Summary/Conclusion: Our results indicate that HLS5 methylation is a frequent event in multiple myeloid neoplasms, especially for AML and MPN, but the exact mechanism needs further investigation.

PB2258

BONE MARROW TRYPTASE LEVEL EVALUATION IN SISTEMIC MASTOCYTOSIS: ROLE IN DIAGNOSIS AND CLASSIFICATION OF DISEASE

M. Rondoni[1,*], G. Poletti[2], V. Cova[3], A. Pezzi[4], G. Cortellini[5], E. Massari[2], C. Zingaretti[6], M. Rosetti[2], F. Monti[2], C. Conti[3], S. Soverini[7], M. Mancini[7], L. Riccioni[8], L. Cardinale[9], G. Martinelli[6], F. Lanza[1]

[1]Section of Hematology & SCT Unit, AUSL Romagna, Ravenna, [2]Hematology Unit. Laboratory of Clinical pathology, [3]Allergology Unit. Laboratory of Clinical Pathology, AUSL Romagna, Pievesestina (FC), [4]AUSL Romagna, Ravenna, [5]Internal medicine and Allergology, AUSL Romagna, Rimini, [6]Hematology/Oncology, IRST IRCCS, Meldola(FC), [7]Department of Experimental Diagnostic and Specialty Medicine-University of Bologna, Institute of Hematology "L. e A. Seràgnoli", Bologna, [8]Pathology Unit, AUSL Romagna, Cesena, [9]Pathology Unit, AUSL Romagna, Ravenna, Italy

Background: Systemic Mastocytosis (SM) is a complex disorder characterized by the accumulation of abnormal mast cells (MCs) in different organs and a wide spectrum of symptoms, derived from abnormal MCs degranulation. The diagnosis is based on serum baseline tryptase (sBT) level, histopathological, morphologic and immunophenotypic evaluation of MCs and D816V mutation. Diagnosis and cure of SM is peculiar, and patients have to be referred to specialized centers. In some cases, with a very small percentage of MCs in bone marrow (BM), diagnosis is difficult, because major diagnostic criteria (MDC) are often missing, sBT may be <20ug/L, and very sensitive methods for detection of D816V mutation are required to avoid false negative results. Once a diagnosis of SM is made, it is mandatory to assess the burden of the disease, its activity, subtype and prognosis, and the appropriate therapy. Evaluation of sBT levels have been associated with the burden of disease, especially in ISM, whereas in ASM and MCL sBT level varies. Tryptase can be easily measured in many laboratories, in contrast to FC and high sensitive molecular methods.
Aims: to explore the utility of bone marrow tryptase (bmT) level for predicting disease diagnosis and behavior.
Methods: We systematically explored the bmT level in 39 patients with suspected SM, with sBT level, histopathological, morphologic and immunophenotypic evaluation of MCs and molecular analysis. The commercial technique Immuno CAP Tryptase System was used. To exclude lower level of bmT due to hemodilution of the sample, we performed the tryptase assay on the same sample used for the FC analysis considering eventual increased T-lymphocyte count. Moreover we evaluated bmT level in 16 BM samples analysed with FC for other hematological/suspected hematological diseases. bmT levels were correlated with all the others diagnostic parameters, including an accurate morphologic examination, and with the final diagnosis and classification according to WHO.
Results: the median bmT level was 230 ug/L in patients with SM diagnosis (IQR 123-471), whereas 14.95 in normal BM (IQR 6.52-26,30) ; p 0.0001. The bmT level is different in the categories of disease (ISM, SSM, ASM and LMC), with low significance (p= 0.0244), due to the predominance of ISM, but importantly this difference is lost considering together ASM and MCL forms *versus* the ISM. bmT level is elevated in patients without MDC (histopathological, presence of aggregates with more than 15 elements) as in the patients with the MDC, with 190.5 ug/L (IQR 95-345) in the first group and 230 (IQR 169-473) in the former, instead of 17.75 (IQR 14.6-19.10) *versus* 34 (IQR 20.6-105) for sBT level (p=0.0073). There is a correlation between bmT Level and percentage of MCs in BM, although less strong than in the case of sBT level. Correlations between bmT Level and morphological aspects of the BM smears show an higher median value in cases with mature forms of MCs (387ug/L *versus* 135.5ug/L, with p=0.0878), but without significance. No correlations were found with CD2 or CD25 expression.
Summary/Conclusion: high bmT level is closely associated with diagnosis of SM. Evaluation of bmT level can be integrated in the diagnostic process of SM due to its feasibility, in particular in ISM, where it can identify ISM with very low percentage of MCs. The variability of bmT Level in advanced forms highlights the need of different parameters for the disease characterization of MCL, ASM, and probably a subset of SSM, as the maturation markers of MCs in FC, or new Molecular alterations.

PB2259

A NOVEL GERMLINE CARL MUTATION AFFECTING AN EVOLUTIONARY CONSERVED REGION OF 3'UTR IN JAK2-NEGATIVE SIBLINGS WITH POLYCYTHEMIA VERA

A. Quattrocchi[1,*], S. Tomassini[2], M.C. Scerpa[1], N. Cenfra[2], A. Ceccherelli[1], S. Pisanò[1], E. De Marinis[1], M. Gentile[1], C. Maiorca[1], G. Cimino[2], C. Nervi[1]

[1]Department of Medical-Surgical Sciences and Biotechnologies, [2]Department of Cellular Biotechnology and Hematology, University La Sapienza - Rome, Latina, Italy

Background: The principal phenotypic driver mutations of MPNs, occurring on JAK2, MPL and Calreticulin (CALR) genes, cause a direct or indirect deregulation of JAK/STAT signalling. Recurrent exon 9 CARL mutants, associated almost exclusively with ETs and PMFs, activate JAK/STAT by interacting with TPO-receptor. CALR mutations generally arise from a +1 frameshift that generates a common novel peptide lacking of KDEL ER-retrieval signal, converting the first 31 bases of CALR 3'UTR into coding sequence. However, the hematopoietic and regulative functions of this 3'UTR region are still unknown.
Aims: We aimed to analyse in 2 siblings the clinical and biological consequences of an unusual deletion of 3'UTR, designated c.1254+10_+33del24, occurring 10 bp downstream the stop codon, thus not altering the coding sequence. Interestingly, both these patients were diagnosed with JAK2-negative PV.
Methods: CALR mutations and mutant allele burden were detected by PCR screening, identified by Sanger sequencing and confirmed by fragment analysis on DNA from granulocytes, CD34+-hematopoietic progenitor cells (HPC) isolated from peripheral blood (PB) and saliva epithelium. CALR mRNA levels was measured by quantitative RT-PCR (ABI-PRISM-7000). CALR, STAT-3/-5 and phopho-STAT-3/-5 protein levels were assessed by immunoblotting both in PB cells and in myeloid cell lines. The colony assays were performed using PB mononuclear cells isolated by Ficoll.

Results: CALR 3'UTR deletion c.1254+10_+33del24 was found in two sibling PV patients. This deletion is located within an evolutionarily conserved region at nucleotides 10-33 of 3'UTR, thus not altering the KDEL domain. Such mutation was detected in granulocytes, $CD34^+$-HPCs and oral epithelial cells from saliva samples, suggesting that the mutation is germline. In all samples, the mutant allele burden was >50%. Both siblings showed an enhanced erythropoiesis, also assessed by BFU-E growth at low erythropoietin conditions. Moreover, these patients and other MPNs cases affected by canonical CALR mutations showed increased CALR mRNA/protein levels and an aberrant activation of the JAK/STAT pathway, respect to healthy donors.
Summary/Conclusion: CALR mutations in MPNs have hitherto been observed only in ETs and PMFs, with a pathogenesis relying on TPO-receptor activation by the mutated novel peptide. Here we report that the mutation c.1254+10_+33del24, deleting 24 bp of CALR 3'UTR, is associated with PV. JAK/STAT signalling activation correlates with increased CALR expression and 3'UTR alteration. Our results suggest that CALR 3'UTR functional impairment, not altering KDEL sequence and operating for instance on RNA intracellular localization, miRNA binding, translational efficiency and mRNA editing, may affect erythropoiesis.

PB2260

INFLAMMATION THROUGH MATRIX METALLOPROTEINASE IN MYELOPROLIFERATIVE NEOPLASM

M. Kovacic[1,*], S. Bjelica[1], B. Beleslin-Čokić[2], D. Djikić[1], D. Leković[3], M. Gotić[3], V. Čokić[1]

[1]Department of molecular oncology, Institute for Medical Research, [2]Clinical for Endocrinology, Diabetes and Metabolic Diseases, Genetic laboratory, [3]Clinical of Hematology, Cliical Center of Serbia, Belgrade, Serbia

Background: Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases involved in remodeling of the extracellular matrix. MMPs have multiple roles in cancer cell proliferation and inflammation, including myeloproliferative neoplasm (MPN).
Aims: The goal of our study is to explore the MMPs level in essential thrombocythemia (ET) and primary myelofibrosis (PMF), during inflammation.
Methods: Using zymography, we analyzed expression of MMP2,3,9 and 13 in plasma of healthy controls (n=5), ET (n=17) and PMF (n=17), according to JAK2V617F, calreticulin (CALR) and thrombopoietin receptor (MPL) mutation status by DNA sequencing. In addition, we determined the level of MMP2 in JAK2V617F mutated human erythroleukemia cell line (HEL) treated with pro-inflammatory IL6 and anti-inflammatory IL10. Also, MMP2 levels were determined in peripherial blood mesenchymal stromal cells of healthy controls, with or without mononuclear cells of PMF origin, treated with IL6 and JAK1/2 inhibitor Ruxolitinib. We divide ET and PMF patients per three groups: *JAK2*V617F positive (JAK2+, n=6), *CALR* positive (CALR+, n=6), and triple (*JAK2*V617F / CALR / MPL) negative (n=5).
Results: The levels of MMP3 and MMP13 were significantly higher in all three groups of PMF patients ($p<0.001$) compared to control and moreover the level of MMP3 and MMP2 were significantly lower ($p<0.01$) in triple negative ET/PMF compared to JAK2+ and CALR+ ET/PMF patients. The MMP9 was significantly higher in triple negative and JAK2+ patients with PMF compared to control ($p<0.01$). The level of MMP2 was significantly higher and in ET JAK2+ and CALR+ compared to control ($p<0.001$). The level of MMP3 was statistically lower in ET triple negative and JAK2+ patients compared to healthy controls ($p<0.01$), in opposite to MMP9 levels. The MMP13 levels were significantly higher and in ET JAK2+ and CALR+ compared to control ($p<0.001$) and triple negative ET ($p<0.01$). IL-6 induced expression of MMP2 in HEL cells after 6 hours ($p<0.01$), but not IL-10. IL-6 induced expression of MMP2 in mesenchymal stromal cells ($p<0.01$), while Ruxolitinib inhibited MMP2 in PMF mononuclear cells in co-culture with mesenchymal stromal cells after 48 hours.
Summary/Conclusion: The examined MMP levels were increased in plasma of ET and PMF patients, with predominance in JAK2+ and CALR+ patients. Inflammation increased MMP2 levels demonstrating JAK1/2 dependence. These observed MMP result may be a potential diagnostic marker of MPN.

PB2261

CALRETICULIN AND CD47 EXPRESSION IN A MDS/MPN CELL LINE MODEL K562: A MODEL FOR OUTCOME OF THERAPEUTIC RESPONSE

K. Boasman*, M.J. Simmonds, C.R. Rinaldi

Life Sciences, University of Lincoln, Lincoln, United Kingdom

Background: Myelodysplastic syndromes (MDS) and Myeloproliferative neoplasms (MPN) are a group of bone marrow neoplasms arising from clonal disorders of haematopoietic stem cells, with the tendency to progress into acute leukaemia. In solid tumours, calreticulin (CALR) overexpression produces a pro-phagocytic signal and is counteracted by concomitant expression of anti-phagocytic CD47, reflecting an apoptosis *vs* survival mechanism in response to chemotherapy. To investigate the role of CALR and CD47 expression during treatment for MDS and MPN we used the intermediate MDS/MPN cell line K562.
Aims: To investigate CALR and CD47 protein expression and location after treatment. Protein expression was analysed within an intermediate MDS/MPN cell line model (K562)
Methods: CALR and CD47 gene expression was measured by Real Time PCR and protein expression by western blotting, before and after treatment with azacytidine (AZA) and ruxolitinib (RUXO). Cells were incubated with 0.05μM/ml drug, re-dosed at 24 hours and harvested at 48 hours. Cell were also fractionised into 4 compartments: membrane, cytoplasm, cytosol and nucleus and CALR and CD47 expression measured in those compartments before and after treatment.
Results: We demonstrated upregulation of both genes, CALR and CD47 and the concomitant overexpression of both CALR and CD47 protein, after incubation with AZA or RUXO. When we analysed the cell fractions, we observed in untreated cells, the highest CALR protein expression is in the cytosol and nucleus (48.4% and 25.4%, respectively) comparing with the membrane and cytoplasm (13.3% and 12.9% respectively). CD47 expression in untreated cells is higher in the membrane and cytoplasm (43.1% and 33.4% respectively) and lower in the nucleus and cytosol (21.3% and 2.2% respectively). Possibly during fractionation CD47 protein residing on the nuclear envelope have been included not representing a common CD47 location. After incubation, cell survival rates were 81.6% in AZA and 89% in RUXO treated cells respectively, comparing with untreated cells, suggesting a slight more cytotoxic effect of AZA. Both drugs reduced CALR expression in the membrane (AZA 8.1%; RUXO 8.2% *vs* untreated 13.3%), but increased its expression in cytosol (AZA 54%; RUXO 51% *vs* untreated 48.4%) and in the cytoplasm (AZA 15.3%; RUXO 13.5% *vs* untreated 12.9%), indicating that pro-phagocytic CALR moves away from the membrane back into the cell. In contrast, CD47 expression significantly increased on the membrane (64.4% *vs* 43.1%) and decrease in the cytoplasm (0.9% *vs* 33.4%) when treated with AZA, while the expression remains virtually unmodified on the membrane (40.4% *vs* 43.1%) and in the cytoplasm (34.5% *vs* 32.5%) after treatment with RUXO.
Summary/Conclusion: In this study, we observed changes in CALR and CD47 localization after exposure to drugs. We identified a very similar pattern of CALR localization which internalised away from the membrane. This opposes previous studies in solid tumours, which show an increase of both CALR and CD47 on the cell membrane in response to chemotherapy. Interestingly the CD47 expression on the cell surface seems to follow a more drug specific trait, increasing significantly when exposed to AZA but decreasing when exposed to RUXO suggesting different ways of priming the immune-response. Further work is now required in MDS and MPN patient's cells to confirm our *in vitro* findings and to evaluate whether the different patterns of CALR and CD47 expression result in different treatment outcomes.

PB2262

GENE EXPRESSION PROFILE IN ESSENTIAL THROMBOCYTHEMIA: FINDING NEW MARKERS FOR TRIPLE NEGATIVE CASES

S. Sánchez Sosa[1,2,*], C. Bilbao Sieyro[1,3], Y. Florido Ortega[1], E. González Pérez[1], M.D.L.N. Sáez Perdomo[1,2], J.M. González Martín[4], R. Stuckey[1], M.Á. Perera-Álvarez[1,2], G. Santana Santana[1], M.D.C. Galindo Rodríguez[1], M.T. Molero Labarta[1,5], J.F. Loro Ferrer[6], M.T. Gómez Casares[1]

[1]Hematology, Hospital Universitario de Gran Canaria Dr. Negrín, [2]Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain, [3]Morfology, Universidad de Las Palmas de Gran Canaria, [4]Unidad de Investigación, Hospital Universitario de Gran Canaria Dr. Negrín, [5]Ciencias médicas, Universidad de Las Palmas de Gran Canaria, [6]Ciencias clínicas, Universidad de Las Palmas de Gran Canaira, Las Palmas de Gran Canaria, Spain

Background: Essential thrombocythemia (ET) is a BCR-ABL1-negative myeloproliferative neoplasm characterized by JAK2, CALR and MPL mutations which trigger the cytokine receptor/JAK2 pathway. Whole exome sequencing studies have shown that in most ET cases, just one mutation in one of the three MPN driver genes is found. In approximately 15% of chronic thrombocytosis cases classified as ET, the genetic cause remains unknown and, since they do not present mutations in any of the three driver genes, they are known as triple negative. Therefore, epigenetic or other transcrip-

tion modulation mechanisms may be implicated. New molecular markers are needed to characterize triple negative cases, for that reason we studied the expression levels of genes related to MPN pathogenesis and evolution such as RB1, ASXL1, TET2, and EZH2.
Aims: To analyze the mRNA expression profiles of CALR, ASXL1, RB1 and TET2 genes in a series of ET patients with known mutational status of JAK2V617F, CALR and MPL.
Methods: Our series consisted of 73 ET patients, 53 females and 20 males, with a mean age of 61 years (range 45–77) diagnosed and treated between 1996–2017 at the Hospital Universitario de Gran Canaria Dr. Negrín. mRNA expression levels were determined by real time PCR in a LightCycler 480 Instrument II (Roche) using ABL as a control gene. Results were normalized with a cDNA pool from the peripheral blood of 10 healthy donors, which was introduced as an internal control in each experiment. The R Core Team software (2017) was used for statistical analyses.
Results: There was a positive, marginally significant, association between EZH2 and ASXL1 expression levels (p= 0.057). EZH2 expression levels were significantly lower in triple negative cases compared to those with mutations in any of the three driver genes (mean 0.75±0.3 SD *vs* 1.03±0.53 SD, respectively; p=0.003, t Student test). Levels of platelets were significantly higher in patients with mutations in any of the three driver genes compared to triple negative cases (777.82±302.48 SD and 652.31±231.4 SD, respectively; p=0.03, Mann–Whitney U test). Levels of hemoglobin were also higher in mutated patients compared to triple negative ET patients (134.91±31.7 SD and 134.03±11.1 respectively, p=0.04, Mann–Whitney U test).
Summary/Conclusion: The positive association between EZH2 and ASXL1 expression could be explained by their collaborative role in H3K27 methylation activity. A low expression level of EZH2 in triple negative cases indicates that EZH2 deficiency may be involved in the pathogenesis of a portion of triple negative cases. Finally, lower levels of platelet/hemoglobin in triple negative cases could reflect a subgroup of ET patients misclassified as triple negative while they may have reactive thrombocytosis.

PB2263

MOLECULAR CHARACTERIZATION OF MYELOPROLIFERATIVE NEOPLASMS IN RUSSIAN COHORT OF PEDIATRIC PATIENTS

M. Gaskova*, A. Panfyorova, N. Ershov, O. Soldatkina, A. Kazakova, A. Maschan, Y. Olshanskaya, N. Smetanina

Dmitry Rogachev National Research Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation

Background: Myeloproliferative neoplasms (MPNs) are a group of clonal hematopoietic stem cell disorders, which are exceedingly rare and poorly understood in children. The reported frequency of *JAK2* (V617F), *MPL*, *CALR* mutations is much lower in pediatric patients than in adults and the underlying genetics remain largely unknown.
Aims: The aim of our study was to characterize mutational profile of pediatric MPNs using next-generation sequencing (NGS).
Methods: The study included 19 patients diagnosed as Ph-negative MPN (11 boys and 8 girls). Essential thrombocythemia (ET) was diagnosed in 12 patients, polycythemia vera (PV) in 7 patients. The median manifestation age was 7.5 years (ranged from 3 months to 21 years). Next-generation sequencing (NGS) was performed using MiSeq (Illumina, USA). Amplicon library was prepared using Human Myeloid Neoplasms Panel, DHS-003Z (Qiagen, Germany), which included 141 genes. Fragment analysis of *CALR* gene exon 9 was performed for the detection of indels using ABI PRISM 3500 (Applied Biosystems).
Results: Classical *JAK2*(V617F) mutation was found in 9 patients: six patients with PV (86%) and two with ET (17%). Also *JAK2* c.1613_1616delACAAinsT in exon 12 was found in one case with PV. Mutations in *CALR* gene exon 9 were identified in four patients with ET (33%). Type 1 *CALR* frameshift mutation, a 52-bp del (p.L367fs*46) was found in two cases and one patient carried type 2 mutation, a 5-bp TTGTC insertion (p.K385fs*47), one patient harbored complex mutation c.1149-1154_delGGACAAinsTCCTTGTC(p.E383fs*48). No additional somatic mutation in epigenetic regulators (*ASXL1, IDH1/2, DMT3A*) specific for adult MPN were observed in our group. Investigation of 22 matched germline and somatic samples in 11 cases revealed the germline nature of the majority of additional mutation in epigenetic regulators (*IDH2, TET2, EZH2, EP300, BCOR, CREBBP*), in JAK-STAT negative regulators (*SH2B3*) and recurrent mismatch repair gene (*MSH6*), annotated as "possibly pathogenic" by predictive software. One JAK2(V617F) positive PV patient carried germline mutation *BLM* c.1642C>T, which was described as the breast and prostate cancer predisposal mutation. This patient also had somatic mutation in *BCOR* c.4934C>G and *MSH2* c.815C>T genes. In triple negative MPN cases additional mutations were very rare. Two patients had germline mutations *ANKRD26* c.2480T>C and *RELN* c.3651C>G and in one patient *TET2* c.5152G>T was found.
Summary/Conclusion: In our study frequency of *JAK2* and *CALR* mutations consisted 47% and 21%, respectively. Mutations in *MPL* gene were not observed. Thus, 32% patients were triple negative. The value of germline mutations in development of MPN should be further evaluated.

PB2264

THE UTILITY OF SERUM TRYPTASE LEVELS FOR THE INDICATION OF BONE MARROW BIOPSY IN THE DIAGNOSIS OF SYSTEMIC MASTOCYTOSIS. EXPERIENCE IN OUR CENTRE

C. Muñoz-Novas*, I. González, M. Infante, C. Heras, M.A. Foncillas, J.-A. Hernandez

Hematology, Hospital Infanta Leonor, Madrid, Spain

Background: Mastocytosis is a rare disease, classically included in the myeloproliferative diseases, and characterized by clonal proliferation of mast cells (MCs) in one or more organs. In the WHO classification of 2016 it is a separate entity. There are two types, cutaneous mastocytosis (CM), affecting exclusively the skin, and systemic mastocytosis (SM), affecting mainly the bone marrow, among other organs. Clinical expressions are due to the anomalous release of mast cell mediators (MCMs) or to the infiltration of MCs into the tissues. MCs release a large amount of mediators during their activation, tryptase, histamine and heparin being the most specific elements which are also measurable in the conventional laboratory. Tryptase is produced almost exclusively by MCs, although it can also be increased in anaphylaxis, myeloid neoplasms and severe renal failure. Normal levels of it are almost undetectable in healthy individuals, up to 11.4 µg/L being normal. Levels >20 µg/L are one of the WHO's minor criteria for the SM diagnosis.
Aims: To evaluate the diagnostic utility of increased levels of typtase as an independent marker in the diagnosis of SM.
Methods: We retrospectively reviewed the 2.877 requests for bone marrow biopsy (BMB) addressed to the Department of Hematology in our hospital between March, 2009 and February, 2017, we selected those whose reason for request was elevated levels of tryptase.

Table 1.

Age	Sex	Tryptase (µg/l)	BMA	BMB
40	woman	22.6	-	-
58	woman	18.4	+	+
84	man	27	-	-
44	man	15	-	-
60	woman	13	-	-
49	man	14	-	-
54	woman	22.9	-	-
77	man	47.6	-	-
44	woman	31	-	-
55	woman	21	-	-
51	man	156	+	+
70	woman	21	-	-
50	woman	13.5	-	-
60	man	36	-	+

Table 1. Clinical and laboratory characteristics of the patients

Symtoms	Pruritu	Allergy	Skin lesions	Cytopenias	Others organs	Asyntomatics
Percentage	71%	65%	64%	7%	7%	35%

Table 2. Symtoms

Results: BMB were performed in 14 patients to rule out SM because of increase of tryptase levels. All these requests came from the Department of Allergy. Of the 14 cases 6 were male and 8 females, with a median age of 57 years old (Table 1). A review of medical records showed that 35% of the patients (5/14) did not present any other indication suggesting SM. The remaining 65% had a past history of allergy: medication (4), chronic urticaria (1), hymenoptera (1), food (1), sun allergy (1), metal (1). In addition, these patients presented different types of skin lesions (Table 2). Serum tryptase levels ranged between 13.5-156 µg/L (mean 32.7), and 10/14 (71%) showed levels >20 µg/L. The BMB was diagnostic of SM in 3/14 (21%). In these cases, atypical MCs were observed in the bone marrow aspirate (BMA), and they were positive for the KIT D816V mutation with an inmunophenotype CD59+, CD25+ and/or CD2+ by flow cytometry. Only 2/3 had tryptase levels >20 µg/L. These 3 cases, in addition to the elevated tryptase levels, had monoclonal IgG kappa and bone lesions. One case had diagnosis of CM before SM and another case had abnormal blood count.

SM was not diagnosed in any case with elevated tryptase levels as the only abnormal parameter.
Summary/Conclusion: In the light of our results, we can conclude that tryptase is a sensitive but not a specific marker for the diagnosis of SM. In the absence of any other clinical evidence, and with slightly elevated tryptase levels, the risk of having SM is very low, so the study of BMB is not useful and could be avoided, while the association of other data suggestive of SM or an increase of other MSMs confers a high risk of suffering SM.

PB2265

TET2 MUTATION IN CELLULAR REPROGRAMMING AND HEMATOPOIETIC DIFFERENTIATION

C.E.G. Limia[1], R. Reis[1], C. Solza[2], A.H.D. Gabriel[3], I.R. Zalcberg[1], A. Di Stefano[4], I. Plo[4], S. Rehen[5], M.H. Bonamino[1,6], B. Monte Mor[1,*]

[1]Brazilian National Cancer Institute, [2]Hospital Universitário Pedro Ernesto, UERJ, Rio de Janeiro, [3]Hospital Universitário Antônio Pedro, Niterói, Brazil, [4]Institute Gustave Roussy, INSERM UMR[1170], Paris, France, [5]Laboratório Nacional de Células Tronco (LANCE), UFRJ, Instituto IDor, [6]Vice Presidência de Pesquisa e Coleções Biológicas (VPPCB), FIOCRUZ, Rio de Janeiro, Brazil

Background: Primary myelofibrosis (PMF) is a myeloproliferative neoplasm characterized by an clonal myeloproliferation, bone marrow fibrosis and extramedular hematopoiesis. In PMF, driver somatic mutations occur in *JAK2*, *MPL* or *CALR* genes. Mutations in epigenetic regulators as *TET2* and *ASXL1* that could lead to loss-of-function were frequently identified. In this context, induced pluripotent stem cells (iPSC) could be used to study clonal heterogeneity and to recapitulate *in vitro* some hematological features of PMF. Tet2 deletion in mouse embryonic fibroblast was shown to reduce cellular reprograming efficiency. Therefore, we asked if it would be possible to obtain iPSC from a *TET2* mutated PMF patient.
Aims: The main goal of this work was to assess the impact of somatic mutations in *CALR* and *TET2* in both cellular reprogramming and hematopoietic differentiation using iPSC.
Methods: This work was approved by INCA Ethics committee and patients signed informed consent. We used next generation sequencing to screen a cohort of Brazilian PMF patients for myeloid somatic mutations. One of the patients granulocytes (P1) were shown by Sanger sequencing to harbor $CALR^{ins5}$ and $TET2^{G898X}$ mutations. CD34+ primary cells were isolated from P1 or a healthy donor control and erythroblasts were differentiated *in vitro*. We generated iPS cells from P1 erytroblasts (P1-iPS) or from the control erytroblasts (C1-iPS) using the Sendai virus system. The pluripotency was confirmed in iPSC colonies by the expression of embryonic stem cells markers (Oct-3/4, Sox-2, SSEA-4, and TRA-1-81). The capacity to form germ layers was evaluated by embryonic body formation and layer specific markers detection by immunohystochemistry. Hematopoietic differentiation was performed on feeder-free culture supplemented with cytokines and $CD43^+CD34^+$ progenitors sorted at day 10-14. Myeloid Colony-Forming Units (CFU) were quantified in methylcellulose assays, under microscopic evaluation on day 12. CFU-Megakaryocytes were scored in plasma clots assays, after 9 days of culture and labeling with anti-CD41a antibody and alkaline phosphatase staining. Granulocytes were differentiated from $CD34^+CD43^+$ progenitors in liquid culture during 20 days.
Results: After cellular reprogramming, 32 P1-iPSC clones were obtained, 20 $CALR^{ins5}$ /*TET2wt* and 12 $CALR^{ins5}$/$TET2^{G898X}$ *homozygous*. The different genotypes observed for iPSC reflect the clonal diversity present in the PMF primary sample. We confirmed that *TET2* WT as well as *TET2* mutated iPSCs displayed pluripotency markers and were able to generate all three germ layers in EB assays, indicating that the G898X mutation did not impair cellular reprogramming. We next sought to study the role of these mutations in the hematopoietic differentiation. We observed that all type of myeloid colonies were generated in methylcellulose assays for P1-iPSC and C1-iPSC. Our preliminary results show that an increased number of granulocyte colonies derived from $CD34^+CD43^+$ progenitors of P1-*TET2* mutated iPSC, when compared both with iPSC P1-*TET2* WT and C1-iPSC. Using plasma clot assay, we observed higher numbers of large CFU-MK colonies derived from P1-iPSC *versus* C1-iPSC.
Summary/Conclusion: Our results suggest that the $TET2^{G898X}$ did not impair cellular reprogramming, since iPSC harboring this mutation displayed all the features of *bona fide* iPS and that mutations in *CALR and TET2* have an impact on hematopoietic differentiation of iPSC.

PB2266

CD271+ MSCS IN MYELOPROLIFERATIVE NEOPLASMS PH-NEGATIVE

M. Sobas[1,*], K. Zduniak[2], T. Wróbel[3], P. Ziółkowski[2]

[1]Hematology, Blood Neoplasms and Bone Marrow Transplantation, [2]Department of Pathology, [3]Department of Hematology, Blood Neoplasms and Bone Marrow Transplantation,, Medical University, Wroclaw, Poland

Background: Myeloproliferative neoplasms Ph-negative (MPN Ph-) are clinically heterogenous group of malignancies, characterized by presence of JAK2 or CALR or MPL mutation in most of the cases. Beside their clinical differences, these neoplasms differ significantly on histological grounds, not only in hematopoietic cells morphology and architecture, but also their microenviroment features. Mesenchymal stromal cells (MSCs) CD271-positive play a major role in hematopoietic stem cells renewal, and have been analysed previously in context of solid tumor propagation and some hematological cancers development.
Aims: To analyse quantity and architecture of CD271-postive MSCs in MPN Ph-.
Methods: We have analysed bone marrow trephine biopsies from 22 MPN Ph- patients: essential thrombocythemia (ET; n=7), polycythemia vera (PV; n=3), myelofibrosis (MF; n=11), prefibrotic myelofibrosis (prePMF; n=1) and 4 with non MPN diagnosis: chronic lymphocytic leukemia (n=1) and reactive disorders (n=3). Clinical, analytical and molecular data were collected. Histological sections were stained immunohistochemically using anti-CD271 antibody. Extension of staining was assesed semiquantitatively in a 3-grade (1-3) manner and correlated with diagnosis and several clinical and laboratory parameters.
Results: Here we present a very preliminary results. The CD271 staining grade 3 was observed in 6 from 11 MF (54.5%), grade 2 was observed in 5 from 11 MF (45.5%) and in 1 from 1 prePMF. The CD271 staining grade 1 was observed only in 1 MF and in all PV, ET and in non-MPN patients. There was no significant relation between CD271 and mutational status (JAK2, CALR), white blood cells, platelet counts, haemoglobin level, LDH level. There was no significant relation between CD271 and IPSS, DIPSS and DIPSS plus risk score.
Summary/Conclusion: Although we have analyzed a very small group of patients, a strong relationship between MF and high CD271+ cells density in bone marrow is visible. It surely requires further investigations, but CD271+ cells density assessment might be helpful in diagnosis of high-grade MPNs. It may also show novel insight into MPNs development and pathology.

PB2267

MUTATIONAL PROFILES AND PHENOTYPIC FEATURES IN JAK2 V617F-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS – A SINGLE CENTRE EXPERIENCE

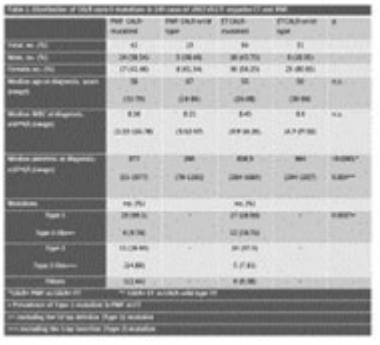
G.F. How*, S.J. Khoo, P.J. Kwok, C. Chuah

Haematology, Singapore General Hospital, Singapore, Singapore

Background: Since the discovery of CALR exon 9 mutations in 2013, the molecular diagnostic of ET and PMF has increased from approximately 60% (with diagnostic markers of JAK2 exon 14 and MPL exon 10 mutations) up to 90%. Generally, patients with mutant CALR exon 9 appear to have a prognostic advantage over those with wild type CALR. However, detection of ASXL1 mutations may enable more accurate assessment of risk stratification of PMF as these mutations are also frequently detected in PMF and appear to have a detrimental prognostic impact.
Aims: The aim of this study was to analyse CALR exon 9 mutation profiles of patients with ET and PMF and their association with ASXL1 exon 12 mutations in PMF.
Methods: For detection of mutations in CALR and ASXL1, CALR exon 9 and ASXL1 exon 12 respectively were PCR-amplified and direct sequencing of the amplicons was performed.
Results: A cohort of 92 (56 females, 36 males) JAK2 V617F-negative ET and PMF patients from a single centre was first screened for CALR mutations. A total of 61% (20/33) of PMF and 47% (28/59) of ET patients were identified with CALR frameshift indel mutations. More than 10 different mutations including several other novel ones were observed whilst Type 1(n=21) and Type 2(n=12) were the common variants detected. There was also an apparent biased incidence of Type 1/Type 1-like mutations in PMF. Overall, CALR mutations occurred more frequently in males than in females (p=0.010). For correlation studies of clinical data with mutation profiles, another 57 CALR-mutated patients (ET, n=36; PMF, n=21) were included. In 105 CALR-mutated patients (64 ET, 41 MF) studied, no significant difference was observed in the white blood cell counts or age between CALR-mutated and CALR-wild type patients within both ET and PMF cohorts. However, platelet count was significantly higher in CALR+ ET patients compared to the wild type cohort (p=0.004) and CALR+ PMF cohort ($p<0.0001$). The higher frequency of Type 1 (CALR1) mutations in PMF than in ET (56% *vs* 27%, p=0.0037) were confirmed in this bigger cohort.

Within PMF, platelet count was statistically higher in patients with Type 2-like (CALR2) mutations than those with CALR1. All CALR+ PMF patients were also screened for ASXL1 exon 12 mutations. Of 41 patients analysed, 7 patients were positive with frameshift mutations and 3 with nonsense mutations. The most frequent mutation was c.1934dupG, which constituted 40% of mutations. Although the study cohort size was small, the patients with ASXL1 mutations had significantly higher white blood cell count than those without (p=0.0035). Age, gender, platelet count and CALR mutation profile did not show any differences between different ASXL1 mutation status.

Table 1.



Summary/Conclusion: Like other studies which reported higher CALR1 prevalence in non-Asian populations, our cohort which comprised mostly Asians also showed similar profile. However, ASXL1 exon 12 mutations which were detected at a frequency of approximately 25% in CALR+ PMF in this study, did not appear to be associated with CALR1 or CALR2. It has been shown in large series that ASXL1 mutations compromised the survival of PMF patients while CALR mutations appearing to attenuate the effect. The interaction of CALR and ASXL1 mutations provides strong evidence for the prognostic relevance of performing *CALR* and *ASXL1* mutation determination in all patients with PMF. This study can also be further expanded to a larger study population with survival analysis of patients based on mutational status and other risk factors.

PB2268

THE MOLECULAR PATHOGENESIS AND THE HEMATOLOGICAL AND CLINICAL ASPECTS OF DRIVER AND SPLICEOSOME MUTATIONS IN POLISH PATIENTS WITH PRIMARY MYELOFIBROSIS

A. Bartoszewska-Kubiak[1,*], K. Matiakowska[2], M. Morgut-Klimkowska[1], E. Bielińska[1], O. Haus[1]

[1]Department of Clinical Genetics, Nicolaus Copernicus University, Faculty of Medicine, [2]Department of Clinical Genetics, Nicolaus Copernicus University, Bydgoszcz, Poland

Background: Primary myelofibrosis (PMF) is a *BCR-ABL1*-negative myeloproliferative neoplasm (MPN). PMF has the most heterogeneous clinical course among MPNs and is characterized by increased proliferation of megakaryocytes, progressive bone marrow fibrosis, extramodullary hematopoiesis, leukoerythroblastosis and shortened survival. Mutations in primary myelofibrosis (PMF) are operationally classified into 2 categories: drivers (*JAK2, MPL, CALR*) and others. Mutations in *JAK2* and *MPL* genes lead to constitutive activation of *JAK2/STAT* signaling pathway that results in increased proliferation of myeloid and magakaryocytic progenitors in absence of TPO and EPO. *CALR* gene encodes calreticulin, a protein which plays role in intracellular signaling, Ca^{2+} storage, regulation of gene expression, cell adhesion, apoptosis and autoimmune response. Mutations of these three genes are reciprocally exclusive. "Triple negative" PMF cases with any of them are associated with poor prognosis. Driver mutations might be accompanied by other mutations whose pathogenetic relevance is even less clear (*ASXL1, SRSF2, IDH1/2, EZH2, TET2, DNMT3A*, and *CBL*). *SRSF2* spliceosome mutations play a crucial role during RNA splicing pathway. Hotspot mutations in the exon 2 have been identified in PMF (3-17%).
Aims: The aim of the study was to evaluate and relate mutational status of *JAK2*, *MPL*, *CALR* genes and splicing factor *SRSF2* in Polish group of PMF patients and find associations between mutated and nonmutated cases.
Methods: *JAK2*V617F and *MPL*W515K/L mutation status were assessed using AS-PCR. Direct sequencing was performed to detect insertion/deletion of exon 9 of *CALR* gene and mutations of exon 2 of *SRSF2* gene. Relationship between the presence or absence ("triple negative") of these mutations and hematological and clinical data of patients was analyzed with Mann Whitney U Test.
Results: Out of the 98 patients, 36 (37%) carried *JAK2*V617F, 25 (26%) *CALR* exon 9 indel, 12 (13%) mutation of exon 2 *SRSF2* gene, 3 (3%) *MPL*W515 mutation and 22 (21%) were "triple negative". Among the three *MPL*-mutated cases 2 samples exhibited splice factor gene mutations *SRSF2* (66%). *JAK2*/V617F was accompanied by *SRSF2* mutation in 4 cases (11%), while *CALR* was rarely combined with splice factor gene mutations (4%). We found significant correlation (0,005) between high plateled (PLT) count and the presence of any mutation (median 207,5) in comparison to PLT in patients without mutation (median 74). An analogical association was detected between patients *CALR*(+) mutation (median PLT 333), and *CALR*(-) mutation (102,5), p=0,018. We have not found any significant relationship between clinical parameters and the presence of analyzed mutations.
Summary/Conclusion: Obtained result may suggest a clinical importance of simultaneous analysis of driver and spliceosome mutations, like *SRSF2*.

PB2269

TYPES OF CALRETICULIN (CALR) MUTATIONS IN MYELOPROLIFERATIVE NEOPLASMS: A COHORT OF PATIENTS FROM PAKISTAN

R.Z Ahmed, M. Rashid*, T. Shamsi

National Institute of Blood Diseases & Bone Marrow Transplantation, Karachi, Pakistan, Karachi, Pakistan

Background: Polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are classic *BCR-ABL*-negative myeloproliferative Neoplasm (MPNs). In December 2013, somatic mutations in exon 9 of Calreticulin (*CALR*) gene were first time identified as the second most prevalent acquired nucleotide changes in *JAK*2 mutation negative ET and PMF patients. *CALR* mutations have important diagnostic and prognostic significance in ET and PMF patients.
Aims: To screen *CALR* mutations in local Pakistani population suspected of having MPNs.
Methods: Clinical and haematological features were obtained from 51 MPN patients (PV=10, ET=17, MF=12 and Undifferentiated MPN=9). *JAK*2V617F mutation was analyzed by ARMS-PCR. *CALR* mutations were identified by bi-directional Sanger sequencing.
Results: *CALR* mutations were detected in 13.7% patients, of which one patient was positive for *JAK*2V617F as well. All those who showed positive results for *CALR* mutations were ET patients (41.17%). Type I mutation was found in 42.8% and among them 2 had two new scattered point mutations (c.1081C>G and c.1086C>G) as well, Type II in 28.5%, and others in 28.5%. Among those patients with other than Type I or Type II mutation, one had novel double deletion of 10bp (c.1130_1139del) and 28bp (1195_1222) and one had reported CALR 12bp deletion (c.1214_1225del). When *CALR* positive and negative ET patients were statistically compared, no differences were observed in haemoglobin, white blood cell, platelet counts, hepatomegaly, and splenomegaly at p-value >0.05.
Summary/Conclusion: In our cohort *CALR* mutations were found associated with ET only. This difference emphasizes to conduct studies with large sample in this region.

PB2270

DOUBLE DELETION IN CALRETICULIN GENE: REPORTING NOVEL CALRETICULIN MUTATIONS IN A PAKISTANI PATIENT WITH ESSENTIAL THROMBOCYTHEMIA

M. Rashid*, R.Z. Ahmed, S. Ahmed, T. Shamsi

National Institute of Blood Diseases & Bone Marrow Transplantation, Karachi, Pakistan, Karachi, Pakistan

Background: The Calreticulin (*CALR*) identification as a recurrently mutated gene in primary myelofibrosis (PMF) and essential thrombocythemia (ET) took several years longer because its mutations were small insertion and deletions and its function in myeloid cell signalling had been less well understood. While 52bp deletions (Type 1) and 5bp insertions (Type 2) constitute >80% of *CALR* mutations, a variety of other insertion-deletion mutations have been reported.
Aims: Here we present *CALR* mutations in one patient with ET that is not reported before. Patient: This is a case of 60 years old, referred with increased platelets count. On physical examination. the liver was palpable 2 fingers and spleen 4 fingers below the costal margin. His platelet count were: $2318x10^9$/L, other parameters were; Hb:7.6 g/dl and TLC count was

13.29x10^9/L. Bone marrow aspirate and trephine biopsy were highly suggestive of MPN most likely ET. Platelet count fluctuates throughout the disease. He took Hydrea (high dose) and also received plateletpheresis.
Methods: For *CALR* exon 9 Traditional polymerase chain reaction was performed on DNA previously extracted from peripheral blood. The amplified product was purified and bidirectionally sequenced.
Results: Electropherogram of *CALR* exon 9 show double deletions, 10bp and 28 bp deletion
Summary/Conclusion: The identification of new *CALR* mutation will improve our understanding of the pathophysiology of MPN and will help to find new therapeutic targets. Still, there is question are those mutations monoclonal? If yes then in cis or Trans configuration? For resolving these issues there need to clone the PCR product in vector and do indirect sequencing.

PB2271

CLINICAL EXOME SEQUENCING REVEALS HOMOZYGOUS VARIANT IN THE RASOPATHY GENE CBL IN A PEDIATRIC PATIENT WITH JUVENILE MYELO-MONOCYTIC LEUKEMIA: A CASE REPORT WITH COMPLEX GENETIC SPECTRUM

A.A. Peer Zada*, M. Samman, H. AlHomaidani

Molecular Pathology, King Fahad Medical City, Riyadh, Saudi Arabia

Background: Juvenile myelomonocytic leukemia (JMML) is a rare clonal myelodysplastic/myeloproliferative neoplasm that occurs in infancy and early childhood, characterized by persistent monocytosis, no Philedelphia chromosome or BCR-ABL1 fusion gene, less than 20% myelo-monoblasts in the marrow and granulocyte-macrophage colony-stimulating factor hypersensitivity among other diagnostic criteria. The median age at presentation is 2 years (range 0.1-11.4) with an incidence of 1.2 per million child per year. Clinical and laboratory diagnostic criteria for JMML have recently incorporated molecular genetic analyses in the form of somatic and/or germline mutations in canonical RAS pathway genes (e.g., PTPN11, NF1, NRAS, KRAS, and CBL).
Aims: We sought to unravel a comprehensive genetic picture using clinical exome sequencing in an 18-month-old Saudi female child from a non-consanguineous marriage who presented with a JMML pathology and showed hematogones with 1% myeloblasts by flow cytometry.
Methods: Diagnostic work-up included pathology including cytomorphology, multicolour flow cytometry, chromosomal and FISH analyses, and array CGH for duplication and deletion analyses. For unraveling genetic etiology, exome sequencing was performed.
Results: Cytomorphology and radiology revealed JMML pathology with hepatosplenomegaly. Immunophenotyping of bone marrow lymphocytes by flow cytometry revealed hematogones with 1% myeloblasts positive for CD34, CD33 and CD13. Multicolour flow revealed T, B and NK lymphocytosis with intact expression of MHC classII antigens on B-cells, 12.6% activated T-cells (CD3+DR+) and intact expression of adhesion molecules. Chromosomal analyses revealed a normal female karyotype with no apparent numerical or structural abnormalities, and FISH analyses for comprehensive AML panel was normal. Deletion and duplication analyses of the Rasopathy panel genes vias aCGH revealed negative results. However, clinical exome sequencing in this patient revealed homozygous and/or heterozygous mutations in canonical RAS pathway genes in addition to gene variants related to acute myeloid leukemia.
Summary/Conclusion: This is a first report from Saudi Arabia and a second only report in the literature using clinical exome sequencing in JMML showing homozygous mutaion in the rasopathy gene CBL and suggests that exome sequencing holds great promise as a diagnostic tool in these patients who have the potential to transform to AML and therefore may warrants quick clinical intervention.

PB2272

CURCUMIN: A NEW THERAPEUTIC STRATEGY FOR JAK2 V617F MUTATED CELLS

J. Petiti*, V. Rosso, C. Panuzzo, D. Cilloni

Clinical and Biological Sciences, University of Turin, Orbassano, Italy

Background: The myeloproliferative neoplasms (MPNs) are chronic myeloid cancers divided in Philadelphia (Ph) positive, chronic myeloid leukemia, or negative: polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Mutations in *JAK2*, *MPL* and *CALR* genes are the biological markers in the most of Ph negative patients. The single base *JAK2 V617F* mutation is most common, with an incidence of 95% in PV and 50-55% in ET and PMF patients. The JAK2 protein has tyrosine kinase activity and the gain-of-function V617F mutation results in a constitutive activation of the JAK/STAT pathway, conferring a proliferative advantage and apoptosis inhibitions. The understanding of the molecular pathogenesis of MPNs has prompted the development of targeted agents, such as JAK inhibitors. Curcumin is the active phytochemical component isolated from the rhizome of the *Curcuma longa* plant. Curcumin is a highly pleiotropic molecule with multiple pharmacological effects, such as anti-inflammatory, anti-microbial, anti-oxidative and anti-proliferative activities. Extensive preclinical trials have indicated curcumin's therapeutic potential against a wide range of human diseases. Furthermore, previous studies showed that curcumin can suppress JAK2/STAT3 signaling pathways in different type of cancer and injuries.
Aims: The purpose of the present study was to investigate anti-proliferative and pro-apoptotic effects of curcumin in MPNs, in particular in *JAK2 V617F* cell lines.
Methods: HEL cell line, JAK2 V617F mutated in homozygosis, has been incubated with different concentrations of curcumin at different time points. Then, apoptosis, proliferation and cell cycle were evaluated. Subsequently, fragmentation of DNA, JAK2/STAT and AKT/mTOR pathways were investigated at both RNA and protein levels.
Results: We identified that curcumin induced apoptosis, cells cycle arrest and inhibition of proliferation in HEL cell lines. Furthermore, we showed JAK2/STAT and AKT/mTOR pathways are affected by curcumin treatments.
Summary/Conclusion: This study evaluates curcumin treatment in JAK2 mutated cell line, suggesting that it could be useful in the future for treatment of *JAK2 V617F* mutated patients.

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PB2273

IMMATURE PLATELET PARAMETERS FOR PREDICTION OF POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA IN PATIENTS WITH ELEVATED PLATELET COUNTS

J. Yoon*, B.K. Jung, J.A. Kwon, C.S. Lim, S.-Y. Yoon

Korea University, Seoul, Korea, Republic Of

Background: Elevated platelet count is characteristic features observed in myeloproliferative disorders, such as Polycythemia vera (PV) and Essential thrombocythemia (ET) but also could be observed in other reactive conditions. Several studies reported elevated numbers of immature platelet population in PV and ET compared to the normal control and yet studies comparing the patients with reactive thrombocytosis have not been performed.
Aims: In this study, we analyzed platelet related parameters including immature platelet fraction (IPF) for prediction of PV and ET in patients with thrombocytosis.
Methods: A retrospective study of IPF, immature platelet count (platelet count x IPF) and other platelet related parameters, such as plateletcrit (PCT), mean platelet volume (MPV) and platelet distribution width (PDW) were conducted using hematology analyzer XE-5000 (Sysmex, Kobe, Japan). A total of 100 whole blood samples from patients with thrombocytosis (platelet count ≥450x10^9/L) were included and among these, 11 were from PV, 13 from ET and 76 were from reactive thrombocytosis.
Results: IPF and immature platelet count were significantly higher in both PV and ET groups compared to the reactive thrombocytosis group. Moreover, PCT, PDW and MPV were also significantly elevated in both groups. Using ROC curve analysis, immature platelet count and PDW showed highest area under curve (both 0.86). In case of immature platelet count and PDW, with cut-off level of 10,243/μL and 10.75 fL, the sensitivities were 91.7% and 83.3%, respectively and specificities were 66.7% and 76.9%, respectively.
Summary/Conclusion: Identification of patients with possibility of PV and ET among the patients with thrombocytosis would allow prompt work up and appropriate treatment. Among the platelet associated parameter, immature platelet count and PDW were the most valuable in discriminating PV and ET from reactive thrombocytosis and would be useful for the differential diagnosis of patients with thrombocytosis.

PB2274

HYDROXYUREA IN PH NEGATIVE MYELOPROLIFERATIVES NEOPLASMS (MPN): EXPERIENCE IN ARGENTINA

G.E. Bendek Del Prete[1], M. Castro Rios[1,*], A. Vicente[1], A. Varela[1], F. Sackman[1], A. Enrico[1], P. Heller[1], B. Moiragui[1], A.V. Galezzi[1], M. Cabrejo[1], E. Nucifora[1], M.P. Cardenas[1], C. Seehaus[1], B. Girardi[1], A. Jorge[1], L. Beligoy[1], A. Marcela[1], A. Manuel[1], R. Benzadon[1], A. Bonacina[1], G. Braidot[1], M.V. Canonino[1], C. Analia[1], C. Maria Fernanda[1], C. Casali[1], V. Castano[1], C. Nicolas[1], C. Lucia[2], V. Celina[2], E. Machiavelli[2], H. Fernandez Grecco[2], L. Ferrari[2], A. Pavlovsky[2], C. Pavlovsky[2], S. Fontana[2], G. Gonzalo[2], M.S. Gomez[2], F. Gonzalez[2], D. Gotta[2], I. Longordo[2], J. Jerez[2], L. Kornblihtt[2], F. Rojas[2], D. Lafalce[2], C. Mahuad[2], J.L. Mardei[2], M. Marquez[2], N. Massachessi[2], L. Melillo[2], M. Perez[2], E. Roveri[2], A. Ruades[2], A. Salto[2], J. Sanchez Avalos[2], Z. Sfeir[2], S. Crucet[2], M. Williams[2], C. Zani[2], M.F. Tosin[2], V. Gilli[2], V. Fernandez[2], A. Galeazzi[2]

[1]Hematologia, [2]Subcomision de Mieloproliferativo SAH, Buenos Aires, Argentina

Background: Hydroxyurea (HU) is the first-line agent recommended as a cytoreductive agent in the treatment of Phi-negative MPNs. The indication is based on the effectiveness in reducing the risk of vascular events in the short term and the risk of transformation to AML and MF in the long term. Its use continues to be controversial in published works regarding the prevention of thrombosis, prolongation of survival and transformation to MF and AML. Being a drug of simple handling in clinical practice, 11-20% of cases of intolerance have been reported and between 13-20% of resistance according to established criteria.
Aims: Describe population of patients with HU, criteria of treatment initiation, vascular complications, remission rate, rates of intolerance, resistance to HU and progression in MPN treated in Argentina in the daily practice.
Methods: Members of the Argentine Society of Hematology (SAH) reported the data obtained from clinical histories of patients with NMP treated with HU. We analyzed, reason for treatment initiation, response, frequency of thrombosis, intolerance rate, resistance and mortality with descriptive statistics. Complete remission was considered for those patients with PV with Hto <45% and with TE with platelets <400000/mm^3 sustained for 12 weeks.
Results: We included 419 patients referred by members of the SAH, diagnosed from 1986 to 2017, of which 417 were analysable, with a median follow-up of 67 (1-372) months; 63% female, mean age 63 years (SD 13.67), 206 patients with PV (49%), 180 TE (43%) and 31 FM (7.4%) Bone marrow biopsy was performed at 81%. Mutations were studied in 80.2%: JAK2V617F POS in PV (90.7%), TE (80%), MF (64%). Of 22 pac JAK2V617F NEG: 9 were CALR type 1 and 6 type 2, 4 MPL POS and 9 triple NEG. Splenomegaly was found in 43.2%. They received aspirin 81.3%. The reason for starting treatment was age (51%), followed by the presence of vascular risk factors (26.6%), thrombocytosis (24.7%) and previous thrombosis (16.8%). 62 patients had venous thrombosis, 48 prior to HU, 8 patients with 2 or more events, 16 had thrombosis with HU. 58 had arterial thrombosis (CVA, TIA, AMI) 48 were previous to HU, 11 during the treatment. 22 patients (20.2%) with major bleeding during HU treatment. Among the patients with PV, 61% maintained Hto less than 45%, and 66% of patients with ET achieved platelets less than 400,000 / mm3. A total of 136 patients (32.6%) with adverse events were reported, the most frequent were cutaneous, cytopenias and gastrointestinal symptoms. The incidence of neoplasms was 1.9%,t refractoriness 5% and 3.8% were intolerant. Progression to AML in 1.6% and to MF 2.6%. The progression-free survival was 282 months. The mortality was 4.8%. The overall survival was 295 months.
Summary/Conclusion: We can conclude that HU was effective in obtaining complete remissions in PV and ET, in reducing the risk of thrombosis. There were frequent side effects, mainly cutaneous ulcers, and a low rate of resistance / intolerance, progression and development of secondary neoplasia. The results obtained show that the evolution and complications of patients with PV JAK2 and JAK2 positive TE are similar and that the low rate of progression is probably related to the follow-up time, since this is directly proportional to the course of each decade of the disease and treatment. The active participation of the members of the SAH in the collection of data provides the basis for the creation of a prospective registry of MNP and the accomplishment of collaborative works within the framework of the SAH in the country and eventually in the region.

PB2275

CONDITIONAL PROBABILITY OF DELAYED RESPONSE IN SYMPTOM AMELIORATION DURING RUXOLITINIB TREATMENT OF PATIENTS WITH MYELOFIBROSIS

S. Koschmieder[1], L. Jacobasch[2], T. Geer[3], C. Schulte[4], J. Wehmeyer[5], B. Gröschl[6], S. Großer[6], R.A. Mesa[7], H.L. Pahl[8,*]

[1]Hematology / Oncology, University Hospital Aachen, Aachen, [2]Onkologische Schwerpunktpraxis, Dresden, [3]Medizinische Klinik III, Diakonie-Klinikum Schwäbisch Hall, Schwäbisch Hall, [4]Gemeinschaftspraxis für Hämatologie & Onkologie, Dortmund, [5]Gemeinschaftspraxis für Hämatologie und Onkologie, Münster, [6]Novartis Pharma GmbH, Nürnberg, Germany, [7]Mays Cancer Center, UT Health San Antonio MD Anderson, San Antonio, United States, [8]Hematology / Oncology, University Hospital Freiburg, Freiburg, Germany

Background: In patients with myelofibrosis (MF), ruxolitinib has proven effective in ameliorating symptoms such as night sweats, itching and abdominal discomfort. Responses are frequently seen within the first month of treatment. To date, however, data on symptom improvement has been reported as decreasing means of symptom scores over time in large patient cohorts. By nature, these data cannot address critical questions arising in clinical practice: if a patient fails to show symptom improvement within the first month or the first three months of treatment, what are the chances that he or she will still respond if treatment is continued?
Aims: Therapeutic decisions for individual patients may therefore be better informed by knowledge of conditional probabilities.
Methods: We thus used conditional probability algorithms to analyze symptom improvement in patients enrolled in the JaKoMo trial, a phase IV prospective, non-interventional study of MF patients receiving ruxolitinib therapy. Symptom burden was measured by the Total Symptom Score (TSS) of the MPN-SAF (Myeloproliferative Neoplasm - Symptom Assesment Form), a scale that measures 10 items and can range from 0-100.
Results: Data at baseline, one month, three and six months follow up were available for 192 MF patients. At baseline, JaKoMo patients reported a TSS of 29.9. Two separate response analyses were conducted. In a first study, response was defined as a change of at least 5 points in the TSS. Following a month of ruxolitinib treatment, 105 (55%) of patients responded by decreasing the total symptom score by 5 points or more. Importantly, of the remaining 87 patients, 23 patients (26.4%) responded during the fol-

lowing 2 months, and these responses were maintained for at least the following 3 months. Of the 64 of patients that had not responded by 3 months, a further 8 (13%) responded at 6 months. We subsequently used a more stringent criterium to define response, a decrease of at least 50% in the TSS of an individual patient. By this criterion, 62 patients (32%) responded following 1 month of treatment, while 130 patients (68%) did not. Of these 130 patients, 26 (20%) responded following two more months of treatment. Likewise, of the 104 remaining patients without a response until 3 months, 17 (16%) responded during the following 3 months. An important strength of our study is that the JaKoMo patient cohort is recruited largely from office-based hematologists, which renders the data more broadly applicable than studies conducted in selected, academic hospital settings.
Summary/Conclusion: Our data demonstrate that a subset of MF patients will experience relief from symptoms under continued ruxolitinib therapy, even if improvement was initially not observed during the first 4 to 12 weeks of treatment. Given the potential myelosuppressive effect of ruxolitinib, these data may be used to weigh risks and benefits in informing therapeutic decisions.

PB2276

IMMUNOHISTOCHEMISTRY AS A TOOL TO DETECT CALRETICULIN MUTATION IN PATIENTS OF PRIMARY MYELOFIBROSIS AND ESSENTIAL THROMBOCYTHEMIA AND ITS COMPARISON WITH POLYMERASE CHAIN REACTION

A. Gupta*, S. Sazawal, M. Mahapatra, H. Pati, S. Tyagi, R. Saxena

Haematology, All India Institute of Medical Sciences, New Delhi, New Delhi, India

Background: Philadelphia negative myeloproliferative neoplasms pose a significant diagnostic challenge. About 30-45% patients of Primary Myelofibrosis (PMF) and Essential Thrombocythemia(ET) are also negative for JAK2 mutation. In recent times calreticulin (CALR) mutations have been identified which can characterise about 50-80% of such patients. However, the heterogeneity of calreticulin mutations demand extensive molecular testing which are not widely available, labour intensive, costly and time consuming. To overcome these limitations immunostaining by immunohistochemistry(IHC) was developed to detect calreticulin mutations.
Aims: 1. Detection of calreticulin mutation by allele specific oligonucleotide (ASO) PCR and IHC in patients of JAK2 negative primary myelofibrosis and essential thrombocythemia; 2. To determine the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of IHC when compared to PCR.
Methods: This is a prospective observational study. We included all suspected cases of PMF and ET both clinically and on bone marrow examination. Cases with positive BCR-ABL and JAK2 V617F mutation were excluded. Eligible cases were screened for CALR mutation by ASO-PCR for type 1 and type 2 mutations. Interpretation was done by comparing bands on gel electrophoresis to the expected product size (wild type CALR: 357bp, CALR type 1 mutation: 302bp and CALR type 2 mutation: 272 bp). For all these cases IHC was simultaneously done on the bone marrow biopsy section using monoclonal CAL2 antibody. Those cases with more than 90% strongly labelled megakaryocytes were taken as positive.
Results: A total of 61 patients were suspected to have PMF or ET on clinical and bone marrow examination. All these patients were negative for BCR-ABL fusion transcript. On further analysis 36 patients were found to have JAK2V617F mutation and were excluded from the study. Remaining 25 cases were analysed for CALR mutation by PCR and IHC. Amongst the 14 cases with positive CALR mutation, 12 were positive by both PCR and IHC. However, two patients who were CALR positive by PCR showed negative immunostaining by IHC. Remaining 11 cases reported negative for CALR mutation by both PCR and IHC. On statistical analysis of IHC performance, we found a sensitivity of 85.71% and specificity of 100% at 95% confidence interval. The positive predictive value (PPV) and negative predictive value (NPV) were 100% and 84.62% respectively.
Summary/Conclusion: IHC is a readily available and less labour-intensive method to identify CALR mutations in patients with PMF and ET with high sensitivity and specificity. In resource limited settings, immunostaining with CAL2 monoclonal antibody may serve as a valuable tool for diagnosing patients with PMF and ET. Limitation of study: The two cases with positive PCR result and negative IHC need to be evaluated by sanger sequencing to exclude the possibility of false positive PCR.

PB2277

COEXISTENCE OF JAK2V617F AND CALR MUTATIONS IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA IN A SINGLE CENTER

M. Saez-Perdomo[1,2,*], E. González Pérez[2], S. Sánchez Sosa[1,2], Y. Florido Ortega[2], C. Bilbao Sieyro[2,3], R. Stuckey[2], S. de la Iglesia Iñigo[2], A. Sáez Salinas[2], G. Santana Santana[2], A. Segura Diaz[2], M.T. Molero Labarta[2,4], M.T. Gómez Casares[2]

[1]Universidad de Las Palmas de Gran Canaria, [2]Hematology, Hospital Universitario de Gran Canaria Dr. Negrín, [3]Morfology, [4]Ciencias médicas, Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain

Background: Classic BCR-ABL1-negative MPNs include PV, ET, and PMF with JAK2V617F as the most common mutation. JAK2V617F can be detected in about 95% of patients with PV while the remaining 5% of PV patients carry a somatic mutation of JAK2 exon 12. Approximately one-third of patients with ET or PMF do not carry any mutation in JAK2 or MPL. In December 2013, mutations were described in CALR in 67–71 and 56–88% of JAK2 and MPL negative patients with ET and PMF, respectively. Various studies have found differences in the clinical features of patients with CALR mutations compared with those harboring JAK2 mutations, such as a lower risk of thrombosis, lower hemoglobin and leukocyte counts, higher platelet count and longer survival for CALR-mutated ET patients. CALR mutations have been reported to be mutually exclusive with JAK2V617F or MPL mutations. However, recently, a few studies have reported the coexistence of JAK2 and CALR mutations in MPN.
Aims: The aim of the study is to evaluate the coexistence of CALR and JAK2 in our center.
Methods: Our series consisted of 122 ET patients, 88 females and 34 males, diagnosed between 1996 and 2017 with a mean age at diagnosis of 56.62±17.65. JAK2 mutation screening was carried out using real time PCR with FRET probes and CALR and MPL analysis using high resolution melting. Mutations were confirmed by Sanger sequencing (ABI 3130 Thermo Fisher).
Results: Among the 115 ET patients studied for the presence of the 3 mutations we found 40.9% (n=47) JAK2+/MPL-/CALR-, 12.2% (n=14) JAK2-/MPL-/CALR+, 44.4% (n=51) JAK2-/MPL-/CALR-, 1.7% (n=2) JAK2+/MPL-/CALR+ and 0.8% (n=1) JAK2-/MPL+/CALR-. Both patients JAK2+/CALR+ presented clinical features similar to the others patients with ET due to a history of gradual elevation of platelet counts. These 2 patients had no associated symptoms, splenomegaly or previous hemorrhagic or thrombotic episodes. The peripheral blood count for one of these patients had a plt count of $1018x10^6$/L, hgb 13.7g/dl, WBC count $12.22x10^9$/L and was considered IP low risk; the other patient had a plt count of $523x10^6$/L, hgb 12.5g/dl and WBC count $6.05x10^9$/L, with a recent diagnosis as IP high risk. Both patients were treated with acetylsalicylic acid. One of them progressed to MF after 7 years of being diagnosed and both are still alive.
Summary/Conclusion: Since CALR mutations discovery, their presence has been proposed to be mutually exclusive with JAK2 and MPL mutations. However, CALR and JAK2 co-mutations have been reported in a few MPN cases across different ethnic groups with a frequency of around 1%, which agrees with the incidence of our series (1.64%), although 2 groups have reported frequencies higher than 4% in Asian patients. To date, we have not observed the coexistence of MPL and CALR/JAK mutations in any MPN patient. This discrepancy could either be due to differences in methodology for the detection of *CALR* mutations, a different mutational spectrum in Caucasian and Asian populations, or the fact that the majority of groups follow a diagnostic workup flow for MPN with the assumption that mutations are mutually exclusive. Co-mutated patients might represent a new subtype in MPNs; in fact, many groups have suggested that *JAK2/CALR* double positive might have a different phenotype and clinical course, distinct from *JAK2*-positive or *CALR*-positive subgroups. Thus identification of the true frequency of these patients is important for defining the prognosis, risk factors and outcomes of these MPN subgroups. Our results suggest that these mutations should be analyzed independently and simultaneously at diagnosis in all MPN patients.

PB2278

INTRODUCTION OF AN NGS GENE PANEL INTO CLINICAL SERVICE FOR MYELOPROLIFERATIVE NEOPLASMS

A. Skowronska*, J. Bryon, S. Clokie, Y. Wallis, J. Mason, K. Reay, C. Corbett, M. Griffiths

West Midlands Regional Genetics Laboratory, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, United Kingdom

Background: In the West Midlands region of the UK, all patients with a suspected myeloproliferative neoplasm (MPN) have access to quantitative analysis of *JAK2* V617F by droplet digital PCR as standard of care. The British Committee for Standards in Haematology recommends that suspected MPN cases have investigation of *JAK2* exon 12, *CALR* and *MPL* genes if *JAK2* V617F is negative.

Aims: The aim of the project was to improve the MPN service by substituting sequential analysis of individual target regions within the *JAK2, CALR* and *MPL* genes with a single assay, and to increase the number of genes available for analysis.
Methods: A commercial next generation sequencing (NGS) gene panel (Oxford Gene Technology, SureSeq Myeloid Panel), coupled with the Illumina MiSeq platform was validated and implemented. The gene panel utilises hybridization based enrichment technology and consists of 25 MPN–related genes. During the validation stage the following were enriched and analysed: 29 positive control samples with 30 known pathogenic variants, 30 negative control samples without known pathogenic variants in the *JAK2, CALR and MPL* genes, and 24 MPN samples of unknown mutational status. Thus so far over 1500 clinical samples have been analysed and reported since the service was introduced in October 2016.
Results: The panel has successfully identified: a large range of known pathogenic variants at high sensitivity (*JAK2* V617F variant allele frequency 1%, *CALR* Type I frameshift variant allele frequency 3%), a potential alternative driver mutation in a known low level *JAK2* V617F positive patient, a rare *MPL* exon 4 pathogenic variant and also the detection of low level *CALR* pathogenic variants, which would not have been detected by Sanger sequencing analysis. In one patient the panel identified the presence of two different *JAK2* exon 14 pathogenic variants in cis (*JAK2* V617F and *JAK2* C618R). The *JAK2* C618R prevented the hybridization of the probe binding site of the *JAK2* V617F ddPCR assay which had led to a false negative result by ddPCR. The validation procedure also explored coverage and limits of sensitivity, potential chemistry specific artefacts and identified common polymorphisms for all 25 genes.
Summary/Conclusion: The panel has replaced the current sequential analysis of *CALR, MPL* and *JAK2* exon 12 in *JAK2* V617F negative patients and reduced turn-around-times with increased accuracy and sensitivity compared to Sanger sequencing and fragment analysis. Our current clinical service operates on a two tier system whereby clinicians can request analysis of the full 25 gene panel or a 4 gene subset (*JAK2, CALR, MPL, CBL* as an *in silico* analysis).

PB2279

ADULT ONSET LANGERHANS CELL HISTIYOCYTOSIS: A SINGLE CENTER EXPERIENCE

E. Arslan Davulcu[1,*], Y. Ulusoy[1], H. Bülbül[1], N. Soyer[1], S. Kamer[2], F. Şahin[1], F. Vural[1], M. Tobu[1], N. Özsan[3], M. Hekimgil[3], M. Tombuloğlu[1], G. Saydam[1]

[1]Hematology, Ege University Medical Faculty Hematology Department, [2]Radiation Oncology, Ege University Medical Faculty Radiation Oncology Department, [3]Pathology, Ege University Medical Faculty Pathology Department, İzmir, Turkey

Background: Langerhans cell histiyocytosis (LCH) is a rare disease of histiocytes that is more prevalent in childhood. The etiology is unknown. Any organ or system may be affected in LCH. Bones, skin, and pituitary gland are frequently involved. Clinical presentation may be diverse according to the organ affected. Treatment modality depends on the extent of the disease. With mild symptoms and no risk organ involvement methotraxate, azathiopurin, thalidomide can be used or patient can be carefully observed without therapy. Symptomatic and mutisystemic LCH with or without risk organ involvement require chemotherapy.
Aims: Our aim is to evaluate clinical characteristics and outcome of adult onset LCH patients in our instutition.
Methods: We retrospectively evaluated adult patients diagnosed as LCH at Ege University Hospital between 2000-2017. All patients were screened by total bone X-ray survey and/or bone scintigraphy, abdominal and chest computerized tomography, cranial magnetic resonance imaging and some of the patients by positron emission tomography. Bone marrow aspiration and biopsy were performed in all patients in order to evaluate the possible bone marrow infiltration. Complete blood count, blood chemistry, erythrocyte sedimentation rate, coagulation studies, thyroid stimulating hormone and free T4, other pituitary gland hormones if pituitary gland is involved, and urine strip test were analyzed. Patients were stratified according to extent of the disease:Single system LCH (SS-LCH): One organ or system involved. It may be uni- or multifocal,Multisystem LCH (MS-LCH): Two or more organs/systems involved.Choice of treatment, dose and duration of drugs, radiotherapy fields and dosage, side effects of the treatment modalities were recorded. Outcome of the patients were evaluated.
Results: A total of 27 patients were diagnosed and treated in our hospital. Mean age at diagnosis was 38.3 years, 19 of them were male. There were 8 patients with MS-LCH, 13 with unifocal SS-LCH, and 6 with multifocal SS-LCH. Patients' characteristics were summarized in the tables 1, 2 and 3. All of our patients are alive. There was only one treatment toxicity, diabetes insipidus, in a patient with pons and mastoid lesion after radiotherapy. Most of MS-LCH and multifocal SS-LCH patients received systemic treatment, whereas unifocal SS-LCH.

Table 1. Patient characteristics with MS-LCH.

Patient No	Gender	Age	Involved organ/tissue	Chemotherapy	Radiotherapy	Treatment toxicity	Follow-up period (years)	Outcome
1	M	36	Lung, Skin	Cladribine (6 courses)	-	-	4	Alive
2	M	40	Thyroid, Gum	Chemotherapy for concomitant thyroidpapillary carcinoma	-	-	3	Alive
3	M	32	Skin, Gum	Cladribine (6 courses)	-	-	12	Alive
4	M	38	Bone (parietal), Lung	Cladribine (5 courses)	-	-	1	Alive
5	F	47	Bone (left mandible, left tibia), Pons,Lung	-	Pons-mastoid 10x2 gy,tibia 10x2 gy	Diabetes insipidus	8	Alive
6	F	47	Bone (left parietal, left femur), Skin	Cladribine (6 courses)	-	-	6	Alive
7	F	24	Bone (right femur), External ear (right)	-	10 GY for femur,10 gy for ear	-	6	Alive
8	M	24	Bone (Right frontal, right femur), Lung	Cladribine (6 courses)	5x190 cgy for frontal bone,1080 gy for femur	-	3	Alive

Table 2.

Summary/Conclusion: LCH is a rare and heterogeneous disease of adult patients. Systemic evaluation is crucial because treatment desicion depends on organ-tissue involvement. Treatment should be individualized and multi-disciplinary in order to reach good outcome.

PB2280

MORBIDITY AND MORTALITY OF PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS: A TERTIARY CENTER EXPERIENCE

E. Papadakis, V. Papadopoulos*, K. Kokoviadou, A. Banti, A. Kioumi

Hematology Department, Papageorgiou General Hospital, Thessaloniki, Greece

Background: The main causes of morbidity and mortality in patients with myeloproliferative neoplasms are thrombohemorrhagic events in polycycthemia vera (PV) and essential thrombocythemia (ET) and transformation to acute leukemia (most frequent in primary myelofibrosis – PMF) (Frederiksen *et al.* Blood 2011;118:6515). The introduction of tyrosine kinase inhibitors (TKIs) has changed the clinical course of chronic myeloid leukemia (CML) from a slowly progressive disease into a chronic one with near-normal life expectancy (Bower *et al.* JCO 2016;34:2851). Moreover the cardiovascular complications of TKIs have raised concerns about their effect on hemostasis (Moslehi *et al.* JCO 2015;33:4210).
Aims: The aim of the study is to assess the incidence of the main causes of morbidity and mortality collectively in patients with MPNs.
Methods: A retrospective study was conducted in patients with MPNs in a tertiary center. Mortality rates were calculated, as well as incidence rates for thrombotic and hemorrhagic events, transformation to acute leukemia and occurrence of solid tumors.

Results: A total of 224 patients, with median age at diagnosis 64.5 years (range 17-92) were included in the analysis. The initial diagnosis was CML (13%), ET (51%), PV (28%), PMF (5%) and myeloproliferative/myelodysplastic syndrom in 2%. Mean duration of follow-up was 8 years. In 20% of patients the presenting symptom was a thrombotic episode (arterial, venous or splenic infarct). During the follow-up, 83 events of interest were recorded. In the whole cohort, the incidence rate (in events per 100 patients per 10 years) of thrombosis was 13.42, hemorrhage 1.68, transformation to another hematologic malignancy 11.74, occurrence of solid tumor 5.59 and other events 13.98. Other events for which patients received medical attention included atypical chest pain, joint pain, arrythmias, pleural effusion and infections. Incidence of major events per diagnosis are presented in table 1. Cumulative incidence of thrombotic complications is illustrated in figure 1.

Table 1. Incidence rate (per 100 patient-years) and 95% confidence interval (CI) for the major events during follow up for different myeloproliferative neoplasms.

	CML	ET	PV	PMF
Thrombosis	2.39 (0.9-6.3)	1.27 (0.75-2.13)	1.29 (0.58-2.85)	0
Hemorrhage	0	0.27 (0.09-0.84)	0	0
Hematological malignancy	0.6 (0.08-4.2)	0.82 (0.43-1.56)	1.07 (0.45-2.57)	2.21 (0.32-15.37)
Solid tumor	0.6 (0.08-4.2)	0.36 (0.14-0.96)	1.07 (0.45-2.57)	0

All-cause mortality rates in deaths per 100 patient-years (95% CI) were for CML 1.8 (0.6-5.5), ET 0.9 (0.5-1.7), PV 1.9 (1-3.7), PMF 6.6 (2.2-19.8). Transformation to acute leukemia accounted for 46.7% of all deaths, solid tumors for 16.7% and other causes (cardiac death, sepsis) for 33.3% of deaths. All patients with atypical CML or other myeloproliferative/myelodysplastic syndrom died at a median of 16.4 months from diagnosis, while their disease had transformed to AML at a median of 14 months from initial diagnosis.

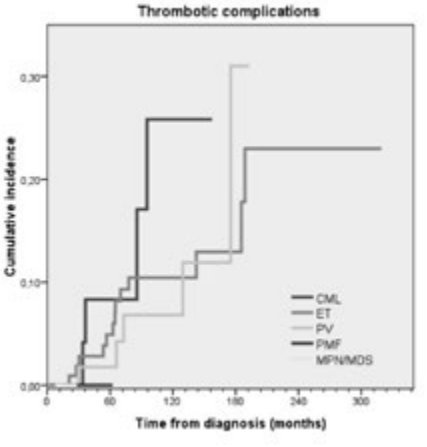

Figure 1.

Summary/Conclusion: Thrombotic complications account for the main burden of morbidity not only in patients with ET and PV, but also for CML patients on TKIs. Transformation to acute leukemia is the leading cause of death. It is crucial that future studies incorporate cardiovascular risk factors and estimate the effect of therapeutic interventions in both the thrombotic risk and the progression free survival.

PB2281

HETEROGENEITY AMONG DIFFERENT MYELOPROLIFERATIVE NEOPLASMS ASSOCIATED WITH SPLANCHNIC VEIN THROMBOSIS

D. Cattaneo[1,*], N. Orofino[1], C. Bucelli[1], V. Mattiello[1], P. Bianchi[1], U. Gianelli[2], A. Cortelezzi[1], A. Iurlo[3]

[1]Hematology, IRCCS Ca' Granda - Maggiore Policlinico Hospital Foundation, [2]Pathophysiology and Transplantation, University of Milan, and IRCCS Ca' Granda Maggiore Policlinico Hospital Foundation, [3]Hematology, IRCCS Ca' Granda Maggiore Policlinico Hospital Foundation, Milano, Italy

Background: In *BCR-ABL1*-negative myeloproliferative neoplasms (MPNs) incidence of major thrombotic events ranges from 1.75 to 5.5% events patient-years according to the specific MPN subtype. Venous thrombosis account for about 30-40% of these complications and can occur also at unusual sites, including the splanchnic circulation (SVT) with a prevalence ranging between 1 and 23%.
Aims: To evaluate differences in MPNs associated with SVT.
Methods: We reported a consecutive monocentric series of 38 patients with a diagnosis of *BCR-ABL1*-negative MPN, who developed a SVT at diagnosis or during the follow-up between 1979 and 2016.
Results: We identified 18.4% of PV, 26.3% of ET, 34.2% of MF and 21.1% of MPN-U. The latter were all diagnosed at the time of SVT onset, and characterized most frequently by a PV- or PMF-like bone marrow morphology but lacked the clinical phenotype required for a complete diagnosis. The majority of the cases (81.5%) bear *JAK2*V617F mutation; however, five patients were characterized by other molecular markers, *i.e. MPL* mutations in three patients, and *CALR* type 1 mutation in the remaining two cases. Among patients with a previous diagnosis of MPN who developed SVT during the follow-up, a cytoreductive treatment was already on-going in 53.8% of the cases, whereas it was then started in all but four of the remaining cases, due to young age and a blood cell count in the normal range or even below. After thrombotic index event, anticoagulants were started in 29 patients (76.3%), including six cases (15.8%) with direct oral anticoagulants (DOACs). At a median follow-up from MPN diagnosis of 12.3 years, six deaths were recorded: it was due to leukemic transformation in four patients, intracranial bleeding and infectious complication in one patient each. According to the literature, 44.7% of the patients in our series suffered from recurrent vascular events, either involving the arterial (21.1%), or the venous district (23.7%): in particular, five patients experienced a recurrent SVT.
Summary/Conclusion: In this report patients with a diagnosis of MPN-U seem to represent a distinct clinical entity when compared to the other MPN subtypes. In particular, in all MPN-U cases, SVT was the initial manifestation which led to the diagnosis of the underlying MPN. They were all characterized by the presence of *JAK2*V617F mutation, except one case which bear an *MPL*W515L mutation, and showed a normal karyotype. In addition, any of these patients neither developed clinical features which could enable physicians to re-classify them among one of the so-called classical MPN subtype even according to the WHO 2017 classification, nor experienced a leukemic evolution. Being aware of the limits of the present study, we can speculate that SVT associated with MPN-U represents a disease with a more indolent course as compared with other cases associated with full-diagnosed MPNs which more frequently developed during the follow-up. Notably, all the cases of leukemic transformation were reported among patients with a previous MF diagnosis after a median follow-up of 17.9 years. Furthermore, about half of our patients developed recurrent vascular events, confirming the limited efficacy of conventional therapeutic approach in these particular patients. However, it is interesting to underline that none of the six patients treated with DOACs developed another thrombotic complication, so probably representing a more valid strategy in this setting.

PB2282

THE EPIDEMIOLOGY AND PRESENTING CLINICAL CHARACTERISTICS OF MYELOPROLIFERATIVE NEOPLASMS IN MALAYSIA

Y.Y. Yap[1,*], J. Sathar[1], K.B. Law[2], S.C. Edmund[2]

[1]Department of Haematology in Ampang Hospital, [2]Clinical Research Centre, Ministry of Health, Ampang, Malaysia

Background: The evolution of molecular studies in myeloproliferative neoplasms (MPN) has enlightened us the understanding of this complex disease consisting of polycythaemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). The epidemiology is well described in the western world but not in Asian countries like Malaysia.
Aims: To research the epidemiology of MPN in Malaysia in correlation with the clinical parameters and molecular studies.
Methods: This national registry of MPN was conducted from year 2009 to 2015 in Malaysia with description of clinical demographic in correlation to JAK2 V617F mutation, thrombosis, haemorrhagic complications and blood counts.
Results: A total of 1010 patients were registered over a period of 5 years. The mean age was 54 years with male predominance. The ethnic distribution revealed that Chinese had a relatively high weighted incidence proportion (43.2%), followed by Indian (23.8%), Malay (15.8%) and other ethnic groups (17.2%). The types of MPN reported were 40.4% of ET (n=408), 38.1% of PV (n=385), 9.2% of PMF (n=93), 3.1% of hypereosinophilic syndrome (HES) (n=31) and 7.9% of unclassifiable MPN (MPN-U) (n=80). Splenomegaly was only palpable clinically in 32.2% of patients. The positive JAK2 V617F mutation was present in 644 patients with 46.6% in PV, 36.0% in ET, 9.0% in PMF, and 7.4% in MPN-U, and had significantly

lower haemoglobin (p<0.001), haematocrit (p<0.001) and white blood cells (WBC) (p<0.001) than those with negative mutation. Significant differences in platelet and WBC count were detected in ethnic groups and MPN subtypes. There were more arterial thrombosis events seen in those with JAK2 V617F mutation as compared to venous thrombosis events (23.1% *vs* 4.4%). The bleeding rate was only 6.6%. Among the risk factors, previous thrombosis, old age (≥60 years) and hypertension were significantly correlated to positive JAK2 V617F mutation. The arterial thrombosis event is associated with higher presenting HB, HCT and PLT while the bleeding event is associated with lower presenting HB, HCT but higher PLT. The presence of JAK2 V617F mutation is associated with higher risk of arterial thrombosis.

Summary/Conclusion: Chinese ethnicity is associated with higher rates of MPN. The previous history of thrombosis, old age (≥60 years) and hypertension are risk factors that can be corelated to JAK2 V617F mutation in MPN patients. This study is instrumental for policy makers and funding agencies to ensure preventive strategies can be implemented in future.

PB2283

THE EVALUATION OF OXIDATIVE STRESS IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND VASCULAR EVENTS

M.-A. Gaman[1,*], C. Moisa[2], A.M. Gaman[3,4]

[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Bucharest, [2]Hematology, Emergency County Hospital Slatina, Slatina, [3]Pathophysiology, University of Medicine and Pharmacy of Craiova, [4]Hematology, Filantropia City Hospital of Craiova, Craiova, Romania

Background: Essential thrombocythemia (ET) is characterized by stem cell-derived clonal myeloproliferation with mutually exclusive "driver" mutations (JAK2, CALR, and MPL). Patients with ET have a high risk of thrombotic and hemorrhagic complications, and also a high risk of leukemic transformation. Increased levels of oxidative stress have been reported in patients with hematological disorders, including essential thrombocythemia. We previously reported that JAK2V617F-positive cases registered higher values of reactive oxygen species than healthy controls. Higher levels of oxidative stress markers have also been found in patients with essential thrombocythemia that also associated vascular events.[1-9]

Aims: To evaluate the antioxidant capacity in patients with ET and to observe if JAK2V617F-positive cases associate a lower antioxidant capacity and a higher risk for vascular events.

Methods: We evaluated 27 Romanian patients diagnosed with ET according to the 2016 revised WHO criteria. Informed consent was obtained from all patients enrolled. JAK2V617F mutation was detected by allele specific PCR testing. The antioxidant capacity was measured using a multidetection microplate reader FLUOstar Omega and a Sigma-Aldrich detection assay kit. Results were compared both to healthy controls and to each other. We compared the parameters of patients with ET and vascular events *versus* patients with ET and no vascular events. The exclusion criteria of this study were the presence of any condition associated with an increased oxidative stress status or the use of exogenous antioxidants. Statistical data analysis was performed using the student T-test (p-value ≤0.05 considered statistically significant).

Results: The study group involved 15 females and 12 males (median age=54 years). Eight patients had vascular events: six patients had arterial or venous thrombosis (one of them associated thrombophilia) and two patients had hemorrhage. Fourteen patients were JAK2V617F-positive. All patients with vascular events were JAK2V617F-positive. All patients with ET had a lower antioxidant capacity compared to healthy controls (p≤0.05). The antioxidant capacity was significantly decreased in patients with ET and vascular events compared to patients with ET and no vascular events (p≤0.05). The patients with arterial thrombosis associated the presence of JAK2V617F, older age at diagnosis, leukocytosis, higher hemoglobin levels, and a lower antioxidant capacity (p≤0.05).

Summary/Conclusion: In our study group, ET patients had a lower antioxidant capacity than healthy controls. The history of vascular in ET patients was associated with a lower antioxidant capacity than ET patients with no previous vascular events or healthy controls. We may hypothesize that, via a decreased antioxidant capacity, oxidative stress is related to vascular events in ET in addition to the JAK2V617F status.

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PB2284

IDENTIFICATION OF NEW CALR AND ASXL1 MUTATIONS IN PATIENTS WITH MYELOFIBROSIS BY TARGETED NGS PANEL

T. Subbotina[1,2,*], P. Nicolaeva[1], M. Mikhalev[3,4], M. Smelyanskaya[5], N. Oreshkova[1,6], R. Shaikhutdinova[1], E. Dunaeva[7], K. Mironov[7], G. Shipulin[7], V. Khorzhevskyi[4,8], A. Petkevich[9], V. Govorovskiy[10], V. Lutynskiy[10]

[1]Siberian Federal University, [2]The Federal State-Financed Institution Federal Siberian Research Clinical Centre under the Federal Medical Biological Agency, [3]Municipal Budget Health Service Institution «City Clinical Hospital № 7», [4]Krasnoyarsk branch of the Federal State budgetary Institution «National Research Center for Hematology» Department of Health, [5]Krasnoyarsk regional hospital, [6]Sukachev Institute of Forest, Siberian Branch of Russian Academy of Sciences, Krasnoyarsk, [7]Federal Budget Institute of Science «Central Research Institute for Epidemiology», Moscow, [8]Krasnoyarsk State Regional Bureau of Pathology, Krasnoyarsk, [9]N. N. Blokhin Russian Cancer Research Center, [10]Algimed Ltd, Moscow, Russian Federation

Background: There are well-known somatic driver mutations in genes JAK2, MPL, CALR associated with Ph-negative myeloproliferative neoplasms (MPN). Also somatic mutations in other genes (ASXL1, TET2, etc) are involved in the formation of the disease phenotype. Probably, there are some new mutations associated with MPN.

Aims: To carry out an advanced search for mutations in JAK2, MPL, CALR and ASXL1 using a next-generation sequencing–based method, with a MYELOID TUMOR SOLUTIONTM BY SOPHiA GENETICS panel for the patients with primary myelofibrosis (PMF).

Methods: 4 patients with PMF were included in this study, their written informed consent for scientific evaluations were obtained. The diagnosis of all patients was confirmed by bone marrow trephine biopsies histological examination. Amplicon libraries were prepared by Myeloid Solution sequencing panel (SOPHiA GENETICS, Switzeland) and paired-end sequencing runs were performed on a MiSeq (Illumina, USA). MiSeq Reagent Kit v3 (600-cycle, Illumina) was used for direct sequencing. Dry bench was performed by SOPHiA DDM. In order to confirm the variants in ROI Sanger or pyrosequencing were performed.

Table 1.

Table 1. Characteristics of 4 patients with PMF

№	Age of manifestation (years)	Sex	Disease	HU	Duration of the disease (mo)	Driver MPN mutations (allele burden, %)	ASXL1 mutations (allele burden, %)
1	63	M	PMF	Yes	24	MPL c.1544G>T; p.W515L (75%)	New Mutation: c. 2445_2449 del GTCTC; p.L815fs*5 (24%)
2	44	F	PMF	Yes	168	New CALR Mutations: 52nt 1128_1129insCTTTGCCTC; p.Lys377 Leu fs*5 (24%) 2) c. 1031_1133 del AGA; p. Glu 341 del (24%)	c. 1934_1935 ins G; p. G646 fs*12 (35%)
3	43	F	PMF	Yes	107	JAK2 (V617F) From 40% (2014) to 80% (2017)	New Mutation: c. 1772_1773 ins AA; p. Y591 fs*1 (31%)
4	56	M	PMF→ CMML	Yes	36	JAK2 (V617F) before the burden was up to 60%, now the mutation is not detected	c. 1934_1935 ins G; p. G646 fs*12 (46%)

Results: Characteristics of 4 patients with PMF are reported in Table 1. Patient 1 carries a known driver somatic mutation of MPL and new (here and below: not yet included in the COSMIC website) mutation in ASXL1. As a result of this mutation, there is a 5-bp deletion and a 2-bp frameshift that results in a mutant protein with a novel short C-terminus. Two new monoallelic driver mutations in oncogenic region of CALR gene were found in patient 2. The new mutant CALR fragment contains all the mutant amino acid sequence of the type I mutant L367fs*46 and nine altered and mainly positively charged amino acids LCLRRRRQR, therefore this new sequence has at least the same importance for oncogenicity as the type I CALR mutation. Also this patient carries ASXL1 mutation. Patient 2 suffers from PMF during 14 years without severe complications, which possibly means that mutation in ASXL1 simultaneously with new identified CALR mutation does not influence on survival rates. Patient 3 has V617F mutation and new mutation in ASXL1. There is a 2-bp insertion and a 2-bp frameshift that results in a mutant protein with a novel short C-terminus. The patient 4 had V617F mutation before October 2017. Currently, this mutation is not

detected, which is probably due to the replacement of the V617F clone by another pathological clone. There are also somatic and germline mutations in genes KIT, SETBP1, TET, CBL, EZH2, SRSF2, which may be associated with chronic and acute leucosis (Greenman, 2007; Makishima, 2013; Hirsch, 2016) and ASXL1 mutation. Since August 2017 there is a worsening of the patient`s state and in the same time decreasing of allele burden of V617F mutation following complete extinction. There is a pathomorphologic data for transformaition of PMF into chronic meylomonocytic leucosis (CMML). Mentioned transformation is possibly intermediate step between PMF and acute leucosis partly due to epigenetic status caused by ASXL1 mutation.
Summary/Conclusion: Newly identified mutations in CALR and ASXL1 with high probability have diagnostic and prognostic meaning for patients with MPN.

PB2285

THE MPN-10 SCORE AND DRIVER MUTATIONS IN THAI MYELOPROLIFERATIVE NEOPLASMS PATIENTS

N. Cherdchoo*, S. Kongkiatkamon, C. Polprasert, P. Rojnuckarin

Department of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Bangkok, Thailand

Background: Recent advances in molecular diagnostics found that the majority of classic myeloproliferative neoplasms (MPNs) harbor characteristic driver mutations. The JAK2 protein transmits signals of various cytokine receptors, while CALR and MPL are involved only in the thrombopoietin receptor signaling. This may contribute to different cytokines-related symptoms that impact quality of life. The MPN Symptom Assessment Form total symptom score (MPN-SAF TSS) or MPN-10 score is a useful tool to assess symptom burden. However, the score has not been applied to clinical practice in Thailand.
Aims: To determine the MPN-10 scores and correlate them with clinical characteristics, genetic mutations and outcomes of MPNs patients.
Methods: The baseline characteristics, MPN-10 score and outcomes of MPNs patients diagnosed according to the WHO criteria at King Chulalongkorn Memorial Hospital, Thailand between 2014 and 2017 were reviewed.

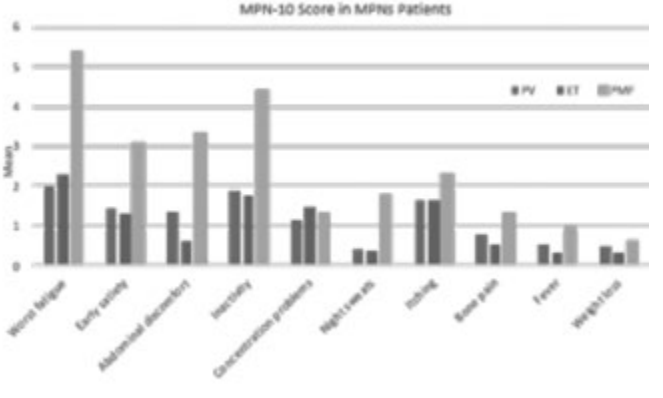

Figure 1.

Results: There were 146 patients enrolled. Forty-seven patients were diagnosed as polycythemia vera (PV), 72 as essential thrombocythemia (ET), and 27 as primary myelofibrosis (PMF). One hundred and thirty-two patients (90%) had driver mutations. PV patients harbored *JAK2V617F* mutation in 46 (97.8%) and *JAK2exon12* mutation in 1 (2.1%). ET patients had *JAK2V617F* mutation in 41 (56.9%), *CALR* mutation in 17 (23.6%), *MPL* mutation in 1 (1.3%) and triple negative mutation in 11 (15.2%), while PMF harbored *JAK2V617F* mutation in 20 (74.0%), *CALR* mutation in 4 (14.8%) and triple negative mutation in 3 (11.1%). Interestingly, the mean MPN-10 score of Thai PV and ET patients were significantly lower than those of Western population 11.7±11.4 *vs* 21.8±16.3, p=0.002, and 10.7±9.7 *vs* 18.7±15.3, p=0.0014 for PV and ET respectively. The mean MPN-10 score of Thai patients *vs* Western MF patients was 24.4±12.5 *vs* 25.3±17.2, respectively, p=0.862. In ET, the MPN-10 scores were not different between cases with *JAK2* vs. *CALR* mutations. In addition, a MPN-10 score is not associated with survival (p=0.643).
Summary/Conclusion: PMF had a higher symptom burden than PV and ET. MPN-10 scores in Thai PV and ET were significantly lower than those of Western patients. MPN-10 scores were not associated with driver mutations and could not predict survival in Thai MPNs patients.

PB2286

RUXOLITINIB IN MYELOFIBROSIS: THE NORTHERN IRELAND EXPERIENCE

J. Russell[1,*], A. Daly[2], C. Arnold[2], M. Bowers[3], K. Boyd[4], R. Cuthbert[2], M. El-Agnaf[3], P. Elder[5], H. Eswedi[4], D. Finnegan[2], J. Hamilton[3], D. Hull[4], S. McCloskey[6], C. McConville[4], F. McNicholl[5], Y.-L. Ong[3], P. Windrum[6], M.F. McMullin[2]

[1]Cambridge University Hospitals NHS Foundation Trust, Cambridge, [2]Belfast City Hospital, Belfast, [3]Ulster Hospital, Dundonald, [4]Craigavon Area Hospital, Portadown, [5]Altnagelvin Area Hospital, Derry, [6]Antrim Area Hospital, Antrim, United Kingdom

Background: Ruxolitinib (RUX), the oral Janus kinase (JAK)1/2 inhibitor, is approved for treating disease-related splenomegaly or symptoms in adults with primary myelofibrosis (PMF), post-essential thrombocythaemia (PET)-MF, or post-polycythaemia vera (PPV)-MF. In March 2016, RUX was commissioned in Northern Ireland (NI) for higher-risk MF patients (IPSS 2+) and had only been available through clinical trials or special funding requests before then.
Aims: To assess the clinical outcomes of MF patients treated in NI and to compare the efficacy and safety of RUX with best alternative therapy (BAT) in the 'real-world' setting.
Methods: We performed a multicentre retrospective analysis of MF patients referred to a large tertiary cancer centre, from 1996 to 2017. Clinical data were obtained from electronic care records, laboratory and pharmacy databases. Survival analysis was estimated using the Kaplan-Meier method and standard log-rank test.
Results: We identified 47 patients, 46 of which were included in the whole cohort analysis (1 was excluded due to insufficient data). The median age at diagnosis was 65 years (35–83) and 63% were male. There were 24 (52%) PMF, 9 (20%) PET-MF, 11 (24%) PPV- MF, 2 (4%) post-myeloproliferative neoplasm-unclassified (PMPN)-MF patients, comprising 35% high-risk, 28% intermediate-2 (int- 2), 35% intermediate-1 (int-1) and 2% low risk patients. A driver mutation was detected in 89% (78% JAK 2+; 11% CALR+). The whole cohort median survival was 8.5 years (0.5–21.5). Of the 46 patients, 28 (61%) received RUX. Five were excluded from further analysis (2 had received another JAK inhibitor; 3 had stem cell transplantation). Of the remaining 41, 24 (59%) received RUX; 10 (24%) as first-line therapy. Baseline demographics were similar between patients treated with RUX or BAT. The RUX group comprised 29% high-, 38% int-2 and 33% int-1 risk patients, and had a significant 5-year overall survival (OS) advantage from time of diagnosis *vs* BAT; 77% (95% CI 53–90) *vs* 57% (95% CI 27–79); p=0.044. Median survival in the BAT group was 6.3 years for int-1, 1.4 years for int-2, and 2.1 years for high-risk patients. Median survival in the RUX group was 1.8 years for high-risk and was not reached for int-1 and int-2 risk patients. There was no survival difference between int-1 and int-2 risk RUX patients; however, int-1 risk RUX patients had a notable survival advantage *vs* int-1 risk BAT patients; 5y OS 100% *vs* 86% (95% CI 33–98); p=0.045. In the RUX group, 1y OS was 82% (58–93) with median follow-up of 14 months (1-50). The estimated discontinuation rate was 31% (95% CI 16–55) at 1 year. At the time of analysis, 10 patients had stopped RUX; 5 died, 4 progressed/no response, and 1 due to adverse effects (AE). Two (8%) transformed to acute myeloid leukaemia. The most common grade 3/4 AEs were thrombocytopaenia (25%) and anaemia (54%), with 33% requiring dose reduction or interruption. The most common non-haematological AEs were infection (42%). Data collection of spleen response and symptom control is underway but incomplete.
Summary/Conclusion: In summary, our whole cohort data demonstrate similar outcomes for MF patients treated in NI compared with contemporary clinical trials. Notably, there was a significant survival advantage for patients treated with RUX *vs* BAT in all risk groups, including intermediate-1 risk patients who at present do not meet funding criteria to receive RUX. This data must be interpreted with caution due to the limited follow-up but it suggests a potentially unmet clinical need in lower-risk MF patients that requires further study.

PB2287

THE RELEVANCE, EFFICACY AND SAFETY OF ERYTHROPOIETIC STIMULATING AGENTS (ESAS) IN ANEMIA CONTROL: A RETRO SPECTIVE ANALYSIS IN MYELOFIBROSIS PATIENTS IN A SINGLE HOSPITAL

H.N. Fernandez-Leyva[1,*], J. Arnold[1], N. Sargant[2], N. Rayman[2], R. Ashok[2]

[1]Haematology, Basingstoke and North Hampshire Hospital, winchester, [2]Haematology, Basingstoke and North Hampshire Hospital, Basingstoke, United Kingdom

Background: Primary myelofibrosis (PMF) is characterized by the dysregulated proliferation of myeloid cells including megakaryocytes and myeloid and erythroid progenitors in the bone marrow (BM), resulting in ineffective erythropoiesis and the reactive deposition of fibrous connective tissue (reticulin/collagen) often with osteosclerosis. The related myeloproliferative neoplasms essential thrombocythemia (ET) and polycythemia vera (PV) can both undergo delayed disease transformation into a fibrotic state: post-ET myelofibrosis (post-ET MF) or post- PV MF, respectively. Individuals affected by MF face unique challenges, even compared to other MPN patients.
Aims: The main aim of this study is to evaluate clinical features and hematological parameters, of patients with PMF on ESAs. The secondary aim is to assess response rates and duration of response in terms of IWG-MRT.
Methods: This is a retrospective analysis of clinical and laboratory features, ESAs treatment modalities, and outcomes of PMF patients evaluated between July 2015 until December 2017. Continuous and categorical variables were tested as SPSS Statistics analysis: We conducted a review of the medical data of the patient on ESAs Respondents were recruited via pharmacy software data. In total, **11** patients with primary MF or MF post-thrombocythemia / polycythemia vera (post-ET/PV: n=**6**) received ESAs in monotherapy for anemia (Hb <10g/dL). The concomitant use of cytoreduces treatment was allowed. According to criteria of the IWG-MRT, a favorable response is considered the cessation of the transfusion requirements in patients with transfusional dependence or the increase in Hb>2g/dL in those who were not transfused, maintained for at least 12 weeks.
Results: Patients had a diagnosed of PMF approximately 2.5 years ago. Most (59.1%) were male and predominant Caucasian population (97.6%). A total of 11 patients (64.7%) obtained clinical improvement of the anemia, after a median of 3.8 months of treatment. In the univariate analysis, the baseline factors associated with a higher probability of response were: female gender, MF post-ET/PV, leukocytes >10x10^9/L, EPO levels <125U/L, serum ferritin levels <200ng/mL, grade 1-2 marrow fibrosis and absence of cytogenetic alterations or previous transfusions. Based on the binary logistic regression analysis, the clinical factors with independent prognostic significance associated with the response were the patient's gender, the number of leukocytes and the ferritin levels. The median duration of the response was 18.2 months (IQR]: 9.5-21.4). After a median follow-up from the start of the 24-month treatment, 5 patients (29.4%) had discontinued ESAs.
Summary/Conclusion: The results of the study demonstrated that ESAs significantly improved the anaemia in about half of patients with MF and induced a durable response rates [risk ratio (RR): 2.77, 95% confidence interval (CI): 2.01–3.86, P=0.03; RR: 7.52, 95% CI: 3.81-6.2, P=0.05; respectively]. Some clinical factors can help predict the likelihood of response to treatment. An early initiation of ESAs could offer better results. The potential benefit is more significant in transfusion independent patient with baseline Haemoglobin levels of 90 g/L. A specific educational program needs to develop specifically for this population. The results of the research promote a development of comprehensive local guidelines.

PB2288

EXPRESSION OF CALRETICULIN AND CD47 IN MYELOPROLIFERATIVE NEOPLASMS

K. Boasman*, M.J. Simmonds, C.R. Rinaldi

Life Sciences, University of Lincoln, Lincoln, United Kingdom

Background: Myeloproliferative neoplasms (MPN) are chronic myeloid cancers that are characterized by the overproduction of mature blood cells, and that may evolve into acute myeloid leukaemia. In solid tumours, calreticulin (CALR) overexpression produces a pro-phagocytic signal and is counteracted by concomitant expression of anti- phagocytic CD47, reflecting an apoptosis *vs* survival mechanism.
Aims: To investigate the expression and cellular location of CALR and CD47 in patients with MPN in comparison with healthy controls.
Methods: Mononuclear cells were obtained from peripheral blood of 6 MPN patient samples (2 Polycythaemia Vera, 1 Myelofibrosis, 3 Essential Thrombocythemia), along with 3 healthy controls by FICOLL separation.Cells were fractionised into 4 compartments: Membrane, cytoplasm, cytosol and nucleus. Proteins were extracted using TRIzol extraction andCALR and CD47 protein expression was analysed by western blotting.
Results: Overall CALR expression was unchanged in MPN samples comparing with controls (3.23 *vs* 2.95 fold, respectively) (Fold changes are seen through normalization against the housekeeping protein). In contrast, CD47 significantly increased in MPN samples *vs* controls (3.66 *vs* 0.06 fold, respectively). CALR and CD47 showed similar patterns of cellular localization in controls: membrane (51% and 58.7%, respectively), cytosol (38.4% and 36.2%, respectively), cytoplasm (9.6% and 4.5%, respectively), and nucleus (1% and 0.7%, respectively). In MPN samples CALR and CD47 moved into the membrane (CALR=77.2%; CD47=66.6%) and cytoplasm (CALR=12.5%; CD47=14.8%), reducing in cytosol (CALR=5.4%; CD47=16.4%) and no changes in nuclear expression (CALR=1.3%; CD47=2.2%) were observed.
Summary/Conclusion: CD47, but not CALR, is overexpressed in patients with MPN comparing with controls. This opposes previous studies in solid tumours, which show significant increases of both CALR and CD47, suggesting a role for CD47 as a strong anti-phagocytic signal responsible for immune survival in MPN. We have also shown that in contrast to cells from healthy controls, MPN cells mainly express CALR and CD47 on the cell surface with possibly a slightly higher relative CALR expression. No differences in CALR and CD47 expression was noted in different MPN subtypes however a larger cohort of patients, treated and untreated, is required to confirm our findings and identify CALR/CD47 pattern changes in response to therapies.

PB2289

FUNCTIONAL IRON DEFICIENCY AND INFLAMMATION IS COMMON IN MYELOFIBROSIS AND CONTRIBUTES TO ANAEMIA AND IMPAIRMENT OF QUALITY OF LIFE

G. Birgegård[1,*], E. Ahlstrand[2], E. Ejerblad[3], W. Ghanima[4], H. Hasselbalch[5], C. Nielsen[6], H. Knutsen[7], J. Samuelsson[8], A. Lindholm Sörensen[6,9], B. Andreasson[10]

[1]Inst for Medical Sciences, Uppsala University, Uppsala, [2]Department of Haematology, Örebro University Hospital, örebro, [3]Dept of Medical Sciences,, Uppsala University, Uppsala, Sweden, [4]Department of Research, Østfold hospital,, Østfold, Norway, [5]Department of Haematology, Zealand University Hospital, Roskilde, Roskilde, [6]Institute for Inflammation Research, Center for Rheumatology and Spine Diseases, Copenhagen, Denmark, [7]Department of Haematology, Oslo University, Oslo, Norway, [8]Department of Haematology, University Hospital, Linköping, Sweden, [9]Department of Haematology, Zealand University Hospital, Roskilde, Denmark, [10]Department of Haematology, Specialistmedicin, Uddevalla Sjukhus, Uddevalla, Sweden

Background: Anaemia is a major problem in myelofibrosis (MF). The cause of anaemia is not completely understood. Recent studies have shown inflammatory activity in myelofibrosis. Inflammation influences iron metabolism. Cytokines upregulate hepcidin, causing a retention of iron in macrophages. This leads to sequestration of iron in the macrophages, a lowered transferrin saturation and reduced availability of iron for erythropoiesis. This is named functional iron deficiency (FID) and in malignancy is characterized by transferrin saturation (TSAT) <20% and a normal or elevated S-ferritin. FID is common in malignancies and is treatable with intravenous iron.
Aims: We aimed to investigate if FID and inflammation are common in MF and whether they may contribute to anaemia and influence Quality of life (QoL).
Methods: We recruited 80 patients with MF, 22 with ET (JAK2V617 was found in 60 and 61% in MF and ET, respectively) from 6 centers in the Nordic area. Blood values, iron variables and inflammatory markers were analyzed. Quality of life was investigated with MPN-SAF.
Results: MF 53 of the 80 MF patients were anaemic (Hb <120g/L in women, <130g/L in men). 14 of these were transfusion dependent with haemosiderosis (S-ferritin >500mg/L). These were excluded from the analysis of FID. Among the rest of the anaemic patients (n=39) FID (TSAT <20%, S-ferritin <500ug/L) was found in 35% *vs* 23.8% of non-anaemic (NS). In MF patients with FID, 70.6% were anaemic, *vs* 29.4% in patients with TSAT >20 (p=0.3). Among non-anaemic MF patients, 23.8% had a TSAT <20%, *vs* 35.3% of the anaemic patients. Hepcidin was significantly higher in patients with anaemia, 50.6 *vs* 24,4mg/L (p=0,01). 51% of women with MF had increased hepcidin levels *vs* 9% in men. There was a significant negative correlation between Hb and inflammatory markers in MF patients: TNFα, IL-6 and CRP(p<0.01 to 0.02), also LD (p=0.004) and Hepcidin (p=0.03). TSAT correlated with CRP (p<0.001). In all MF patients, hepcidin was higher in patients with S-ferritin >500mg/L (p<0.001), as was IL-6 (p=0.02), IL-2 (p=0.03), and CRP(p=0.001). For the whole group (MF+ET) there was a correlation between TSAT and QoL scores (Brief fatigue inventory (BFI) p=0.03, Total symptom score (TSS) p=0.002) as well as between inflammatory markers IL-6 and CRP and QoL scores(p=0.002-0.02 for BFI, overall QoL and TSS). Cytokine levels (IL-2, IL-6, TNFα and CRP) correlated positively with hepcidin (p values <0.001 to 0.01) in the whole group (MF+ET). **ET** In ET patients, TSAT was below 20% in 4 patients, but 3/4 had S-ferritin in the low range, indicating iron deficiency. The one ET patient with anaemia had a true iron deficiency with low S-ferritin. Only one ET patient with low TSAT could be classified as FID.
Summary/Conclusion: FID is common in MF but not in ET and patients with FID are more likely to be anaemic. MF is an inflammatory state and Hb correlated negatively with cytokine and hepcidin levels. Cytokine levels

correlated positively with hepcidin. The inflammatory activity and TSAT correlated with QoL. Our results indicate that the inflammatory state of MF disturbs iron turnover and contributes to anaemia development and impairment of QoL. FID should be sought for in anaemic MF patients. It is possible that MF patients with FID like other cancer patients may respond to IV iron.

PB2290

ESSENTIAL THROMBOCYTHEMIA AND SECONDARY THROMBOCYTOSIS IN CHILDREN OF BELARUS

T. Kozarezova*, N. Klimkovich

Department of pediatric oncology and hematology, Byelorussian Medical Academy of Post-Graduate Education, Minsk, Belarus

Background: Essential thrombocythemia (ET) is a disorder of adult-aged patients. The incidence in adults is 2/100 000 per year, but is approximately 1/10 000 000 per year in children. The low incidence recorded in children suggests that the biology of the disease could be different compared with the adult forms. Furthermore, ET can present in pediatric age as sporadic or familial diseases.
Aims: Analysis of etiology and clinical course of primary and secondary thrombocytosis in children.
Methods: Retrospective analysis was performed in 127 children (68 boys and 59 girls at the age of 5-10 years) with a diagnosis of thrombocytosis, observed between 2013 and 2017 at the Belarusian Research Center for pediatric oncology, hematology and immunology. The study was participated by all patients who were found thrombocytosis over 500x10^9/L (platelet count 680-1200x10^9/L).
Results: Among all patients, 122 children (96%) had secondary thrombocytosis (median platelet count 774 (680...940) x10^9/L), which is a result of excessive megacariopoyesis and thrombopoiesis in the course of various disorders, both hematologic and non-hematologic. The most common causes of secondary thrombocytosis were infections: viral (cytomegalovirus, parvovirus B19, herpesvirus type VI) and bacterial (Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Haemophilus influenzae, Streptococcus pyogenes, Streptococcus pneumoniae, Pseudomonas aeruginosa), bleeding (Crohn's disease, gastric and intestinal ulcers) and iron deficiency anemia. In all cases of secondary thrombocytosis, the platelet number normalized after curing the underlying diseases during the 2-14 month period. Other 5 patients (4%) after elimination of the relationship of thrombocytosis with taken medication and after elimination of the foci of infection in case of suspicion of primary thrombocytosis (median platelet count 1027 (925...1200) x10^9/L), performed myelogram, cytogenetic and molecular examination of bone marrow. Cytological study of the bone marrow: hypercellular bone marrow, increased proliferation of megakaryocytes (MK), with an increase in the number of large mature cells. Cells of the erythroid and myeloid lines are within normal limits. There is no shift towards unripe forms. Histological examination of the bone marrow showed an increase in the number of MK, large MK with excessive cytoplasm and a hyperbular nucleus. Molecular examination of bone marrow found a JAK2 V617F mutation in all 5 children with ET. Other types of mutations, characteristic of myeloproliferative diseases, are not identified. All patients with ET had not familial MPD. Because criteria for the treatment of ET are not specific for children, therapeutic choices are highly heterogeneous. Specific treatment included interferon alfa and hydroxyurea, and low-dose aspirin. In all the analyzed cases of ET in children, no clinical symptoms of disorders of hemostasis were ound. No patient experienced thromboembolic complications associated with thrombocytosis.
Summary/Conclusion: Myeloproliferative marker a JAK2 V617F mutation, and clonal hemopoiesis assay, are useful diagnostic tests in children with sporadic forms ET. Larger samples are obviously needed and of biologic markers to conclusions for diagnosis and treatment of ET in children.

PB2291

LONG TERM CLINICAL OUTCOMES OF MPN PATIENTS WITH JAK2, CALR AND MPL MUTATIONS IN BOSNIA AND HERZEGOVINA: 17 YEARS FOLLOW-UP

E. Islamagic[1,*], H. Komic[1], I. Eminovic[1], S. Kurtovic[2], A. Hasic[1], L. Mehinovic[1], Z. Halilovic[2], E. Suljovic Hadzimesic[2], A. Kurtovic-Kozaric[2]

[1]Faculty of Science, University of Sarajevo, [2]Clinical Center of the University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Background: Philadelphia-negative classical myeloproliferative neoplasms (MPNs) are a heterogenous group of disorders characterized by cellular proliferation of one or more hematopoietic cell lines. In 2016, after revision of the *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues,* the detection of somatic mutations in three driver genes, *JAK2*, *CALR* and *MPL* was introduced as the major diagnostic criteria for MPN patients. Molecular profiling in combination with morphological and hematologic abnormalities will allow accurate evaluation of MPN subtypes and monitoring of response to the treatment.
Aims: In this study we analyzed clinical outcomes of MPN patients with detected *JAK2, CALR* and *MPL* mutations from Bosnia and Herzegovina as well as the correlation between clinical parameters, presence of the mutation and disease severity.
Methods: MPN patients (n=90) who were diagnosed in the period from January 2000 to September 2017 and treated at the Clinical Center of the University of Sarajevo Bosnia and Herzegovina, were included in this study. Patients were categorized according to four different MPN subtypes: polycythemia vera (PV, n=29), essential thrombocythemia (ET, n=40), primary myelofibrosis (PMF, n=3) and MPN-unclassifiable (n=18). Standard patients' variables were collected including full blood count, bone marrow characteristics, hepato/splenomegaly, and overall survival. qPCR was performed for *JAK2*^{V617F} detection. PCR and agarose gel electrophoresis were used for detection of *CALR* mutation including wild-type *CALR* (product: 357 bp), type 1 (product: 305 bp) and type 2 (product: 272 bp) mutations. Detection of *MPL* mutation was performed using capillary sequencing, to detect type 1 (W515L) and type 2 (W515K) mutations. Survival probabilities were estimated with the Kaplan-Meier method and compared using the log-rank test.
Results: Ninety MPN patients were enrolled in this study (50% were males). Median follow-up period was 63 months, and the median age at diagnosis was 61 years. Regarding clinical characteristics of studied cohort, 10% of patients had hypercellular bone marrow, while splenomegaly and hepatomegaly were detected in 16% (14/90) and 7% (6/90) of patients, respectively. The overall *JAK2*, *CALR* and *MPL* mutation frequencies in our cohort were 60%, 10%, and 3%, respectively. For PV patients (n=29), 76% carried *JAK2*^{V617F} mutation. For ET patients (n=29), 59% of patients were positive for *JAK2*^{V617F}, 24% of patients carried *CALR* type 1 or 2 mutation, and 3% carried *MPL* type 2 mutation, while 14% of patients were triple negative. *CALR* type 2 mutation was slightly more predominant than *CALR* type 1. In PMF patients (n=3), 33% of patients carried *JAK2*^{V617F}, 33% of patients carried *CALR* and 33% of patients carried *MPL* type 1 mutation. In the group of MPN-unclassifiable patients, 44% (8/18) carried *JAK2*^{V617F} mutation. Out of 90 patients, 18 died during the observed period of time (PV n=8, ET n= 3; PMF n=0, MPN-u n=7). In all MPN subtypes, Kaplan-Meier survival analysis showed that there were no statistically significant differences in overall survival between patients with and without detected driver mutation (p>0.05).
Summary/Conclusion: Determination of the mutational status of *JAK2, CALR* and *MPL* is important for diagnosis and treatment. We found that overall survival did not depend on the presence of the tested mutations in MPN patients.

PB2292

OXIDATIVE STRESS IN PRIMARY AND SECONDARY MYELOFIBROSIS: IS THERE A ROLE FOR THE JAK2V617F MUTATION OR EXTRAMEDULLARY HEMATOPOIESIS SITES?

M.-A. Gaman[1,*], A.M. Gaman[2,3]

[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Bucharest, [2]Pathophysiology, University of Medicine and Pharmacy of Craiova, [3]Hematology, Filantropia City Hospital of Craiova, Craiova, Romania

Background: Several studies have suggested the involvement of oxidative stress in aging and in many hematological disorders, including BCR-ABL1-negative myeloproliferative neoplasms. However, information is scarce about the oxidative status of patients with primary myelofibrosis (PMF) or myelofibrosis secondary to polycythemia vera (PV) or essential thrombocythemia (ET)[1-9].
Aims: The major aim of this study is to evaluate the levels of reactive oxygen species and the total antioxidant capacity in patients with PMF and post-ET or post-PV myelofibrosis compared to healthy volunteers (control group). The minor aim is to observe whether the presence of the JAK2V617F mutation or of extramedullary hematopoiesis (EH) sites influenced the oxidative status of the study group.
Methods: We enrolled 10 patients with PMF or post-ET/post-PV myelofibrosis, hospitalized in the Clinic of Hematology, Filantropia City Hospital Craiova, and 20 healthy volunteers (control group). Informed consent was obtained from all subjects involved. Oxidative stress was evaluated using a

CR3000 analyzer from a single drop of capillary blood. Reactive oxygen species were evaluated by FORT (Free Oxygen Radicals Testing) and the total antioxidant capacity by the FORD (Free Oxygen Radicals Defense) assays. The normal range for the FORT assay is <2.3mmol/L H_2O_2 and the normal range for the FORD assay is 1.07-1.53mmol/L. The JAK2V617F mutation was detected by amplification refractory mutation system–polymerase chain reaction. EH sites were detected using computed tomography (CT), magnetic resonance imaging (MRI), cutaneous biopsy or scintigraphy. Statistical data analysis was performed using the student T-test and a p-value ≤0.05 was considered significant.

Results: The study group involved 10 patients (median age =63.0±11.61 years, male-to-female ratio =3:7) and 20 healthy controls (median age =64.1±2.26 years). Three patients had been diagnosed with PMF and seven patients developed post-PV or post-ET myelofibrosis. In PMF, scintigraphy revealed one lienal and one renal EH site and skin biopsy revealed one cutaneous EH site. In post-PV or post-ET myelofibrosis, CT detected one osseous EH site, MRI detected one lienal EH site and both CT and MRI confirmed a hepatic EH site. Data regarding the oxidative status are presented as mean value ± standard deviation in the attached table.

Table 1.

Group	FORT	p-value	FORD	p-value
Normal range	≤ 2.300		1.07 – 1.53	
Control group	2.162 ± 0.375	p<0.05	1.405 ± 0.439	p<0.05
Study group	3.080 ± 0.553		0.535 ± 0.272	
PMF	2.567 ± 0.250	p<0.05	0.777 ± 0.232	p<0.05
Secondary MF	3.300 ± 0.500		0.431 ± 0.217	
EH +	3.150 ± 0.621	NS	0.532 ± 0.296	NS
EH –	2.975 ± 0.409		0.540 ± 0.233	
JAK2V617F +	3.433 ± 0.354	p<0.05	0.348 ± 0.072	p<0.05
JAK2V617F –	2.550 ± 0.328		0.815 ± 0.217	

Summary/Conclusion: We found an unbalanced oxidative status in the study population compared to controls: levels of FORT were increased, whereas FORD levels were decreased. Post-PV or post-ET myelofibrosis cases and JAK2V617F-positive cases had higher FORT and lower FORD values than PMF cases and JAK2V617F-negative cases (p≤0.05). The presence of EH sites did not have an impact of oxidative stress status. We may hypothesize that oxidative stress is involved in the pathogenesis of PMF and post-PV or post-ET myelofibrosis and that the presence of the JAK2V617F mutation plays a role in the increase in oxidative stress levels.

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PB2293

EFFICACY AND SAFETY OF RUXOLITINIB IN 132 TURKISH PATIENTS WITH CHRONIC MYELOPROLIFERATIVE NEOPLASMS: A MULTICENTER AND RETROSPECTIVE ANALYSIS

G. Saydam[1,*], N. Soyer[1], M. Turgut[2], I.C. Haznedaroglu[3], Y. Fergun[4], I. Aydogdu[5], A. Pir[6], V. Karakus[7], G. Ozgur[8], C. Kis[9], F. Ceran[10], G. Ilhan[11], M. Ozkan[12], M. Arslaner[13], I. Ince[13], I. Yavasoglu[14], F. Gediz[4], M. Sonmez[6], B. Guvenc[9], G. Ozet[10], E. Kaya[12], M. Tobu[1], F. Vural[1], F. Sahin[1]

[1]Hematology, Ege University Faculty Of Medicine, IZMIR, [2]Hematology, OMU Faculty of Medicine, Samsun, [3]Hematology, Hacettepe Medical Faculty, Internal Medicine, Ankara, [4]Hematology, Ataturk Research and Training Hospital, IZMIR, [5]Hematology, CBU Medical Faculty, Manisa, [6]Hematology, KaTU Medical Faculty, Internal Medicine, Trabzon, [7]Hematology, Sitki Kocman Mugla University, Mugla, [8]Hematology, GATA, Ankara, [9]Hematology, Cukurova University, Adana, [10]Hematology, Numune Research and Training Hospital, Ankara, [11]Hematology, Mustafa Kemal Univ, Hatay, [12]Hematology, Inonu Univ. Medical Faculty, Malatya, [13]Hematology, Dr Ersin Arslan Research and Training Hospital, Gaziantep, [14]Hematology, AMU Internal Medicine, Aydin, Turkey

Background: Ruxolitinib has been approved for the treatment of patients with high- or intermediate-risk myelofibrosis with symptomatic splenomegaly. The aim of this study is to assess the efficacy and safety of ruxolitinib in patients with chronic myeloproliferative neoplasms.

Aims: The aim of this study is to assess the efficacy and safety of ruxolitinib in patients with chronic myeloproliferative neoplasms and clarify the existing treatment strategies of these patients.

Methods: Across all of Turkey, 14 centers were enrolled in the study. We retrospectively evaluated 132 patients who treated with ruxolitinib. Ethical committee aproval was obtained.

Results: Among 132 MPN patients, 72 (54.5%) of them were male, 60 (45.5%) cases were female. The median age of the patients at the time of diagnosis was 58 (26- 83). Twenty (15.2%) of the patients were post- ET MF, 32 were (24.2%) post-PV MF and 80 (60.6%) were diagnosed as PMF. There was thrombosis and bleeding history before diagnosis in 15.2% and 8.3% of the patients, respectively. Only two patients had concomitant cancer history. Antiplatelet, androgen, steroid and erythrocyte stimulating agent treatments were present in 59.9%, 12.1%, 7.6% and 2.3% patients, respectively. Splenectomy was performed only in 3.8% of the patients. There was JAK2 mutation and MPL mutation positivity in 66.9% and 14.6% of patients, respectively. Conventional cytogenetic analysis was applied in 31 patients. Twenty three patients had normal karyotype and 8 patients had complex karyotype. Leukemic transformation was observed in 6 (4.5%) patients. Only one patient was undergone hematopoietic stem cell transplantation. The median white blood cell, platelet and hemoglobin ($x10^3$/µl) counts were 10.75 (0.8- 61.3), 346 (42- 1920) and 10.8 (6. 6- 20.7), respectively. All patients had first line cytoreductive treatment. Hydroxyurea (85.2%) was the most common drug as first line treatment. Anagrelide (n=18) and interferon (n=14) were the most common drugs as second line treatment. Eight patients were used third line treatment. Interferon was the most common drug. Twenty patients were lost. Exitus reasons were pneumonia/sepsis in 10 patients, myocardial infarction (n=1), acute respiratory distress syndrome (n=1), bleeding (n=1), and cholangiocellular carcinoma (n=1) in other patients. The median dose of ruxolitinib was 30 (10- 40) mg at the beginning of the therapy. Dose change was made in 39 (43.8%) of 89 patients. Forty one (39%) of 105 patients had adverse events. Thirty five (33.3%) of 105 had hematological adverse events (n=21, anemia and n=19, thrombocytopenia). Eighteen (17.1%) of 105 patients had non-hematological adverse events. The most common events were infection (n=4), elevated liver enzymes (n=4) and fatigue (n=4). Others were zona zoster (n=2), abdominal discomfort (n=2), rash (n=1) and dizziness (n=1). Eighty eight (82.2%) of 107 patients who had constitutional symptoms were improved. The median spleen sizes before and after ruxolitinib treatment were 220 (110-350) mm *versus* 193 (110-270), respectively.

Summary/Conclusion: We observed a reduction in spleen size after ruxolitinib treatment in Turkish patients with MPN and constitutional symptoms were improved. Ruxolitinib is both safe and efficacious in Turkish patients with MPNs.

PB2294

DECITABINE AS SALVAGE TREATMENT FOR AML TRANSFORMATION OF CHRONIC MYELOPROLIFERATIVE NEOPLASM IN ELDERLY AND UNFIT PATIENTS

P. Niscola*, E. Abruzzese, A. Tendas, B. Neri, M.M. Trawinska, L. Cupelli, M. Palombi, L. Scaramucci, M. Giovannini, G. Catalano, P. de Fabritiis

Hematology Unit, S.Eugenio Hospital, Rome, Italy

Background: Myelodysplastic /myeloproliferative neoplasms, such as chronic myelomonocytic leukemia (CMML), and Philadelphia-chromosome negative chronic myeloproliferative neoplasms (MPNs) transformed to acute myeloid leukemia (AML) are associated with a poor response to available therapeutic options and a very dismal outcome, being only a minority of patients suitable for intensive chemotherapy and allogenic stem cell transplantation. Although a beneficial clinical activity by hypomethylating compounds have been suggested by some clinical studies, the role of these agents in this difficult setting has not been clearly established.

Aims: To report the outcome of 4 patients with AML transformation from essential thrombocytemia (1), polycythemia Vera (1) and JAK-2 positive CMML with bone marrow (BM) fibrosis, who were started on decitabine (25 mg/m2, 5 days every 4 weeks) at our institution between January 2015 and February 2018.

Methods: Clinical parameters, treatment and follow-up data were retrospectively collected and analyzed. There were 4 (3 male) patients with a median age of 71 (67-75). All patients were transfusion-dependent at the start of hypomethylating therapy. Abnormal karyotype (-7) were detected

in one patients whereas the remaining three showed no cytogenetic abnormalities. Previous therapies included phlebotomy (1), hydroxyurea (3) and pipobroman (1); 1 patient was treatment-naïve. The time interval from the MPNs and CMML diagnoses to AML transformation was of 10 (2-27) years.The median number of decitabine courses was 14 (11-24). Other than expected therapy related cytopenias, which were well manageable with conventional measures, treatment was well-tolerated and no remarkable side effects were recorded. Broad spectrum antibacterial and anti-fungal agents were given according to the duration and the degree of neutropenia.
Results: Responses were assessed according to 2006 IWG criteria after almost 4 courses of decitabine, Out of the 4 patients, 2 (50%) achieved complete remission (CR) and 2 (50%) a partial remissions (PR); in one of latter PR patients, the disappearance of extramedullary (nodal and lung) AML involvements was observed by six cycles of epigenetic courses. After a median follow-up of 12 (11-28) months, 2 patients, both having obtained CR and including that presenting the -7 abnormal karyotype, are still alive: overall survivals from the start of decitabine were of 11,+15,18 and + 28 respectively. Two patients progressed from the achieved PR and deceased because of clinical complications of overt AML.
Summary/Conclusion: Despite the limited number of cases, our experience reflected a real-life treatment scenario with encouraging results, given that all patients responded to decitabine which was tolerable and efficacious in elderly, comorbid and pretreated AML patients with an unfavorable prognostic profile long disease history of chronic MPNs and CMML with BM fibrosis. The efficacy of decitabine in this difficult to treat setting, although not durable in our patients who have achieved a PR, may represent an useful clinical basis for potential applications of this agent with other clinically active synergistic compounds, such as JAK2 inhibitors, are needed to improve overall outcome into future clinical trials which should include molecular profiling tools for an accurate selection of suitable patients.

PB2295

ASSESSMENT OF THE EFFICACY OF LOW-DOSE ASPIRIN BY THROMBOELASTOMETRY IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

E. Cacciola[1,*], E. Gentilini Cacciola[2], V. Vecchio[2], R. Cacciola[3]

[1]Medical and Surgical Science and ADvanced Technologies, [2]Institute of Haemostasis, Catania, Italy, [3]Experimental and Clinical Medicine, Institute of Haemostasis, Catania, Italy

Background: The essential thrombocythemia (ET) is a myeloid neoplasm characterized by platelet hyperreactivity and thrombosis. The daily low-dose aspirin (ASA) is a cornerstone in the prevention of the thrombotic events. In the ET an accelerated platelet turnover translates in a renewal of the drug target shortening the duration of cyclooxygenase (COX-1) inhibition and may dictate new dosing strategies particularly in ASA "low-responders" patients.
Aims: Therefore, we evaluated platelet count, β-thromboglobulin (β-TG) and platelet factor 4 (PF4), as markers of platelet activation, the clotting time (CT), clot formation time (CFT) and maximum clot firmness (MCF), as indicators of aspirinated platelet contribution to clot formation/firmness.
Methods: We studied 60 patients (20 men, 40 women; mean age 51 years, range 32-70) with ET according to WHO criteria. The mean duration of disease was 11 years. All patients were on ASA 100 mg once daily. Of the 60 patients, 45 were on anagrelide hydrochloride (daily dose 1.5 mg) (10 men, 35 women), 15 were on hydroxyurea (daily dose 2 mg) (10 men 5 women). None had inherited or acquired thrombotic risk factors. Sixty subjects served as controls. Platelets were measured by automated analyzer. β-TG and PF4 were determined by ELISA. CT, CFT and MCF were measured by ROTEM delta.
Results: The mean platelet count was 455±200x10^9/L. All patients had normal β-TG and PF4 (12±5 IU/ml and 4±1 IU/ml), normal CT (CT, unit: s. n.v. 100-240 s) (110±20 s), normal CFT (CFT, unit: s, n.v. 30-110 s) (45±5s) and normal MCF (MCF, unit: mm, n.v. 50-72mm) (61±2mm).
Summary/Conclusion: These findings suggest that in ET patients the daily low-dose ASA represents an optimal dosing strategy and that thromboelastometry may be an useful tool to confirm the efficacy of the low-dose ASA in ET patients.

PB2296

ESSENTIAL THROMBOCYTOSIS IN CHILDREN

C. Albayrak*, D. Albayrak

Pediatric Hematology Department, Ondokuz Mayis University Medical Faculty, Samsun, Turkey

Background: In childhood age, sporadic essential thrombocytosis is a very rare disease and its frequency has been reported as 1/1,000,000. WHO 2008 Essential Thrombocytosis Diagnostic Criteria; (iron deficiency, megaloblastic anemia, acute phase reactors, trauma, operation), absence of myeloproliferative neoplasm and thrombocytosis in the family, and the presence of thrombocytosis in WHO the absence of myeloid neoplasm criteria.
Aims: Seven cases of sporadic essential thrombocytosis diagnosed in our Pediatric Hematology Clinic were presented in this study.
Methods: Six of the patients are girls, one is a boy. Median referral age was 13 years (minimum 5 months, maximum 15 years). Application complaints; adolescent boys were found incidentally, with headache, dizziness, and tinnitus while young children had no complaints. No thrombus was detected in any patient. The median platelet count was 1442x10^9/L (963 lowest, 2438 highest).
Results: There was an increase in megakaryocytes in bone marrow aspiration, no cytogenetic abnormality. In one case Jak-2 (V617F) mutation, in two cases CALR mutation was detected. There was no mutation of MPL (W515L) in any case. One type 2 mutation known to one of the CALR mutation cases, and a new mutation that was not previously defined. No clonality was detected in the other four cases. Three cases of mutation detection and two cases of undetectable disease are followed by hydroxyurea treatment. Two other cases use low-dose aspirin. Follow-up is between six months and nine years. No complications developed.
Summary/Conclusion: Thrombocytosis is a common problem in childhood age group. Generally, reactive and secondary causes are detected. Essential thrombocytosis is a diagnosis that should come to our mind after other causes have been ruled out. Mutation studies should be carried out in children with WHO 2008 criteria. In adults, Jak-2 (V617F), CALR and MPL (W515L) mutations occur in 90% of cases, whereas in childhood, these three mutations occur only in 25% of cases. The high number of cases in which no mutation is detected indicates that new candidate genes must be searched and studied.

PB2297

THE MORTALITY OUTCOMES AND SURVIVAL PATTERNS OF MPN REGISTRY IN MALAYSIA

Y.Y. Yap[1,*], J. Sathar[1], K.B. Law[2], S.C. Edmund[2]

[1]Department of Haematology in Ampang Hospital, [2]Clinical Research Centre, Ministry of Health, Ampang, Malaysia

Background: The prognostication of myeloproliferative neoplasm (MPN) has been always challenging even in the advent of Janus kinase 2 (JAK2 V617F) molecular studies. The survival pattern of MPN in developing country like Malaysia is still awaiting discovery.
Aims: To investigate the mortality outcome in relation to demographic, clinical presentation and clinical blood parameters.
Methods: This was a retrospective national registry conducted from year 2009 to 2015 in Malaysia with description of mortality outcome in correlation to demographic, MPN subtypes, JAK2 V617F mutation, thrombosis, haemorrhagic events and blood parameters.
Results: A total of 865 patients were included for survival analysis. The mean survival duration was estimated to be 23.2 years (95% CI:20.739, 25.684). There were 154 deaths with 57.8% of males and 42.2% of females. The ethnic distribution among the decease were 50% of Malay, 40.9% of Chinese, 7.1% of Indian and 1.9% of others. JAK2 V617F was present in 61.1% while absent in 14.9% of mortality cases. The causes of death were mainly non-haematological related (55.2%) while haematological related was only 23.4%. The MPN sub-types like essential thrombocythemia (ET) had the best mortality outcome followed by polycythemia vera (PV), hypereosinophilia syndrome (HES), MPN-unclassifiable (MPN-U) and primary myelofibrosis (PMF) which was associated with odds of death of 1.2 times in comparison to others, p<0.001. The JAK2 V617F mutation had no influence in the overall survival (OS). The haemoglobin <12g/dL, haematocrit <35%, platelet <150 x10^9/dL and white blood count <5 x10^9/dL were significantly associated with the inferior OS (p<0.001). The arterial or venous thrombosis had no effect on the OS while those with bleeding events had a marginally significant better outcome (p=0.04). The grade 2 and above bone marrow fibrosis had a worse OS compared to the grade 1 and below bone marrow fibrosis. Patients presented with vasomotor symptoms were found to do better than those patients without (p=0.003).
Summary/Conclusion: The ET had the best OS while PMF had the worst OS. This is in conjunction with low blood counts, worsening bone marrow fibrosis and without vasomotor symptoms. The survival outcome of the MPN registry is instrumental for future policy development of effective healthcare in Malaysia.

PB2298

INDIVIDUALIZING PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS BY INTEGRATING GENETICS, AND CELLS COUNTS

M. Rashid*, R.Z Ahmed, U. Zaidi, T. Shamsi

National Institute of Blood Diseases & Bone Marrow Transplantation, Karachi, Pakistan, Karachi, Pakistan

Background: When evaluating classical myeloproliferative neoplasms (MPNs), the detection of Janus Kinase 2 (*JAK*2), Calreticulin (*CALR*), or Myeloproliferative Leukemia Virus Oncogene (*MPL*) mutations confirms the presence of an underlying MPN but their absence does not rule out the possibility.
Aims: The current study was to analyze the prevalence of *JAK*2 and *CALR* gene mutations in patients with MPN; secondary was to evaluate the impact of gene mutations on cell counts of MPN at diagnosis.
Methods: Our study population consisted of 90 patients with clinically suspected as MPN, 30 polycythemia (PV), 36 essential thrombocythemia (ET), 4 primary myelofibrosis (PMF) and 9 unclassified myeloproliferative neoplasms (U-MPN). Male predominance was found in PV patients 76.6%, ET patients were found in young age group (mean 45 years) and more splenomegaly was observed in PMF patients. *JAK*2V617F mutation was analyzed by ARMS-PCR while *CALR* mutations were identified by bi-directional Sanger sequencing.
Results: *CALR* mutations was not found in PV, in ET group frequency of *CALR* and *JAK*2 mutations were same 24.2%. *CALR* mutant ET was associated with younger age (mean 45 year), higher platelet counts (mean= 1251x10^9/L), lower leukocyte counts (mean= 11.x10^9/L) and also lower haemoglobin (median =1.7 g/dL) in comparison with *JAK2* V617F-positive ET. More splenomegaly was also observed in *CALR* mutated ET.

Table 1.

Total MPN patients 90				
	PV (30)	ET (36)	PMF (15)	U-MPN (9)
Gender Male, no. (%)	23 (76.6)	20 (55)	8 (53.3)	6 (66.6)
Female	7 (23.3)	16 (44.4)	7 (46.6)	3 (33.3)
Age at diagnosis in years, (mean + SD)	55 ± 10.8	45 ± 17	45 ±19.1	53 ± 20.4
Haemoglobin (g/L), (mean + SD)	17 ± 2.5	12.1 ± 2.4	9.3 ± 1.9	14.2 ± 3.6
White Cell Count (x 109/L), mean	17.9 ± 12.6	12.4 ± 6.6	9.8 ± 7.7	36.2 ± 30.5
Platelet count(x 109/L), (mean + SD)	458 ± 2.5	1148 ± 439	112 ± 92	695 ± 506
Splenomegaly, no (%)	5/30 (16.6)	10/35 (28.5)	12/13 (92)	6/9 (66.6)
Hepatomegaly, no (%)	2/30 (6.6)	3/35 (8.5)	3/13 (23)	3/9 (33.3)
JAK2V617F mutation, Tested (n)	30	33	9	6
mutation-positive, n (%)	25 (83.3)	8 (24.2)	2 (22.2)	4 (66.6)
CALR mutation, Tested (n)	29	33	15	9
mutation-positive n (%)	0 (0%)	8 (24.2)	3 (20)	1 (11.1)
*BCR-ABL*1 mutation, Tested n (%)	15	19	13	6
mutation-positive	2 (13.3)	1 (5.2)	2 (15.3)	0 (0)

1. One patient had both coexistence of *CALR* exon 9 mutation with *JAK*2V617F in ET. 2. One patient's marrow status show myelofibrosis, *BCR/ABL* gene fusion by FISH was positive in 20% cells and *CALR* sequenced show 52bp deletion. 3. One patient with pancytopenia, show a novel homozygous mutation c.1139delA;p.E380fs50*. 4. One patient of ET shows 10 bp deletion (c.1 120_1129del) along with 28bp deletion.
Summary/Conclusion: *CALR* positive patients are phenotypically distinct from *JAK*2 positive patients. There is need to include a wide variety of bioinformatics assessments.

PB2299

SPLANCHNIC THROMBOSES IN MYELOPROLIFERATIVE DISORDERS EXPERIENCE OF THE HEMATOLOGY CENTER OF FUNDENI CLINICAL INSTITUTE

I. Ursuleac[1,2,*], R. Dragan[2], A. Bardas[2], C. Constantin[2], C. Dobrea[1,2], D. Coriu[1,2]

[1]'Carol Davila' University of Medicine and Pharmacy, [2]Hematology, Fundeni Clinical Institute, Bucharest, Romania

Background: Myeloproliferative disorders are known to cause splanchnic thromboses. Risk factors for thromboses in myeloproliferative neoplasms are: age >60years, previous thromboses, JAK2V617F mutation positivity, associated thrombophilic status, pancytosis, and classical risk factors (obesity, dislipidemia, diabetes, smoking, use of oral contraceptives).
Aims: Evaluating risk factors for splanchnic thromboses in a group of pacients with myeloproliferative neoplasms, unicentric analysis over 7 years (2010-2017) in Fundeni Hematology Clinic.
Methods: Clinical and epidemiological retrospective study of 20 cases of patients diagnosed with myeloproliferative neoplasms and visceral thromboses.
Results: Clinical and epidemiological data: 20 patients diagnosed with myeloproliferative neoplasms and visceral thromboses (14 women, 6 men) of median age 34.5 years (20-55 years): 4 cases of polycythemia vera, 7 essential thrombocytemia, 7 myelofibrosis, 2 unclassified myeloproliferative disorder. In 18 cases JAK2V617F mutation was present, 1 case negative and 1 was not tested. 10 patients with suprahepatic vein thrombosis, the majority positive for JAK2V617F mutation (9 cases). Portal vein thrombosis was found in 3 cases, all of them positive for JAK2V617F mutation, and associations of suprahepatic vein thrombosis with portal vein thrombosis was found in 7 cases. Pancytosis (Ht>44%, Le>11.000/mmc, Tr>450.000/mmc) was found in 5 cases, association of Ht>44% and Tr>450.000/mmc in 4 cases. Association between Ht>44% and leucocytosis over 11.000/mmc was found in 1 case. Normal blood cell counts values were detected in 4 cases. We identified the following as additional risk factors for thrombosis: smoking (6 cases), dislipidemia (3), use of oral contraceptives (2), thrombophilia (5). Two or more risk factors were present in 5 cases. Anticoagulant therapy was associated with cytoreductive therapy in 18 cases and 2 patients required only anticoagulant therapy. Recurrent thromboses were found in 2 cases. Death caused by progression of hematological disease occurred in 3 cases; all other patiens are hematologically stable under specific treatment. Due to progressive hepatic disease, 3 patients received liver transplants and 2 patients underwent transjugular intrahepatic portosystemic shunt.
Summary/Conclusion: Presence of splanchnic thrombosis is highly suggestive for the diagnosis of myeloproliferative neoplasms - 12 of our patients were diagnosed simultaneously for both pathologies, and in 8 patients the thrombotic episode occurred after the diagnosis of hematological disease. In the studied group of 20 cases, JAK2V617F mutation was identified in 18 cases. Most frequent myeloproliferative neoplasms were essential thrombocytemia and myelofibrosis, 7 cases each. Cytoreductive therapy associated with anticoagulant therapy represented therapeutical option for 18 cases. The evolution of thrombosis depends on the therapeutical response of the hematological disorder and also on the possibility for correcting the associated risk factors.

PB2300

A SINGLE-CENTER RETROSPECTIVE ANALYSIS OF AUTOIMMUNE MYELOFIBROSIS (AIMF). AIMF ASSOCIATED WITH COMMON VARIABLE IMMUNODEFICIENCY AND RELAPSING-REMITTING MULTIPLE SCLEROSIS

M. García-Recio[1,*], L. Lo Riso[1], A.M. Quintero[2], A. Formica[1], S.A. Jimenez[1], A. Ortuño[1], B. Lopez[1], R. Ramos[2], J. Ginés[3], M.A. Durán[1]

[1]Hematology, [2]Pathology, [3]Pharmacy, HUSE, Palma, Spain

Background: Autoimmune myelofibrosis (AIMF) is a rare cause of marrow fibrosis. It has been reported in association with autoimmune disorders, like systemic lupus erythematosus among others (Vergara-Lluiri *et al.* 2014). Our study presents two cases of common variable immunodeficiency (CVID) associated AIMF and one case of relapsing-remitting multiple sclerosis (RRMS) associated AIMF. Autoimmunity in the context of CVID occur in 20–30% of patients being cytopenias the most common autoimmune condition (Xiao *et al.* 2014).
Aims: To review the incidence and evolution of patients diagnosed with AIMF in our center.
Methods: We retrospectively reviewed patients from the Pathology service data base and medical histories.
Results: From 2000 to 2018, 34 patients were newly diagnosed of MF. Three (8.8%) were AIMF: two women (66%) diagnosed with CVID and one male (33%) diagnosed with RRMS. Median age at diagnosis was 38. One patient (33%) died due to an infection. Patients evolution and pathology (Table 1). Case A: A 25-year-old female diagnosed with CVID in 2010 in the setting of severe bicytopenia (600 neutrophils/uL and 6.000 platelets/uL), and mild/moderate infections. Despite autoimmunity tests were negative, direct coombs test was positive and has remained so ever since. Bone marrow biopsy (BMB) was compatible with AIMF. Immunoglobulins (Ig) 0,5g/Kg/21 days were given with CBC normalization. Corticoids were added and slowly tapered. Interestingly, while before each cycle platelets dropped under 10.000/uL, neutrophils sustainably normalized. Eltrombopag was iniciated in April/2011 with good results (>50.000 platelets/uL pre-Ig). Six years later, pre-Ig platelets account dropped to <10.000/uL. We switched to Romiplostim (raised to 4mcg/kg in the last visit) and increased Ig to 1g/kg with encouraging results (11.000 platelets/uL pre-Ig in the last visit). Case B: A 43-year-old female diagnosed in 2003 CVID and receiving Ig 0,5g/kg/21 days and corticosteroids, was sent to our clinic in 2010 due to grade IV neutropenia and severe infections. Neutrophils oscillated between 300/uL and 1000/uL and platelets progressively dropped to 25-30.000/uL. The patient presented with positive antibodies

anti-parietal cell (1/160), vitamin B12 in normal range. BMB was compatible with AIMF. In August/2017 due to grade IV bicytopenia, Ig were increased to every 14 days and mycophenolate was started maintaining neutrophils >600/uL and platelets >40.000/uL. Case C: A 38-year-old male diagnosed with RRME in 2008, last treatment alemtuzumab (June/2016) presented with asymptomatic grade IV thrombocytopenia in May/2017. Dexamethasone 40mg for 4 days (2 cycles) was started raising platelets to 253.000/uL. However, within a month, he presented with severe pancytopenia. Positive anti-neutrophil cytoplasmic antibodies were detected. BMB was compatible with AIMF. He received rituximab (2 doses) and Ig 1g/Kg/2days with no response. In September/2017 erythropoietin and plasma exchange was initiated with normalization of the platelet account (1 plasma volume was replaced with 0.5% human albumin diary (x4), then once a week). Neutrophils oscillated between 20 and 2.000/uL (good response to G-CSF and plasma exchange intensification). On February/2018 he presented with sepsis (gripeA) and died in the intensive care unit.

Table 1.

Table 1. Patients characteristics, pathology findings and evolution.

	Case A	Case B	Case C
Patients Characteristics			
Age at diagnosis	25	43	38
Sex	Female	Female	Male
Associated conditions	Common variable immunodeficiency	Common variable immunodeficiency	Relapsing-remitting multiple sclerosis
Autoimmunity tests	Negative[1]. Direct coombs: +++	Positive antibodies anti-parietal cell (1/160).	Positive anti-neutrophil cytoplasmic antibody.
Peripheral Blood Smear			
WBCs	Decreased	Decreased	Decreased
RBCs	Rare teardrop cells	Rare teardrop cells	Rare teardrop cells
Platelets	Decreased Giant forms	Decreased Giant forms	Decreased Normal
Bone Marrow Biopsy			
Cellularity	Augmented	Augmented	Augmented
Megakaryocytes	Augmented	Augmented	Slightly Augmented
Megakaryocytes morphology	Normal, no clustered	Normal, no clustered	Normal, no clustered
Myeloid precursors	Augmented	Augmented	Augmented
Erythroid precursors	Augmented	Slightly Augmented	Decreased
Lymphoid aggregates	Isolated interstitial, predominance LT	Paratrabecular, intratrabecular, predominance of T lymphocytes.	Nodular interstitial, predominance of T lymphocytes.
Plasma Cells	Isolated	Slightly Augmented	Augmented
Dysplasia	Absent	Absent	Mild
Intratrabecular hematopoiesis	Observed	Observed	Absent
Reticulin fibrosis	Grade 1-2	Grade 2	Grade 1 - 2
Treatment and follow-up			
Frontline	Corticosteroids and immunoglobulins	Corticosteroids and immunoglobulins	Corticosteroids and immunoglobulins
Response to frontline	WBCs: Normal. Platelets: No response.	WBCs: >600 Platelets: >40.000	No response.
Second line	Eltrombopag	Not required.	Plasma Exchange

Summary/Conclusion: AIMF is a rare disease of which we present previously unreported cases associated with CVID and RRMS. Thrombopoietin agonists can be considered as a therapeutic option for these patients.

PB2301

PLASMA CONCENTRATIONS OF INTERLEUKIN 6 AND INTER LEUKIN 8 MAY BE RELATED TO POOR SURVIVAL AND ARE NOT AFFECTED BY CYTOSTATIC THERAPY IN PATIENTS WITH PRIMARY MYELOFIBROSIS FROM UKRAINE

R. Lozynskyy[1,*], O. Danysh[2], M. Voronyak[3], M. Lozynska[4], K. Susida[5], M. Kokoruz[6], Y. Vyhovska[7], Z. Maslyak[2], V. Orel[8], O. Abrahamovych[1]

[1]Internal Medicine #1, Danylo Halytsky Lviv National Medical University, [2]Hematology, Institute of Blood Pathology and Transfusion Medicine of National Academy of Medical Sciences, [3]Molecular Genetic Laboratory, Institute of Blood Pathology and Transfusion Medicine of National Academy of Medical Sciences of Ukraine, [4]Diagnostics of Hereditary Pathology, Institute of Hereditary Pathology of National Academy of Medical Sciences of Ukraine, [5]Hematology, Institute of Blood Pathology and Transfusion Medicine of National Academy of Medical Sciences of Ukraine, [6]Molecular Genetic Laboratory, Institute of Blood Pathology and Transfusion Medicine of National Academy of Medical Sciences, [7]Hematology, Danylo Halytsky Lviv National Medical University, [8]Consulting Polyclinic, Lviv Regional Clinical Hospital, Lviv, Ukraine

Background: The levels of different cytokines including VEGF, interleukin (IL) 6, IL-8 (CXCL8), and other molecules involved in inflammation were shown to be elevated in patients with primary myelofibrosis (PMF), a myeloproliferative neoplasm that still remains incurable without allogeneic hematopoietic stem cell transplantation. However, most of the studies give scarce data about clinical and genetic correlations with cytokine levels. So, the prognostic significance of cytokine concentrations is still unclear, especially under different palliative treatment strategies, including hydroxyurea and interferon-alpha, and in different populations.
Aims: To evaluate the plasma levels and clinical correlations of VEGF, IL-8, IL-6 and TNF-alpha and in patients with primary myelofibrosis from Ukraine.
Methods: The total of 45 patients clinically diagnosed with PMF were examined for plasma levels of VEGF, IL-8, IL-6 and TNF-alpha levels using ELISA on admission and after a year of follow-up. Control group consisted of 35 healthy persons. The study group included 21 patients with early PMF, the others had overt PMF according to WHO 2016 classification. Histological, cytogenetic and molecular genetic studies were performed for PMF patients. All the PMF patients received treatment with either hydroxyurea or interferon-alpha. Statistical differences were analyzed by the Mann-Whitney test.
Results: The upper quartile level of all studied cytokines in the group of patients with PMF exceeded the maximal levels of healthy donors. However, only VEGF and IL-8 levels of the patients from the study group were significantly increased (p=0.016 and p=0.044 respectively) comparing to the controls. Moreover, the levels of VEGF and IL-8 were higher than maximal for control group in only 37.8% and 46.7% of the patients with PMF. There were no differences found in the levels of the cytokines in patients with and without *JAK2*V617F mutation, but the TNF-alpha levels were significantly increased (p=0.001) in the patients bearing either *MPL*515L or *MPL*515K mutations. The level of IL-8 was significantly higher (p=0.048) in patients with cytogenetic abnormalities. There were no statistically significant differences in the cytokine levels between early and overt PMF groups. The levels of the cytokines had no significant differences between Dynamic International Prognostic Scoring System Plus risk groups. However, all 5 patients that died during the follow-up had significantly higher level of both IL-8 (p=0.0004) and IL-6 (p=0.02). The VEGF level was decreased at the follow-up on either hydroxyurea or interferon-alpha treatment in 93.3% of patients respectively, and the TNF-alpha levels increased in 88.9% of the patients, even if they were within the control group range at presentation. At the same time the IL-8 and IL-6 levels were not significantly changed under the same treatment during the follow-up.
Summary/Conclusion: The levels of VEGF and IL-8 may be increased in some patients with PMF, but this is not a disease specific feature, because most of the patients have the level of the cytokines within the range of values observed in healthy individuals. It is possible that the treatment with hydroxyurea and interferon-alpha could change the levels of VEGF and TNF-alpha, however the IL-8 and IL-6 levels do not change significantly after the year of follow-up on the same therapy and may be related with inferior prognosis. As the higher IL-8 level correlates with the presence of cytogenetic abnormalities, it is possible to suppose that cytogenetically abnormal cells may cause more intensive production of this cytokine.

PB2302

EPIDEMIOLOGICAL DATA ON POLYCYTHEMIA VERA IN ALBANIA (1999-2014)

A. Perolla[1,*], T. Caja[1], P. Pulluqi[2], E. Calliku[3], A. Ivanaj[4], A. Cili[4], A. Pushi[5]

[1]Internal Medicine, Hematology, [2]internal Medicine, [3]Internal Medicine ,Hematology, [4]internal Medicine Hematology, UHC " Mother Teresa", [5]Internal Medicine Hematology, UHC" Mother Teresa", Tirana, Albania

Background: Polycythemia Vera (PV) is a chronic myeloproliferative disease characterized by the elevation of the number of red blood cells. It is more common in males over 60 years old, but it can be present in every age group. An uncontrolled PV can induce a large number of thrombotic events putting in a high risk the lives of those patients.
Aims: To evaluate the incidence and sex-related incidence of PV in Albanian population during the period of time from 1999-2014 and to assess the emergency events at the moment of the diagnosis.
Methods: We collected and studied the data of all the patients more than 18 years old hospitalised and diagnosed with PV in the UHC "Mother Teresa" during the period from 1999-2014. SSPS 20.0 was used for the statistical analyses.
Results: According to the data collected and analyzed in the period from 1999-2014, the total number of the patients diagnosed with PV was 119 patients. From all the patients diagnosed with PV, 71 (60%) were males and 48 (40%) females. Only 23% of them have been presented in the Emergency Unit. 61% of all those patients presented in the Emergency Unit presented cardiovascular diseases (arterial hypertension, CAD, myocardial infarction), 12% neurological complications (stroke, convulsions etc..) 12% VTE (pulmonary, portal system, etc), 8% diabetes mellitus untreated (only one patient presented in the emergency with primary hypothyreosis) and 7% melena. The annual incidence of PV in Albania results in about 0,24

new cases per 100,000 inhabitants with an augmentation during 2006 (0,56 new cases per 100,000 inhabitants). The incidence was higher in males compared to females with a ratio 1,51/1. The annual incidence for males and females was respectively 0,29 new cases/100 000 inhabitants and 0,19 new PV cases/100000 inhabitants.
Summary/Conclusion: PV is a disease presented in Albania with about 7,4new cases per 100.000 inhabitants and with an incidence of around 0,24 new cases per 100,000 inhabitants affecting more males than females with a ratio 1,51/1. Mostly the patients went to the haematologist through the consultations and only 23% of them was diagnosed after presenting in the Emergency Unit predominantly with cardiovascular complications, before being diagnosed with PV. The correct diagnosis and management of those patients reduces the risk of thrombotic events and improves the prognosis.

PB2303

MLPA ASSAY USED FOR VERIFICATION OF RARE FINDING: SPORADIC PRESENCE OF TWO CALR MUTATIONS IN ONE PMF PATIENT

H. Drimalova[1,*], R. Sitkova[1], A. Buricova[2], D. Adamova[2], L. Stejskal[2], K. Horka[1]

[1]Department of medical genetics, Laboratory of molecular biology, AGEL Laboratories, Novy Jicin, [2]Hematology and Transfusion Departement, Silesian Hospital in Opava, Czech Republic

Background: Recently, somatic mutations in *CALR* gene have been demonstrated to be the second most common driver mutations in essential thrombocythemia (ET) and primary myelofibrosis (PMF). Approximately 50 different indels have been found in exon 9 of the *CALR* gene but more than 80% of *CALR* mutated patients possess one of the two most frequent variants: a 52-bp deletion (p.Leu367fs*46; type 1) and a 5-bp insertion (p.Lys385fs*47; type 2 mutation). An 80-year-old male patient with thrombocythemia was investigated for *CALR* mutation to confirm his clinical diagnosis of primary myelofibrosis. The patient carried both of the two most frequent types of *CALR* mutations (type 1 and type 2).

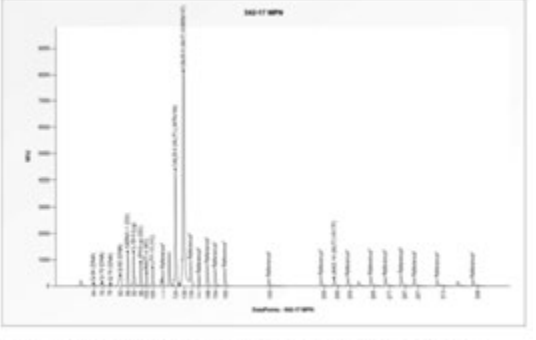

Figure 1. Capillary electrophoresis pattern of the patient DNA analysed with SALSA MLPA probemix P520-A2 MPN mix 2. |

Figure 1.

Aims: Verification of rare findings in *CALR* gene by MLPA assay and their interpretation considering the recent studies and clinical data of the patient.
Methods: In our laboratory the routine detection of the *CALR* mutations is performed by a fragment analysis on capillary electrophoresis. Positive results are confirmed by Sanger sequencing. However, sequencing data were not clear in this case (presence of deletion and insertion in one amplicon). Therefore we chose SALSA MLPA probemix P520-A2 MPN mix 2 for successfull verification of our findings (Figure 1).
Results: Presence of *CALR* mutation was one of the three major WHO diagnostic criteria of prePMF for this patient. Mostly, ET and PMF patients carry only one *CALR* mutation. Suprisingly, we found a patient with two variants: type 1 and type 2 mutation. MLPA and Sanger sequencing were used to verify our findings gained from fragment analysis, but only MLPA assay clearly confirmed the presence of two mutations. In our patient anamnesis the several diseases of urinary system were mentioned (subglandular hypospadia, kidney cysts, carcinoma of kidney) and the patient underwent radiotherapy due to carcinoma of prostate gland in 2010. The PMF diagnosis was established in 2017 with subsequent moderate progress.
Summary/Conclusion: The moderate progress of PMF is associated with prognostic benefit, which is described in patients with *CALR* mutation, particularly with type 1 cases. Both types of *CALR* mutaions in one patient have been already mentioned but without any clarification of its development or impact on the PMF prognosis. In the case of our patient, it seems to be moderate progress of PMF yet. As generally known, radioactivity increased a risk of myeloproliferative neoplasms. Therefore we suppose that the radiotherapy could influenced the development of PMF with the sporadic presence of two *CALR* mutations.

PB2304

A RETROSPECTIVE STUDY OF PATIENTS WITH POLYCYTHEMIA PRESENTING TO A COMMUNITY HAEMATOLOGIST: NOT ALL INCREASED HEMOGLOBIN IS POLYCYTHEMIA VERA

N. Gupta*, A. Garg, F. Naim

Clinical Hematology and bone marrow/stem cell department, Sir Ganga Ram Hospital, New Delhi, India

Background: As per new WHO 2016 classification, polycythemia vera (PV) is suspected once hemoglobin is above 16.5gm/Dl (HCT >49%) in males and 16gm/dL (HCT 48%) in females. JAK 2 V617F mutation or similar mutations in JAK 2 gene are seen in almost all of the patients with polycythemia vera. Bone marrow examination is required to make a diagnosis of PV and to differentiate it from other myeloproliferative neoplasms. High hemoglobin values more than the above ranges are commonly seen in clinical haematology practice.
Aims: Aim of present study was to evaluate patients presenting with raised hemoglobin and differentiate ideopathic polycythemia and its clinical course from polycythemia vera.
Methods: Retrospective review of patients referred to the haematologist in Sir Ganga Ram Hospital, New Delhi, India, for high hemoglobin levels from January 2015 to December 2017 was performed. Demographic characteristics, history (including smoking and alcohol) and general physical examination were noted. All patients were tested for JAK 2 V617F mutation, erythropoietin levels (EPO) and ultrasound whole abdomen for splenohepatomegaly. Patients with PV were treated with phlebotomy, low dose aspirin and cytoreductive therapy with hydroxyurea as per standard guidelines.
Results: A total of 70 patients were found, 65 males: 5 females, 14/70 (20%) were JAK2 V617F positive and rest 56/70 (80%) were negative (table 1). JAK 2 Exon 12 mutations were done in 31 of 56 JAK 2 V617F negative patients and all were negative. Bone marrow examination was done in 28 of 56 JAK 2 V617F negative patients and was normal in all except 2 patients in which panmyelosis was seen. Palpable splenomegaly was seen in 9/14() patients with JAK 2 V617F positive patients while splenomegaly was absent in all JAK2 negative patients. Phlebotomy was done if Hemoglobin was more than 17g/dL in JAK 2 negative patients and low dose aspirin was given if hypertension/hypercholesterolemia/diabetes was present. None of JAK 2 negative patient developed thromboembolism or AML/myelofibrosis during study period with a median follow up of 13.5 months while 2 patients died in JAK 2 positive group (one due to cerebrovasucular accident and other due to development of myelofibrosis.

Table 1. Comparision of jak2 positive and jak 2 negative patients with polycythemia.

	JAK 2 POSITIVE	JAK 2 NEGATIVE
PATIENTS- 70	14/70 (20%)	56 (80%)
MALE :FEMALE	54:2	11:3
AGE; MEDIAN (RANGE) YEARS	59.5 (25-76)	36 (20-67)
HYPERTENSION	8/14 (57%)	30/56 (53%)
SPLENOMEGALY	9/14	0/56
HISTORY OTHROMBOSIS (ARTERIAL AND VENOUS)	4/14 (28.5%)	3/56 (5.35%)
FOLLOW UP IN MONTHS; MEDIAN (RANGE)	13.5 (3-36)	13.5 (3-36)

Summary/Conclusion: Most of the patients referred for isolated increase in hemoglobin were young males without splenomegaly, had high prevalence of hypertension and were JAK 2 negative. Bone marrow examination was not performed in most of these patients and were treated as idiopathic erythrocytosis and managed conservatively with optimal clinical outcomes. There is a need to review the requirement of doing bone marrow examination in such patients.

PB2305

MANAGEMENT OF SYSTEMIC MASTOCYTOSIS IN A SINGLE CENTER EXPERIENCE

M. Criscuolo[1,*], L. Fianchi[1], A. Maraglino[1], A. Buonomo[2], P. Chiusolo[1], A. Bacigalupo[1], L. Pagano[1]

[1]Hematology, [2]Allergology, Catholic University of Sacred Heart Fondazione Policlinico Universitario A. Gemelli, Rome, Italy

Background: Mastocytosis is an extremely rare neoplasm originating from abnormal proliferation and tissue infiltration of clonal mast cells. The WHO classification recognizes cutaneous and systemic mastocitosis (SM), the former localized to skin and the latter further classified as indolent or aggressive, based on organ infiltration. Moreover, SM can be associated to another clonal hematological disease (SM-AHD), typically myelodysplastic syndromes (MDS) or acute myeloid leukemia (AML): in these cases the prognosis is similar to the predominant neoplasm.
Aims: The aim of the study is to analyze the characteristics of patients with both indolent and aggressive systemic mastocytosis (SM) and the triggering factors for treatment. We also reviewed the clinical course and treatment of patients with SM-AHD.
Methods: We retrospectively reviewed the charts and collected clinical and laboratory data of 20 patients diagnosed with SM and 9 patients diagnosed with SM-AHD from January 2008 through December 2017. We focused on onset symptoms, clinical characteristics, organ damage and indication to treatment.
Results: SM: Eighteen patients (90%) sought medical evaluation because of cutaneous lesions or anaphylaxis, and 2 (10%) because of bone pain or fractures. In 12 patients (60%) SM was diagnosed after a skin and bone marrow biopsy revealed mast cell infiltration. Six patients (30%) presented with anaphylaxis and high triptase levels: as reported in literature, both characteristics are strongly associated with SM, and both underwent a bone marrow examination. While bone marrow biopsy was always positive for mast cell infiltration, CKIT D816V mutation was detected in 12 patients only. Skeleton X-ray and 18F-FDG PET/CT were performed in 16 patients: 9 (56%) presented a radiological bone involvement but only 4 of them (25%) showed increased glucose uptake. Ten patients were diagnosed with indolent SM, and did not need systemic treatment: they were given antihistamines to control symptoms. Ten patients were diagnosed with aggressive SM: 3 started tirosin kinase inhibitors (TKI) for uncontrolled degranulation causing anaphylaxis and flushing; 5 started Interferon α (IFNα) considering bone pain due to fractures or severe osteoporosis; 2 started cladribine for diffuse bone and systemic involvement. Four patients needed further therapy for transient benefit or primarily poor control of clinical conditions. Of these 20 patients, 18 are surviving at 0.5-89.5 months. SM-AHD: These patients were all diagnosed after performing bone marrow evaluation for cytopenia. Two patients were diagnosed with AML: considering age >65 y.o., they were both treated with intermediate dose citarabine associated to TKI, and died after a median of 3 months. The other 7 patients were diagnosed with MDS: 3 had skin lesion and few symptoms related to uncontrolled degranulation, none presented with bone pain or skeleton alteration at X-ray. Regarding treatment, 2 patients were treated with TKI and 1 with IFNα for underlying SM, and 4 patients with best supportive care for low risk MDS; median overall survival was 23.9 months.
Summary/Conclusion: SM is difficult to recognize because it presents with generic symptoms, as anaphylaxis, osteoporosis and fractures, or flushing and gastritis. Nonetheless, it is important to investigate unexplained bone fragility, wasp venom anaphylaxis or non-catecholamine associated flushing, possibly suggesting an underlying mastocytosis. SM-AHD are mainly treated for the predominant non-mast cell disease, associated with antihistamines for symptoms control in few cases.

PB2306

CHARACTERISTICS OF PATIENTS WITH JAK2 EXON 12 MUTATED POLYCYTHEMIA VERA

T. Subbotina[1,2,*], E. Dunaeva[3], K. Mironov[3], A. Kharsekina[1], E. Vasiyliev[4], M. Mikhalev[5], M. Osadchaya[6], M. Smelyanskaya[4], V. Khorzhevskyi[7], G. Shipulin[3]

[1]Siberian Federal University, [2]The Federal State-Financed Institution Federal Siberian Research Clinical Centre under the Federal Medical Biological Agency, Krasnoyarsk, [3]Federal Budget Institute of Science «Central Research Institute for Epidemiology», Moscow, [4]Krasnoyarsk regional hospital, [5]Municipal Budget Health Service Institution «City Clinical Hospital № [7]», [6]Krasnoyarsk Territory Department of Health Regional state budget health facility «Krasnoyarsk interdistrict clinic №[1]», [7]Krasnoyarsk State Regional Bureau of Pathology, Krasnoyarsk, Russian Federation

Background: JAK2 exon 12 somatic mutations are highly specific to confirm the diagnosis of polycythemia vera (PV) (Pardanani A *et al.*, Leukemia, 2007). It is known patients with JAK2 exon 12 mutated PV have isolated erythrocytosis and a younger age than patients with JAK2 V617F mutated PV (Scott L *et al.*, N Engl J Med, 2007; Passamonti F *et al.*, Blood, 2011).
Aims: This study aims to assess the clinical and molecular characteristics of patients with JAK2 exon 12 mutated PV from Krasnoyarsk region, Russia.
Methods: 6 patients with JAK2 exon 12 mutated PV were involved in this study. The informed consents of these patients were obtained. The PV diagnosis of 1, 2, 3, 5 and 6th patient was confirmed by bone marrow trephine biopsies histological examination. JAK2 exon 12 mutation analysis was performed by heteroduplex analysis with separation of the PCR product by electrophoresis on non-denaturing PAGE (Subbotina T *et al.*, Haematologica 2017). The identification of the JAK2 exon 12 mutation types and allele burden measurement was carried out by pyrosequencing (Subbotina T *et al.*, Haematologica 2014). Clinical and laboratory data were collected from the time of initial diagnosis to the present. JAK2 exon 12 variance^{MUT} was calculated as a measure of relative changes in allele burden between the baseline and follow-up sample (Theocharides A *et al.*, Haematologica, 2008).
Results: Some clinical and laboratory characteristics of 6 patients with JAK2 exon 12 mutated PV are reported in Table 1. The subjects were both men and women. Age of disease onset in subjects was from 28 to 72 year. Three out of these patients became ill before the age of 30 years. Duration of the disease is different: from 3 to 12 years. All 6 patients have an increased number of red blood cells, along with an accompanying increase in the concentration of hemoglobin and hematocrit level in the peripheral blood. Some of them had moderate increase number of leukocytes and platelets in the disease dynamics. All 6 patients have different mutation variant in the 12 exon of the JAK2: N542-E543del (c.1624_1629delAATGAA); I540-E543delinsKK (.1619_1627 TCAgAAATg>AAA); N542_E543del (c.1623_1628delAAATGA); R541_E543>K (c.1622_1627delGAAATG); F537_K539>L (c.1611_1616delTCACAA); H538_K539>L (c.1612_1616CACAA>TT), accordingly. These mutations have been already described and included in the COSMIC website. Two out of six patients also have a mutation JAK2V617. JAK2 exon 12 allele burdens in the sample from 1 patient are significantly increased in the disease dynamics. N°1,2,5 patients in the anamnesis had splenomegaly. N°2,4,5 patients in the anamnesis had any thrombotic events. N°2-4 patients were treated phlebotomy only and did not receive any cytoreductive treatment to date. 5 and 6 patients receive hydroxyurea (HU). N°1 patient was treated only with phlebotomy until 2016. Since 2017 cytoreductive therapy with HU has been started, but this causes to the development of hydroxyurea-induced thrombocytopenia to I-II degree. Carrying out of bone marrow trephine biopsies histological examination in 2015 years was confirmed PV and in 2017 – fibrosis manifestations. Thus, in N°1 patient simultaneously with the allele burden increase develops disease progression – the initial manifestations of the transition of the PV to myelofibrosis. Thrombotic events have not been identified.

Table 1.

Table 1. Characteristics of 6 patients with JAK2 exon 12-mutated PV

№	Age of manifestation (years)	Sex	Disease Duration of the disease (mo)	HU	JAK2 V617F	JAK2 12 exon	% JAK2 12 exon mutation baseline	% JAK2 12 exon mutation last sample	JAK2 12 exon variance MUT	Time between two acute events (mo)
1	61	M	PV*/33	Yes	20g	N542_E543 del	15	50	+233	33
2	48	M	PV*/38	No	<1%	I540_E543 delinsKK	13	8	0,5	27
3	27	M	PV/132	No	20g	N542_E543 del	15	19	0,5	84
4	~72	F	PV/64	No	30g	R541_E543 >K	33	25	0,5	42
5	29	M	PV/132	Yes	<1%	F537_K539 >L	31	26	0,5	7
6	28	F	PV/84	Yes	30g	H538_K53 9 >L	10	11	0,5	4

* – The allele burden was determined at the primary address to the doctor with MPN symptoms

Summary/Conclusion: We confirm an earlier age of disease onset, as well as the presence of isolated erythrocytosis in patients with JAK2 exon 12 mutated PV. The increase of the allele burden level detected in one patient is accompanied with disease progression.

PB2307

JAK2V617F MUTATION AS A RISK FACTOR FOR INCIDENCE OF THROMBOTIC COMPLICATIONS IN PATIENTS WITH PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

E. Simonovic*, V. Colic

Internal Medicine, General Hospital Leskovac, Leskovac, Serbia

Background: Myeloproliferative neoplasms (MPN) are the group of clonal, malignant hematopoietic stem cell disorders, characterized by the proliferation of one or more blood lines with normal or nearly normal maturing in the bone marrow and in extramedullar hematopoietic organs. The following factors give contribution to the incidence of thrombosis: increasing level of products that are formed in the activation of platelets (thromboxane, p-selectin); increased production of microparticles that are parts of various cell membrane structures of platelet origin; JAK2V617F mutation. In

patients with MPN there is increased activity of the coagulation system due to the resistance to the anticoagulant function of thrombomodulin.
Aims: The aim of this study is to monitor JAK2V617F mutation as a potential risk factor for the incidence of thrombotic complications in patients with Philadelphia negative myeloproliferative neoplasms.
Methods: During the six-year period we monitored the occurrence of thrombotic complications in 157 patients of both sexes, aged between 30 and 87 years, being diagnosed with Ph-myeloproliferative neoplasm. Patients were classified into the following groups: 1. Group with polycythemia vera (PV) (68); 2. Group with essential thrombocythemia (ET) (36); 3. Group with idiopathic myelofibrosis (IMF) (26); 4. Group with unclassified myeloproliferative neoplasm (MPNs) (27). Among possible risk factors, we monitored the presence of JAK2V617F mutation, as well as the age of patients, deviations in counts of leukocytes, the presence of cardiovascular comorbidity, and the presence of diabetes mellitus. We used methods of clinical, laboratory, endoscopy, ultrasound and CT scans.
Results: JAK2V617F mutation was statistically more significantly present in patients with PV (about 81%). In patients with ET it was noticed 58%, in patients with IMF about 38%, and in group of patients with MPNs about 48%. The highest percentage of thrombotic complications (arterial and venous) was found in the group of patients with ET, which was statistically more significant relative to PV. Thrombotic complications were in both groups more frequent in percentage with JAK2V617F positive patients, but without statistical significance. It is believed that activated neutrophils bind to platelets by influencing the increased expression of tissue factor activity, as well as the activation and damage of the endothelial cells, especially with JAK2V617F positive patients. In patients with IMF, thrombotic complications were statistically more significantly present in JAK2V617F positive patients. In patients with MPNs, thrombotic complications were more common in JAK2V617F positive patients, but with significant deviations reported in the count of leukocytes in terms of leukocytosis. Thrombotic complications in all groups, except in the IMF group, were more commonly present in patients with cardiovascular comorbidity and diabetes mellitus, but the statistical significance was present only in the group with PV and ET.
Summary/Conclusion: JAK2V617F mutation can be considered as potential risk factors for thrombosis in patients with Philadelphia negative myeloproliferative disorders. Other risk factors must be also considered, like leukocytosis populations, cardiovascular diseases and diabetes mellitus. Further monitoring of those patients and a larger number of subjects is needed.

PB2308

A NATURAL LANGUAGE PROCESSING ALGORITHM TO AUTOMATICALLY CATEGORIZE DISEASE TYPE AND STATUS FROM FREE-TEXT BONE MARROW MORPHOLOGY REPORTS

Y.-F. Wang[1,*], J.-L. Li[2], B.-S. Ko[3], C.-C. Lee[2], J.-L. Tang[1,3]

[1]Tai-Cheng Stem Cell Therapy Center, National Taiwan University, Taipei, [2]Electrical Engineering, National Tsing Hua University, Hsinchu, [3]Hematology Division, Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan, Republic of China

Background: Bone marrow (BM) aspiration is a standard diagnostic and evaluation tool utilized by physicians together with other exams to make clinical decisions for all major hematologic diseases. The morphology report includes many important morphological features of the specimens. However, these findings are often documented in an unstructured free-text format, making it extremely difficult to extract and compare information from different reports. Recently, successful applications of natural language processing (NLP) techniques for automatic categorizations of findings on pathology or electronic medical reports have been shown for diseases such as breast cancer diagnoses.
Aims: We aim to develop NLP algorithms to automatically extract key clinical information to support physicians in the clinical decision and further research for hematologic malignancies.
Methods: A total of 12549 free-text BM morphology reports dated from 2007 to 2016 were collected from the National Taiwan University Hospital. Each report consists of information on the following key clinical classifications in a free-text format: specimen quality, disease types and disease status. We first achieved word normalization of these reports through a series lexical pre-processing using Wordnet lexicon corpus. We then derived a document vector as a summary representation of each report. This vector includes a fixed dimension equals to the sum total of unique *uni- bi- tri-*gram word count in our BM dataset, and each i^{th} element reflects the importance weighting for each word in the given report. The importance weighting is computed based on term-frequency inverse-document frequency (tf(t,d)*idf(d,t)). Term frequency, tf(t,d), is the number of occurrences of i^{th} word in the d^{th} report. And, idf(d,t)=log(n/(df(d,t)+1) where df(d,t) indicates the total number of report containing i^{th} word and n is the total number of reports. We finally trained machine learning algorithms to automatically learn to extract key clinical information using class-balanced support vector machine (SVM) with this tf-idf document vectors. We evaluated our approach using a three-fold cross-validation. That is, for each fold, 9790 reports were used as training set and the remaining 2759 reports were used as the blind testing set and we iterated this for three times. The accuracy was measured by comparing the concordance rate between the algorithm categorization and the expert categorization on the testing set.
Results: All three NLP algorithms achieved high categorization performance. The disease type classification achieved 0.95 accuracies in 9 out of 11 types of categories (Figure 1). In addition, the other two algorithms achieved overall 0.98 accuracies in specimen quality classification and 0.95 in disease status classification (data not shown). Among the 11 disease type classification tasks, the algorithm achieved accuracy lower than 0.95 in 3 categories: "Lymphoma", "Other" and "Unsure Diagnosis". The relatively lower accuracy of the "Lymphoma" class may be attributed to the fact that it was used to include all subtypes of lymphoma. Similarly, the "Others" class was used as the "garbage" class where multiple diagnostics decision other than malignancies could be made, and the "Unsure Diagnosis" reflect uncertain clinical diagnostic decisions.

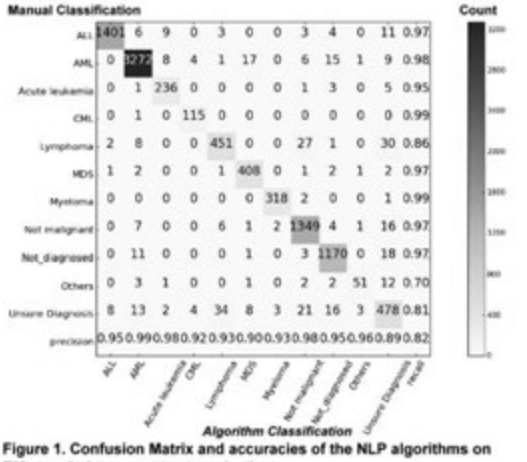

Figure 1. Confusion Matrix and accuracies of the NLP algorithms on BM morphology report categorization
Confusion matrix, precision and recall of 11-class disease type categorization.
Precision: Positive predictive value, Recall: True Positive Rate. The overall accuracy was 0.82.
Abbreviation: ALL: Acute Lymphoblastic Leukemia; AML: Acute Myeloid Leukemia; CML: Chronic Myeloid Leukemia; MDS: Myelodysplastic Syndrome; CR: Complete Response

Figure 1.

Summary/Conclusion: Our NLP algorithms could efficiently and accurately extract important information with high accuracy from a large scale free-text bone marrow morphology reports. Further research is needed to evaluate the algorithm performance on reports from other centers.

PB2309

THE INCIDENCE OF EARLY AND RECURRENT THROMBOTIC EVENTS IN EGYPTIAN MYELOPROLIFERATIVE NEOPLASMS (MPN) PATIENTS

T.E.E. Abouzeid[1,*], S. Elashwah[1], T. El- Gohary[2], R. Farag[3], R. Ghonaim[4]

[1]Hematology, Mansoura oncology center, Mansoura, [2]Oncology, Zagazig university, Zagazig, [3]Ophalmology, mansoura ophthalmic center, mansoura, [4]Clinical pathology, Zagazig university, Zagazig, Egypt

Background: Thrombotic events are considered a major complication in the follow-up, and treatment of Philadelphia negative Myeloproliferative Neoplasms (MPN), particularly polycythemia vera and essential thrombocythemia, associated with high morbidity and mortality, as reported in several retrospective studies.
Aims: To study the incidence of thrombotic events at diagnosis and during follow up of Egyptian MPN patients.
Methods: We studied 102 adult Egyptian patients diagnosed with Ph (-) ve MPN (M/F 49/53, mean age 50.13 ±12.1 years), at Mansoura Oncology center during the period from May 2012 till December 2017. Of them 47(46.1%) had Polycythemia Vera(PV), 27(26.5%) Essential Thrombocytosis(ET),22(21.6%) Myelofibrosis(MF), and 6(5.9%) Unclassified.
Results: Out of the 102 patient observed during the period of the study 32(31.45) patients was having thrombotic episodes, out of these 26 (25.5%) at diagnosis, 6 (5.9%) patient at follow up and 4 (3.9%) is said to have

recurrent thrombotic events. These thrombotic events were 15 arterial (6 cereberal.3 lower limb, 2 upper limbs. 2 hepatic, and I coronary), 13 venous (7 portal, 4splenis, and 2 lower limbs), and 4 patients were having combined arterio-venous thrombosis. As to the incidence of early thrombosis in the different MPNs, they were 14/47 (29.8%) in PV patients,8/27 (29.6%) in ET patients and 7/22 (31.8%) in PMF patients and 3/6(50%) unclassified patients. The median time from diagnosis to thrombotic event was also similar in the different MPNs (p=0.311). Several clinical features at diagnosis (age, gender, Hb levels, WBC and PLT counts, JAK-2 V617F mutation and previous thrombotic events) were evaluated for a role in predicting thrombotic events: only age and previous thrombotic events were significant. The overall survival was affected significantly with the presence of thrombosis (**P=0.097**), while the JAKII V617F positivity has no survival affection.
Summary/Conclusion: The incidence of early and recurrent thrombosis seems to be without any difference among ET, PV and PMF. Only age and previous thrombotic events had a predictive role, affecting the overall survival.

PB2310

BLOOD AND BONE MARROW CULTURES FOR THE DIAGNOSIS AND MANAGEMENT OF PRIMARY MYELOFIBROSIS

T. Saralidze[1,*], I. Mamatsashvili[1], T. Svanidze[2], T. Saralidze[3], T. Shvelidze[4], N. Gogokhia[5]

[1]Department of Internal Medicine, Tbilisi State Medical University, [2]I.Chavchavadze Av.[41]. Flat [16], Tbilisi State Medical University, department of Internal Medicine, [3]Department of Medicine, David Tvildiani Medical University, [4]Clinical laboratory, IUNONA Medical Center, [5]Clinical Laboratory, Tbilisi State Medical University, Tbilisi, Georgia

Background: Though WHO criteria for the prefibrotic and overt fibrotic stages of primary myelofibrosis (PMF) is well established, diagnosis and management of this disease often remains a challenging problem. Pathogenesis of anemia or cytopenia, that accompanies PMF, or development of post MF acute myeloid leukemia (AML) is also frequently unclear.
Aims: Our aim was to reveal peculiarities of hematopoiesis and main reasons of cytopenia in patients with PMF.
Methods: We studied blood and bone marrow cell (BMC) cultures of 7 patients with PMF in fibrotic stage using the method worked out by us. This method enables to observe hematopoiesis and detect proliferating clone in patients with hematological malignances during 3-5-7-14-21-days of cultivation of BMC. In 3-day-blood leukocyte cultures we studied macrophage-lymphocyte rosettes (MLRos) formation that reflects immune reactivity of organism. Increase of the amount of MLRos *in vitro* points to immune sensitization or immune conflict, while its decrease points to low immune reactivity of organism.

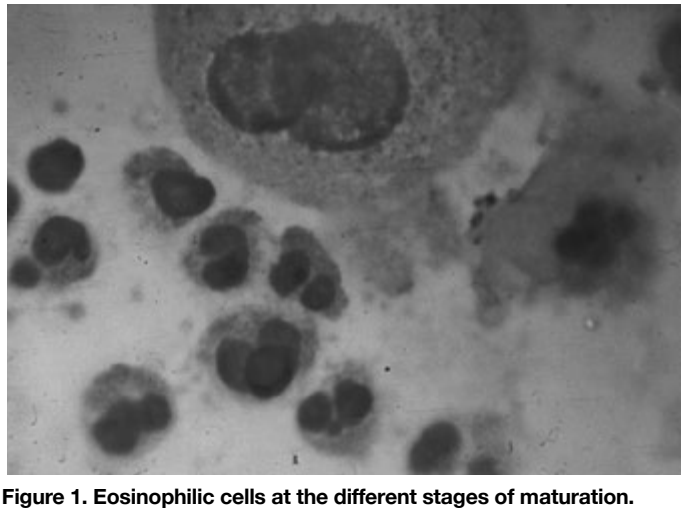

Figure 1. Eosinophilic cells at the different stages of maturation. Hypolobulated megakaryocyte (above) sequestrating platelets. 21-day-culture of bone marrow aspirate of a patient with primary myelofibrosis.

Results: Patients age was in the range of 40-70, 4- men, 3- women, all had anemia with reticolocytosis, 4 had thrombocytopenia, 1- leukopenia, except the last patient leukocytes were in the range of 8-12x10^9/L with moderate neutrophil leukocytosis and left shift to myelocytes and promyelocytes. All patients had splenomegaly (from 5-7 up to 15-18 cm), 5 had hepatomegaly (up to 5cm). BM biopsy showed megakaryocytic proliferation with reticulin and collagen fibrosis grades 2 or 3. Spleen aspirate showed myeloid metaplasia. In 3-5-days BMC cultures poor growth of myeloid cells, atypical megacaryocytes with hypolobulated nuclei and decreased erythropoiesis was observed; In 7-14-21-day cultures mainly growth of reticular stromal cells was seen, besides in one case growth of eosinophilic cells was observed (Fig.1). In 5 cases anti-erythrocyte and in 3 cases antiplatelet antibodies were also detected. In blood cultures of all patients amount of MLRos was increased significantly up to 75-85%, showing autoimmune character of cytopenia, while in norm it equals 37,4±2,2%. As above mentioned patients didn't have high leukocytosis and prominently progressive hepatosplenomegaly, they were effectively treated with prednisone 30-60mg/day. Because of frequent recurrence of cytopenia in 2 cases was done splenectomy. 1patient died after operation because of sepsis, second one was in remission for 5 years. 4 patients are alive more than 6 years and receive low dosage of steroids in case of cytopenia recurrence. 2 patients died because of disease progression. One of them developed post MF AML. Notable that in BMC culture of this patient proliferation of myeloid blast cells was revealed a month before clinical onset of AML.
Summary/Conclusion: Results of our data show that MLRos formation *in vitro* can be successfully applied for the detection of immune conflict in the pathogenesis of cytopenia in patients with PMF while use of BMC cultures is reasonable for observation and estimation of hematopoiesis *in vitro* that will help to precise diagnosis and hold tailored treatment in each case. Growth of eosinophilic cells in long-term culture confirm myeloproliferative nature of the PMF, while clonal expansion of blast cells *in vitro* predicts development of post MF AML.

PB2311

EVALUATION OF THROMBOTIC RISK IN CHRONIC MYELOPROLIFERATIVE NEOPLASMS PATIENTS

V. Popov[1,*], M. Andreescu[1], M. Omer[1], A. Trifa[2], O. Patrinoiu[1], F. Raicu[3], H. Bumbea[1]

[1]Hematology, Colentina Clinical Hospital, Bucharest, [2]Genetics, UMF Iuliu Hatieganu, Cluj Napoca, [3]Genetics, UMF Carol Davila, Bucharest, Romania

Background: Patients with chronic myeloproliferative neoplasms (MPNs) have thrombotic complications. Many reports showed that JAK2 mutation is involved in thrombosis pathogenesis. Genetic thrombophilia characterized by presence of more than 1 mutation PAI-1 4G-4G, MTHFR 677TT, V Leiden 506Q, and prothrombin 20210A could be an important risk factor.
Aims: The aim of our study was to evaluate the frequencies of these mutation in MPNs patients and association with thrombosis risk
Methods: This retrospective study included 75 patients with MPNs (36 female and 39 male), median age 65.1 (female-61.7, male 67.1). Mutational analyses identified in 52 patients presence of JAK2 mutation,14 patients have CALR mutation presence and 9 patients have no mutation. Thrombophilia testing was performed including mutations: PAI-1, MTHFR 677TT, V Leiden and prothrombin 20210A. The group of patients was splitted in group with thrombotic complications (heart infarct, pulmonary embolia, stroke, splanhnic thrombosis) represented by 21 patients and without thrombosis – 54 patients
Results: Thrombosis was present in MPNs patients JAK2 positive. Patients with CALR mutation had not thrombosis in their medical history, although they have higher count of platelet compared with MPNs patient with JAK2 mutation present (CALR group PLT 1036 x 109/L *vs* JAK2 group 847 x 109/L). Patients with thrombotic complication have not more frequent association with genetic mutations MTHFR, prothrombin or V Leiden mutations, 17/21 patients compared with 21/54 patients.
Summary/Conclusion: JAK2V617F mutation is an important risk factor for the pathogenesis of thrombosis in MPN. Association with another genetic mutations like V Leiden factor, PAI1 or prothrombin 20210A mutation could increased the risk of thrombosis but in our study we did not obtained this. We have to check these findings in a higher lot of patients

PB2312

THE SPECTRUM OF THROMBOSIS IN POLYCYTHEMIA VERA: EXPERIENCE OF A TERTIARY HOSPITAL

M. Arcas-Vega, S. Lakhwani*, A. Martín-Martín, P. Machado, S. Martín-Batista, M.T. Hernández, J.M. Raya

Hematology, Hosp. Univ de Canarias, Santa Cruz Tenerife, Spain

Background: Thrombosis is one of the most important complication among

patients with polycythemia vera (PV) and, in the bibliography, roughly one-fifth of patients with PV are diagnosed with an arterial or venous thrombotic event as a presenting feature. Fatal cardiovascular events contribute to the increased mortality rate among patients with PV and thrombotic events are so inextricably tied to the pathogenesis of PV that they are used in guiding patient risk stratification and treatment recommendations. In addition to several conventional risk factors for thrombosis, clinical data have implicated increased hematocrit and red blood cell adhesiveness, activated platelets, leukocytosis, and elevated *JAK2V617F* allele burden.
Aims: The goal of our study was to analyze the incidence and the different moment and form of presentation of thrombosis in patients with PV in our centre.
Methods: A retrospective analysis was conducted based on the review of medical records of the adult patients diagnosed of polycythemia vera in our centre from 1986 to 2017. Clinical and biological data were recorded.
Results: A total of 51 patients were analyzed (28 men and 23 women). Mean age at diagnosis was 61 years old (range 28-83). We found a total of 19 patients (37,2%) who suffered a thrombosis before or after PV diagnosis. Among patients with thrombosis before the diagnosis of PV different scenarios have been found: 1) Two patients developed a thrombotic phenomena that led to diagnosis of PV. Both suffered a stroke and one had heterozygous JAK2 mutation while the other one had mutation of exon2-JAK2. 2) Seven patients developed a thrombosis prior to PV diagnosis and without erythrocytosis [4 strokes, 1 acute myocardial infarction (AMI) and 2 pulmonary embolisms]. All of them had heterozygous JAK2 mutation except one that had homozygous mutation. 3) Four patients had a thrombosis before PV diagnosis but after the evidence of erythrocytosis (2 strokes and 2 AMI). Time from thrombosis until hematologist evaluation range from 1 to 29 months (mean 14,5 months). Time from evidence of polycythemia until hematologist evaluation range from 2 to 123 months (mean 43 months). All these patients had heterocygous JAK2 mutation. On other hand, six patients developed a thrombotic phenomenon after diagnosis (3 strokes, 2 AMI and 1 deep venous thrombosis). Two patients presented homozygous mutation for JAK2 and four heterozygous mutation. Four patients had leukocitosis at diagnosis also. About treatment, three patients were receiving hydroxyurea and two patients phlebotomy when thrombosis occurred, but two male patients had an hematocrit above 45%. Of note, five of these patients were without aspirin prophilaxis when developed the thrombosis .
Summary/Conclusion: In our series 37,2% of patients with PV developed thrombosis, especially before the diagnosis. All of them had *JAK2-V617F* mutation except one who had exon12-JAK2 mutation. Four thrombosis (21%) developed after erythrocytosis was observed but before the patient was evaluated by an hematologist. We wonder if they could have been avoided. After diagnosis, the suspension of aspirin prophylaxis by any reason, seems to be an important factor to develop thrombosis.

PB2313

SECONDARY MALIGNANCIES IN BCR-ABL NEGATIVE CHRONIC MYELOPROLIFERATIVE DISEASES

E. Arslan Davulcu*, H. Bülbül, Y. Ulusoy, F.D. Atilla, N. Soyer, F. Sahin, F. Vural, M. Tobu, M. Tombuloglu, G. Saydam

Hematology, Ege University Medical Faculty Hematology Department, Izmir, Turkey

Background: Myelofibrosis (MF), polycytemia vera (PV), and essential thrombocytosis (ET) are BCR-ABL negative chronic myeloproliferative diseases (CMPD) which may transform to acute myeloid leukemia or myelodysplastic syndrome. Furthermore, lymphoid malignancies are more prevalent in CMPD than normal population. Non-hematological malignancies are also increased in CMPD and those are related to several different etiologic factors.
Aims: Our aim is to evaluate secondary malignancies in our CMPD patients.
Methods: BCR-ABL negative CMPD patients diagnosed and followed up in Ege University Hematology department between 2000 and 2017 are retrospectively evaluated. Patients diagnosis, time of diagnosis, age, gender, mutational status regarding JAK-2, MPL and calreticulin, treatment, treatment side effects, physical examination findings, follow-up time and outcome are recorded. Patients who developed secondary malignancy are evaluated about their cancer treatment and outcome.
Results: There are 264 CMPD (138 female, 126 male) patients investigated. Distribution according to diseases are: 153 ET, 77 PV, 26 MF and 8 other CMPD. During follow-up, secondary malignancy occurred in 5 of our patients (1.89%): **Patient 1**: A male post-polycytemia MF patient, diagnosed at the age of 68. JAK-2 mutation is positive. He has been followed up since 10 years and receiving hydroxyurea 500-1500 mg per day. At the seventh year of CMPD, he was diagnosed as squamous cell skin cancer. After surgical resection, he is still in remission. **Patient 2**: A male ET patient, diagnosed at the age of 63. JAK-2, MPL and calreticulin mutations are negative. He has been followed up since 7 years. He received only hydroxyurea 1000-1500 mg per day for 6 years, hydroxyurea and anagrelide 1 mg per day combination for the last 1 year. At the fifth year of CMPD, he was diagnosed as squamous cell skin cancer on his ear. He treated only with cryotherapy and still in remission. **Patient 3**: A female ET patient, diagnosed at the age of 54. JAK-2, MPL and calreticulin mutations are negative. She has never received cytoreductive treatment. She has been followed up since 7 years. At the fifth year of CMPD, she was diagnosed as licen planus and after local therapies, her lesion is still stable. **Patient 4**: A male ET patient, diagnosed at the age of 54. JAK-2 mutation is positive. He has been followed up since 12 years. He received hydroxyurea for sometime after diagnosis but than swithced to anagrelide 2-4 mg per day since 10 years. At the eleventh year of CMPD, he was diagnosed as thyroid papillary carcinoma. After surgery,he was treated with radioactive iodine and now in remission. **Patient 5**: A female ET patient, diagnosed at the age of 43 after ischaemic cerebrovascular event. JAK-2, MPL and calreticulin mutations are negative. She has been followed up since 14 years. One year after diagnosis she received only hydroxyurea but during last 13 years she has switched to anagrelide 0.5-2 mg per day treatment. At the sixth year of CMPD, she was diagnosed as invaziveductal carcinoma and after mastectomy she received hormonotherapy. She is still in remission.
Summary/Conclusion: There are a number of evidences supporting that secondary malignancies are increased in CMPD patients compared to normal population. Although several factors, such as hydroxiurea, are blamed for that increase, the etiologic connection is yet unclear. Skin cancers have been the leading secondary malignancy (60%) in our study group. It is wise to keep in mind the reality of secondary cancers, during CMPD patients hematological management.

PB2314

RUXOLITINIB AND VITAMIN K ANTAGONISTS(VKA) IS SAFE AND EFFECTIVE AND SHOWS A FASTER RECANALIZATION AND AN ANTINFLAMMATORY EFFECT IN PRIMARY MYELOFIBROSIS(PMF) WITH SPLANCHNIC VEIN THROMBOSIS(SVT)

G. Giordano[1,*], A. Parente[2], D. Berardi[3], D. Castaldi[2], M.A. Cinotti[2], F. Vedruccio[2], V. Susca[4], L. Petrella[4], G. Berardi[5], M. Magri[6], G. Niro[6], R. Gigli[6]

[1]Medicine, Regional Hospital "A. Cardarelli", [2]Medicine, General Medicine, Campobasso, [3]Medicine, University La Sapienza, Rome, [4]Medicine, University of Molise, [5]Family Medicine, General Medicine, [6]Laboratory Medicine, Regional Hospital "A. Cardarelli", Campobasso, Italy

Background: PMF often causes SVT. Data regarding outcome of this subset of patients, mainly if treated with VKA plus ruxolitinib, are very few.
Aims: Aim of this case series is to analyze if use of ruxolitinib +VKA is safe and effective in treatment of patients with PMF with IPSS INT-2.
Methods: This study is a retrospective study. 4female patients, median age47 (R35-55), with PMF INT-2 and with SVT (2portal, 2mesenteric +splenic +portal), median Hb11.5g/dl (R11-12.5), PLT90000/mcl (R70000-100000), WBC10000/mcl (R4000-11000), peripheral blood blasts 1%(R1-2), 1 patient heterozygous for factor V Leiden received ruxolitinib 20mg/day +warfarin. All patients were Jak-2 mutated (GROUP1). In an historical cohort 6patients 2male, 4female median age60 (R45-65), 3PV, 1ET, 1PMF, 1 paroxysmal nocturnal hemoglobinuria and with SVT (4portal, 2splenic +portal), median Hb12.5g/dl (R10-13.5), PLT 150000/mcl (R110000-200000), WBC 8000/mcl (R6000-10000), peripheral blood blasts 0%(R0-1), 1 patient heterozygous for factor V Leiden, 1 for prothrombin G20210, received Hydroxyurea 1000mg (R500-1500)/day +warfarin. 3 patients were Jak-2 mutated (GROUP2). In an other historical cohort 6patients 4male, 2female median age60 (R55-70), 3liver cirrhosis, 3solid cancer and with SVT (5portal, 1splenic +portal), median Hb11.5g/dl (R9-12.5), PLT 100000/mcl (R90000-130000), WBC 4000/mcl (R2000-9000), peripheral blood blasts 0%,received only warfarin. 1 patient was Jak-2 mutated (GROUP3). All patients received an abdominal vascular doppler echography, C reactive protein(CRP) and D-Dimers(DD) mesuration at0, 3and6 months of follow-up. In patients receiving ruxolitinib circulating endothelial cells(CEC) were measured at same time (in flow cytometry with BDantibody CD146).
Results: Patients of GROUP1 showed a complete resolution of SVT in 2 cases and a partial portal recanalization in 2 cases after 3 months, without any thrombosis relapse or progression after 6 months; patients of GROUP2 showed a complete resolution of SVT in 2 cases, a partial portal recanalization in 2 cases and no resolution in 2 cases only after 6 months; patients

of GROUP3 showed a partial portal recanalization in 3 cases, no resolution in 2 cases and 1 progression only after 6 months.MedianCRP after0, 3and6 months(mg/l) was respectively inGROUP1 27(R18-33),8(R3-10),6(R3-7),inGROUP2 30(R20-35),18(R12-22),10(R7-15),in GROUP3 38(R28-40),35(R27-42),33(R22-35). MedianDD after0, 3and6 months(ng/ml) were respectively in GROUP1 6000(R4500-7000), 500(R300-1000), 130(R200-300), inGROUP2 4500(R3200-5500), 900(R850-2100), 400(R350-800), in GROUP3 2800 (R1300-3200), 4700(R3700-4900), 3500(R3000-4800). MedianCEC/ml inGROUP1 after0, 3and6 months(mg/l) were respectively 1500(R800-5500), 800(R600-1800), 500(R400-1200). InGROUP1 all patients showed reduction of spleen dimension and improuvement of systemic symptoms, InGROUP2 3 patients showed reduction of spleen dimension and0 improuvement of systemic symptoms, InGROUP3 no patients showed reduction of spleen dimension and improuvement of systemic symptoms. No patient showed side effects treatment related.
Summary/Conclusion: Ruxolitinib and VKA is safe and effective and shows a faster recanalization and an antinflammatory effect in patients with PMF with SVT. These results needs confirmation on a larger cohort of patients.

PB2315

ESSENTIAL THROMBOCYTHEMIA ASSOCIATED WITH MASTOCYTOSIS

E. Cosi*, M. Pizzi, M. Cancian, I. Bertozzi, C. Santarossa, F. Fabris, M.L. Randi

Department of Medicine, University of Padua, Padova, Italy

Background: Systemic mastocytosis with an associated hematological neoplasm (SM-AHN) is the second most common subtype of SM (estimated frequency 21-44%). In the majority of patients with SM-AHN, a myeloid stem cell malignancy is diagnosed but association of essential thrombocythemia (ET) and SM is anecdotal
Aims: In this work we described a case of Essential Thrmobocythemia associated with Mastocytosis completely diagnosed according to the more recent WHO 2016 criteria and we performed a review of all reported cases in literature about this association.

Table 1.

Sex/Age	Plts x10^9/L	Clinical findings at presentation	Molecular biology	Treatment	FU	Reference
F/65	1373	Maculo-papular skin lesions Bone lesions	-	HU	Normal plts	Krsnik (1991)
F/55	900	Bone lesions	-	none	Stable	Le Tourneau (1991)
M/64	900	Superficial adenopathy	-	HU	NO SM (3y)	
F/22	1000	Maculo-papular skin lesions	-	HU	NO SM (3y)	
M/60	1000	Bone lesions	-	HU	Normal plts. Stable 6 y	
M/68	940	Mild SM	-	HU	Normal plts. Stable 3 y	
F/31	820	Mild spleen enlargement	*JAK2V617F cKITD816V*	IFN-a	Normal plts. Stable BM after 3 y	Dobrea (2012)

Methods: We describe a 62 years-old man with thrombocytosis (600-750x10^9/L), longstanding hepato-splenomegaly and skin lesions compatible with maculopapular lesions at arms and legs roots (urticaria pigmentosa) treated with phototherapy. The patient had no lymphadenopathy. An abdomen ultrasound revealed fatty liver and splenomegaly (largest dimension 18.7cm); in addition, lateral X-ray pictures of the thoraco-lumbar spine showed multiple moderate vertebral fractures. Hemoglobin and white cells count were normal; despite a normal ferritin, homozygosity for *HFE H63D* mutation was detected. We searched for and found *JAK2V617F* mutation (allele burden 9.3%). The bone marrow (BM) biopsy, showing normal cellularity with normal M:E ratio (3:1), preserved myeloid and erythroid maturation, increased megakaryopoiesis (6-7 megakaryocytes/HPF), large/giant megakaryocytes with hypersegmented nuclei disposed in loose clusters, confirmed the hypothesis of ET. Perivascular and paratrabecular aggregates of spindle-shaped mast cells (MCs) were also present. These cells featured fine metachromatic granules and were intermingled with eosinophils and mature-looking lymphocytes. By immunohistochemistry, MCs were positive for triptase and CD117, with aberrant expression of CD25 and CD2. An increase in bone marrow reticulin fibers (MF grade 2) was present in MC-infiltrated areas. Cytogenetic analysis disclosed a normal male karyotype (46, XY). Elevated serum triptase level (123ng/mL) and oncogenic *KIT D816V mutation* definitely established the diagnosis of SM. No mutation in *TET2, ASXL1, SRSF2, RUNX1* and *CBL* genes was found. Treatment with low-dose Aspirin and Hydroxycarbamide (HU) was started and the platelets count rapidly falls to normal values. Two years after diagnosis our patient is alive and in good clinical conditions.
Results: Only seven other patients are described in English literature with ET-SM.
Summary/Conclusion: This is the first reported case of ET-SM diagnosed in agreement with the WHO 2016 criteria. Although SM-AHN are considered risk factor for reduced survival, mainly in patients older than 60 years of age, when SM coexists with ET, the less aggressive form among Myeloproliferative Neoplasms (MPN) that does not reduce the patients' expected survival, prognosis may be doubtful and the absence of mutation in other genes seems to confirm this observation. In the few ET-SM described cases as in our one, HU resulted an efficient and safety treatment.

PB2316

THERAPY OF PATIENTS WITH PREFIBROTIC STAGE OF PRIMARY MYELOFIBROSIS: LONG-TERM EFFECTS

E. Burnasheva[1,*], Y. Shatokhin[1], I. Snezhko[1], O. Matveeva[2], E. Grankina[1], A. Matsuga[3], A. Zeltser[4], E. Kuzub[1], O. Shatokhina[5], I. Eliseev[1], E. Osipyan[1]

[1]Hematology, Rostov State Medical University, [2]Out-patient department, Clinical and Diagnostic Centre "Health", [3]Hematology, City Hospital №7, [4]Laboratory of Medical Genetics, Rostov State Medical University, [5]Oncohematology, Rostov Research Institute of Oncology, Rostov-on-Don, Russian Federation

Background: The use of ruxolitinib, interferon alfa and cytostatic agents is aimed at improving quality of life, prevention of progression and complications in patients with primary myelofibrosis (PMF). It is quite difficult to identify what kind of therapy is the most efective in patients with prefibrotic stage of PMF.
Aims: To assess the effect of different therapy regimens on the course of the disease in patients with prefibrotic stage of PMF.
Methods: We analyzed 32 Jak-positive patients with PMF (diagnosed according to the World Health Organisation Classification 2016). A total of 32 patients were included in this study. The age of subject ranged from 32 to 64 years. 8 patients were males and 24 - females. The mean follow-up was 42±9 month. Bone marrow biopsy control was performed in all the patients in 31±7 month. Estimation of spleen and liver size with ultrasound was made every 6 month. Patients were divided into three groups: 8 received 6±2 courses of cladribine and then interferon alfa, 10 were treated by interferon alfa and 14 received monotherapy by hydroxycarbamid.
Results: Bone marrow biopsy control showed fibrotic stage of PMF in all groups of patients. However there was difference in spleen size. Initially there was no significant difference in spleen size in all groups of patients (range 257±34 by 109±31 mm). By the end of follow-up average size of spleen were 157±24 by 64±12 mm, 167±33 by 79±27 mm and 199±21 by 92±29 in first, second and third group respectively.
Summary/Conclusion: Therapy with cladribine, hydroxycarbamide and interferon alfa doesn't influence the evolution of PMF. Cladribinein patients with prefibrotic stage of PMF significantly improves the quality of life due to more effective reduction of splenomegaly.

PB2317

RISK FACTORS FOR INCIDENCE OF HEMORRHAGIC COMPLICATIONS IN PATIENTS WITH POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA

E. Simonovic[1,*], V. Colic[1], L. Macukanovic-Golubovic[2]

[1]Internal Medicine, General Hospital Leskovac, Leskovac, [2]Internal Medicine, Clinic of Haematology, Niš, Serbia

Background: Polycythemia vera (PV) is a clonal chronic, progressive myeloproliferative disease, resulting from transformation of pluripotent hematopoietic stem cell. Essential thrombocythemia (ET) is a clonal disorder of unknown aetiology that involves multipotent hematopoietic stem cell, and it is characterized by increased formation of megakaryocytes in the bone marrow and distinctively increased platelet counts in peripheral blood without apparent cause. Hemorrhagic syndrome is the kind of complications occurring in about a quarter of patients with PV and reaching even 60% in patients with ET. Bleeding occurs due to ineffective megakaryocytopoiesis, retention of platelets in the large spleen, qualitative platelet disorders, acquired deficiency of factors V and vWF, disseminated intravascular coagulation.
Aims: The aim of this study is to monitor the count of erythrocytes, leukocytes and platelets, as well as hemoglobin and hematocrit values, as well as the presence of JAK2V617F mutation as potential risk factors for the incidence of hemorrhagic complications in patients with polycythemia vera and essential thrombocythemia.

Methods: During the five-year period we monitored the occurrence of hemorrhagic complications in 66 patients (of both sexes, aged between 30 and 78 years), being diagnosed with PV and 28 patients (of both sexes, aged between 38 and 79 years), being diagnosed with ET. We used methods of clinical, laboratory, ultrasound and CT scans. The following possible risk factors were monitored: counts of erythrocytes, leukocytes and platelets, hemoglobin and hematocrit values, as well as the presence of JAK2V617F mutation. Aspirin administration was also monitored for prophylaxis of thrombotic complications.
Results: The highest percentage of hemorrhagic complications were in the group of patients with ET and then in the group with PV. In both groups, the incidence of hemorrhagic complications in patients older than 65 years of age was higher. The highest erythrocyte count, the highest hemoglobin and hematocrit values, as well as the highest leukocyte count was recorded in the group of patients with PV. The highest platelet count was found in the group of patients with ET. Hemorrhagic complications were more frequent in patients with platelet count over $1000x10^9$/L. JAK2V617F mutation was more commonly reported in patients with PV (89%). In those patients, the hemorrhagic complications were also slightly more frequent than in the group of JAK2 negative patients. In patients with ET, the percentage of JAK2V617F positive patients was 48. There was no statistical significance regarding the occurrence of hemorrhagic complications between JAK2 positive and JAK2 negative patients. About 70% of patients with PV and ET were put on prophylactic administration of Aspirin. Studies have shown that statistically significant number of patients from both groups, who were on the prophylactic administration of Aspirin, showed some of the hemorrhagic complications.
Summary/Conclusion: The platelet count can be considered as a significant parameter for monitoring the risk of hemorrhagic complications in patients with ET and PV. The presence of JAK2V617F mutation as a risk factor for the development of hemorrhagic complications can be considered in the context of other risk factors. Monitoring of patients who were on the prophylactic administration of Aspirin had special importance, particularly when other risk factors for prevalence of hemorrhagic complications were present.

PB2318

REAL-LIFE EXPERIENCE WITH RUXOLITINIB IN POLYCYTHEMIA VERA PATIENTS RESISTANT OR INTOLERANT TO HYDROXYUREA

E. González Pérez*, M.D.L.N. Sáez Perdomo, R. Stuckey, M.D.M. Perera Álvarez, S. Sánchez Sosa, C. Bilbao Sieyro, Y. Florido Ortega, A. Segura Díaz, M.T. Molero Labarta, M.T. Gómez Casares

Hematology Service, Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Spain

Background: Polycythemia vera (PV) is a Philadelphia chromosome-negative chronic myeloproliferative neoplasm characterized by mutation in Janus Kinase 2 (JAK2V617F), which is present in 95%–97% of PV patients. The most commonly used first-line cytoreductive agent is hydroxyurea (HU), although 1 in 4 patients become intolerant or resistant to HU. Until 2016, interferon was the main second line treatment. However, the discovery of JAK2V617F mutation and the realization of the critical role that the JAK-STAT pathway has in the pathogenesis of the disease led to the development of the JAK1/2 inhibitor ruxolitinib. Use of ruxolitinib as a second line therapy has been shown to be efficacious in clinical trials; however, few real-life experiences have been reported to date.
Aims: The main aim of this study was a preliminary analysis of the effects of ruxolitinib treatment in HU-resistant or intolerant PV patients in a single center.
Methods: Five PV patients at our hematology service were hydroxyurea resistant or intolerant and were not currently included in clinical trials. Their date of diagnosis ranged from 1999 to 2014. Analytical parameters were extracted from routine analysis. The presence of the JAK2V617F mutation was detected by RT-PCR.
Results: Of the 5 PV patients resistant or intolerant to hydroxyurea, 3 were women (60%). Three (60%) were aged 60 years and over at the date of diagnosis (mean age at diagnosis 60.4±1.67 years). Of the 5 PV patients, 3 (60%) were resistant to HU and 2 (40%) were intolerant. All patients presented the JAK2V617F mutation, with a mean allelic burden of 54.75±31.76%. Maximum HU doses were 2 g/24 h (mean range 1.5±0.71 g/24 h), and mean time with HU treatment was 6.4±6.19 years. The mean hematological parameters of the PV patients at diagnosis, at time of commencement with ruxolitinib, and date of last analysis is shown in Table 1. In general, adverse effects after commencement with ruxolitinib treatment reported were grade 1 or 2. Most common nonheamatological adverse events were asthenia, headache and pruritus. No patients has suffered any infections after of commencement with ruxolitinib treatment. With ruxolitinib, pruritus and asthenia symptoms improved for all patients, except in one patient who suffered a worsering of asthenia after commencement with ruxolitinib, but also an improvement of it after few months of treatment.. At the hematological level, there was a generalized recovery to normal levels with ruxolitinib treatment, especially platelet concentration. At the last analysis, no patients had progressed to secondary myelofibrosis and all patients were still alive.

Table 1.

	Hematocrit (%)	Hemoglobin (g/dL)	Platelets (10^3/µL)	Leukocytes (10^3/µL)	Spleen (cm)
Diagnosis date	53.4±9.79	16.94±3.25	509.8±108.20	11.76±6.28	0±0
Commencement with ruxolitinib	41.16±2,79	12.74±1.11	291.66±351.54	19.21±15.58	0.96±2.14
Last analysis	36,2±4,26	12.14±1.01	234.2±141.49	9.63±4.11	0±0

Summary/Conclusion: Five HU-resistant or intolerant PV patients treated with ruxolitinib had a generalized improvement at the hematological level and an improvement of pruritus and asthenia symptoms. The outcomes of the outlined studies suggest that ruxolitinib is generally well tolerated as a second line treatment HU-resistant or intolerant PV patients. This preliminary study will be extended to include further patients to confirm these results.

PB2319

TREATMENT WITH RUXOLITINIB FOR MYELOID MYELOFIBROSIS THE EXPERIENCE OF SINGLE CENTRE

A.-M. Moldovianu*, R. Stoia, D. Coriu

Hematology, Institutul Clinic Fundeni, Bucharest, Romania

Background: Myeloid myelofibrosis is a malignant hematologic disease characterized by clonal transformation of the stem cell precursors resulting in medullary myelofibrosis and extramedullary hematopoiesis. Ruxolitinib is a current therapeutic option for patients presenting with splenomegaly and constitutional symptoms.
Aims: The objectives of our work is to analize the results of ruxolitinib treated MF patients in Fundeni Clinical Institute.
Methods: Between January 2014 and December 2017 in our institution, 39 patients diagnosed with primary or secondary myelofibrosis were treated with ruxolitinib. Patients were stratified into risk groups according to the dynamic international prognostic scoring system. We analized the patient data regarding the dose of ruxolitinib, required support treatment including transfusions, required concomitant medication, treatment tolerance, side effects and causes of death. The response evaluation was done by assessing the evolution of constitutional symptoms and splenomegaly.
Results: From 39 MF patients treated with ruxolitinib, 37 were considered evaluable. Median follow-up period was 17 months (3-34 months). 27 (72,97%) patients are still on treatment. There were 10 (27,02%) cases of treatment stop due to: death in 6 (16,21%) patients and treatment failure in 4 (10.81%) patients. The main benefit from ruxolitinib treatment was, as our results showed, the improvement of constitutional symptoms in 31 (83,78%) of the patients. The effects on splenomegaly were similar to those described in the literature. 27 (72,92%) of the patients had a reduction of the spleen size more than 30% at one time in the follow-up period. The toxicities were especially hematologic and the majority were non-severe (grade 1-2). Dose reduction was necesary most frequently due to thrombocytopenia. There were 6 deaths, 4 of them possibly treatment related: 3 cases of respiratory infection and one case of urosepsis. Two patients had acute leukemia transformation of the disease.
Summary/Conclusion: Even there are no studies demonstrating the impact of ruxolitinib treatment on overall survival in myelofibrosis patients, its benefits on reducing splenomegaly and improving the constitutional symptoms make ruxolitinib the best available therapy as a symptomatic treatment.

PB2320

A HITHERTO UNDESCRIBED VHL SINGLE NUCLEOTIDE POLYMORPHISM INDUCED/RELATED POLYCYTHAEMIA ENTITY, CLINICAL FEATURES AND FAMILIAR CHARACTERISTICS. CLINICAL APPROACH

M. Udvardy*, G. Remenyi, Z. Simon, A. Illes

Department of Haematology, Bone Marrow and Cell Therapy Unit, Debrecen Medical University, Debrecen, Hungary

Background: At our outpatient we recorded 5 cases of young patients (tweens or tweens) who had remarkably high hemoglobin levels (175-200g/l range) and had mutation of Jak2, Jak exon 12,mpl W 515. None of them were heavy smoker. Their erythropoietin levels were in the lower normal range. Their erythrocytosis was isolated, no splenomegaly, minimal skin signs were detected. Interestingly a strong familiarity documented high heaemoglobin levels in young relatives were also detected along more frequent other oncological disorders.
Aims: Our aims were: 1. To find aetiologic factors for this unusually early polycthaemia syndroma, familiarity and oncologiaval associations; 2. try to characterise clinically this unusual cases and the clinical course; 3. try to establish therapy and followup for this peculiar clinical settings.
Methods: Considering oilycytheaemia, familiarity and oncogical disease associated cases we performed von Hippel Lindau gene (VHL) sequenations form tha plasmna of patients of some of first degree relatives. VHL sequenations were performed PCR amplification of VHL gene coding regions Intron sequences followed by direct fluorescent sequenation. This work had been performed by Dr Istvan Balogh, University Lab. Medicine institute, his work is kindly acknowledged None of the samples so far sequenced revealed any of the true, classical VHL gene mutation, but a polymorphism of the intron (IVS1-195-nt) before the 1st exon: namely rs779805 G>A, similar to what had been described in chinese population without blood count abnormalities (RCC, prostatic ,large bowel cancer association.
Results: In all proband cases, and 1st degree relatives we have found the same VHL gene single nucleotide polymorphism in homozygous or heterozagous form, namely rs779805 G>A. We were able to document high isolated hemoglobin levels in parents, siblings, nephiew, grandson, etc. Some of them had clear renal cell cancer (which also aoccurs in true VHL syndrome), melanoma, large bowel cancer, benign osteoclastomoa, Hodgkin. Leukocytosis, high platelet counts were not observed. Splenomegaly or skin signs were mild or absent. Due to the short observation period we did not find vascular complications, or MPN type transformations. Our treatment was cautious phlebotomy.
Summary/Conclusion: A Chinese survey clearly documented that rs779805 G>A, VHL gene single nucleotide polymorphism can be morre frequently associared with renal,large bowel, or skin cancer. They found this polymorphism much less in caucasians. none of these surves mention polycythemia or any other haematological abnormalities. So our findings might be considered as new ones. We do suggest to perform VHL sequenation in young peorple with otherwise unexplained polycythemia, especially with familiarity. We are going to open avenues cooperative efforts, how to proceed clinically with this cohort of oatients, and perform in depth genetical background analysis.

Figure 1: Native thiols/ Total Thiols percent ratio for PV group and control group

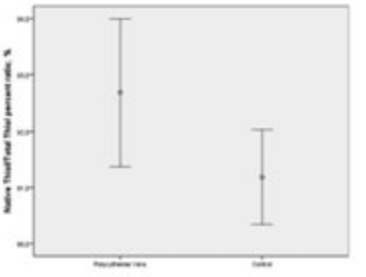

Figure 1.

PB2321

THIOL – DISULPHIDE HOMEOSTASIS IN POLYCYTHEMIA VERA

A. Senturk Yikilmaz[1,*], S. Akinci[2], S.M. Bakanay[1], F. Mustafayev[3], M. Alışık[4], S. Maral[2], Ö. Erel[4], I. Dilek[1]

[1]Department of Hematology, Yıldırım Beyazıt University,, [2]Department of Hematology, [3]Department of Internal Medicine, atatürk education and research hospital, [4]Department of Medical Biochemistry, Yıldırım Beyazıt University,, ANKARA, Turkey

Background: The balance of the thiol-disulphide homeostasis shifted to reductive thiol side in the Polycythemia Vera (PV).
Aims: This study aims to demonstrate in PV patients the thiol disulphide homeostasis which is known to play a role in cell proliferation, apoptosis and various steps of cell cycle.
Methods: Forty-two PV patients and 47 healthy controls were included in the study. Serum total (–SH + –S–S–) and native (–SH) thiol levels were measured in all subjects. The amount of dynamic disulphide bonds and, the ratio of (–S–S–) and (–S–S–) × 100/(–SH), (–S–S–) × 100/(–SH + –S–S), and –SH ×100/(–SH + –S–S–) were calculated with automatic method. The data obtained from the patient group were compared with the control group.
Results: Both groups were similar in terms of age and gender distribution. Compared with the control group, PV group had significantly higher native thiol, total thiol and nativ/total thiol levels.
Summary/Conclusion: In accordance with the nature of the disease, thiol balance in PV patients was in favor of proliferation. Increased total thiol (–SH + –S–S), native thiol (–SH) levels and native thiol/total thiol ratio might be associated with uncontrolled proliferation. his change can provocate proliferation status of the disease and/or may be secondary to the disease.

Non-Hodgkin lymphoma – Biology & Translational Research

PB2322

NEW POTENTIAL CANDIDATE GENES IN MANTLE CELL LYMPHOMA

O. Cédile[1,2,*], M.C. Hansen[1,2], L.H. Ebbesen[3], H.H.N. Bentzen[3], M. Thomassen[4], T.A. Kruse[4], S. Kavan[4], M.B. Møller[2,5], T.K. Kristensen[2,5], J. Haaber[1], N. Abildgaard[1], C.G. Nyvold[1,2]

[1]Department of Haematology, [2]Haematology-Pathology Research Laboratory, Odense University Hospital, Odense C, [3]Department of Haematology, Aarhus University Hospital, Aarhus, [4]Department of Clinical Genetics, [5]Department of Pathology, Odense University Hospital, Odense C, Denmark

Background: Mantle cell lymphoma (MCL) is a B cell non-Hodgkin lymphoma characterized by the translocation of the cell cycle regulator cyclin D1 (CCND1) under control of the immunoglobulin heavy chain (IGH) locus leading to the constitutive overexpression of CCND1 and cell cycle deregulation. The survival of MCL patients is still poor, especially for patients resistant to frontline therapy. Despite the remissions observed in patients, relapses often occur with disseminated lymphoma and are often more difficult to treat. There is a need for a better understanding of the clonal heterogeneity in MCL and to identify new genes, which could be targeted by novel drugs or be used as biomarkers to predict response to treatment.
Aims: We previously showed the genetic complexity and clonal evolution in MCL by exome analysis in paired samples at diagnosis and relapse and identified new mutations in genes involved in B-cell signaling pathways. In the current study, we have investigated the heterogeneity in gene expression in the same cohort of patients.
Methods: Malignant B cells at diagnosis and relapse from 4 MCL patients were sorted as presented at the EHA meeting in 2017 and subjected to total RNA sequencing together with CD19+ enriched B cells from 3 healthy donors. Six genes aberrantly expressed in MCL samples compared to healthy CD19+ B cells were selected and validated by qPCR in independent cohorts of diagnostic samples from MCL and chronic lymphocytic leukemia (CLL) patients. Peripheral blood mononuclear cells from 20 MCL and 20 CLL patients as well as CD19+ enriched B cells from 10 healthy donors were included. Exemption from informed consent was approved by the National Ethical Committee.
Results: The transcriptome analysis pointed to 19 upregulated genes. The expression of these genes varied between patients but also between paired diagnosis and relapse samples. This suggested clonal evolution or malignant progression and inter-patient heterogeneity, supporting our previous study. We selected 6 upregulated genes that were mainly associated to B cell signaling (CD1c, BLNK, MAP4K1, CCDC50, LILRA4 and PTPRJ) and explored the expression levels in MCL, CLL and healthy CD19+ B cells. While BLNK and CCDC50 were previously reported as highly expressed in MCL, we showed that BLNK expression was similar in MCL and CLL but slightly decreased compared to healthy CD19+ B cells. No difference was observed between MCL, CLL and healthy CD19+ B cells for the CCDC50 or MAP4K1 genes. CD1c was detected in MCL and CLL, although downregulated compared to healthy CD19+ B cells, with a lower expression in CLL than in MCL. This supports its expression in malignant B cells. Few studies detected PTPRJ and LILRA4 in MCL. We showed that PTPRJ was upregulated in MCL and CLL compared to healthy CD19+ B cells with a higher expression in MCL than in CLL. Interestingly, both MCL and CLL displayed 2 clear and different LILRA4 expression levels where the expression in healthy CD19+ B cells displayed an intermediate level.
Summary/Conclusion: Our transcriptome analysis supports the genetic complexity and the clonal evolution in MCL. It also identifies the genes CD1c, PTPRJ and LILRA4 to be aberrantly expressed in MCL with differential regulation of CD1c and PTPRJ in MCL and CLL. This study proposes new candidate genes to target with drugs or to use as biomarkers in MCL.

PB2323

THE ROLE OF MULTIPARAMETER FLOW CYTOMETRY IN THE WORK-UP OF IGM-MONOCLONAL GAMMOPATHIES

A. Mestice*, P. Curci, A. Vitucci, M. Urbano, V. Carluccio, S. Zucano, P. Carluccio, T. Perrone, A. Giordano, G. Specchia, R. Rizzi

Hematology Section, Dept. of Emergencies and Organ Transplantation, Bari University Medical School, Bari, Italy

Background: IgM-Monoclonal Gammopathy of Undetermined Significance (MGUS) accounts for 15 to 20% of all MGUS cases and it poses a unique diagnostic challenge as it can be associated with a broad spectrum of pathological processes including both B-cell lymphoproliferative disorders (LPD), and monoclonal-IgM related-disorders (IgM-RD). Among all, IgM-MGUS mostly progresses into Waldenström's macroglobulinemia (WM). According to the II International Workshop on WM (IWWM), IgM-MGUS and WM can be differentiated by absence *versus* presence of marrow infiltration by malignant B-cells, while smoldering WM (sWM) from sympomatic WM are differentiated by presence *versus* absence of clinical findings due to bone marrow (BM) infiltration.
Aims: Based on previous literature data, we aimed to evaluate the contribute of multiparameter flow cytometry (MFC) to discriminate between IgM-MGUS and WM by searching for clonal B-lymphocytes in BM and PB samples.
Methods: A total of 102 patients (64/38, M/F) with a median age of 70 yrs (36-89) were investigated. They were selected among patients with an IgM monoclonal gammopathy not associated with B-cell LPD other than WM. Median serum monoclonal (M)-protein level was 1.0 g/dl (0.2-3.9) and light-chain was kappa in 75% of cases. According to the II IWWM criteria, 52 patients were diagnosed as having MGUS, 23 sWM, and 27 WM. Fifty-one BM aspirates and 85 PB samples were immunophenotyped for evaluation of lymphocyte subsets and detection of clonal restriction by Ig K and λ light chain analyses on B-cell surface, irrespectively from B-cell proportion; all PB samples resulted positive for clonal restriction, and all BM aspirates were further analyzed with a large panel of MoAbs; data acquisition was performed on a Beckman Coulter Navios flow cytometer and analyses were performed by using Kaluza software.
Results: The median percentage of mature B-cells was found 1.8 (0-50) in PB, and 16 (1.3-50) in BM samples. Overall, in 56 out of 104 patients, clonal B-cells were detected; in particular, clonal restriction was demonstrated in 79% of BM and 40% of PB samples. The FCM results are summarized in the following table with regard to the different diagnosis. Of note, the identification of clonal populations required accurate gating strategies in 5 PB and 1 BM samples because of the presence of low clonal B-cell number within total B-lymphocytes.

Table 1.

	MGUS		sWM		WM	
	PB	BM	PB	BM	PB	BM
Number	47	12	16	15	32	24
Median lymphocytes % (range)	25 (12-38)	24 (8-41)	28 (7-51)	21 (12-34)	29 (10-65)	39 (13-61)
Median B-lymphocytes % (range)	3 (0-14)	3 (1-16)	1.5 (0-25)	4 (1-7)	1.4 (0-50)	26 (4-50)
Cases with clonal restriction (%)	9 (19)	5 (42)	10 (62)	12 (80)	15 (68)	24 (100)
Cases with involved/uninvolved light chain >10	3 (6)	4 (33)	3 (19)	5 (33)	7 (32)	21 (87)

Summary/Conclusion: Our results suggest a pivotal role of MFC in the diagnostic work-up of patients with IgM monoclonal gammopathies. Despite in most cases malignant cells display a non-specific phenotype, accurate gating strategies can enable to identify low-sized clonal populations within normal B-cell background in BM as well as in PB samples. In addition, further studies could establish a possible role of PB FCM studies in the management of patients with IgM monoclonal gammopathies.

PB2324

SARCOPENIA IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA IS CAUSED BY THE DEFICIENCY OF ESSENTIAL AMINO ACID TRYPTOPHAN

S. Ninomiya[1,*], N. Nakamura[1], H. Nakamura[1], J. Kitagawa[1], T. Hara[1], K. Saito[2], M. Shimizu[1], H. Tsurumi[1,3]

[1]Hematology, Gifu University Graduate School Of Medicine, [2]Internal Medicine, Gihoku Kosei Hospital, Gifu, [3]Hematology, Matsunami General Hospital, Hashima, Japan

Background: Sarcopenia, which is defined as the depletion of skeletal muscle, is associated with unfavorable outcomes in patients with some types of cancer including hematological malignancies. We previously reported that sarcopenia was a poor prognostic factor in patients with diffuse large B-cell lymphoma (DLBCL). However, the mechanism of development of sarcopenia has not been fully understood. Essential amino acid tryptophan is metabolized by indoleamine 2,3-dioxygenase (IDO) via kynurenine pathway. IDO is one of the immunosuppressive factor in tumor microenvironment. IDO was expressed in DLBCL tumor sites, and high IDO expression was a poor prognostic factor.
Aims: We hypothesized that the sarcopenia could be caused by the deficiency of essential amino acid tryptophan.

Methods: We retrospectively analyzed patients with DLBCL who treated in Gifu University Hospital. Skeletal muscle was measured by the analysis of CT images at the L3 level when they were diagnosed. The surface of muscular tissues was selected according to the CT Hounsfield unit. This value was normalized for stature in order to calculate the L3 skeletal muscle index (L3 SMI, cm2/m2). Serum concentrations of tryptophan and tryptophan metabolites, such as kynurenine were measured by HPLC. C2C12 myoblast cells and IDO-expressed lymphoma cell line (Raji-IDO) were used for investigating the effect of IDO on muscle.
Results: The value of L3 SMI was ranged from 27.2 to 52.2 cm^2/m^2. The levels of serum tryptophan was ranged from 20.2 to 105.7 μM, and serum kynurenine was ranged from 0.678 to 6.78 μM. We found that there was a positive correlation between the value of L3 SMI and the serum tryptophan level in DLBCL patients (p=0.015). These results indicated that tryptophan could be involved in clinically maintaining muscle mass. Therefore, we examined the effect of tryptophan and kynurenine on proliferation and myotube formation of C2C12 myoblast cells. We found that tryptophan depletion and kynurenine supplementation significantly reduced diameter of myotube from 248 to 110 μm and the number of myotube from 24.8 to 3.5 per field during 7 days. Interestingly, the depletion of leucine did not inhibit the proliferation and differentiation of C2C12 cells. Next, C2C12 were co-cultured with Raji cells expressing IDO for 7 days. The proliferation of C2C12 cells were inhibited by IDO-expressing cells, which metabolite tryptophan and produced kynurenines.
Summary/Conclusion: Sarcopenia in patients with DLBCL was caused by deficiency of tryptophan, which is metabolized by IDO. The reason why IDO positive DLBCL have poor prognosis might be not only tumor immunosuppression but also sarcopenia. Tryptophan supplementation and IDO inhibitors may prevent sarcopenia and improve the prognosis of DLBCL patients.

PB2325

DETECTION OF MINOR BLOOD INVOLVEMENT IN MYCOSIS FUNGOIDES: PROGNOSTIC IMPLICATIONS

M.T. Cedena*, F. Mirás, L. Montejano, R. Sánchez, R. Ayala, J. Martínez-López

Hematology, H. 12 Octubre, Madrid, Spain

Background: Mycosis fungoides (MF) is the most frequent cutaneous T-cell lymphoma, generally with indolent course. It is accepted as a marker of poor prognosis a tumor load in peripheral blood of more than 1,000 cells/microL or more than 20% of the lymphocytes (B2), and equivalent to the nodal involvement (1). Less evidence exists on whether the detection of a lower tumor load in peripheral blood (B1<1,000 cel/microL) may have prognostic implications. In these cases of low tumor load, the manual Sézary count is difficult and requires a high experience. Techniques such as flow cytometry (FCM) and PCR (TCR gamma rearrangement) can help us to detect the presence of peripheral blood disease. Recently, the EORTC lymphoma cutaneous task force has recommended the FCM definition of blood-class even in patch/plaque/tumor MF (2), although the clinical implication of this minor blood involvement is to be defined.
Aims: To assess the degree of agreement between the results in the detection of Sézary cells in peripheral blood between FCM and PCR and the prognostic significance of the detection of low tumor load (stage B1).
Methods: The results obtained from 49 patients diagnosed of MF were retrospectively studied. The presence of circulating Sézary cells was analyzed by two techniques. For FCM, according to EORTC recommendations (1), presence of circulating Sézary cells was considered when the CD4/CD8 ratio was ≥10, or when the percentage of CD4 T negative for CD7>40% and/or for CD26>30%. The TCR gamma rearrangement was performed in parallel to identify clonal T cells. In addition, staging and survival data were collected.
Results: We identified 49 patients with MF, a median age 62 (19-88) years and 60% males. Samples of peripheral blood were sent in parallel for analysis by both techniques, at different times of the disease. Samples of 22/49 patients (45%) presented circulating Sézary cells according to FCM: in 7/22 sample with lymphocytes CD4 T>5,000/uL; in 6/22 samples, ratio CD4/CD8>10; in 10/22, CD3dim o negative; in 16/22, CD7dim or negative; in 21/22 samples, CD26 was negative. In 27/49 (55%) peripheral involvement was detected by either or both methods, FCM or PCR. The degree of agreement was moderate (kappa =0.457, p=0.001). There were discrepancies in results in 13/49 (27%) patients (8 positive exclusively for FCM and 5 positive only for PCR). All patients but one with positive samples only by FCM presented CD4 T<5,000/microL, ratio CD4/CD8<10, and CD7 or CD26 negativity were between 35-60%. It is known that a subtype of central memory CD4 T cells shows similar phenotype (CD2dim, CD7dim or negative, CD3dim, CD26negative) and certain overlap between normal T cells and Sézary cells could exit. With a median follow-up of 16.5 (1-48) months after the study, there were significant differences in survival between the group of peripheral blood involvement B2 with respect to the other two groups, without involvement (B0) or with low tumor burden (B1) (p= 0.039) (Figure 1). No significant differences were found in survival between stage B0 and stage B1.

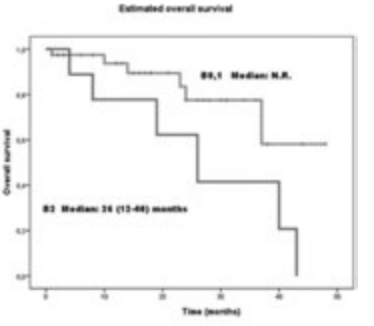

Figure 1.

Summary/Conclusion: FCM is a useful tool for detection of low load of circulating Sézary cells, being CD26 negative the more sensitive marker to discriminate them. Because its phenotyping overlaps with normal cells, false positive could be discarded with an additional assay, such as PCR. The detection of a minimal expression in peripheral blood (<1,000 cells/microL) does not seem to impact the survival of these patients in our series.

References
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PB2326

POTENT MYC AND RUNX COLLABORATION IN LYMPHOMA PERTURBS T-CELL RECEPTOR SIGNALLING AND ATTENUATES P53 TRANSACTIVATION

J. Hay[1,2,*], K. Gilroy[2,3], C. Huser[2], A. Kilbey[2,4], A. McCallum[2], E. Cameron[5], J. Neil[2]

[1]Paul O'Gorman Leukaemia Research Centre, [2]Molecular Oncology Laboratory, Centre for Virus Research, [3]Institute of Cancer Sciences, [4]Beatson Institute for Cancer Research, [5]School of Veterinary Medicine, University of Glasgow, Glasgow, United Kingdom

Background: Over-expression of MYC and RUNX family members collaborates strongly to drive lymphoma development, through their ability to neutralize their fail-safe methods of growth arrest and apoptosis. Although transgenic *Runx2* and *Myc* can independently collaborate with mutational loss of *p53* to induce T-cell lymphomas, their oncogenic cooperation overcomes the need to lose *p53* function.
Aims: To further investigate the potent oncogenic collaboration of *Runx2* and *Myc* and its mechanisms of neutralizing pressure for mutational loss of the *p53* pathway in vivo.
Methods: This oncogene collaboration has been studied further through gene expression array analysis of premalignant thymic tissue from a *Runx2/Myc* transgenic mouse model (GIM). Gene expression in 10 day old pre-lymphomatous *Runx2/Myc* thymus was examined compared to normal age-matched controls.
Results: The transcriptome of *Runx2/Myc* thymus shows predominantly gene up-regulation, and the most significantly altered genes are enriched for Myc and Runx binding motifs in the promoter proximal and enhancer regions respectively, suggesting indirect co-regulation of key target genes. Notably, screening of known post-translational modifiers of *p53* function reveals that the most significantly up-regulated gene is *Smyd2*. A role for SMYD2, a lysine methyltransferase, was further supported by its ability to block RUNX-induced senescence in primary fibroblasts. *Runx2/Myc* lymphoma lines lacking *p53* are resistant to a potent SMYD2 inhibitor (BAY-598) but our findings do not exclude an essential role for SMYD2 *in vivo*.
Summary/Conclusion: We have previously observed potent collaboration of *Runx2* and *Myc* in lymphoma development, and their ability to bypass the need for genetic loss of the *p53* pathway. To better understand this collaboration, we employed a gene expression microarray approach. This study showed a predominance of up-regulated genes in the *Runx2/Myc* (GIM)

mice compared to controls. Analysis of a panel of established *p53* modifiers showed up-regulation of several genes, including *Smyd2* and *Prmt5*. A role for SMYD2, a lysine methyltransferase, was further supported by its ability to block RUNX-induced senescence in primary fibroblasts. *Runx2/Myc* lymphoma lines lacking *p53* are resistant to a potent SMYD2 inhibitor (BAY-598) but our findings do not exclude an essential role for SMYD2 *in vivo*.

PB2327

THREE-DIMENSIONAL CULTURING INDUCES CHEMORESISTANCE AND INVASIVE CAPACITY OF MOUSE LYMPHOMA CELLS BY UPREGULATION TIAM-1/NOTCH

M. Ikram*

Anatomy, Pusan National University, Swat, Pakistan

Background: Lymphomas are a heterogeneous group of lymphoproliferative disorders of B and T cell origin that are treated with chemotherapy drugs with variable success rate that has virtually not changed over decades due to their more complex nature than solid malignancies. Till date new classes of chemotherapy and metabolic drugs have emerged, however durable responses to these conventional and new therapies are achieved in a limited number of cancer patients, with many individuals experiencing resistance to the drugs.
Aims: The paucity in our understanding of what regulates the drug resistance phenotype and establishing a predictive indicator is, in great part, due to the lack of adequate *ex vivo* lymphoma organoids models to accurately study the effect of microenvironmental signaling in which Non Hodgkin lymphomas cells arise and properly studied. Therefore, is a need to develop 3D tissues models that mimic the NHL microenvironment model for NHL biology and drug responses.
Methods: 3D cells culture methods including hydrogel, viability assay like WST based analysis, Annexin/PI staining, RT-PCRs for genes analysis, confocal microscopy and western blot analysis for protein expression analysis, Flow cytometric analysis for single cells sorting and cancer stem cell analysis, knock down effects by using specific inhibitions or designing siRNA of specific signaling molecule.
Results: we explored strategies to enhance chemosensitivity to doxorubicin, an important chemotherapeutic drug widely used for the treatment of hematological malignancies. Lymphoma cells grown in this model exhibited excellent biomimetic properties compared to conventional 2D culture including (1) enhanced chemotherapy resistance, (2) suppressed rate of apoptosis, (3) upregulated expression of drug resistance genes (MDR1, MRP1, BCRP and HIF- 1α), (4) elevated levels of tumor aggressiveness factors including Notch (Notch-1, -2, -3, and -4) and its downstream molecules (Hes-1 and Hey-1), VEGF and MMPs (MMP-2 and MMP-9), and (5) enrichment of a lymphoma stem cell population. Tiam1, a potential biomarker of tumor progression, metastasis, and chemoresistance, was activated in our 3D lymphoma model. Remarkably, we identified two synergistic therapeutic oncotargets, Tiam1 and Notch, as a strategy to combat resistance against doxorubicin in EL4 T and A20 B lymphoma.
Summary/Conclusion: Therefore, our data suggest that our 3D lymphoma model is a promising *in vitro* research platform for studying lymphoma biology and therapeutic approaches.

PB2328

FUNCTIONAL SCREEN FOR 177LU-LILOTOMAB SATETRAXETAN DRUG COMBINATIONS FOR TREATMENT OF AGGRESSIVE DIFFUSE LARGE B-CELL LYMPHOMA

G.E. Rødland[1], K. Melhus[2], J. Dahle[2], R. Syljuåsen[1], S. Patzke[2,*]

[1]Radiation Biology, OUH Norwegian Radium Hospital, [2]Research and Development, Nordic Nanovector ASA, Oslo, Norway

Background: CD37 is an internalizing transmembrane glycoprotein widely expressed on mature B-cells and B-cell malignancies. The next generation anti-CD37 radioimmunoconjugate (RIC) ^{177}Lu-lilotomab satetraxetan (Betalutin®), containing the beta-emitting radionuclide lutetium-177, is currently being tested as one-time injection therapy in a clinical phase 2b trial for follicular lymphoma (FL) and phase 1 trial for diffuse large B-cell (DLBCL) non-Hodgkin B-cell lymphomas.
Aims: The present work is to identify proliferation inhibiting drug combinations with ^{177}Lu-lilotomab satetraxetan in radioimmunotherapy resistant DLBCL cell lines.
Methods: Cell cycle progression and DNA damage induction in response to ^{177}Lu-lilotomab satetraxetan treatment was studied by flow cytometry in six human DLBCL cell lines. Two identified treatment resistant cell lines were treated for 18 hours with ^{177}Lu-lilotomab satetraxetan (600 MBq/mg; 1 or 0.5µg/ml), washed, and seeded on micro-well plates pre-printed with a drug library of 384 approved anti-cancer compounds at 10, 100, and 1000 nM f.c. (Selleck). Cell viability was monitored at days 3 to 6 post seeding using a RealTime-Glo™ MT Cell Viability Assay (Promega). Drug combinations were scored by effect size compared to drug or ^{177}Lu-lilotomab satetraxetan treatment alone.
Results: We identify two aggressive activated B-cell like DLBCL cell lines, U-2932 and RIVA, as resistant to treatment with ^{177}Lu-lilotomab satetraxetan (1 µg/ml) *in vitro*. Both cell lines arrest in G_2-phase with high amount of DNA damage (γH2AX staining) 18h after treatment, but display more than 80% viability six days post treatment. Two independent consecutive screens identify both cell line specific and shared compounds, which in combination with ^{177}Lu-lilotomab satetraxetan result in growth inhibition greater than the additive effect of single treatments alone. Compounds scoring as hit in both investigated cell lines are considered candidates for combination therapy with ^{177}Lu-lilotomab satetraxetan. Redundant hits are enriched in inhibitors targeting cell cycle kinases that regulate transition through mitosis, such as CDK1/2, AURKA, PLK1, as well as enzymes with important roles in DNA integrity surveillance and repair, such as Topoisomerases. Several identified compounds are in clinical trials for treatment of non-Hodgkin's lymphomas. Selected hits, comprising antimitotic small molecule kinase inhibitors, are currently under further functional characterization.
Summary/Conclusion: ^{177}Lu-lilotomab satetraxetan shows promising activity against different DLBCL cell lines, but treatment resistance in an *in vitro* assay is evident for triple hit lymphoma cell lines U-2932 and RIVA. Combinatorial drug screening identifies ^{177}Lu-lilotomab satetraxetan resistance reversing targets. Candidate compounds under clinical development for lymphoma treatment present particularly interesting leads for further functional characterization in mouse xenograft models of human lymphoma and new avenues for future radioimmuno-combination therapy.

PB2329

STRONG PD-1 EXPRESSION ON TUMOR-INFILTRATING T-CELLS IN DIFFUSE LARGE B CELL AND FOLLICULAR LYMPHOMA

S. Bellesi[1,*], F. Corrente[1], A. Cuccaro[1], F. D'Alò[1], P.L. Puggioni[1], E. Maiolo[1], M. Martini[2], L.M. Larocca[2], V. De Stefano[1], S. Hohaus[1]

[1]Istituto di Ematologia, Università Cattolica del Sacro Cuore, [2]Istituto di Anatomia Patologica, Università Cattolica del Sacro Cuore, ROME, Italy

Background: Blocking the PD-1 immune checkpoint is a therapeutic strategy with unprecedented clinical efficacy in the treatment of advanced cancers including hematological malignancies. Evaluation of PD1 expression in lymphoma biopsies has been studied using immunohistochemistry.
Aims: The aim of the present study was to assess the expression of PD-1 in lymph node suspensions of 67 patients with lymphomas compared to reactive and metastatic lymph nodes using flow cytometry (FCM).

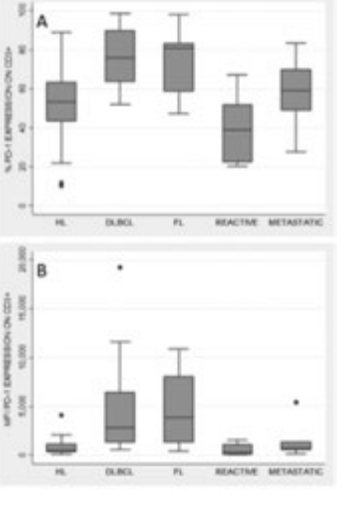


Figure 1.

Methods: Lymph node biopsies were obtained at diagnosis from 16 patients with diffuse large B-cell lymphoma (DLBCL), 11 patients with follicular

lymphoma (FL), 27 patients with Hodgkin lymphoma (HL), 6 patients with metastatic carcinomas and 7 patients with reactive lymph nodes. Lymph node suspensions were prepared by mechanical disaggregation of solid tissue using the Medimachine system (BD Biosciences). PD-1 (CD279, clone EH1 2.1, BD Biosciences) expression was assessed on CD3+, CD4+ and CD8+ T-lymphocytes. Fluorescence Minus One (FMO) was used in setting the gate for positive events. Data were acquired on BD FACSCantoII flow cytometer (BD Biosciences) and analyzed using BD FACSDiva software (BD Biosciences). Data were expressed as percentage of expressing cells and median fluorescence intensity (MFI).

Results: PD-1 was expressed on 53% (median, range: 10% to 89%) of tumor-infiltrating T cells in HL, which was not statistically different from PD1 expression on T cells from reactive (39%, p=0.149) and metastatic lymph nodes (59%, p=0.241). In contrast, T cells from lymph node biopsies from both DLBCL (n=16) and FL (n=11) showed higher expression of PD-1 on CD3+ T-cells (76% and 81%, respectively), when compared to both HL (53%, p<0.01) and to reactive lymph nodes (39%, p<0.01) (Fig. A). The higher PD-1 expression on CD3+ population of DLBCL and FL was also confirmed when analyzing the median fluorescence intensity. MFI on tumor-infiltrating T cells was 2855 (median, range: 582 to 19238) in DLBCL and 3889 (median, range: 451 to 10895) in FL, while it was only 536 (median, range: 88 to 4085) in HL and 339 (median, range: 69 to 1573) in reactive lymph nodes (p<0.001) (Fig. B). The pattern of PD1 expression on the CD3+ T cells was stable across CD4+ and CD8+ T-cells subpopulations in all samples. No significant difference was observed in PD1 expression on tumor-infiltrating T cells between DLBCL and FL.

Summary/Conclusion: Flow cytometric evaluation yields rapid and quantifiable information on PD1 expression of tumor-infiltrating T cells. We found strong PD1 expression on tumor-infiltrating T cells in lymph node biopsies of patients with newly diagnosed B-cell Non-Hodgkin lymphomas, both of diffuse large B cell and follicular type. This expression was stronger than on T cells from HL biopsies and reactive lymph nodes. Further studies are needed to assess the utility of flow cytometric evaluation of PD1 expression as a biomarker for immune checkpoint inhibition.

PB2330

BONE MARROW STROMAL MICROENVIRONMENT PRECURSOR CELLS IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA WITHOUT BONE MARROW INVOLVEMENT

E. Fastova*, N. Petinati, N. Sats, N. Drize, A. Magomedova, S. Kravchenko, V. Savchenko

National Research Center for Hematology, Moscow, Russian Federation

Background: Bone marrow (BM) is not affected in most patients with Diffuse Large B-cell Lymphoma (DLBCL). In the onset of the disease, histological examination shows no changes in the BM stroma. Patients, depending on IPI, are treated either by R±CHOP courses or by the program R±mNHL-BFM-90. Immediately after the course of treatment and in the distant periods (6-12 years), changes in the bone marrow stroma are observed. There is no data about alterations in stromal precursor cells (multipotent mesenchymal stromal cells /MSCs/ and colony forming unit fibroblasts /CFU-F/).

Aims: The aim of the study was to investigate the changes in MSCs and CFU-F from the BM of patients with DLBCL at the onset of the disease and 6-12 years after treatment.

Methods: The study included 10 patients at the onset of the disease, 20 after treatment with R±mNHL-BFM-90 and 11 with R±CHOP. In all patients BM was taken after informed consent. From the BM, MSCs were isolated by the standard method and the concentration of CFU-F was determined. The relative expression level (REL) of various genes in MSCs was determined by real-time PCR. As a control 31 MSC samples from the BM of healthy donors selected for each group of patients according to age were used.

Results: DLBCL patients' stromal progenitor cells differ from those of donors. In primary patients, the concentration of CFU-F in the BM is reduced by 30%. After R±CHOP, it is 2 times reduced compared with donors and 1.6 times with primary patients. After R±mNHL-BFM-90 it is 1.5 times higher than that of donors and 2 times higher than in patients after R±CHOP. The total cellular production of MSCs in primary patients is 2.3 times higher than that of donors. Many years after R±CHOP, it almost reaches the level of donors, after R±mNHL-BFM-90, it remains 1.7 times higher. Activation of the stromal microenvironment occurs. The expression of *FGF2* in MSCs is reduced in primary patients and years after treatment. The REL of *FGFR1* is also lower in patients compared to donors. The REL of *FGFR2* is doubled in primary patients compared to donors and remains significantly increased years after the treatment. The REL of *ICAM1* and *MMP2*, involved in the adhesion of hematopoietic cells, are reduced by 2 times, which indicates irreversible changes in the ability to maintain hematopoiesis irrespective of the treatment. Osteopontin (*SPP1)* regulates the quiescent state of hematopoietic stem cells (HSC) in a niche. In primary patients, the REL of this gene is increased almost 3 times in comparison with donors. In patients after R±CHOP, it decreases, and after treatment with R±mNHL-BFM-90, it rises even more. Apparently patients MSCs react to the lymphoid tumor and use the mechanisms of HSCs protection. The level of osteocalcin (*BGLAP)* expression is reduced in primary patients and remains low for years regardless of the treatment. Patients bone tissue suffers and these changes are not restored. The REL of interleukin 6 (IL6) is reduced in primary patients by 3.5 times in comparison with donors. Years after chemotherapy, it remains reduced 2 times regardless of the treatment. IL6 is a growth factor for lymphoid cells and simultaneously a pro-inflammatory cytokine. Its decrease can also be attributed to the protective mechanisms used by the MSCs.

Summary/Conclusion: Stromal BM precursor cells respond to a lymphoid tumor even if there is no BM involvement. Analysis of all factors shows that many years after treatment, the characteristics of MSCs are only partially restored. Supported by grant from the Russian Foundation for Basic Research, Project 17-00-00170.

PB2331

FINE-NEEDLE-ASPIRATION: IS IT A USEFUL DIAGNOSTIC TOOL?

Á. Gil[1,*], D. Subirá[2], F. Barriopedro[1], R. Martínez[1], T. Miguel[3], A. Rosell[4], H. Guillén[1], A. Pérez[1], A. Vázquez[1], S. Herrero[1], J. Arbeteta[1], N. Golbano[1], D. Morales[1], L.M. Juárez[1], D. de Miguel[1]

[1]Hematology, [2]Immunology, [3]Internal Medicine, [4]Pathology, Hospital de Guadalajara, Guadalajara, Spain

Background: The diagnosis of NHL is based on histological findings from biopsies, but clinicians often perform fine-needle aspirations (FNA). This rapid and cost-effective procedure provides material for both cytology (Cy) and flow cytometry immunophenotyping (FCI) studies.

Aims: To determine the accuracy of the combined use of Cy and FCI in samples obtained by FNA to discriminate between reactive/benign processes (RP) and neoplasia (N). For this purpose, FNA report was correlated with clinical follow-up and histology from patients submitted to excisional biopsies to achieve the definitive diagnosis (Image 1).

Methods: From March 2010 to December 2016, 380 FNA specimens from 357 patients were obtained from lymphoid tissues (n=219), transbronchial aspirations (n=95) and extranodal sites (n=66). Median follow-up from the time of FNA study was 35.5 months (0-95.4). Conventional cytological techniques were applied. For FCI, a stabilisating reagent was added to fresh samples (≥16 hours) before using a standard stain-lyse-wash protocol. For screening, mAbs against CD45, CD3, CD4, CD8, CD19, CD20, CD22, CD56, kappa and lambda were used. Additional reagents were only included if the screening test showed data of malignancy. Results from Cy&FCI were considered as concordant when they matched together with their discrimination between RP and N, further classified as lymphoma, carcinoma, other hematological (OHN), and other neoplasms (ON).

Table 1.

			Results from FNA samples							
			Concordant Cy&FCI		Only one technique Cy		Only one technique FCI		Misdiagnosed Cy&FCI	
Definitive diagnosis	n	%	n	%	n	%	n	%	n	%
Reactive/ Benign	229	63.97	185	80.8	29	12.66	12	5.24	3	1.31
NHL	43	12.01	35	81.4	2	4.65	3	6.98	3	6.98
Hodgkin lymphoma	9	2.51	0		8	88.89	0		1	11.11
OHN	7	1.96	5	71.4	0		2	28.57	0	
Carcinoma	66	18.44	20	30.3	30	45.45	1	1.52	15	22.73
ON	2	0.56	1	50.0	0		0		1	50.0
Others*	2	0.56	2	100.0	0		0		0	
TOTAL	358		248	69.27	69	19.27	18	5.03	23	6.42

Results: Twenty-two samples were discarded because loss of patient s follow-up (n=7), or necrosis/insufficient material for study (n=15). In 30 of the remaining 358 samples, material was adequate for either Cy or FCI, and 328 samples (91.6%) were properly evaluated by both methods. A concordant result between Cy&FCI correlated with the definitive diagnosis in 69.3% of samples, mostly RP (80.8%) (Image 1). Within RP, 3 double Cy&FCI false positive results were reported (1.3%); in contrast, 16/68 solid tumors (23.5%) were misdiagnosed by both techniques. Regarding the hematological diseases, an exact identification was done by Cy&FCI in 81.4% of all NHL (n=35) and 71.4% of OHN. Interestingly, 33 out of 35 NHL (94.3%) were new

diagnosis, and FCI data were concordant with the definitive histological subclassification in 26/33 NHL (78.8%). In contrast, both Cy&FCI reported a false negative result in 1 Hodgkin lymphoma (HL) and 3 NHL (6.98%). Thirteen samples (3.6%) had discrepant Cy&FCI results: Cy correctly identified 2 carcinoma, 2 NHL and 3 RP; FCI correctly identified 3 low-grade NHL and 3 RP. The sensitivity, specificity, and positive and negative predictive values of concordant Cy&FCI results were 81.2%, 98.4%, 95.4% and 92.9% respectively for all type of malignancies as compared to 90.9%, 98.9%, 93% and 98.5% for hematological neoplasms only.
Summary/Conclusion: Adequate material for both Cy&FCI studies was obtained in most samples of our series. Their combined use didn t preclude discrepant results, but the high correlation between a concordant negative Cy&FCI result and RP may avoid unnecessary biopsies. Regarding neoplasms, there was a much better correlation between the results of Cy&FCI in hematological diseases than in carcinomas. The diagnosis of HL relied on Cy only, but the feasibility of FCI for grading NHL allows a prompt start-up of extension studies until achievement of definitive diagnosis with a second diagnostic procedure.

PB2332

IGVH GENES IN SPLENIC MARGINAL ZONE LYMPHOMA COMPLICATED WITH AUTOIMMUNE HEMOLYTIC ANEMIA

H.L. Julhakyan*, B. Biderman, A. Sudarikov

National Research Center for Hematology, Russian Federation, Moscow, Russian Federation

Background: Splenic marginal zone lymphoma (SMZL) is an indolent B-cell lymphoma frequently associated with monoclonal gammopathy and autoimmune disorders. Therefore, whereas it is well known that the prevalence of autoimmune hemolytic anemia (AIHA) is highest in the more advanced stages of the disease and may depend on the type of treatment administered.
Aims: The ame of this study was to investigate association between autoimmune hemolytic anemia (AIHA) and IgVH genes status.
Methods: We searched in our database of 118 SMZL patients consecutively referred from 2005 to 2017. We identified 9 SMZL patients (7,6%) who developed overt AIHA. All patients met the SMZL WHO diagnostic criteria. AIHA was defined with standard criteria: a fall in hemoglobin level of at least 2 g/dL, associated with a positive direct Coombs test and/or increased reticulocyte count, and a rise in indirect bilirubin with no other causes of anemia identified. *IGHV* mutational status was determined by Sanger sequencing. Cases with 98% or higher *IGHV* gene homology to germinal sequences were considered unmutated. We performed a case-control study comparing the 9 patients with SMZL and AIHA with 10 SMZL patients without AIHA.
Results: A higher prevalence of unmutated *IgVH* was found in patients with AIHA (7 of 9, 77,8%). In comparison only 1 of 10 (10%) controls showed unmutated *IgVH*, as expected in an unselected cohort. The Pirson criteria significant in cases with unmutated *IgVH* in SMZL with AIHA (p<0,05). No significant difference was observed in VH family distribution between the two groups. There was a tendency towards more common tumor progression in patients with unmutated *IgVH* genes with AIHA than in those with mutated ones without AIHA. In all cases SMZL with AIHA were present monoclonal secretion of paraprotein.
Summary/Conclusion: Our data show that AIHA is associated with unmutated status in SMZL patients and that patients with early onset of AIHA have a shorter survival. Larger prospective cohorts are needed in order to verify this observation, and to tell us whether the association is directly causal or whether it reflects an increased tumor bulk at the time of AIHA development.

PB2333

IGHV GENES AND STEREOTYPIC RECEPTORS IN DIFFERENT B-CELL MALIGNANCIES

B. Biderman[1,*], H. Julakyan[1], L. Al-Radi[1], T. Trishkina[1], E. Nikitin[2], P. Butylin[3], A. Rumyantsev[3], E. Stadnik[3], S. Kulikov[1], A. Sudarikov[1]

[1]National Research Center for Hematology, [2]S.P.Botkin Clinical Hospital, Moscow, [3]V.A.Almazov North-West Federal Medical Research Center, Saint Petersburg, Russian Federation

Background: Immunoglobulin heavy chain V-gene (IGHV) mutational status is known to be a key factor for the long-term prognosis in B-cell chronic lymphocytic leukemia (B-CLL). In addition, the diversity of rearranged IGHV genes in B-CLL cells is very limited and significantly different from that of normal B cells. Recent data obtained suggest narrowing of the IGHV repertoire not only in B-CLL but also in other B-cell lymphomas (such as hairy cell leukemia, marginal zone lymphoma, etc.).
Aims: To compare the sequences of IGHV genes in patients with various B-cell malignancies.
Methods: The study included 790 patients with B-CLL, 45 with splenic marginal zone lymphoma (SMZL) and 33 with hairy cell leukemia (HCL). Rearranged IGHV genes for 683 patients observed at the National Research Center for Hematology (2006-2017) and for 185 patients observed at Almazov North-West Federal Medical Research Center (2012-2016) were sequenced according to Sanger procedure and analyzed as described in [Biderman *et al*., 2012].
Results: Rearranged IGHV gene repertoire for B-cell malignancies was compared. V-gene family usage was different for B-CLL, HCL and SMZL. For example, VH3 genes are most often (60%) expressed in HCL, VH4 and VH1 genes are less common - 21% and 12%, respectively. In SMZL, half of all cases represent VH1 genes (51%), 27% - VH3 genes and 15% - VH4. In the case of B-CLL, 45% are VH3, 30% and 17.5% are VH1 and VH4 respectively. While typical distribution for normal B cells is VH3 - 57%, VH1 - 20%, VH4 - 18% [Fais *et al*., 1998]. Other VH genes usage was also rare for the cases investigated (1% -2%), similar to that for normal B cells. For SMZL patients from our sample with VH1 family genes all cases (with the exception of one) represent IGHV1-2 gene (49% of the total sample). In addition, there is a very high similarity of the nucleotide sequences of the CDR3 region for several of these cases (Pic. 1). On the other hand, in B-CLL this gene is relatively rare and occurs in 5% of cases. Furthermore in B-CLL this gene is preferably unmutated (84%), while in SMZL the frequencies of mutated and unmutated cases are similar. It should be noted that IGHV1-2 gene usage was not detected for HCL patients in our sample. In B-CLL, the most common gene was IGHV1-69 (18%), it also participates in the formation of the most common stereotypic antigenic receptors (CLL # 3, 5, 6). Two cases (6%) with IGHV1-69 gene usage were detected in HCL patients, and none in SMZL patients. No stereotypic antigenic receptors described for B-CLL so far were found in our sample of SMZL and HCL patients.

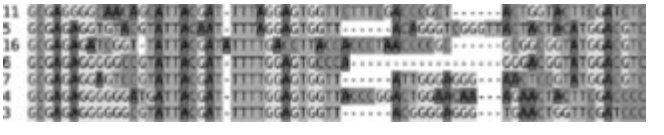


Figure 1.

Summary/Conclusion: The narrowing of the IGHV gene repertoire in B-CLL, HCL and SMZL suggests antigen stimulation of B-cells could play an important role in the development of these diseases. At the same time differences in IGHV gene repertoire between these B-cell malignancies may indicate that different antigens may be involved. Unfortunately, our sample of patients with SMZL and HCL are much smaller than that for B-CLL. Further studies with extended samples of SMZL and HCL may be beneficial to reveal possible prognostic factors and probably CDR3 stereotype data for these diseases.

PB2334

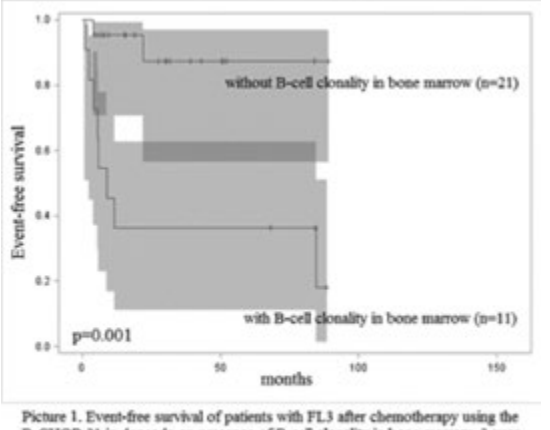
HISTOLOGICAL AND MOLECULAR BONE MARROW INVOLVEMENT IN FOLLICULAR LYMPHOMA 3 GRADE

L.V. Plastinina*, A.M. Kovrigina, E.S. Nesterova, F.A. Babayeva, K.A. Sychevskaia, A.B. Sudarikov, S.M. Kulikov, S.K. Kravchenko

National Research Center for Hematology, Moscow, Russian Federation

Background: Bone marrow (BM) is one of the most frequent extranodal sites of follicular lymphoma (FL). BM involvement is an unfavorable prognostic factor in FL included in FLIPI2. Detection of B-cell clonality in the BM suggests a specific BM involvement, but is not sufficient to confirm of the BM involvement in a tumor process.
Aims: To characterize morphological and molecular (B-cell clonality) features of BM involvement in FL 3 grade and assess their prognostic value in patients who received chemotherapy with R-CHOP-21.
Methods: In this retrospective analysis 89 primary patients with FL3 (median age was 54 years, range 21-78 years, f:m=1:1,5) were observed in the «National Research Center for Hematology» Moscow, Russian Federation (2001-2016) were included. B involvement in FL3 was studied by histological slides of bone marrow biopsy (BMC) with staining H&E in 48 patients. Three variants of the tumor lymphoid cells in the BM were determined in 46 available speciments: a) small-cell (BM infiltration predomi-

nantly centrocytes); b) large-cell (BM infiltration predominantly centroblasts); c) mixed-cell (BM infiltration by centrocytes and centroblasts in different ratios). B-cell clonality was studied in 49 patients. A multivariate analysis was performed in groups of patients with R-CHOP-21 chemotherapy. The analysis included a number of clinical and laboratory parameters - age, gender, stage by Ann-Arbor, FLIPI and others, including histological (n=54) or molecular (n=32) BM involvement.
Results: B involvement in FL3 was detected by BMB in 48 (54%) of 89 patients – 54% in FL3A, 39% in FL3B, 56% in FL 3(A+B) grade. Small-cell BM involvement detected in 22 (48%) patients, large-cell – in 14 (30%) patients, mixed-cell – in 10 (22%). Large-cell BM involvement was more common in FL3B in comparison with FL3A (57% *vs.* 20%, p=0,06). Patients with BM involvement had more often involvement of other extranodal sites besides BM (79% *vs.* 54%, p=0,01), three times more often had thrombocytopenia (p=0,07), four times more often had monoclonal secretion of paraprotein (in blood and/or urine, p=0,06) than patients without BM involvement. By multivariate analysis we did not reveal an unfavorable prognostic value of BM involvement by histological study (p=0,058). B-cell clonality was detected in 21 (43%) of 49 patients. According to the multivariate analysis the detection of B-cell clonality in the BM had an independent unfavorable prognostic value for the overall (HR-10,367, p=0,001) and event-free survival (HR-8,0, p=0,001) (picture 1), Me 30 MEC.



Picture 1. Event-free survival of patients with FL3 after chemotherapy using the R-CHOP-21 in depends on presence of B-cell clonality in bone marrow. 3-year EFS in the group with B-cell clonality is 36% vs. 87% in the group without B-cell clonality (p=0,001).

Figure 1.

Summary/Conclusion: There are 3 morphological variants of BM involvement in FL3. BM involvement in FL3 is associated with other extranodal sites tumor involvement. Detection of B-cell clonality in the BM is independent unfavorable prognostic factor for the overall and event-free survival (on R-CHOP-21 therapy).

PB2335

ACTIVATION INDUCED CYTIDINE DEAMINASE IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

L. Vanhinsbergh[1,*], P. Patten[2], S. Kassam[2], A. Ramsay[2], S. Al Sarraj[3], C. Shah[2]

[1]Haematology, NHS, [2]Haematology, [3]Histopathology, Kings College, London, United Kingdom

Background: Primary Central Nervous System Lymphoma (PCNSL) is a distinct subtype of extranodal non-Hodgkin lymphoma (NHL) confined to the brain, leptomeninges, eyes and spinal cord. It accounts for approximately 1% of all lymphomas and 3% of all brain tumours and has a dichotomous, frequently poor outcome despite recent novel treatment approaches. Specific antigen (Ag) recognition by PCNSL tumour cells through their B cell receptors (BCR) has recently been reported. Further, evidence for Ag selection and drive of the BCR of PCNSL cells is shown by marked restricted immunoglobulin heavy chain variable gene (*IGHV*) usage. Activation induced deaminase (AID), required for both class switching and somatic hypermutation (SHM) of the BCR, is oncogenic in NHL. Evidence for AID activity in PCNSL is demonstrated by marked and ongoing SHM of the BCR and mutations in oncogenes that show aberrant SHM mutation patterns.
Aims: Investigate the role of AID in PCNSL.
Methods: We have reviewed AID expression by immunohistochemistry (IHC) in 38 PCNSL biopsies. Additionally we successfully analysed the *IGHV* of some of these biopsies using the LymphoTrack® Dx IGH FR1 assay (Invivoscribe).
Results: We have demonstrated ongoing expression (positive/focal) in 24 of which 23 also express MYC protein (>30% positive cells). AID negative biopsies lack MYC positivity in 9 of 14 of cases, overall showing a strong correlation between AID and MYC (p=0.0001, Fischer's exact test). In three PCNSL biopsies further analysed we have found that transition mutations in or near WRCY motifs, typical of an AID mutation footprint, predominate (80% mutations found, n=10).
Summary/Conclusion: This data supports a role for AID mediated antigenic drive in the pathogenesis of PCNSL with a strong correlation to MYC, a recognised tumour driver. Further work is required to explore the role of the tumour microenvironment and potential antigens.

PB2336

IGVH SOMATIC MUTATION PROFILE IN SPLENIC LYMPHOMAS

H.L. Julhakyan*, B. Biderman, L. Al-Radi, I. Yakutik, A. Sudarikov

National Research Center for Hematology, Russian Federation, Moscow, Russian Federation

Background: Splenic Lymphomas are rare diseases often misdiagnosed as hairy cell leukemia (HCL), splenic diffuse red pulp lymphoma (SDRPL) and splenic marginal zone lymphoma (SMZL). Criteria for differential diagnosis of these diseases are still controversial and require further elaboration.
Aims: The aim of our study is to determine the immunoglobulin variable heavy chain (IGHV) gene usage and somatic mutation patterns in a series of SDRPL and SMZL patients.
Methods: We studied 10 patients with SDRPL and 24 patients with SMZL (2014-2017 years). Diagnosis was based on standard WHO criteria. In all patients, the diagnosis was based on peripheral blood and BM findings. The baseline clinical and laboratory features as well as follow-up and outcome were recorded for every patient. IGHV mutational status was determined by Sanger sequencing. Cases with 98% or higher IGHV gene homology to germinal sequences were considered unmutated.
Results: Two cases (20%) of SDRPL were shown to bear unmutated IGHV genes whereas 8 (80%) - mutated. In 5 cases (50%) genes of VH3 family were found; in other 5 (50%) - genes of VH4 family. No preference for certain VH genes (from families 3 or 4) were observed. VH1 gene usage was not detected. In 9 cases (37.5%) of SMZL unmutated IGHV genes were detected and in 15 (62,5%) - mutated. VH1 gene family usage was found in 14 (58%) SMZL patients; most of them (12 patients, 86%) carrying IGHV1-2 gene (unmutated in 6 cases and mutated also in 6 cases). VH3 gene family usage was found in 7 SMZL patients (3 cases represent distinct IGHV3-7 gene) and 3 cases had different genes from VH4 family.
Summary/Conclusion: Despite the limited number of cases analyzed it should be concluded that IGHV mutational status may not be used to distinguish between SDRPL and SMZL since both diseases are shown to be associated with mutated as well as unmutated IGHV genes. However IGHV1-2 gene usage may strongly indicate SMZL. These findings have to be confirmed on extended sets of disease cases.

PB2337

MICROVASCULAR DENSITY, CD68 AND TRYPTASE EXPRESSION IN HUMAN DIFFUSE LARGE B-CELL LYMPHOMA

D. Ribatti[1,*], G. Ingravallo[2], F. Gaudio[2], T. Perrone[2], R. Tamma[1], B. Nico[1], E. Maiorano[2], G. Specchia[2]

[1]Department of Basic Medical Sciences, Neurosciences and Sensory Organs, [2]DETO, University of Bari Medical School, BARI, Italy

Background: Diffuse Large B-cell Lymphoma (DLBCL) is the most common form of Non-Hodgkin lymphoma characterized by clinical and biological heterogeneity attributable both to the tumor cells and the complex tumor-microenvironment surrounding them. Tumor-associated macrophages (TAMs) and mast cells are two major components of the tumor inflammatory infiltrate with a definite role in enhancing tumor angiogenesis.
Aims: In this study, we have investigated CD68 and tryptase expression and their relationship with microvascular density (MVD) in chemo-resistant and chemo-sensitive patients affected by DLBCL.
Methods: This retrospective study reviewed data from 29 patients diagnosed with DLBCL. Paraffin-embedded tissues representatives of the DLBCL cases were sectioned and the slides were then incubated with antibodies against CD68, tryptase and CD31. For each case, three slides stained for CD68, tryptase and CD31 expression were scanned using the whole-slide scanning platform Aperio Scanscope CS. CD68 and tryptase expression were assessed

with the Positive Pixel Count algorithm embedded in the Aperio ImageScope software and reported as a percentage of positivity, defined as the number of positively stained pixels on the total of pixels of the image. Fields were selected in areas with the most intensive CD68 and tryptase expression.To quantify MVD, the microvessel analysis algorithm embedded in the Aperio ImageScope software was used. Fields were selected in the areas of highest vascularization and only vessels with defined shape and lumen were considered. Statistical significance of CD68, tryptase and CD31 expression between responders and non-responders groups was assessed with the unpaired t-test. Correlation analysis between CD68 and MVD and tryptase and MVD was performed with the Spearman non parametric correlation test.
Results: CD68 and tryptase expression as well as MVD were increased in chemo-resistant patients when compared with chemo-sensitive patients. Tryptase expression showed a positive correlation with MVD, supporting a role for mast cell in DLBCL tumor angiogenesis, while CD68 correlation with MVD was not significant, indicating a different role for TAMs than angiogenesis in DLBCL.
Summary/Conclusion: Overall, this study is one of the first to compare the expression levels of CD68 and tryptase to MVD in DLBCL. Our results show that although CD68 expression is increased in non-responder DLBCL cases, this increased expression is not correlated with the increase in MVD, while tryptase increased expression in non-responders group appear to be correlated significantly with MVD, strengthening the association between mast cells and tumor angiogenesis.

PB2338

LOW CONCENTRATION OF VENETOCLAX INDUCES APOPTOSIS OF HIGH-GRADE B-CELL LYMPHOMA CELLS

A. Uchida*, Y. Isobe, Y. Uemura, J. Asano, I. Miura

Division of Hematology and Oncology, Department of Internal Medicine, St. Marianna University School of Medicine, Kawasaki, Japan

Background: High-grade B-cell lymphoma with recurrent chromosomal translocations of 8q24/*MYC* and 18q21/*BCL2* (HGBL-MBR) is a subtype of aggressive lymphoma with poor prognosis in spite of showing germinal center B-cell (GCB) phenotype. New therapeutic strategies against HGBL-MBR are needed due to the resistance to conventional chemoimmunotherapy.
Aims: We examined *in vitro* sensitivity of HGBL-MBR cells to topo-II inhibitor doxorubicin, bromodomain inhibitor JQ-1, BH3 mimetics venetoclax, and Mcl-1 inhibitor S63845.
Methods: Three GCB-type cell lines (Karpas231, OCI-Ly8, and BJAB) were used. The translocations were confirmed by FISH analysis, and the expressions of Myc and Bcl-2 family proteins were assessed by Western blot analysis. At 24 and 48 hours after treatment with each drug, the cell count and viability were evaluated by dye exclusion test. Annexin V binding assay was also performed to detect apoptotic changes.
Results: FISH analyses confirmed that Karpas231 and OCI-Ly8 have both *IGH-BCL2* fusion and a split of *MYC* and that BJAB has only *IGH-MYC* fusion. Although the expression levels of Myc and Bcl-xL were varied, those of Bcl-2 were similarly high in the three lines. Compared with BJAB, Karpas231 and OCI-Ly8 showed the minimal expression of Mcl-1. Doxorubicin and JQ-1 suppressed the proliferation in a dose-dependent manner in the three lines. Although venetoclax showed growth suppression in both HGBL-MBR cell lines but not in BJAB, S63845 had the minimal effect only in BJAB. After treatment with 200 nM of venetoclax, annexin V-positive cells increased above 70% of total cells in Karpas231 and OCI-ly8, while apoptotic changes were hardly detected in BJAB (1.3%). After treatment with venetoclax, dephosphorylation of Bcl-2 rapidly occurred in both HGBL-MBR cell lines. Furthermore, the sensitivity of BJAB to venetoclax was restored in combination with 100 nM of S63845, suggesting that the resistance to venetoclax in BJAB seemed to be attributed to Mcl-1.
Summary/Conclusion: Our data indicate that the anti-apoptotic activity of both HGBL-MBR cell lines more strongly depends on Bcl-2 than Mcl-1, and that venetoclax may be a promising agent to overcome the resistance to conventional chemoimmunotherapy in HGBL-MBR.

PB2339

INTEGRATION OF BIOINFORMATICS AND EXPERIMENTS TO IDENTIFY TP53 AS A POTENTIAL TARGET IN EMODIN INHIBITING NON–HODGKIN'S LYMPHOMA

J. Hu*, Y. Chen, M. Lin, Y. Cao, Z. Wu

Department of Hematology, Fujian Institute of Hematology, Fujian Medical University Union Hospital, Fuzhou, China

Background: Non–Hodgkin's Lymphoma (NHL) was a group of lymphoid malignancies with unsatisfactory treatment effect in some aggressive subtypes. Emodin was an anthraquinone with potent anti-cancer activities. However, the molecular mechanism of Emodin repressing aggressive NHL remains to be revealed in detail.
Aims: To explore the active mechanisms of Emodin in aggressive NHL using a bioinformatics method-Functional-activity network (FAN) analysis and subsequently to verify those bioinformatics findings by *in vitro* cell experiments.
Methods: The initial target of Emodin, DPTs were acquired by DrugBank. Protein Interaction Network Analysis Platform (PINA) was employed to predict the DPT-protein interaction. Protein interaction chart and network derived from the integrated data was forecasted using String JAVA consortium. Top 10 pathways in Emodin associated network were validated and those which were associated with NHL were investigated by KEGG website and selected for further analysis. The cBio Cancer Genomics Portal was applied to investigate the connection of Emodin related-genes in NHL studies. Subsequently, effect of E35, a novel derivative of emodin were investigated on the diffuse large B cell lymphoma (DLBCL) cell lines SU-DHL4. The effect of E35 on SU-DHL4 cell vitality was tested by 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetra-zolium bromide (MTT) and Colony formation assay. Cell apoptosis following E35 treatment was assessed by Annexin V 7-AAD and PE staining. Western blot analysis was used to confirm those molecular targets predicted by FAN analysis.
Results: Emodin was queried on Drugbank database and 4 of its primary direct protein targets (DPT) were identified. Then the 489 DPTs-associated proteins were predicted on Protein Interaction Network Analysis Platform (PINA). Emodin-DPTs and DPTs-targeted proteins/genes were categorized as Emodin-related proteins/genes and submitted to String database for expanding the protein network and KEGG pathways analysis. 3 signal pathways, Pathway in cancer, Viral carcinogenesis and Transcriptional misregulation in cancer, were identified as significant association with Emodin treatment in NHL. Advanced integrated analysis exhibited the bridging that functionally connected Emodin action to key target protein TP53 in NHL. Subsequently, treatment of E35 on SU-DHL4 cells suppressed cell proliferation and induced apoptosis in a time- and dose-dependent manner, with an IC50 of 0.87±0.10μM at 48h. Annenxin-V PE and 7-AAD staining indicated raised apoptosis phenomenon with the increasing dose of E35. Apoptotic cells without intervention were 7.70%±1.55%, while 5μM and 10μM E35 treatment for 24h brought them up to 23.01±2.70% 50.23±1.90%. E35 declined TP53 protein expression and inhibited PI3K/AKT pathway in a dose-dependent manner.

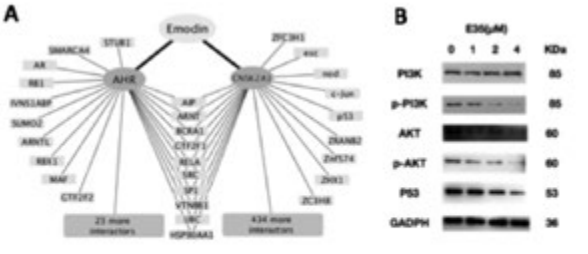


Figure. (A)Emodin-targeted protein interaction network. Drug: Emodin (color in yellow), primary direct protein targets (DPT): CSNK2A1, AHR (color in green) and secondary DPT-targeted protein: color in pastel orange and blue. **(B) Effect of E35 on SU-DHL-4 cells.** Detection of the TP53 protein and PI3K/AKT pathway.

Figure 1.

Summary/Conclusion: Bioinformatics tool FAN analysis could predict key target protein TP53 in Emodin intervention in DLBCL. Further experiments confirmed the bioinformatics findings. All of above showed that combined bioinformatics analysis and experiments offered a novel approach for understanding of mechanisms of Emodin and its derivative action in NHL with convenience and integrity.

PB2340

NOVEL THIENOPYRIDINES AS POTENT INDUCERS OF CELL CYCLE ARREST IN MATURE B-CELL NEOPLASMS

C. Broughton[1,*], M. El Mohtadi[1], C. Graham[1], J. Ashworth[1], K. Whitehead[1], M. van Rensburg[2], L. Pilkington[2], D. Barker[2], N. Dempsey-Hibbert[1]

[1]School of Healthcare Science, Manchester Metropolitan University, Manchester

Metropolitan Univesity, Manchester, United Kingdom, [2]School of Chemical Sciences, University of Auckland, Auckland, New Zealand

Background: Thienopyridines are a family of compounds well known for their anti-platelet activity, and are commonly used to prevent thrombotic events. However, attention has recently turned to their anti-cancer properties. Mechanistic investigations have explored their mode of action, with PLCγ, Jak2 and also Lck suggested as direct targets, while mitotic spindle inhibition via micro-tubule de-stabilisation has also been demonstrated. Mature B-cell malignancies are infamously difficult to treat. Traditionally, treatment approaches have included Microtubule Target Agents (MTA) such as vincristine and vinblastine that bind to tubulin and disrupt microtubule dynamics. MTA have been hugely successful and are among the most important anti-cancer drugs. However, neurotoxicity, neutropenia and the development of drug resistance can be limiting factors. A greater understanding of the mechanisms of action of MTAs will enhance their use and effectiveness and aid in the identification of newer compounds with this action.
Aims: The aim of this study was to assess the effects of six novel thienopyridine derivatives synthesized by our group on B-cell apoptosis, cell cycle arrest and mitotic disruption with the intention of identifying a new family of compound for use in the treatment of mature B-cell neoplasms
Methods: The Burkitt lymphoma mature B-cell line, Daudi, was used in this study. Cells were seeded at a standard density dependent on the downstream assay to be performed and treated with varying concentrations (1-100μM) of thienopyridine derivative (DJ0081, DJ0199, DJ0206, DJ0209, DJ0014 and DJ0021) for varying culture times. Cellular biochemical activity was assessed using the MTS assay, while apoptosis and necrosis were observed using the Annexin V/Propidium Iodide flow cytometry assay. Cell cycle arrest was determined by flow cytometric Propidium Iodide cell cycle assay, and alpha-tubulin expression was visualised using confocal microscopy.
Results: Five of the novel compounds demonstrated a significant reduction in biochemical activity after 48h and 72h of treatment at 10μM with three of the compounds having affects at 1μM, indicating cytostasis/cell death. As expected, compound DJ0021 (synthesised as the control compound) did not significantly affect cell viability. At these same concentrations and time points, significant apoptosis was induced, with very few necrotic events observed by flow cytometry. Cell cycle analysis demonstrated a significant G2 arrest and tetraploidy following just 24h treatment with 10μM, and more marked G2 arrest at 48 and 72h treatment, while microtubule formation was found to be disrupted by all five compounds.
Summary/Conclusion: The study demonstrates the anti-mitotic activity of these thienopyridine derivatives and their potential for use in the treatment of mature B-cell malignancies.

PB2341

LYMPH NODE ASSESSMENT BY CYTOLOGY AND MULTIPARAMETRIC FLOW CYTOMETRY: A PROSPECTIVE STUDY OF 176 SAMPLES

Z. Diez Gallarreta*, F. Marco De Lucas, P. Isusi Gorbea, Á. Blanco González, J. Uriarte Elguezábal, B. Dávila De Las Fuentes, S. Herráez Rodríguez, I. Leal Martínez, C. Alonso Caballero

Hematology, Hospital Universitario Basurto, Bilbao, Spain

Background: Histopathologic study is considered nowadays the technique of choice for the diagnosis of pathology in lymph nodes. However, it has limitations: subjective analysis, limited reproducibility, reduced amount of reagents for determining antigenic expression and considerable consumption of time and human resources. Cytomorphologic examination and multiparametric flow citometry (C-FCM) may solve some of these obstacles: faster diagnosis and multiparametric analysis with qualitative and quantitative characterization of different cell antigens expression. Limitations of this technique are: impossibility to evaluate lymph node architecture and cellular damage during processing.
Aims: Our aim was to evaluate in our centre the diagnostic value of C-FCM for oncohematologic diseases in lymphoid tissues and its accuracy in subclassifying Non Hodgkin Lymphomas (NHL), apart from indicating in which circumstances this technique was insufficient.
Methods: From 2015 to 2017, we prospectively analyzed by C-FCM 176 lymph tissue biopsy specimens, mainly obtained (76.7% (135/176)) by large needle biopsy of lymph node. Every sample was evaluated by cytomorphology of touch imprints stained with May-Grümwald-Giemsa and by FCM. Likewise, a part of the same sample was evaluated by histopathology. Median age was 60 years (1-91): 94 male (53.4%) and 82 female (46.6%). 54 (31%) had previous lymphoma history. Statistical analysis was performed employing G-STAT program (version 2.0.1, Biometrics Department of GlaxoSmithKline).
Results: C-FMC detected neoplastic disorders in 120 (68.2%) samples: 82 NHL (67.8%), 11 Hodgkin Lymphomas (HL, 9%), 26 malignant non hematologic neoplasms (NHN 21.5%) and 1 Acute Myeloid Leukemia (0.8%). Sensitivity and specificity were 85% and 83%. Positive predictive value (PPV) and negative predictive value (NPV) were 94.2% and 63.6%, respectively. Sensitivity and NPV increased to 93% and 86% when HL, NHN and T-cell / histiocyte-rich-B-cell lymphomas (TCRBL) were excluded from the analysis. 71/82 LNH (86.6%) were defined according to the 2008 World Health Organization (WHO) classification of hematolymphoid neoplasms, with a concordance of 78% with respect to the histopathological study.

Table 1.

Concordance Between C-CFM and Histopathologic Study in B-NHL According to the WHO Classification

WHO Classification	C-FCM	Histopathologic Confirmation	Concordance (%)	Discordance
Diffuse Large B Cell Lymphoma (DLBCL)	30	22	73.4	7 FL[1], 1 unspecified B-NHL
Burkitt Lymphoma	3	3	100	None
Small Lymphocytic Lymphoma (SLL)	10	7	70	1 HL[2], 1 MZL, 1 Follicular hyperplasia[3]
Follicular Lymphoma (FL)	19	14	73.7	4 DLBCL, 1 Follicular hyperplasia
Mantle Cell Lymphoma	4	4	100	None
Marginal Zone Lymphoma (MZL)	3	3	100	None
Angioimmunoblastic T Lymphoma	2	2	100	None

[1]There are 4 out of 7 FL in which the diagnosis of DLBCL was confirmed afterwards by other techniques (PET with high SUV)
[2]Richter Syndrome Hodgkin Variant [3]SLL was confirmed later in another sample

Summary/Conclusion: C-FCM provides very valuable information regarding diagnosis of pathology in lymph nodes, showing high sensitivity and specificity. C-FCM is especially useful in NHL, allowing their sub-classification according to WHO criteria with a concordance of 78%. Moreover, being a faster technique compared to histopathology, while keeping a high PPV, it makes it easier to take quick therapeutic decisions, which is relevant in aggressive diseases. However, in NHN, HL and TCRBL, the utility of this technique relies on excluding the most usual NHL diagnosis, remaining histopathology the gold standard for diagnosis.

PB2342

THE UTILITY OF THE FORWARD SCATTER PARAMETER IN THE FLOW-CYTOMETRIC EVALUATION OF LYMPH NODE CELL SUSPENSION TO IDENTIFY B CELL LYMPHOMAS

F. Corrente[1,*], S. Bellesi[1], P.L. Puggioni[1], F. D'Alò[1], A. Cuccaro[1], E. Maiolo[1], F. Attili[2], E. Meacci[3], E. Scarano[4], C. Lodoli[5], F. Pacelli[5], L.M. Larocca[6], V. De Stefano[1], S. Hohaus[1]

[1]Istituto di Ematologia, Università Cattolica del Sacro Cuore, [2]Istituto di Gastroenterologia, Università Cattolica del Sacro Cuore, [3]Istituto di Chirurgia Toracica, Università Cattolica del Sacro Cuore, [4]Istituto di Otorinolaringoiatria, Università Cattolica del Sacro Cuore, [5]Istituto di Chirurgia Generale, Università Cattolica del Sacro Cuore, [6]Istituto di Anatomia Patologica, Università Cattolica del Sacro Cuore, Rome, Italy

Background: Flow-cytometric assessment on lymph node biopsies has been reported to be useful in the diagnosis of non-Hodgkin lymphomas (NHL). Diffuse large B-cell lymphoma (DLBCL) is the most common form of B-NHL among adults. The forward-scatter is a useful flow-cytometric parameter to study the lymphoma cell size.
Aims: The aim of the present study was to assess the diagnostic relevance of forward-scatter in identification of DLBCL on lymph node suspension samples.
Methods: Lymph node suspensions were prepared from biopsies obtained by surgical resection or by ultrasound-guided endoscopic core biopsies. We used the Medimachine system (BD Biosciences) for mechanical disaggregation of the solid tissue. The screening panel included CD19, CD20, CD10, Kappa, Lambda, CD5, CD3, CD4 and CD8. For B-cells the normal range of Kappa/Lambda ratio was >0.8 or <3.0. A clear clonality, CD10+ or CD5+ expression on B cell populations or absence of surface immunoglobulin light chain were suggestive of B-NHL. The Forward-scatter Area (FSC-A) was evaluated on B- and T-cells of lymph node suspensions and expressed as

median value. Data were acquired on BD FACSCantoII flow cytometer (BD Biosciences) and analyzed by BD FACSDiva software (BD Biosciences). We defined the ratio between FSC-A of the pathological B-cell population and the FCS-A of CD3+ T-cells as FSC ratio.
Results: In this study we included 41 lymph node suspensions with a pathological B cell population. Immunohistochemical diagnosis was DLBCL in 23 patients, follicular lymphoma (FL) in 12 patients, mantle cell lymphoma (MCL) in 3 patients, marginal zone lymphoma (MZL) in 2 patients and small lymphocytic lymphoma (SLL) in 1 patients. Subsequent immunohistochemical analysis proved that all B-NHL were correctly identified (100% sensitivity) by flow-cytometry analysis. B-cell pathological populations were CD19 and CD20 positive and presented a clear clonal light chain restriction in 33/41 cases, while 8/41 (6 DLBCL, 1 FL, 1 MZL) did not show any light chain expression. CD10 was expressed in all FL cases and in 4/23 DLBCL. CD5 was present in the patients with MCL and in 1 case of DLBCL. DLBCL cases showed a higher FSC ratio (see fig. 1) compared to other B-NHL (1.60 *vs* 1.04, P <0.001). By calculating the receiver operating characteristic (ROC) curve, the best cut-off for FSC ratio was 1.23, with an AUC=0.94 (95% CI 0.85 to 1.00). This cut-off value provided a sensitivity of 96%, a specificity of 79%. The positive predictive value was 86% and a negative predictive value of 96%.

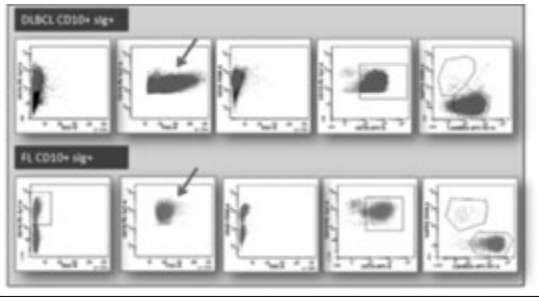


Figure 1.

Summary/Conclusion: The FSC ratio between the pathological B lymphoma cell population to the normal CD3+ T cells is a simple parameter, that is routinely acquired during flow cytometry and can be helpful in discerning between DLBCL and low-grade lymphomas and mantle cell lymphoma. This information may be helpful to direct the immunohistochemical work-up.

PB2343

THE COMPREHENSIVE DESCRIPTION OF TP53 GENE ABERRATIONS IN DLBCL

E. Voropaeva[1,*], T. Pospelova[2], M. Voevoda[1], V. Maximov[1]

[1]"Research Institute of Internal and Preventive Medicine" - Branch of IC&G SB RAS., [2]Novosibirsk State Madical University, Novosibirsk, Russian Federation

Background: At present, there is no comprehensive description of aberrations in the *TP53* gene in DLBCL. *TP53* mutations is the most studied aspect. A comparative analysis of *TP53* mutations presented in the special databases shows that their frequency and spectrum can vary for the same type of the tumor depending on the study population. Information about the Russian population in the current version of the IARC *TP53* mutation database is not provided.
Aims: The purpose of the present study was to carry out a complex analysis of the frequency and spectrum of mutations in 5-8 exons, the frequency of loss heterozygosity and methylation of the promoter of the *TP53* gene in DLBCL.
Methods: Genomic DNA was isolated from formalin-embedded paraffin blocks of lymph nodes and extranodal tumor lesions biopsies of 92 patients with DLBCL by phenol-chloroform extraction method using guanidine. The tissue sections containing at least 70-80% of the tumor cells were taken. Direct Sequencing by Sanger of the *TP53* gene was performed according to the IARC protocol. Analysis of the methylation status of the *TP53* gene was performed by methyl-specific PCR. Estimation of loss of heterozygosity (LOH) in the gene *TP53* was carried out at the microsatellite locus D17S796.
Results: A quarter (24.3%) of patients had mutations in the coding sequences of the gene, which is consistent with the literature data. Four patients had multiple mutations. The distribution of the mutations was as follows: 1 (3%) mutation leading to splicing failure, 11 (33%) intron with an unknown effect, 12 (37%) missense, 6 (18%) - synonymous, 2 (6%) - nonsense type, 1 (3%) - a mutation leading to a shift in the reading frame in the *TP53* gene. 5 (15.6%) of substitutions was GC AT substitutions in CpG islets. In 9 tumor samples, rs78378222 was detected, which leads to a violation of the polyadenylation signal and disruption of mRNA translation. According to the IARC *TP53* mutation database, the codons 248, 273, 175, 245, 281, 244, 305, 249 and 297 are the "hot spots" in the *TP53* gene in DLBCL. In the samples of patients from Russian cohort, only a mutation in 244 codons was identified (p.G244S). However, mutations p.W146R, p.T155I, p.V272E, p.R213X was verified in two cases of each. The frequency of methylation of the *TP53* gene promoter was 4/69 (5.8%), and the LOH frequency was 10/74 (13.5%).
Summary/Conclusion: In total 32.4% of DLBCL samples had *TP53* aberrations with proven oncogenic potential. Complex analysis made it possible to clarify that the insufficiency of the function of the *TP53* gene in DLBCL can be formed according to the classical "two-stroke" mechanism: almost all cases of LOH were combined either with somatic mutations, germ-line marker rs78378222 or methylation of the *TP53* gene promoter.

PB2344

THE IMPAIRED PRODUCTION OF TNF, TGF BETA 1 AS AN IMPORTANT REASON OF DEFECTIVE NATURAL KILLER ABILITY IN NON-HODGKIN LYMPHOMA PATIENTS WITH SPLEEN AFFECTION

V. Barilka[1,*], V. Matlan[2], O. Shalay[1], Y. Yevstachevych[1], S. Prymak[1], V. Loginsky[1]

[1]Immunology and Genetics of Blood Neoplasms Lviv, Ukraine, SI Institute of Blood Pathology and Transfusion Medicine of the NAMS of Ukraine, [2]Department of Hematology and Transfusiology, Danylo Halytsky National Medical University, Lviv, Ministry of Health of Ukraine, Lviv, Ukraine

Background: The microenvironment of non-Hodgkin lymphoma (NHL) may assists to distribution and survival of neoplastic cells with simultaneous development of immune tolerance of natural killer (NK) by broken secretion of tumor necrosis factor (TNF) and transforming growth factor beta 1 (TGFβ1).
Aims: o detect the cytotoxicity ability (CA) of NK from mononuclear cells (MN) of peripheral blood (MNPB), MN of lymphatic nodes from the gate of spleen (MNLNs), MN from spleen tissue (MNS) of 10 NHL patients (pts) with spleen affections. To detect the CA NK from peripheral blood (as an effector cells) of NHL pts *vs.* autological MNS, MNLNs (as a target). To prepare supernatants (Sp) of the MNPB, MNLN and MNS after 24 h incubation cells in RPMI 1640 and to detect the concentration of cytokines in Sp and plasma.
Methods: The peripheral blood gained before breakfast. The tissues both of lymph nodes and spleen obtained after surgical remover. The concentrations of TNF and TGFβ1 were determinatend by biological methods. The CA of NK was estimated vs. K562 and autological NHL cells marked with ^{3}H-methylthymidine. Control group consisted of 15 healthy persons and 8 pts with reactive hyperplasia of spleen (RHS) and 7 pts - with non-specific reactive lymphadenitis (nSRL).
Results: The level of TNF in both plasma and Sp MNPB of NHL pts were higher, than in the plasma and Sp MNPB of the healthy donors (p<0,001). The TNF levels in both Sp of MNS (0,23±0,12 ng/ml) and MNLNs (0,19±0,07 ng/ml) of NHL pts did not differ statistically and were exceeded by analogical data in RHS pts and nSRL. The level of TGF β1 in plasma (3,68±0,73 ng/ml) NHL pts was higher (3,730±0,900 ng/ml) than in control group (p<0,05). TGFβ1 in Sp of MNPB was two times lower that of control (p<0,05). The level of TGFβ1 in Sp of MCS of NHL pts was for certain below (4,860±0,610 ng/ml), than in Sp of MCS of RHS pts (8,330±0,531 ng/ml; p<0,05). The CA of NK from NS *vs* K562 was higher (29,27±7,92%) than in both NLNs (15,85±3,80%; p<0,05) and NPB in NHL pts (16,69±2,26%; p<0,05). The CA of NK from peripheral blood of NHL pts was for certain below (14,695±2,26%) than in healthy donors (22,241±0,84%; p<0,05). The CA of NK from NLNs was lower *vs* 562 cells line, than CA of NK from MNS of NHL pts with spleen affections. The of NK from blood *vs* the autological NLNs and MNS were in both cases for certain below, than CA of NK *vs* the of 562 cell lines (p<0,01). The CA of NK from blood NHL pts *vs* autological of MNS (5,36±1,64%) and CLNs (7,26±1,60%) were statisticall below, than data of autological CA of NK in the RHS pts (30,175±2,94; p<0,001). The CA of NK from blood of NHL pts negatively correlated with the ncentration of TNF in both plasma (r= - 0,50) and Sp of MNPB (r= - 0,55). It should be noted that the level of TNF in plasma of pts with RHS positively correlated with CA of NK from NS (r=0,40). The CA of NK from MNPB of NHL pts negatively correlated with the concentration of TGF β1 in plasma (r=-0,619) and negatively with the TGF β1 in Sp from MNS (r=-0,620).

Summary/Conclusion: Concentration of TNF and TGFβ1 in plasma of NHL pts with spleen affection for certain higher, than in the healthy persons. NK from blood of NHL pts *vs* own neoplastic cells was lower than stranger 562. These results could specify on tolerance of cellular immunity in the NHL pts. Thus, impaired secretion of TNF and TGF β1 may has an important role in the regulation of ability NK vs. neoplastics cells and can be important markers for modulation of immune responses in NHL pts with spleen affection.

PB2345

CLONAL EVOLUTION OF FOLLICULAR LYMPHOMA CELLS FROM ONSET TO RELAPSE

K. Sychevskaia*, B. Biderman, S. Kravchenko, L. Plastinina, S. Smirnova, F. Babayeva, E. Nikulina, J. Mangasarova, A. Sudarikov

National Research Center for Hematology, Moscow, Russian Federation

Background: Though complete clinical remissions could be induced in Follicular lymphoma (FL) early and late relapses are relatively often. Key events in tumorogenesis in FL while predecessors of tumor cells migrate from bone marrow to the germinal center of the lymph node follicle include V(D)J-recombination, overexpression of the BCL-2 gene and somatic hypermutation. However, the origin of residual cells that may contribute to the development of relapses in FL still not known.
Aims: To compare FL clonal tumor cells in disease onset and relapse by means of sequencing and mutational analysis of clonally rearranged genes of immunoglobulins heavy chains (IGHV).

Table 1.

Table1. Clinical and laboratory data of FL patients

	Onset	1st relapse	2nd relapse
Patient C	08/2014 10 mutations	07/2016 12 mutations (+2)	-
Patient K	06/2010 10 mutations	12/2013 13 mutations (+3)	-
Patient R	03/2007 18 mutations	05/2009 20 mutations (+2)	-
Patient H	08/2012 10 mutations	03/2013 10 mutations	08/2013 10 mutations

Methods: Four FL patients diagnosed and treated at the National Research Center for Hematology, who subsequently relapsed were included in the study. Clinical and laboratory data are summarized in Table 1. B-cell clonality testing and IGHV sequencing was performed according to BIOMED-2 protocol. Fragment analysis and Sanger sequencing was performed on ABI PRISM 3100 genetic analyzer, followed by comparison with the germinal sequence databases www.imgt.org and www.ncbi.nlm.nih.gov/igblast.
Results: B-cell clonality testing and IGHV sequencing at diagnosis and in relapse were done for all 4 patients. Clonal peaks of identical length at diagnosis and in relapse were identified in all 4 cases. Somatic mutations were identified in all cases (90-95% homology with germinal sequence). In three cases additional somatic mutations (from 2 to 3) were identified in relapse while preserving those found in the debut of disease (Table 1). In 3 cases of acquisition of new somatic mutations, patients had late relapses (from 13 months to 21), whereas in one patient with early relapse the nucleotide sequence of the tumor clone remained the same. The data obtained indicate that the tumor cells in relapse originate from the mature clonal cells found in disease onset, and not from the earlier progenitor cell during parallel development.
Summary/Conclusion: The data obtained strongly indicate that the late relapse substrate is a mature tumor cell of the debut, which repeatedly passes the differentiation stage in the germinal center of the follicle, acquiring new somatic mutations. The possibility of tumor cells migration between the lymph node and the bone marrow had been shown for FL. One can speculate that the cells of the primary tumor clone may survive chemotherapy in the bone marrow niches and in recurrence return to the lymph node under the influence of an unknown stimulus, while entering a new cycle of somatic hypermutation. In contrast to the late, the development of early relapses may be due to incomplete eradication of FL cells in the sites of primary tumor lesion, as indicated by the stability of the nucleotide sequence. Further studies with extended samples of FL patients are required to obtain data of more statistical power.

PB2346

EZH2 GENE UPREGULATION IN PRIMARY TESTICULAR AND CENTRAL NERVOUS SYSTEM DIFFUSE LARGE B-CELL LYMPHOMA: A POTENTIAL TARGET FOR TREATMENT IN IMMUNE-PRIVILEGED SITES?

G. Koenig[1], B.V. Chakravarthi[1], J. Gordetsky[1], G. Netto[1], M. Rodriguez-Pena[1], D. Correia-Salles[2], L. Schwartz[3], J. Jorns[4], S. Varambally[1], D. Peker[1,*]

[1]University of Alabama at Birmingham, Birmingham, [2]Johns Hopkins University, Baltimore, [3]University of Pennsylvania, Philadelphia, [4]Medical College of Wisconsin, Madison, United States

Background: Upregulation of the enhancer of zeste homolog-2 (*EZH2*), a histone N-methyltransferase, is a well-established in cancer development through various mechanisms. *EZH2* has recently become a major target in cancer treatment. Primary central nervous system (CNS) and testicular diffuse large B-cell lymphomas (DLBCL) are rare but aggressive diseases with poor outcome due to therapeutic challenges.
Aims: In the present study, we investigated the EZH2 expression status in these two distinct variants.
Methods: A retrospective chart review, from 2003 to 2013, identified cases with a diagnosis of primary testicular and CNS DLBCL. Immunodeficiency-related, EBV-associated, and MYC gene positive LBCL were excluded from the analysis. A formalin-fixed-paraffin-embedded tissue samples were analyzed using quantitative real-time PCR (Taqman assay) for RNA expression levels of EZH2, using custom designed primers in commercially available kits. Control samples included three benign lymph nodes free of any neoplastic processes.
Results: Twenty-seven cases had adequate RNA extracted for analysis and PCR results were available in 18 cases; testis (n=11) and primary central nervous system (n=7). The median age was 65 years (range 41- 71 years), with a male to female ratio of 6 (M=15, F=3). Median overall survival (MOS) was 27months (2 to 74 months). Immunophenotypic cell-of-origin subtype was determined in 15 cases using Han's algorithm, six cases were germinal center B-cell (GCB) type (33%) and nine cases were non-GCB type (50%). Eleven cases showed higher expression of EZH2 when compared to normal lymph nodes. Five of these cases were testicular lymphoma (45%) while six cases were CNS origin (85%). The remaining seven cases did not show increased expression of EZH2.
Summary/Conclusion: Results suggest that EZH2 gene upregulation might be playing an important role, particularly in primary CNS DLBCL and possibly in testicular DLBCL; therefore might be a potential target of treatment. Larger studies are needed to establish the prognostic and therapeutic utility of RNA expression level of the EZH2 in these aggressive forms of DLBCL.

PB2347

CORRELATION BETWEEN CLINICAL ANT PATHOLOGICAL VARIABLES WITH IMMUNOHISTOCHEMICALLY DIFFERENTIATED MOLECULAR SUBTYPES OF DIFFUSE LARGE B-CELL LYMPHOMA

E. Hadzimesic Suljovic[1,*], S. Radovic[2], N. Obralic[1], A. Kozaric[1], N. Bilalovic[1], A. Sofo-Hafizović[1]

[1]Clinical Center University of Sarajevo, Hematology Clinic, [2]Medical Faculty University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Background: Gene expression profiling has made a distinction between the two pathological entities of the DLBCL, ABC DLBCL and GCB-DLBCL. Genetic profiling is still far from routine clinical practice, so using immunohistochemical methods, identification of GCB and ABC phenotype has been translated into clinically feasible approach by developing algorithms for defining the molecular subtypes based on the expression of immunohistochemical markers defining subgroups as GCB and non-GCB. *I*PI (International Prognostic Index) remains the most accurate prognostic model due to inconsistent results of prognostic models based on biomarkers, and research focus is directed to upgrading the IPI models and to correlations between clinical parameters and biomarkers with biological characteristics of the disease.
Aims: To determine the correlation between clinical and pathological variables with immunohistochemically differentiated molecular subtypes of DLBCL.
Methods: We have analyzed 50 patients with DLBCL for the expression of bcl-2, bcl-6, CD10, MUM1 and Ki-67 Patients were divided into two groups, the non-GCB, GCB group or favorable group 1 and unfavorable group 2, according to Hans's algorithm and Muris's algorithm. Clinical-pathological, biochemical parameters of disease have been correlated with

subgroups of DLBCL and biomarkers individually. The impact of the expression of bcl-2, bcl-6, CD10, MUM1 and Ki67 on IPI-highest score in multiple regression analysis, afterwards in regression equation and variance analyse.
Results: Patients younger than 60 years are significantly more non-GCB subtype, avarage age 46.29 years. CD10 and MUM1 are significantly more present in non-GCB subtype and in Group2. Positive correlation between bcl6 and extranodal localisation was proved (p>0.05). In the analysis of correlation of pathological variables and immunohistohemically determined molecular subtypes, difference in the size of the nucleus as well as the correlation between the subtypes to Hans (p<0.05), but not Muris (p>0.05) was demonstrated. 91.7% of non-GCB patients had the size of nucleus II. Difference in the incidence rate of more than one extranodal site in non-GCB groups per Hans was found (p=0.045). POsitive correlation was found between increased values of lactat dehidrogenase and beta2mikcroglobulin and Group2. GCB phenotype showed positive correlation with good-risk patients identified by IPI. Multiple regressional analysis proved impact of biomarkers on IPI. The mutual impact of bcl-2, bcl-6, MUM1, Ki67 is significantlyrelated to poor-risk IPI patients
Summary/Conclusion: A more aggressive type of the disease and more severe clinical features are significantly more common among patients in the subgroup of non-GCB or group 2, DLBCL. Non-GCB subgroup and group 2 were associated with a higher clinical stage (III / IV), lower Karnofsky index or higher ECOG performance status, number of extranodal localization of more than one locus, elevated lactate dehydrogenase value, increased value of Ki67 and increased value of beta2microglobulin.

Platelets disorders

PB2348

GENETIC MUTATION WITH BAT SCORING ASSESSMENT IN GLANZMANN THROMBASTHENIA PATIENTS

M. Abid*, M. Borhany, N. Fatima, S. Ahmed, T. Shamsi

Hematology department, National Institute of Blood Disease and Bone Marrow Transplantation, Karachi, Pakistan

Background: Glanzmann thrombasthenia is a platelet function disorder that is caused by an abnormality in the genes for glycoprotein IIb/IIIa receptor. The receptor is either absent or does not function properly as a result of which the platelets do not have the ability to attract each other and the coagulation factors and thus leads to impaired clot formation. A number of bleeding assessment tools (BATs) has been developed to standardize the bleeding history. To improve the diagnostic accuracy and sensitivity by predicting the future risk of bleeding.
Aims: To evaluate the genetic mutation and BAT scoring assessment of diagnosed GT patients.
Methods: This was an observational study, conducted at the National Institute of Blood disease Karachi. Diagnosed GT patients of all age group of either gender were included in this study. Polymerase chain reaction (PCR) was used to amplify all coding regions of the *ITGA2B* and *ITGB3* genes (using reference sequences NM_000419 and NM_000212, respectively). ABI-3500 was used for genomic analysis. ISTH-BAT questionnaire assessment was applied to evaluate bleeding score.
Results: A total of 11 patients were included in this study. At diagnosis, female to male ratio was 7:4. The median age was 7 years with range of 2-17 years. Consanguineous marriages with positive family history were reported in the study. Missense, nonsense, deletion, insertion and splice site were the types of mutations observed in our study. Of these mutations, missense (54.5%) was the most common. The study was assessed in the GT patients with respect to ISTH-BAT scoring with mean BAT score of the patient at diagnosis was 9.27±4.1. The clinical manifestations observed in our study were epistaxis and gums bleed (82%) each, GI bleed (27%), menorrhgia (18%) and hematoma and hemarthrosis (9%).
Summary/Conclusion: Mutational analysis is a key component of a complete diagnosis of GT with respect to The ISTH-BAT is a useful tool for documenting bleeding symptoms.

PB2349

ADULT PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) TREATED WITH THROMBOPOIETIN RECEPTOR AGONISTS: A RETROSPECTIVE STUDY USING DATA FROM THE ITP REGISTRY OF THE HELLENIC SOCIETY OF HEMATOLOGY

C. Pontikoglou[1,*], E. Stavroulaki[2], V. Kaliafentaki[1], V. Tzikoulis[1], P. Kanellou[2], A. Patrinos[3], T. Chatzilygeroudi[3], A. Kourakli[3], A. Symeonidis[3], M. Dimou[4], M. Matzourani[5], N.-A. Viniou[5], P. Panayiotidis[4], S. Hontropoulos[6], G. Kanavos[6], A. Galanopoulos[6], T. Marinakis[6], D. Liapi[2], G. Tsirakis[7], A. Kolovou[7], A. Syrigou[8], E. Gavriilaki[8], A. Anagnostopoulos[8], P. Lampropoulou[9], A. Loidoris[9], I. Patsias[9], A. Megalakaki[9], E. Vlachaki[10], M. Papaioannnou[11], G. Kaiafa[12], M. Bobola[13], A. Kostourou[13], S. Giannouli[14], I. Kotsianidis[15], G. Vassilopoulos[16], M. Protopappa[17], E. Hatzimichael[18], P. Zikos[19], G. Chalkiadakis[20], H. A. Papadaki[1]

[1]Department of Hematology, University of Crete School of Medicine, [2]Department of Hematology, Venizeleio-Pananeio General Hospital, Heraklion, Crete, [3]Hematology Division, Department of Internal Medicine, University of Patras, Patras, [41]st Propedeutic Department of Internal Medicine, [51]st Department of Internal Medicine, Laikon General Hospital, National and Kapodistrian University, [6]Department of Clinical Hematology, G. Gennimatas District General Hospital, Athens, [7]Hematology Department, General Hospital of Chania, Chania, [8]Hematology Department-BMT Unit, George Papanikolaou Hospital, Thessaloniki, [9]Department of Hematology, Metaxa Anticancer Hospital, Piraeus, [102]nd Department of Internal Medicine, Aristotle University of Thessaloniki, Hippokration General Hospital, [111]st Internal Medicine Department, [121]st Propedeutic Internal Medicine Department, Aristotle University of Thessaloniki, AHEPA University Hospital, Thessaloniki, [13]Department of Hematology - Blood donation, Pamakaristos General Hospital, [142]nd Department of Internal Medicine, National and Kapodistrian University of Athens, Athens, [15]Department of Hematology, Democritus University of Thrace, Alexandroupolis, [16]Department of Hematology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa,

[17]Department of Hematology, General Hospital of Serres, Serres, [18]Department of Hematology, University of Ioannina, Ioannina, [19]Department of Hematology, General Hospital of Patras "St. Andrew", Patras, [20]Information Systems Unit Center of Information and Communications Technologies, University of Crete, Heraklion, Crete, Greece

Background: ITP is a heterogeneous autoimmune disorder mediated by platelet (PLT) antibodies thought to accelerate PLT destruction while inhibiting also their production. ITP features in the general Greek population have not been properly investigated.
Aims: To access systematically, for the first time, the characteristics of adult patients (pts) receiving thrombopoietin receptor agonists (TPO-RAs), *i.e.* Romiplostim (R) and Eltrombopag (E), using data from the national database (ITP registry) operated under the auspices of the Hellenic Society of Hematology.
Methods: The greek ITP registry recruits pts (n=1167, to date) nationally through a network of 19 centers. In the present study we retrospectively analyzed data from adult ITP pts diagnosed from 1979 to 2017, who received R and E.
Results: The total number of evaluable adult pts was 69 (29 M; 40F) and the median age at diagnosis was 54 years (20-88). R was administered in 27 pts and E in 42 pts, with a median age of 59.8 years and 58.6 years, respectively. 94% of the pts started R from 2009 to 2013 and 95% started E from 2012 to 2016. The median time from ITP diagnosis to R and E administration was 12 months (0.5-360) and 8 months (0.4-265) respectively. The mean number of prior ITP therapies was 2.3 (1-5) in the R- and 2 (1-7) in the E-cohort (P=0.061). Similar proportions of pts in the R- and E-cohort, respectively, had received corticosteroids (26/27 & 4/42), intravenous immunoglobulin (20/27 & 24/42), rituximab (5/27 & 3/42), vinblastine (3/27 & 2/42), immunosuppressants (2/27 & 2/42), vincristine (1/27 & 1/42) and had undergone splenectomy (3/27 & 2/42). Anti-RhD immunoglobulin was administered in 1/27 pts in the R-cohort and Danazol in 1/42 pts in the E-cohort. The median PLT number at R or E initiation was 20.42x10^9/L and 25.03x10^9/L respectively, P=0.254. Similar PLT counts were observed between pts receiving R and E at 6 months (163.9x10^9/L & 151x10^9/L, respectively, P= 0.8937) and at 12 months (166.5x10^9/L & 157.4x10^9/L respectively, P=0.7236). Patients remained on R and E treatment for a median of 23.5 months (0.5-106) and 15.5 months (0.4-75), respectively. There was no difference in complete response (CR, ie PLT count above 100x10^9/L) to R and E (62% & 59%, respectively, P=0.192) or partial response, *i.e.* PLT count 30-100x10^9/L, (19% & 22%, respectively, P=0,7264). 14(52%) pts in the R-cohort discontinued R: 2 in CR stopped R maintaining a sustained remission after discontinuation, 6 had no response, 2 due to adverse effects (gastrointestinal disorders, headache) and 4 underwent splenectomy. After R discontinuation, 10 pts received E, 8 of whom responded. In 2 non-responders additional therapies included corticosteroids, vinblastine, intravenous IgG and Rituximab. 14 (33%) pts in the E-cohort discontinued E: 4 in CR stopped E obtaining a sustained remission after discontinuation, 5 had no response, 4 due to adverse effects (hepatotoxicity, pulmonary embolism, retinal artery thrombosis) & 1 underwent splenectomy. Following E discontinuation 4 pts received R, 1 of whom responded. Other treatments included intravenous immunoglobulin, danazol, splenectomy and corticosteroids.
Summary/Conclusion: Our real-life multicenter retrospective analysis on the use of TPO-RAs suggests that both R and E have acceptable toxicity profiles and are highly effective in adult ITP pts failing 1 or more lines of therapy. TPO-RA switch is a feasible strategy that can be beneficial, at least in some cases.

PB2350

PERIOPERATIVE USE OF ELTROMBOPAG IN PATIENTS WITH CHRONIC THROMBOCYTOPENIA. A SINGLE-CENTER EXPERIENCE

E. Landete[1,*], A. Domingo[1], G. Pérez-Rus[1], A.M. Rodríguez-Huerta[1], D. Carbonell[1,2], A.M. Pérez-Corral[1,2], J. Anguita[1,2], J.L. Díez-Martín[1,2], C. Pascual[1,2]

[1]Department of Hematology, Gregorio Marañón General University Hospital (HGUGM), [2]Gregorio Marañón Health Research Institute (IiSGM), Madrid, Spain

Background: Patients with moderate-severe chronic thrombocytopenia have an increased risk of bleeding during surgical procedures. Perioperative management of these patients may be difficult. Eltrombopag is a well established treatment for chronic immune thrombocytopenia (ITP). During the last years, new indications for this agent have been proposed. Eltrombopag may be an interesting option for patients with different thrombocytopenic disorders requiring surgical interventions.
Aims: The aim of this study was to evaluate the efficacy and safety of perioperative use of Eltrombopag in thrombocytopenic patients undergoing invasive interventions.
Methods: We retrospectively analyzed a total of 12 procedures carried out in 10 patients with chronic thrombocytopenia who received Eltrombopag. One patient was Jehovah's Witness. We included patients with chronic ITP and thrombocytopenia due to liver disease. The data was obtained from the hospital computer records. Patients and procedure characteristics are shown at table 1. Eltrombopag was administered at a median initial dose of 25mg/day (IQR, 25-50). To evaluate treatment efectiveness based on platelet response we used the International Working Group Consensus Criteria for ITP, defyning "Partial Response" and "Response" as platelet count ≥30x10^9 or ≥100x10^9/L in absence of bleeding, respectively, and "Non response" as platelet count <30x10^9/L or less than 2-fold increase of baseline platelet count or bleeding. Statistic analysis was performed using SPSS software, version 21.0.
Results: Median platelet count at initiation of Eltrombopag was 32x10^9/L (IQR, 28-56x10^9/L). All patients achieved response over a median of 12 days (IQR, 7-14). Median platelet count before intervention was 116x10^9/L (IQR, 104-126x10^9/L). There were no procedure cancellations due to thrombocytopenia. No patient needed rescue treatments or platelet transfusion during procedures. No several bleeding or thromboembolic event was recorded. No adverse effect was registered in our cohort. After surgical intervention, all patients discontinued Eltrombopag progressively with no rebound effect.

Table 1.

Summary/Conclusion: Eltrombopag may be an effective perioperative treatment for patients with thrombocytopenia of a variety of etiologies. This agent increases platelet count in a short period of time, what may reduce the risk of bleeding of patients undergoing elective invasive interventions. It is a good alternative for patients with ITP who are not candidates to classical therapies as corticoids or immunoglobulins and its use may avoid perioperative platelet transfusion. Treatment with Eltrombopag may be discontinued safely after procedures in patients not requiring chronic treatment. Properly designed clinical trials are needed to verify these findings.

PB2351

INCIDENCE OF THROMBOTIC MICROANGIOPATHY IN HOSPITAL UNIVERSITARIO SON ESPASES. THE ROLE OF THE HEMATOLOGY LABORATORY AND THE MULTIDISCIPLINARY TEAM IN THE TMA EARLY DIAGNOSIS

B. Lopez-Andrade[1,*], M.C. Ballester[1], M.A. Duran[1], L. Lo Riso[1], J.M. Sanchez-Raga[1], A. Perez[1], M. Uriol[2], S. Cabello[2], M. Garcia-Recio[1], G. Aspas[1], A. Sampol[1]

[1]Haematology, [2]Nephrology, H. Universitario Son Espases, Palma mallorca, Spain

Background: Thrombotic microangiopathy (TMA) is a rare and life-threatening condition that can arise secondary to different diseases or mechanisms. The best known is the thrombotic thrombocytopenic purpura (TTP) a severe deficiency of ADAMTS13 in which an early treatment associates good prognosis, Hemolytic uremic syndrome (HUS) and Secondary TMA were the potential multiple etiologies can further delay diagnosis and treatment. The early detection of TMA can be done by both the clinical physician and hematology laboratory. In May of 2016 we started the implementation of the multidisciplinary team (MDT) in our center, in which the laboratory

screens for early detection and works with the MDT when a TMA is suspected either by clinical or laboratory findings; decreasing the time for the diagnosis and treatment.
Aims: Retrospectively evaluate the improvement in the response time and detection of TMA cases with the joint effort of the MDT and the Hematology laboratory, in a single center.
Methods: The MDT working group takes in physicians from Intensive care unit, Nephrology, Hematology, Farmacy, Immunology, and other services who follow the criteria for the diagnostic of TMA and discuss all potential TMA cases via a smartphone chat App, to elaborate the differential diagnoses and to recommend the best treatment option at every case. The screening evaluation is performed by a hematologist present in full time in the laboratory since 2015, either by critical results or a call from a suspected TMA case from the MDT. The laboratory screening and team workflow is described in figure 1. We report 44 cases from 2010-2017 (Including adults and children) with a median age 40-year-old. (1-76) from before and after the MDT group (table 1). The Response time (RT) was retrospectively evaluated from the laboratory records, from the first day that a TMA diagnosis was feasible up to the day it was diagnosed.
Results: In the cohort of 44 cases there has been a dramatic increase in the detection of cases after the MDT group and screening was stablished, 2010 to Apr16 (15cases) *versus* >May16-Feb18 (29 cases) an increase of 65% of the diagnosis after the MDT. The median response time decreased from 11 days (1.5-12) before the MDT to 0 days (0-2) (*P*=0.03) after the MDT. (table 1).

Table 1. Overall TMA diagnosis before and after MDT.

Cases	TMA	Atypical HUS	HUS	TTP	TMA transplant related	Mean Response time
2010– Apr 2016	15	10	0	2	3	11 days (1.5 – 12)
May 2016 – Feb 2018	29	10	11	4	4	0 days (0-2)
TOTAL	44	20	11	6	7	----------

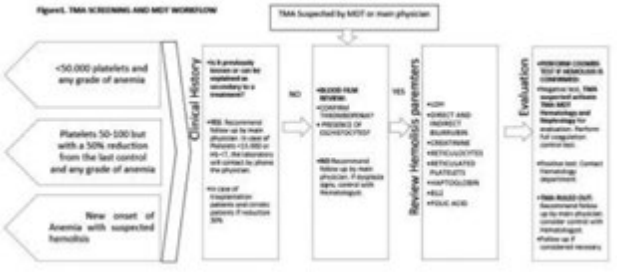


Figure 1.

Summary/Conclusion: The MDT communication with the laboratory and vice versa increases the awareness and detection of TMA, in which is fundamental to have a hematologist present in the laboratory and a trained staff to early recognize these alterations. The implementation of the MDT has improved the probability to early detect a TMA at our center. We believe that the Increase in the number of cases is probably related to missed or not diagnosed cases in the pre MDT era.

PB2352

ACCURACY EVALUATION OF LOW PLATELET COUNTS ON A NOVEL SLIDE-BASED INTEGRATED HEMATOLOGY ANALYZER

V.H. van der Velden[1,*], K. Ropar[2], D. Bracco[2], T. Allen[2], H. Russcher[1]

[1]Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands, [2]Roche Diagnostics, Westborough, United States

Background: The **cobas m** 511 integrated hematology analyzer is a novel slide-based system that performs a CBC, WBC differential, reticulocyte count, and nucleated RBC count using digital analysis of a microscope slide.
Aims: To assess the accuracy of low platelet (PLT) counts on the **cobas m** 511 compared with the Sysmex® XN-10 Automated Hematology Analyzer and BD FACSCanto™ Flow Cytometer in a single-center study.
Methods: Residual whole blood samples (n=115) in the PLT range 1-505 x10^3/µL (based on flow cytometery using CD61, CD41a, and CD42b antibodies) were randomly processed on each system. Sysmex samples were set to automatically reflex to PLT-F function, which is marketed as more reliable for thrombocytic or problematic samples than the standard impedance PLT-I function. Three analyses were performed: **cobas m** 511 and Sysmex Analyzer *versus* flow cytometer (reference), and **cobas m** 511 *versus* Sysmex Analyzer (reference). Data were compared using three thresholds and results included if a valid PLT result was obtained on both systems.
Results: All low PLT samples had valid results with the **cobas m** 511 and flow cytometry. Two (1.7%) samples were flagged with the Sysmex Analyzer (PLT-F) indicating unreliable results. The **cobas m** 511 demonstrated excellent accuracy compared with flow cytometry, with ≥93.6% sensitivity, specificity, and agreement, and good accuracy compared with Sysmex Analyzer (**Table 1**). Attempts were made to also study results for the Sysmex Analyzer PLT-I function; however, the vast majority of samples (100% for PLT counts ≤10, 80% for PLT 11-20, and 28% for PLT 21-50 x10^3/µL) had flags indicating unreliable values using this function which prevented any further comparison.

Table 1. Accuracy analyses.

	PLT threshold (10^3/µL)	Sensitivity (%)	Specificity (%)	Agreement (%)
cobas m 511 vs. flow cytometer (N = 115)	10	95.5	96.8	96.5
	20	93.6	94.1	93.9
	50	98.6	97.6	98.3
Sysmex Analyzer (PLT-F) vs. flow cytometer (N = 113)	10	100.0	96.7	97.3
	20	100.0	92.5	95.6
	50	100.0	97.6	99.1
cobas m 511 vs. Sysmex Analyzer (PLT-F) (N = 113)	10	88.0	97.7	95.6
	20	94.1	100.0	97.3
	50	100.0	100.0	100.0

Summary/Conclusion: These data demonstrate the robustness and accuracy of platelet counts reported by the **cobas m** 511 system. Many samples were flagged using the Sysmex PLT-I function that, in routine use, would have reflexed to the PLT-F function. All samples on the **cobas m** 511 system had valid PLT results.

PB2353

FREQUENCY OF AUTOIMMUNE THYROIDITIS IN CHILDREN WITH CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA

T.N. Tahtakesen Güçer*, E. Yılmaz, G. Aydoğan, N.N. Ozdemir

Pediatric Hematology Oncology, Kanuni Sultan Suleyman Education and Research Hospital, Istanbul, Turkey

Background: Chronic immune trombocytopenic purpura (ITP) is defined as an unexplained isolated thrombocytopenia that persists more than 12 months. Rheumatologic diseases, immunodeficiencies and thyroid diseases may coexist with chronic ITP due to immune dysregulation.
Aims: To investigate the frequency of autoimmune thyroiditis in patients with chronic ITP.
Methods: A total of 142 patients with chronic ITP with a follow up period of more than one year between 1995-2018 were included. Thyroid function tests, thyroid autoantibodies, thyroid ultrasonography were investigated.
Results: Seventy-three were female (51.4%) and 69 were male (48.6%). Mean age of patients was 13.2±5.4 years (range 2.4-28.1 years) and the mean age at diagnosis was 89±51 months (range 2.1-200 months). Thyroid function tests and thyroid autoantibodies were investigated in 129 patients. There was no clinical findings of either hypo / hyperthyroidism in any patient however, at least one value of fT4, TSH, anti TPO,or anti TG values was abnormal in 40 (31%) of investigated cases. Twentynine patients (22.4%) were put on follow-up by pediatric endocrinology department with a diagnosis of Hashimoto thyroiditis. Thyroid hormone replacement therapy was started in one patient. Anti TPO values of 22 patients (15.5%) and Anti-TG values of 18 patients (12.7%) were high. The majority of patients with high thyroid autoantibodies were female (67.5%).
Summary/Conclusion: We found that thyroid autoantibody positivity without clinical findings was a common finding in children with chronic ITP. According to a comprehensive study conducted in the USA, AntiTPO positivity is 10.4% and AntiTG positivity is 11.3% in general population. When compared with these rates, the frequency of thyroid autoantibody positivity and autoimmune thyroiditis was higher in children with chronic ITP in our study. As a conclusion hematologists managing children with chronic ITP should be vigilant about the regular evaluation with thyroid function tests and thyroid autoantibodies.

PB2354

WASP MUTATIONS: A SINGLE GENE DEFECT, LEADING TO VARIOUS CLINICAL AND HEMATOLOGICAL PHENOTYPES

K. Kiaras[1,2,*], H. Farajifard[3,4], Z. Aryan[3,4], B.S. Shamsian[5], R. Sherkat[6], I. Mohammadzadeh[7], J. Ghaffari[8], M.T. Arzanian[3], M. Sadeghi Shabestari[9], R. Molatefi[10,11], N. Rezaei[1,3,4]

[1]Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), [2]Research Center for Immunodeficiencies, Children's Medical Center, [3]Department of Immunology, School of Medicine, [4]Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, [5]Pediatric Congenital Hematologic Disorders Research Center, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, [6]Acquired Immunodeficiency Research Center, Al-Zahra Hospital, Isfahan University of Medical Sciences, Isfahan, [7]Noncommunicable Pediatric Diseases Research Center, Amirkola Hospital, Babol University of Medical Sciences, Babol, [8]Department of Pediatrics, Mazandaran University of Medical Sciences, Sari, [9]Department of Pediatrics, Children's Hospital, Tabriz University of Medical Sciences, Tabriz, [10]Department of Allergy and Clinical Immunology, Rasool e Akram Hospital, Iran University of Medical Sciences, Tehran, [11]Department of Pediatrics, Bo-Ali children's Hospital, Ardabil University of Medical Sciences, Ardabil, Iran, Islamic Republic Of

Background: Wiskott-Aldrich Syndrome (WAS) is an inherited x-linked defect, with characteristic features of thrombocytopenia, eczema and recurrent infections that result from ineffectiveness of the immune system. Mutations in *WASP* gene lead to WAS; however, mutations in that gene could also be seen in non-syndromic conditions such as isolated thrombocytopenia or neutropenia.
Aims: This study was performed to highlight the importance of Wiskott-Aldrich syndrome and to emphasize the need to have this diagnosis in mind if encountering a patient with microthrombocytopenia and eczema.
Methods: Herein, four patients with mutations in WASP gene are presented, who were referred to the Primary Immunodeficiency Diseases Referral Clinic of Children's Medical Center. Although direct sequencing of WASP gene was performed for three patients with classical phenotype of WAS, exome sequencing method was carried out for the remaining one, as his clinical phenotypes did not fulfil WAS criteria.
Results: One of the patients had initial signs of dysentery, eczema and petechiae at the age of 4 months; the diagnosis was immediately made, as his uncle had the diagnosis of WAS. He is under treatment with intravenous immunoglobulin (IVIG) and is awaiting a matching donor for hematopoietic stem cell transplantation (HSCT). Another one, who unfortunately passed away four months after HSCT, because of EBV infection of pharynx, had had the same initial presentations at the age of 6 months, but there had been a diagnosis lag of two and a half years before the confirmation of WAS diagnosis. The other two patients, on the other hand, did not initially have any clinical presentations and low platelet count was discovered during a routine check-up. They were diagnosed with autoimmune thrombocytopenia (ITP) at first and there was a 1.5 and a 9 year diagnosis lag. They are also under treatment with IVIG since diagnosis. The first recorded platelet count for these four patients varied from under 10,000 to 90,000. The mutations found in 3 of the patients were already known mutations in *WASP* gene, including c.121 C>T, c.91G>A, c.397 G>A. However, a novel mutation was detected in the 4th patient.
Summary/Conclusion: We wish to call attention to this uncommon, yet highly important disease. Although small size platelets and thrombocytopenia associated with recurrent infections and eczema make a suspicion of WAS, mutations in *WASP* gene should also be considered in those with isolated thrombocytopenia as well. It seems that there is still a long way from fully comprehending the genetics underlying the disease and there is plenty of scope for more research on therapeutic approached.

PB2355

IMMUNE MEDIATED THROMBOCYTOPAENIA IN CHILDREN: WHAT HAPPENS AFTER THE REFERRAL? A 7 YEAR RETROSPECTIVE REVIEW IN A TERTIARY PAEDIATRIC HAEMATOLOGY CENTRE

R. Koshy*, B. Nolan

Paediatric Haematology, Our Lady's Hospital For Sick Children, Dublin, Ireland

Background: Immune thrombocytopaenia (ITP) develops secondary to the production of auto-antibodies against platelets leading to isolated thrombocytopaenia. The cause is unknown in most cases, although ITP may be triggered by a viral illness. It is characterised by spontaneous bruising, petechiae and mucosal bleeding. ITP resolves in approximately 90% of children without intervention. However, a minority will continue to have low platelet counts for over 1 year (chronic ITP). Treatment is indicated for severe or prolonged bleeding. The majority of children do not require treatment. This condition can result in considerable concern from diagnosing clinicians and parents alike in both acute and chronic settings.
Aims: This study aims to perform a retrospective review of all children referred /attending our haematology department with a diagnosis of ITP and report on outcomes, treatment tolerability and describe the relationship, if any, between chronic ITP and the progression to development of auto-immune disease in a cohort of paediatric patients.
Methods: A retrospective review of patients referred to the Haematology Department between 2010-2017 with a platelet count <100x10^9 platelets per litre. Demographics, disease-related data, relevant auto-immune history and laboratory results were collected from medical records entered and analysed using Excel.
Results: Demographics: 140 patients were identified, 71 male and 69 female. At diagnosis, 78 were between 0 and 4 years of age, 35 between 5 and 10 years and 27 between 10 and 15 years. **Preceding events:** 3 patients had history of recent vaccinations. 6 patients had Epstein Barr Virus associated ITP. **Course of thrombocytopenia**: In 79 patients, platelet count recovered in less than 6 months. 43 patients had chronic ITP : 19/78 between 0 and 4 years of age, 13 / 35 between 5 and 9 years of age, and 11 / 27 between 10 and 15 years of age. Platelet count returned to normal in 17 patients who had ITP for more than 12 months. 3 patients developed recurrent ITP after initial recovery of platelet count. **Bleeding:** 27 patients received treatment for bleeding : 24 for epistaxis, 1 for haematuria, 2 for GI bleeds. 4 patients received precautionary treatment following trauma, 7 received treatment for dental/surgical procedures. 7 of 37 patients who received intravenous immunoglobulin developed signs and symptoms suggestive of aseptic meningitis. Two patient received platelets (one: Poland, one: Letterkenny) No patients received red cells. **Treatment of thrombocytopenia.** 8 patients received Prednisolone with non sustained increase in platelet count. 3/8 received Rituximab treatment. **Auto-immune disease.** 5 patients had co-existing / progression to autoimmune conditions : 1 hyperthyroidism, 1 mixed connective tissue disease and auto-immune hepatitis, 1 systemic lupus erythematosus ,1 autoimmune neutropaenia, IgA deficiency and coeliac disease and 1 evolving connective tissue disease/SICCA syndrome.
Summary/Conclusion: In conclusion, this data illustrates the diversity of ITP in children. Data here may not reflect the true history of ITP. Data may be skewed to chronic or atypical patients referred to one tertiary centre. We would recommend that a national registry be established to capture data on all children presenting with ITP in Ireland.

PB2356

LONG-TERM ELTROMBOPAG IN PEDIATRIC PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA (CITP): AN EXTENSION STUDY OF RUSSIAN PATIENTS FROM PETIT2

E. Donyush[1,*], A. Petrova[2], N. Schedrova[3], M. Goldbrunner[4], O. Ocak Arikan[4], J. Maier[4], V. Lebedev[5]

[1]Izmaylovskaya Children's City Clinical Hospital, Moscow, [2]Children City Hospital #[1], Saint-Petersburg, [3]Novartis Pharma LLC, Moscow, Russian Federation, [4]Novartis Pharma AG, Basel, Switzerland, [5]GUZ Regional Children's Clinical Hospital, Krasnodar, Russian Federation

Background: cITP is an acquired autoimmune disorder defined by low platelet counts for >12 months from diagnosis, resulting in an ongoing risk of significant bleeding (Rodeghiero *et al. Blood* 2009). Common therapy includes immunosuppression with corticosteroids; however, associated complications may be problematic, particularly for young children. The randomized, multicenter, placebo-controlled PETIT (Bussel *et al. Lancet Haematol* 2015) and PETIT2 (Grainger *et al. Lancet* 2015) trials in patients 1-17 years old demonstrated eltrombopag (EPAG), an oral thrombopoietin receptor agonist, as a therapeutic option for children with previously treated cITP. However, long-term data in pediatric patients are limited. Here we report outcomes from an open-label, Phase III extension study of pediatric patients who completed PETIT2 from four centers in Russia.
Aims: To evaluate the long-term safety and tolerability of EPAG in pediatric patients with previously treated cITP.
Methods: Patients aged ≥1-18 years previously enrolled in PETIT2 with clinical benefit from EPAG, were enrolled in the extension. Patients/guardians provided written informed consent. Screening was followed by a single-arm treatment period. Starting EPAG dose was based on the patient's dose at the end of PETIT2, and adjusted according to platelet count and the investigator's clinical judgement (max 75 mg/day) to maintain a safe hemostatic range (~50–200x10^9/L). Frequency and severity of adverse events (AEs) were recorded (graded using CTCAE version 4.03), and hematology and blood chemistry regularly monitored. Patients were considered to have completed the study and EPAG discontinued within 3 months once the patient reached 18 years of age or EPAG received local regulatory approval for pediatric cITP.
Results: Of nine patients enrolled (4 female, 5 male; median 9 [range 4-15] years old), four (44.4%) completed the study and five (55.6%) discontinued (patient decision, n=2; lack of efficacy, n=2; AE [autoimmune hepatitis], n=1). Median duration of exposure in the extension was 24.6 (range 3-48) months; all nine patients received EPAG for ≥3 months. 8/9 patients started

on or were escalated to 75 mg/day. AEs were reported in eight (88.9%) patients (Table), most commonly nasopharyngitis (n=3), epistaxis (n=2), and headache (n=2). No AE was considered EPAG related by the investigator. Serious AEs were reported in three (33.3%) patients: autoimmune hepatitis (n=1), epistaxis (n=1), and scleral hemorrhage (n=1); none were considered treatment related by the investigator. The autoimmune hepatitis case occurred in a 6 year old after ~3 months on EPAG, while on 75 mg/day; assessments at the time revealed Grade 3 alanine and aspartate aminotransferase (ALT/AST) elevations with normal bilirubin, ALT/AST normalized over the next 3 weeks following discontinuation. Overall, platelet counts fluctuated, but were generally maintained within a safe hemostatic range; patient numbers per timepoint were low.

Table 1.

Table. Adverse events (AEs) by preferred term and maximum severity

Preferred term	All grades, n (%)	Grade ≥3, n (%)
Nasopharyngitis	3 (33.3)	0
Epistaxis	2 (22.2)	0
Headache	2 (22.2)	0
Autoimmune hepatitis	1 (11.1)	1 (11.1)
Blood bilirubin increased	1 (11.1)	0
Iron deficiency	1 (11.1)	0
Pharyngitis	1 (11.1)	0
Scleral hemorrhage	1 (11.1)	0
Tonsillitis	1 (11.1)	0

A patient with multiple severity grades for an AE is only counted under the maximum grade

Only AEs occurring during treatment or within 30 days of the last study treatment are reported

Summary/Conclusion: This open-label, Phase III extension study evaluated the long-term safety of EPAG in pediatric cITP patients who previously participated in PETIT2. Patients continued to receive benefit from EPAG with a safety profile consistent with that observed in the PETIT studies and the known safety profile for EPAG. Only one patient discontinued treatment because of an AE (autoimmune hepatitis), considered unrelated to EPAG by the investigator. There were no unexpected safety findings, indicating a favorable benefit–risk profile for EPAG in the long-term treatment of pediatric cITP patients.

PB2357

Abstract withdrawn.

PB2358

THE USEFULNESS OF THE IMMATURE PLATELET FRACTION IN THE DIAGNOSIS OF ITP AND OTHER HEMATOLOGIC DISEASES

D.J. Park[1,*], S. Kim[1], Y. Kim[1], J.-A. Kim[2], Y.H. Park[2]

[1]Laboratory Medicine, [2]Division of Hematology, Department of Internal medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic Of

Background: The immature platelet fraction (IPF) is a predictive factor in increased platelet production associated with platelet immunomediated consumption or platelet destruction which results from the suppression of bone marrow production.
Aims: We evaluated the value of immature platelet fraction (IPF) in distinguish between immune thrombocytopenic purpura (ITP) and aplastic anemia (AA). Additionally, in order to evaluate its potential usefulness as a diagnostic marker, we detected IPF in other hematologic diseases including myelodysplastic syndrome (MDS) and myeloproliferative neoplasms such as polycythemia vera (PV), essential thrombocythemia (ET) and chronic myelogenous leukemia (CML).
Methods: 10 patients with ITP were compared with 12 patients with AA, 8 patients with MDS, 6 patients with ET, 4 patients with PV, 8 patients with CML and 80 age- and sex- matched healthy controls. Complete blood count tests including IPF, platelet distribution width (PDW), mean platelet volume (MPV), platelet large cell ratio (P-LCR), plateletcrit (PCT) were performed using Sysmex XN-2000 (Sysmex, Kobe, Japan).
Results: Mean IPF was 16.1% in patients with ITP, 4.0% in patients with AA, 11.2% in patients with MDS, 2.5% in patients with ET, 3.2% in patients with PV, 7.2% in patients with CML and 3.1% in the control group ($p<0.05$). ITP patients showed high IPF, PDW, MPV, and P-LCR compared with other groups, while PCT was lower in ITP patients than other groups.
Summary/Conclusion: We found highly elevated IPF in disorders related to increased platelet production, particularly associated with platelet destruction such as ITP. Especially in thrombocytopenic patients, IPF showed significant differences between ITP and AA. MDS patients resulting from the decreased platelet production associated with bone marrow suppression showed slightly increased IPF rather than healthy controls. ET and PV showed normal ITP comparing with healthy controls. Interestingly, CML with fibrosis 2 cases revealed significantly higher IPF (mean:15.1%) than CML without fibrosis 6 cases (mean:4.6%). Besides ITP, we found elevated IPF in MDS and CML. The IPF is highly recommended to evaluate the thrombopoietic status of bone marrow in thrombocytopenia patients.

PB2359

EVALUATION OF PLATELET RECEPTOR EXPRESSION IN CHRONIC LYMPHOID LEUKEMIA TREATED WITH IBRUTINIB

V. Popov[1], I. Dumitru[2], C.O. Matei[3], B.M. Matei[4], M. Omer[1], R. Leu[5], M.G. Moisescu[4], T. Savopol[6], E. Kovacs[5], H. Bumbea[7,*]

[1]Hematology, Colentina Clinical Hospital, [2]Hematology, Emergency University Hospital, [3]Biophysics and Cellular Biotechnology, [4]Biophysics and Cellular Biotechnology, UMF Carol Davila, [5]Biophysics and Cellular Biotechnology, Carol Davila University of Medicine and Pharmacy, [6]Biophysics and Cellular Biotechnology, Carol Davila University of Medicine and Pharmacy, [7]Hematology Emergency University Hospital, UMF Carol Davila, Bucharest, Romania

Background: Patients with chronic lymphoid leukemia (CLL) treated with Ibrutinib have increased bleeding risk. This complication is associated to platelets abnormalities. Platelet aggregation induced by different reagents is significantly decreased compared to controls. Ibrutinib inhibits platelet integrin αIIbβ3, outside-in signaling and thrombus stability. Membrane fluidity (MF) has an important role in the expression of platelet receptors and in modulating the activity of membrane proteins.
Aims: The aim of our study was to determine whether treatment with Ibrutinib influences the platelet MF and platelet receptor expression.
Methods: We present a retrospective study on 12 cases of CLL patients treated with Ibrutinib who were admitted in Colentina Clinical Hospital Bucharest, compared to a control group consisting in 10 untreated CLL patients. Platelet MF was measured by fluorescence anisotropy using TMA-DPH marker. Expression of platelet receptor was assessed by flowcytometry. We evaluated CD41/CD61 and CD42a/CD42b expression.
Results: Patients with CLL treated with Ibrutinib had a a higher platelet MF than in control group ($p=0.001$). 3 CLL patients associated 17p mutation. In this group the level of MF was lower than in patients who had no 17p mutation, without statistical significance. The expression of CD41/CD61 and CD 42a/CD42b was lower in CLL group treated with Ibrutinib compared to untreated CLL patients group ($p=0.04$). We also assessed the variation coefficient that represents the spread of intensity of fluorescence signal. This was higher in CLL patients group treated with Ibrutinib compared to control, for both types of analyzed receptors (CD41/CD61 and CD42a/CD42b). Our results confirm that MF and platelet receptors expression are influenced by Ibrutinib.
Summary/Conclusion: Administration of Ibrutinib in CLL patients is associated with higher MF and lower expression of platelet receptor with high spread of fluorescence. The modification of fluidity of platelet membrane probably could influence the expression of platelet receptors and quality of signaling. We have to check these findings on a larger group of CLL patients treated with Ibrutinib.

PB2360

IMMUNE THROMBOCYTOPENIA AND VITAMIN D DEFICIENCY

C. Tham*, A. Luqmani, M. Atta, D. Paul, C. Vladescu, E. Lucchini, A. Sayed, N. Cooper

Department of Clinical Haematology, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom

Background: In our practice we have observed a number of patients with immune thrombocytopenia (ITP) whose platelet count has increased after vitamin D replacement alone, prompting us to investigate this further. Vitamin D deficiency has been linked with the development of autoimmune disease. This pathophysiological mechanism may also apply to patients with ITP, in which case, correcting vitamin D deficiency would be a safe way of aiding treatment.
Aims: Assess whether there is a relationship between ITP and vitamin D deficiency in both adults and children.
Methods: Adult and pediatric ITP patient cohorts were retrospectively analysed, comprising of 148 and 34 patients respectively. The pediatric

cohort was further divided into acute (11) and chronic (23) groups. Vitamin D laboratory tests were reviewed and classified as – replete (>70nmol/L), insufficient (40-70nmol/L) or deficient (<40nmol/L). Platelet counts taken at the same time as vitamin D testing were also recorded. Thrombocytopenias were categorised as mild (>30x10^9/L), moderate (10-30x10^9/L) or severe (<10x10^9/L). Those with repeat vitamin D tests were categorised as – increased, unchanged or decreased vitamin D level, based on category (replete, insufficient or deficient) rather than numerical value.
Results: In the pediatric chronic ITP group, of the 20 patients tested, the mean vitamin D level was 46.4nmol/L (SD ±25.8). 40% of these were deficient and 35% insufficient. In the acute ITP group 6 patients were tested, the mean vitamin D level was 47.5 nmol/L (SD ±29.6). Overall, 83.3% of those tested were either deficient or insufficient and this did not correlate with severity of thrombocytopenia. In the adult cohort of 148 patients, 117 (mean age 45 years) had vitamin D levels and platelet counts tested. The mean level was 53.8nmol/L (SD ±31.7). Of these, 35% were deficient, 41% insufficient and 24% replete. When stratified by severity of thrombocytopenia, there was no significant difference between groups. There were significantly more patients deficient in winter and spring compared to in summer and autumn. Plotting numerical values of vitamin D level against platelet count showed little correlation, with a Pearson R value of -0.0048. 47 patients underwent repeat testing for vitamin D levels. Vitamin D levels increased in 17, remained unchanged in 24 and decreased in 6 patients. When stratified by change in platelet count (by category, rather than numerical value), 5 patients showed an increase in both vitamin D and platelet count, 2 of which were on no other treatment for ITP. This was not a consistent change with others having a fall in platelet count despite an increase in vitamin D levels.

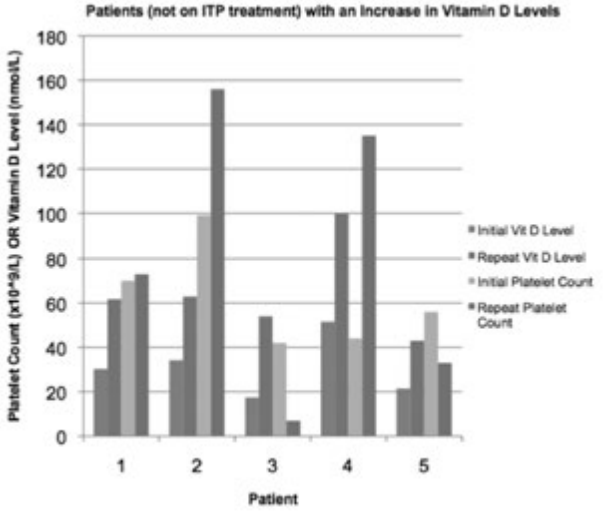


Figure 1.

Summary/Conclusion: From our data, a small number of patients had an increase in their platelet count with vitamin D replacement alone. However, this was not a consistent effect and there was no overall correlation between vitamin D deficiency and thrombocytopenia. Nonetheless, this is a heterogeneous group of patients, on a variety of treatments which were not accounted for. Vitamin D deficiency is a significant problem in our cohort of children and adults with ITP. Vitamin D is of clear importance in regulation of the immune system with an apparent protective effect against infections. The role of vitamin D in autoimmune conditions is harder to establish given the heterogeneity of disease and the variability of clinical trials performed in this area. However, given the low sunlight in the UK and the importance of vitamin D for overall immunity, we recommend testing for and replacing vitamin D, particularly over winter months.

PB2361

DISCONTINUATION OF TREATMENT WITH TROMBOPOIETIN RECEPTOR ANALOGS IN PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA (ITP): IS IT POSSIBLE?

M. Yuste Platero[1,*], L. Bermejo Martínez[2], D. Morillo Giles[1], R. Martos Martínez[1], E. González Arias[1], P. Beltrán Álvarez[2], A. Pascual Martínez[2], P. Llamas Sillero[3]

[1]Hospital General de Villalba, Hematology, Collado Villalba, [2]Hospital Infanta Elena, Hematology, Valdemoro, [3]Hospital Fundación Jiménez Díaz, Hematology, Madrid, Spain

Background: Primary immune thrombocytopenia (ITP) is an autoimmune disease in which, along with a greater destruction of platelets, an insufficient marrow production is observed. This second mechanism confirms the existence of thrombopoietin levels (TPO); which reinforces the hypothesis that thrombopoietin levels were elevated in this population group. To improve the number of platelets in ITP, a group of drugs, the thrombopoietin receptor agonists (TPO-RA), were developed. Two clinical trials led to the approval of eltrombopag (RAISE and EXTEND studies), without determining the time of treatment, as well as the possibility of temporary interruptions of those patients who reached platelet numbers in stable ranges.
Aims: To analyze if the successful suspension of the drug is possible, for those patients who reach adequate platelet counts.
Methods: Prospective study conducted between January/2017 and February/2018, in which 2 patients diagnosed with primary immune thrombocytopenia are included. We analyze the time of active treatment, as well as the period of suspension of therapy. The cut-off point of the platelet count was considered optimal was 100x10^9/mm^3, and was defined by recommendations of clinical guidelines and patient safety and validated according to the laboratory standards of our center (Range of normality: 150x10^9/mm^3-450x10^9/mm^3).
Results: Two patients diagnosed with ITP on treatment with thrombopoietin analogues were included. The first case is a 75-year-old woman, with comorbidities that made difficult the treatment with corticosteroids at full doses (diabetes mellitus and recurrent infections). At diagnosis 15x10^9/mm^3 platelets. First-line treatment with poor tolerance is started, suspending it and starting in May 2017 second-line treatment with eltrombopag. The treatment was maintained for 10 weeks at standard doses with very good tolerance and progressive rise in platelet counts, reaching stable numbers in the different analytical controls. Given the stability, it was decided to suspend treatment and close clinical and analytical follow-up, maintaining platelet numbers above 100x10^9/mm^3 to date. The current period without treatment is 7 months. The second case is an 80-year-old woman with a diagnosis of ITP that debuted with severe thrombocytopenia and associated clinical bleeding. First-line treatment with corticosteroids was initiated at high doses and IVIGs cycle, being refractory without reaching adequate platelet counts. In August/2017, second-line treatment with thromboprotein receptor agonists (eltrombopag) was started until October 2017, with analytical and clinical stability. On that date it was decided to suspend treatment by stable platelet counts. The patient continues without active treatment and asymptomatic from the point of view of bleeding complications. The current period without treatment is 4 months.
Summary/Conclusion: Currently there is published data on the interruption of the TPO-RA, but objective data and standardized recommendations have not yet been provided. It has not been possible to identify the predictive factors related to the response and maintenance or withdrawal of the drug. These two cases demonstrate and support that not only a stable response can be achieved in patients diagnosed with ITP, but that the withdrawal of the drug, once adequate platelet ranges have been reached, is possible, but always with the need for a clinical and analytical follow-up close of these patients.

PB2362

CLINICAL AND LABORATORY PARAMETERS IN CHILDREN WITH ITP

O. Tanous[1,*], A. Koren[2], T. Dujovny[3], H. Eitam[4], R. Colodner[4], C. Levin[3]

[1]Ward A, [2]hematology unit, [3]pediatric hematology unit, [4]yetzhak rabin [20], Emek Medical Center, Afula, Israel

Background: Immune Thrombocytopenia (ITP) is one of the most common hematological abnormalities in children; despite the extremely low platelet count the symptoms are generally mild. Some studies have argued that a compensatory pro-coagulant plasmatic state with a protective effect exist in these patients. Our study aims to investigate this pro-coagulant state in pediatric ITP patients.
Aims: Our study aims to investigate the bleeding tendency and pro-coagulant state in pediatric thrombocytopenic patients.
Methods: Pediatric ITP patients treated at Emek Medical Center were included. Bleeding score and laboratory parameters including platelet (Plt) count, PT- aPTT, von Willebrand factor (vWF) and Factor VIII activity at 2 different points, disease onset and during follow-up, were assessed.
Results: Thirty seven patients were included (24M/13F); median age was 3.3 (0.7-22.7) yr. Eight pts (22%) had a bleeding score of 3 (more than skin) or more. Five patients were treated with steroids or IVIG. Patients with an acute disease (12 mo), (p<0.025). The severity of bleeding was lower

in Arab pts than in Jewish ones (p<0.02). Seventeen pts were evaluated at two time-points. The mean Plt count and vWF were 25.3 K/µl, 165.6% at point 1 and 199.1K/µl, 146.6% at point 2; respectively. Platelet count significantly increased and vWF significantly decreased between time points 1 and 2 among our patients (Z=-3.41, p<0.001; Z=2.23, p<0.03). A compensatory pro-coagulant state with increased vWF was found in our cohort at enrollment and declined when platelet count increased. This might explain the scarcity of severe bleeding in pediatric ITP patients.
Summary/Conclusion: A compensatory pro-coagulant state with increased vWF was found in our cohort at enrollment and declined when platelet count increased. This might explain the scarcity of severe bleeding in pediatric ITP patients.

PB2363

NON –CATHATER THROMBOEMBOLIC EVENTS IN CHILDREN

Z. Salcioglu*, G. Aydoğan, F. Akıcı, E. Yılmaz, G.N. Özdemir, E. Uysalol, C. Bayram

[1]Pediatric Hematology-Oncology, Kanuni Sultan Suleyman Education And Research Hospital, Istanbul, Turkey

Background: Although the incidence of thromboembolic events has increased over the last decade, non catheter-related thromboembolism is uncommanly seen in childhood.
Aims: This study was aimed to analyze the data of 93 thromboembolic events in children who were followed up in our hematology–oncology clinic between 2000-2017, retrospectively.
Methods: Information of 93 patients were retrieved from patient files and from the records contained in the electronic information processing environment created after 2005.
Results: Fifthy four of the patients were males and 39 were females. The age range was between four months-16 years. Presenting ages of the patients were changing from one day to 12 years. Of the children 11 (11.8%) of the cases were neonates, 23 (24.7%) were infants less than 1 year old, and 59 (63.5%) were children over than one year old. Thromboembolic events were mostly located in central nervous system 54 (58.1%), deep venous system of the limbs 20 (21.6%), portal vein 9(9.8%), renal vein 2 (2.1%), intracardiac 2 (2.1%), inferior vena cava 2 (2.1%), peripheral artery 2 (2.1%) and pulmonary embolism 2 (2.1%). Inherited risk factors were present in 55 (48.4%) of the children. Twenty of the patients carried two risk factors (21.5%). FV Leiden heterozygosity was the most common inherited risk factor. Acquired risk factors were present in 35 (37.6%) of the children. Systemic infection was the most common underlying risk factor. Acquired and inherited risk factors were present simultaneously in 20 (21.5%) of the patients. Treatment included low-molecular-weight heparin (n=67), coumadin (n=12), heparin (n=8), aspirin(n=6).
Summary/Conclusion: Thrombosis in children is an important complication with high morbidity and mortality. Thrombosis in children is gaining increased awareness, as advanced medical care has increased treatment intensity of hospitalized pediatric patients. Better predictors of prognosis in relation to risk factors, treatment and prophylaxis are therefore urgently needed. Future respective studies may help to assess the risk profile and therapy.

PB2364

THE IMPACT OF GESTATIONAL THROMBOCYTOPENIA IN NEWBORNS

S. Cristina[1,*], P. Monica[1], B. Ema[1], C. Sonia[1], L. Diana[1], S. Miruna[2], G. Alexandru[1], P. Ovidiu[1]

[1]Hematology, University of Medicine and Pharmacy "Victor Babes" Timisoara, [2]Hematology, Municipality Emergency Hospital of Timisoara, Timisoara, Romania

Background: Thrombocytopenia develops rarely during pregnancy or in the immediate postpartum period. It may develop secondary as a biomarker of a coexisting disorder or it may be also idiopathic.
Aims: The aim of this study was to determine the impact that gestational thrombocytopenia (GT) has on the pregnancy evolution as well as on the newborn's health.
Methods: This is a retrospective study on 40 women who developed thrombocytopenia during pregnancy. Data was obtained from their medical files. We selected data from when they were at least 30 weeks of pregnancy.
Results: From the total of 40 women, 26 (65%) developed mild thrombocytopenia (platelets over 100.000) and 14 (35%) developed mild thrombocytopenia (platelets between 50.000 and 100.000). From the total of patients who developed mild thrombocytopenia, 73,07% presented a very mild oscilation in the platelets values before giving birth while 26,93% presented a significant decrease in platelets count with about 50.000. The situation was different in case of women who developed moderate anemia. The majority of them (71,42%) developed severe thrombocytopenia and cortison therapy was given. What concerns the newborns of mothers presenting mild anemia, only 15,38% of them presented thrombocytopenia and it recovered in a few weeks without therapy. On the other hand, the majority of newborns (57,14%) whose mothers presented moderate thrombocytopenia developed persistent thrombocytopenia with platelets in continuous decrease and further investigations were performed.
Summary/Conclusion: This study reveals that newborns from mothers who developed moderate gestational thrombocytopenia have better chances to develop thrombocytopenia.

Quality of life, palliative care, ethics and health economics

PB2365

MONTELUKAST AND GEMFIBROZIL IMPROVES SYMPTOM CONTROL AND ENHANCES THE RESPONSE TO ORAL CHEMOTHERAPY IN HAEMATOLOGICAL MALIGNANCIES WITH A POOR PROGNOSIS

S. O'Mara[1,2,3,*]

[1]Haematology, Laverty Pathology, [2]Tamara Hospital, [3]New England Northwest Haematology, Tamworth, Australia

Background: Many malignancies occur in elderly and unfit patients who are not suitable for agressive chemotherapy due to side effects, poor response and poor survival with standard therapies. These patients often receive some benefit from orally administered palliative treatment regimens. A phase 1/2 clinical trial (MOM trial ANZCTR) was undertaken using Montelukast and Gemfibrozil to enhance the response to existing chemotherapy in Multiple Myeloma (reported elsewhere). One of the observations was that the patients felt significantly better on montelukast treatment and appeared to have less chemotherapy toxicity. Montelukast and Gemfibrozil was added to a number of oral treatment regimens administered with palliative intent to see whether side effects of treatment as well as the disease symptoms would improve.
Aims: To test whether the addition of Montelukast and Gemfibrozil to palliative treatment regimens improved the disease response, symptoms, treatment side effects and functional performance status.
Methods: All patients gave informed consent. Age range 67 -92. Median 84 yo. 14 patients on palliative oral treatment regimens for a variety of haematological malignancies(AML, CLL, NHL, Richters) were given Montelukast 30mg twice daily as well as 600mg Gemfibrozil twice daily. The effect on transfusions, admissions to hospital, clinical response as well as general well being was measured. Treatment regimens included low dose oral chlorambucil in CLL. Single agent 6-TG or busulphan in non responsive AML. Oral prednisolone, cyclophosphamide and etoposide in agressive nhl. Two patients with solid tumours received no additional chemotherapy.
Results: 2/4 patients on palliative AML treatment have responded. Patient 1 became transfusion independent after 1 month. Patient 2 has had a response in peripheral blood after failing 6 months treatment with Azacytidine. 5/5 patients with CLL on 2 mg of oral chlorambucil per day have had a marked improvement in lymphocyte counts. 1/1 patient with waldenstroms macroglobulinaemia has become transfusion independent and shown response after previously having no resopnse to single agent chlorambucil. 1/1 patients with Richters transformation of CLL has had complete resoloution of B symptoms and complete resoloution on PET scan measured at 2 months. 1/1 patient with myeloma with bone marrow failure has become transfusion independent, improved white cell counts and improved performance status from 4 to 2. 1/1 with progressive disease following chemotherapy for lung cancer has had an improvement in performance status from 3 to1 as well as a reduction in tumour size. 2/2 patients with agressive lymphoma have had a complete response in less than 4 weeks with transformed lymphoma and angioimmunoblastic nhl. 1/1 patient with end stage airways disease and advanced non responsive GIST tumour went from ECOG 4 to ECOG 2 after 3 weeks of treatment. Fourteen of sixteen patients have shown demonstrable benefit.
Summary/Conclusion: Montelukast and Gemfibrozil both given alone and when added to palliative chemotherapy appears to improve the response to treatment and symptoms in the majority of patients. It appears to reduce transfusion dependency. There has been a marked improvement in performance status. There have also been some highly significant responses in otherwise incurable poor prognosis malignancies. As these improvements are ongoing, it is planned to update the results at the congress.

PB2366

METABOLIC PROFILE OF CHILDREN AND ADOLESCENTS FOLLOWING TREATMENT FOR HEMATOLOGIC MALIGNANCIES

D. Apostolaki[1,*], K. Katsibardi[2], V. Efhymiou[1], F. Bacopoulou[1], G. Avgerinou[2], A. Kattamis[2]

[1]Center for Adolescent Medicine and UNESCO Chair on Adolescent Health Care, First Department of Pediatrics, National and Kapodistrian University of Athens, Aghia Sophia Children's Hospital, [2]Hematology-Oncology Unit, First Department of Pediatrics, "Aghia Sophia" Children's Hospital, University of Athens, Athens, Greece

Background: Metabolic disorders have been increasingly recognized in childhood cancer survivors. Dyslipidemia, glucose intolerance, hyperinsulinism and insulin resistance may play a role in the development of metabolic syndrome and later cardiovascular disease in these patients. Contributing factors to metabolic disturbances include cancer type and treatment, associated hormonal disorders, disturbed energy intake and/or physical inactivity as well as family history of metabolic disorders and obesity.
Aims: To assess the metabolic profile of children and adolescents after completing their treatment for hematologic malignancies.
Methods: Children and adolescents with hematologic malignancies (2 groups: leukemia or lymphoma), at least 6 months post-treatment, underwent measurement of height and weight, Tanner staging for sexual maturity, liver ultrasonography and fasting blood lipid profile assessment for cholesterol (C), triglycerides (TG), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), apolipoproteins A_1 (apoA_1) and B (apoB), lipoprotein a (Lpa) and uric acid (UA). Body mass index (BMI) and homeostatic model assessment for insulin resistance (HOMA-IR) were calculated for each patient.
Results: A total of 30 patients, 18 with acute lymphoblastic or acute myelogenous leukemia (15 and 3, respectively), (mean age±SD; 12.7±3.8 years) and 12 with Hodgkin or non-Hodgkin lymphoma (10 and 2, respectively) (mean age±SD, 14.5±3.3 years) participated in the study. Patients had completed treatment for leukemia before 21.6±13.6 months and for lymphoma before 19.3±11.5 months. Among patients with leukemia 23.5% had normal weight and 76.5% were overweight or obese, whereas among patients with lymphoma, 58.3% had normal weight and 41.7% were overweight or obese. Serum concentrations (mean±SD) of lipids in patients with leukemia were C 168.5±32.1 mg/dl, LDL 95.6±26.8 mg/dl, HDL 57.5±13 mg/dl, TG 76.9±30.5 mg/dl, apoA_1 148.5±29.2 mg/dl, apoB 86.9±21.7 mg/dl, UA 4.8±1.7 mg/dl and for Lpa median (interquartile range): 4.7 (37.5) mg/dl. Respective concentrations for patients with lymphoma were C 166±27.6 mg/dl, LDL 89.3±21.4 mg/dl, HDL 63.3±14.7 mg/dl, TG 67.1±26 mg/dl, apoA_1 127.2±45.4 mg/dl, apoB 78.0±11.6 mg/dl, UA 4.5±1.5 mg/dl and Lpa 2.9 (6.4) mg/dl. HOMA-IR (mean±SD) was 2.1±1.5 or 3.1±1.9, for patients with leukemia or lymphoma respectively. There was no statistically significant difference in the metabolic profile between the 2 groups. Among patients with leukemia, serum TG were significantly higher (P=0.011) in those with fatty infiltration of liver (100.8±32 mg/dl) than patients without fatty liver (63.8±21.2 mg/dl). In patients with lymphoma, UA concentrations and HOMA-IR were higher (P=0.042 and P=0.031 respectively) in overweight or obese patients (5.5±1.4 mg/dl and 4.4±1.6 respectively) than in normal weight patients (3.8±1.2 mg/dl and 1.5±0.2 respectively). There were no statistically significant differences in the lipid profile between genders for both groups.
Summary/Conclusion: Serum TG were associated with fatty infiltration of the liver in patients with leukemia whereas UA and HOMA-IR were associated with BMI-category in patients with lymphoma. Larger studies are needed to fully elucidate the metabolic disturbances in young survivors of hematologic malignancies.

PB2367

QUALITY OF LIFE OF TRANSFUSION DEPENDENT THALASSEMIA PATIENTS IN GREECE. A MULTICENTRE STUDY

P. Klonizakis[1,*], C. Apostolou[1], I. Christodoulou[1], D. Andriopoulos[1], K. Kafantari[1], D. Pantelidou[2], S. Kokkota[2], M. Diamantidis[3], A. Kourakli[4], M. Sarigianni[1], R. Klaassen[5], E. Vlachaki[1]

[1]Aristotle University of Thessaloniki, [2]Thalassemia Unit, AXEPA General Hospital, Thessaloniki, [3]Thalassemia Unit, General Hospital of Larisa, Larisa, [4]Thalassemia and Hemoglobinopathies' Center, University Hospital of Patras, Patra, Greece, [5]University of Ottawa Children's Hospital of Eastern Ontario, Ontario, Canada

Background: In Greece, the Quality of Life (QoL) in Transfusion-Dependent Thalassemia (TDT) has been studied in only small groups of patients and all assessments involved the administration of generic QoL questionnaires. TranQol, is a novel, disease-specific QoL questionnaire for TDT patients that has been recently validated and will be administered in this study.
Aims: The aim of this study is to evaluate the QoL of TDT patients in Greece using a novel, disease-specific QoL questionnaire (TranQol) in combination with a generic QoL questionnaire (SF-36).
Methods: In this multicentre study, we recruited 288 consecutive adult TDT patients from four Thalassemia Centers in Greece, according to specific inclusion and exclusion criteria. The participants had a mean age of 32±7 years, 42% were male and 58% were female. Demographic and clinical characteristics were retrieved from the patients' medical records. All participants completed a set of two QoL questionnaires, the generic SF-36v2 and the disease-specific TranQol, during their scheduled transfusion visits. The

data from SF-36v2 were used in order to compare the QoL of TDT patients with the healthy population in Greece. The more sensitive TranQol was used to describe possible differences in QoL between the following subgroups of TDT patients 1) type of iron chelation therapy 2) presence of comorbidities 3) level of liver hemosiderosis. Parametric and non-parametric tests will be used to compare data with or without normal distribution, respectively. Statistical significance is defined as $p<0.05$.

Results: Compared to the available data from the healthy population in Greece, the SF-36 scores of TDT patients were lower in all Qol domains which involved Physical Functioning (81% *vs* 50%), Role Physical (79% *vs* 50%), Bodily Pain (73% *vs* 52%), General Health (67% *vs* 47%), Vitality (66% *vs* 55%), Social Functioning (82% *vs* 50%), Role Emotional (82% *vs* 47%) and Mental Health (68% *vs* 49%). The mean summary score of TranQol was 71%±15, whereas the scores of the domains physical health, emotional health, sexuality, family functioning and school/career where 71%±15, 67%±17, 77%±23, 74%±19 and 75%±21 respectively. The mean TranQol summary score was lower for patients treated with Deferiprone compared to patients treated with Deferoxamine (mean difference=11%, 95% C.I: 2-21), Deferasirox (mean difference=12%, 95% C.I: 15-21) or with the combination of Deferiprone-Deferoxamine (mean difference=12%, 95% C.I :15-21). Assessment of comorbidities revealed that patients with osteoporosis have significantly lower TranQol scores (70%±14 *vs* 74%±13). The TranQol mean scores did not differ between groups with different levels of liver iron but patients with mild liver hemosiderosis had lower physical health scores compared to patients with normal levels of liver iron (mean difference=7%, 95% C.I: 6-14).

Summary/Conclusion: This is the first QoL study in a representative sample of the TDT population in Greece. Compared to the healthy population, TDT patients have a lower QoL. The use of a disease-specific QoL questionnaire revealed distinct groups of TDT patients with worse QoL. Our findings could help the physicians to early recognize these groups of TDT patients and help them improve their QoL. In the era of novel treatments for TDT patients, each new drug should be evaluated in terms of both clinical efficacy and improvement in health-related QoL. Our results may provide a baseline index score of QoL in TDT patients to be compared with relevant outcomes from future clinical trials.

PB2368

COST OF HEMATOLOGIC CANCERS IN INDIA: A HOSPITAL INPATIENT CARE ANALYSIS

C. Cherian Philip[1,*], S. Singh[1], A. Mathew[1], A. Jindal[2], N. Kakkar[3], M.J. John[1]

[1]Clinical Haematology, [2]Immunohematology and Blood Transfusion, [3]Laboratory Haematology, Christian Medical College & Hospital, Ludhiana, India

Background: Health care expenditure in India remains a challenge. Majorly dependent on out of pocket expenditure and private health care, these expenses are now a leading cause of poverty in India and reason to abstain from treatment(1,2). Despite an acknowledged increase in burden of cancers in India, data on the costs of care are lacking. This information is essential for national health care planning and resource allocation. In identifying with this goal we analysed the inpatient hospital care costs for the four most common hematologic cancers from our centre.

Aims: To estimate the inpatient hospital care costs for ALL, AML, NHL and Multiple Myeloma at a tertiary care centre In India.

Methods: Cost data was collected for each hospital inpatient admission beginning July 2015 through January 2018. This was obtained from a prospectively maintained registry which also included baseline demographic information. Hematologic cancers were defined by the WHO classification of tumors of hematopoietic and lymphoid tissues. Inpatient stay was categorized as private room and general ward. The General Ward accommodates five patients in a room with a shared amenities and piped oxygen, suction facility. Private rooms accommodate a single patient and furnished with additional amenities. Patients who were moved to an intensive care unit were not included in this analysis. Standard chemotherapy protocols, antibiotics and antifungal policies with standard of care diagnostic tests were adhered and administered to all patients in an inpatient ward without specialized air handling facilities. The trigger for component transfusion was platelet count of $<10x10^9$/l and Hb<7g/dL.

Results: A total of 461 patients were admitted in this period. Characteristics of these patients are detailed in table1. The median distance from home to hospital was 61 km (range: 10-78) and 73(15.8%) patients travelled from another state. Acute Lymphoblastic leukemia was the most frequent diagnosis for admission in 163 (35.4%) patients followed by Non Hodgkin Lymphoma in 144(31.2%). Patients in the private ward were more likely to have availed a health insurance (6.38% *vs* 2.1%, P<0.01). The median cost of care per inpatient stay was 46027.50 (€575) [€ 260-€1369].

Table 1.

Table 1. Characteristics of patients with hematologic cancers		
Variable	**N (461) n (%)/ Median (IQR)/Mean (±SD)**	
Age	47 (21-60)	
Durations of admission (days)	4 (2-8)	
Payment (Out of pocket)	394(85.5)	
Gender(Male)	285(61.8)	
Comparison across admission categories		
-	**General N (284)**	**Private N (177)**
Age~	46 (20-59)	49 (24-63)
Durations of admission (days)~	4 (1-7)	5 (2-11)
Disease-wise inpatient expenditure (Mean)*		
Multiple Myeloma	₹ 108546 (€1356)	₹ 119646 (€1495)
AML	₹ 99636 (€1245)	₹ 341782 (€4272)
ALL	₹ 74580 (€932)	₹ 209160 (€2614)
NHL	₹ 37476 (€468)	₹ 142473 (€1780)

*₹ 80= €1; ~ P>0.05; # inpatient expenditure included the cost of drugs, antibiotic usage, supportive care with component transfusion and growth factors, hospital room charges, investigation charges and consultation fees.

Summary/Conclusion: In our analysis, the majority of inpatient hospital expenses are borne out-of-pocket. With a GNI per capita of €1346 and multiple admissions required for chemotherapy administration or complications in hematological cancers; the costs of inpatient care places a high burden on patients. This is despite reduced costs of care when compared globally(3). Variations in treatment protocols across treatment centres in different countries limit the generalization of our findings. However, it is likely that this data is broadly representative of the experience of many tertiary centres within India and also likely to represent many developing countries from where there is limited data. Universal health coverage and better allocation of resources is needed for patients with hematological cancers in India.

PB2369

THE OTHER SIDE OF THE MOON: PATIENTS' PERCEPTION OF IMATINIB AND DASATINIB SYMPTOM BURDEN FOR CHRONIC MYELOID LEUKEMIA. RESULTS OF THE PATIENT'S JOURNEY FROM THE REAL-LIFE SOLARIS PROJECT

F. Stagno[1,*], M. Annunziata[2], M. Iovine[3], P. Danise[4], M.M. Spiezia[5], M.R. Esposito[6], A. Russo Rossi[7], V. Accurso[8], A. Malato[9], R. Vallone[10], G. Mineo[11], G. Romano[12], F. Palmieri[13], M. Pizzuti[14], F. Di Raimondo[1], G. Specchia[15], L. Luciano[16]

[1]Hematology Section, AOU Policlinico - V. Emanuele, CATANIA, [2]Hematology Unit, Cardarelli Hospital, Naples, [3]Hematology Unit, A.O. S. Anna e S. Sebastiano, Caserta, [4]Hematology Unit, A.O. Tortora, Pagani, [5]Hematology Unit, P.O. S. Giovanni Bosco, [6]Hematology Unit, P.O. S. Gennaro, Naples, [7]Hematology and BMT Unit, Policlinico Consorziale Bari, Bari, [8]Hematology Unit, A.O.U. Policlinico Giaccone, [9]Hematology Unit, Ospedale Civile Cervello, Palermo, [10]Hematology Unit, Benevento, [11]Hematology Unit, Ospedale Civile S. Vincenzo, Taormina, [12]Eubea, Naples, [13]Hematology Unit, P.O. S. Giuseppe Moscati, Avellino, [14]Hematology Unit, A.O. S. Carlo, Potenza, [15]Hematology and BMT Unit, University of Bari, Bari, [16]Hematology Department, II University Federico II, Naples, Italy

Background: The outstanding availability and clinical success of tyrosine kinase inhibitors has turned CML into a chronic disease in which patients need to both tolerate and adhere to continuing treatment for many years. Nonetheless this great clinical achievement has not been paralleled by a full understanding of the impact of the CML disease from the patient viewpoint. This new scenario changed both physician's behaviour and patient's perceptions about the disease and influence clinical decision-making. There is now evidence that patient-reported outcomes, including self-reported symptoms, might provide useful information beyond traditional disease factors.

Aims: Aim of the SOLARIS project was both to develop and investigate a simplified health-related quality-of-life (QoL) assessment to detect real-life information from the patient about the symptom burden and on the administered treatment.

Methods: Therefore 244 CML patients in chronic phase (M 60%, F 40%, median age 61yrs) were enrolled: 175 of them were on Imatinib (IM) therapy and 69 on Dasatinib (DAS), either as first or second line treatment. All patients provided written informed consent. More than 45% of them received concomitant medications; elderly patients (over 65yrs) represented 41.4% of the total population. A simplified and easy to use QoL questionnaire with 9 single-item and another with 14 single-item was given to patients receiving IM- or DAS-therapy respectively. A small cohort of patients (19) shifted from IM-therapy to DAS. Fisher exact test was performed to evaluate any possible differences between the two treatment groups (IM *vs* DAS).

Results: CML patients treated with IM reported a high incidence of periorbital edemas with ocular side effects (70%), muscle cramps (79%), bone and joint pain (51%), fatigue (61%). Interestingly, most of the patients receiving DAS-therapy reported fatigue as the main symptom (70%). When compared, we found that diarrhea (p=0.001), periorbital edemas with ocular side effects (p=0.00007) and muscle cramps (p=0.000006) clearly emerged among the treatment symptom burden as main differences favouring DAS, whereas no other difference among symptoms was detected. Moreover, those patients who changed to DAS showed an improvement of most of the side effects with the exception of fatigue.
Summary/Conclusion: In conclusion we are aware that our questionnaire is not validated and that some items are missing, however we believe that a short and easy to use questionnaire and real-life spontaneous questions might be helpful in understanding patients' perception on symptom burden and treatment in CML.

PB2370

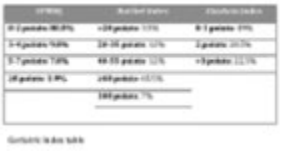
PROFILE OF "OLDEST OLD" PATIENTS WITH ANEMIA IN A HEMATOLOGY-GERIATRIC INTERDISCIPLINARY CONSULTATION

L. Lacalle[1,*], N. Fernández[2], N. Espinosa[3], S. Pinzon[4], M.P. Olier[5], M.J. Agustín[5], M. Clerencia[2], M.P. Delgado[4], M.Á. Montañés[4], M.V. Recasens[4]

[1]Hematology, Hospital San Jorge, Huesca, [2]Geriatrics, Hospital Universitario Miguel Servet, Zaragoza, [3]Hematology, Instituto de Investigación Biomédica de Salamanca, Salamanca, [4]Hematology, [5]Pharmacy, Hospital Universitario Miguel Servet, Zaragoza, Spain

Background: Anemia is a frequent entity arising with age, mainly in people over 85 years old, known as "oldest old". Nowadays, this segment of the population is growing, and consequently it will increase along next decades. In our center (Miguel Servet Universitary Hospital), an interdisciplinary Hematology-Geriatrics consultation has been created in order to attend these patients.
Aims: To analyze, in a 15 months follow-up, the profile of elderly patients with anemia in order to optimize their treatment and improve their life quality.
Methods: It is a descriptive, observational and prospective analysis of patients assessed in Hematology-Geriatrics consultation in a tertiary hospital. Period of study: from October 2016 to January 2018. Functional status was determined by Barthel Index. Comorbidity was measured by Charlson Index. Cognitive function was measured by Pfeiffer questionnaire (SPMSQ). Nutrional risk was evaluated by a Mini-Nutritional short version (Mini-MNA).Data collection was included in a SPSS base for clinical parameters and epidemiological variables.
Results: 69 patients were analyzed (47%male-53% female), with a mean age of 86 years old. 208 consecutive consultations have been attended with a mean of 3 reviews per patient. All of them had multi-pathological requirements and were polymedicated. Geriatric syndrome was detected in 80% of patients, and only 53.2% of them required treatment. 19.5% of the patients showed cognitive impairment. 15% of the patients had severe or complete dependence for activities of daily living (ADL), 12.5% had moderate dependence, and 72.5% of them had mild dependence. Through the brief Charlson Index, we have objectified high comorbidity (>3points) in 22.5% of our patients. The most frequent causes of anemia were iron deficiency (71%), vitamin B_{12} deficiency (52%), renal failure (52%) and most of them presented multifactorial causes of anemia (68%). The percentage of patients requiring transfusion support was 34.8% and erythropoiesis-stimulating agents, as subcutaneous erythropoietin, were prescribed in 33% of them (alpha: 88%, beta: 12%).

Table 1.



Summary/Conclusion: Our patient profile is a 86-year-old female, multipathological and polymelicated with multifactorial anemia, that presents mild dependency and moderate comorbidity, with no cognitive impairment. Carrying out a comprehensive geriatric assessment to these elderly patients with anemia, has allowed us to optimize their treatment, by reducing invasive tests and by avoiding refer them to other hospital services.

PB2371

EVALUATION OF FERRIC MALTOL AS ALTERNATIVE TO PARENTERAL IRON THERAPY IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE (IBD) IN TERMS OF COSTS REDUCTIONS AND HEALTHCARE HUMAN RESOURCE UTILISATION

S. Lovato[1,2,*], P. Oppong[3], A. Akbar[3]

[1]Imperial College London, [2]Postgraduate, [3]Gastroenterology, London North West University Healthcare NHS Trust, London, United Kingdom

Background: In 2016 Ferric maltol (Feraccru), was approved, as alternative to parenteral iron, as second line treatment for iron deficiency anaemia (IDA) in patients with inflammatory bowel diseases (IBD) non responsive or intolerant to oral iron. Two clinical trials (AEGIS-1 & AEGIS-2), showed a statistically significant improvement in haemoglobin (Hb) with good tolerability.Both oral and parenteral iron are effective in correcting IDA, however parenteral iron is often used in IBD patients as oral iron is poorly tolerated. Parenteral iron is more expensive, and the cost of administration in a healthcare setting and nursing time required must also be considered.
Aims: We wanted to assess impact of the introduction of ferric maltol in our NHS Trust on the hospital budget and the nursing time.
Methods: We consider the following costs: 3 months of ferric maltol £168.78, iron infusion (drug plus appointment cost) £570.68. Nursing time for each infusion was estimated as 75 minutes. First we analysed how many of our patients who were prescribed ferric maltol needed to switch to parenteral iron. For these patients we calculated the cost of both therapies as £739.46.We calculated how many parenteral iron infusions were administered to IBD patients in a period of 12 months before the introduction of ferric maltol. As last we calculated the potential savings, if ferric maltol prescription was extended to any patients with IDA.
Results: In a period of 9 months we prescribed ferric maltol for 28 patients. If all the patients were treated with parenteral iron the total expense would have been £15960. As 4 (14%) of these had to stop the drug and have parenteral iron the cost have been calculated as £7005 with a saving of £8955 and 56% of cost reduction. In addition there was saving of 30 hours or 3.75 days in nursing time. If these savings were projected to a period of 12 months the calculation would be of £11940 and 5 days. In a period of 12 months IBD patients received 169 iron infusions at a total cost of £99.445. If all these patients were started on ferric maltol and only 14% of them needed parenteral iron the total cost would have been £42.220. The nursing time would decrease from 221 hours to 30 hours with a saving of 22.5 days. If we apply the same considerations to all the parenteral iron infusion, we estimated that in a 12 months period were issued around 800 parenteral iron prescriptions, the estimated saving with ferric maltol would have been £257.604 and 107.7 days.

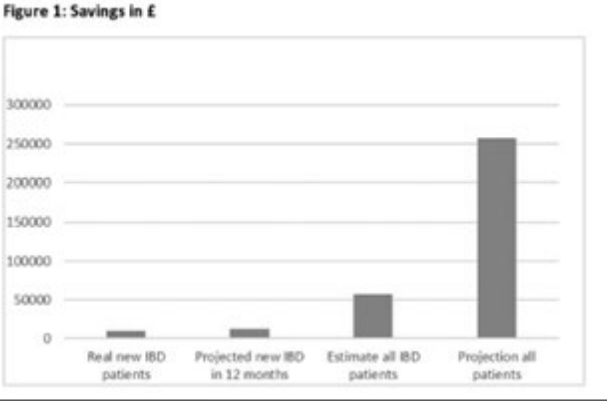


Figure 1.

Summary/Conclusion: Using Ferric maltol in our NHS Trust has been cost effective reducing costs and saving nursing time. Only new patients with IDA were prescribed ferric maltol, the patients who were already on IV iron maintenance continued with the parenteral therapy. The cost effectiveness will improve if we could switch to ferric maltol all the patients who were on parenteral iron maintenance and if ferric maltol was approved for treatment of any cause of iron deficiency. We recognise some limitation of this analysis. First the percentage of patients who had to stop the drug and needed parenteral iron could be underestimated because as patients prescribed ferric maltol towards the end of the study period had shorter follow up. Second, the number of appointments for iron infusion could have been overestimated as some of the patients could have been admitted. Last not all the patients, particularly those with active IBD could qualify for oral therapy

and some could refuse it. Further data are needed to clarify these points and better estimate the real impact of this intervention.

PB2372

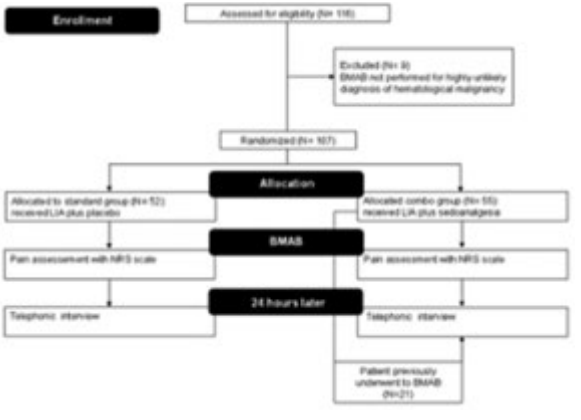
COMBINED ORAL ADMINISTRATION OF ANALGESIA AND ANXIOLYSIS FOR PAIN ASSOCIATED WITH BONE MARROW ASPIRATION AND BIOPSY

C. Cerchione*, M. Picardi, N. Pugliese, R. Della Pepa, A. Gravetti, A. Casoria, D. Nappi, G. Ciancia, F. Pane, V. Martinelli

Hematology - Department of Clinical Medicine and Surgery, University Federico II, Naples, Napoli, Italy

Background: Bone marrow aspiration and biopsy (BMAB) is a painful procedure, and the commonly adopted local infiltration anesthesia (LIA) with lidocaine is unable to relieve the pain during the most uncomfortable phases, or the anticipatory anxiety related to pain recalling thereafter. As there are no formal guidelines for adding a sedoanalgesic premedication before beginning the BMAB, many combinations have been adopted by several authors.
Aims: Our randomized and patient blinded trial aimed to evaluate, as primary end point, the efficacy and safety of opioid and benzodiazepine agent combination plus LIA in patients who underwent BMAB for hematological malignancies. Two secondary end points were: 1) to define if patients who already underwent to BMAB without LIA prefer sedoanalgesia; 2) to demonstrate if sedoanalgesia can influence the quality of the biological specimen harvested.
Methods: Patients were randomly assigned into two arms for receiving either placebo plus LIA (standard group, 48,6%) or oral fentanyl citrate 200 mcg plus oral midazolam 5 mg in addition to LIA (combo-group, 51,4%) during BMAB. Pre-procedural anxiety and procedural pain were assessed according to the Numered Rating Scale (NRS: 0-10), dividing the time of the procedure into five intervals (T0, T1, T2a, T2b, and T3) and evaluating discomfort grade during each moment of procedure in both groups. Cognitive function was measured before and 30 minutes after the procedure. Possible side effects were recorded, as well as the adequacy of tissue samples harvested. A telephone interview was performed 24 hours later. A total number of one-hundred-sixteen (n=116) were enrolled in the study. Nine (n=9) patients did not meet inclusion criteria and were excluded. Fifty-two (n=52) patients were randomized and assigned to standard group and fifty-five (n=55) to combo group (Figure 1).
Results: At T2b and T3 (corresponding to the biopsy time and time after the biopsy, respectively) there was a significantly lower (p<0.05) perception of pain in the patients who received sedoanalgesia (combo-group) compared to those who did not (standard group). Moreover, 100% of the patients in combo group who had previously undergone this procedure without premedication reported that they would prefer sedoanalgesia for the subsequent procedures, thus confirming the effectiveness of this combination also in relieving anticipatory anxiety. Finally, the histological specimen was found to be high in quality, as defined by standards.

Table 1.



Summary/Conclusion: Administration of oral analgesia and anxiolysis is a safe and feasible option to be used in outpatient setting; sedoanalgesia is very effective in reducing pain during the biopsy and it diminishes the anticipatory anxiety related to a painful procedure. Patients should have the possibility to choose between local anesthesia alone or sedoanalgesia plus local anesthesia.

PB2373

RESULTS OF A MULTICENTER UNIVERSAL NEWBORN SCREENING PROGRAM FOR SICKLE CELL DISEASE IN ITALY: A CALL TO ACTION

R. Colombatti[1,*], M. Martella[1], L. Cattaneo[2], G. Viola[1], A. Cappellari[1], C. Bergamo[1], S. Azzena[1], E. Baraldi[3], B. Dalla Barba[3], U. Trafojer[3], P. Corti[2], M. Uggeri[2], A. Biondi[2], G. Basso[1], C. Zorloni[2], P. Tagliabue[2], M. Bracchi[2], N. Masera[2], L. Sainati[1]

[1]Clinic of Pediatric Hematology Oncology, Department of Child and Maternal Health, Azienda Ospedaliera-Università di Padova, Padova, Italy, Padova, [2]Clinica Pediatrica, Università degli studi Milano-Bicocca-Fondazione MBBM, Monza, [3]Neonatal Care Unit, Department of Child and Maternal Health, Azienda Ospedaliera-Università di Padova, Padova, Italy, Padova, Italy

Background: Sickle Cell Disease (SCD) is a chronic and complex multisystem disorder requiring comprehensive care with newborn screening (NBS), health education, management of acute and chronic complications. Early diagnosis through NBS programs allows timely implementation of preventive measures such as penicillin prophylaxis, adequate health care measures (i.e spleen palpation), stroke prevention programs and hydroxyurea treatment. In spite of evidence of the above benefits of NBS for SCD and the inclusion of NBS as first step for comprehensive care in international guidelines, Italy still lacks a national SCD NBS program and policy on blood disorders. Pilot single center screening programs and a regional targeted screening have been implemented so far, but more evidence is needed to impact health policies.
Aims: To evaluate feasibility and efficacy of a multi center universal newborn screening for SCD in the frame work of a public-private partnership; to determine SCD epidemiology in the areas of Padova and Monza (North Italy) as rationale to support the need for a national universal SCD NBS.
Methods: Two public tertiary care university hospitals, Reference Centers for Pediatric Hematologic Disorders- including SCD, partnered with local charities to organize the NBS program. Guthrie cards for HPLC analysis were collected for newborns in both centers' nursery and NICU, after informed consent from the mothers. The analysis were done in the Padova lab. Confirmation test with molecular genetics was performed for HPLC positive samples. SCD patients were enrolled in comprehensive care programs; S carriers were offered genetic counseling extended to the family.

Table 1. The incidence of SCD patients, traits and other Hb abnormalities.

	Newborn	Positive test	Newborn SCD	Newborn S carriers	Other hemoglobinopathies
Padova (PD)	2821	37 (1.27%)	3 (0.10%)	28 (0.99%)	6 (0.21%)
Monza (MZ)	2618	23 (0.88%)	1 (0.038%)	9 (0.34%)	13 (0.5%)
MZ + PD	5439	60 (1.1%)	4 (0.07%)	37 (0.68%)	19 (0.34%)

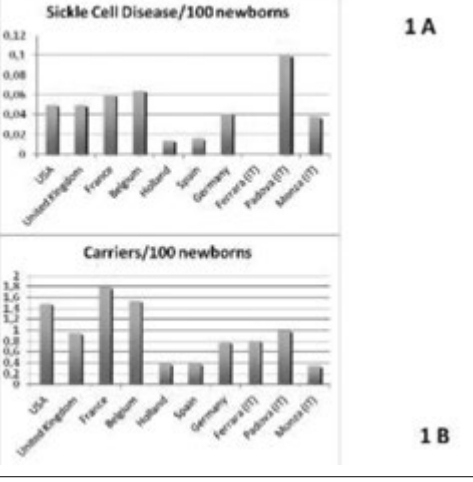


Figure 1.

Results: 5466 newborns were enrolled and for 5439 informed consents were obtained. All the samples were adequate for analysis. A similar families origin was seen in the two centers (65% Italians, 9% Mixed Couples, 26% Immigrants), but Padova had higher percentage of immigrants (>60%) coming from areas at high risk of hemoglobinopathies. The incidence of SCD patients, traits and other Hb abnormalities are showed in Table 1. Compared to other SCD NBS programs in USA and Europe, our results show similar incidence of SCD patients and carriers (Figures 1 A and B). SCD patients were all Sub-Saharian Africans, while HbS and other variants car-

riers were: 15% and 23% Caucasians (Italian and Albanians); 10% and 47% from North Africa-India-South America respectively.
Summary/Conclusion: Our results demonstrate the feasibility of a multicentric NBS program for SCD; give information on HbS epidemiology in two Northern Italian Areas and support previous European recommendation for a universal NBS program for SCD in Italy: a high incidence of patients and carriers was detected, with high percentage of carriers of non Sub-Saharian African origin, impossible to identify in a targeted NBS which is therefore not not adequate in our context.

PB2374

TIME FROM SYMPTOM ONSET TO DIAGNOSIS AND TREATMENT AMONG HEMATOLOGICAL MALIGNANCIES: DISEASE, PATIENT AND HEALTH SYSTEM RELATED INFLUENCING FACTORS

A. Dapkevičiūtė[1,2,*], L. Griškevičius[1,2], V. Šapoka[1,2], E. Martynova[3], V. Pečeliūnas[1,2]

[1]Vilnius university hospital Santaros klinikos, [2]Vilnius University, Faculty of medicine, Vilnius, Lithuania, [3]Quantitative psychology program, Arizona State University, Arizona, United States

Background: Diagnostic delay causes unfavourable outcomes among cancer patients. This subject has been widely analysed in solid tumours. However, data regarding haematological malignancies diagnostic delay is scarce worldwide and has never been analysed in Lithuania.
Aims: We aimed to evaluate diagnostic intervals, factors influencing their duration and prolonged time to diagnosis negative effect on clinical outcomes among multiple myeloma and lymphoma patients.
Methods: Patients were asked to participate in the study during their scheduled visits in outpatient clinic at a single institution if they had multiple myeloma or lymphoma diagnosis (ICD codes - C90, C81-C84). All participants signed Informed consent form. Following time intervals were evaluated: A - time from symptom onset to registration for a medical consultation, B - from registration to first medical consultation, C - from first medical consultation to diagnosis, D - from diagnosis to treatment initiation, E - from symptom onset to diagnosis, F - time from symptom onset to treatment initiation. Interval durations and majority of influencing factors were assessed based on face-to-face questionnaire. Data of disease characteristics was collected from medical records.

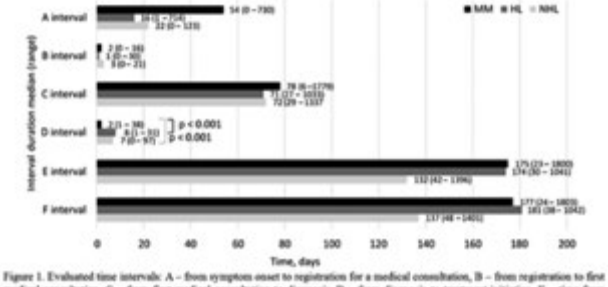


Figure 1. Evaluated time intervals: A – from symptom onset to registration for a medical consultation, B – from registration to first medical consultation, C – from first medical consultation to diagnosis, D – from diagnosis to treatment initiation, E – time from symptom onset to diagnosis, F – time from symptom onset to treatment initiation. MM – multiple myeloma, HL – Hodgkin lymphoma, NHL – non Hodgkin lymphoma.

Figure 1.

Results: 100 patients diagnosed with multiple myeloma (n=53) and lymphomas (n=47) were included. Median interval from symptom onset to registration for a medical consultation (A interval) was 30 (0-730) days, from registration to consultation (B interval) 2 (0-30) days, from first consultation to diagnosis (C interval) 73 (6-1779) days, from diagnosis to treatment (D interval) 5 (0-97) days. Overall time to diagnosis (E interval) was 151 (23-1800) days and did not differ statistically significantly for lymphoma and myeloma patients. Major factors influencing overall diagnostic E interval in multiple linear regression were living in big cities (p=0.008), anxiety and depression (p=0.002), self-medication (p=0.019), more specialists seen before diagnosis (p=0.022). Longer overall time to diagnosis resulted in higher incidence of multiple myeloma complications (p=0.024) and more advanced Durie-Salmon stage (p=0.049) when corrected for age, gender and CIRS comorbidity score. However, longer E interval was not associated with higher ISS stage for myeloma and increased Ann-Arbor stage for lymphomas.
Summary/Conclusion: The most important factors causing delay were living in big cities, anxiety and depression, self-medication, more specialists seen before diagnosis. Diagnostic delay may have negative influence on clinical outcomes for multiple myeloma patients. Median time from symptom onset to diagnosis was nearly 5 months indicating that there is space for improvement. Further studies are needed in order to prepare recommendations directed at shortening time to diagnosis and providing clinical benefits for patients with haematological malignancies.

PB2375

THE RUSSIAN NATIONAL GAUCHER REGISTRY

S. Kulikov[1,*], R. Ponomarev[2], K. Lukina[2], E. Sysoeva[2], E. Lukina[2]

[1]Department of Biostatistics, [2]Department of Orphan Diseases, National Research Center for Hematology, Moscow, Russian Federation

Background: The Department of Orphan diseases of the National Research Center for Hematology has gained a unique experience of treating patients (pts) with Gaucher disease (GD) in the last 10 years. A total of 286 Gaucher pts have been examined and are under follow-up in the Department which constitutes 94,4% of all diagnosed pts in the Russian Federation. Establishment of the national Gaucher registry is crucial for ensuring data integrity, enabling systematization and analysis of clinical data as well as continuous monitoring and long-term follow-up of the pts.
Aims: Demonstrate advantages of the centralized approach to management and monitoring of the pts with orphan diseases.
Methods: GD is an autosomal recessive lysosomal storage disease, caused by deficiency of the enzyme glucocerebrosidase (GBA), required for the degradation of glycosphingolipids. GBA deficiency results in accumulation of its immediate substrates, glucosylceramide and glucosylsphingosine, predominately in lysosomes of cells of the reticuloendothelial system. Clinical manifestations include hepatosplenomegaly, cytopenias and bone involvement. Historically managed by splenectomy, transfusions and orthopaedic surgery, the development of specific therapy in the form of intravenous enzyme replacement therapy (ERT) in the 1990s has resulted in dramatic improvements in hematologic and visceral disease and provided the possibility to prevent the development of debilitating bone complications. GD is an extremely heterogeneous condition with severe early manifestations in some patients, whereas others may exhibit a lifelong asymptomatic course. Thus, an individualized approach and a multidisciplinary team of specialists are needed to assess the severity of the disease and provide an adequate treatment and monitoring. Therefore, all adult Gaucher pts are examined and followed up in the National Research Center for Hematology. From 2007 to 2018, a total of 286 Gaucher pts have been examined and are under follow-up in the Department of Orphan diseases of the National Research Center for Hematology. 99% of these pts have been receiving pathogenetic treatment for the period of 1 – 15 yrs. The development and implementation of the national registry allowed us to create a platform for the registration of the whole population of adult Gaucher pts in the Russian Federation. The registry captures detailed laboratory, clinical and radiological data of all pts obtained both before the initiation of the treatment and on treatment, thus representing a convenient tool for comprehensive data analysis.
Results: The data accumulated in the national Gaucher registry allowed us to analyze the long-term results of splenectomy in Gaucher pts which was the primary treatment option in the pre-ERT era. It was demonstrated that splenectomy leads to a regress of cytopenia, however, at the same time it is the risk factor for severe bone involvement. The priority field of our further research is the development of optimal, scientifically and economically justified treatment regimen in Gaucher pts who achieved treatment goals.
Summary/Conclusion: Centralized examination and management of Gaucher pts in the Federal Research Center enabled us to accumulate, organize and systematize all available data on the patient population of the Russian Federation. The national registry represents a valuable tool for epidemiological research and provides a data source to carry out the analysis of both short and long-term results of the pathogenetic treatment.

PB2376

A REVIEW OF CLINICAL PRACTICE REGARDING DNACPR DECISION MAKING, DISCUSSION AND IMPLEMENTATION

A. Burge[1,*], J. de Vos[2]

[1]Acute Medicine, [2]Haematology, Royal Surrey County Hospital, Guildford, United Kingdom

Background: Cardiopulmonary resuscitation (CPR) was introduced in the 1960's as a treatment in the context of sudden cardiac arrest due to arrhythmias, most commonly due to acute Myocardial Infarction. As awareness spread and equipment improved it became more common in other situations, and it has become increasingly recognised that this is not always appropriate. In fact, it can even expose patients to invasive and intensive physical treat-

ment which may at best be briefly successful. Anticipatory decisions are a way to prevent palliative patients from being subjected to the trauma and indignity of CPR where there is no real prospect of benefit. However it is clearly a difficult and potentially distressing topic for patients, families and healthcare professionals alike. Guidance exists in the UK in the form of 'Decisions relating to cardiopulmonary resuscitation guidance 2016' which states 'Whenever possible making specific anticipatory decisions about whether or not to attempt CPR is an important part of good-quality care for any person who is approaching the end of life and/or is at risk of cardiorespiratory arrest. This is a highly important aspect of patient care, and is an area of continual improvement for in order to ensure ensuring the best care for dying patients and their families.

Aims: To analyse the DNACPR discussions taking place in the context of acute admissions to a tertiary oncology and haematology centre in order to try and improve clinical practice. Areas included were: intent of current treatment, reason for acute admission, evidence of previous resuscitation discussions in out-patient clinics or the community, the timing of DNACPR decisions in the context of the acute admission and time of death, grade of doctors carrying out discussions and the clinical context in which this was taking place.

Methods: Retrospective analysis of 41 sets of notes of deceased Haemato-Oncology and Oncology patients, identified through monthly divisional mortality and morbidity meetings.

Results: 66% of patients identified were undergoing palliative treatment at the time of admission, and the most common reasons for admission included general decline/disease progression (29%)and infection (32%). Overall 15% of patients had evidence of previous DNACPR decisions *or* end of life discussions prior to admission. 51% of patients who passed away had DNACPR decisions made within 3 days of admission and 49% of patients died within one week of admission. In 61% of patients this discussion was carried out by a junior doctor and 56% of patients were acutely or critically unwell at the time of discussion.

Summary/Conclusion: DNACPR are a key element of good and patient-centred care, allowing patients to die with dignity and have input into their end of life care. This review shows that significant changes in practice are required in order to improve the care delivered to patients with incurable malignant diagnoses. It highlights the importance of the early recognition of the palliative patient or patients in whom CPR would be unlikely to be successful. Equally important is the understanding and recognition by physicians as well as the communication to patients and relatives, that DNACPR decisions do not affect ongoing cancer treatment; ideally these discussions are carried out by a senior member of the team who knows the patient well. This review has increased DNACPR awareness, discussions and trasparency in our department. In this era of ever-developing and increasing lines of often palliative treatments these discussions and decisions are more relevant than ever.

PB2377

NEPA (NETUPITANT PLUS PALONOSETRON) FOR THE PREVENTION OF CHEMOTHERAPY INDUCED NAUSEA AND VOMITING IN PATIENTS RECEIVING STEM CELL TRANSPLANTATION

M. Corrochano[1,*], M.E. Moreno[2], I. Garcia-Cadenas[1], A. Barata[3], A. Feliu[2], A. Esquirol[1], E. Rodriguez[2], R. Martino[1], J. Sierra[1]

[1]Hematology, [2]Pharmacy, [3]Clinical psychology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

Background: Chemotherapy-induced nausea and vomiting (CINV) is a significant side effect during stem cell transplantation (SCT) despite prophylactic therapy. NEPA (Netupitant plus palonosetron) has proven to be highly effective in controlling these symptoms in patients with solid tumors; however, data are scarce in SCT recipients.

Aims: To evaluate the efficacy and safety of NEPA in two different cohorts of SCT patients.

Methods: Two cohorts of patients were analysed: those undergoing high emetogenic conditioning regimens before SCT and those receiving high doses of cyclophosphamide as graft *vs* host disease (GVHD) prophylaxis.

On the first day of chemotherapy (CT), the cohort of patients receiving high emetogenic conditioning regimens (group number 1) were prescribed an oral fixed-dose combination of NEPA (300 mg netupitant, 0.5mg palonosetron) together with 8 mg of dexamethasone (DXM) followed by daily DXM at a dose of 8 mg whilst receiving CT and 4 mg in the subsequent 48 hours. On the other hand, the cohort of patients being prescribed GVHD prophylaxis based on high dose cyclophosphamide (group number 2), were given NEPA on day +3 after infusion (first day of cyclophosphamide) and DXM was not administered before day +5.

Results: Twenty-one patients were included in the study: 13 in group number 1 and 8 in group number 2. Baseline characteristics are detailed in Table 1. In regard to patients in group number 1, during the acute phase of treatment (days in which patients receive CT), 6 (46%) out of the 13 patients achieved complete response (CR, defined as no emesis or no need for antiemetic rescue) and 4 (31%) partial response (PR, defined as presence of mild to moderate nausea but no emesis). The maximum number of emetic episodes experienced per day was of 2 in two patients. Six patients required additional treatment for breakthrough emesis and were all successfully controlled with single standard doses of olanzapine (n=2), metoclopramide (n=3) or lorazepam (n=1). Responses improved during the delayed phase (up to 72 hours after the last dose of chemotherapy) where 9 patients (69%) achieved CR and 3 patients (23%) achieved PR. In respect to patients in group number 2, during the acute phase (period of time whilst receiving cychophosphamide postSCT), 4 patients (57%) achieved CR and 5 patients (71%) PR. Four patients were successfully controlled for breakthrough emesis during this time with single standard doses of metoclopramide. Regarding the delayed phase, 5 patients achieved CR (62.5%) and 4 (50%) PR. 4 patients required single standard doses of metoclopramide (n=2) and olanzapine (n=2) for breakthrough emesis with appropriate response. Of the 21 patients evaluated, 11 (52%) had a history of significant nausea and emesis with prior chemotherapies, 7 (33, 3%) of which did not develop emesis with the NEPA regimen. Non-hematologic adverse effects attributed to the study medications were minor (hiccups n=3, somnolence n=3, hyperglycemia n=10, constipation n=3); all of which gave no further complications.

Table 1.

	Group 1/N=13 (100%)	Group 2/N=8 (100%)
Gender: Male	8 (61,5%)	5 (62,5%)
Age	Median44 (17-60)	Median54(29-60)
ECOG performance status		
0 - 1	13 (100%)	7 (87,5%)
2		1 (12,5%)
Baseline disease		
Acute leukemia	5 (38,4%)	3 (37,5%)
Non hodgkyn lymphoma	6 (46%)	3 (37,5%)
Hodkyn lymphoma	2 (15,3%)	1 (12,5%)
Chronic myeloid leukemia	-	1 (12,5%)
Disease phase: Advanced	5 (38,4%)	3 (37,5%)
Conditioning chemoterapy		
CyTBI	4 (30,7%)	-
BEAM	6 (46%)	-
CBV	2 (15,3%)	-
BuCy	1 (7,6%)	-
Flu-TBI	-	1 (12,5%)
Flu-Bu	-	1 (12,5%)
Flu-Mel	-	3 (37,5%)
TBF	-	3 (37,5%)
Type of SCT		
Allogenic	5 (38,4%)	8 (100%)

CyTBI: cyclophosphamide and total body irradiation; BEAM: carmustine, etoposide, cytarabine and melphalan; CBV: cyclophosphamide, carmustine and etoposide; BuCy: busulfan and cyclophosphamide; Flu-TBI: fludarabine and total body irradiation; Flu-Bu: fludarabine and busulfan; Flu-Mel: fludarabine and melphalan; TBF: thiotepa, busulfan and fludarabine.

Summary/Conclusion: NEPA-based antiemetic regimens seem to offer encouraging results in terms of prophylaxis of CINV in the SCT setting, including different procedures (autologous and allogeneic transplants) and new options such as Cy-post for GVHD prevention. Based on these promising results in terms of efficacy and safety, enrolment of new patients is currently ongoing.

PB2378

REFERRALS TO HEMATOLOGICAL CONSULTATIONS FOR EOSINOPHILIA – DIFFERENCES BETWEEN GENERAL AND UNIVERSITY HOSPITAL PATIENTS.

H. Holik[1,*], B. Coha[1], I. Vučinić Ljubičić[1], I. Radman-Livaja[2], S. Bašić-Kinda[2], P. Rončević[2], B. Miklaušić[3], I. Franić Šimić[4], I. Horvat[4], R. Zadro[4,5], I. Aurer[2,6]

[1]Department of Hematology and Oncology, General Hospital Dr. Josip Benčević, Slavonski Brod, [2]Division of Hematology, Department of Internal Medicine, University Hospital Centre Zagreb, Zagreb, [3]Department of Infectious Diseases, General Hospital Dr. Josip Benčević, Slavonski Brod, [4]Department of Laboratory Diagnostics, University Hospital Centre Zagreb, [5]Faculty of Pharmacy and

Biochemistry, [6]Medical School, University of Zagreb, Zagreb, Croatia

Background: Eosinophilia is mostly caused by allergic or infectious disorders. Only a small proportion are primary clonal needing hematological treatment. Despite that, patients with eosinophilia are frequently referred to hematologists for diagnostic evaluation.
Aims: Analyze characteristics of patients with eosinophilia referred for hematologic evaluation in different hospital types.
Methods: Patients referred to hematologic evaluation for eosinophilia in 2016 were identified by searching electronic data bases of a general and a university hospital. Individual patient data were then extracted from their respective files.
Results: We identified 75 patients fulfilling entry criteria, 20 in the general and 55 in the university hospital. Median age was 58; 36 were male and 39 female. Seven had severe eosinophilia, 30 moderate and 38 mild eosinophilia. Ten patients (13%) had myeloid neoplasms, one with PDGFRA and one with PDGFRB rearrangement; 6 had other myeloproliferative neoplasms and 2 myelodysplasia. Twenty-three patients (31%) had allergic and autoimmune disorders (e.g. asthma, rheumatoid arthritis, polyarteritis nodosa, bullosus pemphigoid, psoriasis, coeliac disease, discoid lupus erythematosus, eosinophilic fasciitis and other), 6 (8%) paraneoplastic syndrome, (metastatic lung, ovarian, gastric cancer, lymphoma), 12 (16%) parasitosis (strongyloidiasis, ascariasis, toxoplasmosis, trichinellosis, echinococcosis) 12 (16%) had drug-induced or postinfectious eosinophilia (reaction to lenalidomide, rituximab, phenoxymethylpenicillin, vancomycin which presented with drug rash with eosinophilia and systemic symptoms syndrome (DRESS)) and in 12 (16%) the cause remained unknown. Patients referred to the general and university hospital had similar demographic characteristics and severity but different causes of eosinophilia with the most pronounced differences in frequency of parasitosis (35% *vs* 9%), allergic and autoimmune disorders (20% *vs* 35%) and unknown causes (10% *vs* 18%).
Summary/Conclusion: Less than 20% of patients referred to hematologic evaluation for eosinophilia have primary hematological disorders. Most have other underlying disorders, including autoimmune diseases and cancer. However, a significant proportion, especially in patients living in less urbanized areas, have parasitic infestations which must be excluded before treatment with steroids is initiated. Finally, in a significant proportion of patients the cause remains unknown, more frequently in a university hospital setting.

PB2379

PAIN AND SERIOUS ADVERSE EVENT WITH BONE MARROW ASPIRATION AND BIOPSY: A SINGLE CENTRE EXPERIENCE FROM INDIA

C. Cherian Philip[1,*], S. Singh[1], A. Mathew[1], A. Jindal[2], N. Kakkar[3], M.J. John[1]

[1]Clinical Haematology, [2]Immunohematology and Blood Transfusion, [3]Laboratory Haematology, Christian Medical College & Hospital, Ludhiana, India

Background: The bone marrow aspiration and biopsy (BMAB) is an important and frequent investigative tool for hematological disorders. It serves an important role in establishing diagnosis and prognosis. Although thought to be safe and well tolerated; little is known about the complications and degree of pain experienced by patients undergoing BMAB.
Aims: We attempted to estimate the frequency of serious adverse events and level of pain and experienced by patients undergoing BMAB at our centre.
Methods: We conducted a prospective analysis on patients who underwent BMAB from January 2015 through December 2017 at our tertiary care centre. An informed consent was taken pre-procedure. The procedure was performed by variable operators namely; consultant, residents and physician assistants. All patients where premedicated with tramadol intravenous pre procedure and the preferred approach was from the posterior superior iliac (PSIS) approach through a left lateral approach. Post procedure each patient was asked to define the pain by the Wong-Baker grimace scale. In the day care setting; the patient was observed for upto 60 minutes prior to being sent home. A serious adverse event was considered as one requiring a prolonged observation beyond routine practice or leading to or extending of admission to manage adverse events following and related to the BMAB.
Results: A total of 665 BMAB procedures were performed in this period (Table1). The most frequent indication was for staging of lymphoma in 171(26.2%) patients. Sixteen patients (2.4%) did not report any pain. Four serious adverse events were reported, representing 0.6% of total reported procedures. The major serious adverse event was haemorrhage, which comprised 2 of the 4 serious adverse events. Both the haemorrhage event related to a posterior Ilial Artery psedoaneurysm. One was managed conservatively while the second one required surgical excision of the retroperitoneal hematoma and ligation of right internal iliac artery. Remainder events related to persistent vomiting and severe aching pain in the ipsilateral leg. There was no difference in pain levels [2 (1-10)] or significant complications when performed by variable operators, P>0.05. Patients who experienced greater pain (Pain score >3) had a longer duration and more than two attempts to complete the BMAB.

Table 1.

Table 1: Baseline features of patients undergoing BMAB

Variable	Patients (N=665) n (%) Median (IQR)/Mean ±SD
Age	50 (30-62)
Gender (male)	415(62.4)
Pain score	3 (2-4)
Duration of procedure (mins)	24.6 (± 9.8)
Number of attempts to complete the BMAB	2 (1-2)
Operator (Physician Assistant)	459 (69.0)

Summary/Conclusion: In our analysis BMAB is associated with a low level of perceived pain and complications. The procedure appears relatively safe and the level of pain and attempts do not appear related to the operator. Current strategy appears effective and the further attempts to control pain for this procedure may not be indicated.Serious adverse events following BMAB though rare, but nevertheless can have considerable impact on individual patients.There is a potential for dangerous adverse events following BMAB.

PB2380

PREVALENCE OF METABOLIC SYNDROME AND SARCOPENIA AS LONG-TERM LATE EFFECTS IN LYMPHOMA SURVIVORS

A. Daniele*, C. Minoia, G. Lerario, S. Ciavarella, I. Abbate, R. Divella, E. Savino, A. Guarini

IRCCs National Centre Institute "Giovanni Paolo II, Bari, Italy

Background: Metabolic syndrome and sarcopenia could often occur in long-term cancer survivors. The first is characterized by a set of cardiovascular risk factors defined by the Cholesterol Education Program Adult Treatment Panel III, which requires simultaneous presence of at least 3, abdominal obesity, hypertension, hyperglycemia, hypertriglyceridemia and low to high density lipoprotein cholesterol (HDL-C), sarcopenia is characterized by a progressive lean mass loss that causes asthenia and malnutrition.
Aims: The aim of this study was to evaluate the prevalence of metabolic syndrome and sarcopenia in lymphoma survivors and provide adequate nutritional support according to Mediterranean Diet.
Methods: Since November 2016 to March 2017, we enrolled 41 consecutive patients (19 women and 22 men) aged between 24 and 76 years in continuous remission of lymphoma for at least 3 years and in current follow-up at our Institutionwithin the "CCM2014 project supported by the Italian Ministry of Health. Nutritional status was assessment by anthropometry (arm, wrist, waist, thigh and calf circumference), plicometry (according to Durnin Womerslay) and body mass index, while glucose, HDL-cholesterol, triglycerides were tested by immunometric assay; For each patient, a customized food plan has been developed based the Mediterranean Diet and they were followed every four weeks.
Results: 16/41 (39.0%) of patients of both gender presented a status of obesity (mild, moderate and severe),12/41 (29,2%) were overweight, 11/41 (26,8%) were normal weight and 2/41(4.8%) were underweight; In the women the waist circumference mean was 60.4 cm (range:70-116), while for men the mean was 90.27 cm (range: 69-142). Considering the parameters that characterize the metabolic syndrome, 15/41 patients (36,5%) had at least 3 of these, significantly associated with status of obesity or overweight ($p<0.001$); Regarding the evaluation of the compartments by plicometry, a significant loss of lean mass and consequent increase in fat mass was and malnutrition observed in the obese and overweight patients respect to normal weight ($p <0.001$).
Summary/Conclusion: More than 60% of long-term lymphoma survivors have a moderate or severe weight gain and 36% have metabolic syndrome associated to sarcopenia; these preliminary data suggest that an early nutritional intervention associated with adequate physical activity could reduce the risk of onset of both complications in lymphoma survivors.

PB2381

TEACHING HAEMATOLOGY TO MEDICAL STUDENTS: WHAT DO WE TEACH AND WHAT THEY WANT TO BE TAUGHT

S. Lovato[1,2,*], B. Bhatkal[1,2], J. Arnold[1,2]

[1]Imperial College London, [2]Postgraduate, London North West University Healthcare NHS Trust, London, United Kingdom

Background: In the UK Haematology is part of medical school curriculum, yeaching is delivered through lectures but in some cases a "clinical attachment" in Haematology will be part of their clinical experience. In 2017 we piloted a project on 24 third year medical students to understand if they were able to interpret full blood count results and to recognise some haematological conditions. We found that the understanding of general haematology conditions was greater that haemato-oncology conditions. Using a "Team Based Learning" (TBL) approach, where students could discuss the cases in small groups, did improve their knowledge.
Aims: We wanted to validate the results of the pilot study extending the teaching session to more students. We also wanted to understand what they thought was the most important thing they learnt.
Methods: Four teaching session were conducted for 83 students in total. The teaching session consisted in 10 multiple choice questions on clinical cases including full blood count results. The students first completed the test individually, then they discussed the answers in small groups. At the end of the session students were asked to complete a feedback form.
Results: This study confirmed that students have better knowledge of non-malignant condition, haemato-oncology is poorly understood. The specific results for each topic are shown in Figure 1. Anaemias due to iron deficiency, thalassemia trait and B12 or folate deficiency and MDS were correctly diagnosed by more than 50% of the students, the only non-malignant condition that confused the students was reactive lymphocytosis due to viral infection. Less than half of the students could recognise malignant conditions except for AML. When the cases were discussed in small groups the percentage improved significantly, with the exception of AML, but still less than half of the students could recognise reactive lymphocytosis due to viral infection, CML, Lymphoma and Multiple Myeloma. Students were asked in the feedback form what was the most useful topic they learnt, the results are shown in Table 1. Most of the students appreciated the practical aspect of the session: interpreting blood results in a clinical scenario context; they also appreciated revising topics covered by the course attended during the previous year and they found the teaching on lymphomas and leukaemia the most useful.

Figure 1: percentage of correct answers for each topic

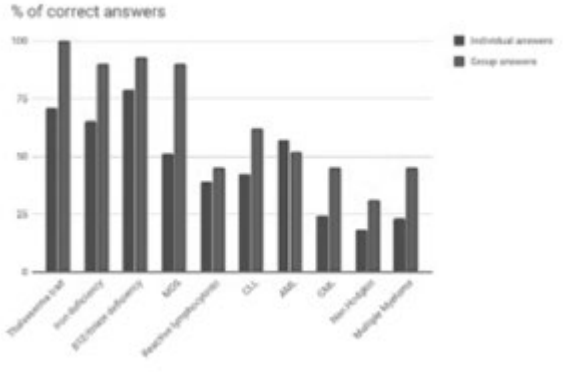


Figure 1.

Table 1.

Table 1: What was the most useful thing students learnt?

Students' answer	Number of students
Lymphomas	16
Interpreting full blood count results	13
Revision of haematology course	13
Differential of Leukaemias	8
Blood test results applied to clinical scenarios	6
CRAB criteria in Multiple Myeloma	5
Differential of Anaemias	3
Myelodysplastic Syndrome	3

Summary/Conclusion: This study confirmed what we found last year in the pilot study, medical students have a satisfactory understanding of non malignant haematology conditions but knowledge in haemato-oncology is often poor. What students appreciated is to be able to apply their theoretical knowledge to practical scenario, this reflect an understanding of the fact that interpreting blood results is an essential skill for doctors. The conditions that most of the students could not recognise where also indicated as the most appreciated topics of the teaching session. Using TBL session as revision was useful to improve students' performance. We are planning to continue this project to confirm the results.

PB2382

MYELOPROLIFERATIVE NEOPLASMS AND ACCEPTANCE AND COMMITMENT THERAPY: AN ONGOING FEASIBILITY STUDY AIMED AT SYMPTOM MITIGATION AND QUALITY OF LIFE

L. Padrnos[1,*], M. Buras[1], H. Kosiorek[1], R. Scherber[2], E. Knight[1], A. Cuc[1], C. Stonnington[1], A. Dueck[1], Y. Geda[1], R. Mesa[2]

[1]Mayo Clinic Hospital Arizona, Scottsdale, [2]UT Health San Antonio, San Antonio, United States

Background: Patients with myeloproliferative neoplasms experience significant symptom burden, including fatigue, pruritus and pain. The high symptom burden has been shown to be associated with decreased quality of life, sleep difficulties and mood disturbances. Acceptance and Commitment Therapy (ACT) is an evidence based psychological intervention targeting emotions, thoughts and behavior. ACT employs 6 core psychological processes to attain psychological flexibility: acceptance, flexible attention to the present moment, values clarification, committed action, self as context and defusion. Several Randomized Clinical Trials have reported the efficacy of ACT targeting mood, pain, and medical conditions such as Diabetes Mellitus, but it has never been investigated in patients with MPN. We launched a feasibility study to the ACT intervention among MPN patients to assess the ability to improve symptom burden, physical function and mitigate decreased QOL.
Aims: To evaluate the impact of Acceptance and Commitment Therapy in MPN patients.
Methods: 10 participant intervention includes 6 one-hour, in person therapy sessions with an ACT-certified psychologist. Assessments are based on validated questionnaires to assess MPN specific symptoms, global health, perceived stress, fatigue and degree of acceptance.
Results: We enrolled 5 MPN patients to date, the 4 patients who completed the pre and post surveys were evaluable for review. Most of the patients (N=3) were female and with an average age of 63.5 years. Two were actively working outside of the home and two were retired. One patient's MPN diagnosis came 1-3 years ago, the remaining 3 patients were diagnosed more than 5 years ago. Most of the patients endorsed constitutional symptoms (N=3), with fatigue being the most bothersome (N=3). At baseline, at least half of participants (≥2) reported feeling not able to control worrying, lack of interest or pleasure in activities, feeling depressed or hopeless, feeling bad about themselves, trouble concentrating several days a week, feeling nervous, worrying "too much", trouble relaxing, irritability, trouble falling asleep, and feeling tired several days a week. After the intervention, there was a decrease in degree of fatigue experienced at the time of documentation (median change (MC) -2.0) and over the past 24 hours (MC -1.5). In the post-intervention setting, there was a decrease in frequency of participant reported negative experiences including distress, negative emotions, difficulty with handling situations. Additionally, there was an improvement in patient reported assessment of quality of life, mental health and satisfaction with social roles and activities.
Summary/Conclusion: Patients with MPNs have a high degree of physical stress from their disease, psychological distress from the impact of their disease on their ability to function within their social network, and emotional stress of having a hematologic malignancy. ACT may be a feasible option to improve patient experience with predominantly incurable myeloproliferative neoplasm. In addition to developing disease modifying pharmaceutical interventions, the health care system must also develop innovative methods of addressing symptoms and the psychological distress of a hematologic malignancy.

PB2383

REAL-WORLD UTILIZATION OF MULTIPLE MYELOMA TREATMENTS IN THE ERA OF NOVEL THERAPIES: WHAT IS THE CURRENT STANDARD OF CARE?

D. Singer[1], E. Thiel[2], D. Irwin[2], M. Moynihan[2], C. McKay[1], E. Maiese[1,*]

[1]Janssen Scientific Affairs LLC, Horsham, PA, [2]IBM Watson Health, Ann Arbor, MI, United States

Background: Historically, few FDA approved treatments for multiple myeloma (MM) were available, with bortezomib and lenalidomide considered the standard of care. Recently, several new treatments for MM have been approved by FDA. With only a short time on the market, little is known about how the new MM treatments are being utilized in routine clinical practice and how the standard of care may be evolving.
Aims: To address this gap in understanding, this real-world study described the characteristics of patients initiating a new line of therapy (LOT) and characteristics of the new LOTs.
Methods: This retrospective study utilized data from IBM Explorys Research Database to identify MM patients with no record of stem cell transplant and ≥1 record for MM treatment between 11/2015 - 9/2017 (study period). An algorithm was used to identify new LOTs based on MM therapies (excluding corticosteroids) initiated during the study period. Descriptive analyses characterized utilization of all MM therapies and combinations.
Results: A total of 2615 patients started a new LOT during the study period. 1753 (67.0%) patients who started a new LOT were ≥65 years and 1169 (44.7%) were female. 1629 (62.3%) patients started 1st LOT, 426 (16.3%) started 2nd LOT, 235 (9.0%) started 3rd LOT, and 325 (12.4%) started 4th or more LOT. Among the new LOTs, the most common regimens were lenalidomide (n=733 (28.0%)), bortezomib (n=463 (17.7%)), bortezomib +lenalidomide (n=345 (13.2%)), daratumumab (n=219 (8.4%)), and bortezomib+cyclophosphamide (n=187 (7.2%)). Less common regimens included pomalidomide (n=75 (2.8%)) and carfilzomib (n=44 (1.7%)).
Summary/Conclusion: This real-world study suggests that the MM treatment paradigm is evolving to include newer therapies, however, bortezomib and lenalidomide remain common regimens. As disease recurrence is often more aggressive and survival shorter with each subsequent LOT, critical research is in progress to understand the real-world effectiveness of new treatments and how use in earlier LOTs *vs* later LOTs may impact effectiveness.

PB2384

PREVALENCE OF ANXIETY AND DEPRESSION AMONG CAREGIVERS OF CANCER PATIENTS IN MALAYSIA

K. Tey[1,*], R. Abdul Malik[2], M. Saad[2], G.F. Ho[2], J. Paul[2], G.G. Gan[3]

[1]Pharmacology, [2]Oncology, [3]Haematology, University Malaya Medical Centre, Kuala Lumpur, Malaysia

Background: Cancer is the third most common cause of death in Malaysia according to the National Cancer Registry in 2007. It is well documented that many cancer patients experienced significant symptoms of anxiety and depression. The shift of cancer patients' care is now mainly towards home care and outpatient treatment and hence increasingly, family members are becoming more involved in the care of these patients. Good social support from close relationship especially from spouse or family members is one of the important factors which have a protective effect on risk of anxiety and depression. Many studies had reported that caregivers also experience psychological distress. It was reported that as high as 38.1% of caregivers had anxiety and 82.2% had depression. However, there is limited information available in Malaysia which specifically report on the prevalence of psychological distress of the caregivers.
Aims: This study aims to determine the prevalence of anxiety and depression of all cancer patients and their caregivers in a teaching institution in Malaysia.
Methods: This is a cross-sectional study where family caregivers and patients who had been diagnosed with cancers within the last 12 months were recruited in University Malaya Medical Centre. Their psychological symptoms were assessed using Hospital Anxiety and Depression scale (HADS) and scores of >7 was used to demonstrate symptoms of anxiety or depression. Multi-dimensional Scale of Perceived Social Support (MSPSS) was used to identify the social support factors perceived by the patients.
Results: A total of 109 patients with underlying diagnosis of cancer and their caregivers (109) were recruited from September 2016 to December 2017. The age of patient's ranges from 18 years to 91 years (mean 52.94±SD 16.49), while the mean age of caregivers is 47.3 years (ranges 17 to 80). Majority of the patients (52%) were male, while most of the caregivers (64%) were female. Majority of patients were Chinese (56%), followed by Malay (32%) and Indian (10%). There were almost equal proportion of patients interviewed who had solid tumors and haematological malignancies. The prevalence rate of patients with symptoms of anxiety and depression was 33.9% and 39.4% respectively. The prevalence rate of caregivers with symptoms of anxiety and depression was 38.5% and 27.5% respectively. There was no significant association of anxiety and depression with the demographic factors of patients. Greater proportion of the female caregivers (45%) reported symptoms of anxiety compared to male caregivers (25.6%), P<0.05. There was significantly higher prevalence of anxiety if the caregivers are the only person providing care to patients, 51.0% *vs* 29.7%, P<0.05. There was significant correlation of anxiety and depression of patients and caregivers, P<0.05. The mean total scores of perceived social support of the patients were relatively high is 69.64; the mean score of significant others subscale was 24.27, family subscale was 25 and friends subscale was 20.39. There was no significant association of the perceived support with risk of anxiety or depression in patients.
Summary/Conclusion: This study demonstrated that family caregivers experienced high prevalence of anxiety and depression. It would appear that there was a correlation between patients and caregivers' psychological distress. Therefore, it is important to ensure adequate support be provided to caregivers.

PB2385

HEALTH-RELATED QUALITY OF LIFE IN CAREGIVER OF HEMOPHILIA PATIENTS

G. Ferrer Garrido*, J.M. Calvo Villas, N. Fernández Mosteirin, P. Delgado Beltrán

Hematología clínica, Hospital Universitario Miguel Servet, Zaragoza, Spain

Background: The care demands of children with chronic diseases can affect caregivers' health by imposing caregiving burden to them. In regard to caregiving burden of hemophilia, the levels of overload are high due to the frequent hospital dependence as well as the home management of bleeding. The development of inhibitors is related to a greater effect on the caregiver.
Aims: To analyze caregiving burden in hemophilia patients aged <25 years in a Reference Centre of Congenital Coagulopathies.
Methods: A questionnaire on health and quality of life for adults was given to both caregivers of hemophila patients <25 years of age controlled in our centre and a control group of caregivers of healthy children. The validated questionnaire used was the Short Form Health Survey (SF-36) of 36 items. This form includes 8 dimensions of health and quality of life: physical health, physical role, corporal pain, general health, vitality, social function, emotional role and mental health. The score is from 0% (worst quality) to 100% (best quality).
Results: The results of questionnaire of three groups of caregivers are shown in table and graph below: 1) Caregivers of children with severe hemophilia (SH); 2) Caregivers of children with mild hemophilia (MH); 3) Caregivers of healthy children (Control). The groups were homogeneous in their demographic characteristics. No relevant differences were observed neither the 3 main groups nor among the parents of each group.

Participating caregivers	Number both sexes	Number mothers	Number fathers	Participating children	Number
Total number	35	20	15	Total number	31
Caregivers of healthy children (control)	13	8	5	Healthy children Median 13 years (range 3-24)	17
Caregivers of hemophiliac children	22	12	10	Hemophiliac children Median 9,5 years (range 1-24)	14
Caregivers of children with MH	9	5	4	Children with MH	6
Caregivers of children with SH	13	7	6	Children with SH (on prophylaxis)	8 (2 with inhibitors presence)

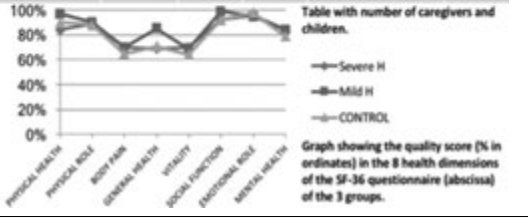


Table with number of caregivers and children.

Graph showing the quality score (% in ordinates) in the 8 health dimensions of the SF-36 questionnaire (abscissa) of the 3 groups.

Figure 1.

Summary/Conclusion: 1. We have not found significant impact on the health related quality of life of caregivers of hemophilia children in relation to controls. 2. Mild clinical hemophilia profile, capable monitoring and compliance with the prophylaxis regimens have a positive impact on both health and quality of life of caregivers. 3. It is advisable to enlarge the sample size in futher studies to validate our results.

PB2386

ACTIONS THAT MAKE ANTICOAGULANT THERAPY SAFER: 10 YEARS ON - DO WE COMPLY? EVALUATING WARFARIN USE AND APPROPRIATENESS AT A LONDON ACUTE HOSPITAL

D. Harding[1,*], J. Kotecha[1], S. Patel[2]

[1]General Internal Medicine, Chelsea and Westminster Hospital NHS Foundation Trust, [2]Pharmacy, Chelsea andWestminster Hospital NHS Foundation Trust, London, United Kingdom

Background: Anticoagulant prescribing, dispensing and administration continue to be a source of preventable near misses and harmful events in healthcare settings. Patients often have complex comorbidities and polypharmacy; which can mean they are more vulnerable to potential over- or under- anticoagulation and the resulting adverse effects. Breakdowns in communication between staff or confusion about warfarin prescribing, monitoring and administration have been cited as a major factor in adverse events.
Aims: The National Patient Safety Agency (NPSA) issued guidance aimed at preventing harm as a result of anticoagulant use in 2007 - this project assesses how well this hospital adheres to this guidance ten years on from its release.
Methods: A literature review was performed and audit standards were agreed with the multi-disciplinary team. Data was collected from electronic patient and prescribing records as well as patient notes, during April-May 2017. Approval was granted by the local governance department.
Results: 84% of patients on warfarin had a documented indication and target INR range. 39% of patients had significantly deranged INR; appropriate action was taken in 67% of cases. Prescription times varied greatly; 49% were prescribed on time (before 2pm), 17% were prescribed after 5pm. Administration times varied; 47% were administered before 4pm, 4.5% administered after 8pm.
Summary/Conclusion: There are still improvements to be made. The variation in standards are undoubtedly multifactorial; late return of INR results, heavy workload of ward teams, failure to handover to on-call teams, and the use of agency nursing staff (without e-prescription access) are some of the contributory factors postulated at this hospital. A plan is in place to change practice and improve compliance with NPSA. The audit highlighted the requirement to maintain training for all medical staff, especially as the introduction of direct oral anticoagulants means junior staff are perhaps less familiar with warfarin use nowadays.

PB2387

STRONG ASSOCIATION BETWEEN HERPES SIMPLEX VIRUS AND CHEMOTHERAPY-INDUCED ORAL MUCOSITIS IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES UNDERGOING INTENSIVE CHEMOTHERAPY OR STEM CELL TRANSPLANTATION

J. Hong[1,*], H.-K. Park[2], H. Park[3], D.-Y. Shin[1], Y. Koh[1], S.-S. Yoon[1], J.-Y. Choi[4], Y. Choi[5], I. Kim[1]

[1]Department of Internal Medicine, Seoul National University Hospital, [2]Department of Oral Medicine and Oral Diagnosis, Seoul National University Dental Hospital, Seoul, [3]Department of Internal Medicine, Inha University Hospital, Incheon, [4]Department of Preventive Medicine, Seoul National University College of Medicine, [5]Department of Immunology and Molecular Microbiology, School of Dentistry and Dental Research Institute, Seoul National University, Seoul, Korea, Republic Of

Background: A link between oral cavity infections and chemotherapy-induced oral mucositis (CIOM) in patients with hematological malignancies (HMs) undergoing intensive chemotherapy (IC) or hematopoietic stem cell transplantation (HSCT) has been suggested. However, conclusive data are lacking, and there are no current guidelines for the prophylactic use of antimicrobials to prevent CIOM in these populations.
Aims: The aim of the current study was to prospectively determine the prevalence of HSV reactivation and colonization with *Candida* as well as the relationship between such oral microbial factors and CIOM development in patients with HM undergoing IC or HSCT.
Methods: Patients aged ≥19 years with HM undergoing IC or HSCT were enrolled. Each patient was evaluated for HSV and *Candida* in the oral cavity along with CIOM at baseline as well as the second, third, and fourth weeks. To evaluate the reactivation of HSV-1 and -2 in oral keratinocytes, a sample was obtained by placing a sterilized Transfer Membrane on the buccal mucosa. If CIOM developed, the sampling site included the CIOM lesions. DNA was isolated from the sampled membrane and reactivation of HSV-1 and -2 was determined by PCR using the HSV 1/2 PCR kit. At every evaluation (baseline, week 2, week 3, and week 4), CIOM presence was estimated and graded according to the WHO oral toxicity scale and the NCI-CTCAE), version 3.0. For the semiquantitative evaluation of the severity of CIOM, the reticulation, erythema, and ulceration (REU) scoring method for oral lichen planus or oral lichenoid lesions was applied.
Results: Seventy presentations among 56 patients were analyzed. CIOM was observed in 23 presentations (32.9%), with a higher incidence associated with HSCT (17 of 35 presentations, 48.6%) than with IC (6 of 35 presentations, 8.6%). Reactivation of HSV-1 was significantly associated with an increased incidence of CIOM and a higher 'reticulation, erythema, and ulceration' score after adjusting for age, sex, type of disease, and treatment stage. A higher HSV-1 viral load was associated with increased and more severe incidents of CIOM. The presence of *Candida* was not associated with CIOM.
Summary/Conclusion: HSV reactivation in the oral cavity is highly associated with CIOM in patients with HM undergoing high-dose chemotherapy, independent of age, sex, treatment stage, and type of disease. A prospective trial to evaluate the effect of prophylactic acyclovir on CIOM prevention in HM patients receiving induction chemotherapy or autologous HSCT is therefore warranted.

PB2388

QUALITY OF LIFE OF PATIENTS WITH TYPE 1 GAUCHER DISEASE AFTER LONG-TERM ENZYME REPLACEMENT THERAPY

H. Li*, Z. Long, Y. Du, M. Chen, B. Han

Hematology, Peking Union Medical College Hospital,China, Beijing, China

Background: Chronic condition and weakening clinical manifestations of type 1 Gaucher disease (GD1) can significantly affect patients' quality of life (QOL). Given the extraordinary cost and degree of uncertainty in predicting the course of condition, understanding the potential benefits of ERT pertaining to improvements in psychosocial parameters and observable symptoms is important.
Aims: This study investigates survival status and QOL in GD1 patients who underwent long-term imiglucerase enzyme replacement therapy (ERT) and identifies possible relevant factors affecting QOL.
Methods: Factors affecting QOL were analyzed based on clinical data, survey of living conditions, and SF-36 questionnaires of GD1 patients receiving ERT (20–40 U/kg intravenously, twice a week).
Results: Median age of onset, start of ERT, and current median age of the 22 GD1 patients (13 men, 9 women) were 6 (1–38), 26 (6–41), and 40.5 (24–52) years, respectively. Of these, 68.2% lived in developing cities, 86.4% did not receive college education, and 77.3% were with annual income <$4500. Median ERT duration was 15.75 (7–22) years. All dimensions but mental health (MH) of QOL in GD1 patients were significantly poor ($P<0.05$) compared with that of normal Chinese population based on SF-36 scores. History of splenectomy before treatment was an adverse factor affecting patients' physical health ($P<0.05$) but not mental health ($P>0.05$). ERT at early ages was beneficial in increasing patients' physical and mental health ($P<0.05$). Presence of splenomegaly and bone involvement despite ERT had no effect ($P>0.05$).
Summary/Conclusion: Compared with normal Chinese population, GD1 patients were associated with reduced QOL, which is consistent with their current living condition, poor education, and income status. History of splenectomy and age at the beginning of ERT are key factors affecting QOL.

PB2389

THE CHANGE OF QUALITY OF LIFE IN 52 NON-SEVERE APLASTIC ANEMIA PATIENTS AFTER CYCLOSPORINE A THERAPY

F. Chen[1,*], Z. Guo[1], L. Zhang[2], F. Ye[2], H. Li[1], Z. Long[1], C. Yang[1], M. Chen[1], B. Han[1]

[1]Hematology, Peking Union Medical College Hospital, [2]Hematology, Chuiyangliu Hospital affiliated to Tsinghua University, Beijing, China

Background: Aplastic anemia is an immune-mediated bone marrow failure syndrome that impairs the quality of patients' life greatly and immunosuppressive therapy is one of the effective treatment. Few studies have been conducted on the quality of life of AA patients.
Aims: To explore the change of quality of life (QoL) in non-severe aplastic anemia (nSAA) patients before and after 2 years of cyclosporine A therapy, and the possible factors which may affect the QoL.
Methods: Patients with *de novo* non-severe AA from Jan,2014 to Jan, 2016 who had been treated with only cyclosporine A (CsA) for at least 2 years in the out clinic of Peking Union Medical College Hospital were asked to fill-

in the SF36 form before and after CsA treatment. Data from nSAA were compared with those of normal controls and patients' information like age, sex, education, annual income, insurance type, compliance were collected, disease severity and treatment effect were also evaluated.
Results: 52 patients were included in our study with 27 51.9% males and 25 48.1% females. The medium age were 48-year-old 21 ~85 year-old . After two years of treatment, 15 28.8% patients achieved CR 25 48.1% achieve PR and 12 23.1% patients had no effects (NR) The overall response ORR were 76.9% Before therapy, the scores of SF36 in nSAA patients were significantly lower than that of normal controls either in physical or mental component summaries P 0.05 . After 2 years of therapy, however, nSAA patients had significant improvement of mental component summaries and recovered to normal with even higher score in VT and MH compared with normal controls, although they still had lower scores in physical component summaries compared with normal ones. No associations were found between QoL and age, sex, education level, family income, type of payment, adherence of patients, or severity of disease onset. Patients with CR and PR had shown significant improvement in QoL.
Summary/Conclusion: Patients wih nCAA had impaired QoL compared with normal. Treatment of CsA can improved the QoL, especially in mental component summaries. Patients can benefit from the treatment regardless of their social statues or severity of disease and patients need to achieve at least PR to see significant improvement.

PB2390

PHARMACOECONOMIC EVALUATION OF MANAGEMENT OF DASATINIB AND IMATINIB THERAPY SIDE EFFECTS IN CHRONIC MYELOID LEUKEMIA. MULTICENTRIC RETROSPECTIVE STUDY

G. Giordano[1,*], A. Parente[2], D. Berardi[3], D. Castaldi[2], M.A. Cinotti[2], F. Vedruccio[2], V. Susca[4], L. Petrella[4], G. Berardi[5], M. Magri[6], G. Niro[6], R. Gigli[6]

[1]Medicine, Regional Hospital "A. Cardarelli", [2]Medicine, General Medicine, Campobasso, [3]Medicine, University La Sapienza, Rome, [4]Medicine, University Of Molise, [5]Family Medicine, General Medicine, [6]Laboratory Medicine, Regional Hospital "A. Cardarelli", Campobasso, Italy

Background: About10% of patients with chronic myeloid leukemia(CML) treated with thyrosine kinase inhibitor(TKI) show side effects.
Aims: Aim of this study is to evaluate the side effects management expense in patients receiving imatinib or dasatinib.
Methods: This study is a retrospective multicentric study. 13patients, M/F:8/5,median age75(R60-78) received imatinib 400mg/day and 15patients, M/F:9/6,median age68(R50-72) received dasatinib100mg/day. During an observation period of 7years 3imatinib patients showed 1acute heart failure and hepatotoxicityG4,1chronic diarrheaG3, 1acute recurrent abdominal painG4 and 3dasatinib patient showed 1osteomuscular painG4, 1gastrointestinal bleedingG4, 1pleural effusion. For each patient, the overall cost of side effects management during the entire follow-up period was calculated. This cost was then divided by the days of hospital admission, in order to give an average daily patient treatment cost. Then in each group the median of average daily costs was performed. Cost for each diagnostic and therapeutic intervention was considered in conformity of Italian National Health Service.
Results: In imatinib group the median days of hospitalization were30(R7-60), with a median daily expense of366€(R330-461),a median complication management cost of€10980and a median hospitalization cost for each patient of€22500. In dasatinib group the median days of hospitalization were15(R3-30), with a median daily expense of275€(R195-370),a median complication management cost of€4125and a median hospitalization cost for each patient of€11250. For each patient the median saving in dasatinib side effect management respect to imatinib group is €11250for hospitalization and €6855for complication management. In dasatinib group the saving is about €90/day. All imatinib patient with side effects changed therapy with a IInd generation TKI with an increase in follow-up test expense of about€13/day for the first 6months.
Summary/Conclusion: Management of side effects of dasatinib seems to be cheaper than imatinib. These data need confirmation on a larger cohort of patients.

PB2391

SINGLE WEIGHT-BASED DOSE OF RASBURICASE FOLLOWED BY ALLOPURINOL FOR SPONTANEOUS TUMOR LYSIS SYNDROME IN HEMATOLOGICAL MALIGNANCIES – A SUCCESSFUL AND COST SAVING COMBINATION

F. Mousinho[1,*], T. Mendes[1], P. Sousa e Santos[1], E. Viegas[2], B. Madureira[3], F. Falcão[2], F. Lima[1]

[1]Clinical Hematology, [2]Pharmacy Department; Faculty of Pharmacy - Universidade de Lisboa, [3]Pharmacy Department, Hospital de São Francisco Xavier, Centro Hospitalar de Lisboa Ocidental, Lisboa, Portugal

Background: Allopurinol, an inhibitor of xanthine oxidase, has long been considered the standard agent for the management of hyperuricemia in patients with preexisting hyperuricemia from spontaneous tumor lysis syndrome (TLS), or patients at high risk for developing TLS during chemotherapy (CT). Rasburicase is a recombinant urate oxidase enzyme which represent an effective alternative to allopurinol in rapidly reducing uric acid (UA) levels, improving patients electrolyte status, and reversing renal impairment, and the recommended dose is 0.2 mg/kg/day administered intravenously for up to five days, with a very high cost.
Aims: To evaluate and characterize a single weight-based dose of rasburicase followed by oral allopurinol for treatment of preexisting hyperuricemia related with TLS and in patients at high-risk for TLS, as well as to determine the effect on serum creatinine (Cr).
Methods: Retrospective medical record review, between February 2012 to August 2017, from patients with *de novo* or relapse/progressive hematological malignancies who received a single weight-based dose of rasburicase (0.2mg/kg, intravenously). Subsequent control of UA and Cr were recorded at baseline and monitored daily after initial rasburicase administration.
Results: A total of 32 administrations in 30 adult patients with *de novo* disease (19) and with relapse/progressive disease (13), and rasburicase was started the day before of CT protocol. Two patients received 2 administrations, but at different phases of the disease. At the time of administration 96.9% of the cases had hyperuricemia (>6.0 mg/dL), and 3.1% had a high-risk of TLS. The median baseline UA and Cr levels were 11.0 mg/dL (3.6-22.3) and 1.57 mg/dL (0.65-5.67), respectively. All patients responded to rasburicase presenting undetectable UA levels (<0.2-0.5 mg/dL) 24 hours after rasburicase administration, and detectable UA levels from the 5th day and at that time successfully started allopurinol 300mg daily. The 7th day median of UA and Cr levels in the available patients was 3.65 mg/dL (0.7-9.7) and 1.15 mg/dL (0.41-3.52), respectively. No patient needed additional dose of rasburicase, as no one required renal replacement therapy TLS related, during CT. The use of rasburicase in single weight-based dose led to a saving of € 72891,88 compared to 5 days of administration.
Summary/Conclusion: According to our results, the use of a single weight-based dose of rasburicase has demonstrated to be well tolerated, and seems to be an effective and inexpensive therapeutic strategy in managing hyperuricemia secondary to TLS, which should be considered in an era of high health costs due to innovatory therapies directed at molecular targets.

PB2392

A 4-YEAR EXPERIENCE IN PALLIATIVE CARE OF THE ONCO-HEMATOLOGY PATIENT

M. Couto[1,*], I. Oliveira[1], N. Domingues[1], M. Mariz[1], J. Ferraz-Gonçalves[2]

[1]Onco-Hematology, [2]Palliative Care, Instituto Português de Oncologia do Porto, F.G., E.P.E., Porto, Portugal

Background: Most Palliative Care (PC) patients have oncologic disease, being liquid tumors a part of it (around 7%). According to the literature, the latter should be individualized from solid tumors: they usually have more advanced stages of disease at diagnosis, more symptomatology expressed along their course and there is a shorter interval between the last treatment and the referral date or even death. There are few PC units in Europe with significant number of Onco-hematology (OH) patients admitted and characterized.
Aims: Review of all OH patients referred to the PC Service of one oncologic institution along four years, to understand their profile, the invasive medical decisions prescribed in their last month of life and how PC referral and care could be improved.
Methods: Clinical records of all OH patients referred to PC between 2014 and 2017 were reviewed and characterized (demography and disease, treatments, relevant medical decisions taken in the last month of life by PC and survival).
Results: A sample of 179 patients was reviewed and characterized: 94 males (52.5%), median age of 71 years [19-99]; 48.6% had Non-Hodgkin Lymphoma, 26.3% had Multiple Myeloma, 10.6% had Acute leukemia, 14.5% had other OH diseases. For those who were treated for their OH disease (n=158, median number of 2 lines [1-8]), 96.2% underwent chemotherapy, 28.5% radiotherapy and 21.5% underwent hematopoietic stem cell transplant. The referral was heterogeneous among physicians (27.4% from one physician). Most patients were observed first inpatient (55.3%), 17.9% in PC outpatient consult, 1.7% refused PC, 1.1% were transferred to another unit, one had home care and 23.5% died before being observed. At the end of this study, 98.3% died (89.2% in the hospital, 10.8% outside the hospi-

tal). The median time between the end of treatment and referral do PC was 45 days and between referral and death was 17 days. Medical prescription of PC patients in the last month of life was reviewed in the 176 patients who died: 39 patients were blood transfused (22 erythrocyte units and 17 platelets concentrates), 7 were prescribed with antibiotics and 3 with antifungal agents, 3 did a Computerized Tomography, 2 went through thoracentesis, 2 did PC radiotherapy, 2 were intervened surgically in the operation room; some procedures were done only once in the population studied, as echography, paracentesis, central venous catheterization, cystostomy and catheter positioning, hemodialysis, parenteral nutrition, nasogastric tube placed, Percutaneous Endoscopic Gastrostomy placement.
Summary/Conclusion: OH patients should be referred earlier to PC in order to better benefit from this planned and specialized care, preferentially followed in the out-patient consult. This decision requires a more integrative work between PC and OH physicians since diagnosis. In the last month of life a variety of invasive procedures were done, which should be minimized (for the lack of benefit and negative impact in patient's quality of life).

PB2393

TREATMENT PATTERN AND UNMET NEED IN ADULTS WITH PHILADELPHIA POSITIVE (PH+) RELAPSED OR REFRACTORY (R/R) B-CELL PRECURSOR (BCP) ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN EU-5 COUNTRIES

X. Zhang[1,*], L. Zhang[2], M. Gijsen[2], Z. Cong[1]

[1]Global Health Economics, Amgen Inc., Thousand Oaks, United States, [2]PRMA Consulting, Fleet, United Kingdom

Background: Ph+ R/R BCP ALL is a rare disease with poor prognosis. Tyrosine kinase inhibitors (TKIs) are usually recommended for these patients but exact treatment patterns are unclear.
Aims: This study was aimed to understand the current treatment pattern and unmet need in adults with Ph+ R/R BCP ALL in EU-5 countries.
Methods: A Delphi-based methodology was employed: a survey sent to clinicians and a country-specific panel were used to discuss the survey results to generate point estimates. Physicians must be board certified with at least 5 years' experience in R/R ALL. The percentage of using each treatment was estimated for patients who were relapsed/refractory to at least one 2nd-generation TKI or were intolerant to 2nd-generation TKIs and intolerant/refractory to imatinib. Limitations of the current treatments for Ph+ R/R ALL were examined.
Results: Four physicians from the UK, 4 from Germany, and 3 from each of the remaining 3 countries were enrolled with a median of 10 years' experience in R/R ALL. Regimens are widely distributed in each country and across the countries (Table). TKI (mainly ponatinib) combined with chemotherapy is the most commonly used regimen. Most physicians agree or strongly agree with these limitations of the current treatments: The risk of developing resistance to TKIs is very high; Survival is short, particularly once the disease becomes resistant to TKIs; Chemotherapy alone does not significantly extend overall survival; Patients may experience different adverse events (AEs) with each TKI.

Table 1.

Regimen	Proportion of Patients (%)				
	France	Germany	Italy	Spain	UK
TKI only		15	86	17	10
TKI + Clofarabine based chemo				1	15
TKI + FLAG-IDA based chemo			2	10	50
TKI + Hyper-CVAD	20		7	10	
TKI + Other chemo (e.g. vincristine + steroids)	67	35		17	25
TKI + Blinatumomab	7	7			
Blinatumomab		29		22	
Inotuzumab	6	14		5	
Chemo only			5		
Best supportive care only				18	
TKI					
Ponatinib	60	71	100	90	100
Dasatinib	7	15		10	
Nilotinib	33	14.5			

Summary/Conclusion: TKI plus chemotherapy is the most commonly used regimen for adults with Ph+ R/R BCP ALL in the EU-5 countries. There remains significant unmet need due to limited survival, resistance to TKIs and AEs associated with the current treatments.

PB2394

QUALITATIVE STUDY INVESTIGATING THE PERCEPTIONS OF MYELOPROLIFERATIVE NEOPLASM PATIENTS PARTICIPATING IN AN ONLINE YOGA INTERVENTION

J. Huberty[1,*], R. Eckert[2], L. Larkey[3], K. Gowin[4], J. Mitchell[5], R. Mesa[2]

[1]School of Nutrition and Health Promotion, Arizona State university, Phoenix, [2]Mays Cancer Center, University of Texas San Antonio, San Antonio, [3]College of Nursing and Health Innovation, Arizona State university, [4]Division of Hematology and Oncology, Mayo Clinic, Phoenix, [5]Jules Mitchell Yoga, Hermosa Beach, United States

Background: Myeloproliferative neoplasms (MPNs) are rare hematological malignancies with a significant symptom burden (e.g., fatigue, sleep disturbances, depressive symptoms, anxiety, etc.) that is often left unresolved despite the recent advances in pharmacologic therapy. Yoga is a non-pharmacologic strategy that has been shown to improve symptom burden in other cancers, with preliminary evidence that online yoga may be effective for improving fatigue, sleep disturbance, anxiety, depression, and total symptom burden in MPN patients. Online yoga helps to address many of the commonly reported barriers of cancer patients to in-person interventions and may make yoga more accessible to MPN patients due to the rarity of the disease and limited in-person opportunities for MPN patients. An exploration of MPN patient perceptions of participation in online yoga is needed to tailor interventions to patient needs and inform future studies.
Aims: The purpose of this study was to explore the perceptions of MPN patients participating in a 12-week online yoga intervention.
Methods: This paper represents the combined qualitative interview data gathered from two studies. Participants were asked to complete 60 min/week of online, home-based yoga (via Udaya.com) for 12 weeks. Participants were also asked to participate in a 15-20 min phone interview at post-intervention. The interview consisted of 10 open-ended questions aimed at identifying patient perceptions, attitudes, and feelings towards the yoga intervention. The qualitative data was coded in NVivo 11 for content analysis.
Results: The total sample included 39 MPN patients, of which 87% (n=34/39) were female and the mean age was 60.3+/-7.3 years. Online yoga was well-accepted and liked among MPN patients. MPN patients reported physical and mental health benefits, including improved physical activity levels (n=27/39), reduced fatigue (n=20/39), improved sleep (n=16/39), and reduced stress (n=14/39). Participants liked the convenience and flexibility of being able to do yoga at home (n=21/39). MPN patients also cited some negative aspects of the yoga intervention, including the perceived difficulty of the yoga classes (n=9/39), the lack of feedback and accountability associated with home-based yoga (n=16/39), and some minor technical issues with the streaming of online yoga videos (n=11/39). The majority of MPN patients (n=24/39) had some intention to continue participating in yoga of some form (*i.e.*, online or in-person) and nearly all (n=35/39) would recommend yoga for other MPN patients.
Summary/Conclusion: Online yoga provides a feasible and attractive format through which to deliver a non-pharmacologic intervention for symptom management among MPN patients. Although the overall yoga intervention was perceived positively by MPN patient participants, opportunities for improvements in the delivery were identified. Randomized controlled trials are needed to confirm the effects of online yoga on MPN patient symptom burden. The qualitative findings presented here may help to inform the development and design of future trials.

PB2395

PEDIATRIC REFERENCE RANGES FOR A NOVEL SLIDE-BASED INTEGRATED HEMATOLOGY ANALYZER

J. Tabor*, D. Bracco, T. Allen, D. Zahniser

Roche Diagnostics, Westborough, United States

Background: The **cobas m** 511 system is a novel slide-based automated hematology analyzer that performs a CBC, WBC differential, reticulocyte count, and nucleated RBC count using automated digital microscopy.
Aims: To determine pediatric reference ranges for the 26 parameters measured by the system in a single-center study.
Methods: Residual EDTA whole blood samples were obtained from 245 healthy subjects covering four age ranges (6–≤24 months, 2–≤6 years, 6–≤12 years, and, separately for males and females, 12–≤18 years). A hematologist reviewed the subjects' medical records to rule out the presence of pathologies or therapies known to affect blood cell counts. Samples were analyzed on the **cobas m** 511 system and reference ranges were calculated as the central 95% of values obtained for each parameter according to CLSI guideline EP28-A3c.
Results: Reference ranges for each pediatric cohort for the 26 blood count parameters measured by the system are presented in **Table 1**.
Summary/Conclusion: The observed reference range for the 26 parameters analyzed on the **cobas m** 511 Hematology Analyzer, and the age and sex differences, are consistent with reference ranges determined for other automated hematology analyzers.

Table 1. Pediatric reference ranges.

Parameter [units]	6–≤24 months	2–≤6 years	6–≤12 years	12–≤18 years (females/males)
WBC [10^3/µL]	5.06-14.94	4.46-13.42	4.28-12.68	3.86-11.03 / 3.87-12.53
RBC [10^6/µL]	3.97-4.98	4.01-5.02	3.99-4.99	3.88-5.18 / 4.09-5.34
HGB [g/dL]	10.8-13.3	11.0-13.6	11.6-14.1	11.1-15.0 / 12.3-15.9
HCT [%]	31.7-38.3	32.7-40.1	33.5-42.1	33.4-44.0 / 36.6-47.7
MCV [fL]	72.4-86.0	72.5-87.5	71.6-94.6	71.9-95.4 / 79.7-96.5
MCH [pg]	23.9-29.3	23.2-29.9	24.0-32.0	24.8-31.9 / 26.5-31.7
MCHC [g/dL]	32.4-35.1	32.4-34.9	32.4-35.4	32.2-35.1 / 32.5-34.5
RDW [%]	12.3-16.3	12.8-16.3	12.3-14.7	12.1-16.7 / 12.1-14.3
RDW-SD [fL]	35.3-46.0	36.6-45.8	35.2-44.7	37.0-47.2 / 37.4-45.6
PLT [10^3/µL]	222-551	213-579	199-420	184-409 / 138-409
MPV [fL]	7.4-10.3	7.4-10.0	8.0-11.6	8.3-11.9 / 8.2-12.9
#NRBC [10^3/µL]	0.00-0.01	0.00-0.01	0.00-0.02	0.00-0.01 / 0.00-0.03
#NEUT [10^3/µL]	1.45-5.64	1.18-6.51	1.63-6.90	1.69-6.70 / 1.50-9.55
#LYMPH [10^3/µL]	2.68-9.85	2.27-6.86	1.20-5.82	1.40-3.40 / 1.27-3.24
#MONO [10^3/µL]	0.38-1.51	0.34-1.07	0.29-0.91	0.22-1.09 / 0.35-1.30
#EO [10^3/µL]	0.02-0.91	0.07-1.82	0.01-1.37	0.01-0.51 / 0.02-0.64
#BASO [10^3/µL]	0.00-0.16	0.00-0.12	0.00-0.13	0.00-0.12 / 0.01-0.12
#RET [10^6/µL]	0.02-0.06	0.01-0.06	0.02-0.08	0.02-0.06 / 0.01-0.08
HGB-RET [pg]	24.1-34.3	27.1-35.3	27.1-36.1	28.4-36.8 / 29.4-37.9

Data for%NRBC,%NEUT,%LYMPH,%MONO,%EO,%BASO, and%RET not shown.

PB2396

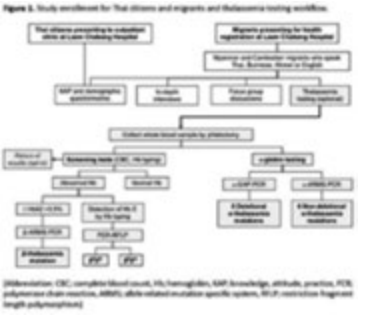
KNOWLEDGE, CULTURAL, AND STRUCTURAL BARRIERS TO THALASSEMIA SCREENING IN MIGRANT POPULATIONS IN THAILAND

J. Xu[1,*], W. Tanongsaksakul[2], T. Suksangpleng[3], S. Ekwattanakit[3], S. Riolueang[3], B. Kaiser[4], N. Thielman[1,4], M. Telen[1], V. Viprakasit[3,5]

[1]Department of Medicine, Duke University, Durham, United States, [2]Department of Paediatrics, Laem Chabang Hospital, Laem Chabang, [3]Siriraj-Thalassaemia Center, Mahidol University, Bangkok, Thailand, [4]Duke Global Health Institute, Duke University, Durham, United States, [5]Department of Paediatrics, Mahidol University, Bangkok, Thailand

Background: Thalassemia is an inherited disorder characterized by reduced α and/or β globin chains, leading to severe anemia, ineffective erythropoiesis, and other complications. Although originally prevalent in the malaria belt region, thalassemia has become a global problem due to population migration, including in Europe and North America. Thailand has one of the highest burdens of thalassemia in the world, and in response, has developed a national thalassemia prevention and control program. Using public education, genetic counseling, and prenatal screening and diagnosis, the program has achieved moderate success in lowering the incidence of new severe thalassemia cases. However, it excludes nearly 4 million largely low-skilled migrant workers from neighboring Myanmar, Cambodia, and Laos, where thalassemia is also prevalent. Thus, increasing migration may worsen the burden of severe thalassemia in Thailand, as well as in other regions of the world. It is of global interest, therefore, to identify strategies for thalassemia prevention and control in migrants. Nevertheless, few studies have explored barriers to thalassemia screening among Southeast Asian migrants. Both quantitative and qualitative methods are needed to investigate how migrants' knowledge, attitudes, beliefs, and behaviors may affect the success of interventions targeted to this population. We have designed and are currently conducting a mixed-methods study of barriers to thalassemia screening in Myanmar and Cambodian migrant workers in the industrial region of Laem Chabang, Chonburi, Thailand.
Aims: To compare levels of awareness and knowledge of thalassemia in migrants and Thai citizens; to characterize migrant attitudes and beliefs surrounding thalassemia screening; and to determine the feasibility of large-scale thalassemia screening in migrant communities in Thailand.
Methods: Written informed consent is being obtained from 200 Myanmar or Cambodian migrants and 200 Thai citizens (age 18-49 years) residing in Chonburi. Subjects are sampled for demographic and KAP (knowledge, attitudes, and practices) surveys on thalassemia. Myanmar or Cambodian subjects fluent in Thai, Burmese, or Khmer, presenting for routine health registration, and Thai subjects presenting for outpatient visits are included. Pregnant women and their partners are excluded. Descriptive statistics, tabulations and Chi-Square analysis will be used to compare socio-demographic variables and levels of thalassemia awareness between migrant and Thai subjects. Thai-speaking migrants will also be sampled purposively for in-depth interviews (IDI) and focus group discussions (FGD) to explore beliefs underlying migrant attitudes, as well as sensitive topics such as prenatal screening, termination of pregnancy, stigma, and access to healthcare. FGD will be stratified by gender and country of origin. Qualitative data will be coded and inductive thematic analysis used to identify relevant recurrent themes. Finally, as a feasibility study, migrants will be offered comprehensive thalassemia testing (Figure 1), providing preliminary estimates of thalassemia prevalence and screening uptake among migrants.
Results: This mixed-methods study will evaluate knowledge of thalassemia and identify key barriers to implementing thalassemia screening in migrant populations.

Table 1.



Summary/Conclusion: Novel insights gained may inform future thalassemia education and screening efforts for migrants in Thailand and have broader implications for the screening and care of migrants with thalassemia. This is an emerging issue that requires a global solution.

PB2397

INFLUENCE OF ABO BLOOD GROUP ON VON WILLEBRAND FACTOR TESTS IN HEALTHY SAUDI BLOOD DONORS

A. Alharbi[1], S. Bakr[2,3,*], A.-K. Al-Momen[4], K. Al-Saleh[5], R. Nasr[6], H. Kohgear[6], T. Owaidah[6]

[1]Department of Pathology and Laboratory Medicine, King Faisal Specialist Hospital & RC, [2]College of Medicine, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia, [3]College of Medicine, Fayoum University, Fayoum, Egypt, [4]College of Medicine, King Saud University[4], [5]College of Medicine, King Saud University, [6]Department of Pathology and Laboratory Medicine, King Faisal Specialist Hospital & RC, Riyadh, Saudi Arabia

Background: Von Willebrand disease is a common bleeding disorder. The wide variation in VWF levels between and within normal individuals highlights the clinical challenge of defining its cutoff value. Although studies on the influence of ethnicity on ABO phenotypes and the levels of VWF have been carried out on different ethnicities, there is a lack of such data among Arab population.
Aims: We aimed to evaluate the correlation of ABO phenotypes with all the parameters of the minimal test panel of VWF including (VWF:Ag, VWF:RCo, VWF:CB and FVIII:C), tested in a normal Arab population, and to estimate ABO specific normal reference range.
Methods: Blood samples were collected from 87 healthy donors in Riyadh to determine levels of factor VIII and VWF panel between the various ABO phenotypes.
Results: The highest mean values of factor VIII:C (128 U/dL), VWF:Ag (125 U/dL), VWF:RCo (109 U/dL), and VWF:CB (91 U/dL) were observed with type AB and the lowest mean values of factor VIII:C (81 U/dL), VWF:Ag (85 U/dL), VWF:RCo (73 U/dL), and VWF:CB (70 U/dL) corresponded to type O.
Summary/Conclusion: ABO phenotypes significantly influence plasma levels of VWF parameters in Arab nations as seen with other ethnicity. Hence, ABO specific normal ranges of the minimal test panel of VWFand factor VIII:C are essential for the appropriate prediction of mild VWD. Further study including a larger categorized sample size is required to generalize the test panel on the Arab population.

PB2398

OBSERVATIONAL STUDY TO DEVELOP A TREATMENT–RELATED PATIENT-REPORTED OUTCOME MEASURE IN GAUCHER DISEASE (QOL-ONE PRO1G)

M. Di Rocco[1], M.D. Cappellini[2], F. Carubbi[3], M. Alpa[4], G. Andria[5], A. Barbato[5], A. Burlina[6], F. Giona[7], G. Giuffrida[8], D. Roccatello[9], L.M. Borin[10], M. Spada[11], F. Papadia[12], S. Linari[13], S. Salek[14], T. Ionova[15], E.N. Oliva[16,*]

[1]Departments of Pediatrics and Neuroscience, Gaslini Institute, Genoa, [2]Department of Clinical Sciences and Community Health, Università degli studi di Milano, Milan, [3]Department of Medicine, Endocrinology, Metabolism and Geriatrics, NOSE U.O. Medicina ad indirizzo metabolico-nutrizionistico, University of Modena and Reggio Emilia, Modena, [4]Center of Research on Immunopathology and Rare Diseases (CMID),, San Giovanni Bosco Hospital and University of Turin, Turin,

[5]Department of Clinical Medicine and Surgery, Federico II University Medical School, Naples, [6]Unit of Metabolic Diseases, Azienda Ospedaliera Universitaria, Padua, [7] Department of Cellular Biotechnologies and Hematology, Sapienza University, Rome, [8]Division of Hematology, Department of Clinical and Molecular Biomedicine, University of Catania, Ospedale Ferrarotto, Catania, [9]Center of Research on Immunopathology and Rare Diseases (CMID), San Giovanni Bosco Hospital and University of Turin, Turin, [10]Department of Hematology, San Gerardo di Monza Hospital, Monza, [11]Dipartimento di Scienze Pediatriche, A.O. Città della Salute e della Scienza di Torino - Ospedale Regina Margherita, Turin, [12]U.O.C. Malattie Metaboliche e Genetiche; D.A.I. Pediatria, Ospedale Pediatrico Giovanni XXIII, Bari, [13]Center for Bleeding Disorders and Coagulation, Careggi University Hospital, Florence, Italy, [14]School of Life and Medical Sciences, University of Hertfordshire, Hatfield, United Kingdom, [15]National Medical Surgical Centre and Multinational Centre for Quality of Life Research, University Clinic, St.Petersburg State Universit, St. Petersburg, Rwanda, [16]Grande Ospedale Metropolitano Bianchi Melacrino Morelli, REGGIO CALABRIA, Italy

Background: Gaucher disease (GD) is a rare autosomal recessive lysosomal disorder that results in the accumulation of sphingolipids in the body's tissues due to glucocerebrosidase enzyme deficiency. GD manifests with vast clinical heterogeneity. Type 1 is the most common form (non neurophatic) with a wide spectrum of signs and symptoms at presentation: some are very mild, others may present variably enlarged liver and spleen which may cause abdominal discomfort. Trombocytopenia and anemia are very common at diagnosis and may be associated with signs and symptoms (bruising and bleeding, tiredness, vertigo, dyspnea and reduced physical functioning). Over 20% of patients experience bone pain or fractures. Treatment is indicated for patients with type 1 GD who exhibit clinical signs and symptoms of the disease, including anemia, thrombocytopenia, skeletal disease, or visceromegaly. Assessment of the impact of illness on physical, mental, and social functioning is an essential element of clinical diagnosis, a major determinant of therapeutic choices and efficacy, and a guide to longer-term care. Furthermore, it is known that patient reported outcomes (PROs) may influence various changes in intervention. There are many generic instruments available to measure the impact of disease on patient's health-related quality of life (HRQoL). However, there is no PRO measure that has been developed specifically for the use in GD. Such a measure would help clinicians to gain a more in-depth understanding of the impact of GD on patients and inform clinical decision making, leading to better patient care and compliance.
Aims: The aim of this study is to develop and validate an instrument designed to measure the impact of GD and treatment on individual patients' PROs. Primary endpoints are the generation of items to construct a PRO instrument for patients with GD and its psychometric evaluation.
Methods: This is an observational multicentre study. Participants will be GD patients aged ≥18 years attending referral centers. The study will be divided in 6 stages: Stage 1 - conceptualisation of PROs in GD patients before the data collection, in order to lay a conceptual foundation for the new instrument; Stage 2 - qualitative interviews; Stage 3 - item generation; Stage 4 - pre-testing; Stage 5 - item reduction; Stage 6 - validation of the final questionnaire. The total number of patients estimated to be assessed is approximately 100.
Results: The results of first stages of the study will be presented. The issues related to PROs of patients with GD will be researched and conceptualized. The interview guidance on HRQoL and symptom issues related to disease and its treatment will be developed and the interviewer will conduct interviews in accordance with this guidance. Emerging themes on HRQoL and symptoms from the interviews will be reported and are expected to fall into the four main HRQoL domains - physical, social, environmental and psychological. Outcomes of qualitative interviews will be analyzed.
Summary/Conclusion: The user-friendly questionnaire to evaluate impact and symptoms of GD will meet the minimum standards set out by the FDA for PROs and HRQoL instruments that include intrinsic characteristics (reliability and validity), responsiveness, sensitivity to change in health states and adequate sample size (*FDA Guidance, 2006; https://www.fda.gov/downloads/drugs/guidances/ucm193282.pdf*). The instructions on the questionnaire will be easy to use, and the instrument will be short in length, self-explanatory, take a short time to complete, be easy to use and put minimal burden on the patient.

PB2399

NEW HEMATOLOGICAL RESEARCH PAPER TO DEVELOP AN INTERNATIONAL PROSPECTIVE REGISTRY FOR GD THAT CAN BE USED TO PROVIDE A CLEAR PICTURE OF THE CURRENT WORLD-WIDE MANAGEMENT OF GD

S. Salek[1,*], A. Symeonidis[2], C. Bradley[3]

[1]Institute of Medicines Development, University of Hertfordshire, Hartford, United Kingdom, [2]Hematology, university of patras, patras, Greece, [3]Dacro research group, university of glasgow, glasgow, United Kingdom

Background: Gaucher Disease (GD) is a rare disease, with 75% of GD patients being diagnosed and managed by hematologists. In the majority of cases the appropriate diagnosis is established incidentally, following a bone marrow examination, as part of the investigation process for unexplained splenomegaly, thrombocytopenia or anemia. Despite this, many of the common causes of misdiagnosis of GD when a bone marrow examination is not performed, are hematological in nature, with hematological malignancy, idiopathic thrombocytopenia and anemia of chronic disease being the more likely misdiagnosis.
Aims: If we are to truly find out what is happening in GD and address any possible GD health disparities world-wide, we need to know and understand where we are with regards to current treatment and care services for GD. If these services fall short, how can we improve their effectiveness and thus improve the quality of service that is given.
Methods: This new research initiative will provide a real-world picture of treatment and outcomes by studying clinical characteristics, disease-management and relevant clinical outcomes *i.e.* determine what is happening in the countries surveyed and pinpoint reasons for this. Moreover, by integrating the essential data from all resources we will create recommendations for the appropriate patient data collection even for local registries outside Europe, thus harmonizing diagnostic and registration strategies and contributing to better scientific communication and collaboration worldwide. This research initiative will be devised and conducted, in order to determine whether consecutive patients with known or suspected GD during a hospital admission were being investigated and treated in accordance with guidelines. It will also provides an opportunity to obtain comparative data on the characteristics and outcome of patients with GD, in terms of comparative data about GD rates, morbidity, mortality and survival data after treatment.
Current local and national registries will be used as a platform to collect detailed clinical information relevant to clinical practice and thus create a more clinically orientated International GD Registry.
Results: This future important research initiative will help us develop a truly International Prospective Registry for GD that can be used to provide a clear picture of the current world-wide management of GD. This will help us to: Promote research of GD and Develop both practical and clinical research recommendations in GD. Develop educational initiatives to allow the sharing of knowledge and best practice. Create a dataset of preserved biological material, potentially available for research purposes.
Summary/Conclusion: We aim to remove any world-wide GD health disparities by building up a partnership of all sectors involved in GD care *i.e.* multi-disciplinary teams, medical societies and co-op groups, politicians, the pharmaceutical industry, GD patient groups, the private sector and the wider community of people involved in the multifaceted aspects and phases of GD care.

PB2400

TERTIARY PREVENTION FOR PATIENTS WITH MULTIPLE MYELOMA

P. Kotoucek[1,*], R. Bezergianov[2], R. Enright[3], W. Nagi[1], Z. Ondrusova[4], A. Orfao[5], Z. Stefanikova[4], J. Sedlak[6]

[1]Haematology, Broomfield Hospital, Chelmsford, United Kingdom, [2]Counseling Services, Arizona State University, Arizona, [3]Educational Sciences, University of Wisconsin-Madison, Madison, United States, [4]Slovak Myeloma Society, Bratislava, Slovakia, [5]Salamanca Cancer Centre, Salamanca, Spain, [6]Biomedical Centre, Slovak Academy of Sciences, Bratislava, Slovakia

Background: Myeloma (MM) is a cancer derived from the immune system. The immune system is a part of the Psycho-neuro-endocrine-immune system (PNI) (Adler and Cohen, 1975). The noetic dimension has been defined and described as the highest level of the psychological function (Frankl, 2014) so influences the whole PNI. Present research in understanding the biology of myeloma is focused mainly on pre-myeloma disorder, monoclonal gammopathy of undetermined significance (MGUS). Interactions of MGUS cells with the immune system, bone cells, and others in the bone marrow niche may be key regulators of malignant transformation. These interactions involve a bi-directional crosstalk leading to both growth-supporting and inhibitory signals (Dhodapkar, 2016). Over-saturation of antigen-presenting cells by bioactive lipid antigens can be one of the intrinsic mechanisms in patients with myeloma with high BMI or Gaucher disease, an inherited metabolic disorder. One of the extrinsic mechanisms promoting myeloma development is suppression of the immune system via inhibitory signals from PNI during long term stress or conflicts, such as loss of a family member, long term resentment or long term deprivation of acceptance or a lack of meaning of life, ie existential emptiness (Enright, 2012, Frankl, 2014).

Aims: We have organised psycho-immunology research in the Slovak Myeloma Society based on case reports of patients and/or family members, correlating psychological factors and responses to chemotherapy, including autologous stem cell transplant.
Methods: We have examined the relationship between responses to a phenomenology questionnaire, particularly about the meaning of life, and the response to chemotherapy of patients from the national conference of the Slovak Myeloma Society (SMyS) in September 2014. 46 patients responded to an anonymous questionnaire which consisted of 7 questions concerning: 1) Age, 2) Gender, 3) Status of MM disease, 4) Status and timing of the most recent chemotherapy, 5) Meaning of life before myeloma, 6) Meaning of life with myeloma, 7) Qualitative changes of meaning of life before and after being diagnosed with MM.
Results: Results: N=46 patients; Female n=28; median age 62 years, age range 30-70 years; Male n=18; median age 64.5 years, age range 45-79 years. 57% of the patients had MM in CR (complete remission) or responding to chemotherapy with improving the parameters of meaning of their life (MoL). 4% of the patients had progressing MM with worsening MoL parameters. 22% of the patients had progressive MM but with improving MoL parameters. 17% of the patients had MM in CR or responding to chemotherapy but with deteriorating MoL parameters.
Summary/Conclusion: Results above raise an important question about implementing efficient integrative treatments, like logotherapy (Frankl, 2014) or Forgiveness Therapy (Enright, 2012) targeting PNI into treatment of patients with multiple myeloma (Rosenthal and Dean-Clower, 2005) and their tertiary prevention. There is clear evidence that patients living with myeloma more than 13 years without chemotherapy have a constellation of unique immune changes favouring both immune cytotoxicity and recovery of B-cell production and homing, suggesting improved immune surveillance. (Pessoa de Magalhães *et al*., 2013). Tertiary prevention for patients with myeloma should target both intrinsic and extrinsic factors with the intention of keeping efficient immune surveillance even better than in healthy volunteers to prevent recurrence of disease (Chang *et al*., 2016, Tariman *et al.* 2016, Demark-Wahnefried *et al.* 2008).

PB2401

GENERAL HEALTH IN OLDER ADULTS WITH NON-HODGKIN LYMPHOMA USING A COMPREHENSIVE GERIATRIC ASSESSMENT

D. Mercante[1,*], J. Musacchio[2], Y. Hökerberg[1], I. Mattos[1]

[1]Escola Nacional de Saúde Pública, FIOCRUZ, [2]Instituto de Pesquisa, Oncoclínicas, Rio de Janeiro, Brazil

Background: Older adults develop their aging process in a very heterogeneous way and so, considering only chronological age to decide for the best treatment is not effective. To assist in these decisions, Oncogeriatric specialists suggest the use of the Comprehensive Geriatric Assessment (CGA) that classifies this group in terms of general health assessment. The patients recognized as fit by CGA could be referred to an intensive therapy, whereas the unfit should have their impairments identified and reverted, as possible, to get hold of a better treatment, improving prognosis.
Aims: The aim was assess the general health in older adults with non-Hodgkin Lymphoma (LNH) using CGA and analyze her association with endpoints related to medical therapy decisions.
Methods: A cross-sectional study was carried out using data of older adults diagnosed with NHL who were submitted to a structured survey while in an appointment in National Cancer Institute, between February and July 2013. After applying inclusion and exclusion criteria, 125 patients comprised the study sample. Sociodemographic, clinical, pathological and related to treatment variables were analyzed, after classification in fit or unfit health condition, using three different criteria of CGA. The elements included in CGA were the assessments of functionality, comorbidity, emotional and cognitive function, nutrition, medication use and social support.
Results: Patients had a median of 68 years. There was a slight predominance of the female gender and of aggressive lymphomas in advanced stage. The prevalence of fit patients ranged from 25 to 76%, according to the criteria of CGA chosen, with age and education being factors that affected this result. With regard to treatment, 76% were submitted to an intensive therapy, using anthracycline agents, but 26% of those had to decrease dose or discontinue treatment. In relation to these outcomes, CGA categorization showed a stronger association than age, although none were statistically significant.
Summary/Conclusion: A careful review of the literature and exploration of the present data leads to the conclusion that general health assessment, using CGA, and its domains in the approach of older adults with cancer, has a well-established role. On account of this being a cross-sectional study, with incident and prevalent cases and a small sample size, further studies will be required to reach better conclusions.

Sickle cell disease

PB2402

GLYCERYL TRINITRATE FOR THE TREATMENT OF SICKLE CELL LEG ULCERS

M. Boustani[1,2], C. Roignant[2], B. Kuk[2], G. De Luna[2], A. Habibi[2,3], E. Foïs[2], K. Ezzedine[4,5], F. Galactéros[1,2,3], P. Bartolucci[1,2,3,*]

[1]Université Paris Est Créteil, Faculté de médecine, [2]Unité des Maladies Génétiques du Globule Rouge, centre de référence « syndromes drépanocytaires majeurs », médecine interne, Hôpital Universitaire Henri Mondor, [3]Institut Mondor de Recherche Biomédicale (IMRB), Institut National de la Santé et de le Recherche Médicale (INSERM) U[955], DHU A-TVB, F-[94010], [4]Service de dermatologie, Hôpital Universitaire Henri Mondor, [5]Université Paris Est Créteil, Faculté de médecine, EpiDermE, Créteil, France

Background: Sickle cell disease is the most frequent genetic disorder in France with an incidence of 1/2364 birth in 2010. It is a multisystemic disease with various clinical presentations, with the vaso-occlusive crisis being the most common. Several therapeutic strategies have already been tested, such as the use of hydroxycarbamide, red cell exchange, bone marrow transplantation, and recently, gene therapy. All the progress made, contributed to lower the morbimortality of the disease. Sickle cell leg ulcer (SCLU) is a common complication affecting almost 1/5th of patients Europe and has a major impact on patients' quality of life, mainly due to the chronic pain it induces and its slow healing process, leading eventually to social marginalization and occupational deprivation. However, there is no clear recommendation regarding the specific treatment of SCLU. Hemolytic anemia-induced phenotypes, tend to have low nitric oxide bioavailability which alters vasoreactivity, and thus encourages endothelial activation and the development of an ischemic vasculopathy.
Aims: The purpose of our study was to evaluate Glyceryl Trinitrate efficacy in the treatment of leg ulcers.
Methods: We conducted a retrospective study in adult patients with sickle cell disease, presenting a SCLU of more than 1 cm^2, treated in our center from January 2015 till December 2017. Patients with other potential causes of leg ulcers (Diabetes, arterial insufficiency, systemic diseases, venous insufficiency) were excluded. Ulcers were either treated using a regular dressing or with an additional puff of Trinitrate 0.30 mg sprayed on the ulcer on a weekly basis, at each wound dressing change, and photographic follow up was implemented for and was analyzed with Pictzar software. Our primary endpoint was healing speed (decrease of ulcer surface/day) and secondary endpoint was the total duration of healing. Mann-Withney test was used to compare medians.
Results: Seventeen patients were included: 16 had homozygous Sickle cell disease (SS) and 1 heterozygous patient (Sβ0 thalassemia). Six SS patients (2 men et 4 women) with a mean age of 47 years old received Trinitrate spray. Ten homozygous and one heterozygous patients (10 men) with a mean age of 47 years old, didn't receive topical Trinitrate. There was no significant difference (*p value*=0.3) between median leg ulcer size at the inclusion (5.75 *vs* 2.4 cm^2). Faster healing was seen in the Trinitrate group but did not attain statistical significance (0.11 cm^2/day *vs* 0.005 cm^2/day; *p value*=0.08,). Median duration of ulcer healing was 100 days in the control group, and 56 days in the Trinitrate group, although one patient still had a persistent ulcer by the end of the follow-up.
Summary/Conclusion: Our retrospective study, on a small number of patients, shows a tendency towards leg healing hastening when spraying Trinitrate on the ulcer during wound care. A randomized controlled study on a larger scale of patient is necessary to confirm this result. Other vasodilatory treatments may also be tested following a similar methodology.

PB2403

Abstract withdrawn.

PB2404

INTERLEUKIN-1B AND INTERLEUKIN-6 GENETIC POLYMORPHISMS AND SICKLE CELL DISEASE: AN EGYPTIAN STUDY

M. El-Ghamrawy[1,*], M. Khorsheid[2], O. Ibrahim[3], A. Gad[2]

[1]Pediatrics, Pediatric Hematology and BMT Unit, [2]Clinical Pathology Department, Faculty of Medicine, Cairo University, [3]Child Health Department, National Research Center, Cairo, Egypt

Background: Sickle cell disease (SCD) is one of the most common monogenic red cell disorders worldwide. IL-1 and IL-6 have pivotal role in pathogenesis of many acute and chronic diseases, their genetic alterations have been considered as molecular contributors for several inflammatory disorders.
Aims: To detect the frequency of Interleukin-1ß (IL-1ß +3954 C/T) and Interleukin-6 (IL-6-174 G/C) polymorphisms in a cohort of Egyptian SCD patients and to study their possible impact on the clinical course of the disease in a cohort of pediatric SCD patients.
Methods: Eighty four pediatric Egyptian SCD patients (54 males) with a mean age of 11.08±5.9 years followed up at Pediatric Hematology and BMT Unit, Children Hospital, Cairo University were enrolled. Hundred age and sex matched unrelated healthy children were included in as a control group. Genotyping of IL-1β +3954 C/T and IL-6 -174 G/C polymorphisms was performed by PCR-RFLP assay. Informed consents were obtained from the parents or legal guardians of patients before enrollment and the study was approved by the Research Ethics Committee of Faculty of Medicine, Cairo University.
Results: Genotypic frequencies of IL-1β +3954 C/ T in studied group (n=84) were 38.1% for the heteromutant genotype and 15.5% for homomutant genotype. For IL-6-174 G/C, 58.2% and 12% of SCD patients had heteromutant and homomutant genotypes respectively. There was no statistical difference in the distribution of polymorphic genotypes between SCD and controls Polymorphic genotypes of IL-6-174 G/C were associated with frequent and severe attacks of vaso-occlusion (VOC) requiring hospitalization (p=0.023 and 0.03 respectively), while there was no statistical difference between SCD patients harboring wild or polymorphic genotypes of IL-1β +3954 C/T regarding gender, frequency and severity of VOC or disease-related complications.
Summary/Conclusion: Our study provides evidence of the possible role of IL-6, as an inflammatory marker, in the vaso-occlusive subphenotype of SCD and marking a more unfavorable disease phenotype. IL-6 -174 G/C polymorphism could be considered as a molecular predictor for recurrent, severe attacks of VOC in Egyptian SCD patients. Further investigations with larger cohorts are recommended for better characterization of patients prone for complications and for identification of novel molecular markers that could modulate disease morbidity and mortality.

PB2405

HBS/BETA+ THALASSEMIA PATIENTS LIVING IN ITALY: A SURVEY OF "MILD" SCD PATIENTS

L.D. Notarangelo[1], A. Agostini[1], P. Samperi[2], A. Barone[3], F. Arcioni[4], A. Pegoraro[5], B. Filippini[6], M. Nardi[7], G.C. Del Vecchio[8], L. Sainati[9], N. Masera[10], T. Casini[11], P. Giordano[8], R. Colombatti[9], G. Russo[2,12,*]

[1]Paediatric Onco-haematology Unit, Children's Hospital, Spedali Civili, Brescia, [2]Pediatric Onco-Hematology Unit, AOU Policlinico Vittorio Emanuele, Catania, [3]Department of Pediatric Onco-Hematology, University Hospital, Parma, [4]Pediatric Onco-Hematology Unit, S. Maria Della Misericordia Hospital, Perugia, [5]Pediatric Hematology Oncology Unit, Ospedale Donna Bambino, Verona, [6]Pediatric Unit, Hospital, Rimini, [7]Department of Pediatrics, University of Pisa, Pisa, [8]Department of Paediatrics "B. Trambusti", Azienda Policlinico-Giovanni XXIII, Bari, [9]Department of Child and Maternal Health, Clinic of Pediatric Hematology Oncology, Azienda Ospedaliera-Universita` di Padova, Padova, [10]Department of Pediatric Clinic, San Gerardo Hospital, University of Milano-Bicocca, Monza, [11]Department of Pediatric Oncology-Hematology, Meyer Children's Hospital, Firenze, [12]Clinical and Experimental Medicine, University of Catania, Catania, Italy

Background: HbS/beta+ thalassemia (S/beta+ th) patients usually suffer from a mild form of the disease; nevertheless, there are many grey areas concerning the clinical decision-making process, since most evidence is derived from SS and S/beta0 patients who suffer from a severe clinical condition. Specific protocols of care are lacking. We started our project on the observation of a patient with S/beta+ th, who experienced a moderate/severe form of the disease, with unexpected high number of vaso-occlusive crise (VOC) episodes.
Aims: To report on a subpopulation of Sickle Cell Disease (SCD) patients, *i.e.* S/beta+ th.
Methods: We prepared an excel database in order to collect essential clinical data from such patients. The file has been used in 11 centres which are part of the AIEOP (Associazione Italiana di Ematologia ed Oncologia Pediatrica).
Results: We collected data from 34 patients (20 males), aged 1.7-55 years (median 11.6). Country of parents' origin were Albania (21), Italy (18), Ivory Coast (6), Senegal (4), Burkina Faso (4), Brasil, Santo Domingo, India, Tanzania and Ghana (2 each). The most frequent thalassemia mutations were IVS-6 T>C (7 patients) and IVS-I-110 G>A (6 patients). Diagnosis of SCD was obtained as prenatal diagnosis in 5 patients, for the remaining 31, median age at diagnosis was 3.1 years; the event that lead to diagnosis was a clinical occurrence related to SCD in 16/32 patients; for 16/32 diagnosis was either fortuitous or due to familiarity, unknown in the remaining 2 patients. Anaemia was mild, median Hb concentration being 9.5 g/dl. Current clinical appearance was related mainly to VOC, with a median 0.5 episodes/year (range 0-6); other manifestations included splenic sequestration in 3/32 patients, acute chest syndrome in 2/34, osteomyelitis ind 1/34 and stroke in 1/30. Out of 26 patients screened for Transcranial Doppler (TCD), it was reported 1 conditional and 1 abnormal pattern, respectively. 10/33 patients were on hydroxyurea; 4/28 on regular transfusion regimen. Antibiotic prophylaxis was prescribed to 19/33 patients. No genotype-phenotype correlation was found.
Summary/Conclusion: Our "real-life" data show a heterogeneous clinical spectrum, with different policies regarding therapy (none, hydroxyurea, transfusion) and antibiotic prophylaxis in the various centres. Clinical data reveal a subgroup of patients with a more severe clinical phenotype, requiring disease modifying treatments, like the severe genotypes, and raise the need to reconsider the routine follow up clinical protocol currently applied to S/beta+ th patients, including TCD.

PB2406

TRANSFER FROM PEDIATRIC TO ADULT CARE IN THE SETTING OF HAEMOGLOBINOPATHIES IN ANTWERP, BELGIUM

K. Saevels[1,*], A. Devos[2], M. Philippe[2], V.D.V. Ann[1]

[1]Haematology, [2]Pediatrics, UZA, Edegem, Belgium

Background: The complexity of haemoglobinopathies, resulting in multi-organ dysfunction, necessitates a carefully integrated care regimen. Unlike normal red cells, diseased cells tend to form clumps and obstruct blood flow. This results in extreme pain crises, severe haemolytic anaemia and chronic damage to vital organs due to oxygen deficiency, infections and iron overload. Today, because of early preventive screening and treatment in red blood cell clinics, more than ninety percent of patients reach adulthood. With increased life span, patients encounter multiple transitional care episodes. These are particularly challenging since they increase morbidity and mortality. Good transition and access to primary care coupled with a comprehensive care in red blood cell diseases can improve outcomes.
Aims: To understand and to promote successful transfer from pediatric to adult care in the setting of hemoglobinopathies in the Antwerp region.
Methods: Patient demographics: At the University Hospital of Antwerp a total of 80 patients with severe haemoglobinopathies were followed on a routinely base in 2017 on a paediatric ward (n=50) and an adult ward (n=30). Haemoglobinopathies were divided in sickle cell disease (HbSS (n=56); HbSC (n=10) and HbS/β-thalassemia (n=7) (n=73) and β-thalassemia major (n=7).
Methods: We did a survey amongst 5 adolescents (15 to 18 years) and 5 young adults (18 to 30 years) during their transition to adult care. All patients were asked what has to be improved in the current protocol of transititional care to enhance their quality of self-care.
Results: All patients asked for a mobile medical card and an illustrated booklet with crucial information to help them to communicate their physical, psychological and social needs. This card with medical history, chronic medication, transplant related topics and hematology contact information improves communication with caregivers and healthcare professionals worldwide. Also the card has to expanded with a personal pain plan, sport advice and information on specific situations at school and professional environments. To make sure that the information is valuable we collaborate intensively with some of the dedicated young patients, to move forward with this patient-centered care project.
Summary/Conclusion: Successful transfer from paediatric to adult care has its foundation in a strong collaboration amongst the adolescents and young adults, their family and friends and the health care system to support building skills and positive disease management. Patients are encouraged to become empowered about their health and appropriate medical care. This is especially important in the new and rapidly evolving era of new therapies like T-cell depleted haplo-identical haematopoietic stem cell transplantation for severe and advanced stage sickle cell disease and gene therapy for β-thalassaemia. Incorporation in daily medical practice of patient-centered care evolves quickly and helps future clinical trials for haemoglobinopathies in Belgium.

PB2407

ASSOCIATION BETWEEN BCL11A, HSB1L-MYB AND XMNI ГG-158 (C/T) GENETIC POLYMORPHISMS AND HEMOGLOBIN F IN EGYPTIAN SICKLE CELL DISEASE PATIENTS

M. El-Ghamrawy[1,*], M. Khorsheid[2], M. Abdel-Hady[1], E. Michael[3]

[1]Pediatrics, Pediatric Hematology and BMT Unit, [2]Clinical Pathology Department, [3]Pediatrics, Faculty of Medicine, Cairo University, Cairo, Egypt

Background: Sickle cell disease (SCD) is a monogenic disease associated with multisystem morbidity. Clinical severity of SCD is extremely variable, and reasons for this heterogeneity are not fully understood. Inter-individual variation in hemoglobin F (HbF) level is likely one of the main modifiers that contribute to the clinical heterogeneity observed in SCD patients as it inhibits HbS polymerization and reduces the mean corpuscular HbS concentration. Previous studies showed association of variants at 3 major genomic loci with HbF levels.
Aims: To investigate the prevalence of BCL11A (rs11886868), HSB1L-MYB (rs9382268) and *Xmn*I γG-158 (C/T) genetic polymorphisms in a cohort of Egyptian SCD patients and to clarify the possible association between these polymorphisms and HbF level before and after hydroxyurea (HU) therapy.
Methods: One hundred Egyptian SCD patients (53 females) with a mean age of 13.68±8.91 years followed up at Pediatric Hematology and BMT Unit, Children Hospital, Cairo University were enrolled. Hundred age and sex matched unrelated healthy children were included as a control group. Genotyping of the studied single nucleotide polymorphisms (SNPs) was performed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay. Informed consents were obtained from the parents or legal guardians of patients before enrollment and the study was approved by the Research Ethics Committee of Faculty of Medicine, Cairo University.
Results: The distribution of the studied SNPs did not differ between SCD patients and controls except for the heteromutant genotypes of BCL11A which was significantly higher in SCD patients (p=0.13). For the HSB1L-MYB, BCL11A and Xmn1, baseline HbF level was higher in patients having the polymorphic genotypes than those with the wild genotypes, yet the difference between them did not reach a statistically significant level (p=0.47, 0.95 and 0.19 respectively). Fold change of HbF after HU therapy did not differ between patients harboring the wild or the variant genotypes for each SNP either alone or when more than one SNP co-existed (p=0.12, 0.91 and 1 for HSB1L-MYB, BCL11A and Xmn1 respectively). for HSB1L-MYB, BCL11A and Xmn1 respectively for base line HbF level and p=0.15, 0.14 and 0.35 for HSB1L-MYB, BCL11A and Xmn1 respectively for steady state HbF level).
Summary/Conclusion: *HSB1L and BCL11A* genetic polymorphisms had no positive impact on HbF level. Identification of regulators of HbF expression might be promising and mechanisms mediating this switching process could lead to better, less toxic, and more effective strategies for HbF induction.

PB2408

DISTRIBUTION OF IL4 VNTR POLYMORPHISMS AMONG SUDANESE PATIENTS WITH SICKLE CELL DIEASE

T. Alabid*

Heamatology and immuneheamatology, University of Khartoum, Khartoum, Sudan

Background: : Sickle cell disease (SCD) is an inherited disease associated with the chronic inflammatory condition indicated by elevated levels of inflammatory cytokines and increased Th17 response. These inflammatory pathways may be directly regulated by genetic polymorphisms and could be associated with different outcomes of the disease. Interleukin-4 (IL-4) is the main cytokine of T helper 2 lymphocytes, which has a key role in the regulation of humoral immune responses. The expression level of IL-4 gene could change by the 70 bp variable number of tandem repeat (VNTR) polymorphism that found in its third intron. In Africa (SCD) is one of the most common inherited disorders of hemoglobin and varies in frequency between different areas in Sudan.

Table. IL-4 VNTR genotypes and alleles distributions among SCD patients and controls.

Genotype	Patients N (%)	Control N (%)	P-value	OR(95% CI)
RP1/RP1	53(53.0)	59(59.0)	0.393	0.78(0.48 - 1.37)
RP1/RP2	31(31.0)	0	0.000**	-
RP2/RP2	16(16.0)	41(41.0)	0.000**	0.27(0.14 - 0.53)
Alleles RP1	137(68.5)	63(31.5)	0.048*	1.511(1.002 - 2.278)
Alleles RP2	118(59.0)	82(41.0)		

** P-value<0.05 statistical significantly **P-value<0.001 highly significantly different, OR=Odd Ratio, CI= Confident interval.*

Aims: The aim of this study was to detect the distribution of IL-4(VNTR) alleles among Sudanese patients with SCA and to investigate the possible associations with the development of stroke among these patients.
Methods: One hundred patients (50 male and 50 female; 8.34±6.67 years) with SCD and one hundred (47 male and 53 female; 8.64±4.99 years) healthy blood donors were evaluated. The polymorphisms were performed by PCR analysis and described as genotype frequencies.
Results: The entire RP1/RP2 genotype carriers were SCD patients. A significant difference in the distribution of RP2/RP2 genotype was found among patients (16%) and controls (41%) (P =0. 000) and it was only found among western Sudan tribes .RP1/RP1 genotype frequency was almost the same between (patients: controls), (53%: 59%). RP1 allele can be considered as risk factor for SCD as it was found 1.5 fold time to be in SCD patients than in controls (O.R =1.51).
Summary/Conclusion: In this study, there were three main genotypes of IL-4 identified (RP1/RP1, RP1/RP2, RP2/RP2) in the patients and controls. The risk of sickle cell patients was higher in individuals with RP1/RP2 genotype than controls. In addition, the frequency of allele 1 was higher in sickle cell anemia patients (68.5%), but this difference was weak evidence, therefore, this allele could be a risk factor for sickle cell patients (O.R =1.5).The distribution of SCD among Sudanese population directly affect the frequency of IL4 VNTR among different tribes. Studies in Sudan reported the presence of SCD in western tribes mainly. The RP1 allele could be a risk factor for sickle cell anemia patients. No statistically significant difference between tribes in VNTR allele's distribution, but Darfour and Kordofan tribes showed high frequency of RP2 allele.

PB2409

GENDER-RELATED DIFFERENCES IN SICKLE CELL DISEASE: A RETROSPECTIVE STUDY IN A PEDIATRIC POPULATION

G. Ceglie[1,*], I. Tarissi de Jacobis[1], F. de Gennaro[1], M. Di Mauro[1], M. Quaranta[1], C. Baronci[2], A. Villani[1], G. Palumbo[2]

[1]University Department of Pediatrics (DPUO), [2]Department of Oncohematology, Ospedale Pediatrico Bambino Gesù, Rome, Italy

Background: Sickle cell disease (SCD) is the most common monogenic disease worldwide. The disease mainly affects people of African, Caribbean, Middle Eastern, Eastern Mediterranean and Asian origin, but its prevalence is increasing in Europe, probably as a result of human migration. The incidence of SCD is not gender related, since it is transmitted as an autosomal recessive disorder. However, there have been reports of sex related differences in SCD mortality and morbility in adult patients. No studies are currently available about gender heterogeneity in the pediatric population.
Aims: The aim of this study is to find gender-related differences in the clinical course of SCD in a pediatric population.
Methods: In our study, we retrospectively analyzed the clinical records of 39 pediatric patients with a diagnosis of SCD (hemoglobin SS genotype), 23 males (59%) and 16 females (41%). We studied the gender differences analyzing the frequencies of the pain crisis per year, the number of blood transfusion per year, cardiac sequelae and severe complications (e.g. infectious, cardiovascular).
Results: Pain crisis frequency per year were significantly increased in the male population with a mean number of crisis per year of 1.6 *versus* 0.6 in the female population (p=0.04). Also, severe complications were mostly found in the male population; in fact, in 4 cases of osteomyelitis, 3 occurred in male patients and 1 in female patient. Moreover, we observed one case of transient ischemic attack and one of portal vein thrombosis, both of them occurred in male subjects. To evaluate cardiac complications of the disease, we analyzed the echocardiographic findings, available in 31 patients (17 males and 14 females). SCD-related cardiac complications were observed mainly in the male population: among 13 patients with echocardiographic alterations, 10 of them were boys (77%) and 3 were girls (23%) (p=0.04, Fisher's test). The number of blood transfusions per year was not different in males and females. The age at diagnosis was lower in males (median age of 2 years) than in girls (median age of 4 years), without reaching statistical significance (p=0.08).
Summary/Conclusion: Our data support the hypothesis that gender could play a role in determining the clinical course of SCD. The higher morbility in males is a well-known feature of SCD in adults but this is, to our knowledge, the first study that confirms this finding in a pediatric population. In our cohort both pain crisis frequency and severe complications of the disease were found to be more frequent in males than in females. We also found that the median age at diagnosis is higher in girls, probably explaining the lower total number of females in our population. These two findings support the hypothesis of a less severe clinical phenotype occurring in girls, so that the milder course of the disease leads to a later diagnosis in this group of

patients. These sex-related differences, in part observed in the adult population, have, until now, been attributed to hormonal variations that are physiologically found in the two sexes after puberty. On the other hand, in a pediatric population, other factors must be responsible for these differences and should be addressed to in further studies. These findings might also be valuable to integrate gender as a factor in the risk assessment of these patients at diagnosis, and possibly guide therapeutic decisions, with the aim of personalizing the therapy.

PB2410

NEWBORN SCREENING FOR SICKLE CELL DISEASE: EXPERIENCE IN BALEARIC ISLANDS 2 YEARS AFTER OF THE IMPLEMENTATION OF THE SCREENING PROGRAM

L. Lo Riso[1], A. Ortuño Cabrero[1,*], J. Robles Bauza[2], M. Garcia-Recio[1], B. Robredo Rodriguez[1], B. Lopez Andrade[1], J.M. Sanchez-Raga[1], A. Sampol Mayol[1], M.A. Duran Pastor[1]

[1]Hematology and hemotherapy, [2]Clinical analysis laboratory, Hospital Universitario Son Espases, Palma, Spain

Background: Migratory flows changed the distribution of the incidence of hemoglobinopathies. Nowadays Sickle cell disease (SCD) is more frequent en the mediterranean area than beta or alpha thalassemia, until now typical in this area. The increase in the incidence of SCD and the need of an early treatment due to frequent and severe complications in the firsts years of life, have determined, by the National Health System, the inclusion of the deteccion of hemoglobinopathies as part of the newborn's mandatory screening for endocrinal and metabolic pathologies.
Aims: This study presents data obtained after 23 months since this screening program in the Balearic islands.
Methods: Blood samples are obtained by newborn's heel-pricks between the 2nd and 5th day of life, and they are analyzed in Son Espases Hospital's Clinical Analysis Laboratory by high performance liquid chromatography (HPLC) with VARIANT system (BioRad). Positive cases for hemoglobin (Hb) variants were confirmed by peripheral blood samples from both parents (in case of FAS, FAC, FAX) or from both parents and newborn (in case of homozygous for FS or double heterozygous FSC or FSb). Samples were analyzed in the Eritropathology Laboratory in Son Espases Hospital by HPLC (Biorad-10) and Capillary Electrophoresis (Sebia Minicup).After checking results, an appointment is scheduled for the affected family with Hematology department, and there they are given information about the pathology, genetic counseling and a recommendations guide from the Spanish Society of Pediatric Hematology and Oncology. In homozygous cases, antibiotic prophylaxis is prescribed and a follow-up visit is scheduled. Data from this screening program are filed in a registry controlled by the Balearic Islands Ministry of Health.
Results: 19197 samples were analyzed from Balearic Autonomous Community obtained between 25/04/2016 and 28/02/18. 105 cases were detected with some Hb variant (Table 1), with 3 of them homozygous for HbS.Due to the family hemoglobinopathy study another two cases (1 homozygous HbS, 1homozygous HbC), and 3 families were both parents were either heterozygous HbS or HbC were detected.

Table1. Hemoglobinopathy incidence in Balearic islands 25/04/2016 and 28/02/2018.

Number of samples analyzed 19.197		
	Number of cases	1 every…
FAS	79	243,00
FAC	20	959.85
FAD	2	9599.50
Hb Burt	1	19197.00
FS	3	6399.00
Total	105	182.83

Summary/Conclusion: Incidence of homozygous HbS in Balearic islands is similar to the one calculated in other observational studies among different Spanish territories, although we were able to detect a greater number of heterozygous Hb variants than in those other regions.The screening for hemoglobinopathies has been useful in the detection of cases affected of sickle cells disease in newborn and family members not yet diagnosed, and the instauration of early treatment.

PB2411

SICKLE CELL SYNDROMES (SCS) IN SOUTHERN TUNISIA: A COHORT OF 76 CASES

K. Faten[1,*], H. sondes[1], L. lamia[2], M. Abir[1], M. Ghorbel[1], F. Safi[3], M. Naifar[4], H. Mnif[5], F. Makni[4], M. Hachicha[2], A. Mahfoudh[3], E. Moez[1]

[1]Hematology Department, [2]pediatric Department, [3]pediatric resucitation department, CHU Hédi Chaker Sfax Tunisia, [4]biochemestry department, chu habibi bourguiba, [5]blood transfusion center, Chu Hbib Bourguiba, sfax, Tunisia

Background: Hemoglobin sickle cell disease is one of the most frequent hemoglobinopathies in Tunisia.The sickle cell syndromes (SCS) representing by patients with homozygosity for HbS, patients with hemoglobin SC(HbSC, combined heterozygosity for hemoglobins S and C) and patients with sickle cell-beta thalassemia, is still a health problem because of their severe clinical manifestations and their complications.
Aims: The objective of this study is to describe the clinical profile, treatment and complications of these patients in the south Tunisian.
Methods: Our study is retrospective having interested the patients with SCS diagnosed and taken care in three centers of the university hospital Hedi chaker of Sfax: Pediatrics Department, Pediatrics Emergency Resuscitation (PUR) and the Hematology Department of CHU Hedi Chaker of Sfax for a period of 35 years (from 1979 to 2014). The diagnosis and classification of the type of SCS is based on clinic data, hemogram, hemoglobin electrophoresis and the family survey.
Results: We collected 76 cases of SCS: 44 cases followed in hematology department, 23 cases in pediatric center and 9 patients in pediatric intensive care unit. The mean age at diagnosis was 8 years (extremes: 1month - 49 years). Sixty-five of our patients (85.5%) were white, 11 (14.5%) were black. Inbreeding (consanguinity) was observed in 37 cases (48.7%). SS homozygous sickle cell disease was noted in 68.4%, hemoglobin SC in one patient (1.3%) and Sß thalassemia in 9 patients.SCS diagnosis was made following anemic syndrome in 46% of cases, a vasoocclusive crisis in 34% of cases, a complication in 4% of cases and hand-foot syndrome in 1.3% of cases.The main acute complications observed in our patients were vaso-occlusive attacks (67%), infections (44.2%) and aggravation of anemia (24.7%). chronic complications were vesicular lithiasis in 20 patients with osteonecrosis of the femoral head in 5 patients.The symptomatic treatment consisted of antibioticprophylaxis in 85% of the patients, hyperhydration performed in 47 cases (hospitalized for vaso-occlusive attacks), blood transfusion in 40 patients and transfusion exchange in 11 patients(a preoperative preparation in 6 cases,pregnant women in 2 cases and a serious vasoocclusive accident in 3 patients.the background treatment consisted of a transfusion exchange program in 9 patients and Hydroxyurea in 17 patients.
Summary/Conclusion: Homozygous sickle cell disease was the most common type of sickle cell disease in our series (68.4%), as also described in most of the series in the literature. The diagnosis of major sickle cell syndrome remains relatively late in our series (8 year) compared to literature. The frequency of vaso-occlusive seizures is the same as that reported in the series, while the infections are a little less due to antibiotic prophylaxis.The rate of anemia, however, remains more important than in the series. Vesicular lithiasis was the most frequently described chronic complication in our study, a little more common than in the literature. Background treatment has improved treatment outcomes and decreased the incidence of vasoconstriction and hospitalization. The bone marrow allograft, not made in our patients remains a pillar in the process that should be introduced in our institution.

PB2412

IMPACT OF SICKLE CELL DISEASE ON WORK, SCHOOL, RELATION SHIPS, AND SOCIAL LIFE: RESULTS FROM IN-DEPTH INTERVIEWS WITH PATIENTS

P. Stebbins[1], C. Saucier[1], M. Bailey[2,*], D. D'Alessio[2], A. Mitchell[1], M. White[1], K. Raymond[1]

[1]Optum, Johnston, [2]Novartis Pharmaceuticals Corporation, East Hanover, United States

Background: Sickle cell disease (SCD) affects approximately 100,000 individuals in the US. There are few published studies which document the impact SCD has on patients' day to day activities and relationships. Qualitative research methods are ideal to gain an in-depth understanding of the ways in which SCD symptoms impact patients' lives.
Aims: To describe SCD patients' self-reported impacts of SCD symptoms/pain crises on their work, school, relationships, and social life.
Methods: Twelve patients (aged >=12) who experienced one or more sickle cell-related pain crises in the past year were interviewed either in person

(n=9) or by telephone (n=3) using a qualitative, exploratory interview approach.
Results: Of the eight adult (aged >=18) patients interviewed, five reported having left the workforce due to the effects of their SCD and three remained employed. Three of the eight patients described having worked during pain crises despite pain and fatigue; two reported needing to make accommodations during their work day. Of the five currently unemployed, three had voluntarily retired and two had been displaced as a direct result of SCD. Of the three currently employed patients, two reported being restricted in their ability to perform their duties. Of the adolescent patients interviewed (n=4, age ranged from 12-16), all reported one or more pain crisis episodes in which he or she missed between one and four days of school, and all reported experiencing presenteeism. Two reported that symptoms and absences due to crises resulted in getting behind on school work. All four reported missing gym and other physical activities at school to manage symptoms. All 12 patients reported family and social relationships as either sources of stress, support, or both during pain crises. Seven of 12 described having one or more close friends or family members who did not understand pain crises, and labelled patients as "lazy" or "a crybaby." Four of 12 patients reported minimizing contact with others during a pain crisis. Three of the eight adult patients reported experiencing anxiety at having to cancel plans at the last moment either due to the onset of a pain crisis or to prevent being exposed to a sick loved one for fear of the exposure triggering a crisis. Six of 8 adult patients reported having friends, family, and neighbors who offered support, planned recreational outings, and assisted with childcare, transportation, and errands. Adolescents did not report direct impacts on their relationships; however, three of the four reported envy of siblings and peers who could participate in activities such as sports and field trips while they could not due to SCD.
Summary/Conclusion: Living with SCD has a significant impact on patient's physical and emotional well-being and social functioning for both adults and adolescents. Absenteeism and job loss due to SCD was common among the adult patients interviewed. Missing school and having difficulty completing schoolwork due to symptoms and crises may have future consequences for adolescents. The tendency to self-isolate due to a lack of understanding about SCD and the debilitating nature of pain crises amongst friends and family members further add to the social and economic consequences of SCD. Having a better understanding of patients' experiences can help improve treatment for SCD and reduce its impact on a patient's day to day life.

PB2413

BARRIERS TO TREATMENT FOR PATIENTS WITH SICKLE CELL DISEASE: RESULTS FROM IN-DEPTH INTERVIEWS WITH PATIENTS AND CAREGIVERS

P. Stebbins[1], M. Bailey[2,*], D. D'Alessio[2], C. Saucier[1], K. Raymond[1], M. White[1]

[1]Optum, Johnston, [2]Novartis Pharmaceuticals Corporation, East Hanover, United States

Background: Previously published research on sickle cell disease (SCD) demonstrates that patients experiencing a SCD-related pain crisis often treat themselves at home rather than seek medical care outside the home (e.g. medical professional such as a physician's office or walk-in clinic, or by emergency services). Rapid initiation of treatment outside the home for patients experiencing a SCD-related pain crisis can be critical to minimize the risk of organ damage and reduce overall pain. What are the perceptions and treatment experiences that shape the decision to seek treatment outside the home for a SCD-related pain crisis?
Aims: To describe SCD patient and caregiver self-reported perceptions of barriers to seeking treatment outside the home for SCD-related pain crises.
Methods: Twelve patients (>=12 years of age) and six caregivers of patients <12 years of age with SCD were interviewed either in person (n=14) or by telephone (n=4) using a qualitative, exploratory interview approach. Patients had all experienced one or more sickle cell-related pain crisis in the past year.
Results: All patients interviewed (n=12) and one-third of caregivers (n=2) reported deliberately delaying seeking treatment outside the home when experiencing a SCD-related pain crisis. They also reported using a variety of pain crisis prevention and minimization strategies to delay care outside the home as long as possible. Strategies included: rest, hydration, music, meditation, and increased medication use. All patients described seeking treatment outside the home as typically the last resort; while the majority of caregivers of pediatric patients were more likely to initiate care inside the home, but seek care outside the home as pain levels escalated. Of the subset of study participants who shared reasons for delaying seeking treatment outside the home (n=7), the most commonly reported reasons to delay or avoid treatment outside the home were: receiving stigmatizing attitudes from doctors and other treatment professionals or not being believed about pain levels (n=6) and long wait times in emergency rooms (n=4; participants could report more than one reason). Additional factors included: inadequate or ineffective treatment options (n=3), a lack of knowledge on SCD-related pain crises and SCD in general amongst (non-specialist) clinicians (n=2), and perceived racial discrimination (n=2). Most caregivers (n=4) reported a lack of education and/or knowledge about how to identify and mitigate a pain crisis, which delayed their ability to seek treatment outside the home.
Summary/Conclusion: Although this study was conducted with a small sample in the US, the findings emphasized the importance of understanding patient and caregiver perceptions of barriers and treatment experienced outside the home for SCD-related pain crises. Educating medical professionals, particularly non-specialists and emergency care personnel, about SCD may help reduce some of the barriers experienced by these patients and caregivers, ultimately helping patients by providing appropriate and timely pain relief and minimizing potential organ damage and other complications. In addition, it is important to ensure patients and caregivers are making treatment related decisions, about when to seek treatment outside the home, based on medical need rather than perceptions of medical professionals.

PB2414

IMPACT OF HYDROXYUREA ON NEPHROTIC SYNDROME SECONDARY TO DREPANOCYTOSIS - A CASE REPORT

I. Oliveira[1,*], J. de Amaral Melo[2], M.J. Palaré[3], A. Ferrão[3], C. Simão[4], A. Morais[3]

[1]Serviço de Pediatria, Hospital de São Bernardo, [2]Serviço de Hematologia e Transplantação de Medula, Departamento de Pediatria, Centro Hospitalar de Lisboa Norte E.P.E., [3]Unidade de Hematologia, [4]Unidade Pluriassistencial, Departamento de Pediatria, Centro Hospitalar de Lisboa Norte E.P.E, Lisboa, Portugal

Background: Nephropathy is a common complication of sickle cell anemia (SCA) and an important poor prognostic factor among these patients. It can rarely present as a nephrotic syndrome.
Aims: We aimed to describe the impact of hydroxyureia on nephrotic syndrome secondary to drepanocytosis in an adolescent.
Methods: Clinical case presentation.
Results: A 12-year-old African girl from São Tomé and Príncipe was diagnosed with sickle cell disease 6 years earlier. She remained asymptomatic and without any organ involvement up until the age of 11 when she moved to Portugal and presented with an inaugural nephrotic syndrome. Her initial urinary protein/creatinine ratio was 18,3mg/dL (normal <0,15) and she was started on prednisolone 60mg/m^2/day. A renal biopsy was done which revealed a membranoproliferative glomerulonephritis with hemosiderin deposits in the tubular epithelium. However, she maintained a nephrotic proteinuria with episodic but exuberant malleolar swelling which brought her great discomfort and motivated frequent school absences. A minimum urine protein/creatinine ratio of 8mg/dL was reached while on prednisolone and enalapril. After the discontinuation of corticosteroid, she maintained a clinical and laboratory deterioration. Nine months after the inaugural episode, she was started on hydroxyurea to delay the progression of the renal disease. Since then, she showed regression of the edema, her serum albumin normalized and her urinary protein/creatinine ratio decreased (16,5mg/dL at the beginning of the treatment with hydroxyurea and 6,7mg/dL after 6 months). There was an increase in the fetal hemoglobin from 9% to 18%. She remains on both enalapril and hydroxyurea with no further complications. Ophthalmic and cardiac evaluations were normal and she has a normal middle cerebral artery flow velocity on the transcranial doppler.
Summary/Conclusion: The best approach to prevent the progression of nephropathy in patients with sickle cell disease remains to be determined. In this case, the use of corticosteroids and a renin-angiotensin-aldosterone inhibitor reduced the urinary protein/creatinine ratio, but the patient maintained a nephrotic proteinuria with episodes of severe edema. The initiation of hydroxyurea was effective in controlling the progression of the secondary nephropathy. We believe this was a result of limiting intraparenchymal sickling and thus decreasing the glomerular dysfunction. Starting treatment with hydroxyurea early on should therefore be considered in the treatment of renal disease in children with SCA.

Stem cell transplantation – Clinical

PB2415

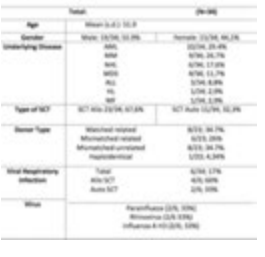
T CELL EXHAUSTION PROFILE (CD4+/CD28- CD8+/CD28-) IN BONE MARROW TRANSPLANTATION. CLINICO-EPIDEMIOLOGICAL FINDINGS

M. Villanueva[1,*], A. Figuera[1], Y. Perez[2], C. Cuellar[1], J.M. Galvan[3], G. Silva[1], M.C. Marcos[4], J.C. Nazario[5], L. Cardenoso[6], R. De La Camara[1], C. Munoz[2]

[1]Hematology, [2]Immunology, [3]Internal Medicine, [4]Pneumology, Hospital Univesitario de La Princesa, [5]Innovation Unit, Hospital Clínico San Carlos, [6]Microbiology, Hospital Univesitario de La Princesa, Madrid, Spain

Background: Recipients of allogeneic stem cells grafts have clonally expanded CD8+/CD28- and CD4+/CD28- T lymphocytes during the early period after SCT, this cellular dynamic is probably associated with the acquisition of a toxic phenotype who over produce granzime and perforin. This scenario predisposes to continuous inflammation, increase T cell cytotoxic, NK activity (NK Per+ Gran+) and favor the appearance of senescent lymphocytes. On the other hand, viruses are recognized as the predominant pathogens leading to pneumonia after allo SCT (lack data in auto SCT). The direct consequences of respiratory viral infection (RVI), subsequent cellular injury and altered host immunity could also initiate a cascade of immunologic events (T cell exhaustion, inflammation).
Aims: The present study aimed to evaluate the dynamics of T cell exhaustion profile CD4+/CD28- Per+/Gran+, CD8+/CD28- Per+/Gran+ NK Per+ Gran+ during the first 100 days after SCT recipients (autologous, allogenic and patients with RVI).
Methods: Informed consent was obtained from all the patients before blood samples were collected. In a prospective study, peripheral blood samples were obtained previous BMT and at day +100 post BMT from 34 patients (11 autologous, 23 allogenic). To evaluate the expression of CD4+/CD28- Per+/Gran+, CD8+/CD28- Per+/Gran+, flow cytometry analysis was performed: 100µL of PB was labeled, with a panel of 8 monoclonal antibodies: PERFORIN FITC, GRANZIME PE, CD4 PerCP, CD28 APC, CD8 APC-H7, CD16/56 V450 and CD45 V500.The molecular detection of RV were tested with the CLART® Pneumovir assay (Clinical Array Technology, Genomica, Spain) based on the principle of multiplex polymerase chain reaction and DNA microarray.Statistical analysis was performed with IBM SPSS v24.
Results: Thirty-four patients were evaluated from November 2016 to December 2017 at the University Hospital of La Princesa, Madrid Spain. The patients characteristics are shown in Table 1.The median percentages of baseline CD8+/CD28- cell line was 9,09% (range, 4,1-14,7) and the median percentages of CD8+/CD28-cell line at +100d were 29,2% (range, 13,3 -38,9). Likewise, significant differences were found (p=0.001). We compared between the group of patients who had a RVI and the group without RVI: We found differences between patients with VRI and those who did not have VRI in the CD8+/CD28- +100d cell line, the median percentages of the RVI patients were 58.43% (range, 42.72-68.43) and the median percentages in the uninfected were 29.26% (range, 13.26-38.94) (p=0.002). In the other cell lines, we did not find any statistically significant association.

Table1.



Summary/Conclusion: In the present work, we show a dynamic change in the exhausted phenotype of T cell lymphocytes CD8+/CD28- throughout the SCT. There are a statistically significant differences when analyzing patients infected with RV *vs* non-infected patients (baseline *vs* + 100d). In agreement wtih previous reports, there was a marked increased od CD8+/CD28- cell fraction early after SCT. Exposure to certain VR in the first 100 days after TPH may contribute the appearance of populations of exhausted T lymphocytes CD8+/CD28- favoring a sustained inflammatory environment and probably works as a trigger in certain immunological complications (immune-disregulation). It is necessary to evaluate these patients over time and see their correlation with other clinico-epidemiological variables in the SCT (aGVHD, cGVHD, pulmonary functional tests, bronchiolitis obliterans, CMV reactivations).

PB2416

THE EFFICACY AND SAFETY PROFILES OF UPFRONT AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA: FOCUSED ON RISK FACTORS FOR SURVIVAL AND CONDITIONING REGIMENS

H.-J. Shin[1,*], D.S. Kim[2], S.-H. Jung[3], Y. Ko[4], H.-Y. Yhim[5], W.S. Lee[6], Y.J. Lee[7], J.S. Hong[4], H.J. Kang[8], S.Y. Oh[9], Y.-S. Choi[10], H.S. Lee[11], J.-H. Lee[12], Y. Park[8], B.B. Park[13], Y. Do[14], J.S. Kim[15]

[1]Pusan National University Hospital, Busan, [2]Korea University School of Medicine, Seoul, [3]Chonnam National University Hwasun Hospital, Hwasun, [4]Seoul National University Hospital, Seoul, [5]Chonbuk National University Hospital, Jeonju, [6]Busan Paik Hospital, Busan, [7]Kyungpook National University Hospital, Daegu, [8]Korea Cancer Center Hospital, Seoul, [9]Dong-A University College of Medicine, Busan, [10]Ulsan University Hospital, Ulsan, [11]Kosin University College of Medicine, Busan, [12]Asan Medical Center, [13]Hanyang University College of Medicine, Seoul, [14]Dongsan Medical Center, Daegu, [15]Yonsei University College of Medicine, Seoul, Korea, Republic Of

Background: The role of upfront ASCT in patients with diffuse large B cell lymphoma is still controversial.
Aims: We evaluated the effectiveness and safety profiles of upfront ASCT with 194 patients of DLBCL.
Methods: Total 151 patients (77.80%) achieved complete remission after ASCT and overall response rate was 84.0%.
Results: Treatment related mortality was 3.6% and 66 patients (34.0%) had progression or relapse. The 5-year overall survival rates and progression free survival rates were 72.3% and 54.6%. There were no significant differences in OS (p=0.371) and PFS (p=0.572) between BCNU conditioning group (BEAM) and busulfan conditioning groups (BuCyE or BuMelE). Neutrophil engraftment was significantly faster in busulfan group than in BCNU group (p=0.011). But, the frequency of mucositis was higher in busulfan groups than in BCNU group (p=0.03). Among the busulfan group, BuMelE group showed lower recurrence rate and higher rate of achievement of CR after ASCT than BuCyE group. Multivariate analysis for OS showed that performance status ≥2 (p<.001), non-rituximab induction therapy (p=0.011) and BuCyE conditioning (p=0.016) were poor prognostic factors. In addition, PS ≥2 (p=0.018), non-rituximab induction therapy (p=0.003) and non-CR status before ASCT (p=0.029) were significantly associated with poor prognosis in PFS. The following factors; stage, bulky disease, high LDH, bone marrow involvement and high risk IPI did not affect survival significantly.
Summary/Conclusion: In conclusion, it is considered that upfront ASCT can overcome the poor prognosis of high risk DLBCL patients. And busulfan based conditioning, especially BuMelE, is an effective conditioning regimen, showing similar efficacy and safety profile as BEAM conditioning.

PB2417

EFFICIENCY OF CD34+-SELECTED STEM CELL BOOSTS FOR PRIMARY POOR GRAFT FUNCTION AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH APLASTIC ANEMIA

R. Bogdanov*, N.K. Steckel, D.W. Beelen

Department of Bone Marrow Transplantation, University Hospital of Essen, Essen, Germany

Background: An important point for success of allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a sufficient and durable reconstitution of hematopoietic donor stem cells. Primary poor graft function (PPGF) after allo-HSCT is characterized by persistent pancytopenia, immunodeficiency and dependence on blood transfusions, despite of a complete donor chimerism. Absence of a regulatory effect of bone marrow stromal microenvironment in patients with aplastic anemia, even in case of using bone marrow as a source of stem cells, increases the probability of development of PPGF.
Aims: The purpose of this analysis is to assess safety, efficacy and survival after CD34+-selected stem cell boost in patients (pts) with PPGF after allo-HSCT for aplastic anemia.

Methods: The analysis comprises data of 13 pts with aplastic anemia after allo-HSCT from HLA-matched sibling (n=7) or unrelated (n= 6) donors, who underwent allo-HSCT between 2008 and 2017. The median age of the 10 male and 3 female pts was 32 years (range, 17 to 66). The immunoablative conditioning regimen consisting of 4x50mg/kg cyclophosphamide and 3x30mg/kg rabbit ATG was performed in all pts before allo-HSCT. In case of unrelated donors, 2 Gy total nodal irradiation was additionaly administerd. Bone marrow (n=12) or peripheral blood stem cells (n=1) with the a median of $2.65x10^6$ $CD34^+$ cells/kg bodyweight (BW)(range, 1.5 to 4.7) were transplanted. PPGF was defined as transfusion-dependent thrombocytopenia with platelet counts <20.000/µl, or transfusion-dependent anemia or leucopenia with neutrophil counts <1.000/µl. PPGF was diagnosed at a median of 47 days (range, 22 to 100) after allo-HSCT by bone marrow biopsy. The median neutrophil count at time of stem cell boost administration was $0.3x10^9$/L (range, 0.14 to 1.8), the median platelet count $15x10^9$/L (range, 7 to 30), and the median hemoglobin concentration 8 g/dL (range, 7 to 9), respectively. Immunomagnetically selected $CD34^+$ stem cells from original donors were used without further immunosuppressive prophylaxis. Cell counts were as follows: $5.48x10^6$ $CD34^+$ cells/kg BW (range, 0.95 to 13.2), $0.22x10^4$ $CD3^+$ cells/kg BW (range, 0.16 to 0.8), $2,12x10^4$ $CD20^+$ cells/kg BW (range, 0.55 to 3.55). Hematological engraftment was defined as an increase of neutrophils by 1.000/µl, of platelets by 50.000/µl, and hemoglobin by 8.5g/dL.
Results: The rate of complete hematological engraftment was 85% (11 of 13 pts). A partial response in 1 or 2 hematopoietic cell lines was noted in 2 pts (15%). The median time to hematological engraftment was 17 days after stem cells boost. The current actuarial 9-year survival rate is 76% (Fig.1). In only one case, 8 months after stem cell boost, a relapse of aplastic anemia developed, requiring a second allo-HSCT. Three pts died as a result of infectious complications. Acute graft-versus-host disease was diagnosed in 3 pts (23%): grade I (skin, liver) and chronic GvHD in 5 pts (38%) (limited n=4, extensive n=1).

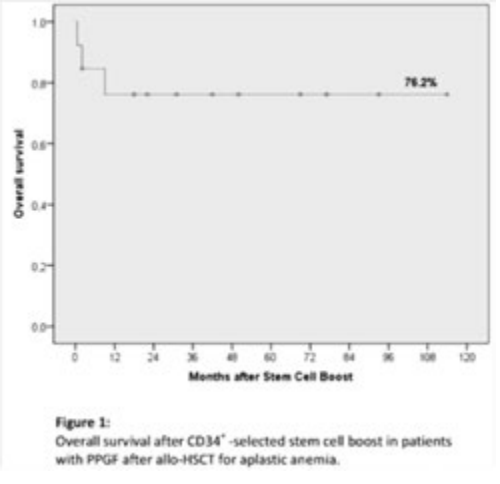


Figure 1.

Summary/Conclusion: The use of $CD34^+$-selected peripheral blood stem cell boosts from the original donor allows a rapid regeneration of hematopoiesis in the majority of aplastic anemia patients with PPGF after allo-HSCT. Infectious complications might be decreased by deciding on early stem cell boost and improved anti-infectious prophylaxis in pts with PPGF.

PB2418

ALLOGENEIC TRANSPLANTATION FOR HODGKIN'S LYMPHOMA IN THE BRENTUXIMAB VEDOTIN ERA: A RETROSPECTIVE ANALYSIS OF THE RETE EMATOLOGICA PUGLIESE (REP) EXPERIENCE

F. Gaudio[1,*], P. Mazza[2], A.M. Carella[3], D. Pastore[4], A. Mele[5], G. Palazzo[2], P. Carluccio[1], G. Pisapia[2], N. Cascavilla[3], G. Specchia[1]

[1]Hematology, Bari, [2]Hematology, Taranto, [3]Hematology, San giovanni Rotondo (FG), [4]Hematology, Brindisi, [5]Hematology, Tricase (LE), Italy

Background: Patients with Hodgkin's lymphoma (HL) progression after autologous stem cell transplantation (SCT) have a very poor outcome. Brentuximab vedotin (BV), an anti-CD30 targeting antibody-drug conjugate, has been studied in this patients setting. Allogeneic SCT is the only strategy with a curative potential.
Aims: This study reports a retrospective analysis of the multicenter experience of the Rete Ematologica Pugliese (REP) over the past 16 years with the aim of comparing the patient characteristics and outcomes of 22 BV pretreated patients with those of 45 patients who received reduced intensity conditioning (RIC) allogeneic SCT without prior BV, in the time period fetore the drug was available (pre-BV era).
Methods: 67 patients with a histologically confirmed diagnosis of HL who underwent allogeneic SCT from 2000 to 2016 were retrospectively studied. The median age was 34 years (range 16-57 years) and 36 (54%) were male. At the time of allogeneic SCT, 28 (42%) patients had chemosensitive disease and 39 (58%) were chemorefractory. All the patients received reduced-intensity conditioning, 52% received matched sibling donor and 48% matched-unrelated donor grafts.
Results: Of the 26 patients with chemosensitive disease, 18 (70%) achieved CR, 7 (27%) had PR or stable disease and 1 (3%) had progressive disease. Of the 36 patients with chemorefractory disease 7 achieved CR (20%), 26 had PR or stable disease (72%) and 3 (8%) had progressive disease. Following transplantation, 40 patients relapsed or progressed at a median time of 6.3 months (range 1- 59 months) post-transplant. After a median follow-up of 38 months (range 3-195 months) 41 patients remain alive and 26 have died. There were no significant differences between groups in terms of engraftment or acute/chronic GVHD incidence. For the BV-treated group, the 2-year PFS was 59%, 2-year OS was 72%, 1-year NRM was 10%, and the 2-year relapse/progression incidence was 24%. In the no-BV group the 2-year PFS was 42%, 2-year OS was 60%, 1-year NRM was 18%. The cumulative incidence of relapse/progression at two years was 58%. Patients in the BV group had a lower median SCT-CI score, higher percentages of patients in CR and fewer peri-transplant toxicities.
Summary/Conclusion: Allogeneic SCT may be an effective salvage strategy for patients who suffer relapse after autologous SCT. Use of the salvage treatment BV yields improved responses over conventional multi-agent chemotherapy with less toxicity, thereby providing better candidates for allogeneic SCT. The impressive activity of novel regimens, such as PD1 inhibitors, has the potential to further enhance responses and survival in HL patient.

PB2419

POST HARVEST PRODUCT HPC AS A COST-EFFECTIVE SURROGATE MARKER FOR CD34+ CELL COUNT IN ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANT SETTING

A. Jamal[1,*], M.T. Khan[2], S. Parveen[3], Q. Rizvi[1], T. Farzana[1], U. Zaidi[1], M. Borhany[1], S. Munzir[4], I. Ghafoor[5], M. Saud[4], S. Ansari[1], T. Shamsi[1,5]

[1]Clinical Haematology & BMT, National Institute of Blood Disease and Bone Marrow Transplantation, [2]School of Public Health, Dow University of Health Sciences, [3]Research and Development, [4]Transplant Immunology, [5]Apheresis and Stem Cells, National Institute of Blood Disease and Bone Marrow Transplantation, Karachi, Pakistan

Background: Allogeneic haematopoietic stem cells transplant provides the only chance of cure for various haematological disorders. Success of bone marrow transplant relies on the infusion of appropriate dose of haematopoietic progenitor cells (HPC) *i.e.* a minimum CD34+ cell count of $>2x10^6$ CD34+ cells/kg of recipient's body weight. Enumeration of CD34+ cells by flowcytometry is a universally accepted reliable indicator of HPC. However, it is an operator-dependent costly procedure with a turnaround time of few hours. Sysmex automated haematology analyzers (XN-1000) segregate an immature myeloid population referred to as haematopoietic progenitor cells (XN-HPC).It is a non-operator dependent cost-effective procedure with a turnaround time of 90 seconds. Incipient statistics of various studies have suggested good correlation between XN-HPC and CD34+ cell count.
Aims: To elucidate the role of XN-HPC as a reliable and cost-effective surrogate of CD34+ cells in post-harvest products in allogeneic bone marrow transplant setting.
Methods: A cross sectional analytical study was conducted at the National Institute of Blood Disease & Bone Marrow Transplantation from Dec'2012 - Dec'2016. Eighty four patients, with various benign and malignant haematological diseases, and their matched related donors were recruited in the study. Donors were primed with injection GCSF 10ug/kg body weight subcutaneously for 4 days to mobilize stem cells. Peripheral blood apheresis was performed on day 5 to achieve the minimum target of $>2x10^6$ CD34+ cells/kg of recipient's body weight. HPC, and CD34+ cell count of peripheral blood apheresis (post-harvest) product was performed by Sysmex XN-1000, and BD FACSCalibur respectively. Statistical analyses were performed using STATA version 11. Spearman's rank correlation coefficient (p) and Receiver Operating Characteristic (ROC) curve were plotted to identify HPC value to optimally distinguish the cut-off of ≥2million CD34+ cells/kg of recipient's body weight.

Results: Eighty-four healthy donors underwent peripheral blood stem cell harvest. Median age of the donors was 21 years. The most common benign and malignant diseases were Aplastic anemia (27%) and Acute myeloid leukemia (11%) respectively. Multiple XN-HPC cut-off values were computed for the CD34+ cell count of ≥2 × 10^6/kg of recipient's body weight to establish evaluable sensitivity and specificity of XN-HPC. The XN-HPC cut-off of 0.049×10^3/ul has a sensitivity and specificity of 96.3% and 09% respectively, whereas the HPC cut-off of 0.87×10^3/ul has a sensitivity and specificity of 47.5% and 82% respectively. Despite a weak correlation between HPC and CD34 (p=0.18, p-value=0.104), the ROC curve analysis showed area under the curve (AUC) of 0.71 (95% CI; 0.58-0.84, p-value=0.023) highlighting the possible potential of XN-HPC to serve as a cost effective surrogate for CD34+ cell count.

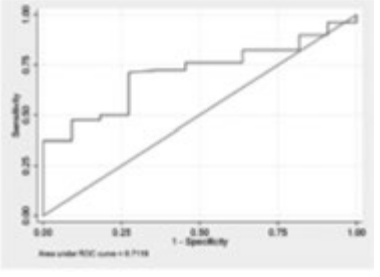


Figure 1.

Summary/Conclusion: Our results demonstrated a statistically significant p value of <0.05 that points towards the possible prognostication potential of XN-HPC for CD34+ cell count, in post-harvest products, in allogenic bone marrow transplant setting. A larger sample size will help in further elucidating the relationship of post-harvest product XN-HPC and CD34+ cell counts.

PB2420

INFLUENCE OF RABBIT ANTI THYMOCYTE GLOBULIN (ATG) AS PART OF CONDITIONING REGIMEN ON CMV REACTIVATION IN ALLOGENEIC STEM CELL TRANSPLANT RECIPIENTS

U. Zaidi*, S.H. Ansari, S. Parveen, D. Zahid, T. Farzana, T. Shamsi

National institute of blood disease and bone marrow transplantation, Karachi, Pakistan

Background: Anti thymocyte globulin (polyclonal anti T cell antibody preparation) is used in pre transplant conditioning regimen to reduce the risk of GvHD and graft rejection emanating from allo-reactivity of donor and recipient, which is reduced by CD34+ lymphocyte depletion. ATG has improved overall survival due to better GvHD prophylaxis. On the other hand, T-cell depletion regimen (ATG) increases the risk of opportunistic infections and hence increase transplant related mortality. Cytomegalovirus (CMV) infection or antigenemia is a significant risk factor in transplant related morbidity and mortality.
Aims: To determine if GvHD prophylaxis with r-ATG increased CMV reactivation 100 days post-transplant in allogeneic stem cell transplant recipients. The primary outcome will be the incidence of CMV at 100 days.
Methods: This retrospective study evaluated patients who received an allogeneic stem cell transplant for malignant and non malignant hematological disorders from June 2015 to 2017, at National Institute of Blood Disease and Bone Marrow Transplantation. Pre Transplant CMV serology was performed in all donors and recipients. All recipients received leucodepleted blood products and oral Acyclovir for CMV prophylaxis. CMV antigen by PCR was routinely done twice weekly in all patients. For preemptive treatment of CMV ganciclovir was offered to all patients with >100copies/ml.
Results: 100 patients with hematological disorders included: Thalasemia major (n=44), Aplastic anemia (n=30), Fanconi Anemia (n=8), Acute myeloid leukemia (n=7), Chronic myelogenous leukemia (n=2), Paroxysmal nocturnal hemoglobinuria (n=2),acute lymphoblastic leukemia (n=1), Gaucher's Disease (n=1) and Myelodysplastic syndrome (n=1), PRCA (n=1), Sideroblastic Anemia(n=1), severe combined immunodeficiency (n=1), Hemophagocytic lymphohistiocytosis (n=1). Mean age of patient at time of transplantation was 10.45±9.2years, 9 patients underwent haplo-identical and 91 were full matched allergenic bone marrow transplantation. Neutrophil and platelet engraftments were achieved in median days of 13(10-29) and 16(10-42) respectively. CMV reactivation was observed in 38/64(59%) patients who received ATG based conditioning regimen *versus* 14/36(39%) patients who did not receive ATG which was clinically and statistically significant. Median duration of CMV reactivation was 13(range=1-42) days post transplant. Treatment related neutropenia (grade II-III) was observed in 7 patients after ganciclovir therapy.CMV infection (Pneumonitis) was documented in 1 patient. Acute GvHD of gut and skin was observed in 7 and 1 patient respectively. Secondary graft failure was observed in 3/52(5.7%) CMV positive and 2/48 (4.2%) in patients with negative CMV.
Summary/Conclusion: CMV reactivation is increased when r-ATG is used as part of the conditioning regimen for GvHD prophylaxis. Large number of patients and follow-up over longer period of time data is still needed to establish CMV as a risk factor for secondary graft failure in allogeneic transplant recipients.

PB2421

AUTOLOGOUS HEMATOPOIETIC STEM CELLL TRANSPLANTATION AS A SALVAGE THERAPY IN RELAPSED/REFRACTORY DLBCL PATIENTS – A SINGLE CENTRE EXPERIENCE

M. Wach*, M. Cioch, D. Jawniak, J. Manko, A. Walter-Croneck, W. Legiec, E. Wasik-Szczepanek, O. Czabak, A. Szczepanek, M. Hus

Klinika Hematoonkologii i Transplantacji Szpiku, SPSK [1], Lublin, Poland

Background: Diffuse large B-cell lymphoma (DLBCL) is one of the most frequent non-Hodgkin lymphpmas (NHL) accounting for about 30% of all newly diagnosed cases and more than 80% out of aggressive lymphomas. Outcome of initial therapy has improved over the last decade but approximately 10-15% of patients present primary refractory disease and additionally 20-25% out of them will relapse. Salvage chemotherapy followed by autologous hematopoietic stem cell transplantation (AHSCT) is still the standard second-line treatment for refractory/relapsed DLBCL.
Aims: The aim of the study is to present chemosensitive patients with relapsed/refractory DLBCL who underwent AHSCT at our centre between December 1997 and October 2017.
Methods: We evaluated 81 patients; there were 35 male and 46 female. Median age was 42.6 years (range; 18.0-69.7) .Most patients were diagnosed as DLBCL NOS-63 pts. (78%), as Primary Mediastinal Large B-cell Lymphoma-12 pts. (15%), as Primary DLBCL of central nervous system-2 pts. (2%), T-cell /histiocyte –rich large B-cell lymphoma -1 pt.(1%) and as Anaplastic ALK+ large B-cell lymphoma-3 pts. (2%). They received median 2 lines of induction therapies (range; 1-4); most were treated with R-CHOP and R+/R-/-DHAP. 34% patients achieved complete (CR), 53.2% partial remission(PR) and 12.8% had progression before transplantation.
Results: In all 81 patients stem cell collection was performed from peripheral blood (100%). The standard conditioning regimen BEAM(BCNU, Etoposide ,Ara-C and Melphalan) was given to 80 patients, 1 received CBV (BCNU,Cyclophosphamide, Etoposode). A median number of 3.8 CD34+ cells/kg (range; 1.6-8.0) were infused. All patients were engrafted with a median time to achieve an absolute neutrophil count>0.5x109/L of 12 days (range; 8-36) and to platelets>20x10/9/L of 13 days (range: 7-36). The complications grade 3 and 4 seen in transplanted patients inculded; mucositis (n=69), bacterial infection (n=5), viral infection (n=4), hemorrhage of CNS in 2 patients (they alive). Transplant related mortality (TRM) was 6.3%. The overall response rate(OS) was 90%. 62% patients achieved CR, 28% PR and 10% stayed in progrssion after AHSCT.The median overall survival (OS) was 47,1 months (median: 3.13-237.9). The median of disease free survival (DFS) was 40.1 months (median; 2.97-237.9).
Summary/Conclusion: Conclusion: AHSCT is highly effective and safe procedure for refractory/relapsed DLBCL patients .Novel agents incorporated in AHSCT programm will be helpful in every part of it; as an induction chemotherapy, preparative regimen and maintenace therapy to improve the results of DLBCL patients, refractory even to AHSCT.

PB2422

BEAC (CARMUSTINE, ETOPOSIDE, CYTARABINE, AND CYCLOPHOSPHAMIDE) IS A SAFE AND EFFECTIVE CONDITIONING REGIMEN IN AUTOLOGOUS HEMATOPOIETIC CELL TRANSPLANTATION FOR LYMPHOMA

I. Sakellari*, S. Bouziana, E. Gavriilaki, V. Constantinou, D. Mallouri, M. Iskas, A. Vardi, A. Marvaki, I. Batsis, D. Sotiropoulos, A. Anagnostopoulos

Hematology Department - BMT Unit, G. Papanicolaou Hospital, Thessaloniki, Greece

Background: Melphalan and Carmustine (BCNU) are key components of

the gold standard conditioning regimen (BEAM) for autologous hematopoietic cell transplantation (AHCT) in Hodgkin (HL) and Non-Hodgkin (NHL) lymphoma patients. However, given their limited availability over the last decade, we have administered alternative conditioning regimens including intravenous Busulfan (BuEM) instead of BCNU and then, Cyclophosphamide (BEAC) instead of Melphalan. Although BuEM has been well tolerated and highly efficacious compared to the gold standard BEAM regimen, BEAC has not been extensively studied.
Aims: We aimed to determine the safety, toxicity and efficacy of BEAC in AHCT for lymphoma.
Methods: We enrolled consecutive lymphoma patients that received BEAC (Carmustine 300mg/m^2, Etoposide 800mg/m^2, Cytarabine 800mg/m^2, and Cyclophosphamide 140mg/kg) between 2016 and 2017. As a control group, we studied consecutive lymphoma patients that had been transplanted using BuEM (Busilvex 9.6mg/kg, Etoposide 800mg/m^2 and Melphalan 140mg/m^2) in our center between 2011 and 2013.
Results: In total, 33 patients received BEAC and 67 BuEM with no significant difference in baseline characteristics (Table).

Table 1.

	BEAC	BuEM	p-value
Age (years)	42 (17-64)	34 (18-65)	0.146
Hodgkin Lymphoma (%)	64	61	0.813
Relapsed/refractory disease (%)	21	21	0.998
Chemosensitive disease (%)	69	52	0.140
Infused CD34 (x10^6/kg)	6.3±3.7	6.4±3.4	0.900

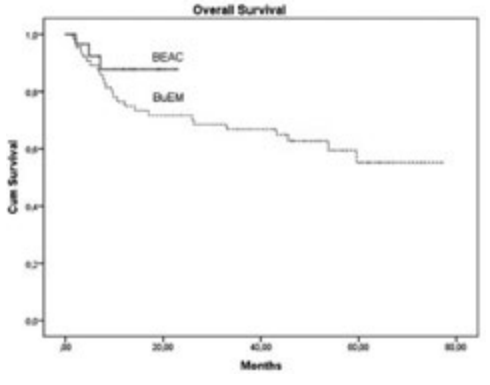


Figure 1.

Days of neutrophil (p=0.657) and platelet (p=0.572) engraftment or transfusion needs (p=0.114 for red blood cell units and p=0.135 for platelets transfused) were also similar between BEAC and BuEM patients. BEAC resulted in significantly lower toxicity regarding infection rate (51.5% *versus* 91%, p<0.001) and WHO grade 3-4 mucositis (p<0.001), gastrointestinal (p=0.025) and liver toxicity (p=0.013). Regarding outcomes, there was no difference in disease status at +100 or at last follow-up. Furthermore, no significant difference was found in the percentage of patients receiving adjuvant therapy post-transplant (p=0.492). Among them, BuEM patients received only radiation; whereas 6 BEAC patients received also adjuvant therapy with brentuximab according to current indications. With a median follow-up of 7.2 (0.8-22.9) months for BEAC and 44.9 (2.3-77.1) for BuEM, relapse mortality was similar between regimens (p=0.626). 1-year overall survival (OS) was 92.4% and 87.6% respectively (Figure, p=0.186). In the multivariate model, OS was significantly associated with diagnosis of HL (p=0.001) and chemosensitive disease (p=0.005).
Summary/Conclusion: BEAC resulted in lower toxicity and similar outcomes compared to BuEM regimen in lymphoma patients with similar pre-transplant characteristics. Outcomes were also comparable to our previously published experience with the gold standard BEAM regimen. Therefore, BEAC emerges as a feasible alternative conditioning regimen in AHCT for lymphoma.

PB2423

MESENCHYMAL STEM CELLS CAN BE DEMONSTRATED BY FLOW CYTOMETRY IN THE G-CSF MOBILISED PERIPHERAL BLOOD OF PATIENTS UNDERGOING PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

A. Jain[1,*], A. Khadwal[1], M.S. Sachdeva[2], P. Malhotra[1], N. Varma[2], S. varma[1], G. Prakash[1], D. Lad[1]

[1]Internal medicine, [2]Haematology, Postgraduate institute of medical education and research, Chandigarh, India

Background: Mesenchymal stem cells (MSCs) possess unique properties of self-renewal, wide differentiation potential and an immunosuppressive effect, are enriched in the bone marrow and their isolation relies on culture in special media.
Aims: Present study aimed to demonstrate MSCs in the peripheral blood (PB) of individuals undergoing hematopoietic stem cell transplant (HSCT) using flow cytometry and correlate their counts with the post-transplant outcomes.
Methods: A total of 33 subjects which included autologous arm (n=25) [patients of MM (n=20), lymphoma (n=5)] and allogeneic arm (n=8) [healthy donors of patients undergoing allogeneic HSCT] during the period extending from 1-1-2016 to 30-06-2017 were prospectively included in the study. Baseline PB sample of the eligible subjects was analyzed for MSCs by flow cytometry. Stem cell mobilization of the subjects was done using granulocyte-colony stimulating growth factor (G-CSF, given for 4 days @ 10mcg/kg/day). Two milliliters of PB sample was analyzed for CD34 and MSC counts using a panel of monoclonal antibodies (CD34-APC, CD45-APC H7, CD73-PE, CD90-FITC and CD105-PE-Cy7) immediately prior to the apheresis. MSCs were defined by an absence of CD34/CD45 and any dual positivity for CD90/73/105. Mononuclear cells were harvested using cell separator and 3 ml of sample from the apheresis product (AP) was analyzed for CD34 and MSC by flow cytometry. Subjects with inadequate CD34 count after the first apheresis (<2x10^6/kg) underwent a second procedure next day after receiving G-CSF (10 mcg/kg/day) and a single dose of plerixafor (0.24mg/kg, given 8 hours prior to the procedure). CD34 and MSC were analyzed by flow cytometry at day 6 (both PB and AP). AP with a minimum of 2.0x10^6/kg CD34 dose was infused to the patient following conditioning. Patients were followed up for a minimum of 100 days post-HSCT and complications in the post-transplant period were recorded till the last follow up. Analyses were conducted using IBM SPSS statistics (version 22.0).
Results: Baseline MSCs were seen in 14 (42%) subjects. The mean baseline MSC (PB) (x10^{-4})% in the autologous and allogeneic group was 19.7±41.83 and 7.9±9.95 respectively (p-0.852). Patients with baseline MSC (PB) had a higher mean MSC (PB) at D5 and D6 (p-<0.001 and 0.030 respectively). Overall 19 (58%) subjects had MSCs at D5 (PB). Administration of more chemotherapy cycles was associated with an absence of D5 MSCs (PB) in patients of MM (p-0.045). A total of 17 (52%) subjects had D5 MSC (AP). Mean D5 MSC (AP) (x10^{-4}%) in the autologous and allogeneic groups was 5.4±8.7 and 25±39 respectively (p-0.019). Patients with D5 MSC (AP) had a trend towards requiring anti-fungal drugs for a greater duration (p-0.051). Only 6 (18%) patients had detectable D6 MSC (PB). Patients with D6 MSC (PB) required anti-fungal drugs for a greater duration (p-0.041) and had a lesser duration of GI mucositis (p-0.015). A total of 6 (18%) subjects had D6 MSC (AP). Post-HSCT events were not associated with the D6 MSC (AP) except for a greater duration of anti-fungal drug requirement (p-0.041). Day-100 outcome of MM patients was significantly associated with D5 MSC (AP) and D6 MSC (PB) (p-0.043 and 0.046 respectively). No significant difference was found between the type of chemotherapy received by MM and lymphoma patients and baseline, D5 (PB/AP) and D6 (PB/AP) MSC.
Summary/Conclusion: MSCs can be demonstrated by flow cytometry in the PB and AP of individuals undergoing HSCT. MSC count in the PB and AP correlates with post-HSCT events.

PB2424

THE PROGNOSTIC ROLE OF E2A-PBX1 EXPRESSION DETECTED BY RQ-PCR IN B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Y. Hong*, X. Zhao, Y. Qin, S. Zhou, Y. Chang, Y. Wang, X. Zhang, L. Xu, X. Huang

Peking University People's Hospital, Beijing, China

Background: The *E2A-PBX1* rearrangement is common in B-cell acute lymphoblastic leukemia (B-ALL). However, whether this fusion gene can be used as a reliable marker for minimal residual disease (MRD) following allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains unknown.
Aims: The aim of this study was to investigate the clinical characteristics of *E2A-PBX1*(immunoglobulin enhancer binding factor-pre-B leukemia) fusion gene in patients with acute lymphoblastic leukemia (ALL) after allogeneic stem cell transplantation (allo- HSCT).
Methods: Clinical data were collected from 28 consecutive B-ALL patients who received allo-HSCT. The *E2A-PBX1* gene was examined by real-time

quantitative polymerase chain reaction (RQ- PCR). The correlation between its expression level and the disease status was analyzed.
Results: The median follow-up was 374d (55-2342d). Of the enrolled patients, 7 (25%) patients died of leukemia relapse. A total of 9 (32.1%) patients experienced relapse at a median of 164d (75-559d) after transplantation. The median expression level in the first positive sample was 0.14% (0.0071-902.4%). The duration from *E2A-PBX1*-positive results to hematological relapse was 74d (30-469d). *E2A-PBX1* expression generally became positive prior to flow cytometry. Patients with positive *E2A-PBX1* gene expression pre-transplantation were more likely to have positive *E2A-PBX1* expression after transplantation.

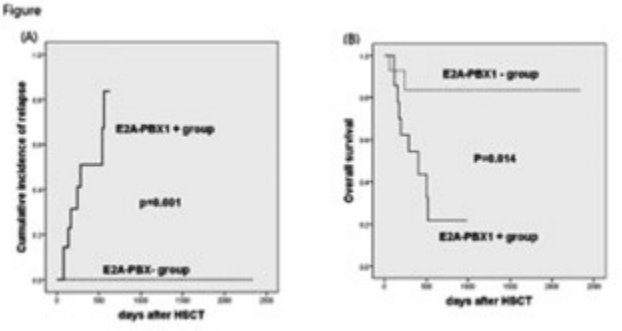


Figure 1.

Summary/Conclusion: *E2A-PBX1* expression determined by real-time quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) could be used to evaluate MRD status after allo-HSCT. Patients with positive *E2A-PBX1* expression after transplant will have a poor prognosis.

PB2425

HIGH DOSE THERAPY AND AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION FOR NON-HODGKIN LYMPHOMA MALAYSIAN HOSPITALS EXPERIENCE

N.A. Anuar[1,*], K. Tey[1], A. Teh[2], M.H.F. Abdul Rahman[2], S.C. Ng[2], P.C. Bee[1], G.G. Gan[1]

[1]Haematology, University of Malaya Medical Centre, Kuala Lumpur, [2]Haematology, Sime Darby Medical Centre, Subang Jaya, Malaysia

Background: High dose therapy (HDT) followed by autologous hematopoietic stem cell transplantation (AHSCT) is recognized as a standard of care for relapsed/refractory Non-Hodgkin Lymphoma (NHL).There are many studies which have demonstrated improved disease free survival(DFS) and overall survival(OS) with AHSCT in these patients. There have been also several studies which reported better outcome in patients with high risk NHL who had upfront AHSCT.
Aims: This study aims to determine if upfront AHSCT do improve patients' clinical outcome in Malaysia.
Methods: This is a retrospective study where patients who had undergone AHSCT from October 1997 to September 2017 in 2 major hospitals in Malaysia were included. Patients' demographic data and clinical information were collected. The risk groups were categorized into 2 groups; whereby IPI score of 0-2 were defined as low risk and IPI score of 3-5 were considered as high risk.
Results: A total of 169 patients (male: female 105:64) were included. Majority of patients had B cell lymphoma (85.8%) while the rest had T cell lymphoma (14.2%). The median age at diagnosis was 46 (ranges from 15-69) years. The median period of diagnosis to AHSCT was 10 (range 3-111) months. Majority of patients (89.3%) were transplanted in complete remission (CR) and the remaining (10.7%) in partial remission (PR). Majority of patients (61%) who were transplanted were in first CR. 68% of patients were categorized as low risk and 32% patients were categorized as high risk. Conditioning regimens used were: BEAM (n=107), Benda-EAM (n=17), BEAC (n=12), TEAM (n=11), TBI-Cyclo (n=6), Thiotepa-BuCy (n=5), CBV (n=4), BuCy (n=4) and C/L-EAM (n=3). The mean CD34+ cell dose infused was $8.8x10^6$/kg (ranges 1.53 to $72.3x10^6$/kg). The median duration of platelet count remaining <$30x10^6$/L and white cell <$1.0x10^9$/L were both 8 days respectively. The mortality rate during follow up was 28%; 3% were due to transplant related mortality and the remaining were from progressive disease. The two years OS for patients transplanted in first CR, second CR, and PR were 87%, 67.8%, 29.4% respectively, (p≤0.0001). The two years event free survival (EFS) for patient transplanted in first CR, second CR, and PR is 81.5%, 52.5%, 17.6% respectively, (p≤0.0001). Using Cox regression analysis, remission status at transplantation was shown to have a significant effect on OS and EFS. Patients who were transplanted at first CR regardless of their IPI risk status had a significantly better OS and EFS compared to the others, p<0.001. The IPI risk status did not demonstrate any significant effect in OS and EFS.
Summary/Conclusion: This study demonstrated that upfront AHSCT should be considered in patients with NHL regardless of their IPI risk status.

PB2426

CYTARABINE IS MORE EFFECTIVE THAN CYCLOPHOSPHAMIDE AS A STEM CELL MOBILIZATION REGIMEN IN MULTIPLE MYELOMA

L. Adamusová[1,2], T. Jelínek[1,2,3,*], M. Šimíček[1,2,3], M. Navrátil[1], M. Kaščák[1], H. Plonková[1], J. Mihályová[1], J. Ďuraš[1], I. Tvrdá[1], Z. Kořístek[1,3], R. Hájek[1,3]

[1]Department of Haematooncology, University Hospital Ostrava, [2]Faculty of Science, [3]Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic

Background: High dose melphalan followed by autologous hematopoietic stem cell transplantation (HSCT) was established as a standard of care for patients under the age of 65 with newly diagnosed multiple myeloma (MM). Current hematopoietic stem cell (HSC) mobilization strategies in patients with MM are based on administration of granulocyte-colony stimulating factor (G-CSF). Intermediate doses (1-5g/m^2) of cyclophosphamide (Cy) are given prior G-CSF in most European transplant centers in order to increase the yield of HSC collection. Nevertheless, mobilization with intermediate doses of cytarabine (AraC) followed by G-CSF was shown to be very effective alternative mobilization regimen (Giebel 2013).
Aims: To retrospectively compare the efficacy and toxicity of two mobilization regimens, Cy+G-CSF and AraC+G-CSF, used in patients with newly diagnosed MM.
Methods: 70 consecutive patients with MM (46 males, 24 females, median age of 62 years, from 37 to 71) mobilized between July 2014 and August 2017 either by Cy+G-CSF (n=30), or by AraC + G-CSF (n=40) were included in the analysis. Cy group: Cy 2,5g/m^2 day 1 followed by G-CSF. AraC group: AraC 400mg/m^2/12h day 1-2 followed by G-CSF. In both regimens the G-CSF was given from day 5 until the end of apheresis at the dose of 10µg/kg/day divided into two daily doses (rounded to the whole ampoules). HSC collections were performed using Spectra Optia cell separator (Terumo BCT, USA). Standard mobilization efficacy (complete blood count, CD34$^+$ cells) and apheresis product quality controls (complete blood count, CD34$^+$ cells, CFU-GM) were performed. The primary endpoint for HSC collection was to harvest ≥$10x10^6$ CD34$^+$/kg, which was considered to have enough HSC for 4 high-dose chemotherapies followed by autologous HSCT.
Results: Peak levels of circulating CD34$^+$ cells in peripheral blood were significantly higher in the AraC group compared to Cy group (368 *vs* 99 CD34$^+$ cells/µL, means, p<0.0001). Mean number of apheresis per patient was 1.2 in AraC group compared to 2.1 in Cy group. Single apheresis was sufficient to collect target amount of CD34$^+$ cells (≥$10x10^6$/kg) in 83% of patients in the AraC group compared to 17% of patients in the Cy group, and ≥$10x10^6$ CD34$^+$/kg was collected in 98% patients in the AraC group compared to 57% of patients in Cy group. CD34$^+$ cell yield was significantly higher in the AraC group compared to Cy group ($27x10^6$ *vs* $5x10^6$ CD34+/kg, means, p<0.0001). Numbers of collected CFU-GM were also significantly higher in the AraC group ($404x10^4$/kg *vs* $109x10^4$/kg, means, p<0.0001). Mobilizations with AraC were well tolerated. It should be emphasized that 50% of patients from AraC group developed Gr.4 thrombocytopenia compared to 7% in Cy group. This resulted into the higher need of platelet transfusions in the AraC group (48% *vs* 10% of patients), however no serious hemorrhagic complications were observed. Times to engraftment after transplantations did not differ between both groups of patients.
Summary/Conclusion: Mobilization regimen AraC + G-CSF was significantly more efficient compared to commonly used mobilization regimen Cy + G-CSF. Safety profile of AraC is acceptable, except of higher rate of Gr. 4 thrombocytopenia, which resulted in need of platelet transfusions in half of patients mobilized by AraC + G-CSF. We conclude, that mobilization with AraC could be considered as a safe and very efficacious alternative to Cy in patients with MM when more than 2 autologous HSCT are anticipated.

PB2427

REDUCED RISK OF SINUSOIDAL OBSTRUCTION SYNDROME OF THE LIVER AFTER BU-CY CONDITIONING BEFORE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION, A RESULT OF PERSONALIZED DOSE ADJUSTMENT

M. Remberger[1], I. El-Serafi[1,2,3,*], P. Ljungman[4], O. Ringdèn[5],

J.K. Törlén[1], M. Sundin[6], A. Björklund[4], J. Winiarski[6], J. Mattsson[1,7]

[1]Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden, [2]Biochemistry, Port-Said University, Port-Said, Egypt, [3]Clinical and Experimental Medicine, Linköping University, Linköping, [4]Medicine, Huddinge, [5]Laboratory Medicine, [6]Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden, [7]Hematology, Oslo University Hospital, Oslo, Norway

Background: Sinusoidal obstruction syndrome/veno-occlusive disease of the liver (SOS/VOD) has been a common and potentially life-threatening complication after allogeneic hematopoietic stem cell transplantation (HSCT).
Aims: To study the effect of busulfan dose adjustment during conditioning prior to HSCT on the patients' risk for developing SOS/VOD and the clinical outcome.
Methods: We focused on patients undergoing myeloablative conditioning with oral busulfan (16mg/kg) and cyclophosphamide (120mg/kg) (Bu-Cy) before allogeneic HSCT during 1990-2015. Since the end of 1990's dose adjustment based on pharmacokinetic analysis has been performed for busulfan. Norethisterone therapy was discontinued in 1998. Most patients had a hematological malignancy. Median age was 34 years (<1-61). A matched related donor was used in 164 patients, a matched unrelated donor was in 166 and a mismatched donor was in 42 patients. Stem cell source was bone marrow (BM) (n=157), peripheral blood stem cells (PBSC) (n=195) and cord blood (CB) (n=20). The majority of patients received cyclosporine and methotrexate as GVHD prophylaxis. SOS was diagnosed according to the Baltimore criteria.
Results: SOS/VOD of the liver was diagnosed in 26 patients (7.0%). Patients receiving PBSC had a SOS/VOD incidence of 3.1% compared to 10.8% and 15.0% in patients given BM and CB, respectively (P=0.006). The incidence of SOS/VOD decreased gradually over time with the highest incidence 1995-99, 14.7% compared to 2.3% during 2010-2014. In multivariate analysis, use of PBSC (P=0.01) and patients in CR1/CP1 (P=0.027) was protective. Overall survival at one year was 46% *vs* 77% in patients with or without SOS/VOD (P<0.001).
Summary/Conclusion: With appropriate management steps the incidence of SOS/VOD may be decreased.

PB2428

MODIFIED BUCY VERSUS BEAM FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION IN LYMPHOMA: CASE PAIR COMPARATIVE ANALYSIS ON TOXICITY AND EFFICACY

H. Huang[1,*], L. Zhang[1], Y. Jiang[1], Z. Jin[1], C. Li[1], J. Ruan[2], D. Wu[1]

[1]Department of Hematology, The First Affiliated Hospital of Soochow University, Suzhou, China, [2]Division of Hematology and Medical Oncology, Weill Cornell Medical College, New York-Presbyterian Hospital, New York, United States

Background: High-dose chemotherapy (HDC) followed by autologous stem cell transplantation (auto-SCT) is superior to conventional chemotherapy in a subgroup of newly diagnosed and relapsed/refractory lymphomas. And BEAM (semustine, etoposide, cytarabine, and melphalan) is one of the most commonly used conditioning regimens. However, the source of melphalan is restricted in China. The conditioning regimen of modified BuCy (busulfan, cyclophosphamide, semustine and cytarabine, mBuCy) has been widely used as the myeloablative regimen for AML and is well tolerated and has shown good efficacy. However, there are limited datum on the efficacy and safety of the mBuCy conditioning regimen in lymphoma auto-SCT. And whether this mBuCy conditioning regimen can be safely used as an alternative to BEAM in lymphoma was uncertain.
Aims: To evaluate the efficacy and toxicity of mBuCy and BEAM regimens.
Methods: From February 2014 to November 2017, a total of 70 Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL) patients underwent HDC with mBuCy (n=27) or BEAM (n=43), followed by auto-SCT in our center. As far as the patient's disease status at time of auto-SCT is concerned, we made a 1:1 match. In total, 27 pairs of patients were analyzed.
Results: In our study, we found that there were no significant differences in median number of infused CD34+ cells/kg, hospitalization duration, and median units of transfused red blood cells and platelets, as well as the time of platelet engraftment between the two groups. However, neutrophil engraftment was somewhat faster in mBuCy group than that in the BEAM group (median days: 9 days *vs* 9.5 days, p=0.038). When it comes to toxicity, the incidence of nausea/vomiting, hepatic impairment, renal impairment, pulmonary infection and treatment-related mortality was found to be similar in the two groups. However, compared with patients conditioned with mBuCy, patients conditioned with BEAM were more likely to develop mucositis and diarrhea (p=0.029; p=0.040). For grade III-IV mucositis and diarrhea, the incidence rate was higher in BEAM (25.9% *vs* 3.7%; 18.5% *vs* 0%). With a median follow-up of 15.2 months (range, 3.0 - 41.3 months) in the mBuCy group and 34.9 months (range, 4.6 - 48.0 months) in the BEAM group, we compared the 2-year PFS (72.0% *vs* 72.0%, p=0.761) and 2-year OS (84.0% *vs* 77.0%, p=0.783) of the regimens mBuCy and BEAM respectively, which indicated that the two groups were equivalent. Univariate analysis revealed that the International Prognostic Index (IPI) at diagnosis (p=0.046) and the level of serum LDH at transplantation (p=0.002) were the variables associated with PFS. For OS, only the level of serum LDH at transplantation (p=0.010) remained significant. In multivariate analysis, only the level of serum LDH was significantly associated with PFS (p= 0.029, HR: 4.52; 95% CI: 1.17-17.56).

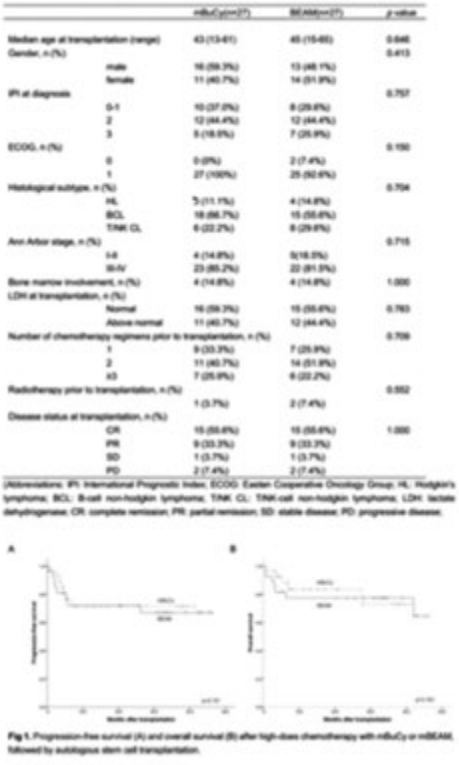


Figure 1.

Summary/Conclusion: Conditioning regimen of mBuCy was found to be well tolerated with acceptable nonhematological toxicity. Patients who underwent auto-SCT after mBuCy had similar survival outcomes and faster neutrophil engraftment than a matched pairs-cohort who underwent auto-SCT after BEAM, indicating that mBuCy can be considered a valid alternative to BEAM.

PB2429

ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION FOR PRIMARY REFRACTORY ACUTE LEUKEMIA PATIENTS: A RETROSPECTIVE GITMO SCORE BASED ANALYSIS

I. Cutini*, C. Nozzoli, A. Gozzini, S. Guidi, I. Donnini, R. Saccardi

Cellular Therapies and Transfusional Medicine Unit, University Hospital, Florence, Florence, Italy

Background: Patients with acute leukemia (AL) who fail to achieve complete remission (CR) have a dismal prognosis. Only few of them can be rescue after allogeneic hematopoietic cell transplantation (HCT). We retrospectively applied the GITMO score for PIF AL patients, that divides patients in 3 different categories; low, intermediate and high risk (Todisco E, BMT 2017).
Aims: Retrospective GITMO score based analysis.
Methods: The study population included 25 patients with AL presented as primary induction failure (PIF) who had received an allogeneic HCT between 1 March 2014 and 30 September 2017 at our institution. Median age was 51 yo. Disease characteristics and prior history of hematological diseased and gender are summarized in Table 1. Patients received myeloablative (n=16) or reduced-intensity conditioning (RIC) regimens (n=9). Median time from diagnosis to transplant was 7 months, (range 0-9 months).
Results: Among PIF AL population 16 belong to low risk group; 7 to intermediate group and 2 to high risk group respectfully. 18 out of 25 were

evaluable on +30 days from transplant: 16 patients achieved complete hematological remission (CHR), 2 patients shown refractory disease. 7 patients died within 2 months (range 0-4 months) from transplant due to multiorgan failure (2 pts), sepsis (3 pts) and engraftment failure (2pts). 12 out of 25 (35%) died due to TRM. Mean OS was 521 days, median OS was 331 days. Only 7 out of 25 patients (28%) are still alive without active disease, mean follow up was 337 days (79-1348 days). Among the three GITMO categories, the mean and median OS was 527 and 364 days, 581 and 341 days, and 262 days for low, intermediate and high risk group respectively (p=0.126). 8 patients experienced aGVHD and 7 patients cGVHD. All patients had received corticosteroids as frontline treatment. Those unresponsive were treated with second-line immunosuppressors. Mean time for developing GVHD was 2 and 4 months for aGVHD and cGVHD respectively. GVHD characteristic was shown in table 1. A trend to a better OS was shown in patients who developed aGVHD (p=0.380) and cGVHD (p=0.219). No impact was shown for CMV sierological status. (p=0,651). No impact was shown for myeloablative *versus* RIC regimens (p=0.983).

Table 1.

	N° 25	%
Age	51 yo (20-66)	
Gender	M 10 F 15	40 60
Diagnosis		
AML	24	96
ALL	1	4
De novo	16	64
Prior history of:		
MDS	2	8
ET	2	8
IMF	1	4
Chemo	1	4
Chemo+RT	1	4
HSCT	2	8
AML RISK (ELN 2017)		
LOW RISK	2	8
INTERMEDIATE RISK	11	44
HIGH RISK	12	48
CMV STATUS		
NEGATIVE	5	20
POSITIVE	20	80
CONDITIONING REGIMEN		
MYELOABLATIVE	16	64
RIC	9	36
PIF GITMO SCORE		
LOW	16	64
INTERMEDIATE	7	28
HIGH	2	8

	Organ	N°
aGVHD	Skin	8
	Global grade 1	5
	Global grade 2	3
	Intestine	2
	Global grade 1	0
	Global grade 2	1
	Global grade 3	1
	liver	0
cGVHD	Mild	2
	Moderate	4
	Severe	1

Table 2

Summary/Conclusion: The clinical outcome of PIF AL patients is poor and only a minor proportion of patients is rescued by HSCT. GITMO score can be used to create a risk score that helps to identify patients who most likely benefit from the procedure. The availability of reliable prognostic factor is particularly important in the era of alternative donor, such as haploidentical source. The small sample size may prevent to assess more significant differences across the population.

PB2430

OUTCOME OF SECOND ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ACUTE LEUKEMIA: A SINGLE CENTER EXPERIENCE

T.N. Yigenoglu*, H. Batgi, B. Uncu Ulu, A. Merdin, S. Bascı, M. Bakırtas, J.V. Yıldız, M.S. Dal, M.K. Cakar, D.K. İskender, A.I.E. Tekgunduz, F. Altuntas

Hematology, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Ankara, Turkey

Background: Patients with acute leukemia who relapse after their first allogeneic hematopoietic stem cell transplantation (allo-HSCT1) have poor prognosis. Second allo-HSCT (allo-HSCT2) from matched related or unrelated donor or haplo-identical transplantation has been already used as a potentially curative treatment for patients who relapsed after their first allogeneic stem cell transplantation. There is still limited information about alternative donor sources on the efficacy of allo-HSCT2.
Aims: To identify prognostic factors affecting the outcome of second HSCT, we performed a retrospective study on our patients with acute leukemia.
Methods: Data from Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Stem Cell Transplantation Unit database were retrospectively analyzed.
Results: A total of 319 patients with acute leukemia had received allo-HSCT1 at our center between March 2009 and May 2017. 78 of them relapsed after their first allogeneic stem cell transplantation and 13 of them underwent their second allogeneic stem cell transplantation. The median interval between allo-HSCT1 and allo-HSCT2 was 240 days (range: 120-1800). Patients had acute myeloid leukemia (AML, n=4), T cell acute lymphoblastic leukemia (T ALL, n=4), Philedelphia positive B cell acute lymphoblastic leukemia (Ph+ B ALL, n=3), Philedelphia negative B cell acute lymphoblastic leukemia (Ph neg ALL, n=2). All of the patients received myeloablative conditioning regimen. Donors at allo-HSCT2 were HLA-matched or 1 antigen HLA-mismatched related donor (MRD, n=11) and unrelated donor (URD, n=2). 7 patients were transplanted from the same donor used in their first transplantation while in 6 patients transplantation was performed from alternative donors.Among 13 patients, 1-year overall survival (OS) and progression-free survival (PFS) rate were 46.1% and 23% respectively. Overall survival rates did not differ between patients with varying age, diagnosis, times of relapse, durations between the first transplantation and the relapse, GVHD grade, donor type(same or different, related or unrelated) and EBMT score.
Summary/Conclusion: The outcome of allo-HSCT2 has been still unsatisfactory because of the high rate of TRM and relapse. Outcome of second allogeneic transplantation from an alternative donor is comparable with the second transplantations performed from the same donor.

PB2431

AUTOLOGOUS STEM CELL TRANSPLANTATION OUTCOME IN NEWLY DIAGNOSED TRANSPLANT-ELIGIBLE MULTIPLE MYELOMA IN SIRIRAJ HOSPITAL, BANGKOK, THAILAND: AN 18-YEAR FOLLOW-UP

N. Tungsuksomboon*, C. Kunacheewa, E. Karoopongse

Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Background: Treatments for multiple myeloma (MM) with induction chemotherapy followed by autologous stem cell transplantation (ASCT) and maintenance therapy have been recommended as the standard approach. Long term follow-up after transplantation and factors associated with progression-free survival (PFS) and overall survival (OS) are not yet elucidated.
Aims: To investigate long term transplantation outcome including OS, PFS and related prognostic factors in patients with newly diagnosed MM who underwent ASCT in a referral university hospital in Thailand.
Methods: A cohort of patients older than 18 years with first diagnosed MM treated with ASCT from 2000 to 2015 were reviewed. We used IMWG criteria to classify the depth of response. All patients received induction regimen(s) comprising 2 or 3 drugs followed by high-dose melphalan before undergoing ASCT. Factors affecting survival were explored using Cox proportional hazard models.
Results: One hundred and twenty-two patients were included. The median follow-up time was 39 (2-115) months. The median age of the cohort was 56 (31-68) years with 45.1% of male. IgG Kappa was the most common type of paraprotein identified. Majority of patients were in ISS stage III (34.4%) followed by stage II and I at 21.3 and 14.8%, respectively. Approximately half of patients (48%) received only 1-drug regimen before ASCT. Most patients (92%) received novel agent(s) for which bortezomib was the most commonly used drug (87%), either as a single agent or in combination. Thalidomide or lenalidomide was used 30% and 18%, respectively. Prior to ASCT; 63.1%, 5.7%, 26.2% and 4.9% of subjects achieved sCR, CR, VGPR and PR, respectively. After ASCT; 68.9%, 4.9%, 23.7% and 3.3% of subjects achieved sCR, CR, VGPR and PR, respectively. There was no transplantation-related mortality. ASCT improved disease response in 22/45(49%) patients who achieved less than sCR before ASCT. After ASCT, 24.6% received consolidation and 52.5% received maintenance therapy. Median OS was 111.3 months while 3-yr OS, 5-yr OS and 10-yr OS were 91%, 74%, and 45% respectively. Median PFS was 53.4 months while 3-yr PFS, 5-yr PFS, and 10-yr PFS were 75%, 43% and 16% respectively. Factors affecting PFS included presence of plasma cells ≥5% in BMA after induction chemotherapy (HR 3.53, 95% CI 1.25-9.93, p=0.017), or after ASCT (HR 4.29, 95% CI 1.23-14.99, p=0.022), achieving less than CR after ASCT (HR 2.32, 95% CI 1.35-3.98, p=0.002), and receiving maintenance therapy (HR 0.35, 95% CI 0.21-0.59, p <0.001). While male (HR 2.10, 95% CI 1.01-4.37, p=0.047) and achieving less than VGPR after ASCT (HR 5.72, 95% CI 1.03-31.67, p=0.046) were affected patients' OS.

Subgroup analysis showed that maintenance therapy had significant benefit for both OS and PFS only in the patients who achieved VGPR after ASCT (OS_HR 0.115, 95% CI 0.03-0.43, p=0.001, PFS_HR 0.12, 95% CI 0.05-0.30, p <0.001). Maintenance with lenalidomide or thalidomide didn't alter PFS (HR 0.71, 95% CI 0.35-2.06, p=0.844). Abnormal SFLC had significantly impacted on PFS (HR 2.15, 95% CI 1.24-3.74, p=0.006).

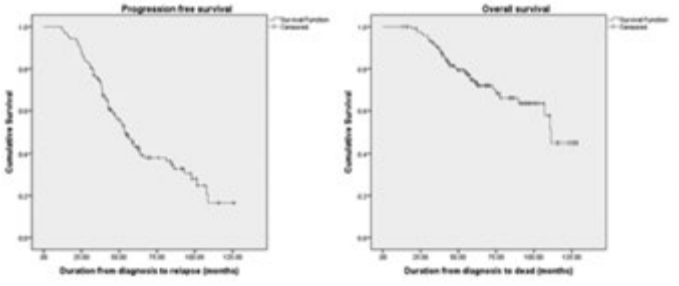


Figure 1.

Summary/Conclusion: We reported almost 10-year overall survival in newly diagnosed multiple myeloma treated with upfront autologous stem cell transplantation. Depth of response, either pre or post stem cell transplantation, determines patients' survival. Moreover, our data revealed that maintenance therapy post transplantation improved clinical outcome, particularly among patients who achieved less than complete response after stem cell transplantation.

PB2432

SEQUENTIAL CLOFARABINE CONTAINING CHEMOTHERAPY WITH REDUCED THIOTEPA BASED CONDITIONING AND T CELL REPLETE HAPLOIDENTICAL STEM CELL TRANSPLANT FOR REFRACTORY MALIGNANCIES: A SINGLE CENTRE EXPERIENCE

A. Garcia Sola*, A. Gallardo, O. Benítez, C. Díaz, M. Ortiz, A. Pascual, A. Rodríguez, M. Pascual

Hospital Regional Universitario de Malaga, Malaga, Spain

Background: A sequential conditioning provides promissing results that has not, otherwise, been validated in haploidentical setting. Only 10-20% of refractory acute myeloid leukaemia (AML) achieve complete remission (CR) with another course of chemotherapy, and less than 20% of OS is reached after a year in this high risk population, this approach could be meant to obtain the best outcome. With similar chemotherapy schemes it has been obtained a 40% OS but still unsatisfactory results due to toxicity.
Aims: We show our experience with clofarabine-based sequential chemotherapy in haploidentical SCT followed by TBF reduced intensity conditioning (RIC-TBF) (thiotepa, busulfan, fludarabine) and post transplant cyclophosphamide (PTCy) plus tacrolimus as graft *versus* host disease prophylaxis (GvHD).
Methods: We collected data retrospectively of 15 cases of refractory/relapsed/progressive haematological malignancies who received clofarabine based sequential conditioning followed by RIC-TBF. As a standard, we have used clofarabine at the dose of 40mg/m^2/day from days -13 to -9 (5 doses), alongside cytarabine 2000 mg/m^2/day. Once the disease burden was reduced, thiotepa 5 mg/Kg/day on days -5 and -4, fludarabine 50 mg/m^2 on days -3 and -2, and busulfan 3.2mg/Kg on day -2 were administered. Only one patient received fludarabine instead of clofarabine for burden mass reducing. As GvHD prophylaxis we combined PTCy 50 mg/Kg/day on days +3 and +4 with tacrolimus in all cases, adding sirolimus in one patient, or mycophenolate mofetil in other two cases. Given the clinical situation, and because all patients lacked a suitable HLA matched donor, an haploidentical donor was selected with a median age of 36 years old (16-62) and a sex ratio male-female of 60%>40%. From our N of 15 patients (median age 39 yrs (6-66), male/female 7/8), the underlying conditions were AML 46.6%, ALL 33.3% and MDS 20%, with disease status pre-transplant of refractoriness in 73.3%, 13.3% relapsed and 13.3% progressed. Sorror HCT -CI scoring was 40%, 40% and 20% for 0, 1 and ≥3 respectively. Disease scoring risk of high/very high (86.7%).
Results: With a median follow up among survivor of 19.3 months an estimated OS and RFS at 13 months of 58.2% and 43.6% respectively. A CR at day +90 was achieved in 80% of the cases (12). In two patients, the test could not be assessed due to early death at days +48 and +57. Four patients (26.7%) relapsed at a median period time of 6 months (range 2-9) and 2 of these died. All together (6 patients) died while 4 of these cases were non leukaemia related. Transplant related mortality of the entire cohort was 26,7%. We had 53.3% of grade ≥3 aGvHD with a median onset at D+55 and cGvHD (at least moderate) in 3 cases (20%) with a median onset of D+302.
Summary/Conclusion: With promising results, even though presenting a small N of 15 patients, a significant reduced disease burden and limited toxicity due to RIC with thiotepa T cell replete haplo SCT, allows to show feasibility with significant OS, RFS rates and a good quality of life (QoL) for patients with a presumed very poor outcome. There are still several challenging goals to achieve, that we need to work on, especially on considering to reduce the incidence of GvHD without incrementing the relapse rate, thus, overall means to improve a QoL, which would be the ultimate target.

PB2433

THE USE OF THE ANTI-EMETIC AKYNZEO FOR AUTOLOGOUS STEM CELL TRANSPLANT PATIENTS RECEIVING HIGH DOSE MELPHALAN : RESULTS OF PILOT STUDY SHOW REDUCTION IN EMESIS, NAUSEA AND LENGTH OF HOSPITAL STAY

R. Hall[1,*], R. Brooks[1], L. Hammond[1], R. Hudson[2]

[1]Haematology, [2]Pharmacy, The Royal Bournemouth Hospital, Bournemouth, United Kingdom

Background: High dose Melphalan is standard conditioning chemotherapy for patients with Myeloma and Lymphoma for autologous stem cell transplant (ASCT). This is a highly emetic regimen and despite the use of the 5-HT3 receptor antagonists, nausea and vomiting continues to be a problem. During 2016 we noticed a high use of subcutaneous infusion of levopromethazine in this patient group. This anti-emetic can result in patient drowsiness, potential injection site infection and demands on nursing time. We performed a pilot study using the new antiemetic Akynzeo (netupitant and palonosetron hydrochloride 300mg/0.5mg) given prior to high dose Melphalan.
Aims: The study aimed to reduce the incidence of nausea and vomiting associated with high dose melphalan chemotherapy using a novel antiemetic regimen. We hope to demonstrate a resultant improvement in nutritional intake and a reduction in length of hospital admission.

Table 1.

Average number days	nausea overall	nausea grade 1	nausea grade 2	vomiting grade1	reduced nutritional intake	sc levo pump use	sc pump inflammation	ondansetron use	metaclopramide use	length hospital admission
standard antiemetic regimen	4.9	2.85	2.9	2.6	8.5	8.33	0.83	17.21	14.13	19.83
Akynzeo antiemetic regimen	0.79	1.5	0.08	0.08	1.84	0	0	0.83	12.69	17.67

Methods: From 29/12/15 to 4/1/17, 24 consecutive patients were treated with high dose Melphalan as part of an ASCT protocol (22 Myeloma patients and 2 lymphoma patients). The first 12 patients were treated using our standard anti-emetic protocol of regular Ondansetron 8mg bd and metaclopromide 10mg tds from D1 chemotherapy. In addition as required doses and continuous sc infusions of Levopromethazine were used as needed. The subsequent 12 patients were given a novel anti-emetic protocol of Akynzeo single dose on D1 chemo with dexamethasone 12mg orally D1 and 8mg orally D2-4 as per Akynzeo product specification. No other regular anti-emetics were used during the ASCT admission period. As required doses of metaclopromide and as required doses and continuous sc infusions of Levopromethazine were utilised. Retrospective analysis was made of all 24 patients. Factors analysed were: number of days of nausea and vomiting documented in medical notes with CTCAE grade. Number of days of reduced nutritional intake, all antiemetic drugs used, use of subcutaneous infusion Levopromethazine, duration of inpatient admission. Results from the 2 cohorts of patients were compared. Anti-emetic drug costs and nursing time required were also considered.
Results: There was a significant reduction seen in days of recorded nausea, vomiting, poor nutritional intake, subcutaneous levopromethazine pump usage and overall length of hospital stay in the pilot study group receiving the novel anti-emetic Akynzeo (see table).
Summary/Conclusion: The anti-emetic regimen of single dose Akynzeo with dexamethasone resulted in a reduction in nausea and vomiting. Cessation of subcutaneous levopromethazine infusion usage and a reduction in length of hospital admission was seen in the Akynzeo group. The Akynzeo anti-emetic regimen drug cost was higher (average £78 *vs* £24 in the standard regimen group) but the saving in length of hospital stay, nursing time and positive patient experience is significant. We now use the Akynzeo regimen as our anti-emetic protocol for all ASCT conditioning regimens.

PB2434

EVALUATION OF THE MEASUREMENT ERROR IN THE CALCULATION OF CHIMERISM FROM THE RATIO OF TANDEM REPEATS (STR) CONTAINING STUTTER PEAKS

U. Chabaeva[1,*], N. Risinskaya[2], N. Kostritsa[2], A. Sudarikov[2], S. Kulikov[1]

[1]Department of biostatistics, [2]Lab of Molecular Hematology, National Research Center for Hematology, Moscow, Russian Federation (Russia), Moscow, Russian Federation

Background: Chimerism analysis plays a key role in the detection of bone marrow engraftment after allogeneic bone marrow transplantation (BMT). The analysis of short tandem repeats (STR) loci from the donor and the recipient DNA and calculation of individual STR markers percentage is one of routine test for chimerism monitoring. One of most often complications are the stutter PCR peaks appearance due to polymerase slippage during amplification of target STRs. The stutter peak may concur with an informative recipient`s marker hindering chimerism estimation based on that locus. This problem seems to be especially serious in case of a sex-matched sibling bone marrow transplantation. Thereby, the absence of "stutter-peaks free" markers hinders mixed chimerism estimation at the point of low recipient hematopoiesis output. Previously we demonstrated the possibility of the universal formulas correction for the chimerism calculation excluding stutter percentage.
Aims: Detection of low levels of mixed chimerism is possible if only recipient peak is greater then expected donor stutter peak plus some threshold because of error of measurement. The aim of this work was to evaluate the portions of variation of peak level caused by person (donor), locus and noise of measurements and then to calculate the threshold according given type II error of stutter-complicated chimerism detection.
Methods: Data includes measurements of stutter peaks (SP) percentage in 10 loci in DNA samples of 23 donor/recipient sets. In each donor/patient set first measurement was done for donor then further stutter percentage was calculated on the DNA samples of patients with verified complete donor chimerism in 6 up to 14 time points. Additionally we made measurements in parallel probes – 8 aliquots for 8 donors in 7 loci in the most recent time point samples. The UNIVARIATE and GLM SAS procedures were used to calculate simple statistics and run analysis of variance.
Results: Relative levels of stutter peaks by locus types for 23 donor/patient sets is shown on Fig.1, multiple points means repeated measurements. The analysis of variance shows that the main sources of variation are the person (F=5.4, p<.0001) and locus(F=12.8, p<.0001) but not time (number) of measurement (F=0.77, p=0.6). We also compared the variance of repeated measurement of SP of donor/patient set and variance in aliquots of last patient's measurements. At that case the analysis of variance shows that the main sources of variation are the person (F=49.45, p<.0001) and locus type (F=45.16, p<.0001) but not aliquots (number of the parallel probes) (F=1.93, p=0.08). The deviations in repeated measurement and in aliquots are close (0.73 *vs* 0.54) and we can regard them as measurement errors. Then we estimate the distribution of errors, for this we take regression residuals (RR) after fitting by donors and locus types. The mean of RR is zero by definition, the standard deviation is 0.73, 95% percentile is 0.86. The threshold for chimerism detection with 5% of type II error can be calculated by formula: Th5=SP+0.86, where SP is value of stutter peak percentage of donor.

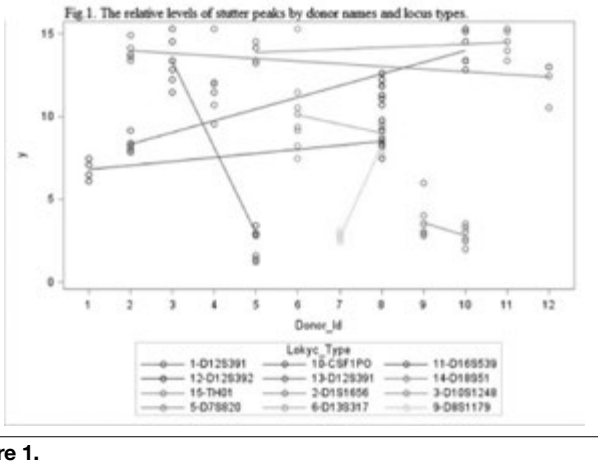


Figure 1.

Summary/Conclusion: We recognized that the stutter peaks are variated mostly by locus type and by person but rather stable in series of measurements in different time points and in several aliquots for the same locus. That means that we can construct the individual chimerism detection rule using donor SP and measurement error which we have evaluated.

PB2435

THE EXPERIENCE USING OF ELTROMBOPAG FOR TREATMENT OF THROMBOCYTOPENIA AFTER ALLOGENEIC HEMATOPOETIC STEM CELL TRANSPLANTATION IN PEDIATRIC PATIENTS

I. Sasmaz[1,2,*], B. Antmen[2], B. Karagun[2], M. Serbest[2]

[1]Pediatric Hematology, Cukurova University, [2]Department of Pediatric Hematology, Bone Marrow Transplantation Unit, Adana Acıbadem Hospital, Adana, Turkey

Background: Thrombocytopenia after allogeneic hematopoetic cell transplantation is a common complication. The underlying causes are often multifactorial in this patient population including impaired platelet production, increased platelet destruction and combination of these mechanisms. Eltrombopag is an oral thrombopoietin receptor agonist which interacts with the transmembrane domain of the receptor on bone marrow megakartocytes and upstream progenitor stem cells. Eltrombopag use has been suggested chronic idiopathic thrombocytopenic purpura and aplastic anemia. There are few reports eltrombopag treatment after allogeneic hematopoetic cell transplantation.
Aims: We aim to report our eltrombopag experiences with 7 pediatric patients treated with eltrombopag for severe thrombocytopenia after allogeneic hematopoetic cell transplantation at our center.
Methods: A total of 198 allogeneic hematopoetic cell transplantation were performed at Acıbadem Adana Hospital Pediatric Bone Marrow Transplantation Unit in Turkey from 2013 to 2017. Seven cases of 198 patients were treated eltrombopag for persistan thrombocytopenia after allogeneic hematopoetic cell transplantation. Bone marrow aspiration and biyopsy were performed in all patients before starting eltrombopag treatment. Medical records and treatment modalities were evaluated retrospectively.
Results: In this study, 7 patients were treated with eltrombopag, age ranging from 5 to 17 years with a average of 12 years. There were 3 patients with thalassemia major, 2 patients with acute lymphoblastic leukemia, one patients with aplastic anemia and one patient with myelodysplastic syndrome. Four patients were males, three were female. Five patients had HCT from an unrelated donor. Two patients had haploidentical transplantation from mother. Five patients had bone marrow transplantation,2 had peripheral blood stem cell transplantation. Eltrombopag was started in 6 patients with prolonged isolated thrombocytopenia and one patient with secondary failure of platelet recovery. The number of megacaryocytes was decreased in 6 patients and was within the normal range in one patient. Before starting eltrombopag treatment 7 patients were dependent on platelet transfusions weekly. Eltrombopag was started at a median 228 days after HSCT (range, 77 to 582 days) in patients with PIT. Eltrombopag were started at the dose of 25-50 mg once daily. Median duration for treatment was 177 days. No serious side effects were seen. The incidence rate of successful platelet recovery to >50000/mikrolitre without transfusion support was 71% (5 of 7 patients).
Summary/Conclusion: Eltrombopag is currently available oral thrombopoietin receptor agonist. We showed that our experience using of eltrombopag is very effective with minimal side effects for persistent thrombocytopenia after allogeneic hematopoetic cell transplantation in pediatric patients who had depended on platelet transfusion.

PB2436

COMPARISON OF MYELOABLATIVE AND REDUCED-INTENSITY CONDITIONING REGIMENS FOR ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

R. Çiftçiler, M. Okay, E. Aladağ, Y. Büyükaşık, H. Göker, H. Demiroğlu*

[1]Hematology, Hacettepe Universty, ankara, Turkey

Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is an e ective therapy for a variety of malignant and non-malignant hematologic disorders. Myeloablative conditioning (MAC) and reduced-intensity conditioning (RIC) regimens may have different clinical outcomes. Several investigators have reported a dose-response relationship between the pretransplant conditioning regimen and long-term outcome after allogeneic hematopoietic transplantation in acute leukemia. MAC regimens are associated with a reduced risk of relapse after HSCT, but it's doubtful. That it translates into prolongation of overall survival due to the increased treatment-related and

nonrelapse mortality. In many studies, RIC has been shown to have similar overall survival (OS) but higher relapse rates compared with MAC regimens in patients with myeloid malignancies undergoing allogeneic HSCT.
Aims: To evaluate long-term outcome of MAC *versus* RIC regimens in patients with acute myeloid leukemia (AML) patients undergoing allogeneic HSCT.
Methods: This study is a retrospective single-center analysis. We retrospectively compared the long term outcome with MAC and RIC regimens in patients who underwent allo-HSCT for AML at Hacettepe University between 2001 and 2017.
Results: We analyzed the survival outcomes after MAC-HSCT *versus* RIC-HSCT among 107 adult patients with AML diagnosed from 2001 through 2017. Of these, 44 patients received a MAC regimen and 63 patients received a fludarabine-based RIC regimen in AML. Median follow-up of 95 months (range, 7-213) for the RIC-HCT group and 34 months (range, 6-153) for the MAC-HCT group. The 6-month estimated survival was found to be higher in RIC patients as compared to MAC patients (93% *versus* 82%), but no statistically significant difference was observed (p: 0,5). The 3-year estimated survival for RIC and MAC patients were 67% and 60%, respectively. In multivariate analysis, the type of conditioning regimen (RIC *vs* MAC) did not influence the progression free survival (p: 0,24). Five of the RIC patients and 9 of the MAC patients developed acute graft *versus* host disease (GvHD). Sixteen of the RIC patients and 6 of the MAC patients developed chronic GvHD. There was no significant difference between two groups in terms of acute and chronic GvHD (p:0,089).

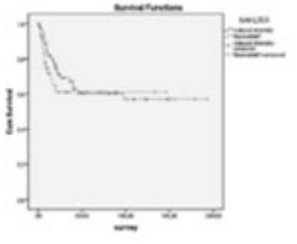


Figure 1.

Summary/Conclusion: This retrospective analysis confirmed that MAC and RIC regimens had a consistently equivalent rate of overall and disease free survival in AML patients with allogeneic HSCT.

PB2437

RELAPSE AFTER ALLOGENIC STEM CELL TRANSPLANT. CAN AZACITIDINE PROLONG SURVIVAL? EXPERIENCE IN OUR CENTER

I.G. García García*, A. Chinea Rodríguez, F. Martín Moro, B.M. Michael Fernández, K. Velázquez Kennedy, C. Martínez Geijo, A. Sanz Ruipérez, J. Marquet Palomares, F.J. López Jiménez

Hematology, Hospital Universitario Ramón y Cajal, Madrid, Spain

Background: Relapse after allogeneic stem cell transplantation (post-allo-SCT) is the main cause of treatment failure. Currently, the incidence of relapse in patients with acute myeloid leukemia (AML) is between 30-40%. The mean overall survival is 3 to 4 months and the overall survival at two years is less than 20%.
Aims: The aim of this study was to define the benefits of Azacitidine (AZA) in relapsed patients post-allo-SCT, looking for an association between the post-relapse overall survival and the response rate to AZA. We also examined FBC values (increase in hemoglobin, neutrophil or platelet counts), cytogenetic abnormalities present at relapse, and variations in chimerism.
Methods: This is a retrospective single-center study, which analyzed 14 patients who received AZA from January 2013 to January 2018 at a dose of 75mg / m². The cytogenetic risk groups were established according to the European Leukemia Net classification. We used the Kaplan-Meier statistical analysis to estimate the postrelapse overall survival, and we analyzed the impact with the Log-Rank and Breslow tests.
Results: The patient's characteristics are shown in Table 1. The mean age was 54 (±12) years. The mean time to relapse post-transplant was 7.4 (±4.9) months. The mean treatment time with AZA was 4 (±2.5) months, with an average of 4 (±2) cycles, and a mean cycle length of 6 (±1) days per cycle. A decrease in the blast percentage occurred in 4 patients, one of them reached a complete remission. We found a statistically significant association ($p<0.05$) between the treatment response measured by blast count decrease, and the overall survival (graph 1). The average duration of the response was 12 months. One of these 4 patients presented a hematological response with an increase in the hemoglobin level, another showed an increase in platelet count, and a third presented an increase in both hemoglobin and in the neutrophil count. Two patients presented a cytogenetic response with post-AZA negativization of high-risk genetic markers: Del (5q) and del (7q) in one patient, and inv (3) (q21q26) in the other. Changes in chimerism occurred in four patients, two of them achieved a complete chimerism. The median overall survival was 7 (3-14) months. However, even patients who presented a hematological response, a decrease in the percentage of blasts, normalization of cytogenetic abnormalities or who achieved a complete chimerism, lost those responses with the progression of the underlying disease. Table1.

Table 1.

Characteristics		N	%	Results postAZA		N	%
Sex	Female	9	64	Blast	Descent without RC	3	21
	Male	5	36		Descent with RC	1	7
					Progressión	10	72
Cytogenetics in AML	Good	0	0	Cytogenetic response	Yes	2	14
	Intermediate	1	7		No	12	86
	Adverse	13	93				
Conditioning	Haploidentical	10	72	Change chimerism	Yes	4	29
	MAC	2	14		No	10	71
	RIC	2	14				
AZA indication	Frank relapse	12	86				
	EMR+	1	2				
	Genetic marker+	1	2				
Early relapse (≤6 months)	Yes	8	57				
	No	6	43				

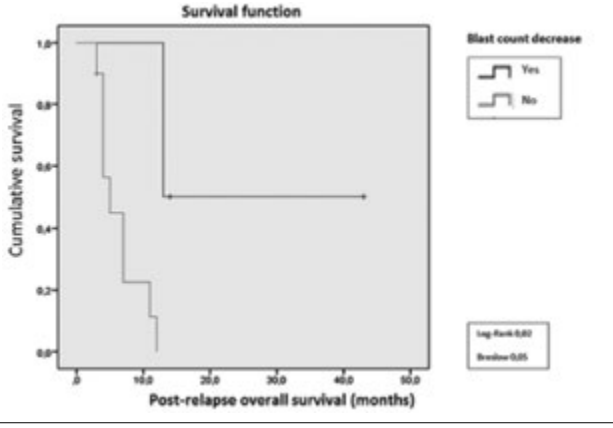


Figure 1.

Summary/Conclusion: AZA is a therapeutic option in post allo-SCT relapsed patients. In our study, the median overall survival was 7 (3-14) months, which is longer than expected in this group of patients. In addition, AZA can modify characteristics of the disease, such as genetic markers of poor prognosis and chimerism. Althought many patients present an initial response, this is then lost with disease progression. The development of new therapeutic strategies is needed in this cohort of patients, to offer a better prognosis in post allo-SCT relapse.

PB2438

IMPACT OF A MULTI-FACETED INTERVENTION BUNDLE ON THE COLONIZATION RATES OF MULTIDRUG RESISTANT BACTERIA IN HAEMATO-ONCOLOGY PATIENTS

N. Denny[1,*], N. Martinez-Cibrian[1], R.J. Shorten[2], E. Tholouli[1], F. Dignan[1], A. Dodgson[3], M. Saif[1]

[1]Department of Haematology, Manchester University Foundation Trust, [2]Public Health Laboratory, Public Health England, [3]Department of Microbiology, Manchester University Foundation Trust, Manchester, United Kingdom

Background: The growing threat of colonization by multidrug resistant organisms (MDRO) presents a particular challenge to haemato-oncology patients receiving chemotherapy or undergoing a haematopoietic stem cell transplantation (HSCT) as they are heavily reliant on antimicrobial agents to prevent and treat overwhelming infection. MDRO include carbapenem producing Enterobacteriaceae (CPE) and vancomycin-resistant Enterococci (VRE) and both can colonise the bowel. Haemato-oncology patients often develop prolonged neutropenia and gastrointestinal mucositis which creates the perfect means for translocation of these MDRO and can lead to systemic infection. There are few studies examining the impact of multi-faceted infec-

tion prevention and control bundles on MDRO colonisation and infection in haemato-oncology patients.
Aims: To assess the impact of the introduction of a bundle of infection prevention and control measures on CPE and VRE colonisation and infection rates in haemato-oncology patients.
Methods: Data on MDRO colonisation and infection of consecutive patients receiving treatment in our haematology ward between April 2012 and December 2017 was collected. An intervention consisting of 15 infection prevention and control measures, such as patient and staff cohorting and the use of a vapourised hydrogen peroxide system was introduced in November 2014. Patients were divided into pre and post intervention cohorts and the incidence of new CPE and VRE colonisation compared. Secondary outcomes include the incidence of MDRO infection and hand hygiene compliance. Statistical analysis was carried out using SPSS version 22 and Mann Whitney U test was used to compare colonisation and infection incidence between pre and post intervention periods.
Results: During the 69 month period of the study, there were 41,594 patient days; 20,768 in the pre-intervention period and 20,826 in the post intervention period. Monthly acquisition of CPE and VRE colonisation for the two study periods is shown in figure 1. The total number of CPE colonisation acquisitions over the whole study period was 105; 82 in the pre-intervention period and 23 in the post intervention period. The incidence density of CPE colonisation acquisition in the pre-intervention period was 3.95 cases per 1000 patient days compared to 1.1 cases per 1000 patient days in the post intervention period (p=0.0001). The total number of VRE colonisation acquisitions over the study period was 279; 157 in the pre-intervention period and 122 in the post-intervention period. The incidence density of VRE colonisation acquisition in the pre-intervention period was 7.6 cases per 1000 patient days compared to 5.9 cases per 1000 patient days in the post intervention period (p=0.12).

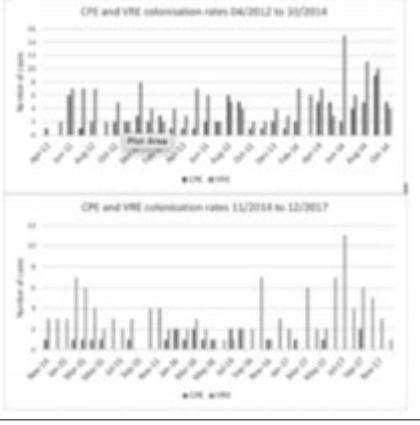


Figure 1.

Summary/Conclusion: The implementation of a multi-faceted bundle of infection prevention and control measures led to a significant reduction in new colonisation with CPE in our haemato-oncology cohort. In contrast, the incidence density of new VRE colonisation showed no statistically significant decrease. This may reflect differences between CPE and VRE in mode of transmission and susceptibility to particular infection control measures. Since the main source of MDRO infection in this cohort is thought to be mucositis related, we expect the significant reduction in CPE colonisation to be matched by a similar reduction in CPE infection. This emphasises the importance of rigorous infection prevention and control measures in protecting our patients in the age of the superbug.

PB2439

OVERALL SURVIVAL AND NON-RELAPSE MORTALITY OF PATIENTS WITH CHRONIC GRAFT VERSUS HOST DISEASE: A SYSTEMATIC LITERATURE REVIEW

T. Ágh[1], M. Csanádi[1], B. Elezbawy[1], T. Webb[2,*], M. Mahler[3], N. Sengupta[3], A. Tordai[4]

[1]Syreon Research Institute, Budapest, Hungary, [2]Janssen Research & Development, High Wycombe, United Kingdom, [3]Janssen Research and Development, Raritan, NJ, United States, [4]Institute of Pathophysiology, Semmelweis University, Budapest, Hungary

Background: Chronic graft-versus-host disease (cGVHD) is a serious and life-threatening complication of allogeneic hematopoietic stem cell transplantation (HSCT) affecting 30% to 70% of patients. It is characterized by systemic inflammation and fibrosis with a heterogeneous presentations affecting a wide range of tissues and organs. cGVHD is the leading cause of non-relapse mortality (NRM) following HSCT, also contributing to morbidity and a decrease in quality of life.
Aims: To perform a systematic literature review of evidence (1) on overall survival (OS) and NRM among cGVHD patients and (2) on the effect of cGVHD on OS and NRM compared to patients without cGVHD.
Methods: A systematic review of English-language articles was conducted in PubMed. Literature search was limited to studies that applied the National Institute of Health (NIH) Consensus Criteria for the diagnosis and staging of cGVHD, published between 2007 and 2017, studied human subjects with a cohort size of at least 100 patients. Included studies were assessed for quality with a NIH quality assessment tool. NRM and OS values measured among cGVHD patients were extracted from the articles. In the evaluation of cGVHD-effect on OR and NRM we extracted data only from those analyses that applied a multivariate study design.
Results: From the screened 1682 publications, 38 studies were included. From the onset of cGVHD, the 1 and 2 year NRM rates ranged from 10% to 26%, and from 10% to 32%, respectively. The 1 and 2 year OS rates from the onset of cGVHD ranged from 66% to 75% and from 59% to 81%, respectively. Studies found that patients with cGVHD had better OS (range of hazard ratio [RoHR]: 0.09 - 0.38), but increased NRM (RoHR: 2.4 - 4.8) compared to patients without cGVHD. Studies where cGVHD was included as a time-dependent covariate in the multivariate analysis found that the improved OS was primarily due to differences in relapse rate (RR). These results showed that patients with cGVHD had significantly lower RR than patients without cGVHD: no cGVHD *vs* classic cGVHD, HR: 0.46; and no cGVHD *vs* mild / moderate / severe cGVHD, RoHR: 0.12 - 0.26. However, higher NRM was observed in several sub-groups of cGVHD patients when they were compared to those without cGVHD: cGVHD after acute GVHD, HR: 4.83; *de novo* cGVHD, HR: 3.52; classic cGVHD, HR: 2.4; and overlap cGVHD, HR: 2.5. Furthermore, higher cGVHD severity was reported to be associated with worse NRM (severe *vs* mild/moderate, RoHR: 3.04 - 3.07). Four studies found a significant association between OS and cGVHD severity and 3 indicated better OS in case of lower cGVHD severity. Overlap GVHD was associated with worse OS (HR: 2.1) and higher NRM (HR: 2.8) compared with classic cGVHD.
Summary/Conclusion: Evidence suggests that the improved OS in cGVHD patients is due to lower risk of relapse, which can be explained with the presence of a potent graft *versus* tumor (GVT) effect. However, NRM was found to be higher in patients with cGVHD and studies indicated an increased risk in the presence of more severe disease. A delicate balance is thought to exist between cGVHD severity as a source of NRM and a favorable effect on decreased RR in the presence of less severe cGVHD. Considering the importance of the GVT effect and higher risk of NRM associated with more severe disease, there exists an unmet medical need for novel approaches to ameliorate the severity of cGVHD whilst preserving the benefits of the GVT effect.

PB2440

IN MULTIPLE MYELOMA, AFTER CTX-BASED PBSC MOBILIZATION, THE USE OF HIGH DOSE G-CSF (10 MCG/KG) IS MORE EFFECTIVE IN RESPECT TO LOW DOSE G-CSF(5 MCG/KG) BUT ONLY IF PLERIXAFOR ON DEMAND IS NOT USED

S. Leotta[1], A. Cupri[1], A. Spadaro[1], G. Sapienza[1], L. Scalise[1], M.G. Michieli[2], G. Milone[1,*]

[1]Hematology, BMT UNIT, Catania, [2]Oncology, BMT UNIT, Aviano, Italy

Background: The use of algorithms to administer PLX has increased the success rate of the PBSC mobilization treatments. However also the increase of the dose of G-CSF from 5 mcg/Kg to 10 mcg/Kg could be important to the end of harvesting an optimal amount of CD34+ cells. It is not known if "high dose G-CSF" and "on demand PLX" show a synergistic effect so that their combined used may be of additive usefulness or, on the contrary, if these two measures do not have a synergy.
Aims: Determine, in Multiple myeloma patients mobilized with cyclophosphamide 2-4 gr/sqm, if the use of high dose G-CSF (10 mcg/Kg) is more effective than lower dose (5 mcg/Kg) and if the possible increase of effectiveness of G-CSF high dose is influenced by the concomitant use of on demand Plerixafor.
Methods: 404 patients were retrospectively studied, all were affected by MM and were in first mobilization attempt. All 404 patients received CTX at 2-4gr/sqm, 180/404 patients were enrolled in on demand PLX (PLX GROUP) and 224/404 were not (NO PLX GROUP). In NO PLX GROUP

112 patients received G-CSF at 10mcg/Kg and 68 received it at dose of 5mcg/Kg. In PLX GROUP 153 received G-CSF at dose of 10mcg/Kg and 71 at dose of 5mcg/Kg.
Results: In NO PLX GROUP, percentage of patients failing to harvest the minimum CD34+ of $2x10^6$/Kg was 19% in patients receiving 5mcg/Kg *versus* 8.8% in patients receiving G-CSF at 10mcg/Kg (p=0.04). Patients receiving G-CSF at dose of 5mcg/Kg reached a harvest $>6x10^6$ CD34+ cells in 42% of cases while this outcome was reached in 72% of patients receiving G-CSF at dose of 10mcg/Kg (p=0.0001). Mean harvested CD34+ cells was $6.3x10^6$/Kg *versus* $13.0x10^6$/Kg (p=0.0001). In PLX GROUP, patients failing the minimum CD34 harvest ($2x10^6$/Kg) was 5.0% and 1.7% in the two groups treated, respectively, with 5 mcg/Kg and in 10mcg/Kg (p=0.08) the optimal threshold of $6x10^6$/Kg was reached In 67% of those receiving G-CSF at 5mcg/Kg and in 73% of patients treated with G-CSF at 10 mcg/Kg (p=not significant). The mean number of harvested CD34+ cells was $10.4x10^6$/Kg *versus* $11.2x10^6$/Kg, respectively in 5mcg/Kg and in 10mcg/Kg groups (p=NS). Thus, high dose G-CSF seems more advantageous when PLX on demand is not used while its efficacy is less evident when PLX on demand is programmed. We therefore wished to study the existence of an interaction between these two factors. In ANOVA test the amount of CD34 cells harvested was used as dependent variable and "dose of G-CSF" and "to be enrolled in a PLX ON DEMAND study" were evaluated. Interaction between the two factors was significant (F=14.1; p=0.0002).
Summary/Conclusion: After CTX based mobilization, a dose of G-CSF of 10 mcg/Kg allows to reach an optimal amount of CD34+ cells ($>6x10^6$/Kg) more frequently in respect to the dose of 5 mcg/Kg. However, this hold true only when PLX is not used. In fact, in patients enrolled in PLX studies, there is no evidence that a higher dose of G-CSF determines further improvement in respect of that obtained with the use of the on demand PLX. Thus, we can conclude that if PLX on demand is used there is no need to increase dose of G-CSF.

PB2441

Abstract withdrawn.

PB2442

PATIENTS NON-COMPLIANCE LEADS TO HIGHER RISK OF ACUTE GVHD

E. Mikhaltsova[1,*], M. Drokov[1], L. Kuzmina[1], O. Pokrovskaya[1], V. Vasilyeva[1], N. Popova[1], D. Dubnyak[1], O. Koroleva[1], A. Sidorova[1], E. Usikova[1], S. Khrushchev[2], Z. Konova[1], A. Dmitrova[1], D. Vybornykh[2], E. Parovichnikova[1], V. Savchenko[1]

[1]BMT department, [2]Laboratory studying Mental and Neurological Disorders in Patients with Hematological Diseases Moscow, National Research Center for Hematology, Moscow, Russian Federation

Background: Non-effective medical therapy is like a "worldwide problem of striking magnitude". Sometimes ineffective medical therapy closely relates with non-compliance with medical recommendations. First 3-4 months after allogeneic stem cell transplantation (allo-HSCT) are crucial for acute graft *versus* host disease (GVHD) developing in patients after allo-HSCT. Here we report the data about an impact of patient non-compliance on aGVHD incidence after allo-HSCT.
Aims: to assess the impact of patient non-compliance with medical recommendations on aGVHD incidence.

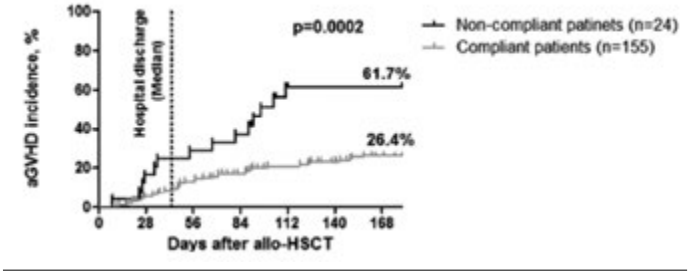


Figure 1.

Methods: We analyzed the data of 179 patients who underwent allo-HSCT between 2009-2017 in National Research Center for Hematology. All patients had an acute leukemia (59- ALL and 120 – AML) and were in complete remission (CR) before allo-HSCT. Physician expert opinion (based on physician-patient interactions and/or clinical interview with mental health specialist) was used to assess patient non-complaince with medical recommendations. Exact Fisher's test was used to analyze the 2 × 2 contingency tables. Kaplan–Meier analysis with log-rank test was used for aGVHD probability analysis and group comparison. A p-value less than 0.05 was considered as significant.
Results: 13% patients were non-compliant with medical recommendations. The groups of compliant and non-compliant patients were absolutely comparable based on various characteristics (diagnosis, sex, age, disease status, transplant source, type of donor). According to our data aGVHD occurred in "non- compliant" group more frequently than in "compliant" group - 61.7% and 26.4% respectively (p=0.0002). All other factors that could affect aGVHD incidence (graft source, HLA-disparity, disease status) were balanced.
Summary/Conclusion: Patient non-compliance with medical recommendations is strongly associate with developing of aGVHD. We are convinced that patient non-compliance an reduce to zero previously conducted the best aGVHD prophylaxis. This study reinforces the importance of this problem for each transplant center and needs to be continued together with clinical psychologists and psychiatrists.

PB2443

DONOR AND RECIPIENT CCR5-DELTA 32, CCR2-64I, SDF1-3'A, IL6 -572 C/G POLYMORPHISMS AND TRANSPLANTATION OUTCOME

N. Risinskaya*, I. Fevraleva, U. Chabaeva, N. Petinati, S. Kulikov, A. Sudarikov, L. Kuzmina, E. Parovichnikova, V. Savchenko

National Research Center for Hematology, Moscow, Russian Federation

Background: Immunological tolerance after bone marrow transplantation (BMT) depends on complex interaction between graft and host cells and includes cytokine production and reception. Both host and graft cells may carry different polymorphic alleles in appropriate genes that may alter these processes. Possible impact of cytokine gene polymorphisms on the BMT outcome have not been thoroughly studied yet.
Aims: To measure possible correlation of overall survival, reconstitution time and level of GVHD with CCR5-delta 32 (rs333), CCR2-64I (rs1799864), SDF1-3'A (rs1801157), IL6 -572 C/G (rs1800796) polymorphisms in host and graft cells.

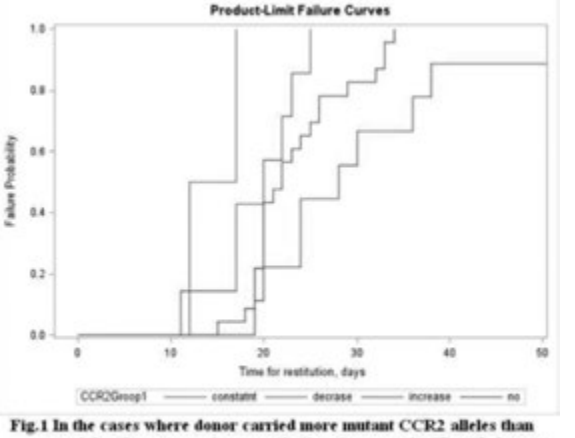


Fig.1 In the cases where donor carried more mutant CCR2 alleles than recipient hematopoesis reconstitution time was significantly longer than in other cases (p<0.0001)

Figure 1.

Methods: Forty three patients 18 to 64 years old (median age 32), 13 males and 30 females who received allogeneic hematopoietic transplantation in the National Research Center for Hematology were included in the study. Stem cell source was bone marrow in 14 cases and mobilized peripheral stem cells in 29 cases. 26 patients were diagnosed with AML, 9 with ALL, 3 with AA, 2 with MDS, 2 with CML and 1 with MPN/CLL. DNA of donors and recipients were analyzed for CCR5-delta 32(rs333), CCR2-64I(rs1799864), SDF1-3'A(rs1801157), IL6 -572 C/G (rs1800796) polymorphisms by means of allele-specific PCR. Genetic data were correlated with overall survival, reconstitution time, frequency of acute or chronic GVHD after BMT. Infectious complications where analyzed at days 0-30 and at days 30-120 after transplantation. SAS software was used for statistical analysis. Kaplan-Meier estimates, Log-Rank test and chi-square were used for group comparison.

Results: Patients were divided according to each polimorphysm issue into 4 groups: 1/ no mutant alleles in both recipient and donor; 2/ equal number of mutant alleles in recipient and donor; 3/ donor has less mutant alleles than recipient (0 *vs* 1, 0 *vs* 2, 1 *vs* 2); 4/ donor has more mutant alleles than recipient. All groups were equal in graft source, diagnosis, disease stage, gender and age. No correlations of most polymorphic alleles with overall survival, reconstitution time, frequency of a GVHD and infectious complications at day 30 were found. In the cases where donor carried more mutant CCR2 alleles than recipient hematopoesis reconstitution time was significantly longer than in other cases (p<0.0001) (Fig 1). Infectious complications were also more frequent at day 30-120 (p<0,0016): 10 of 11 patients who received transplant from a donor carrying more mutant CCR2 alleles than recipient (91%) *vs* 8 of 32 patients in other groups (22-50%).
Summary/Conclusion: Cytokine polymorphic alleles of donors and recipients may alter bone marrow transplantation outcome. Decreased reconstitution rate and increased levels of infectious complications could be registed in patients who received transplant from donors carrying mutant CCR2 alleles. More patient samples and extended cytokine gene panels are required for further studies.

PB2444

CONDITIONING REGIMENS FOR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN LYMPHOMA: COMPARISON BEAM / BEAC-BUCY-GEMBUMEL

E. Colombo*, S. Jarchum, E. Mas, M. Alvarez, M. Balseiro, D. Minoldo, M. Lavarda, M. Rizzi, G. Jarchum

Sanatorio Allende, Cordoba, Argentina

Background: Hematopoietic progenitor cell transplantation (HSCT) represents a potentially curative therapy for certain hematological diseases. Improving conditioning regimen (CR)efficacy and safety is the major variable for results and is an important area of research. Also the world shortage and expense of carmustine has promoted the use of alternative conditioning regimens to the classical BEAM/CBV. Recently at MD Anderson Cancer Center, Dr. Nieto demonstrated that GEMBUMEL improves PFS and OS in patients with HL R/R. GEMBUMEL combines the alkylating activity with the inhibition of DNA repair, producing an additive or synergistic effect. We present here a comparative study of the CR: BUCY, BEAM-BEAC and GEMBUMEL in patients (P) with diagnosis of Lymphoma subjected to autologous HSCT.
Aims: To evaluate the safety and efficacy of these regimens in our experience.
Methods: Retrospective, observational and descriptive work. Patients with diagnosis of Hodgkin's Lymphoma (HL) and non-Hodgkin's Lymphoma (NHL) undergoing autologous HSCT in the 2010-2017 period were included. CR were (all the medication was done intravenous): BEAC (Carmustine 300mg/m^2, Etoposide 1200mg/m^2, Cytarabine 800mg/m^2 Cyclophosphamide 140mg/kg IV) BEAM (Carmustine 300mg/m^2, Etoposide 800mg/m^2, Cytarabine 1600mg/m^2, Melphalan 140mg/m^2) BUCY (Busulfan 12.8mg/kg Cyclophosphamide 120mg/kg) GEMBUMEL (Busulfan 420mg/m^2, Melphalan 120mg/m^2, Gemcitabine 5500mg/m^2). Safety was analyzed assessing: toxicity (according to CTCAE), haematological recovery and transfusional requirement. Progression Free Survival (PFS) and Overall Survival (OS) was evaluated for the efficacy analysis. Descriptive statistics were used for the numerical variables, ANOVA for the categorical ones, Chi-Square test for comparison of proportions and the analysis of PFS and OS by Kaplan Meier curves. A p <0.05 was considered statistically significant.
Results: We analyzed 70 P, with the following distribution according to CR: 1-BUCY (n=16), 2-BEAM / BEAC (n=35) and 3-GEMBUMEL (n=19). Only a statistically significant difference was observed in the relationship between men and women, within their baseline. When grade III-IV of Toxicity was evaluated between the groups, there were statistically significant with p <0.001, mucositis (50% *vs* 54.2% *vs* 100%), hepatitis (0% *vs* 0% *vs* 73.7%) and dermatitis (0% *vs* 0% 36.8%), with no difference in transplantation related mortality. When Efficacy was compared, neutrophil recovery was achieved with a mean of 13, 13.8 and 11 days (p 0.169) and platelets 33, 20 and 18 days (p 0.008), and there were no differences in transfusion requirements. The 12-month PFS was 75, 80 and 78.95% and the OS at 12 months was 87, 94.2 and 94.7%, with no significant statistical differences. The comparison according to disease (HL *vs* NHL) also showed no difference in terms of OS.
Summary/Conclusion: GEMBUMEL CR presented more toxicity, evidencing a higher incidence of mucositis, hepatitis and dermatitis, while BUCY showed late recovery of platelets. There were no differences in PFS or OS between the CR.

PB2445

CLINICAL RESPONSE TO SYSTEMIC TREATMENTS FOR STEROID-REFRACTORY CHRONIC GRAFT VERSUS HOST DISEASE: A SYSTEMATIC LITERATURE REVIEW

T. Ágh[1], M. Csanádi[1], B. Elezbawy[1], T. Webb[2,*], M. Mahler[3], N. Sengupta[3], Z. Vokó[1]

[1]Syreon Research Institute, Budapest, Hungary, [2]Janssen Research & Development, High Wycombe, United Kingdom, [3]Janssen Research and Development, Raritan, NJ, United States

Background: Chronic graft-versus-host disease (cGVHD) is a serious complication of allogeneic hematopoietic stem cell transplantation affecting 30% to 70% of patients. It is the leading cause of late non-relapse mortality, contributes to morbidity and impairs quality of life of transplant patients. Corticosteroids are standard first-line therapy for cGVHD, but around 50% of patients do not respond adequately. Evidence on the comparative efficacy of second-line treatments is limited, partly because, until the 2005 National Institutes of Health (NIH) Consensus Conference (revised in 2014) no uniform criteria for the diagnosis of cGVHD and response were available.
Aims: To systematically review the available clinical trials to investigate the efficacy of systemic treatments on response in patients with steroid-refractory (SR) cGVHD.
Methods: A systematic review of English-language publications was performed using Scopus and Cochrane Central Register of Controlled Trials databases. Literature was searched from 2005 to October 2017. The review was limited to phase 2 to 4 clinical trials that used the NIH Consensus Criteria for diagnosing cGVHD and determining response, and included >25 patients per study arm. Data on response were extracted from the included trials. Overall response rate [ORR] was defined as: (number of patients with complete response [CR] + number of patients with partial response [PR]) / total number of patients treated.
Results: Four phase 2, single arm trials were included. These investigated the efficacy of ibrutinib (n=1), interleukin-2 (IL-2; n=1), imatinib (n=1), and rituximab (n=1). Most trials failed to report response at a fixed time point and reported best response at any time during the follow-up, thus CR and PR data presented below are maximum response rates. At median follow-up of 13.9 months, CR with ibrutinib was 21% and PR was 45% (ORR: 67%; 28/42 patients); 71% (20/28 patients) and 48% (12/25 patients) responders showed a sustained response of ≥20 and ≥32 weeks, respectively. With ibrutinib, 56% of patients with ≥2 involved organs showed a response in ≥2 organs, and 42% of patients with ≥3 involved organs showed a response in ≥3 organs. During 1 year follow-up, CR with rituximab was 22% and PR was 65% (ORR: 87%; 32/37 patients); 21 patients maintained their response for 1 year. Response rates with rituximab were higher in the musculoskeletal system (ORR: 100%), skin (ORR: 77%) and oral cavity (ORR: 71%) than those in the liver (ORR: 44%), eyes (ORR: 43%), gut (ORR: 33%), and lungs (ORR: 9%). Patients treated with IL-2 or imatinib had no CR. By week 12, 61% of patients (20/33 patients) treated with IL-2 had PR; response sites included skin (PR: 27%), joint/fascia (PR: 17%), lung (PR: 15%), liver (PR: 13%), and gastrointestinal tract (PR: 10%). During 6 months, PR with imatinib was 51% (20/39 patients); the best response rates were observed in the gut (PR: 50%), lungs (PR: 35%) and skin (PR: 32%). The definitions of SR cGVHD varied in the included clinical trials.
Summary/Conclusion: Findings suggest that patients with SR cGVHD treated with ibrutinib and rituximab can reach CR. However, none of the included clinical trials reported comparative data demonstrating superior efficacy in response for one particular agent over others. Heterogeneity in the definition of SR cGVHD and follow-up time did not allow direct comparison of results. Further controlled clinical trials with larger cohorts are needed.

PB2446

PATIENTS HAPLOTYPE, DONORS KIR ALLELE AND POSTHSCT EVOLUTION

D. Bratu[1,*], A. Moise[2], I. Constantinescu[3]

[1]Haematology, [2]Department of Immunogenetics and Virusology, Fundeni Clinical Institute, [3]Department of Immunogenetics and Virusology, Fundeni Clinical Institute, Bucharest, Romania

Background: Some new consideration about haplotype of patients with acute leukemia and their binding with genoidentical donors KIR allele in HSCT try to explain few aspects in postHSCT evolution.
Aims: Some new consideration about haplotype of patients with acute leukemia and their binding with genoidentical donors KIR allele in HSCT try to explain few aspects in postHSCT evolution.

Methods: Patients with acute leukemia,lymphoblastic and non-lymphoblastic,aplastic anemia and their genoidentical donors are evaluated(eighteen paars). Haplotype HLA sistematisation follow IPD/ KIR : HLA- A3/A11, HLA-Bw4, HLA-Bw6(Bw4 negative) HLA-C1/C1 homozygot, HLA-C1/C2 heterozygot, HLA-C2/C2 homozygot.

Table 1.

Ligand	Activatory allele	Inhibitory allele
HLA A3/A11	KIR3DL2	KIR2DS4
HLA Bw4	KIR3DL1	KIR3DS1
HLA C1	KIR2DL2, KIR2DL3	KIR2DS4
HLA C2	KIR2DL1	KIR2DS1

The source of HSCT was PBSC.The method used was PCR-SSP (Innotrain DIAGNOSTIK GMBH, Dynal BIOTECH PEL-FREEZE) The complications like graft *versus* host disease acute and chronic, relapse, TMA and the recovery with leucocytes and thrombocytes are followed.
Results: HLA-Bw6 haplotype(7) with 85,71% overall survival, HLA-A3/A11(15) with 53,33%, HLA-C2/Cx(17) with 50.00% and presence of all these haplotype at same patient(6) with 83,33% overall survival demonstrate the protective effect and cumulative effect of some categories of HLA haplotype. Donors KIR haplotype are AB, protective ,evident effect at patients with KIR haplotype AA(4:2 AML,1ALL,1AA) but also with favorable HLA. Patients with HLA-Bw6, HLA-C2/C2,HLA-A3/A11(6) don't develop aGVHD, relapse, TMA, a single patient with HLA-Bw4 develop aGVHD; positive effect also about leucocyte and trombocyte recovery,especially in the presence of actiatory allele (sig, 0,5%). HLA-Bw6 haplotype (Bw4 negative) with donor inhibitory KIR3DL1, confirm missing ligand theory, (83% OS, sig<0,05); with donor activatory KIR3DS1,OS is 100% (sig<0,05).
Summary/Conclusion: Cumulative effect of some patient HLA haplotype with donor KIR haplotype (also patient KIR haplotype)improve overall survival and protect again most complication after HSCT.

PB2447

IMPACT OF PROCALCITONIN IN THE DIFFERENTIAL DIAGNOSIS OF FEBRILE COMPLICATIONS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

S. Butera[1,2], A. Evangelista[3], L. Giaccone[1,2], C. Dellacasa[2], D. Faraci[1,2], L. Brunello[1,2], G. Ciccone[3], G. Mengozzi[4], A. Busca[2], B. Bruno[1,2,*]

[1]Department of Molecular Biotechnology and Health Sciences, University of Torino, [2]Department of Oncology, SSD Trapianto Allogenico di Cellule Staminali, [3]Clinical Epidemiology, [4]Department of Clinical Chemistry, SC Baldi Riberi, A.O.U Città della Salute e della Scienza di Torino, Torino, Italy

Background: Serum procalcitonin (PCT) has been proposed as a promising diagnostic biomarker for severe bacterial infections in patients with febrile episodes following allogeneic haematopoietic stem cell transplantation (allo-HSCT). A reliable biomarker may be particularly helpful during neutropenic fever.
Aims: The purpose of this study was to determine the potential role of PCT in the differential diagnosis of febrile complications in allo-HSCT recipients.
Methods: Febrile episodes and PCT values were retrospectively reviewed in 553 consecutive allografts performed at our Centre, from January 2007 to December 2016. Biomarkers (PCT and C reactive protein) were assessed in correspondence of the febrile event. Fever work-up also included complete blood counts, haemocultures and chest X-ray. Other imaging studies and/or bronchoalveolar lavage were performed as clinically indicated. A total of 3430 determinations of PCT on 355 patients were analysed. Donors were HLA-identical siblings (no.101); haploidentical (no.58); or unrelated (no.209). Association between PCT and clinical outcomes were evaluated by generalized linear model with Gamma distribution and link-log.
Results: Frequency of PCT determinations was higher in the first 36 days after transplantation, with a median at +16 days (IQR: 8-36). Overall 210 PCT determinations (6,1%) were performed at the onset and within the first 48 hours of a documented bacterial infection. PCT values were significantly higher in patients with documented gram negative bacteraemia (mean 8.85 ng/ml) compared to patients with no documented infections, with a mean difference of PCT levels of 6.93 ng/ml (95%CI: 1.62, 12.24; p<0.01). PCT showed a promising diagnostic accuracy for Gram negative infections, as compared with inflammatory fever or Gram positive proven bacteraemia. PCT showed a high negative predictive value for bacteraemia regardless of Gram stain, ranging from 97% at cut off value of 2 ng/ml to 96.9% at a cut off value of 0.5 ng/ml. PCT levels superior to 2 ng/ml can be considered specific of bacteraemia with a specificity of 89,5% for gram positive bacteraemia and of 89.7% for gram negative proven infection. PCT elevations were not significant in patients with documented invasive fungal infections defined by EORTC/MSG criteria (p=0.618), and in patients developing cytokine release syndrome (CRS) after haplo-identical allografts (p=0.207). A mean difference of PCT levels of 6.37 ng/ml was found if anti-thymocyte globulin (ATG) treatment was included as graft-versus-host disease prophylaxis, compared to patients that did not receive ATG. (95%CI: 2.67-10.06; p<0.001). Multi organ failure was significantly associated with an elevation of PCT values with a mean rise of 3.27 ng/ml in the last 96 hours before death. (95%CI: 1.35, 5.18; p<0.001).
Summary/Conclusion: Despite the limitations of the retrospective design of our study and of the low prevalence of proven bacteraemia in correspondence of a PCT determination in our population, PCT may be considered a helpful tool in the differential diagnosis of febrile complications after allo-HSCT. Our findings form the basis for the prospective assessment (in the contest of a clinical trial) of a diagnostic algorithm and scoring system that include PCT determination to evaluate risk of gram negative sepsis and of early mortality in febrile episodes after allografting.

PB2448

SUFU RS17114808 (C>T) POLYMORPHISM DOES NOT INFLUENCE THE OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ADULTS

N. Meggyesi[1,*], P. Kovy[1], K. Balassa[2], L. Varga[3], A. Bors[1], A. Batai[4], E. Torbagyi[4], L. Gopcsa[4], L. Lengyel[4], A. Barta[4], A. Tordai[5], I. Valyi-Nagy[4], T. Masszi[6], H. Andrikovics[1], P. Remenyi[4]

[1]Laboratory of Molecular Genetics, Central Hospital of Southern Pest, National Institute of Hematology and Infectious Diseases, [2]School of PhD Studies, Semmelweis University, [3]Hungarian National Blood Transfusion Service, [4]Department of Hematology and Stem Cell Transplantation, Central Hospital of Southern Pest, National Institute of Hematology and Infectious Diseases, [5]Department of Pathophysiology, [63]rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary

Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the most effective treatment option for certain hematological malignancies. Suppressor of fused (SUFU) is a negative regulator of the hedgehog signaling pathway which is involved in cell proliferation and differentiation, and it interacts with the Glioma transcription factors. Recently, correlation has been shown between the SUFU polymorphism rs17114808 and the incidence of acute graft *versus* host disease (aGvHD).
Aims: The aim of this study was to investigate the role of SUFU rs17114808 (C>T) polymorphism in the outcome of HSCT.
Methods: We examined the association of recipient and donor SUFU rs17114808 C>T and allo-HSCT outcome in a cohort of 407 adult patients who underwent first allo-HSCT between January 2007 and December 2013 at our single center. 208 patients received stem cells from their siblings, 199 patients from matched unrelated donors (MUD). 227 patients was diagnosed with AML (n=155) or ALL (n=72) and 180 patients with other (MDS [n=31], CML [n=22], MPN [n=20], MM [n=25], CLL [n=25], NHL [39], HL [18]) diseases. In 260 cases myeloablative conditioning (MAC), while in 147 cases reduced intensity conditioning (RIC) was applied. For identification of SUFU rs17114808 from genomic DNA LightCycler melting curve analysis (LightCycler 480II, Roche Diagnostics) was performed.
Results: We did not find any association between recipients' SUFU rs17114808 C>T polymorphism and HSCT outcome on the whole cohort. In patients with SUFU rs17114808 CT or TT genotype, aGvHD grade II-IV occurred in 68% (67/99), compared to 32% (98/307) in patients with CC genotype (p=1). Relapse occurred in 29% (29/99) T carrier patients and in 25% (76/307) of patients with CC genotype (p=0.428). Similar findings were observed in case of SUFU rs17114808 C>T polymorphism in donors. The frequency of aGvHD was 29% (22/76) in case of T allele carrier donors and 32% (107/336) in patients with wild type (CC) donor (p=0.682). Donors' SUFU genotype did not influence the relapse rate (22% [17/76] in patients with donors bearing at least one T variant *vs* 27% [91/336] for wild type donors). Overall survival did not differ in subgroups with different genotypes of recipients and donors. Based on tests for interaction, subgroup analysis was performed according to diagnosis (acute leukemia *vs* other) and conditioning regimen (MAC *vs* RIC), but we did not find significant association in the subgroups.
Summary/Conclusion: In contrast with a previous study performed in pediatric HSCT patients, our findings suggest that SUFU rs17114808 C>T polymorphism in adult HSCT recipients and donors does not influence the outcome of HSCT.

PB2449

IMPACT OF AB0 INCOMPATIBILITY ON ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION OUTCOME: A SINGLE-CENTER RETROSPECTIVE STUDY

A. Busani, G. Saporiti, T. Zollner, E. Tagliaferri, F. Grifoni, F.G. Rossi, C. Manera, N.S. Fracchiolla, A. Cortelezzi, F. Onida*

Ematologia - Centro Trapianti Midollo - Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico - University of Milan, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico - University of Milan, MILANO, Italy

Background: Hematopoietic stem cell transplantation (HSCT) is used to treat a range of hematological malignant and non-malignant conditions including autoimmune, metabolic and immunodeficiency diseases. Because major histocompatibility genes are inherited independently of blood group system genes, approximately 40-50% of all allogeneic HSCTs are performed across the AB0-blood group barrier. Due to the widespread expression of AB0 antigens on a variety of human tissues other than erythrocytes, AB0 incompatibility may have an impact on the outcome of allogeneic HSCT that goes beyond the well-known immune-hematological complications such as immediate hemolysis due to the presence of isoagglutinins and delayed hemolysis due to passenger B lymphocytes.
Aims: With this retrospective study, we aimed to assess the impact of AB0 mismatch on allogeneic HSCT outcomes, including non-relapse mortality, overall and relapse-free survival, post-transplant PRC transfusion requirement, as well as relapse rate and incidence of graft- failure and acute GvHD.
Methods: Retrospectively collected data from 169 consecutive patients undergoing allogeneic HSCT between 01/2008 and 10/2017 at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico Hospital in Milan, Italy, were analyzed. Kaplan Meier estimates were used for survival outcomes while cumulative incidences were analyzed by competing risk analysis.
Results: The patient series included 98 AB0-identical, 29 major incompatible, 32 minor AB0-mismatched and 10 bidirectionally incompatible transplants. Mean overall survival for groups of patients undergoing AB0-identical, major AB0 mismatch and minor AB0 mismatch HSCT were 66 months (95% CI [55 ;77]), 47 months (95% CI [28; 65) and 46 months (95% CI [31; 61]), respectively. Non-relapse mortality in the three groups were significantly different by Gray's test with point estimates of 12%, 29% and 26% at 5 years, respectively, whereas no significant differences were observed for relapse rate and graft failure incidence. Although not statistically different, incidence of acute grade III-IV GvHD was twice as high in patients transplanted from minor AB0-mismatched donors than in the AB0 identical group (16% *vs* 8%). Following transplantation, PRC transfusion requirement was significantly higher in the major AB0 mismatch then in the AB0-identical transplanted patients (median 14 *vs* 7, p=0.01). Of note, a weak positive correlation was found between the anti-donor A/B IgG titers measured prior HSCT and the total number of RBC transfusions administered one year after HSCT. One case of PRCA occurred in one 0+ 50-year-old woman who received peripheral blood-derived hematopoietic stem cells from an A+ HLA-identical 32-year-old male sibling after myeloablative conditioning for AML in first complete remission. Anti-A IgG isoagglutinin titers prior to transplantation were 1:256. The patient received a total of 46 RBC products in the year following transplantation and resolution occurred during danazole treatment.
Summary/Conclusion: In our patient cohort, both major and minor AB0 mismatching were associated with a significantly higher NRM. Major ABO mismatching associated to higher PRC transfusion requirement. Possible more frequent occurrence of severe acute GvHD is also suggested in minor AB0-mismatched transplants.

PB2450

AUTOLOGOUS HEMATOPOIETIC CELL TRANSPLANTATION FOR HIV-RELATED LYMPHOMA: A SINGLE CENTER MATCHED CASE-CONTROL STUDY

M.O. Popova*, Y. Rogacheva, A. Nekrasova, I. Tsygankov, K. Lepik, O. Pirogova, E. Darskaya, Y. Zalylov, L. Stelmah, I. Moiseev, S. Bondarenko, N. Mikhaylova, B. Afanasyev

Raisa Gorbacheva Memorial Institute of Children Oncology, Hematology and Transplantation, First Pavlov State Medical University of Saint-Petersburg, Saint-Petersburg, Russian Federation

Background: Human immunodeficiency virus (HIV) infection is associated with an increased incidence of non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL). Throughout the HAART era, autologous stem cell transplantation (ASCT) has been reported as a feasible approach to either rescue or consolidate HIV-related lymphoma patients. However, the number of published comparative studies according to the HIV status is limited.
Aims: An observational prospective study was designed to assess the safety and tolerability of intensive chemotherapy and ASCT for the treatment of HIV-related NHL and HL.
Methods: Since the Jan 2016 nine patients with HIV-related lymphoma who have undergone ASCT were included in prospective singe centre study (study group - HIV group, n=9). The data of the non-HIV-infected patients with lymphoma who have undergone ASCT at the same period of time (control group, n=36) were collected to compare the efficacy and safety of the procedure (1:4). Median follow up time was 12 (2-20) months. The primary end points were overall survival (OS) and relapse rate at 12 months after ASCT. Secondary end points were time to hematopoietic recovery and organ toxicity. Common Terminology Criteria for Adverse Events (CTCAE 4.0) for the toxicity analyse have been used. The underlying diseases in HIV-group were HL n=6 (67%) and NHL n=3 (33%). Conditioning regimen was BEAM with BCNU replacement by Bendamustine 160 mg/m2/day at D-7, D-6. HIV viral load was undetectable (100%); the median number of CD4+ cells was 265 cells/mcl; all patients were on HAART. An assessment of potential differences in patients, disease and treatment characteristics between the 2 groups showed no significant differences.
Results: Overall survival (OS) at 12 months for all patients (n=45) was 93,3%. OS at 12 months was 88,9% in HIV-group *vs* 94,4% (p=0,3) in control group. One patient died later on engraftment in HIV group (cerebral hemorrhage) and two in control group within 1.5 years after ASCT (lymphoma progression). Relapse rate of the underlying disease at 12 months was 14% in HIV-group, in control group – 11% (p=0,8). The median time of leukocytes, neutrophils, and platelets recovery was D+13, D+15, D+15 respectively in HIV-group and D+13, D+16, D+14 in control group. There were no differences in rate of organ (Nephrotoxicity, Hepatotoxicity, Enteropathy and Mucositis) toxicity according to CTCAE.
Summary/Conclusion: One-year overall survival in patients with HIV-related lymphoma was 88,9%, relapse rate – 14% and did not differ from the control group. There were no found significant differences between two groups in hematopoietic recovery and toxicity rate. Preliminary data provide further evidence that HIV status does not affect the outcome of ASCT for lymphoma. Patients with HIV-related lymphoma should be considered candidates for ASCT if they meet standard transplant criteria.

PB2451

IMPACT OF MIXED CHIMERISM IN T REGULATORY CELLS ON RELAPSE RATE IN ACUTE LEUKEMIA PATIENTS AFTER ALLO-HSCT

D. Dubnyak[1,*], N. Risinskaya[2], M. Drokov[1], N. Kostritsa[3], J. Davydova[4], L. Kuzmina[1], N. Kapranov[4], V. Vasilyeva[1], N. Popova[1], E. Mikhaltsova[1], O. Koroleva[1], A. Sidorova[1], E. Usikova[1], Z. Konova[1], I. Galtseva[4], A. Sudarikov[2], E. Parovichnikova[1], V. Savchenko[1]

[1]Bone marrow transplant, [2]Molecular biology laboratory, National Research Center for Hematology, Moscow, Russian Federation, [3]School of Medicine, Lomonosov Moscow State University, [4]Immunophenotyping laboratory, National Research Center for Hematology, Moscow, Russian Federation, Moscow, Russian Federation

Background: It is known that T-regulatory cells (Treg) play an important role in maintaining tolerance after allo-HSCT. Tregs prevent the development of acute graft-versus-host disease (aGVHD) (Beres & Drobyski, 2013). At the same time these T-cell populations can limit the antitumor response (graft-versus-leukemia, GVL) and that limitation may cause relapses. Here we report the analysis of mixed chimerism in T-reg population and the frequency of relapse in patients with acute leukemia (ALL and AML) after allo-HSCT with comparable levels of donor chimerism.
Aims: To evaluate a possible relationship between the mixed chimerism in Treg cells and the rate of relapses in acute leukemia patients after allo-HSCT.
Methods: The study included 31 patients after allo-HSCT (ALL n=6, AML n=25). The median age was 38 years (19-66). Peripheral blood samples for analysis were taken on day 30 after transplantation. Immunomagnetic separation (Miltenyi Biotec, Germany) was used to isolate population with CD4+CD25+ phenotype which is predominantly associated with Treg cells. Extraction of DNA was performed from the obtained cells. Chimerism in DNA samples was determined using the STR-PCR method. The percentage of donor chimerism was calculated using standard procedures (Nollet *et al.*, 2001) Statistical analysis of the data was carried out using SPSS ver 23. (IBM, Chicago, Ill., USA). Exact Fisher's test was used to analyze the 2x2 contingency tables.
Results: In the patients group with less than 11% of cells with host genotype (more than 89% of cells of donor origin) the relapse rate was significantly

higher - 52.6% (10 of 19) than in the other group of patients with 11% and more cells with host genotype - 8.3% (1 of 12), (p=0.02). At the same time donor chimerism in the unselected bone marrow did not significantly differ between the groups (p=0.36) and amounted to 100% (75-100%) and 97.5% (90-100%). The study groups were balanced for all other factors that could affect the relapse rate: disease status, graft source, GVHD.

Table 1.

Cells with host genotype	Relapse	No relapse	p-value
<11%	52.6% (n=10)	47.4% (n=9)	0.02*
≥11%	8.3% (n=1)	91.7% (n=11)	

Table 1. Relapse rate depending number of Treg cells with host genotype

Summary/Conclusion: According to our data we proposed that host's Treg cells are not capable of suppressing GVL that explains significant differences in the frequency of relapses in patients with acute leukemia after allo-HSCT. Predominance of host's Treg cells may serve as a favorable prognostic sign but this hypothesis needs to be confirmed in the largest cohort of patients.

PB2452

HAPLO-IDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) IN HIGH-RISK HEMATOLOGICAL MALIGNANCIES WITH COMBINATION OF UNMANIPULATED BONE MARROW AND PERIPHERAL BLOOD STEM CELLS

D. Ait Ouali*, M. Benakli, F. Mehdid, N. Rahmoune, M. Baazizi, S. Zerkout, K. Tarhi, H. Bouarab, R. Belhadj, F. Harieche, R.-M. Hamladji, R. Ahmed Nacer

Hematology and Bone Marrow Transplantation, Pierre and Marie Curie Center, Algiers, Algeria

Background: The haplo-identical HSCT is currently a rescue procedure in patients (pts) with high risk hematological malignancies when identical HLA donor is not available. This procedure without T-depletion appears to allow rapid immune reconstitution and reduced incidence of graft-versus-host disease (GVHD) and good survivals.
Aims: We propose a retrospective study of 35 pts who benefited from this procedure.
Methods: From May 2013 to January 2017, 36 haplo-identical HSCT were used in 35 pts with hematological malignancies (7 AML, 19 ALL, 5 CML in blast crisis, 1 lymphoblastic NHL, 2 AL biphenotypic, 1 MDS). Median age was 24 years (5-55) and sex-ratio (M/F):2.8. The diagnosis-transplant delay is 32 months (6-138). At the time of the transplant, 21 pts were in second complete remission and 5 pts in active disease. The donors used were parent (father: 17, mother: 3) or siblings (brother: 11, sister: 4) or offsprings (daughter 1). The median age for donors is 39 years (13 -65). The degree of compatibility (HLA A, B and DR) is 3/6 (24 cases), 4/6 (10 cases) and 5/6 (2 cases). CMV status between donor/recipient was high risk in 35 cases. The ABO incompatibility is major in 5 cases, minor in 4 cases. The conditioning regimen associated Busilvex 9.6 mg/kg; Aracytine 8 g/m^2; Cyclophosphamide 3.6 g/m^2 for all pts. The GVHD prophylaxis included the combination Ciclosporin-Methotrexate, Mycophenolate mofetil and Thymoglobulin (10 mg/kg) in 35 pts. Thirty-five pts received an unmanipulated bone marrow (BM) transplant and Peripheral blood stem cells (PBSC) with a median dose infused CD34+ cells: 8.89 10^6/kg (1.43-32), mononuclear cells: 7.67.10^8/kg (0.59-19.2), CD3+ cells: 2.99 10^8/kg (0.04-14.2), CD4+ cells: 1.62 10^8/kg (0.02-7.53), CD8+ cells: 1.46 10^8/kg (0.36-7.53). At September 2017, the minimal follow-up delay was 8 months and maximal 52 months.
Results: Aplasia was observed in all pts with median duration of 20 days (13-32). The median day of neutrophils engraftment was 13 days (11-27). No cases of VOD were observed. One pt presented an early rejection and benefited from a second haplo-identical transplant with another donor. Acute GVHD occurred in 17 pts (48%) including 14 (40%) grade II-IV. Chronic GVHD was seen in 11 pts (39%) with extensive form in 4 pts. Seventeen pts (43%) showed CMV reactivation on average at day 44 (35-67). Eight cases of haemorrhagic cystitis (22%) (one grade 4) are observed on average at day 47 (26-119). Nine pts (25%) relapsed, of which 5 pts were blast crisis at the time of the transplant. After follow-up of 15 months (8-52), 19 pts (54%) are alive and 16 pts (45%) died within 10 pts (28%) from TRM (GVHA digestive: 3, severe infection: 5, haemorrhagic cystitis: 1, TRALI syndrome: 1) and 6 pts from relapse. The overall survival (OS) and disease free survival (DFS) are 51.5% and 41.6% respectively.
Summary/Conclusion: Haplo-identical allograft is, currently, a well-validated procedure in pts with high-risk haematological malignancies who do not have sibling HLA donor as our results show. However GVHD, CMV reactivation and TRM are relatively high with this procedure using combination of primed BM and PBSC.

PB2453

IMPACT OF IFN LAMBA 3/4 SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) ON THE CYTOMEGALOVIRUS (CMV) REACTIVATION IN AUTOLOGOUS STEM CELL TRANSPLANT (AUTO-SCT) PATIENTS

O. Annibali[1,*], E. Riva[2], L. Piccioni[3], V. Tomarchio[1], E. Circhetta[1], C. Sarlo[1], L. Franeschini[4], M. Cantonetti[4], M. Rizzo[4], S. Angeletti[5], G. Avvisati[1]

[1]University Campus Bio-Medico, Hematology stem cell transplantation, [2]University Campus Bio Medico,Virology Unit, [3]University Campus Bio Medico,Virology Unit, [4]University Tor vergata, Hematology, [5]University Campus Bio Medico,[4]Laboratory of Pathology and Microbiology, Rome, Italy

Background: CMV infection represents one of the main cause mortality after SCT. Recently, a protective effect of the T allele of rs12979860 IL-28B SNP against CMV infection in the allogenic stem cell transplantation was suggested. We investigate whether the rs12979860 and rs368234815 (IFNl4) SNPs might affect the incidence of active CMV infection in Autologous stem cell transplantation (Auto-SCT) setting.CMV infection represents one of the main cause mortality after SCT. Recently, a protective effect of the T allele of rs12979860 IL-28B SNP against CMV infection in the allogenic stem cell transplantation was suggested.
Aims: We investigate whether the rs12979860 and rs368234815 (IFNl4) SNPs might affect the incidence of active CMV infection in Autologous stem cell transplantation (Auto-SCT) setting.
Methods: The study included 99 patients who underwent to Auto-SCT. IL28 and IFNl4 SNPs were correlated with CMV reactivation along with other clinical and treatment parameters. CMV reactivation by CMV DNAemia was evaluated once a week until day 100 from Auto-SCT.
Results: CMV reactivation was documented in 50% (TT-DG/DG), 35% (CC-TT/TT) and 29.2% (CT-TT/DG) of the patients respectively. No differences in CMV copies number were recorded at reactivation between different IL28/IFNl4 genotypes. The analysis of patients older than 60 years showed a significantly higher incidence of active CMV infection in the TT-DG/DG (83%) population with respect to CC-TT/TT (21%) and CT-TT/DG (40%) patients.
Summary/Conclusion: Our data suggest a negative role of TT-DG/DG genotype in the CMV reactivation in Auto-SCT. The exposure to rituximab and the pre infusion presence of anti CMV IgG also significantly influenced CMV reactivation.

PB2454

BUSULFAN VS MELPHALAN IN REDUCED INTENSITY CONDITIONING ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION FOR HIGH RISK MDS/AML: OUTCOMES OF A SINGLE UK CENTRE

N. Borges*, L. Duncan, E. Hurst

Northern Centre for Cancer Care, Newcastle upon Tyne, United Kingdom

Background: Reduced intensity conditioning (RIC) allogeneic haematopoietic stem cell transplantation (allo-HSCT) is an effective treatment for high risk MDS and AML, but the optimal conditioning regimen remains debatable. FluMel (Fludarabine 150mg/m^2, melphalan 140mg/m^2) replaced FluBu (Fludarabine 150mg/m^2, Busulfan 9.6mg/kg IV) as the standard RIC regimen for myeloid malignancy in our centre due to perceived elevated transplant-related mortality. All RIC transplants are T-cell depleted with standard doses of alemtuzumab.
Aims: To determine whether the use of busulfan or melphalan with fludarabine in RIC allo-HSCT for MDS/AML had a significant impact on outcomes in our centre.
Methods: Outcomes pertaining to consecutive MDS/AML patients transplanted with fludarabine and either busulfan (FluBu) or melphalan (FluMel) based RIC over a 2-year period in a single centre were retrospectively analysed, including demographics, Karnofsky performance status (KPS), engraftment, graft-versus-host disease (GVHD), and mortality. Statistical analysis was conducted using Student's t-test and Fisher's exact test.
Results: Between May 2015 and May 2017, 44 patients underwent RIC allo-HSCT for MDS/AML. Twenty-two (50%) patients received FluBu and 22 patients received FluMel. In the FluBu group, 9 (41%) patients were male, with an average age of 56 years (range 31-71) and KPS range of 90-100. Ten

(45%) patients died, 5 (50%) of which due to relapse and the remainder (50%) due to infection, within an average of 255 days from transplant (range 37-608). In the FluMel group, 13 (59%) patients were male, with an average age of 57 years (range 40-69), and KPS range of 80-100. Seven (32%) patients died, 2 (29%) of which due to relapse, and the remainder (71%) due to infection, within an average of 198 days from transplant (range 44-428). Average time to engraftment was 15.1 and 15.5 days in the FluBu and FluMel groups, respectively. Eleven (50%) patients in the FluBu group and 9 (41%) in the FluMel group developed GVHD (grades II-IV). There was no significant difference in survival or non-relapse mortality (NRM) between both groups (p=0.5365 and p=0.6221, respectively).
Summary/Conclusion: In a uniform MDS/AML cohort, we have demonstrated no significant difference in outcomes between T-cell depleted fludarabine/busulfan and fludarabine/melphalan RIC allo-HSCT. However, more studies are needed with larger samples and longer follow-up times.

PB2455

RELATIONSHIP BETWEEN THE CONTENT OF CD34 + CELLS AND THEIR COLONY-FORMING CAPACITY IN PATIENTS WITH MULTIPLE MYELOMA IN THE PROCESS OF PREPARING A LEUKAPERESIS PRODUCT FOR AUTOLOGOUS TRANSPLANTATION

J. Chubukina[1,*], V. Balashova[2], V. Rugal[3], N. Semenova[3], I. Kostroma[2], S. Gritsaev[4], T. Glazanova[1], L. Bubnova[1], S. Voloshin[2], S. Bessmeltsev[2]

[1]Laboratory Of Immunohematology, [2]Hematological Department, [3]Laboratory For Study Of Leukemia, [4]Department Of Bone Marrow Transplantation, Russian Research Institute Of Hematology And Transfusiology, Saint-Petesburg, Russian Federation

Background: Restoration of normal hematopoiesis after autologous transplantation of peripheral hematopoietic stem cells (PHSC) depends on a number of factors, including the colony-forming capacity (CFC) of CD34+ cells. The number of colony forming units (CFU) per unit volume of the test material is a characteristic of the functional activity of the product harvested for transplantation.
Aims: To study the correlation between the number of CD34+ cells and the number of CFUs in cell culture in the same samples of the peripheral blood leukapheresis product before and after cryopreservation in patients with multiple myeloma (MM). vity of the product harvested for transplantation.
Methods: Samples of peripheral blood apheresis product before and after cryopreservation in 32 patients with MM who underwent autotransplantation of PHSC have been studied. CFU was studied by culturing cells in a semi-solid culture medium MethoCultH4435 based on methylcellulose. The CD34+ cell count was determined by a standard flow cytometry method. Both indicators were calculated for 10,000 nuclear cells.
Results: In MM patients, the number of CD34+ cells in the apheresis product prior to cryopreservation was on average 1697,0±206,1x10^5 (230,0-4710,0). After thawing of the cellular suspension, the number of CD34+ cells increased almost 1.5-fold and amounted to an average 2473,0±294,7x10^5 (255,0-7500,0). The number of CFUs in the cell culture prior to cryopreservation was on average 396,6±21,5x10^5 (146,0-636,0 colonies). After the transplant was thawed, the number of CFUs decreased to 238,3±19,4x10^5, which is 1,7-fold less then prior to cryopreservation. Comparison of the number of CD34+ cells and CFU in the transplant prior to cryopreservation showed that the number of CD34+ cells was more than 4 times higher than the number of CFUs and, therefore, only about 25% of CD34+ cells were capable to form the colonies. After cryopreservation, there was a significant decrease in CFC of PHSC, whereas the number of CD34+ cells increased in comparison with baseline (due to granulocyte destruction), and was 10 times higher than the number of CFUs. At the same time, the correlation between CD34+ cells and CFU in culture in MM patients both before and after cryopreservation was absent; there was a great variability in these parameters. A correlation was found only between the number of CD34+ cells and CFU-GM before cryopreservation (r=+0,302, p<0,05).
Summary/Conclusion: Thus, to evaluate the effectiveness of cell mobilization and transplant quality, it is expedient to simultaneously use two indicators characterizing PHSC – the content of CD34+ and the colony-forming capacity. Probably, the further study of the immunophenotypic characteristics of CD34+ cells, reflecting the degree of differentiation of PHSC, will help to explain the differences in their functional activity.

PB2456

VINORELBINE FOR STEM CELL MOBILIZATON IN HODGKIN'S LYMPHOMA

A. Kuzyaeva[1,*], V. Shuvaev[2], J. Schubukina[2], M. Mikhaleva[2], A. Garifullin[1], A. Kuvshinov[2], A. Shmidt[2], A. Seltser[2], A. Chechetkin[2], S. Voloshin[2]

[1]Haematology, [2]Federal State Budget Institution Russian Scientific and Research Institute of Hematology and Transfusiology of Federal Medical Biological Agency, St-Petersburg, Russian Federation

Background: Currently high-dose chemotherapy with autologous hematopoietic stem cell transplantation is the standard of treatment for patients with Hodgkin's Lymphoma (HL) who has the first chemosensitive relapse of the disease. At our center, most of the patients with HL are mobilized PBSC when they achieve complete or partial remission after the first line of therapy to have adequate stem cells reserve in case of autologous transplantation. There are many studies devoted to the non-myelosuppressive regimens of mobilization with vinorelbine in patients with multiple myeloma, who showed its efficacy, low toxicity and reduced costs for the preparation of PBSC. Therefore, our center performed study for adaptation of this regimen in patients with other hematological malignancies, like HL. We present a comparison of mobilization regimens using vinorelbine at dose 35mg/m^2 and cyclophosphamide at dose 1.5g/m^2 in homogeneous groups of patients with HL in remission after the first line of chemotherapy.
Aims: To assess the efficacy of mobilization with vinorelbine in patients with HL.
Methods: We have analyzed the data of 18 patients with HL, with similar parameters of the median age and status of the disease, in the complete remission who received first line of therapy and underwent PBSC mobilization. Patients were divided into 2 groups according to the mobilization regimens: group 1 - vinorelbine (n=6), group 2 - cyclophosphamide (n=12). In group 1, the following scheme was used: Day 1 - vinorelbine 35mg/m^2, IV; from Day 4 - filgrastim 10 mg/kg, SC daily. In group 2: Day 1 - cyclophosphamide 1.5 mg/kg, IV; from Day 2 - filgrastim 10 mg/kg SC, daily. The first day of apheresis was initiated at $CD34^+$ cells level of more than 20/μL in peripheral blood.
Results: The median of $CD34^+$ cells in peripheral blood (PB) on the first day of harvest was: 193.5/μL in group 1 and 105/μL in group 2. On the first day of harvest, adequate number of PBSC (>2.0x10^6/kg) was achieved in 100% of patients in group 1 (range 2.4-7.1x10^6/kg, the median 4.85x10^6/kg) and 50% patients from group 2 (range 0.7-7.0x10^6/kg, the median 2.89x10^6/kg). There were need from 1 to 3 harvest (the median 1.5) in order to yield the minimum amount of CD34+ in group 2 patients. The length of hospital stay for patients in group 1 was 9-10 days (median 9), and 9 to 13 days (median 11) for group 2. Neutropenia grade 4 developed in 33% of patients in group 2 and was not noted in any patient in group 1. Significant differences were found between the harvests (p=0.04) and days in hospital (p=0.009) between groups 1 and 2. The number of $CD34^+$ cell/kg harvested in first day had tendency (p=0.06) favor to vinorelbine. There were no statistically significant differences between the groups of patients according to the level of $CD34^+$ cells in the PB on the first day of harvest, the number of $CD34^+$ cells/kg collected on the first day of harvest and the total number of collected $CD34^+$ cells/kg.
Summary/Conclusion: Our results demonstrate that vinorelbine for PBSC mobilization i has comparable efficacy with intermediate doses of cyclophosphamide in patients with HL. At the same time, less hospital days and the number of harvest makes this regimen more cost attractive. Continuation of study vinorelbine in mobilization in hematolgical patients will reduce the frequency of "waste harvest", the costs of collection PBSC and the risk of complications.

PB2457

HAPLOIDENTICAL STEM CELL TRANSPLANTATION IN HIGH RISK HEMATOLOGIC MALIGNANCIES: RISK FACTOR AND OUTCOME ANALYSES IN OUR CENTER

M. Perera Alvarez[1,*], M. Saez-Perdomo[2,3], C. Acosta Fleitas[2], C. Rodríguez Medina[1], E. González Pérez[1], L. Guerra[1], M. Torres Ochando[1], S. Jiménez Bravo de Laguna[1]

[1]Hematology, Hospital Universitario de Gran Canaria Dr. Negrín, [2]Hematology, Hospital Universitario de Gran Canaria Dr Negrín, [3]Universidad de las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain

Background: The allogenic stem cell transplantation (Allo-HSCT) is an efficient treatment for patients with high risk hematologic malignancies. Over the past decades new modalities of Allo-HSCT as Haploidentical stem cell transplantation (Haplo-HSCT) allowed us to offer this therapy to patients who for race reasons, presence of rare haplotypes or rapidly progressive disease course can't find a compatible unrelated donor.
Aims: The aim of this study is to assess the efficacy of Haplo-HSCT on our series evaluating the survival (S), overall mortality (OM) at 100 days and transplant related mortality (TRM).

Methods: We analyzed all the consecutive Haplo-HSCT performed in our center since 2013. The conditioning regimens were decided regarding age, comorbidities and pre-Haplo-HSCT disease status. All patients received high doses of Cy (50 mg/kg on days 3 and 4 posttransplantation) cyclosporine and MMF as CGvHD prophylaxis. We classified the patients before the Haplo-HSCT according to performance status (ECOG), comorbidities (Sorror), disease status (DRI and EBMT). We used SPSS V.23 to calculate the S and OM by the KM test and Chi squared tests.
Results: We performed 43 haplo-HSCT, 25 were males and 18 females with a mean age of 43 (range 16- 68). Diagnosis: Acute myeloid leukemia (24), Acute lymphoid leukemia (3), Hodgkin disease (65), Non Hodgkin lymphoma (2), severe aplastic anemia (2) Chronic lymphoproliferative disease (3). 23/43 patients died throughout the follow-up period with 31% OM at day +100 (fig. 1a). 15/23 patients died due to TRM. 7/8 of the remaining patients died due to disease progression and 5 out of these 7 patients had an active disease pre-Haplo-HSCT. For the analysis of the prognostic indexes we only observed a statistically significant difference by chi squared p=0.004 for TRM between patients with ECOG >2 (100% died due to TRM) and patients <2 (28.2% died due to TRM). Among the patients with acute leukemia (27/43) the overall mortality at day +100 was 28.5%. 16 out of the 27 deceased were in CR pre-Haplo-HSCT and 11/27 had an active disease. Comparing the survival of both groups by KM we observed 48% survival for the patients receiving the Haplo-HSCT with CR against 22% for the patients receiving Haplo-HSCT with active disease (figure 1b) at 12 months, which was mantained through the third year (p=0.1 not statistically significant due to the small sample size).

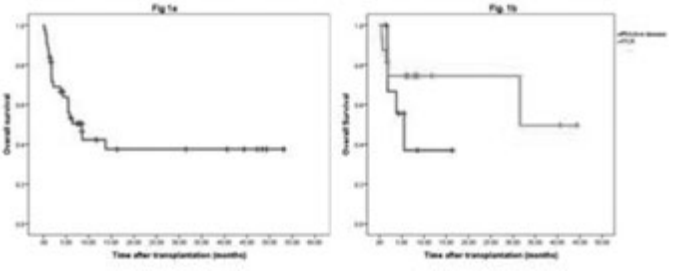


Figure 1.

Summary/Conclusion: Our results revealed ECOG >2 as an independent variable for TRM with statistical signification. Regarding acute leukemias, we were able to rescue 22% patients with refractory disease at 3 years, which agrees with the results previously published, and rises to 50% for patients receiving the Haplo-HSCT with CR.

PB2458

DURATION OF IMIPENEM-CILASTATIN AND/OR PIPERACILLIN TAZOBACTAM IS PREDICTIVE FOR ACUTE GRAFT VS HOST DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

N. Ben Abdeljelil*, W. Chanbeh, A. Barhoumi, L. Essakly, C. Ellouz, O. Ben Dhia, R. El Fatmi, A. Lakhal, D. Belloumi, L. Torjemane, S. Ladeb, T. Ben Othman

Centre National de Greffe de Moelle Osseuse de Tunis, Tunis, Tunisia

Background: Allogeneic stem cell transplantation (ASCT) is associated with a high risk for developing graft-versus-host disease (GVHD).
Aims: The aim of the study is to determine if duration of imipenem-cilastatin and/or piperacillin-tazobactam exposure is an independent risk factor for grade II-IV acute GVHD.
Methods: A retrospective study was conducted in adults patients who underwent ASCT from HLA-identical sibling donors between January 2014 and December 2016 to evaluate the impact of duration of imipenem-cilastatin or piperacillin-tazobactam exposure on grade II-IV acute GVHD. Patients with lymphoid malignancy were conditioned with fludarabine/busulfex/cyclophosphamide or TBI/etoposide. Myeloid malignancies were conditioned with busulfex/cyclophophamide. Patients with aplastic anemia received ATGAM/cyclophosphamide or fludarabine/thymoglobulin/cyclophosphamide regimens. Gut decontamination with polymixin E, gentamicin and amphotericin B was used in all recipients. Graft-versus-host disease prophylaxis consisted of cyclosporine and a short course of methotrexate. No patient received antibiotic prophylaxis. GVHD was confirmed by biopsy and classified according to Glucksberg criteria. Patients who did not receive imipenem-cilastatin or piperacillin-tazobatam or received them less than 24 hours were excluded. The odds ratios and 95% confidence intervals as determined by multivariate analysis for 7 variables (selected based on a P value <0.15 in univariate analysis).
Results: Seventy-nine patients underwent ASCT (47men and 32 women). Median age was 35 years (range, 18-56y). Diagnosis included AML (n=27, 34.2%), ALL (n=23, 29.1%), Aplastic anemia (n=18, 22.8%), other hematologic malignancies (n=11, 13.9%). Stem cell source were BM in 34 patients (43%) and PBSC in 45 patients (57%). Twenty-four patients received piperacillin-tazobactam only (30.4%, group 1), 23 patients received imipenem-cilastatin only (29.1%, group 2) and 32 patients received both (40.5%, group 3).The median duration of antibiotic exposure was 17days (range, 4-64 days). One patient experienced primary graft failure. Non-relapse mortality (NRM) was 17.4%. One patient had early NRM. Twenty-six patients (32.9%) developed grade II-IV acute GVHD at a median of 23 days (range, 14-100 d). The frequency of grade II-IV acute GVHD was comparable in the three groups (33.3%, 23.8% and 40.6% in groups 1, 2 and 3, respectively, p=0.45). Acute gut GVHD occurred in 16 patients (20.7%). Three-year overall survival was 66%. In univariate and multivariate analysis, the risk of acute grade II-IV GVHD increased significantly with duration of antibiotic exposure. Exposure for ≥25 days increases the risk by nearly six times (OR=5.8, 95% CI: 1.8- 18.68, p= 0.003).
Summary/Conclusion: Duration of broad-spectrum antibiotics exposure is associated with an increased risk of acute GVHD. Limiting the use of antibiotics post-ASCT may reduce the occurrence of acute GVHD.

PB2459

ESTIMATED ENERGY REQUIREMENT AND ACTUAL INTAKE IN PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION – A PROSPECTIVE STUDY FROM TERTIARY CARE CENTRE IN NORTH INDIA

K. Mishra*, A. Jandial, B. Barwal, D. Lad, G. Prakash, P. Malhotra, N. Varma, S. Varma, A. Khadwal

Pgimer,Chandigarh, Chandigarh, India

Background: Nutritional status is significantly compromised during the hematopoietic stem cell transplantation (HSCT), which not only postpones the period of engraftment but also plays a role in graft *versus* host disease (GVHD), veno-occlusive disease (VOD) and hospitalization in the ICU. Moreover, given conditioning regimen composed of high-dose chemotherapy or total body irradiation (TBI) triggering various side effects that disturb nutritional intake, such as stomatitis, nausea, vomiting, loss of appetite and diarrhea, it's a challenging task to maintain healthy nutritional status. However, less literature is available regarding nutrition status compromise (average amount and duration of inadequate intake of nutrients) in patients undergoing HSCT which hampers with planning during HSCT. The need for similar data is even more pertinent in developing countries where the average nutritional status of the citizen is poor.
Aims: To assess and compare Estimated Energy Requirement, dietary intake and weight changes from the day of the start of conditioning regimen to 14 days after transplantation or discharge.
Methods: This prospective observational study carried out in the Department of Internal Medicine in association with department of Dietetics PGIMER, Chandigarh. A total of 20 patients (12 auto and 8 allo) undergoing HSCT were included between 2016-2017. Body mass index (BMI) and Mid upper arm circumference (MUAC) was measured daily, and recording of all foods and fluid taken by patients was done using diet chart performa. Based on daily food consumption Calorie, protein, carbohydrate, and fat intake were calculated. The Estimated Energy Requirement (EER) for Maintenance is the dietary energy intake that is predicted to maintain energy balance in healthy individuals or groups of individuals at current levels of body size and level of physical activity. EER was calculated for each patient according to their physical activity level, based on dietary reference intakes (DRI) equations and was compared with actual calorie intake. The Transplantation period was divided into four phases; phase 1(start of conditioning to D-1), phase 2 (D0 to D+7), phase 3(iD+8 to D+14) and phase 4 (at the time of discharge).
Results: The median age was 45.5 years (Range 10-64). The average calories, carbohydrates, protein, and fats intake by all patients undergoing HSCT had decreasing trend during all phases except for the time of discharge. There was a significant effect of duration on weight (p=0.002), BMI (p=0.000), calorie intake (p=0.002) and fats intake during different phases (p <=0.001). There was no significant effect of duration on protein (p=0.128) and carbohydrates intake (p=0.051). None (0%) of the patients met 100% of EER in any phase of transplant duration. Two (10%) at phase 1, eight (40%) at phase 2, 15 (75%) at phase 3 and three (15%) at discharge did not meet even 50% of EER (image).

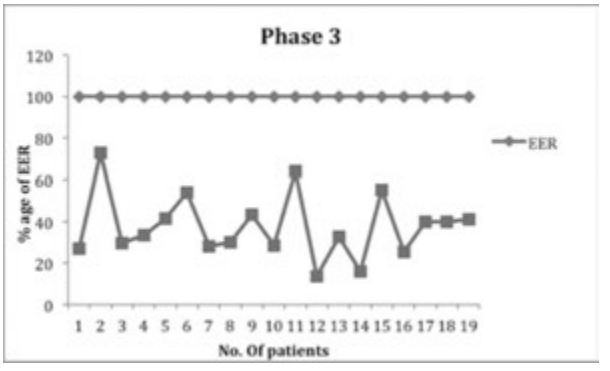


Figure 1.

Summary/Conclusion: There is a significant compromise in nutrients intake in patients undergoing HSCT. The nutritional status continues to worsen unless patient recovers fully and is fit for discharge. Therefore, there is a need to step up our effort to supplement nutrition by giving oral and parenteral supplements. Further study can be done in HSCT patients not meeting EER, analyzing the effect on engraftment, GVHD, VOD and hospitalization stay.

PB2460

DESIGN OF PHASE 3, RANDOMIZED TRIAL OF DEFIBROTIDE FOR PREVENTION OF HEPATIC VENO-OCCLUSIVE DISEASE/ SINUSOIDAL OBSTRUCTION SYNDROME

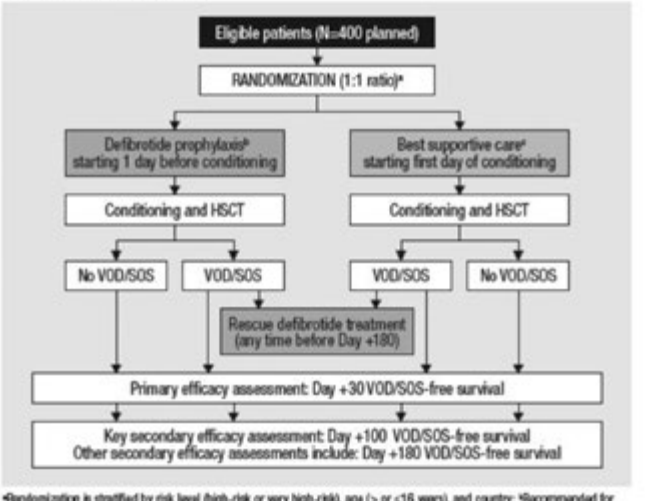
S. Grupp[1,*], A. Pagliuca[2], S. Corbacioglu[3], R. Hume[4], W. Liang[4], W. Tappe[4], P. Richardson[5]

[1]Pediatric Oncology, The Children's Hospital of Philadelphia, Philadelphia, United States, [2]King's College Hospital, London, United Kingdom, [3]University of Regensburg, Regensburg, Germany, [4]Jazz Pharmaceuticals, Palo Alto, [5]Dana-Farber Cancer Institute, Harvard Medical School, Boston, United States

Background: Hepatic veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) is a potentially life-threatening complication of hematopoietic stem cell transplant (HSCT); VOD/SOS with multi-organ dysfunction (MOD) may be associated with >80% mortality. Defibrotide is approved for treatment of severe VOD/SOS post-HSCT in the European Union in patients aged >1 month and for VOD/SOS with renal or pulmonary dysfunction post-HSCT in the United States and Canada, but no drug is approved for VOD/SOS prevention. VOD/SOS risk factors include receiving myeloablative conditioning (MAC) or calicheamicin antibody conjugates. Defibrotide as prophylaxis reduced VOD/SOS incidence in high-risk pediatric patients (Corbacioglu *et al.*, *Lancet*, 2012); data are more limited for adults. A new randomized, parallel group study of defibrotide for prevention of VOD/SOS in high-risk, post-HSCT patients of all ages (≥1 month) is underway (NCT02851407).
Aims: To assess the efficacy and safety of defibrotide prophylaxis plus best supportive care compared with best supportive care alone for prevention of VOD/SOS in adult and pediatric post-HSCT patients at high or very high risk for VOD/SOS.
Methods: HARMONY is a phase 3, multicenter, randomized study being conducted at ~100 sites worldwide (**Figure**). High/Very high-risk patients receive defibrotide prophylaxis with best supportive care or best supportive care alone. VOD/SOS is diagnosed according to modified Seattle criteria, or biopsy; a blinded endpoint adjudication committee validates each diagnosis. Patients in either arm who develop VOD/SOS will receive defibrotide rescue treatment. The planned sample size of 400 provides 90% power to detect a 0.46 hazard ratio for the primary endpoint of VOD/SOS-free survival by Day +30. Study eligibility criteria include: scheduled HSCT and high/very high risk of VOD/SOS. High-risk criteria are MAC plus ≥1 additional risk factor: transaminase >2.5× upper limit of normal (ULN), serum total bilirubin >1.5× ULN, cirrhosis, hepatic fibrosis on biopsy, viral hepatitis within 1 year, prior hepatic irradiation, iron overload, or high-risk stage IV neuroblastoma. Very high-risk criteria are ≥1 of the following: osteopetrosis, familial hemophagocytic lymphohistiocytosis or predefined related disorders undergoing MAC, prior gemtuzumab (dose ≥9 mg/m^2) or inotuzumab (≥1.5 mg/m^2 over 28 days), or Class III high-risk thalassemia. Key exclusion criteria are hemodynamic instability, clinically significant bleeding (or from life-threatening site), or use of medication <24 hours that increases bleeding risk (heparin ≤100 U/kg/day is permitted). The recommended defibrotide dosage is 25 mg/kg/day (in 4 divided doses) for ≥21 days starting the day before conditioning and ending no later than Day +30 post-HSCT. The key secondary outcome is VOD/SOS-free survival by Day +100; other secondary assessments include VOD/SOS-free survival by Day +180, VOD/SOS incidence, disease relapse, and MOD onset and resolution.
Results: As of February 2018, 107 sites are active and 164 patients have been randomized. Enrollment is ongoing.

Table 1.

Figure 1. Study Design



*Randomization is stratified by risk level (high-risk or very high-risk), age (> or ≤16 years), and country; †Recommended for ≥21 days and ending no later than Day +30 post-HSCT, with best supportive care; ‡Given according to institutional guidelines until Day +30 post-HSCT or hospital discharge, whichever is sooner, or diagnosis of VOD/SOS, if applicable.
HSCT, hematopoietic stem cell transplantation; VOD/SOS, veno-occlusive disease/sinusoidal obstruction syndrome.

Summary/Conclusion: HARMONY will provide phase 3, randomized study data on the efficacy and safety of defibrotide for the prevention of VOD/SOS in HSCT patients at high/very high risk for VOD/SOS. These findings will extend previous pediatric study data and provide new evidence in adults.

PB2461

A SINGLE-CENTRE DATA FOR BACTERIAL AND VIRAL INFECTION RATES IN ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS DURING THE FIRST 180 DAYS POST-NEUTROPHIL ENGRAFTMENT

M. Khalifa[1,*], S. Atanze[1], D. Partridge[2], S. Thomas[1], S. Francis[1], S. Stanworth[3], J. Snowden[1], H. Kaur[1]

[1]Haematology, [2]Microbiology, Sheffield Teaching Hospitals, Sheffield, [3]NHS blood and Transplant, John Radcliffe Hospital, Oxford, United Kingdom

Background: Infections are the most common complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT). They are associated with significant morbidity and mortality. In the early post-engraftment bacterial infections and reactivation of latent viruses is frequently seen due to immunosuppressive therapy. There is lack of literature on infection incidence and description of causative pathogens which would be of interest towards any future studies looking at prophylaxis strategies.
Aims: To identify the rates of bacterial and viral infections in allo-HSCT recipients during the first 180 days post-neutrophil engraftment. To compare the infection rates between matched unrelated donors (MUD) and sibling allo-HSCTs.To compare infection rates between myeloablative and reduced intensity conditioning (RIC) allo-HSCTs. To describe the common bacterial and viral organisms causing infection.
Methods: Data was collected retrospectively for all patients that underwent an allo-HSCT from January 2015 to December 2017. All positive blood cultures and viral specimens for each patient were identified for the time period specified using the laboratory database systems. Data was analysed using Prism (Graphpad). Student's t test was used for comparing medians and chi squared for proportions. A p value ≤0.5 was considered significant.
Results: 113 patients received an allo-HSCT during the study period. The median age was 54 (SD 15 years, range 19-72). The majority of donors were matched unrelated donors (MUDs), 64%, with 29% of allograft from sibling donors, 5% haploidentical and 2% with cord blood stem cells. Myeloablative conditioning was used in 19% of allografts whereas (RIC) was used in 81% of transplants. There was no difference in the number of episodes of viral infection in MUD allografts compared to sibling allografts

(MUD median 2, SD 1.4, sibling median 2, SD 1.6.) (p >0.05, unpaired t test). The commonest viral infection was EBV, occurring in 55% of patients overall. EBV infection was significantly more common in sibling allografts at 78% than MUD allografts at 47% (p≤0.05). Infections with respiratory viruses were significantly more common following myeloablative conditioning, with adenovirus and respiratory syncytial virus both seen in 23% of myeloablative allografts, compared to 5% and 3% respectively of RIC allografts (p ≤0.05).Data on p 54% of patients had at least one positive blood culture. There was no difference in the proportion of patients with a positive blood culture in MUD allografts compared to sibling allografts (MUD 50%, sibling 55%) (p>0.05) The organisms isolated were diverse, with a total of 25 different genera/species isolated. The commonest were coagulase negative staphylococci or mixed coagulase negative staphylococci 49% *Pseudomonas sp* in 14%, *Escherichia coli* in 8% and *Klebsiella sp* and *Enterococcus sp* each in 7%.

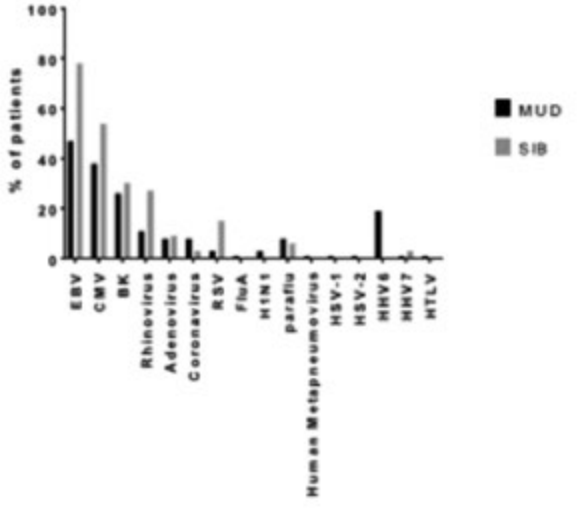


Figure 1.

Summary/Conclusion: We present a comprehensive description of the types and frequencies of bacterial and viral infections in allo-HSCT recipients during the first 180 days post neutrophil engraftment. There are statistically significant differences in patterns of viral reactivation according to donor and conditioning regimen, which may have implications for surveillance, prophylaxis and empirical treatment in these patient groups.

PB2462

COULD THE OUTPATIENT-BASED AUTOLOGOUS STEM CELL TRANSPLANTATION REDUCE THE "FINANCIAL TOXITY" WITHOUT WITHOUT ADVERSELY AFFECTING THE EFFECTIVENESS AND SAFETY OF THE METHOD?

P. Kaloyannidis*, E. Shaibani, N. Al Mokhtar, F. Aburahman, E. Mutahar, M. Al Darweesh, O. Abduljalil, A. Estanislao, B. Jenifer, R. Khalili, J. Apostolidis, K. Al Anezi, H. Al Hashmi

Adult Hematology and Stem Cell Translantation Department, King Fahad Specialist Hospital, Dammam, Saudi Arabia

Background: Given the improvements in the supportive care, autologous hematopoietic stem cell transplantation (AHSCT) could be performed in outpatient basis, for selected patients (pts), offering the benefit of shorter hospitalization, less exposure to hospital pathogens, saving nosocomial beds, demonstrating thus not only a safe but also a cost effective profile.
Aims: To evaluate the feasibility and safety of the outpatient-basis AHSCT approach.
Methods: We retrospectively analyzed outcome of 34 AHSCTs performed on outpatient basis, in a total of 27 pts, previously diagnosed with Hodgkins lymphoma (n=6) or Multiple Myeloma (n=21); 7 pts with MM underwent tandem-AHSCT in the context of the scheduled treatment plan. Nine were females and 18 males aged of a median of 48 (25-68) ys. The eligibility criteria for the outpatient AHSCT, were the standard clinical and laboratory tests, plus psychosocial evaluation, patient's compliance assessment, 24 hours caregiver availability, timely access to the hospital and signed informed consent. The conditioning regimen consisted from single agent Melphalan of 200 (n=25) or 140mg/m^2 (n=9), graft infusion and supportive care were given in an allocated room. The antimicrobial, antifungal and antiviral prophylaxis was administered from day -2 and filgrastim 5mcg/kg from day +5 till neutrophils recovery. If no infection was documented the antimicrobial and antifungal prophylaxis were discontinued upon stable neutrophils recovery while antiviral prophylaxis was continued for 10-12 months. Patients were evaluated daily or every 2 days in the outpatient clinic. The criteria for admission were fever >38^{o}C, intractable nausea/vomiting or diarrhea, mucositis needing total parenteral nutrition and any other toxicity WHO>grade 3.
Results: The median day for neutrohils >1000/mm^3 was 11 (9-18) and for platelets >20000/mm^3 was 11 (0-21); in 2 pts platelets never dropped lower than 25000/mm^3. Totally, 15 admissions were required, 10 for inability for food/fluid uptake due to severe mucositis, 4 for febrile neutropenia and 1 for engraftment syndrome. The infections successfully treated with broad spectrum antibiotics and no pt was admitted to intensive care unit. For the whole 34 ASCTs, the total hospitalization days were 77 (median:1, range 0-12) ,while for the 15 admissions the median hospitalization days were 5 (1-12), which favorably compares with the average of 14 hospitalization days for a single "conventional" ASCT. No other toxicities (WHO>3) were observed. Currently 26/27 pts are alive 9(1-37) months post AHSCT. Foe pts with MM the 2-years overall and progression free survival are 93% and 65% respectively. All six patients with HL are alive 3-12 months post AHSCT.
Summary/Conclusion: Our data indicate that the outpatient-ASCT is a feasible and safe approach provided a caregiver availability, close pts evaluation and adequate supportive care. Keeping in mind the nosocomial complications and the potential high cost of the prolonged hospitalization, it seems that the outpatient ASCT offers lower risk of infections and significant cost saving compared to the "convetional" inpatient ASCT approach.

PB2463

AUTOLOGOUS HEMATOPOIETIC CELL TRANSPLANTATION FOR LYMPHOID MALIGNANCIES IN PATIENTS OF 65 YEARS OLD AND BEYOND. A SINGLE CENTER FEASIBILITY EXPERIENCE

S. Desilets[1], E. Nicolas-Virelizier[1], F. Subtil[2], L. Gilis[1], L. Lebras[1], S. Assaad[1], Y. Guillermin[1], P. Rey[1], M. Michallet[1], A. Belhabri[1], A.-S. Michallet[1,*]

[1]Hematology, Centre Léon Bérard, Lyon, [2]Université Lyon [1], Villeurbanne, France

Background: Autologous hematopoietic cell transplant (auto-HCT) remains a cornerstone consolidation strategy in the front line treatment of Multiple Myeloma (MM) and Lymphomas in young and fit patients. However, with the better toxicity profile of new drugs used in induction phase and in conditioning, as well as the improvement in their management, auto-HCT is more often considered in older patients.
Aims: The aim of this retrospective study was to assess the feasibility and efficacy of consolidation auto-HCT for lymphoid malignancies in patients of 65 years old and beyond.
Methods: Between years 2012 and 2016, 54 patients aged 65 years and beyond, received auto-HCT, median age at transplantation was 67 years (range: 65-71), 23 (43%) were female. Diagnosis was MM in 45 (83%) patients and lymphoma in 9 (17%) patients (2 Diffuse B Cell, 4 mantle cell, 1 follicular, 1 Hodgkin and 1 T cell). Performance status (PS) at transplantation was 0 in 16% of patients, 1 in 78% and 2 in 6%. Auto-HCT was used in first line strategy for the majority of patients (N=45, 83%). For the multiple myeloma patients, the induction treatment was mostly bortezomib-based regimen [VTD (56%), VCD (15%), VRD (5%), VD (5%)] and 5% received lenalidomide and dexamethasone. The lymphoma patients received RDHAP (6 patients), DHAP (1), Brentuximab (1), RCHOP (1). The majority of patients were easily collected for HC without the need of mobilization with plerixafor (78%), the median number of CD34 collected cells was 3.99 million/kg (range 2.04 to 10.96). One MM patient received a tandem auto-HCT. Disease response at transplantation was as follow: in MM patients, 9 (20%) CR, 14 (30%) VGPR, 20 PR and 2 less than PR; in lymphoma patients, 5 (56%) were in complete radiological response and 4 (44%) were in PR. MM patients received Melphalan-based conditioning, 24 (53%) received the standard dose of 200mg/m^2, 12 (27%) received 140mg/m^2 and 9 (20%) received 100mg/m^2. The lymphoma patients were conditioned with either BEAM (N=6), RBEAM (N=2) or TEAM (N=1).
Results: After transplantation, the median time to neutrophils recovery (neutrophils >0.5x10^9/L) was 11 days (range: 9-20) and the median time to platelets recovery (platelet >50x10^9/) was 15 days (range: 10-29). The median duration of hospitalization after transplantation was 17 days (range: 9-28), at hospital discharge 28 (53%) patients had a PS of 1. Main toxicities were gastro-intestinal and febrile neutropenia, 34 (63%) patients had mucitis, of those, 23 (68%) grade 1-2 and 10 (%) grade 3-4. Forty patients had

nausea and vomiting, 21 (52%) were of grade 3-4; 37 patients had diarrhea, 22 (60%) were grade 1-2. Febrile neutropenia was observed in most patients (N=45, 82%). After a median follow up of 32 months, the median progression-free survival rate was 22 months and median overall survival was not reached. At the last follow-up, 45 patients were alive, 9 patients died, all of them had progressive disease (6 were MM), 2 died of septic shock after a chemotherapy treatment.
Summary/Conclusion: We showed that auto-HCT is a feasible and well tolerated treatment option for patient with lymphoid malignancies with 65 years old and beyond, no death related to this procedure, this was mainly due to the good patient selection and the better prevention and management of the procedure-related toxicities and complications.

PB2464

THE EFFECT OF GEOGRAPHICAL DISTANCE BETWEEN A TRANSPLANT CENTER AND PATIENT RESIDENCE WHO UNDERWENT ALLOGENEIC STEM CELL TRANSPLANTATION: A RETROSPECTIVE, UNICENTRIC STUDY

B. Haffar*, R. Massoud, N. Moukalled, A. Zahrelddine, R. Nehme, A. Bazerbachi, J. El Cheikh

Internal Medicine, American University of Beirut, Beirut, Lebanon

Background: Several factors affect the outcome of allogeneic stem cell transplantation (Allo-SCT) in hematologic malignancies patients. Proximity to the transplant center could be an independent factor that affects overall survival (OS). This study has been conducted at American University of Beirut Medical Center (AUBMC), a referral center for the Middle East located in Lebanon.
Aims: Our aim was to determine the differences in the survival between patients residing in Lebanon and those referred from abroad.
Methods: We identified 161 consecutive adults patients who underwent allo-SCT between January 2007 and December 2016. One hundred and three patients (64%) were residing in Lebanon, and 58 patients (36%) were referred from abroad. Follow up on patients residing in Lebanon was done mainly via regular clinic visits, while those residing outside Lebanon was either by clinic appointment, and/or phone calls in case they could not travel to Lebanon. All patients and transplant characteristics are listed in table 1.

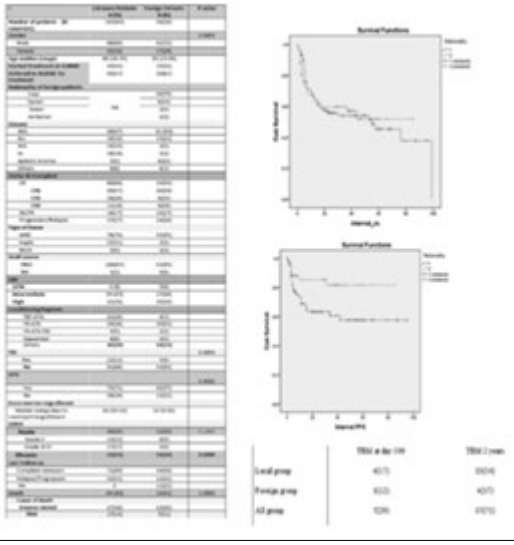


Figure 1.

Results: All patients engrafted. The median time to neutrophil engraftment was 15 days in both groups but with different ranges (10-23) and (9-35) days in local and foreign groups respectively. In Local group 68 (66%) patients were in Complete remission (CR) at transplant, from those 39 (57) were in first CR (CR1), 18 (26) were in second CR (CR2), and 11(16) were third CR (CR3). *vs* 35 (34%) were in progression disease/relapsed disease (PR/RD) and Stable disease/partial response(SD/PR) at transplant, in foreign group 34 (59%) patients were at CR at time of transplant, from those 20 (59) were in CR1, 8(23) were in CR2, and 6(18) were in CR3, while 24 (41%) patients were in PR/RD and (SD/PR) at time of transplant. The incidence of acute graft *versus* host disease (aGVHD) was higher in local group with 30 cases (29%) and 11 cases (19%) in foreign group (P= 0.188). Similarly, the incidence of chronic graft *versus* host disease (cGVHD) in local group was superior to foreign group, with 23 (22%) patients *vs* 11 (19%) patients respectively (P=0.6904). After a median follow up of 16 months (range, 1-99), 59 (57%) and 34 (59%) patients were alive in the local and foreign group respectively. The median OS was 55 months in local groups, but it has not been reached in foreign group. The median progression free survival (PFS) has not been reached in both groups. The cause of death was classified into two categories, disease related and TRM. In the local group, 44 (43%) patients died, from those, 27 (26%) were disease related and 17 (14%) due to TRM. Whereas 24 (41%) patients died in foreign group, from those 12 (21%) deaths were disease related, while 7 (12%) deaths were TRM.
Summary/Conclusion: Our results imply that the geographical distance has no impact on the OS, however, we have found that it might have impact on the PFS, but we should take into consideration that more patients in local group underwent Haploidentical bone marrow transplant. These findings need to be verified in larger scale studies.

PB2465

PROGNOSTIC IMPACT OF MINIMAL RESIDUAL DISEASE IN AML PATIENTS BEFORE ALLOGENEIC STEM CELL TRANSPLANTATION ON DISEASE FREE SURVIVAL

O. Koroleva[1,*], J. Davydova[2], E. Parovichnikova[1], L. Kuzmina[1], I. Galtseva[2], V. Troitskay[3], N. Kapranov[2], E. Mikhaltsova[1], V. Vasilieva[1], D. Dubnyak[1], N. Popova[1], Z. Konova[1], A. Sidorova[1], E. Usikova[1], T. Lobanova[3], M. Drokov[1], V. Savchenko[1]

[1]BMT department, [2]Immunophenotype laboratory, [3]Hematology department, National Research Center for Hematology, Moscow, Russian Federation

Background: It's well known that the disease status before allogeneic stem cell transplantation (allo-HSCT) affects the outcome in adults with acute myeloid leukemia (AML). Multicolor flow cytometry (MFC) is one of the most applicable diagnostic methods to evaluate minimal residual disease (MRD). However, a primary immunophenotype of leukemia cells is often unknown, so the "different from normal" approach to assess MRD could be used in this situation before allo-HSCT.
Aims: To evaluate an impact of MRD by MFC on disease free survival in AML patients after allo-HSCT.

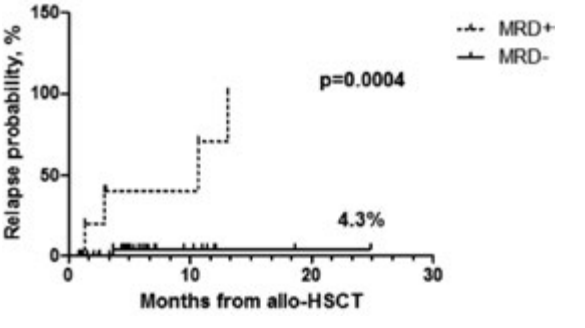


Figure 1.

Methods: MRD status was evaluated in 37 patients with AML. All patients received allo-HSCT in National research center for Hematology between September 2015 and December 2017. A median age was 40 years old (19-66 years). There were 14 males and 23 females. All patients had first complete remission (CR1) confirmed by bone marrow morphology. Patients received allo-HSCT from related (n=16) or unrelated (n=21) donors. The intensity of conditioning was mainly reduced (n=23) rather than myeloablative conditioning (n=14). Bone marrow (BM) as a graft source was used in 25, PBSC – in 12 pts. MRD was measured before allo-HSCT (2 weeks before conditioning) in bone marrow by 6-color flow cytometry (BD FACS Canto II). The panel of monoclonal antibodies included 4 tubes with core 3 markers (CD33, CD34, CD45) and the set of other markers - CD38, CD13, CD117, CD11b, CD99, CD14, CD16, CD66b, CD65, CD56, HLA-DR. The analysis of MRD was performed based on "different from normal" approach without primary immunophenotype of blast cells. Sensitivity of MFC was 0.01% (50 cells with abnormal immunophenotype from total 500 000 cells). MRD >0,01% was considered as "positive".
Results: MRD "positivity" before allo-HSCT was detected in 5 patients, MRD "negativity" - in 32. Leukemia relapse was installed in 1 pt in MRD negativity group, 4 in MRD positivity group. Disease free survival in the MRD negativity group was 77,7% *versus* 32,3% in the MRD "positivity" group, p=0,008. Probability of relapse in MRD "negativity" and in MRD "positivity" groups was 76,6% and 4,3%, respectively, p=0,0004 (Fig1).

Summary/Conclusion: According to our data MRD analysis based on "different from normal" approach is an effective method for AML patients in CR. Moreover, MRD status before allo-HSCT is an important prognostic factor for detection of a minimal population of blast cells for disease-free survival and can be considered as a discriminator for preemptive or preventive therapy.

PB2466

TREATMENTS FOR STEROID-REFRACTORY ACUTE GRAFT *VERSUS* HOST DISEASE: A SYSTEMATIC LITERATURE REVIEW

A. Taylor[1,*], E. Wissinger[2], S. Cadarette[2], K. Ruiz[2], J. Jansson[3], M. Akbari[3]

[1]Takeda Pharmaceuticals International, London, United Kingdom, [2]Xcenda L.L.C, Palm Harbor, [3]Takeda Pharmaceuticals International, Cambridge, United States

Background: Approximately half of patients who develop acute graft-versus-host disease (aGvHD) after an allogeneic hematopoietic stem cell transplant will respond to first-line corticosteroid therapy. Steroid-refractory (SR)-aGvHD has high 6-month mortality rates of up to 80%, particularly when there is gastrointestinal (GI) involvement. There are no approved treatments for SR-aGvHD and success with off-label interventions has been limited and variably reported. There is a high degree of heterogeneity among studies regarding the definitions of SR disease, treatment response, and the timing of assessment of responses. Considering these limitations and the high burden of morbidity in SR-aGvHD, it is important to evaluate the efficacy of treatments currently used in clinical practice, to better understand the factors that may influence clinical outcomes.
Aims: To assess the clinical outcomes of interventions used off-label to treat SR-aGvHD.
Methods: A systematic literature review (SLR) was conducted using MEDLINE and Embase databases to identify studies published in English from January 2006 to August 2017, describing overall survival (OS) or treatment response rates in patients with SR-aGvHD. Any pharmacological intervention(s), including best supportive care, were included; non-pharmacological interventions such as extracorporeal photopheresis and stem cell infusions were excluded.

Table 1.

Results: Sixty-one publications describing treatment of patients with SR-aGvHD and reporting relevant clinical outcomes data were included in the SLR. Interventions utilized in these studies included monoclonal antibodies originally licensed in autoimmune disease such as alemtuzumab, basiliximab, infliximab, tocilizumab, vedolizumab, and combinations thereof, as well as other immunosuppressive agents such as etanercept, mycophenolate mofetil, pentostatin, and ruxolitinib. There was a high degree of heterogeneity in study design, patient population and size, and length of follow-up reported among the publications included in the SLR. Most studies were retrospective chart reviews, and all but 3 were conducted in a single country, most frequently the United States. Most studies focused on patients with Grade II-IV SR-aGvHD, but some investigations included patients with less severe disease, and the proportion of patients with GI involvement reported at baseline was heterogeneous. Data for OS were highly variable across the included publications. Among larger (N>50) studies reporting 1-year OS, rates ranged from 27% in patients treated with thymoglobulin to 62% among those given basiliximab and etanercept in combination. OS rates for patients with GI involvement at baseline, described in a few studies with smaller numbers of patients, were generally lower than for the overall population, 25% *versus* 34%>79% at 6 months, and only 24%>33% at 1 year. Due to limited publications, small numbers of included patients, variability in study designs, and limited reporting of outcomes, no other clear trends for OS could be identified.
Summary/Conclusion: A considerable number of potential off-label treatments has been reported for use in patients with SR-aGvHD. Results are highly variable for clinical outcomes including OS, and long-term survival rates remain disappointingly low, particularly for patients with GI involvement. There remains a need for further research to identify effective treatments for patients with SR-aGvHD.

PB2467

COMPARISON OF PERIPHERAL STEM CELL MOBILIZATION OF PATIENTS WITH MULTIPLE MYELOMA AND LYMPHOMA IN LATE MIDDLE AGE (55-64 YEARS) VERSUS OLD AGE (OLDER THAN 65 YEARS): A SINGLE CENTER EXPERIENCE

Z. Gulbas[1,*], D. Çekdemir[2], I. Dora[1], Ç. Eren[2]

[1]Hematologic oncology and Bone Marrow trssplantation, [2]Anadolu Health Cener, Kocaeli, Turkey

Background: In patients with myeloma and lymphoma, stem cell mobilization can be unsuccessful in 5-30% of the cases. The underlying mechanisms may include qualitative and quantitative differences among hematopoietic stem cells in the bone marrow, differences in the migration ability of hematopoietic stem cells or weakened response to G-CSF.
Aims: to compare of results of peripheral stem cell mobilization of patients with multiple myeloma and lymphoma in late middle age (55-64 years) *versus* old age (older than 65 years).
Methods: Patients were divided into two groups as middle age (55-64 years) (n=101), and old age (older than 65 years) (n=102).We retrospectively reviewed the data of 159 multiple myeloma patients and 44 lymphoma patients who have been mobilized between September 2010 and June 2016 in the bone marrow transplantation center
Results: The median number of peripheral blood CD34+ cell was 24.3×10^9 CD34+ cells/L in the middle age group, 20.0×10^9 CD34+ cells/L in the old age group, and there was no significant difference between the two groups. Two groups do not differ from each other in the terms of neutrophil and platelet engraftment times (p=0.702, and 0.113, respectively). The median number of CD34+ cells collected was 9.55×10^9 CD34+ cells/L in the middle age group, 9.20×10^9 CD34+ cells/L in the old age group, and there was no significant difference between the two groups. Even if the quantity of CD34+ cells collected in elderly patients was lower than in younger patients, this quantity does not reach significance.
Summary/Conclusion: the analysis of our data showed that the age of the patients has no impact on the mobilization of stem cells in lymphoma and myeloma patients. Even if the quantity of CD34+ cells collected in elderly patients was lower than in younger patients, this quantity does not reach significant difference.

PB2468

PRE-TRANSPLANT DISEASE STATUS AS A RESPONSE PREDICTOR IN AGGRESSIVE LYMPHOMAS

S. Marini[1,*], A. Ribeiro[1,2], D. Mota[1], C. Geraldes[1,2], L. Ribeiro[1,3]

[1]Hematology, Centro Hospitalar e Universitário de Coimbra, [2]Faculdade de Medicina, [3]Centro de Investigação em Meio Ambiente, Genética e Oncobiologia, Universidade de Coimbra, Coimbra, Portugal

Background: Autologous stem cell transplantation (ASCT) is currently an important therapeutic strategy. It is used as a consolidation therapy after high-dose chemotherapy in patients with aggressive non-Hodgkin's lymphoma (aNHL). The timing for transplantation depends not only on the lymphoma subtype but also on the disease status prior to the transplant, being indicated in patients with first chemosensitive relapse of aNHL after reaching partial (PR) or complete (CR) responses. However, clinical experience seems to show a worst *outcome* in patients with PR prior to ASCT, wondering wether the intensification of the chemotherapy regimen (until achieving CR) should be an option.
Aims: To compare the outcome of patients with aNHL in PR *versus* CR prior to autologous stem cell transplantation.
Methods: Retrospective longitudinal observational study of patients submitted to ASCT, from january 2007 to july 2017, in a single Portuguese institution. Statistical analysis was performed recurring to descriptive sta-

tistics and survival analysis using the Kaplan-Meier method, stratified by Log-Rank.
Results: We analyzed a sample of 76 patients with a median age at diagnosis of 46 years (17-64 years) and 53 (69.7%) were male. This population included several types of aNHL particularly Diffuse large B-cell lymphoma (DLBCL) n=46 (60.5%), High grade B-cell lymphomas, NOS n=3 (3.9%), Mantle cell lymphoma (MCL) n=22 (28.9%) and T-cell lymphomas n=5 (6.6%). Patients were treated with a median of 2 therapeutic lines (range 1-4) and had a ECOG ≤1, prior to ASCT. The response assessments after day 100, was statistically significant (p<0.001) favoured the transplant, from the 17 patients with PR prior to ASCT, 12 achieved CR. With a median follow-up of 74 months, we observed no statistically significant difference in the median overall survival (OS) of the entire study population, however, in subgroup analysis by lymphoma subtype there was a significant difference in the OS of patients with DLBCL (PR *vs* CR: 106m *vs* NR; p=0.003). Twenty patients relapsed after ASCT, the median time until relapse was 46 months. Progression-free survival (PFS) of the entire cohort, defined from the time of ASCT to the time of relapse, revealed lower time to relapse in patients with PR prior to ASCT compared to patients in CR (86 months *vs* 135 months; p=0.01).
Summary/Conclusion: Patients with DLBCL transplanted in PR after high dose chemotherapy, had a lower overall survival compared to the CR group. This suboptimal prognosis raises the issue whether chemotherapy should be optimized prior to consolidation with ASCT.

PB2469

Abstract withdrawn.

PB2470

A STUDY OF UPFRONT AUTOGRAFT FOLLOWING 3 TO 4 CYCLES OF BORTEZOMIB, LENALIDOMIDE AND DEXAMETHASONE BASED CHEMOTHERAPY IN 52 INDIAN MYELOMA PATIENTS – A TERTIARY CENTRE EXPERIENCE FROM CHENNAI,INDIA

K. Kumar*, C. Subash

Haematology and BMT, Miot Chennai, Chennai, India

Background: In the era of newer immune-modulatory drugs, lot of debate is there on role and timing of Autologous bone marrow Transplantation. We conducted this study to look at the efficacy of RVD combination chemotherapy followed by an Autologous bone marrow transplant in 52 eligible Indian Myeloma patients.
Aims: We conducted this study to look at the efficacy of RVD combination chemotherapy followed by an Autologous bone marrow transplant in 52 eligible Indian Myeloma patients.
Methods: Eligible patients were treated according to this protocol. RVD q 21 days (3 cycles) Lenalidomide 10mg x 14 days every 21 day cycle, Bortezomib 2mg 1,4,8,11, Dexa 20mg 1,2,4,5,8,9,11,12. Then Collection of peripheral blood stem cells (PBSCs) using GCSF+/-Plerixafor. Autologous stem cell transplant: Melphalan 200mg/m^2: Day -1, Re-infusion of PBSCs. Then maintenance Lenalidomide 10mg 21 days every 28 days till progression.
Results: Results: 75% entered the transplant after three cycles of RVD after achieving a minimum of VGPR. The response rated to RVD were more than 95%. All entered the maintenance phase. The median followup was 24 months. In the transplantation group, 8 patients had disease progression, and symptomatic patients received a second-line therapy. Of the patients who were treated for disease progression, three underwent a second transplantation at the time of progression and one underwent Allogeneic transplantation.
Summary/Conclusion: Conclusions: In conclusion, we found that early consolidation therapy with high-dose chemotherapy plus transplantation was associated with longer progression-free survival. This benefit must be weighed against the increased risk of toxic effects associated with high-dose chemotherapy plus transplantation. This is a feasible option in Indian patients both in response rates as well as finances.

PB2471

REVIEW OF THE MANAGEMENT OF HYPOMAGNESAEMIA AND HYPOKALAEMIA IN ALLOGENEIC STEM CELL TRANSPLANT INPATIENTS AT A MAJOR LONDON CENTRE

A. Dervin, O. Tomkins*, K. Thomson

UCLH - University College London Hospitals NHS Foundation Trust, London, United Kingdom

Background: Electrolyte imbalances are common in allogeneic transplant patients, often secondary to gastrointestinal loss and widespread use of calcineurin inhibitors. Hypokalaemia and hypomagnesaemia puts the patient at risk of cardiac arrythmias. There is often variation in the approach taken to electrolyte replacement. At our transplant unit, there is currently no guideline in place to guide prescribing and administration of magnesium and potassium replacement. This leads to apprehension among junior medical staff and nurses, and may lead to prolonged electrolyte derangement through insufficient replacement. There is also signficiant cost associated with repeated electrolyte replacement and monitoring of serum magnesium and potassium.
Aims: We aimed to review current practice of magnesium and potassium replacement at a major London transplant centre. We also aimed to assess the increment rate for low *versus* high concentrations of magnesium and potassium replacement, and to draft a departmental guideline.
Methods: We audited prescriptions of magnesium and potassium for allogenic transplant patients between October and December 2017. Historic medication charts and our pathology results system were accessed. Serum levels pre- and post-replacement were recorded and the 24-hour increment calculated.
Results: Prescription charts for 52 patients were reviewed. Ninety-eight prescriptions of intravenous magnesium were found: nine prescriptions (39.8%) of 8mmol, 7 (7.1%) of 16mmol and 51 (52.0%) of 20mmol. Average serum increment was 0.08, 0.174 and 0.26mmol/L respectively. There was no significant difference in the serum levels pre-replacement between groups, ranging from 0.44 to 0.77 mmol/L. Oral magnesium replacement was not used. For intravenous potassium chloride, 138 prescriptions were found. Patients had pre-replacement levels between 2.4 and 3.9mmol/L. Oral potassium replacement was concomitantly prescribed in 40 cases (28.99%). There were 102 prescriptions for 40mmol potassium, one for 60mmol, 29 for 80 mmol, four for 120mmol and one for 160mmol. The average serum potassium increment at 24 hours was 0.259mmol/L with 40mmol, 0.3 with 60mmol, 0.6 with 80 mmol, 0.85 with 120mmol and 1.1 with 160 mmol. Post-replacement serum potassium was never higher than 4.9mmol/L. Serum magnesium level was checked in 100 of the cases requiring intravenous potassium replacement (72.5%); 75 of these also required magnesium replacement, 30.7% were prescribed only 8mmol.
Summary/Conclusion: We demonstrate that the approach to treatment of hypomagnaesaemia and hypokalaemia at our transplant unit varies significatly. There is a limited increase in serum magnesium level with intravenous doses less than 20mmol, yet lower doses than this are frequently administered. Correction of hypomagnesaemia is also often inadequate in patients with concomitant hypokalaemia. We also demonstrate that increment in serum potassium is limited with doses lower than 80mmol and that it is safe to administer this amount of potassium in hypokalaemic patients without risk of iatrogenic hyperkalaemia. We propose creating a guideline that recommends immediate administration of these higher doses of magnesium sulfate and potassium chloride. We recommend immediate use of 20mmol intravenous magnesium indeficient patients. We also recommed administration of 80mmol potassium if serum concentration is less than 3.0mmol/L. This would minimise risk of arrhythmias and lower the cost of repeated monitoring and administration.

PB2472

ALLOGENEIC BONE MARROW TRANSPLANTATION IN THREE CASES WITH DOCK 8 DEFICIENCY

T. Patiroglu[1,*], M. Cansever[2], M. Karakukcu[1], E. Unal[1], A. Ozcan[1]

[1]Pediatric Hematology, [2]Pediatric Immunology, Erciyes University Medical Faculty, Kayseri, Turkey

Background: Combined immunodeficiency due to dedicator of cytokinesis 8 protein (DOCK8) deficiency is a form of T and B cell immunodeficiency characterized by recurrent cutaneous and sinopulmonary viral infections and susceptibility to cancer. Laboratory findings include elevated IgE level in serum and eosinophilia. T cell lymphopenia and reduced T cell function have been reported. Patients can also have NK and B cell lymphopenia. IgG levels may elevated but specific antibody responses are impaired. IgM and IgA levels may be reduced in DOCK8 immunodeficiency syndrome was called autosomal recessive hyper IgE syndrome (AR-HIES) and considered a rare form of HIES.
Aims: Therefore, we present three cases with DOCK8 immunodeficiency syndrome who they received an HLA-identical sibling donor bone marrow transplant (BMT).

Table 1.

	Case 1	Case 2	Case 3
WBC (mm3)	13100	21450	15940
ANC (mm3)	2150	6010	5500
ALC (mm3)	2490	5860	3810
AEC (mm3)	7356	5860	8607
Ig G (mg/dl)	1670	644	960
IgA(mg/dl)	126	42	109
Ig M(mg/dl)	334	18.9	46.7
IgE(IU/ml)	10300	3080	8080
CD3 (mm3)	1520	10610	1836
CD4(mm3)	470	5960	393
CD8(mm3)	920	2910	721
CD19(mm3)	670	8530	567
CD16+56(mm3)	250	1560	656

Methods: First (age:12 years) and second boys (age:1 year) were sibling and their parents were first cousins. All 3 cases had recurrent pulmonary infection, erythematous and eczematous rash on the whole body and hepatosplenomegaly. Furthermore, first case had bilateral chronic supurative otitis and a mass in left auricula and third female patient (age:10 years) also had the clitoral vegetative mass. Therapeutic measures included antiviral and antibacterial prophylaxis, immunoglobulin replacement and allogeneic BMT. The auricular and clitoral masses after ABMT regressed spontaneously and they are follow-up in our clinic. Laboratory findings of 3 cases are demonstrated in the table 1.
Summary/Conclusion: Early BMT should be strongly considered as a potential curative therapy in DOCK8 immunodeficiency syndrome.

PB2473

HAPLOIDENTICAL TRANSPLANTATION OF HEMATOPOIETIC STEM CELLS IN ADULT PATIENTS WITH HEMOBLASTOSES IN THE REPUBLIC OF KAZAKHSTAN

A. Olifirovich[1,*], V. Kemaikin[2], O. Gainutdinova[2], N. Tabynbaev[2], A. Suleymenova[2], M. Ospanova[2]

[1]Отдел онкогематологии и трансплантации костного мозга, Национальный научный центр онкологии и трансплантологии, Астана, [2]Oncohematology and Bone Marrow Transplantation Department, National Scientific Center for Oncology and Transplantology, Astana, Kazakhstan

Background: A comprehensive analysis of the results of haploidentical hematopoietic stem cell transplantation in adult patients have been performed for the first time during the period from October 2011 to July 2017. The incidence of acute and chronic graft *versus* host disease, overall survival and mortality were assessed.
Aims: To assess the total and relapse-free survival in patients after haploidentical transplantation of hematopoietic stem cells.
Methods: Considering the absence of generally recognized protocol on the date of beginning of haploidentical transplantation of hematopoietic stem cells to be used in the Republic of Kazakhstan, different conditioning and prevention regimens for acute and chronic graft *versus* host disease were used.
Results: At this time, 23 of 64 patients are alive amounting to 36%. The follow-up period is within 4 to 7 days. The median overall survival of the patients followed up is 2.58 months. The curve of overall survival contains tow plateaus. The 1st plateau is patients with relatively short follow-up period equal to 12-20 months. However, the median overall survival was reached in these patients. The 2nd plateau is the group of patients with follow-up period within 22 to 47 months. The cumulative survival percentage is 27%. The median overall survival of the patients followed up is 4.1 months. The cumulative survival percentage is 39%. Despite insignificant differences in the overall survival between the two groups of patients, the percentage and overall survival length are higher in the group of patients with their haploSCT in the 1st complete remission. The follow-up period is not long enough to analyze 5-years overall and relapse-free survival. At present, however, we note the significant difference in the median OS – 2.58 in the general group *vs* 4.1 months in the group of patients with acute leukemias in the 1st remission.
Summary/Conclusion: The obtained results are comparable to those that presented in foreign studies. The frequency of acute GVHD of Grades II-IV was 26.6%, *i.e.* practically comparable to this parameters of 21.9% presented in the study performed by Long H. *et al.* [4]. In relation to chronic GVHD, the parameter observed in our Department is significantly lower than the frequency reported in the above study – 11% *vs* 24.1%. However, at this stage of follow-up we could not conclude that development of chronic GVHD in haploSCT recipients in our Department is significantly more rare. This is explained by the fact that the follow-up period is still insufficient to a valid analysis of this parameter, a part of patients is followed up for less than 100 days. Also, a rather high mortality in the early posttransplantation period was observed. The equivalent comparison of the frequency of chronic GVHD requires a longer follow-up period. The parameter of OS in the general group of patients is lower than this parameter in foreign studies – 29% *vs* 52.6%. This is provided by unfavorable disease status on the date of transplantation and development of severe complications in the early post-transplantation period. However, the analysis of more homogeneous group of recipients with acute leukemias in the 1st complete remission shows a higher OS – 39%.Based on the results of our clinical analysis, the following may be concluded:Considering certain difficulties with organization of non-sibling transplantation of bone marrow in the Republic of Kazakhstan, one of the main tasks at this stage of development of hematological practice in the Republic is developing the haploidentical bone marrow transplantation

PB2474

ELTROMBOPAG FOR THE TREATMENT OF REFRACTORY PURE RED CELL APLASIA (PRCA) AFTER MAJOR ABO INCOMPATIBLE HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

C.M. Dellacasa[1,*], A. Busca[1], S. Aydin[2], G. Iovino[2], L. Giaccone[1], L. Brunello[2], V. Tassi[3], T. Francisci[3], C. Pecoraro[3], S. Manetta[2], L. Godio[4], B. Bruno[1]

[1]Stem Cell Transplant Unit, [2]Hematology, [3]Transfusion Unit, [4]Pathology, AOU Città della Salute e Della Scienza di Torino, Torino, Italy

Background: Pure red cell aplasia (PRCA) occurs in 7,5% to 16% of AB0 major mismatched allogeneic HSCT recipients. Several treatments have been applied but their results are largely variable.
Aims: To describe the use of eltrombopag for the treatment of refractory PRCA after HSCT.
Methods: We describe two PRCA patients resistant to multiple therapeutic options, treated with thrombopoietin mimetic eltrombopag (used off label) at the dose of 75 mg qd for the first two weeks and then increased to 150 mg qd. Both patients received a major ABO-incompatible HSCT (donor A pos, recipient O pos) after myeloablative conditioning regimen. In both cases RBC reduction of the apheretic stem cell product was not performed due to the low hematocrit value.
Results: Patient 1: A 48-year old male with high risk acute myeloid leukemia underwent matched unrelated donor (MUD) HSCT. Pre-transplant anti-A isohemoagglutinin titer was 1:512. Neutrophil engraftment occurred on day + 16; subsequently the patient developed transfusion dependent anemia, and on day +100 the bone marrow biopsy documented the presence of a PRCA with full donor chimerism. The patient was treated with theta-erythropoietin (rHuEPO) 40000 U per week and subsequently received 5 plasma exchange (PEX) procedures without significant response; from day + 234, 4 weekly doses of Rituximab 375 mg/m2/week were administered without any beneficial effect. At 6 months post-HSCT, iron chelation therapy (ICT) with deferasirox (DFX) was started and discontinued after 3 months due to grade III neutropenia. At 14 months post-HSCT, eltrombopag (ETP) was started due to persistent transfusion dependent anemia along with mild leukopenia and thrombocytopenia, leading to rapid improvement of blood cell counts. After 4 months of treatment, ETP was discontinued with an haemoglobin value of 15,4 gr/dl. At the present, the patient is off treatment since 6 months with normal haemoglobin levels and neutrophil counts. The bone marrow biopsy shows a normal cellularity (80%) with good trilinear representation and no evidence of clonal evolution. Patient 2: A 47-year old female with biphenotypic acute leukemia received a MUD HSCT. Patient's anti-A isohemoagglutitin pre transplant titer was 1:256. After engraftment, on day +47 treatment with rHuEPO 40000 U per week was started due to transfusion dependent anemia, with no remarkable response. A bone marrow biopsy on day + 100 was consistent with the diagnosis of PRCA. Chimerism was full donor. PEX procedures were discontinued due to a severe anaphylactic reaction to plasmatic proteins. Rituximab (375 mg/sqm/week for 4 weeks) and 4 doses of Bortezomib (1,4 mg/mq once a week) followed by one dose of Cyclophosphamide (1 gr) were administered without any significant response. At 5 months post HSCT ICT with DFX was started, but stopped after six months due to neutropenia, and replaced by DFO. At 30 months post transplant ETP was started. After 5 weeks the patient became transfusion independent. ETP dose was reduced due to mild thrombocytosis. After 5 months of treatment the bone marrow biopsy showed a moderate trilinear hyperplasia with no evidence of clonal evolution. After 6 months of treatment, ETP was stopped, and the patient mantains normal blood cell counts.
Summary/Conclusion: Our results, even if restricted to a very small proportion of patients, may be considered indicative of a favorable effect of ETP on post transplant unilineage cytopenias such as PRCA. Additional studies are mandatory to confirm our preliminary data.

PB2475

THE OUTCOMES OF HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR THALASSEMIA MAJOR BASED ON A FBCA CONDITIONING REGIMEN

B. Wu[1,*], R. Dong[2], Q. Su[3], J. Xu[4]

[1]Hematology, Shunde Hospital of Southern Medical University, Shunde, [2]Hematology, Shunde Hospital of Southern Medical University, Shunde Hospital of Southern Medical University, [3]Hematology, The first people hospital of Guangzhou, [4]Hematology, Zhujiang hospital of Southern medical university, Guangzhou, China

Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is the sole available curative therapy for patients with thalassemia major. With the progress human leukocyte antigen (HLA) antigen typing technology and supportive care, the outcome of thalassemia major have greatly improved in recent years, even in high risk patients. However, the problem of finding a suitable donor was still a major limit to cure these patients. In the past decades, haploidentical donor was more and more used in hematologic malignancies HSCT. Here, we explored the outcome of haploidentical donor (HD) HSCT to thalassemia major children based on a FBCA conditioning regimen.
Aims: To anlysis the outcomes of haploidentical hematopoietic stem cell transplantation for thalassemia major based on a FBCA conditioning regimen.
Methods: Eghit patients with thalassemia major (age 3-14 year old, meidan 5.5 year old) underwent haploidentical hematopoietic stem cell transplantation. The conditioning regimen was consists of fludarabin(Flu), busulfan(Bu), cyclophosphamide(Cy) and antithymocyte globulin(ATG). All donors were HLA mismatched family members including fathers mothers and sister. ABO blood type between donor and receipt were incompatible in three patients.GVHD prophylaxis included cyclosporine (CSA), and short course of methotrexate (MTX). Chimerism studies were performed with blood nucleated cells whth STR-PCR.
Results: The median of nucleated cell and CD34+ cell dose in the infused product were 9.7×10^8/kg (range, $6.9\text{-}26.7\times10^8$/kg) and 10.1×10^6/kg (range, $8.2\text{-}27.2\times10^6$/Kg), respectively. The median time to achieve absolute neutrophil recovery was in 10 days (range,10 to 15 days), and platelet recovery was in 13 days (range,10 to 102 days).Four patients (50%) have experienced grade I-II acute GVHD; Two patients suffered from grade III-IV (25%) acute GVHD and one of which turned into local chronic GVHD (skin).Chimerism studies were performed for all patients after transplantation. The data showing all patients have achieved full donor chimerism (100%) at post-transplantation +30 day and mixed chimerism status was not observed in all eight patients with the mdeian one year follow up. So far, all of the patients have a stable engraftment and were transfusion independent in daily life.
Summary/Conclusion: The acute and chronic GVHD of haploidentical hematopoietic stem cell transplantation for thalassemia major based on this FBCA conditioning regimen is accpitable and the outcome prompt us to study further.

PB2476

AUTOLOGOUS STEM-CELL TRANSPLANTATION AS FIRST-LINE TREATMENT OF ELDERLY PATIENTS WITH A NEWLY DIAGNOSED MULTIPLE MYELOMA IN THE ERA OF THE NOVEL DRUGS

V. Cabanas Perianes*, M. Moya, B. Navarro, M.J. Moreno, A. Martinez Marin, E. Fernandez Poveda, M. Berenguer, J.J. Cerezo, R. Perez Lopez, A. Sanchez Salinas, A.M. Garcia Hernandez, J. Monserrat, M. Blanquer, J.M. Moraleda

Hematology, Hospital Universitario Virgen De La Arrixaca, Murcia, Spain

Background: We analyzed 24 symptomatic newly diagnosed Multiple Myeloma (NDMM) patients who were 65 years or older receiving induction therapy with novel agents plus ASCT.
Aims: The objectives were to assess the toxicity and efficacy in two cohorts: elderly patients (≥70 years or older) *versus* a younger group (65-69 years).
Methods: The endpoints were: overall response rate (ORR), progression free survival (PFS) and overall survival (OS), adverse effects, time to platelet and neutrophils engraftment and time to discharge. The dose of melphalan conditioning employed were 200 or reduced doses (140, 100 or 70mg/m^2 in tandem) if age ≥70 years, renal failure or Hematopoietic Cell Transplantation-Comorbidity Index over 2 were presented at ASCT moment.
Results: 14 patients ≥70 y and 10 patients between 65-69 years were reported. ORR an OS rate at 1 year was 100%. No differences were observed in terms of time to reach platelets and neutrophils engraphtments, time to discharge and adverse effects. After a median follow-up of 23.5 months, the median PFS was 32 months in the older group, as compared with 43 months in the group down 70 years underwent ASCT (p=0.97).

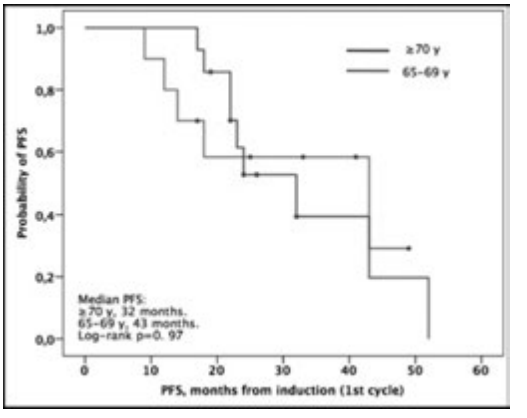


Figure 1.

Summary/Conclusion: ASCT is feasible and safe in NDMM patients aged ≥70 years if an individualized approach is performed.

PB2477

INFLUENCE OF LENALIDOMIDE USED IN INDUCTION PERIOD AND INTENSITY OF MOBILIZATION REGIMEN ON THE NUMBER OF CD34+ CELLS HARVESTED IN PATIENTS WITH MULTIPLE MYELOMA

I. Kostroma[1,*], A. Zhernaykova[1], Z. Chubukina[2], I. Zapreeva[1], N. Semenova[3], S. Bessmeltsev[4], A. Chechetkin[4], S. Gritsaev[1]

[1]Bone marrow transplantation department, [2]Immunologic laboratory, [3]Research laboratory of leukemia, [4]Hematological clinic, Russian Institute of Hematology and Transfusiology, St.Petersburg, Russian Federation

Background: Recovery of hematopoiesis after autologous stem cell transplantation (AutoSCT) depends on the count of the harvested CD34+ cells. It is supposed that count and proliferative capacity of mobilized stem cells are influenced by the kind of previous therapy and variety of chemotherapy agents used in the mobilization regimen.
Aims: The aim of the study was to determine prognostic significance of premobilization lenalidomide apply and the sort of mobilizatrion regimen (cyclophosphomide or vinorelbine) on the number of CD34+ cells in the autotransplant of multiple myeloma (MM) patients.
Methods: We examined the data of autotransplant harvesting in 68 patients retrospectively. Mobilization regimens with vinorelbine 35mg/m^2 (23 patients) and cyclophosphamide 3.0g/m^2 (45 patients) were used. 21 patients (30.9%) were treated with lenalidomide previously. In the group of patients treated with lenalidomide 14 patients were mobilized with vinorelbine and 7 patients with cyclophospamide.
Results: Mobilization failure with the number of CD34+ cells less than $2\text{x}10^6$/kg was fixed in 5 patients (7.4%). There was no any difference in CD34+ cells number in groups of patients according to the age and MM variant. We found the trend with decreasing of CD34+ cells number in patients treated with lenalidomide: $4.1\text{x}10^6$/kg (0.27-23.29) *vs* $6.76\text{x}10^6$/kg (0.58-29.13) in patients who were not treated with lenalidomide; p=0.066. There was significant difference in the count of CD34+ cells harvested after cyclophospomide plus G-CSF and vinorelbine plus G-CSF: $6.8\text{x}10^6$/kg (0.53-29.13) *vs* $3.96\text{x}10^6$/kg (0.27-9.66) accordingly; p=0.022.
Summary/Conclusion: We conclude that vinorelbine plus G-CSF is a mobilization regimen of choice for older patients who were not treated with lenalidomide aggressively in the premobilization period and for whom the single AutoSCT is planned.

PB2478

AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH AGGRESSIVE NON-HODGKIN'S LYMPHOMA

N. Zorina[1,*], N. Minaeva[2], M. Khorobrykh[1], P. Sherstnev[3], S. Utyomov[4], N. Isaeva[5], A. Luchinin[6]

[1]Bone marrow transplantation, [2]Hematologic clinic, [3]Transfusiology, [4]Cryopreservation of blood and tissues, [5]Immunohematology, [6] Clinical research department, Federal State Institute of Science "Kirov Research Institute of

Hematology and Blood Transfusion of the Federal Medical and Biological Agency of Russia", Kirov, Russian Federation, Kirov, Russian Federation

Background: High dose chemotherapy with autologous hematopoietic stem cell transplantation (autoHSCT) in combination with targeted medications is widely used in prognostically unfavorable variants of non-Hodgkin's lymphoma (NHL) treatment.
Aims: To estimate the results of autoHSCT in patients with NHL with unfavorable course.
Methods: 48 autoHSCT to 42 NHL patients (18 – female, 25 – male, age 22-69 years, median 37,5 years) were performed from 1999 till February 2018. Of them: diffuse large B-cell lymphoma (DBCL) in PR1/CR2 – 21 patient, mantle cell lymphoma (MCL) in PR 1/2 – 8, follicular lymphoma (FL) 3B type in CR2 – 4 patients, rimary mediastinal lymphoma – 6 patients,peripheral T-cell lymphoma – 5 patients. Patients were treated with more, than 2 lines of chemotherapy. Hematopoietic stem cells (HSC) were harvested after the second line therapy with G-CSF stimulation. Six patients received 2 autoHSCTs (DBCL 4B in PR1 – 4, FL type 3 in PR2 FLIPI2 -1, primary mediastinal lymphoma – 1). Conditioning regimen BendaEAM was used in 39% of cases, BEAM - in 37,25%, CVB - in 10%, ICE - in 13,75% (plus rituximab in 69,6% autoHSCTs). Transplant – cryopreserved HSCs, counting (2-21)x10^6/kg body weight CD34+ cells, (2,8-5,1)x10^8/kg myelokaryocytes. Leukocyte recovery more, than 1x10^9/l was achieved on 14 day, platelet count more, than 30x10^9/l – on day 17. Most of patients with B-cell lymphomas received 2 year rituximab maintenance therapy after autoHSCT.
Results: At time point February 2018 29 patients were alive, 10 died due to lymphoma progression, 3 – because of infection complications. Transplant failure was revealed in 5,4% of patients, 100 days mortality was 5.4%. Overall 3-year survival (OS) was 73%, 5 – year survival – 67%, 10– year survival – 58%. Overall survival median was 12,7 years. Relapse free 3 – year survival (RFS) was 73%. Median relapse free survival was 98 months. DBCL patients had 3 – year OS 75%, 5 – year OS – 70%, 10-year OS – 63% and median OS 12,7 years. MCL patients had 3-year OS of 75%, 5 year OS – 75%, median OS was not achieved. All patients, who received repeated autoHSCT are alive, in PET-negative CR with surveillance median of 40 months. The most common complications in post-transplant period were 1-2 grade mucositis – 81% of patients. Septic complications were revealed in 8.1% of cases, pulmonary aspergilosis – in 4%. Frequency of infections did not increase after repeated autoHSCT, engraftment occurred at usual time point.
Summary/Conclusion: AutoHSCT method is an effective therapeutic option for patients with high risk NHL.

PB2479

INITIAL THERAPY AND OUTCOMES AFTER STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA PATIENTS

S. Franco*, J. Borras, R. Del Campo, A. Cladera, J.M. Guerra, D. Gómez, I. Herraez, N. Amer, M. Mascaró, J. Bargay

Hematology, Hospital Son Llàtzer, Palma de Mallorca, Spain

Background: Infections are a major cause of morbidity and mortality in patients with Multiple Myeloma (MM). MM related immunodeficiency involves B-cell dysfunction as well as T-cell, dendritic cell, and NK-cell abnormalities. In addition to the immunodeficiency related to myeloma, the type of anti-myeloma therapy used plays a role in the development of infections.
Aims: To compare transplant outcome in terms of risk of infection between MM patients treated with 2 different frontline therapies.
Methods: Out of 87 MM patients from Hospital Son Llàtzer who underwent ASCT from March 2003 to February 2017, we performed a retrospective observational study involving 35 of them. Exclusion criteria: patients with Plasma cell leukemia, concomitant diagnosis of Amyloidosis AL and those who received Busulfan-Melfalan as conditioning regimen. Patients were separated into two groups depending on its frontline therapy (arm A: VBCMP/VBAD *vs* arm B: novel agent combinations). Molecular cytogenetic alterations were not included because data for these were not available in all patients. Hematologic recovery was defined as an absolute neutrophil count ≥500/mm^3 and platelets ≥20,000/mm^3. We analyzed all infections occurred within the first year post-ASCT. In all patients the intensive regimen consisted of Melphalan 200mg/m^2. Data were collected from electronic medical records. Quantitative variables were expressed as median and interquartile range, and the qualitative variables as percentages ($p<0.05$ was considered statistically significant). Differences between groups were analyzed with the Mann-Whitney U test and Chi-square.
Results: Out of 35 patients analyzed, 19 received poly-chemotherapy as frontline treatment, and 16 patients received combinations of novel agents. No differences were observed between groups in regard to age at the time of transplantation (60.9 years old for group A *vs* 59 years old for group B) and HCT-CI. In regards to response before ASCT, the rate of very good partial response or better was slightly higher for group B (86.6% *vs* 63.2%), *p=0.122.* Median CD34+ infused cells was also similar between groups A and B (3.4.10^6/kg *vs* 3.5. 10^6/kg, respectively. There was no significant difference in hematological recovery (12 *vs* 11.5 days) or development of mucositis (94.7% *vs* 100%). Of 35 patients, 19 (54.3%) developed at least one infectious complication. Although not statistically significant, the frequency of infections was higher in group A (68.4%) than in group B (37.5%), *p=0.067.* Median time to first infectious episode was 152 days in group A and 79.5 days in group B, *p=0.282.* Respiratory infections were the most common infections in both groups (33% *vs* 45.5%), *p=0.236.* Of note, 3 patients (16.7%) in group A required hospitalization during the first infectious episode *vs* none in group B, *p=0.153.*
Summary/Conclusion: Infection is a significant cause of morbidity and death in patients with MM. In this analysis, out of 35 patients, 19 (54.3%) developed at least one infectious complication during the first year after ASCT. Although not statistically significant, the frequency of infections and hospitalization was higher in the group that received poly-chemotherapy, suggesting that poly-chemotherapy might induce more immunosuppression. These results are limited because of the small number of patients included.

Table 1.

Table 1. Baseline and outcomes

Parameter	Both groups N=35	Group A N=19	Group B N=16	p value
Sex	Male: 21(60%) Female:14(40%)			
Durie-Salmon		I-3 (15,8%) II-3 (15,8%) III-13 (68,4%)	I-0 II-6 (37,5%) III-10 (62,5%)	0.125
ISS		1=5 (26,3%) 2=10 (52,6%) 3=4 (21,1%)	1=6 (37,5%) 2=6 (37,5%) 3=4 (25%)	0.657
Cr > 2 mg/dl		7 (36,8%)	4 (26,7%)	0.529
Median age at ASCT		60,9 (51,6-72,9)	59 (42,9-68,3)	0.58
Pre-ASCT response		VGPR or better=12 (63,2%)	VGPR or better=13 (86,6%)	0.122
Median HCT-CI		0-1=12 (63,2%) 2-3=4 (21%) 4->=3 (15,8%)	0-1=9 (56,2%) 2-3=5 (31,2%) 4->=2 (12,6%)	0.67
Median CD34+ infused cells		3,4 (1,9-4,8)	3,5 (1,7-7)	1
Hematological recovery median (days)*		12 (10-27)	11,5 (10-13)	0.131
Post-ASCT infection	19 (54,3%)	13 (68,4%)	6 (37,5%)	0.067
Days until first infection reported		152 (19-307)	79,5 (28-194)	0.282

ISS=International Score System; ASCT: autologous stem cell transplantation; HCT-C: hematopoietic cell transplantation co-morbidity index

PB2480

THE ROLE OF MYELOID-DERIVED SUPPRESSOR CELLS IN AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

A. Betsch[1,*], O. Rutgeerts[1], S. Fevery[1], B. Sprangers[2], D. Dierickx[2], M. Beckers[2]

[1]KU Leuven, [2]University Hospitals of Leuven, Leuven, Belgium

Background: Myeloid-derived suppressor cells (MDSC) are a heterogeneous group of immature, immunosuppressive cells that play a role in cancer induction, progression and immune evasion. Two major MDSC subsets exist: polymorphonuclear (PMN-MDSC) and monocytic MDSC (M-MDSC). Recent studies indicated that the immune reconstitution after autologous hematopoietic stem cell transplantation (ASCT) is associated with outcome. Since MDSC are immunosuppressive cells, we hypothesize that they play a role in the immune reconstitution and outcome after ASCT.
Aims: The aim of this study is to define the presence and function of MDSC in ASCT. Therefore, we will phenotypically and functionally analyze different MDSC subsets. Since the content of the peripheral blood stem cell (PBSC) graft is less defined, we will analyze MDSC and other immune cells in the PBSC graft. In addition, the immune reconstitution after ASCT will be studied in relation to the amount of MDSC infused and the kinetics of MDSC after ASCT will be assessed.
Methods: Flow cytometry is used to study the presence of MDSC in peripheral blood mononuclear cells (PBMC) and in the PBSC graft of multiple myeloma and lymphoma patients, undergoing ASCT.
Results: The kinetics of MDSC levels are similar between M-MDSC and PMN-MDSC during the transplantation period. MDSC levels in PBMC and

in the PBSC graft increased after G-CSF mobilization, together with an increased IL-4Rα expression on MDSC. At the time of ASCT, following high-dose chemotherapy, MDSC levels had decreased. After ASCT, MDSC levels recovered again. Interestingly, the PMN-MDSC/M-MDSC ratio was lower in the PBSC graft, compared to PBMC.
Summary/Conclusion: Recent findings have provided proof that immune reconstitution plays a key role in the therapeutic effect of ASCT. Since MDSC are known to be immunosuppressive cells that accumulate after G-CSF mobilization protocols, they may play a role in the immune reconstitution and outcome after ASCT. Indeed, after G-CSF mobilization, both M-MDSC and PMN-levels were increased. Interestingly, IL-4Rα expression on MDSC increased, suggesting a higher immunosuppressive activity of MDSC. In addition, the PMN-MDSC/M-MDSC ratio was decreased in the PBSC graft as compared to PBMC. This may suggest that in the PBSC graft less PMN-MDSC and/or more M-MDSC, which are considered to be the most immunosuppressive population, are present. Further *ex vivo* assays will be performed to study the immunosuppressive capacity of MDSC in PBMC and in the PBSC graft. Moreover, more patients will be included to further study the role of MDSC in ASCT.

PB2481

ABO INCOMPATIBLE BONE MARROW TRANSPLANTS – A TERTIARY CENTRE EXPERIENCE

S. Sen[1,*], S. Tulpule[2], S. Gupta[2], K. Mullanfiroze[1], R. Sawant[3]

[1]Dept of Paediatric Oncology & BMT, [2]Dept of Oncology & BMT, [3]Dept of Transfusion Medicine, Kokilaben Dhirubhai Ambani Hospital, Mumbai, India

Background: Though ABO incompatibility is not a contraindication for BMT, it can be a factor responsible for lower overall survival.
Aims: We present our data on ABO incompatible transplants and their outcomes.
Methods: Of the 87 BMT's done at our centre, 14 were ABO incompatible. The indications for BMT were malignancies like refractory AML or relapsed B ALL and benign haematological conditions like Aplastic Anaemia and Thalassemia Major. Among the ABO incompatible BMTs, 9 were major incompatibility, 5 were minor. For all major and bidirectional blood group incompatibility, we determined the recipient antibody titres, and in all cases where the recipient isoagglutinin titre was more than 1:64, repeated plasma exchange was done till antibody titres were less than 1:16. IVIG in a dose of 0.5 gm/kg was also given to the recipient on Day -1. Red cell depletion of the graft by centrifugation was also undertaken in all cases of major ABO incompatibility.
Results: One patient with major ABO incompatibility had a severe haemolytic reaction following infusion of bone marrow stem cells and transplant had to be aborted. The baseline isoagglutinin level was 1;64 and repeat plasma depletion of the patient was done to reduce the isoagglutinin levels to below 1:4. Peripheral blood stem cells were harvested from the donor which was red cell depleted by centrifugation and this was infused without incident. None of the other cases had any immediate or delayed haemolytic reaction. However 1 patient subsequently died due to graft failure and 2 from sepsis.
Summary/Conclusion: In our series, ABO incompatibility did not seem to have been a major factor behind poor outcome. The mortality in our limited series is possibly due to the aggressive nature of the primary disease like refractory AML and patient condition rather than blood group mismatch per se.

PB2482

THE ROLE OF AUTOLOGOUS STEM-CELL TRANSPLANTATION IN RELAPSED MULTIPLE MYELOMA. OUR SIX YEAR EXPERIENCE

B. Navarro Almenzar*, V. Cabañas Perianes, M. Blanquer Blanquer, M. J. Moreno Belmonte, J.J. Cerezo Manchado, R. Pérez López, M. Berenguer-Piqueras, F. Labbadia, E. Fernández Poveda, A. Martínez Marín, M. Moya Arnao, J.M. Moraleda Jiménez

Hematology, Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain

Background: The role of autologous stem-cell transplantation (ASCT) in relapsed multiple myeloma has not been fully defined and more data are needed.
Aims: To assess the effycacy of second ASCT in patients with relapsed multiple myeloma (MM).
Methods: In this retrospective analysis, we reviewed the charts of the patients who underwent a second ASCT at least 12 months after their first ASCT for relapsed MM at our institution between March 2011 and July 2017. Progression free survival after ASCT, progression free survival after chemotherapy and overall survival were analyzed by the Kaplan-Meier method.
Results: Twenty patients (12 male, 8 female) with a median age of 62 years (48-73) were included. Patients were classified according to the type of myeloma: IgG 8 (40%); IgA 6 (30%); Bence-Jones 5 (25%); IgD 1 (5%). Adverse cytogenetics were present in 10% of the patients, including delp53, t(4; 14) and t(14; 16). HCT-CI was low (<3) in 74% of them: 0 (63%), 1 (11%), 3 (21%), 4 (5%). The median time from the diagnosis of multiple myeloma to the second transplant was 54 months (25-127). The median time from the first transplant to disase was 32.5 months (12-103). The progression-free survival after the first ASCT was <24 months in 30% of patients. PET-CT at the relapse was positive in 14 (71%) patients, and 7 patients (35%) had plasmacytomas. The median number of previous lines of treatment before second ASCT was 2 (2-4). Bortezomib had been administered to 95% of the patients, and immunomodulators to 60%. The last treatment received before the transplant included Lenalidomide in 50% of the cases. The response after salvage therapy prior to transplantation was: stringent complete response 3 (15%); complete response 3 (15%); very good partial response 8 (40%); partial response 6 (30%). Conditioning regimen was Melphalan in 15 patients (75%) and Busulfan-Melfalan in 5 patients (25%). Transplant related mortality was 5%. The second transplantation significantly improved the stringent complete response rate (from 15% to 55%) (p=0.008).). With a median follow up of 33,5 months, the median progression free survival after the second ASCT was 30 months, and 37 months after progression. The progression free survival benefit of the second ASCT was confirmed in all risk subgroups: high risk cytogenetic, time from the first ASCT to relapse <24 m, plasmacytoma at relapse, or response after second ASCT.
Summary/Conclusion: Our results confirm that second ASCT significantly increases the number of stringent complete responses in multiple myeloma patients relapsed after a first ASCT. Studies with larger sample of patients are needed in the era of the novel drugs.

PB2483

HAPLOIDENTICAL BONE MARROW TRANSPLANTS – A TERTIARY CENTRE EXPERIENCE FROM THE DEVELOPING WORLD

S. Sen[1,*], S. Tulpule[2], S. Gupta[2], K. Mullanfiroze[1]

[1]Dept of Paediatric Oncology & BMT, [2]Dept of Oncology & BMT, Kokilaben Dhirubhai Ambani Hospital, Mumbai, India

Background: Bone Marrow Transplantation (BMT) can be a life saving procedure in many conditions, however the lack of a HLA matched sibling or fully matched unrelated donor can be an impediment to proceed for a transplant. Haploidentical transplants using stem cells from familial donors who are only half matched to the recipients has become increasing popular in this context. This is especially relevant in the Indian subcontinent due to the lack of any large stem cell registries and costs associated with obtaining cells from overseas registries.
Aims: Here we report on our experience of haploidentical BMT in our tertiary BMT centre.
Methods: From February 2014 to September 2017, 20 haploidentical bone marrow transplantations were done in 19 patients. The age range was 5 months to 27 years with a median of 9 yrs. 14 male and 5 female patients underwent haploidentical BMT for indications ranging from malignant diseases such as relapsed AML or ALL, CML to benign diseases like Thalassemia Major, Chronic Granulomatous Disease and Aplastic Anaemia. The donors were parents or immediate siblings who were haploidentical to the recipients.
Results: 3 of the 19 died due to transplant related toxicity. 1 child with aplastic anaemia had a first transplant from the father had a graft rejection and a second successful haploidentical BMT was done with the mother as the donor. All received T cell replete stem cells with Post Transplant Cyclophosphamide, MMF and a calcineurin inhibitor for GVHD prophylaxis. All but one (who received bone marrow stem cells) were transfused GCSF stimulated peripheral blood stem cells.
Summary/Conclusion: With a median follow up period of 8 months our data is still young, however, with an overall survival of 84%, haploidentical bone marrow transplant is a promising new option available for those who lack other appropriate donors.

PB2484

MECHANISTIC ANALYSIS OF CYTOKINE RELEASE SYNDROME AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION FOLLOWING IMMUNE CHECKPOINT BLOCKADE; ABERRANT ACTIVATION OF CD8 CTL BEYOND CONTROL OF REGULATORY T CELL

Y. Usui*, Y. Meguri, M. Iwamoto, Y. Sando, M. Sakamoto, Y. Fujiwara,

T. Kondo, K. Tani, K. Saeki, S. Okamoto, N. Asada, H. Nishimori, N. Fujii, E. Kondo, Y. Maeda, K.-I. Matsuoka

Department of Hematology and Oncology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceuticals Sciences, Okayama, Japan

Background: Cytokine release syndrome (CRS) is a severe toxicity which is associated with the elevation of inflammatory cytokines and presumably with the activation of T cells. PD-1 is one of immune checkpoint molecules expressed on activated T cells and its immune regulation is thought to be the important target for anticancer therapy. Recent studies reported that patients received allogeneic stem cell transplantation (Allo-SCT) after PD-1 blockade often developed severe CRS followed by refractory GVHD. However, the safety of the treatment sequence of PD-1 blockade and autologous stem cell transplantation (Auto-SCT) has not been reported so far.
Aims: We demonstrate a case study about severe CRS after Auto-SCT with pretreatment by Nivolumab to characterize the abnormality of lymphoid immune reaction and clinical findings after the therapeutic combination.
Methods: A 63-year-old man was diagnosed with classical Hodgkin lymphoma (HD). His disease was resistant to several lines of chemotherapies including ABVD, DHAP, IDEA (Ifosphamide, Dexamethasone Etoposide and Cytarabine) and Brentuximab Vedotin. However, the disease was progressed with the enlargement of left subclavian lymph node. He received 22 courses of Nivolumab and achieved PR again. Consequently, he received Auto-SCT with LEED. On Day 5, he became febrile to 38.5 and was administered anti-biotics as febrile neutropenia. On Day 6, body temperature rise of up to 40 , erythema of the whole body appeared, and vasopressor was administered due to low blood pressure. On Day 7, since there were few findings suggestive of infection from cultural examinations and imaging tests, it was considered to be a non - infectious fever and we diagnosed as CRS. Symptoms included decreased oxygenation, decreased urine volume, declined renal function (creatinine 2.03mg/dl), increased CRP (21.49mg/dL), increased procalcitonin (63.6ng/mL). Corticosteroid could not improve severe CRS, therefore, on Day 9, Tocilizumab 4mg/kg was administered as CRS. An antipyretic effect was observed immediately after administration, and general condition was gradually improved. On Day 11, neutrophil engraftment was achieved.
Results: We examined the lymphocyte subset of CD4 T, CD8 T, $Foxp3^{+}Helios^{+}$Treg and their expressions of CD62L and CD45RA. The CD8/CD4 ratio was increased at the peak of CRS (68.4%/15.3% of lymphocytes) as compared to the baseline (18.5%/42.2%). Both CD4 and CD8 T cells at CRS dominantly consisted of $CD62L^{-}CD45RA^{-}$Effector-memory phenotype but it was more evident in CD8 T cells (52.3% in CD4 T, 79.5% in CD8 T). After the treatment with Tocilizumab, CD8/CD4 ratio decreased (26.6%/14.9%) and $CD62L^{-}CD45RA^{-}$ Effector-memory phenotype also decreased (22.7% in CD4 T, 64.7% in CD8 T).%$Foxp3^{+}Helios^{+}$Tregs of CD4 T cells was stable in the peritransplant period (9.1% before SCT, 8.3% at the point of CRS and 8.3% 1 month after SCT).
Summary/Conclusion: These results suggested that the sequence of PD-1 blockade and Auto-SCT could induce the aberrant enhancement of CD8-positive effector-memory cytotoxic T cells regardless of the adequate amount of Tregs leading to severe CRS and anti-IL-6 therapy can restore the abnormal lymphocyte activation and CRS symptoms. The careful monitoring of early T cell reconstitution after Auto-SCT following PD-1 blockade might provide a novel strategy to predict and control excessive immune reaction leading to severe CRS.

PB2485

RESCUE HAPLOIDENTICAL STEM CELL TRANSPLANTATION FOR APLASTIC ANAEMIA FOLLOWING PRIMARY GRAFT FAILURE

S. Sen[1,*], S. Tulpule[2], S. Gupta[2], K. Mullanfiroze[1]

[1]Dept of Paediatric Oncology & BMT, [2]Dept of Oncology & BMT, Kokilaben Dhirubhai Ambani Hospital, Mumbai, India

Background: Primary graft failure remains one the important causes of mortality and morbidity in cases of stem cell transplantation. We report on a case of Aplastic Anaemia where an immediate rescue haploidentical stem cell transplantation from a different donor resulted in a successful engraftment.
Aims: To determine whether an immediate transplantation with repeat conditioning chemo-radiation therapy can result in a successful engraftment in a case of primary graft failure.
Methods: A 3yr old male child was diagnosed to have Severe Aplastic Anemia. Unfortunately there were no matched sibling donors and hence a haploidentical transplant using his fathers stem cells was undertaken. He was conditioned with Fludarabine, Cyclophosphamide, ATG and TBI and Post Transplant Cyclophosphamide was used for GVHD prophylaxis. Unfortunately he failed to engraft, and at day +21 we undertook a second rescue haploidentical transplant for the primary graft failure. As he had rejected his fathers stem cells, we decided not to use father the second time around as a donor re-transplant him using his mother stem cells. Though he was profoundly neutropenic with a WBC count of 20, we decided to give him full repeat conditioning with the same protocol as we surmised that he still had a active immune system that had rejected the primary graft. Repeat conditioning using the same protocol was started from Day + 22 and mother's stem cells was infused on Day +28.
Results: He engrafted successfully by Day +12 of the second transplant and though the transplant was complicated by a cutaneous fungal infection and CMV reactivation, child was discharged home with 100% donor engraftment.
Summary/Conclusion: A second haploidentical transplant using repeat conditioning can be used successfully in cases of primary graft failure.

Stem cell transplantation – Experimental

PB2486

OBINUTUZUMAB WITH DHAP DID NOT IMPAIR PERIPHERAL STEM CELL HARVEST: RESULTS OF THE PHASE II PROSPECTIVE FIL GIOTTO TRIAL

L. Rigacci[1,*], B. Puccini[1], C. Visco[2], R. Battistini[3], L. Arcaini[4], M. Gotti[5], F. Elice[2], S. Kovalchuk[1], M. Mian[6], A. Di Rocco[7], L. Flenghi[8], S. Pozzi[9], F. Palombi[10], P. Viero[11], A. Evangelista[12], D. Gioia[13]

[1]Haematology, AOU Careggi, Firenze, [2]Cell Therapy and Hematology, San Bortolo Hospital, Vicenza, [3]Hematology, AO San Camillo Forlanini, Roma, [4]Molecular Medicine and Hematology Division, University of Pavia and IRCCS Policlinico S Matteo, [5] Hematology Division, IRCCS Policlinico S Matteo, Pavia, [6]Hematology-CBMT and Internal Medicine V, Hematology and Oncology, Hospital of Bolzano and Medical University of Innsbruck, Bolzano, [7]Hematology, University La Sapienza, Roma, [8]Hematology, Azienda Ospedaliera, Perugia, [9]Hematology, Medicina Diagnostica Clinica, Modena, [10]Hematology, IFO Regina Elena, Roma, [11]Hematology, Ospedale dell'Angelo, Venezia-Mestre, [12]Epidemiology, Unit of Clinical Epidemiology and CPO Piemonte, Torino, [13]Clinical Trial, Fondazione Italiana Linfomi, Alessandria, Italy

Background: Salvage immune-chemotherapy followed by autologous stem cell transplantation (ASCT) is the standard second line treatment for relapsed and refractory Diffuse Large B Cell Lymphoma (DLBCL). Second line immune-chemotherapy, rituximab plus platinum compound or rituximab plus ifosfamide containing regimens, were able to obtain response and permit sufficient harvest of peripheral stem cell.
Aims: The aim of this phase II study (GIOTTO study, Eudract: 2013-004014-17) was to evaluate the efficacy and safety of new anti-CD20 antibody (Obinutuzumab) in association with DHAP as induction therapy before high dose chemotherapy with ASCT in patients with relapsed/refractory DLBCL. One of the secondary objective of this study was to demonstrate the capacity of hematopoietic cell mobilization of this new immune-chemotherapic regimen.
Methods: Obinutuzumab was administered on day 1,8,15 in association with DHAP in the first cycle and on day 1 in association with DHAP for the other three cycles. Peripheral stem cell collection (PBSCC) had to be performed after the second or third cycle between 12 and 14 days.
Results: From June 2014 to June 2017 29 patients were enrolled, according to clinical characteristics 17 patients were refractory to first line therapy and 12 relapsed after first line treatment. Seventeen patients underwent peripheral blood stem cell (PBSC) mobilization while 12 did not because of disease progression during salvage therapy. Eight out 17 underwent PBSCC after the second cycle and 9 after third cycle. The median number of harvested CD34 positive cells was 5,8x10^6/Kg (range 2-12,24x10^6/Kg) and the median number of aphereses was 2 (range 1-4). No patients needed plerixafor administration. Eight patients were transplanted with a median number of 3,85x10^6/Kg CD34 positive cells and no engraftment failures were reported.
Summary/Conclusion: The use of Obinutuzumab did not impair peripheral stem cell harvest after salvage therapy in relapsed/refractory non Hodgkin's lymphoma.

PB2487

PRE-ANALYTICAL INVESTIGATIONS FOR ESTABLISHING A PROTOCOL FOR TREOSULFAN HANDLING AND ACTIVATION

I. El-Serafi[1,2,*], O. Loy[3], Y. Zhao[3], S. Oerther[3], J. Mattsson[2,4]

[1]Clinical and Experimental Medicine, Linköping University, Linköping, [2]Oncology-Pathology, [3]Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden, [4]Hematology, Oslo University Hospital, Oslo, Norway

Background: Treosulfan is an alkylating agent that is used for the treatment of ovarian cancer and for conditioning prior to stem cell transplantation. It is a prodrug that is activated non-enzymatically to two active metabolites.
Aims: To optimize a protocol for both *in vivo* samples handling and *in vitro* drug preparation.
Methods: Treosulfan stability was tested in biological fluids at different conditions as well as for plasma binding and drug cytotoxicity on cell lines.
Results: Plasma samples can be safely frozen for short period up to 8h, however; for longer periods, samples should be acidified. Urine samples and cell culture media can be safely frozen regardless the pH. For *in vitro* investigations, incubation of treosulfan at 37°C for 24h activated 100% of the drug. Treosulfan binding to plasma proteins was 10% and it was safe to separate the plasma from whole blood without affecting treosulfan concentrations. Whole blood acidification should be avoided for the risk of hemolysis. Finally, the HL-60 cell line was more sensitive to treosulfan compared to K562 cell line and the drug cytotoxicity increased following pre-incubation for 24h at 37°C.
Summary/Conclusion: The stability profiling of treosulfan provided a valuable reference for proper handling of biological samples for both *in vivo* and *in vitro* studies. These results can be utilized as a corner stone for further investigations that will enable better understanding for the drug kinetics and dynamics. Such studies are required in order to personalize treosulfan treatment, reduce its toxicity and improve the clinical outcome.

PB2488

HLA ANTIGEN AND HAPLOTYPE DISTRIBUTION IN MALAYSIAN CANDIDATES FOR HEMATOPOIETIC STEM CELL TRANSPLANTATION

K. Tey[1,*], P.C. Bee[2], Z. Mohamed[1], S. Mohd Zain[1], H.S. Eng[1]

[1]Pharmacology, University of Malaya, [2]Haematology, University of Malaya Medical Centre, Kuala Lumpur, Malaysia

Background: Histocompatibility matching of human leukocyte antigens (HLA) between recipient and their family members is essential to identify suitable donors for allogeneic hematopoietic stem cell transplant (AHSCT). Data on HLA antigen and haplotype frequencies may allow better prediction of the probability of finding HLA-matched donors and consequently enhance the donor search strategy. To our knowledge this is the first haplotype frequency report in Malaysia.
Aims: This study aims to determine the distribution of HLA antigens and haplotypes in patients who had undergone HLA typing for AHSCT in University of Malaya Medical Centre and to review the outcome of the transplant. This study aims to determine the prevalence of anxiety and depression of all cancer patients and their caregivers in a teaching institution in Malaysia.
Methods: This retrospective study involves the analysis of HLA typing results of patients and their potential donors who had undergone HLA matching in University of Malaya Medical Centre from 2003 to 2017. The patients and donors' HLA typing results were collected through their laboratory reports. The Centre routinely tests for HLA antigen/allele group by intermediate resolution solid phase assay. Only the siblings of the patients were tested in view of the patient require full HLA matched donor for transplantation. HLA typing for HLA-A, -B, -DR, and -DQ from each patient and potential donor were collected to derive the haplotype present in the patient's family. The frequency of recurring haplotype was then directly counted and recorded.
Results: A total of 143 patients and their family members (mean no. of siblings=2.7, SD±1.4) were recruited for this study. Majority of the patients were Chinese (62.9%), followed by Malay (29.4%), and Indian (4.9%). While 81 (56.6%) patients found HLA-matched donors, only 42 (29.4%) patients proceeded with AHSCT. Majority of the Malays found a match within their family (69%), followed by Indians (57%), and Chinese (52%). 53.2%, 51.7%, and 25% of Chinese, Malays, and Indians respectively from the matched group proceeded with transplant. No significant association was found between number of siblings screened and number of HLA-matched siblings (p=0.71). Of the 42 patients who proceeded for AHSCT, 47 haplotypes were successfully derived from 11 patients and their families. Of the 47 haplotypes determined, 12.8% were haplotype HLA-A*33-B*58-DRB1*17-DQB1*02, while HLA-A*01-B*27-DRB1*12-DQB1*05:01, HLA-A*02-B*13-DRB1*15-DQB1*06, HLA-A*02-B*46-DRB1*08-DQB1*06, HLA-A*02-B*60-DRB1*09-DQB1*09, HLA-A*11-B*39-DRB1*08-DQB1*06, HLA-A*24-B*15-DRB1*12-DQB1*07 were each 4.26% respectively. No disease relapse occurred within the haplotype determined group, however one death was reported post-transplant. The most frequent antigens observed from the patients and their families of the transplanted group was HLA-A*11 (31.9%), HLA-B*58 (23.4%), HLA-DRB1*12 (19.1%), and HLA-DRB1*15 (19.1%), and HLA-DQB1*6 (27.7%).
Summary/Conclusion: Malaysia is comprised of a multi-ethnic population in which the haplotype distribution may vary among ethnic groups. Though the results suggested the seven most common haplotypes, a larger scale study in healthy population shall be performed to validate the findings.

Thalassemias

PB2489

GLOMERULAR DYSFUNCTION IN ADULT PATIENTS WITH B-THALASSEMIA MAJOR

A.E. Abdelhamid*, S. Shoeib, M. Abdelahafez, H. Elkholy

Internal medicine, Menoufia University, Shebin Alkom, Egypt

Background: β thalassemia major associated nephropathy has recently been a growing matter of concern affecting most aging thalassemia major patients. Serum creatinine is alone an unreliable indicator of renal functions.
Aims: The aim of the present study is to investigate the presence of glomerular dysfunctions in adults with β thalassemia major (β-TM), using early biomarkers of glomerular dysfunctions.
Methods: 50 patients with β-TM were subjected to history taking and clinical examination. Urinary albumin/creatinine ratio (ACR), Serum cystatin-C (SCys-C) levels were measured and CKD-EPI-CysC equation for estimated glomerular filtration rate (eGFR) were calculated. Presence of ACR>300mg/g and/or decreased eGFR cys were considered as glomerular dysfunction.
Results: Cases with HCV infection or diabetes were excluded from our analysis for possible risk factors for glomerular dysfunction, In the 43 patients with β-thalassemia major there were 18 patients with glomerular dysfunction (41.9%). There were significant differences between patients with and patients without glomerular dysfunction as regards age, urine albumin to creatinine ratio, serum Cyst-C, Serum creatinine, estimated GFRcys, hemoglobin and insignificant differences as regads sex, weight, height, BMI, splenectomy, using iron chelators or drugs for osteoporosis, blood requirement (ml/Kg/year) or ferritin level more than 1000.
Summary/Conclusion: our study revealed presence of glomerular dysfunctions in about (41.9%) of adult β-TM patients, age and pretransfusion hemoglobin level are predictors for glomerular dysfunctions. CKD-EPI-CysC equation for eGFR can detect early renal damage in these patients.

PB2490

DO VENTRICULAR DEPOLARIZATION AND REPOLARIZATION DIFFER IN B-THALASSEMIA CARRIERS?

S. Akarsu[1,*], T. Kasar[2]

[1]University of Firat, Faculty of Medicine, Department of Pediatric Haematology, Elazig, [2]Ordu University, Faculty of Medicine, Department of Pediatric Cardiology, Ordu, Turkey

Background: In our population the incidence of β-thalassemia minor (TM) is 2.1 percent. These cases are frequently evaluated as iron deficiency anemia (IDA) and receive iron containing medications. In cases with IDA, and β-thalassemia major, electrocardiographic (ECG) signs reflecting cardiac autonomic dysfunction, and changes in depolarization, and repolarization differ widely.
Aims: We haven't encountered any study related to changes in ECG signs in the literature. So we wanted to investigate corrected QT (QTc), and QTc dispersion (QTcd) in cases with TM which demonstrate clinical manifestations in between IDA, and β-thalassemia major.
Methods: Study population consisted of Group 1 (TM; n:15), Group 2 (IDA; n:17), and Group 3 (healthy control; n:13). Mean ages of Groups 1, 2 and 3 were 10.2±17.4, 13.2±14.5, and 13.5±2.5 months, respectively. In all cases whole blood cell counts, levels of serum iron, iron binding capacity, and ferritin were evaluated, and hemoglobin electrophoresis was performed. Peak heart rate, RR, PR, QRS, QT, QTc and QTcd were calculated using 12-lead ECG. Groups in all 3 groups were compared as for all ECG variables.
Results: QTc intervals were estimated for TM (383.7±40.7 ms), IDA (399.1±20.4 ms), and healthy control (403.8±20.6 ms) groups as indicated. QTc intervals in cases diagnosed as TM were shorter relative to IDA, and healthy control groups. A statistically significant difference was detected between TM, and healthy control groups ($p<0.05$). QTc interval was statistically significantly longer in cases diagnosed as TM (84.6±34.5) ms when compared with cases with IDA (61.7±37.0 ms) ($p<0.05$). The other parametres did not differ between groups.
Summary/Conclusion: Changes in ECG tracings may occur not only in heart diseases, but also it may become manifest as a sign of myocardial ischemia due to anemia. In order to be able to explain some electrocardiographic changes in cases diagnosed as TM caused by etiological factors other than myocardial ischemia, and accumulation of iron, further studies should be carried out with greater number of patients.

PB2491

LONGITUDINAL TREND ANALYSIS OF SERUM TRANSFERRIN RECEPTOR-1 LEVEL IN A COHORT OF 104 PATIENTS AFFECTED BY NON TRANSFUSION-DEPENDENT THALASSEMIA

P. Ricchi*, B. Gargiulo, M. Ammirabile, S. Costantini, A. Spasiano, P. Cinque, A. Filosa

Oncoematologia, AORN Cardarelli, Naples, Italy

Background: Non-transfusion-dependent thalassemias (NTDT) are a wide group of inherited disorders grouped together only by the fact that their phenotypic expression rarely or occasionally requires regular transfusions. The soluble transferrin receptor-1 (sTfR1), that fully reflects the marrow erythropoietic activity, had not only a striking diagnostic accuracy in predicting the risk of extramedullary hematopoiesis (EMH), but also in scoring disease severity. Our prospective data in NTDT patients showed previously that sTfR1 levels were unchanged following iron chelation therapy when ferritin level was clearly and significantly decreased.
Aims: Our aim was to explore the longitudinal trend of sTfR1 levels in a wide cohort of patients with NTDT followed at U.O.S.D. Rare Red Blood Cells Diseases of Ospedale Cardarelli (Naples, Italy).
Methods: Until today, 104 NTDT patients have been undergoing since 2007 the measurement of the sTfR1 levels about twice a year. During the observation period, based on several complications treatment and/or prevention, such as extramedullary hematopoiesis, cardiomiopathy, pulmonary hypertension and fatigue, 16 patients were started on regular blood transfusion (BT) therapy and 12 on hydroxyurea (HU) and 76 have never been treated (controls). Basal and final values were considered those at the first and last assessment for controls (CTRLs), respectively. For patients who were started on transfusion or HU, basal and final values were considered those assessed just before and following about 12 months of both treatments.
Results: Patients treated with BT were older than CTRLs (median value 48.3 *vs* 38.2 years old, $p=0.005$). Patients treated with BT and HU at baseline had higher sTfR1 median levels as compared to CTRLs (11.8 and 8.7 *vs* 5.1 mg/ml; $p<0.001$ and $p<0.001$, respectively). sTfR1 median levels were unchanged (median observation time: 5.78 years) among CTRLs: 5.1 *vs* 4.9 mg/ml (median reduction=-0.2, $p=0.55$). sTfR1 median levels were also unmodified among BT and HU groups, before the start of treatments. Conversely, following the start of treatments, we observed a statistically significant reduction in STfr1 levels in both population. In patients treated with BT, the median levels dropped of 5.6 mg/ml from a median value of 8.1 to a median value of 5.9 mg/ml ($p=0.00023$) with a median 50% of reduction with respect to baseline ($p=0.00003$). Whereas, in patients treated with HU, the median levels dropped of 4 mg/ml to a median value of 8.35 mg/ml ($p=0.005$) with a median 32% of reduction with respect to baseline ($p=0.003$).
Summary/Conclusion: Our data among CTRLs show that the sTfR1 level remains unchanged over a long period of observation and suggest that it could be a reliable marker of the disease since its first measurement. But, in a prospective evaluation, these data highlight that increased sTfR1 levels are associated with the risk of undergoing BT and HU treatment. We found also that these fundamental treatments for the management of patients with NTDT are able to significantly reduce sTfR1 levels. Further studies are needed to evaluate if, consistently with our previous findings, such a conspicuous drop in sTfR1 levels following both treatments could be also predictive of reduction and/or prevention of the complications typical of NTDT patients.

PB2492

HB PALENCIA A NOVEL BETA DELTA GLOBIN GENE FUSION WITH OVEREXPRESSION OF BETA GLOBIN CHAIN

P. Ropero[1,*], J.M. Nieto[1], F.A. González[1], A. Villegas[1], R.B. Martínez[1], C. Vicente[1], J.M. Alonso[2], A.M. Bobes[1], P.A. Velasco[1], A.M. González[1], M.M. Ibarra[1], R.O. Trelles[1], E. Golvano[2], L. Guerrero[2], B. Albarrán[2]

[1]Hematology, Hospital Clínico San Carlos, Madrid, [2]Hematology, Complejo Asistencial Universitario, Palencia, Spain

Background: Non homologous crossing-over between d and β globin genes results in a family of fusion genes that produce the Lepore and anti Lepore type hemoglobins. The Lepore hemoglobins are characterized by globins chains with a δ-amino terminus, a β-carboxyl terminus, and reduced synthesis, resembling that seen in normal δ synthesis. The reciprocal products of the Lepore genes, the anti Lepore type hemoglobins (Miyada, Hong Kong, CHORI…), have β-amino and δ carboxyl terminal and also exhibit reduced synthesis. In other hand the hemoglobin Parchman is a rare variant that hybrid globin chain had a δ-amino terminus, an internal β-chain fragment, and a δ-carboxyl terminus.

Aims: We present a new hemoglobin, the result of a double cross-linking of the δ and β genes or an unequal cross-linking between a Lepore type gene and the β gene. The new fused globin chain has the β-amino end, an internal δ chain fragment and the β carboxy terminus and its synthesis is similar to that of β-globin.
Methods: The propositus was an 80-year-old white man for the hemoglobinopathies study, because to rule out type 2 diabetes, Hb A1c was quantified by ion exchange HPLC (Variant II turbo) and an abnormal peak appeared in a retention time (RT) of 1.15 min which constituted 43.9%. Analysis of hemoglobins was made by HPLC exchange ionic (Variant II), electrophoresis capillary (Sebia Capillarys Flex) and the DNA analysis using PCR; MLPA and automatic sequencing of Sanger of δ and β genes.
Results: Sequencing showed that the 5 'end belongs to the HBB gene up to CD8, from CD9 and up to CD31 to the HBD gene and since the reverse sequencing of the β gene established that from the 3' UTR region and up to CD32 it belonged to the gene HBB, an unequal crossover between the HBD and HBB genes should have been carried out, causing a crossover gene reversion of at least 196 bp and a maximum of 225 bp of the HBD gene in the HBB gene which would give rise to a βδβ hybrid gene. This uncertainty would be due to the fact that although the sequence up to +21 from Cap belongs to the HBB gene, from this position and up to CD8 it could correspond to both the HBB gene and the HBD since this sequence is completely the same in both genes. This recombination probably took place during meiosis.
Summary/Conclusion: In the case of this new variant (Hb Palencia), it is probably the result of a cross-linking between a Lepore type gene (Baltimore or Washington-Boston) and a HBB gene, since the probability of a double cross-linking of the genes HBD and HBB in a region that covers less than 200 bp is extremely low. The normal levels of Hb A2 (2.9%) suggest that in both chromosomes the HBD gene would be intact, allowing a correct synthesis of the δ-globin chain. On the other hand, the percentage of Hb Palencia, around 40%, is similar to that of Hb A, therefore, the synthesis of the βδβ-globin chain should be controlled in a similar way to that of β-globin. And since the propositus does not have a thalassemia phenotype, consequently the β cluster of one chromosome would be strictly normal and that of the other allele would have a normal HBD gene and the β would correspond to the hybrid (βδβ).This Hb Palencia is the first variant due to a hybrid gene, constituted in the 3 'and 5' UTR ends by the sequences and machinery of the HBB gene and a part of the interior by sequences of the HBD gene. This structure would confirm that the sequences of the promoter regions as well as those of the IVS-II are necessary and essential for the synthesis of the β-globin type chain.

PB2493

LABORATORY DIAGNOSIS FOR THALASSAEMIA INTERMEDIA: ARE WE THERE YET?

S. Ansari*, N. Rashid, A. Hanifa, S. Munzir, B. Kaleem, A. Naz, K. Perveen, Z. Hussain, I. Ansari, Q. Jabbar, T. Khan, M. Nadeem, T.S. Shamsi

National Institute of Blood Disease & Bone Marrow Transplantation, Karachi, Pakistan

Background: Differentiation between thalassaemia major and thalassaemia intermedia at presentation is not uniformly characterized, for which an absolute criteria needs to be developed.
Aims: This study investigated the primary and secondary genetic modifiers to develop a laboratory finding by forming different genetic mutational combinations seen among thalassaemia intermedia patients and comparing them with thalassaemia major.
Methods: This cross sectional study analyzed 315thalassaemia intermedia patients. Selection primarily based with hemoglobin between 3-10gm/dl after 2 years of age. 105thalassaemia major patients were recruited on the basis of documented evidence of diagnosis and were receiving blood transfusion therapy regularly. Mutations were identified and various mutational combinations were formed and comparison was done between thalassemia intermedia and major using statistical software STATA 11.1.
Results: The mean age of the total population was 5.9 +/-5.32 years of which 165 (52%) were males and 150 (48%) were females. Of the two groups (thalassemia intermedia and thalassemia major), *IVSI-5, IVSI-1 and Fr 8-9* are more prevalent among the thalassemia intermedia cohort. When comparison was done between the thalassemia intermedia and thalassemia major patients, it showed significant results (p-*value*<0.001) for the presence of *Xmn-1* polymorphism.
Summary/Conclusion: The presence of IVSI-5 homozygous with Xmn-1, IVSI-5 heterozygous with Xmn-1, Cd30 homozygous with or without Xmn-1 and IVSI-1 homozygous or heterozygous either with or without Xmn-1 prove to be strong indicators towards diagnosis of thalassemia intermedia.

PB2494

NOVEL NONSENSE MUTATION IN A1 GLOBIN GENE [HBA1:C.49A>T] RESPONSIBLES FOR A-THALASSEMIA NON DELETION

P. Ropero*, F.A. González, J.M. Nieto, A. Villegas, R.B. Martínez, A.M. Bobes, C. Vicente, P.A. Velasco, A.M. González, M.M. Ibarra, R.O. Trelles

Hematology, Hospital Clínico San Carlos, Madrid, Spain

Background: α-thalasemia is a common disease charcterized mainly deletions of one or several α genes which is known as α-thalassemia deletion. On the other hand, point mutations are also observed in critical regions of the α genes such as the consensus zones of the introns or in the polyadenylation sequences, which can alter the processes of transcription, translation or in the posttradutional processing of the mRNA, originating the so-called α-thalassemia no deletion. So far, more than 100 mutations have been described. One of the least frequent mechanisms is the nonsense mutations, those that generate the substitution of a triplet coding for an amino acid by a stop codon and therefore the protein synthesis stops prematurely. At present 9 mutations of this type have been documented, 6 that affect the HBA2 gene and 3 that affect the HBA1 gene.
Aims: We present a new mutation in CD16 of the HBA1 gene, where the change AAG>TAG generates a stop codon.
Methods: The propositus, a 48-year-old woman from Madrid, was studied because she had maintained microcytosis without iron deficiency. The Hb A_2 and Hb F levels were measured by ion exchange HPLC (VARIANT II). Hemoglobin was studied by capillary zone electrophoresis and ionic exchange HPLC (β-thalassemia Short Program). The most frequent α globin mutations were discarded by multiplex PCR (Alpha-Globin StripAssay ki) and molecular characterization was undertaken using automatic sequencing.
Results: The propositus presented microcytosis with hypochromia and with normal reticulocytes. No abnormal hemoglobins were detected and Hb A2 and Hb F levels were within normality. Molecular characterization of the α1-globin gene by automatic sequencing identified the novel transversion mutation HBA1:c.49A>T, which resulted in an amino acid change from Lys>Stop at codon 16 of exon 1 in the heterozygous state [$\alpha_1$16(A14)Lys>Stop; HBA1:c.49A>T]. This was confirmed by sequencing of the other strand.
Summary/Conclusion: This type of mechanism is very rare among the globin genes, in fact, between the γ genes (A and G) has not been detected, in the β globin gene (HBB) has been identified 18 times (18/913), in the δ gene only in three (3/126) and in the α globin 9 genes (9/799). This new mutation is the fourth described in the HBA1 gene and the tenth nonsense among the alpha globin genes. The first nonsense mutation described in the alpha globin genes was in 1987 in a black family in the USA, since then and until the current 21st century no other had been described, probably as a result of advances and refinement of biology techniques as the automation of genetic sequencing, which has allowed an increase in the ability of analysis. This new mutation has a mild phenotype in the heterozygous state. Short gene products might eventually undergo nonsense mediated decay, while any anomalous short protein will be removed through the ubiquitin mediated proteolytic pathway resulting in the mild phenotype of an α-thal (αTα/αα) genotype. Probably the severe end of the clinical spectrum, when it is inherited with a deletion mutation of two genes.

PB2495

GLOMERULAR AND TUBULAR FUNCTION IN THALASSEMIA PATIENTS

G. Aydogan*, D. Doganci, N. Ozdemir, F. Akici, Z. Salcioglu

Pediatric Hematology, Kanuni Sultan Suleyman Education And Research Hospital, Istanbul, Turkey

Background: Thalassemia is a hereditary hemolytic anemia resulting from defects in globin chains. Transfusion-dependent thalassemia patients are prone to develop renal dysfunction due to iron overload, chronic anemia, and/or chelation therapy.
Aims: The aim of this study was to analyze renal tubular and glomerular function in patients with beta thalasemia major (β-TM).
Methods: Thirty-eight transfusion dependant (β-TM) patients were recruited. Patients were divided into three groups based on serum ferritin levels. Glomerular functions [serum urea, creatinine, cystatin-c, estimated glomerular filtration rate (eGFR) and urinary protein excretion] and tubular functions [serum electrolytes, fractional sodium excretion (FeNa), fractional potassium excretion (FeK), tubular phosphorus reabsorption (TPR), urinary calcium excretion] were assesed in all patients.

Results: In all patients serum urea, creatinine and electrolytes were normal. Cystatin-c was increased in 5.3% of patients, GFR was increased in 28.9% and decreased in 18.4% of patients. Proteinuria (4-40 mg/m2/h) was detected in 36.8%. Protein/creatinine ratio was over 0.2 in 50% of patients. Microalbuminuria (30-300mg/day) was detected in 10.5% of the patients, albumin/creatinine ratio was above 30 mg /gr 18.4% of patients. Calcium excretion over 4mg/kg/day was found in 42.1%, and high calcium/creatinine ratio in 28.9% of the patients. FeNa was detected in 18.4% over 1%, while TPR was found to be decreased in 7.8%. Between β-TM patient groups with ferritin levels below 1000 ng/ml, between 1000-2000 ng/ml, above 2000 ng/ml, glomerular and tubular functions were not significantly different. Also there was no correlation between kidney function tests and ferritin. GFR was found to be significantly lower in patients aged 18 years and older, than patients aged 10-18 years and under 10 years according to age specific references. Creatinine was significantly lower and protein/creatinine ratio was significantly higher in patients younger than 10 years of age.
Summary/Conclusion: Different levels of renal tubular and / or glomerular dysfunction were encountered in this study. Findings suggest that renal injury is associated with increased age. In our study, serum ferritin did not correlated with renal tubular and glomerular functions.

PB2496

Abstract withdrawn.

PB2497

GENE SPECTRUM ANALYSIS OF THALASSEMIA CARRIERS RESIDING IN NORTHERN CHINA

W. Zhou[1,*], Z. Yang[2], Q. Cui[1], L. Qiu[2], B. Han[1]

[1]Peking Union Medical Collage, Chinese Academe of Medical Science, [2]Department of clinical laboratory, Peking Union Medical Collage, Chinese Academe of Medical Science, Beijing, China

Background: Thalassemia is one of the most pervasive monogenic diseases worldwide. In China, southern provinces were known as high incidence areas of thalassemia. But thalassemia is now becoming increasingly common in northern China because of continued migration.
Aims: We compared gene spectrum of α and β-thalassemia in northern and southern China, and compared our data in provinces of different elevations. The results may help to understand the similarities and differences of people who reside in the north China but originate from different areas.
Methods: Positive patients or carriers were analyzed from 2136 suspected α and β-thalassemia carriers who were referred to Peking Union Medical College Hospital from 2012 to 2017 for diagnosis. Gap-PCR and RDB (reverse dot blot) analysis were applied for detections of common α and β-thalassemia gene mutations. Basic clinical data from these patients or carriers were collected. Telephone follow-up survey was conducted on their ancestral information, to confirm whether these northerners have southern lineage.
Results: A total of 1059 carriers (male 299, female 760, age range from 0 to 82 years, mean age 30.2 years) were selected from north dweller, 183 (17.3%) of them with pure northern descent in three generations and the rest 876 (82.7%) carriers with south descent. Most of our people with positive thalassemia gene findings had no or mild symptoms. People who originated from the north origin had higher percentage of β-thalassemia gene mutations compared with people from the south origin (72.8% *vs* 62.4%, χ^2=9.92, P=0.001). Analysis of the individual gene distribution of the south and north did not show significant difference either in α-thalassemia (P=0.221) or β-thalassemia (P=0.979). Differences between gene distributions in provinces with similar average altitudes were relatively small. No significant statistical differences in the frequency of α mutation were found in different altitude levels. But in β thalassemia, the frequency of 6 most common mutations were significantly different in provinces with altitude below 500 meters, about 500-1000 meters, and above 1000 meters (χ^2 test, P<0.05).
Summary/Conclusion: Most of people with positive thalassemia gene findings who reside in the north China are thalassemia carriers. People with north lineage may have higher frequency of β mutation than those originated from the south, but they had similar spectrum of α and β mutations. People lived at different level of altitudes may have different spectrum of β mutations.

PB2498

SERUM FERRITIN TREND AS A PREDICTOR OF LIVER AND CARDIAC IRON OVERLOAD AMONG CHILDREN WITH TRANSFUSION DEPENDENT THALASSEMIA AND SICKLE CELL DISEASE

S. Sahmoud[1,*], M. Azzam[2], S. Elfiky[3], I. Alnasser[4], I. Elghamry[5], A. Gobarah[2], A. Almanea[6]

[1]Pediatric Hematology department, Suez Canal University, [2]Pediatric Departement, [3]Pediatric Hematology department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt, [4]Radiology department, King Fahd Armed Forces Hospital, Jeddah, Saudi Arabia, [5]Pediatric Hematology and Oncology department, Ain Shams University, Cairo, Egypt, [6]Pediatric Hematology and Oncology department, King Fahd Forced Arm Hospital, Jeddah, Saudi Arabia

Background: Chronic blood transfusion cause inevitable iron overload. Conflicting results were found regarding how much serum ferritin can predict tissue iron overload among chronically transfused patients and how much it differs by age.
Aims: to evaluate cardiac (CIO) and liver Iron overload (LIO) in children with sickle cell disease (SCD) and Transfusion dependent thalassemia (TDT) and testing their predictability by using the serum ferritin trend.
Methods: 3 Serum ferritin levels were tested at interval of 6 months, the last one was at the time of MRI T2* examination of both liver and heart. Increasing values of serum ferritin over time was considered an increasing trend.
Results: 90 (43 male, 9.9+/_4.4 year) TDT and 47 (32 male, 11.85 +/_3.82 year) SCD children were included. LIO was higher among TDT children with liver iron concentration of 11.87 mg/g, and 6.58 mg/g (p: 0.0001). Similarly, there was a higher level of CIO in TDT (T2*: 24.38 m/sec) than in SCD (31.97 m/sec) (p: 0.001). Age didn't increase the risk of LIO (OR 0.81, CI 0.46-1.42, p: 0.47), or CIO (OR 1.23, CI 0.74-2.03, p: 0.43) in TDT neither did of LIO (OR 1.054, CI 0.86-1.28, p: 0.61) nor CIO (OR 0.722, CI 0.41-1.28, p: 0.27) in SCD. Percentage of children with increasing trend of serum ferritin was 50% in TDT and 30% in SCD children (p: 0.31). The relationship between serum ferritin trend and CIO/ LIO in TDT (p: 1.0/0.67) and CIO/LIO in SCD (p: 1.0/0.66) was insignificant. In TDT, Serum ferritin at a level of >4036 µg/l could differentiate mod/severe CIO from those with none/mild one (AUC 0.872, p: 0.001). Similar discrimination could be achieved regarding LIO in TDT at a lower level of >2294 µg/l (AUC 0.879, p: 0.001). In SCD Mod/severe LIO could be discriminated from none/mild LIO at a level of >1892 µg/l serum ferritin (AUC 0.822, p: 0.001).
Summary/Conclusion: Serum ferritin level could predict both LIO and CIO in TDT cases, and LIO in SCD. Ferritin trend had no relation to tissue iron overload in both groups.

PB2499

HEMOGLOBINOPATHIES SCREENING IN A EUROPEAN CENTRAL HOSPITAL- RETROSPECTIVE STUDY OF THE LAST 10 YEARS

D. Fonseca E Silva[1,2], C. Sousa Neves[1,*], M. Lima[3,4], M.I. Freitas[1]

[1]Laboratorial Hematology Department, Centro Hospitalar do Porto, [2]Instituto de Ciências Biomédicas Abel Salazar, University of Porto (ICBAS/UP), [3]Unit for Multidisciplinary Research in Biomedicine, Instituto de Ciências Biomédicas Abel Salazar, University of Porto (UMIB/ICBAS/UP), [4]Cytometry Laboratory, Clinical Hematology Department, Centro Hospitalar do Porto, Porto, Portugal

Background: The hemoglobinopathies (HGP) are a group of hereditary disorders in which there is abnormal production or abnormal structure of the hemoglobin (Hb) molecule. These are the most frequent group of genetic disorders worldwide. They are broadly categorized into two major groups: thalassemias and structural variants of hemoglobin. HGP have become much more common recently in northern and central Europe, including Portugal, due to immigration.
Aims: The aim of this study was to analyze the incidence of HGP in all samples screened for HGP in a central hospital during the last 10 years.
Methods: Retrospective study performed in a central and university hospital of Porto, Portugal. A total of 4131 samples of 3886 patients, studied from January 2007 to December 2016, were included. These samples were collected in EDTA-K3 tubes and were analyzed in an automatic hematological counter to obtain red blood cell count with erythrocyte indices. The next step of diagnosis workout consisted on Hb tests: high performance liquid chromatography, electrophoresis and, sometimes solubility test. When detected abnormal structural Hb, samples were tested by molecular biology to identify the Hb variant.
Results: From all the patients studied (n=3886), 19.8% (n=771) had Hb genetic disorders and were subdivided into two main groups: β-thalassemia syndromes (71.5%; n=551) and structural Hb variants (28.5%; n=220). The most frequent HGP were β-thalassemia (n=550 β-thalassemia minor and n=1 β-thalassemia major) and Hb AS (14.5%; n=112), followed by Hb Lepore (5.1%; n=39), Hb SS (2.9%; n=22), Hb AD (2.2%; n=17) and Hb AC (1.0%; n=8). Other rare Hb variants detected were: Hb AE (0.5%; n=4), Hb Indianopolis (0.5%; n=4), Hb Koln (0.4%; n=3), Hb SC (0.4%; n=3), ∅-chain variant Hb A2 (0.3%; n=2), Hb Himeji (0.1%; n=1), Hb Setif

(0.1%; n=1), Hb Strasbourg (0.1%; n=1) and Hb Porto Alegre (0.1%; n=1). Concomitant β-thalassemia and Hb AS was also present in two of our patients (0.3%; n=2).
Summary/Conclusion: Our data are concordant with previous epidemiological studies. It is noteworthy that our sampling included HGP screening samples and not the whole population and therefore we found higher levels of genetic Hb disorders. The α-thalassemia syndromes were not detected by our methods, as characterization of these diseases requires DNA-based α-globin gene testing. For this reason, some of the samples studied for HGP screening may have gone undetected. HGP are a public health issue and therefore early detection and characterization of the HGP is crucial so that appropriate counselling and treatment can be provided to couples through prenatal screening and families who may be at risk of having HGP. The reinforcement of these measures can lead to the reduction of incidence and morbidity associated with these disorders.

PB2500

THE PHENOTYPIC MANIFESTATIONS OF BETA-THALASSEMIC MUTATIONS IN AZERBAIJAN

C. Asadov*, T. Mammadova, E. Abdulalimov, G. Aliyeva, S. Gafarova

Pathology of hemopoesis, Institute of Hematology and Transfusiology, Baku, Azerbaijan

Background: Azerbaijan is one of the countries with the highest prevalence of thalassemias. β-Thalassemia is known for its extremely diverse clinical manifestations. Therefore, determination of factors causing such a diverse clinical presentation has clinical significance, and the major reason for such diversity is the variety of mutations. A number of studies revealed genotype-to-phenotype correlations of β-thalassemia mutations in various populations, and we hereby report the first one conducted in an Azerbaijani population.
Aims: The aim of our study was to reveal genotype-to-phenotype correlations of the most common β-thalassemia mutations in an Azerbaijani population.
Methods: 48 patients (23 heterozygous, 23 homozygous, 2 compound heterozygous β-thalassemia) with known genotypes were included in the study. Evaluations of RBC count, hemoglobin concentration, hematocrit, MCV, MCH, and MCHC were performed with a Sysmex XT2000i hematology analyzer. All samples were tested for hemoglobin fractions by high-performance liquid chromatography via the VARIANT II Hemoglobin Testing System (Bio-Rad). DNA amplification was performed on a C-1000 thermocycler (Bio-Rad). Detection of mutations by reverse dot-blot hybridization was performed with commercial test kits (β-Globin StripAssay Kit, ViennaLab) according to the manufacturer's instructions. One-way ANOVA followed by Tukey's honestly significant difference test was used to analyze between-group differences.
Results: The classical β-thalassemia carrier pattern of increased RBC count and low hemoglobin and erythrocyte indices (MCV, MCH, and MCHC) was observed in almost all patients. Increased HbA_2 concentrations and fetal hemoglobin within the normal range (<2%) were observed in most cases. There was a statistically significant difference ($p<0.05$) in RBC, MCV, and MCH parameters of IVS-I-6 patients compared to the groups with codon 8 and IVS-II-1. The former was associated with comparably lower RBC and higher MCV and MCH mean values. Based on this pattern of hematologic data, it can be concluded that IVS-I-6 presents a milder phenotype compared to codon 8 and IVS-II-1 mutations. No statistically significant difference was obtained between codon 8 and IVS-II-1 mutations ($p>0.05$). The data of homozygous individuals were also observed to correlate with the type of the mutation. Statistically significant between-group differences ($p<0.05$) were observed for MCV, MCH, and MCHC. Contrary to the heterozygous individuals, lower mean values of these parameters were observed in the group of homozygous IVS-I-6 patients, possibly due to their less frequent transfusions, compared to codon 8 and IVS-II-1 patients. The results obtained indicate that clinical presentation varies between different β-globin gene mutations: individuals with IVS-I-6 (T>C) mutations showed milder presentation than those with codon 8 (-AA) and IVS-II-1 (G>A), which is associated with the molecular basis of the mutations.
Summary/Conclusion: Our study revealed genotype-to-phenotype correlations of the most prevalent β-thalassemia mutations of the Azerbaijani population. According to our data, hematologic parameters and consequently the clinical presentation are closely related to the type of the mutation, especially in homozygous patients. Our findings can provide a better prediction of clinical manifestation by early identification of the type of the β-thalassemia mutations.

PB2501

THE MAIN ENDOCRINE HEMOCHROMATOSIS COMPLICATIONS DURING HOMOZYGOUS B-THALASSEMIAS: 18 YEARS SINGLE ALGERIAN EXPERIENCE

G. Yamina*, D. Malika, A. Mohamed Rafik, S. Mohamed, B. Salah Eddine

Hématology, hospital dr Mohamed Seghir Nekkache, ALGIERS, Algeria

Background: Endocrine abnormalities are common in thalassemia patients, despite the initiation of an early adapted chelation treatment, the main complications that can occur, hypothyroidism, diabetes, growth retardation, hypogonadism, hypoparathyroidism, adrenal insufficiency.
Aims: Clinical, biological and evolutionary evaluation of the main endocrine complications of polytransfused homozygous β-thalassemia patients over a period of 18 years.
Methods: This is a retrospective study over 18 years [January 1999 - December 2016], we collected 35 homozygous thalassemic β : major form (MF)=17 cases, intermediate form (IF)=18 cases. The search for endocrine complications is done on a regular basis: clinical examination, biological assessment, hormonal assessment, radiological assessment. Then the patient will be referred to the endocrinologist for proper treatment.
Results: The average age of diagnosis of β thalassemia in major form was 11 months [6 to 46 months], in the Intermediate form was 28 months [6 to 78 months]. The current median age was 17 years [30 months to 47 years], with a sex-ratio=1.05 (18 males/17 females). Endocrine complications are found in 40% of cases (MF=10, IF=4): (hypothyroidism n=5, hypogonadism n=5, diabetes n=4), these various complications are detailed below: **Hypothyroidism**: it is infra-clinical in 5 cases (MF=3, IF=2), the average age of discovery is 24 years [13 to 33 years], the thyroid balance: average TSH of 5.73 mIU / ml [4.96 to 6.61], average FT4 of 81 µg /l [77 to 88], these patients are monitored regularly and there is a stabilization of thyroid status. **Hypogonadism**: n=5 (MF=4, IF=1), the average age is 26 years [17 to 32 years], primary amenorrhea is noted in 3 cases, the hormone balance found: GH average of 1.38µUI / ml [0.88 to 1.86], average FSH of 0.26 IU / l [0.18 to 0.68], average oestradiol of 53 ng / l [33 to 72], 2 patients are treated by patch estrogen with a good evolution in a patient after 12 months of hormonal treatment. **Diabetes:** n=4 (FM=3, FI=1), average age of 32 years [22 to 47 years], average Hb A1c of 8.1% [7.5 to 8.9], these patients are on a low-carbohydrate diet combined with insulin therapy with regular monitoring in diabetology. Note that all our patients are under a deferasirox chelator treatment at the dosage of 20 to 30 mg / kg / day with regular monitoring of ferritin levels. The median follow-up of our patients is 16 years [13 to 47 years].
Summary/Conclusion: Endocrine complications during β-thalassemia are manifestations mainly associated with haemochromatosis resulting from direct involvement of the glandular parenchyma or the hypothalamic-pituitary axis. The frequency of these complications increases with age of patients, they are the most common complications, noted in 40% of cases. When complications are established, management should focus on interrupting the evolutionary process and treating symptoms. It is for this reason that the realization of an early detection in endocrine environment is essential in order to diagnose the beginning endocrinous attacks (intolerance to sugar, rough hypothyroidism) and to intensify the chelating treatment

PB2502

EVALUATION OF THE EFFECTIVENESS OF EXJADE IN PATIENTS WITH B-THALASSEMIA MAJOR DURING 3 YEARS IN GEORGIA

Z. Mtvarelidze*, M. Kvezereli-Kopadze

Hematology, Children s New Clinic, Tbilisi, Georgia

Background: B-Thalassemia is the most common hemoglobinophaty in Georgia. Carrier rate is 3,79%, and annual affected births of homozygote β-thalassemia are **about 20 new births per year.** One of the most important problems of treatment of this pathology is excretion excess iron with iron chelator therapy. In recent years for this purpose, usage of Exjade, but its efficiency criteria's and distant results not known yet.
Aims: This study was undertaken to evaluate the effectiveness of Exjade in patients with transfusion-dependent β-thalassemia major during 3 years in Georgia.
Methods: 16 children, aged 3 to 17 years, with transfusion-dependent β-thalassemia phenotype, were enrolled in a trial. The starting dose of Exjade was 20-30mg/kg per day given orally once a day. Baseline and serial laboratory tests were performed: detailed history and physical examination, complete blood count with red blood cell indices, reticulocyte count, hemoglobin electrophoresis, and ferritin level measurement monthly, liver and

renal function tests, bilirubin, urine analysis. Response to therapy was evaluated after 6 month, 1, 2, 3 years of treatment.
Results: No adverse effects were observed at a dose of 20-30mg/kg day. Positive clinical-laboratory effect was noticed in 13 patients: the general condition of children improved, increased intervals between hemotransfusions after 6 month of treatment. The decrease in ferritin levels started in 4-5 months of treatment. Taking into account the ferritin level, in some cases, treatment was temporarily stopped, or, dose of Exjade was decreased after a year of treatment. In 3 cases, the treatment was ineffective, despite Exjade dose was increased.
Summary/Conclusion: Accurate assessment of excessive iron stores is essential to optimal therapy. In our cases the Exjade is safe and effective in 81,2% of patients with transfusion-dependent β-thalassemia major. It is noted, that the result is achieved with continuous, regular Exjade therapy and such patients are under monthly monitoring of serum ferritin level. We also should consider that Exjade is characterized by a later effect (after 4-5 months of treatment). Exjade therapy should start as soon as the patient becomes significantly iron overloaded, this correlates with the serum ferritin>1,000 ng/ml. In some cases, his ineffectiveness is due by irregular/late start of treatment (the level of ferritin exceeds 2,000 ng/ml).Combination chelation may be an effective treatment strategy for patients with insufficient response to monotherapy. In rare cases, determining the causes of Exjade inefficiency is very difficult, that's why further studies in this direction are needed.

PB2503

MUTATIONAL PROFILE OF HEMOGLOBINOPATHIES IN A THIRD LEVEL HOSPITAL

S.F. Pinzon[1,*], V. Recasens[1], M.A. Montañes[1], F.A. Gonzalez[2], P. Ropero[2], C. Rodríguez-Vigil[3], A.J. Garcia[1], P. Delgado[1], N. Espinosa Lara[1], L. Lacalle Aicua[1], G. Ferrer Garrido[1], A. Madinaveitia[4], E. Salvador Ruperez[1], M. Villalba Montaner[1], A. Gomez Martinez[1], S. Martin-consuegra Ramos[1], C.F. Hernandez Mata[1]

[1]Hematology, Hospital Universitario Miguel Servet, zaragoza, [2]Hematology, Hospital Clínico San Carlos, Madrid, [3]Pediatrician, Hospital Universitario Miguel Servet, zaragoza, [4]Hematology, Hospital Universitario Miguel Servet, Madrid, Spain

Background: Hemoglobinopathies are the most common, potentially deadly monogenic disorders in the world. It is estimated that 7% of the world population are carriers. Global migration in the modern period has led to a continual spread of these anomalies so that they are rapidly becoming more common in the industrialized regions of Europe.
Aims: Description of the mutational spectrum of thalassemias and other hemoglobinopathies in a third hospital level
Methods: A cross-sectional study of patients diagnosed of hemoglobinopathies in a tertiary hospital. Period of study: January 2016- December 2017. Hemoglobin fractions were determined by capillary electrophoresis techniques Minicap® CDT (Sebia, Lisses, France). Molecular study was carried out in selected cases at the Hospital Clínico San Carlos in Madrid. The study of thalassemia was carried out on the most prevalent mutations by Gap-PCR and in those without detected mutation, genetic screening of the HBA1 / HBA2 or HBB genes was performed and the MLPA technique allowing the study of the exons of the HBB and HBA1 / HBA2 genes.
Results: Based on this series of patients, α-thalassemia is identified as the most prevalent hemoglobinopathy in our patients and -α , deletion the most frequent deletion found in these patients. In our series prevalence demonstrates great variability, and the mutations expose big differences with respect to other prevalence studies. The study of these mutations is important for diagnosis, individual clinical prognosis and future planning of the management of possible complications and epidemiological records, these records being the way to develop future cohort studies and the possibility of comparison or integration with other registries because hemoglobin defects are of widely diverse genetic and clinical types, specialized laboratory analysis is needed to diagnose them correctly and provide a basis for proper therapeutic decisions.
Summary/Conclusion: Based on this series of patients, α-thalassemia is identified as the most prevalent hemoglobinopathy in our patients and -α , deletion the most frequent deletion found in these patients. In our series prevalence demonstrates great variability, and the mutations expose big differences with respect to other prevalence studies. The study of these mutations is important for diagnosis, individual clinical prognosis and future planning of the management of possible complications and epidemiological records, these records being the way to develop future cohort studies and the possibility of comparison or integration with other registries because hemoglobin defects are of widely diverse genetic and clinical types, specialized laboratory analysis is needed to diagnose them correctly and provide a basis for proper therapeutic decisions.

Table 1.

HEMOGLOBINOPATIES	FREQUENCY
Thalassemia - α - β - α β - δβ	40 - 30 (75%) - 7(17,5%) - 2 (2%) - 1(1%)
Hemoglobinopaties	11
Sickle cell disease	22

A-THALASSEMIA MUTATIONS	FREQUENCY
-α3.7	27
large deletions	1
alpha	2

PB2504

COMPARISON OF GENE MUTATION SPECTRUM OF THALASSEMIA IN DIFFERENT PART OF CHINA AND SOUTHEAST ASIA

Y. Zhuo[1], C. Quexuan[2,*], Z. Wenzhe[2], Q. Ling[1], H. Bing[2]

[1]Clinical laboratory, [2]Hematology, Peking Union Medical Collage Hospital, Beijing, China

Background: Thalassemia is a common genetic disorder. High prevalence of thalassemia is found in South China, Southeast Asia, India, Middle East and Mediterranean. Thalassemia is usually thought to exist only in southern China, but more and more cases from northern China have been reported recently.
Aims: We compared gene spectrum of α and β-thalassemia in northern and southern China in our group, and compared our data with the largest meta-analysis in southern China, and data from Southeast Asian countries. The results may help to understand the similarities and differences of people from different area and different ethnic groups.
Methods: During 2012 to 2017, suspected thalassemia people were detected for common α and β-thalassemia mutations by gap-PCR and reverse dot blot (RDB) analysis in Peking Union Medical College hospital (PUMCH). 1059 people who carried thalassemia genes were analyzed retrospectively. We picked mutated individuals with northern identity card numbers and conducted telephone follow-up survey in order to collect their ancestral information. Besides, we used 'thalassemia', 'mutation', and Southeast Asian countries as keywords to search potential related studies in PubMed and EMbase.
Results: All carriers included in our study resided in northern China. Among them, 17.3% were native northerners and 82.7% were immigrants from southern China. Although significant difference was found between our data and data from the meta-analysis literature of southern China in both α and β-thalassemia, we also found some similarities between them. Similar gene mutation spectrum were found between Malaysia Chinese and Guangdong people, while other ethnic people in Southeast Asia had totally different gene spectrum from that of Chinese people.
Summary/Conclusion: Chinese People originated from north may have lower percentage of α-thalassemia mutations. Chinese people in different area had similar gene mutation profile and Chinese people had significantly different gene spectrum from other ethnic people in Southeast Asia.

PB2505

EVALUATION OF CARDIAC IRON ACCUMULATION IN THE PATIENTS WITH BETA-THALASSEMIA MAJOR AND ITS CORRELATION WITH SERUM FERRITIN LEVEL:SINGLE-CENTRE RESULTS FROM TURKEY

S. Erdinc[1], M. Söker[2,*], A. Gözü Pirincciogluu[3], H. Uzel[2], K. Yılmaz[3], F. Karakoç[4], S. Söker[5]

[1]Pediatrics, Ergani Public Hospital, [2]Pediatric Hematology and Oncology, [3]Pediatrics, Dicle University Medical Faculty, [4]Pediatrics, Sbu Gazi Yasargil Research And Training Hospital, [5]histology And Embriology, Dicle University Medical Faculty, Diyarbakır, Turkey

Background: Cardiac complications due to iron accumulation in the patients with beta-thalassemia major (B-TM) are the main causes of death.
Aims: The study aims to demonstrate the relationship between cardiac T2* MRI and serum ferritin accumulation in children with B-TM.
Methods: The study involves 27 patients (11 females, 40.7% and 16 males, 59.3%). The ages of the patients range from 9 to 19 years (13.5±3). All of

the patients included in the study received the chelation treatment.
Results: The mean value of ferritin was 2794±2162 ng/ml (509-10795 ng/ml). The mean cardiac T2* MRI value was 28.2±9.9 ms (5.42-41.9 ms) while the mean liver T2* MRI value was 5.2±6.6 ms. The mean value of pre-transfusion hemoglobin was 8.8±0.7 g/dl. Statistical significance was found between the cardiac T2* value and age (p=0.019). Five patients with the cardiac iron accumulation had relatively higher ferritin levels but with no significant correlation.
Summary/Conclusion: In conclusion, it was seen that the cardiac iron accumulation in the patients with B-TM was age-related. Serum ferritin levels were not found to be useful enough to show cardiac iron accumulation.

PB2506

ZINC DEFECIENCY AND INSULIN RESISTANCE IN THALASSEMIA CHILDREN IN ISMAILIA, EGYPT

S. Sahmoud[1,*], S. El Sharkawy[2], A. Atef[2]

[1]Pediatric Hematology department, Suez Canal University, [2]Pediatric Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt

Background: In Egypt there are 10,000 registered thalassemia (thal) cases and more than 20,000 non-registered cases. In patients without thal the diabetogenic effects of altered zinc status had been observed while almost 9% of the Egyptian population at risk of zinc deficiency.
Aims: This study aimed at finding out the relationship between the zinc status and insulin resistance in thal children.
Methods: 40 thal children (21 males, 9.9 +/_3.9 years) and 40 healthy ones (20 males, 11.4 +/_ 3.1 years) where tested for serum zinc level, oral glucose tolerance test, fasting blood glucose and serum insulin with calculation of HOMA-IR.
Results: Mean zinc level wasn't significantly different between thal (74.63+/-19.37) and non thal children (81.03+/-15.44) (p=0.106), but zinc deficiency were more prevalent in thal children than the healthy ones with 32.5, and 15% respectively (p=0.023). Only insignificant percentage of the study (8%) and the control groups (2%) had impaired glucose tolerance test (p=0.087). Using HOMA-IR test 20 and 22.5% of thal children had intermediate and resistant levels compared to only 12.5 and 2.5% of non thal children (p=0.01). No significant correlation was found between the serum zinc level and HOMA- IR (r -0.023, p=0.86).
Summary/Conclusion: zinc deficiency and insulin resistance were more common in thal children, but zinc deficiency didn't significantly increase the risk of insulin resistance.

PB2507

THE USE OF RED BLOOD CELL INDICES FOR SCREENING B-THALASSEMIA

Y. Zhilenkova*

Clinical chemistry, The North-West State Medical University n. a. I. I. Mechnikov, Saint-Petersburg, Russian Federation

Background: Microcytic anemia is frequently due to iron deficiency anemia (IDA) or thalassemia. The differentiation between thalassemia and IDA has important clinical implications.
Aims: To study the possibility of using erythrocyte indices as laboratory markers for screening thalassemia.
Methods: The study included 48 patients with a beta-thalassemia minor (26 male and 22 female, 2 to 58 years old) and 50 patients with IDA with Hb>90 g/l (22 male and 28 female, 2 to 54 years). The Sysmex XN-2000 analyzer was used to determine: Hb, RBC, MCV, MCH, RDW,% Micro,% Hypo-He. The erythrocyte indices were calculated: E=MCV - (10xRBC), E&F=MCV - RBC - 5xHb - 3.4, G&K=MCV_2×RDW/100 × Hb; M=MCV/RBC; Si=MCV-RBC-3xHb; S=MCH/RBC; RDWDI=MCV × RDW/RBC; M-H =%MicroR -%Hypo-He; M-H-RDW =%MicroR -%Hypo-He - RDW-CV. The concentration of iron and serum ferritin was determined using a Cobas-6000 analyzer. Thalassemia was confirmed by determining hemoglobin fractions (HbA_2>3.2%) by capillary electrophoresis (Minicap, Sebia).
Results: The most sensitive were M-H-RDW (97.8%) and M-H (95.4%), G&K (90.1%) and Si (90.1%), specificity - M (97.5%), Si (97.5) and MH-RDW (96.5%). The best Youden index, which combines information on sensitivity and specificity, were in the indices M-H-RDW (94.3) and M-H (89.9).
Summary/Conclusion: The M-H-RDW and M-H indices, which are calculated using the new hematological parameters available on Sysmex analyzers, can be highly reliable for thalassemia screening.

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PB2508

GENETIC POLYMORPHISMS AS RISK FACTORS OF ENDOTHELIAL DYSFUNCTION IN PREGNANT WOMEN

N. Korsakova[1,*], S. Kapustin[1], N. Silina[1], O. Matvienko[1], O. Golovina[1], A. Nikolaeva[2], L. Papayan[1]

[1]Russian Research Institute of Hematology and Transfusiology, [2]Antenatal clinic 22, Saint-Petersburg, Russian Federation

Background: Normal vascular system function is of highest importance in pregnancy. Endothelial dysfunction (ED) is an early nonspecific vascular trouble mark, leading eventually to various pathologies including thrombotic disorders. At present, technology of noninvasive quantitative ED evaluation by means of EndoPAT device is developed, that gives the value of Reactive Hyperemia Index (RHI). Allelic polymorphisms of genes participating in vascular tone, lipid metabolism and thromboresistance regulation might influence ED development, predisposing progression of states involved in ED pathogenesis, *e.g.* arterial hypertension and dyslipidemia.
Aims: To evaluate genetic polymorphisms impact in ED development in pregnant women.
Methods: The study included 18 pregnant women with confirmed diagnosis of arterial hypertension, gestational or type II diabetes mellitus, and/or obesity. ED evaluation was performed with use of EndoPAT 2000 device, RHI value of ≤1,67 was considered as ED mark. Control group consisted of 200 healthy persons. Molecular genetic typing was performed by PCR for following genetic polymorphisms: angiotensinogen (AGT) T704C, angiotensin-converting enzyme (ACE) Ins/Del, angiotensin II receptor type 1 (ATGR1) A1166C, G-protein β3 subunit (GNB3) C825T, apolipoprotein E (ApoE) E2/E3/E4 and endothelial NO-sinthase (eNOS) T786C. STATISTICA 6.0 was used. Median (Me) and 95% confidence interval (CI) for RHI were calculated. Fisher exact test was used to assess the differences in genotype frequencies. Odds ratios (OR) and their 95% CI were calculated.
Results: The patients had the following RHI values: Me 1,4, 95% CI 1,1-1,8, that confirms ED presence. Genotype frequencies in pregnant women with ED are represented in Table 1.

Table 1. Genotype frequencies in pregnant women with ED.

	Pregnants with ED,% (n=18)	Controls,% (n=200)	OR; 95%CI		Pregnants with ED,% (n=18)	Controls,% (n=200)	OR; 95%CI
AGT 704 C/C	38,9	25,5	1,9; 0,7-5,1	**Apo E3/E4**	**38,9***	17,0	**3,1; 1,1-8,6**
AGT 704 T/C	33,3	46,0	0,6; 0,2-1,6	Apo E2/E4	5,6	1,0	5,8; 0,5-67,6
AGT 704 T/T	27,8	28,5	1,0; 0,3-2,8	Apo E3/E3	50,0	61,0	0,6; 0,2-1,7
ACE Del/Del	**50,0***	26,5	**2,8; 1,0-7,4**	Apo E2/E3	5,6	18,5	0,3; 0,03-2,0
ACE Ins/Del	27,8	48,5	0,4; 0,1-1,2	Apo E2/E2	0	0,5	0
ACE Ins/Ins	22,2	25,0	0,9; 0,3-2,7	Apo E4/E4	0	2,0	0
ATGR1 1166 C/C	5,6	7,0	0,8; 0,1-6,3	eNOS 786 C/C	11,1	13,0	0,8; 0,2-3,9
ATGR1 1166 A/C	38,9	37,0	1,1; 0,4-2,9	eNOS 786 T/C	27,8	47,0	0,4; 0,1-1,3
ATGR1 1166 A/A	55,6	56,0	1,0; 0,4-2,6	eNOS 786 T/T	61,1	40.0	2,4; 0,9-6,3
GNB3 82 5 T/T	11,1	11,0	1,0; 0,2-4,7				
GNB3 82 5 C/T	66,7	44,5	2,5; 0,9-6,9	* significant difference, p<0,05.			
GNB3 82 5 C/C	22,2	44,5	0,4; 0,1-1,1				

Summary/Conclusion: Pregnant women with ED had significantly higher ACE Del/Del and Apo E3/E4 genotypes frequencies, OR values indicate the direct correlation of these genotypes with ED development in pregnants. Unexpectedly homozygous and heterozygous eNOS 786 C allelle carriers as a tendency were found less frequently among pregnant women with ED, whereas eNOS 786 T/T genotype frequency had a tendency to be higher in pregnants with ED, showing invert correlation (not statistically significant) of eNOS 786 C allelle carriage with ED development in pregnancy.

PB2509

MULTI-ORGAN THROMBOSIS AS A MANIFESTATION OF IDIOPATHIC HYPEREOSINOPHILIC SYNDROME IN A CHILD – A CASE REPORT

S. Oberoi*, T. Brown, I. Vanan

Pediatric Hematology Oncology, Cancer Care Manitoba, Winnipeg, Canada

Background: Idiopathic hypereosinophilic syndrome (HES) is a disease characterized by an absolute eosinophil count (AEC) greater than 1500/mm^3 lasting for more than 6 months in the absence of any other known cause of eosinophilia and with the evidence of eosinophil mediated tissue damage. HES presenting as multi-organ thrombosis is rare in children.
Aims: To report a pediatric case with idiopathic HES who presented with pulmonary embolism and deep venous thrombosis of lower limbs.
Methods: A retrospective review of patient's electronic chart was conducted to retrieve clinical and laboratory data.
Results: A 14-year-old boy presented with shortness of breath, chest pain and swelling of the left leg to pediatric emergency. A Doppler ultrasound of lower limbs revealed thrombosis of the left femoral vein extending in to the left popliteal vein. A chest CT scan demonstrated segmental pulmonary embolism along with pleural effusion. He was started on Dalteparin and antibiotics. A complete blood count showed an AEC of 7770/mm^3 and a platelet count of 79,000/mm^3. The patient subsequently developed persistent fever along with worsening respiratory distress. His right lower limb became swollen and tender. A repeat Doppler ultrasound of lower limbs showed a new extensive occlusive clot of right femoral vein and popliteal vein. A worsening pleural effusion was noted on chest imaging. Marked eosinophilia was detected in pleural fluid (eosinophils-21%). His AEC was persistently elevated (Range: 6020/mm^3-8250/mm^3). A full diagnostic work up for infectious, immunological and rheumatolgical causes of eosinophilia came back negative. Bone marrow aspirate and biopsy were normocellular with marked non-dysplastic eosinophilia (23%). Bone marrow cytogenetics and flow cytometry were normal. BCR/ABL and JAK2 V617F mutations were negative by polymerase chain reaction (PCR). FIP1L1, CHIC2, PDGFRA and PDGFRB gene regions were normal by PCR. No evidence of B- or T-cell clonality was seen. He was started on methylprednisolone at a dose of 30mg/kg for 3 days with the working diagnosis of idiopathic HES. His clinical status improved significantly within 24 hours of starting steroids. Peripheral blood AEC normalized (AEC-60/mm3) within 3 days. Due to persistently sub-therapeutic anti-Xa levels, Dalteparin was changed to Apixaban. Oral prednisolone was started at dose of 2mg/kg/day following the pulse dose of methylprednisolone and was tapered based on his clinical status and AEC. Assessments done three months in to treatment showed resolving pulmonary emboli with normal pulmonary function and persistence of old thrombus in right and left leg with minimal collaterals. Clinically, he continues to do well on Apixaban and minimum doses of prednisolone.
Summary/Conclusion: Hypereosinophilic syndrome can present as multiple life-threatening thromboses in children. One should be aware of the thromboembolic complications of HES.

PB2510

DEEP VEIN THROMBOSIS IN THE INPATIENT : A TERTIARY CARE ANALYSIS FROM INDIA

C. Cherian Philip[1,*], S. Rathore[2], S. Singh[1], A. Mathew[1], A. Jindal[3], N. Kakkar[4], M.J. John[1]

[1]Clinical Haematology, [2]Radiodiagnosis, [3]Immunohematology and Blood Transfusion, [4]Laboratory Haematology, Christian Medical College & Hospital, Ludhiana, India

Background: Venous thromboembolism and DVT is recognized as the most common cause of inpatient death in the west and there exists established guidelines to direct its management. The incidence of Deep Vein Thrombosis however has been traditionally considered low in Asia. There exists no definitive scientific explanation to this and recent data challenge this concept. The limited data on the prevalence of deep vein thrombosis in India is both conflicting and scarce. This information is important to sensitize health care professionals and influence treatment strategies. In identifying with this goal we analysed the inpatient burden of DVT from our centre.
Aims: We attempted to estimate the prevalence of DVT among inpatients in our tertiary care centre.
Methods: We conducted a retrospective analysis of patients hospitalized between January 2014 through December 2016 to our academic hospital with 700 beds. Patients with potential venous thromboembolism were identified as those who underwent duplex ultrasound and CT Pulmonary angiogram (CTPA) examinations during that period. The diagnosis was confirmed by a review of the patients' inpatient records. Major bleed was defined as one requiring transfusions. Death and repeat thrombosis were defined as events.
Results: A total of 106074 patients were admitted in these period and184 examinations (duplex of the leg and CTPA) were done. Of these 26 patients were excluded (16- arterial thrombus and 10 – no details on type of thrombus) for this analysis. Characteristics of the included patients are detailed in table1. The prevalence of DVT was 158 of 106074 patients (0.15%). The median length of hospitalization was 10 (5-15) days. Four (2.6%) patients had a pulmonary embolism. While hospitalized and diagnosed with a DVT; 18 (11.4%) had undergone a major surgery; 25 (15.8%) patients were diagnosed with a malignancy; 16 (10.1%) were related to their central line and 11 (7%) patients were on pharmacological prophylaxis. A thrombosis workup was done in 10 (6.3%) patients and swelling of the leg was the major complaint in 91 (82.0%). The patients [55 (45.8%)] were mostly treated by a Vitamin K antagonist bridged with a LMWH.Seven (4.4%) patients required an IVC filter or embolectomy. There was one documented history of major bleed (Upper gastrointestinal bleed) and 20 deaths. The Overall (OS) and Event-free survival (EFS) at one year were 83.2%±3.9% and 74.1%±5.0% respectively.

Table 1.

Table 1. Characteristics of patients with DVT	
Variable	**N (158) n (%)/ Median (IQR)/Mean (±SD)**
Age	55(42-66)
Gender(Male)	95 (60.1)
Durations of admission (days)	10 (5-15)
Hb(g/dL)	10.7 (±2.6)
TLC (x 10^9/L)	10.0 (6.7 - 15.2)
Platelets (x 10^9/L)	228 (157 - 327)
Comorbidities (Hypertension)	39 (24.7)
Pregnancy (Yes)	5 (3.2)
Smoking (Yes)	5 (3.2)
Family History of Thrombosis (Yes)	4 (2.5)
Recent trauma (Yes)	7 (4.4)

Summary/Conclusion: Our analysis supports the belief that the prevalence of DVT in the Indian setting is low. An underlying malignancy is the most commonly identifiable cause of a DVT. Major bleed post treatment and primary prophylaxis are infrequent in inpatients. Racial differences and low levels of suspicion with resulting investigations might account for this variation in prevalence. Multicentric ,prospective data collection along with biological studies within India is needed to establish and explain this difference.

PB2511

PLASMA LEVELS OF SOME HEMOSTATIC MARKERS IN PATIENTS WITH ACUTE CORONARY SYNDROMES

Y. Tyravska[1,*], T. Vovk[2], N. Raksha[2], V. Lizogub[1]

[1]Internal medicine#4, Bogomolets National Medical University, [2]Department of Biochemistry, Educational and Scientific Center 'Institute of Biology and medicine', Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

Background: Disturbances in the plasma levels of tissue plasminogen activator (t-PA) and its inhibitor (PAI-1) as well as in fibrinolysis have been implicated in the pathogenesis and development of ischemic heart disease, particularly acute coronary syndrome.
Aims: The main goal of the current study was to compare some hemostasis parameters of the patients with acute myocardial infarction and patients with unstable angina.
Methods: Blood plasma was taken from 35 healthy donors and 9 patients with acute myocardial infarction (AMI), 14 patients with new onset unstable angina (NOUA), 21 patients with progressive unstable angina (PUA). Plasma levels of tissue-type plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1) and von Willebrand factor (vWF) were measured by enzyme-linked immunosorbent assays. Soluble fibrin monomer complex (SFMC) was determined using the phosphate method with 0.1 M phosphate-buffered saline containing sodium citrate and 0.2% 6-aminohexanoic acid with following determination (Zaichko N.V., 2006).
Results: Tissue-type plasminogen activator is an enzyme that converts the inactive proenzyme plasminogen to the active protease plasmin – the main executer of fibrinolysis. The obtained data showed that the plasma concentration of t-PA was in 1.43 times lower in the patients with AMI and NOUA than in the group of the healthy donors. The decreased of plasma level of t-PA may reduce fibrinolytic activity and attenuate removal of the thrombus and may ultimately lead to acute myocardial infarction in some patients with unstable angina. On the other hand, the t-PA level in patients of PAU group was at the control level. The catalytic activity of t-PA in the bloodstream is mainly regulated by the binding with some inhibitors. Therefore,

the plasma level of the primarily inhibitor PAI-1 was also estimated. There were significant elevations of PAI-1 in patients with AMI, NOUA and PUA. According to the obtained results the level of PAI-1 was in 16 times higher than the control value for 30% of patients with NOUA and for 70% of patients with PUA. For all the patients of AMI group PAI-1level was in 11 times higher than this in the group of the healthy donors. The elevated level of PAI-1 results in deficient plasminogen activation and is strongly associated with a predisposition to thrombosis. To estimate the thrombotic conditions and the degree of blood coagulation system activation SFMC concentration was determined. SFMC is formed in the early-activated state of blood coagulation and its appearance is expected to be a parameter for the diagnosis of thrombus formation. The conducted study revealed significant elevation of SFMC level in all patients of AMI group (in 10.6 times), NOUA group (in 12.6 times) and in 60% of the patients with PUA (in 11.5 times). Despite the well-known fact the involvement of endothelial dysfunction in the pathogenesis of cardiovascular disease no significant differences of the vWF's concentration in patients with AMI and NOUA have been detected. In contrast, about 60% of the patients with PUA have plasma level of vWF in 1.36 times higher comparing to the group of healthy donors.
Summary/Conclusion: The obtained findings suggest the strong impairment of the hemostasis balance in patients with acute coronary syndromes that appears as elevation of level of PAI-1, depletion of level of t-PA and accumulation of SFMC.

PB2512

THROMBOLYTİC TREATMENT IN A CHILD WITH OF DIFFUSE VENOUS THROMBOSIS: BEHCET'S DISEASE

D. Seven Karaman[1], E. Zengin[2], B. Özoğlu[1], A. Tekin Yılmaz[3], N. Uyar[4], S. Aylan Gelen[2,*], A. Sakarya Güneş[5], K. Babaoğlu[6], A. Uncuoğlu[4], N. Sarper[2]

[1]Kocaeli University Medical School Pediatry, [2]Kocaeli University Medical School Pediatric Haematology, [3]Kocaeli University Medical School Pediatric Infection, [4]Kocaeli University Medical School Pediatric Gastroenterohepatology, [5]Kocaeli University Medical School Pediatric Neurology, [6]Kocaeli University Medical School Pediatric Cardiology, Kocaeli, Turkey

Background: Behçet's disease is characterized by recurrent oral aphthous ulcers, genital ulcers, and uveitis. Involvement of large vessels, central nervous system, and gastrointestinal tract, and thrombotic events are less frequent but can be life threatening.
Aims: Here we report the therapeutic process as a case report of an 12 years old male patient presenting with neurological symptoms and diffuse venous thrombosis with Behçet's disease..
Methods: A 12 years old male patient with three days of fever was referred to our center, because of hepatomegaly, pleural effusion and persistent fever. In history; 3 years ago he applied to a hospital with double vision, inward shift in both eyes and bilateral papillary edema. Pseudotumor cerebri was diagnosed. Cranial MRI was re-evaluated, right sinus venous thrombosis was detected, thrombophilia screening was normal. Anticoagulant treatment was used for 1 year. In last 5 years, painful aphthous lesions occurred 3-10 times a year and were healing in 3-5 days. There was no similar disease in the family history. In physical examination; he had fever and tachypne. The skin was pale, right lung baseline respiratory sounds were decreased, and there was 1/6 systolic murmur on mesocardiac region. Superficial veins on the abdomen were apparent, liver was 5 cm palpable. Ampicillin sulbactam and clarithromycin was started for pneumonia-parapneumonic effusion. Abdominal USG was found normal except hepatomegaly. On the 10th day, pleural effusion was reduced, the fever persisted, the liver continued to grow, and the anemia deepened. After the diagnosis of thrombus in right atrium and vena cava inferior, treatment was changed to ceftriaxone and gentamicin with the diagnosis of infective endocarditis and unfractionated heparin was started. Abdominal and thoracic CT angiography was performed in terms of thromboembolism. Bilateral pulmonary embolism, infarct in parenchyma, filling defect in right atrium was observed. Vena cava inferior, bilateral iliac veins, vena porta, vena hepatica were obliterated. Liver perfusion deterioration and gall bladder hydrops were observed.
Results: The tissue plasminogen activator (tPA) was started at a dose of 0.3 mg/kg/h in addition to heparin upon approval from his family. Due to nosebleed dose was reduced to 0.25 mg/kg/hour, when the nosebleed stopped the dose was increased to 0.3 mg/kg/hour again. At the end of 16th hour, Doppler USG revealed normal in the portal vein, hepatic vein and vena cava inferior. At the 19th hour of tPA therapy, the thrombus in the right atrium was narrowed, but the thrombus continued in the vena cava inferior. On the second day of tPA treatment, upper gastrointestinal tract bleeding was observed in the patient. Erythrocyte suspension transfused, antacid and proton pump inhibitor started. On the 46th hour of the therapy thrombus in both the right atrium and the vena cava inferior was dissolved and then tPA and unfractionated heparin was terminated. The abdominal tendency disappeared, the veins on the abdominal wall have become silky and the liver has become smaller. Enoxiparin 1 mg/kg/dose was started at 12 hours interval. Methylprednisolone and azathioprine initiated. Anticoagulant treatment was changed to Coumadin.
Summary/Conclusion: In pediatric patients, tPA is the agent for thrombolytic upon approval from the parents. There is minimal experience with thrombolytic therapy in children. A survey showed no consensus in indications for thrombolysis, dose, mode of delivery, or duration of therapy. Thrombolytic therapy in patients is life saving for life or limb threatening diffuse thrombosis.

PB2513

RESULTS OF USING IDARUCIZUMAB IN SIX PATIENTS

E. Morente Constantín[1,*], C. Ferrer Chaves[2], P. Romero Garcia[3], A. Biedma López[2], J. Berruga Oñate[2], M.A. Garcia Ruiz[1], M. Rivas Luque[1], M. Jurado Chacón[1]

[1]Servicio de Hematología y Hemoterapia, Hospital Universitario Virgen de las Nieves, Granada, [2]Servicio de Hematología y Hemoterapia, Hospital San Juan de la Cruz, Úbeda, [3]UCI, Hospital de Santa Barbara, Soria, Spain

Background: Patients who are receiving oral anticoagulant therapy may benefit from anticoagulant reversal if they present life-threatening bleeding or undergoing urgent intervention. Idarucizumab (Praxbind, Boehringer Ingelheim) is a specific reversal agent for dabigatran. It is a humanized monoclonal antibody fragment that binds dabigatran and it is indicated in adult patients when a rapid reversal of its anticoagulant effect is required.
Aims: This is a descriptive study to determine whether idarucizumab would be able to reverse the anticoagulant effect of dabigatran in patients who are about to undergo an urgent procedure.
Methods: The six clinical cases of patients treated with idarucizumab are described. **CASE 1**: A 70-year-old woman receiving Dabigatran 150mg/12h who comes to the emergency due to postoperative metrorrhagia. Due to the need of urgent surgery, idarazizumab was given. Hysterectomy with a double annexectomy was made. 20 days later, Dabigatran 150mg/12h was reintroduced without further complications. **CASE 2:** A 55-year-old male receiving anticoagulant Dabigatran 150mg/12h, who came to the emergency with complicated renal colic. It was decided to implant double J-catheter, for which we administered idarazizumab. Dabigatran was restarted in 24h at its usual dose, with no new complications thus far. **CASE 3:** A 77-year-old woman treated with Dabigatran 110mg/12h goes to the emergency room with urinary tract infection and rectal bleeding. She was treated with Idarucizumab. 1h after its administration, the diarrhoea continues without evidence of bleeding. 24h later, she died due to multi-organ failure but without evidence of bleeding. **CASE 4:** A 88-year-old woman treated with Dabigatran 110mg/12h goes to the emergency room with 24-hours rectal bleeding. Given the persistence of rectal bleeding 13h after admission, it was started reversal treatment with Idarucizumab. After 1 hour of administration, there is no evidence of bleeding and there is evidence of normalization of clotting times. **CASO 5:** A 86-year-old woman treated with Dabigatran 110mg/12h comes to the emergency room with 48-hours rectorragia. Given the situation of severity with active bleeding, in addition to establishing measures of hydration and transfusion, it was decided to start reversal treatment with Idarucizumab. Improving her general condition progressively, 16h after its administration, there was an increase in the coagulation times, recovered gradually. **CASE 6:** A 76-year-old male, treated with Dabigatran 150mg/12h comes to the emergency room with dizziness and melenic depositions of 3 days of evolution. Although rectal bleeding was not present at this time, it was decided to start reversal treatment with Idarucizumab 2h after admission. 3h later, there was no evidence of bleeding and the clotting times were normal. 12h later, there was an increase in coagulation times. Gastrointestinal endoscopy was performed with no evidence of blood remnants or apparent lesions.
Results: The use of idarucizumab permitted rapid and safe intervention of patients. They had normal hemostasis during the procedure. 5 g dose of idarucizumab was sufficient and reversal was maintained for 24 hours.
Summary/Conclusion: Idarucizumab is integrated into protocol for the emergency management of patients on dabigatran. Idarucizumab is effective for dabigatran reversal among patients who have uncontrolled bleeding or will be undergoing urgent surgery.

PB2514

MAY-THURNER SYNDROME AND RECURRENT DEEP VENOUS THROMBOSIS IN TWO PEDIATRIC CASE TREATED WITH DABIGATRAN ETEXILATE

M. Cetin, S. Aytac*

Pediatric Hematology, Hacettepe University, Ankara, Turkey

Background: May-Thurner Syndrome (MTS) also known as iliac vein compression syndrome is a predisposition factor for venous thromboembolism. It occurs as a result of compression of the left common iliac vein between the right common iliac artery and the vertebrae. As a result of venous congestion deep venous thrombosis develop in the left lower extremity. MTS is diagnosed in 2-5% of all patients being evaluated for chronic venous insufficiency of the lower extremity. It is reported that MTS was more common in women and the mean age at presentation is 42.6±16.9 years.
Aims: Here, we present two adolescent girl who was initially diagnosed as lower extremity DVT and received LMWH however their symptomps were progress under the treatment of LMWH, they both experience recurrence. Because of swelling and pain of the leg they were both re-evaluated and diagnosed as postthrombotic syndrome. However their symptomps did not resolve and PO dabigatran etexilate was given.
Methods: Two adolescent girl(15 and 16 years old) who were initially diagnosed as left lower extremity DVT with postphylebitic syndrome, treated with dabigatran etexilate and their distinctive symptomps eventually disappear with this treatment however MR angiography did show MTS. Althogh they both had a history of recurrence since PO dabigatran etexilate treatment they had neither recurrence nor aggravation of symptomps for 2 years.
Results: Several treatment modalities have been used to treat MTS mostly based on surgical and/or endovascular interventions in the past. More recently medical treatment approaches especially for patients with DVT were considered.
Summary/Conclusion: In conclusion leg swelling and pain in adolescent girls with left lower extremities DVT should be evaluated for MTS.

PB2515

EXPERIENCE IN REAL-LIFE ANTICOAGULANT TREATMENT WITH EDOXABAN IN PATIENTS WITH ATRIAL FIBRILLATION AND SECONDARY PREVENTION OF DEEP VEIN THROMBOSIS WITH A FOLLOW-UP OF LAST YEAR

M.A. Garcia Ruiz[1,*], M. Rivas Luque[1], P. Romero Garcia[2], E. Morente Constantín[1], P. Garrido Collado[1], M. Jurado Chacon[1]

[1]Servicio de Hematología y Hemoterapia, Hospital Universitario Virgen de las Nieves, Granada, [2]UCI, Hospital de Santa Barbara, Soria, Spain

Background: Atrial fibrillation (AF) is associated with the risk of ischemic stroke. Oral anticoagulation therapy is a well-established treatment for preventing strokes in patients with moderate to high risk. Non-vitamin K antagonist oral anticoagulants (NOACs) have been recommended as alternatives to Anti-Vitamin K (AVK) for stroke prevention in patients with non-valvular AF (NVAF) and Deep Vein Thrombosis (DVT). Edoxaban (Lixiana®, Daiichi-Sankyo) is an oral, reversible, direct factor Xa. The dose consists of 60mg/24h and 30mg/24h (reduced dose) for estimated creatinine clearance of 15-50mL/min, body weight <60 kg or concomitant use of P-glycoprotein inhibitors.
Aims: Primary Objective: To evaluate the efficacy of Edoxaban in the prevention of stroke and Embolism Systemic. Secondary objective: To assess the safety, in function of the major bleeding largest defined according to the criteria of the International Society of Thrombosis and Hemostasis.
Methods: This is a descriptive study. We have included a total of 211 patients with NVAF (199 patients) and DVT (12 patients). The mean CHA2DS2-VASc (risk of stroke) of patients studied was of 3.74 in the total population and 4.18 in reduced dosage population. The mean HAS-BLED (risk of bleeding) of the studied patients was 3.01 in the total population and 3.25 in the reduced dosage population. We have included 163 cases with doses of 60mg/24h and 48 cases at a dose of 30mg/24h (22,7% of total patients. It was about 109 women (51,66%) and 102 men (48,34%), aged between 42 and 95 years old (mean age 72.5). The main reason of suspension of AVK and replacement by Edoxaban was poor control of INR (199 patients) and diathesis bleeding (12 patients). Of the total number of included patients, 41 had presented a previous stroke under treatment with AVK. The treatment time has been between 3 and 13 months, with an average of 7.65 months of follow-up.
Results: It has not been reported any case of Stroke, Systemic Embolism, or Major during the follow-up time of the patients treated. Only one case of non-major clinically relevant bleeding (rectal bleed) which required changing to another NOAC. One case of small bruise in a patient treated with a 60 mg dose /24h which had a creatinine clearance of 44ml/min, so the dose was reduced to 30mg/24h. One patient presented self-limited hemoptysis that did not require suspension of the treatment. One patient with a subdural hematoma, after traumatic brain injury, which treatment was suspended. During the study, a patient was diagnosed with lung cancer and was discontinued anticoagulant treatment, and replaced by low molecular weight heparin. The patient died later by this pathology oncology. Three patients died due to respiratory disease (Influenza and respiratory insufficiency), not being related to the anticoagulant medication.
Summary/Conclusion: The results obtained confirm the efficacy and safety of Edoxaban in the prevention of stroke in the NVAF and DVT. This is a good sample of 211 patients and more than seven months of follow-up, despite the short period of time since the use approval of the drug in our country (18 months). The results support the results of the trials. We believe that there is a good alternative to oral anticoagulation in patients with NVAF with efficacy in the prevention of stroke and Systemic Embolism, and with a good safety profile with a clinical benefit net at the expense of mainly absence of bleeding. Subsequent studies will provide data confirmation.

PB2516

RITUXIMAB FOR REFRACTORY THROMBOTIC THROMBOCY TOPENIC PURPURA IN CRITICALLY ILL PATIENT

S. Mohamed[1,*], M. Yassin[1], A. El Buzidi[2], M. Abdelaty[2], A. Akkari[2], A. Nashwa[1]

[1]Hematology, [2]MICU, HGH, DOHA, Qatar

Background: Thrombotic thrombocytopenic purpura (TTP) is characterized by thrombocytopenia and microangiopathic hemolytic anemia (MAHA) without an obvious cause, and may include fever, mild renal failure, and neurologic deficits. Refractory TTP defined by some authors as any one of the following: 1-TTP for which treatment with plasma exchange (PEX) and glucocorticoids fails to produce a satisfactory response. 2-new neurologic abnormalities during PEX that are not attributable to another cause such as bleeding or infection. 3- Exacerbation of symptoms or laboratory findings occurs during PEX or within the first 30 days of stopping PEX.
Aims: To show outcome of two difficult cases of refractory TTP to daily PEX plus rituximab twice weekly.
Methods: The first case is a 32 years old male patient, known case of ulcerative colitis, the second case is a 26 years old male healthy before both, diagnosed him with of TTP based on clinical and laboratory findings and started on PEX and prednisolone.They were not responding initially and their hospital stay complicated with renal failure, ARDS and seizures so both patients intubated and admitted in intensive care unit. We added rituximab to the treatment, and as desperate measures we made rituximab twice weekly. After several weeks improve gradually. Both of them were extubated, and later sent to the medical word for further rehabilitation.
Results: The pathophysiology is lack of protease which cleavage von willbrand large multimeric. The protease called ADAMT- 13 and it could be absent congenitally or acquired. Severe deficiency of ADAMT-13 less than 5% specific for idiopathic TTP. The established management is plasma exchange (PE) along with steroids 80% of patients to survive, however, the result is still there are refractory and relapse cases. There is increase of usage of Rituximab as salvage therapy in cases of TTP. Mischelle *et al*., reported four cases and systemic review of literature. The found patient who received Rituximab in relapse or refractory, their median age is 42, most were females 78%, median doses 4, given once weekly and dose was 375mg/m^2. Complete remission achieved in 95% within 11- 35 days. According to our knowledge rituximab twice weekly agents was never used before. Our two patients were refectory to PE although it was going on daily for more than 2-3 weeks along with steroids.
Summary/Conclusion: TTP is a serious hematological malignancy with considerable amount of refractory cases. Rituximab addition to PE and steroids is good strategy. We suggest it can be used twice weekly safely, although further studies needed to proof efficacy Young patients and healthy deserves all extraordinary measures, which include not only rituximab but continue PE daily with giving up on patient.

PB2517

THE ELEVATION OF VON WILLEBRAND FACTOR (VWF) IN THE PATIENTS WITH MALIGNANT LYMPHOMA TREATED WITH RITUXIMAB (R)-CHOP THERAPY

N. Shimizu[1,*], R. Oka[1], S. Nakamura[1], Y. Watanabe[1], S. Tanaka[1], Y. Sato[1], H. Imamura[1], H. Kawana[1], T. Yamaguchi[1], M. Ohira[1], A. Saiki[1], H. Bujo[2], K. Shirai[3], I. Tatsuno[1]

[1]Center for Diabetes, Metabolism and Endocrinology, [2]Department of Clinical Laboratory Medicine, Toho University Sakura Medical Center, Sakura City, [3]Department of Internal Medicine, Mihama Hospital, Chiba, Japan

Background: An increased incidence of atherosclerosis has been noted in

cancer survivors. In past, there only were a few reports on the relation between atherosclerosis and chemotherapy. As a mechanism for the progression of atherosclerosis with chemotherapy, the reduction of nitric oxide from endothelial cells has been reported apart from complications of metabolic diseases. We have also reported a case who was 68-year-old female with follicular lymphoma, clinical stage IVA showing the plaque formation of carotid artery and the elevation of cardio-ankle vascular index (CAVI) after the eight courses of R-CHOP therapy (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) without complication of diabetes mellitus and hyperlipidemia.
Aims: So, herein we evaluate the risk for the development of atherosclerosis with chemotherapy using an arterial stiffness parameter, the CAVI, the findings of carotid artery ultrasonography and the value of von Willebrand factor (vWF) as an endothelial damage in the patients with malignant lymphoma who were treated with R-CHOP therapy.
Methods: We enrolled eight patients who were diagnosed B-cell malignant lymphoma and finished with treating with R-CHOP therapy from December of 2016 to August of 2017. We evaluated the atherosclerosis with CAVI index, carotid artery ultrasonography and the value of vWF during each consecutive chemotherapy. The Ethics Committee in Toho University approved this study, and we obtained informed consent.
Results: Seven men and one woman were registered (the median age, 72.5 years old). Every eight patients achieved complete remission after chemotherapy. Two patients were treated with 6th course, one patient with 7th course and the other patients were with 8th course of R-CHOP therapy. Two patients had not evaluated the vWF after final therapy, and we missed to check the value of vWF in some patients during the treatment, so evaluable number of patients were shown as in Figure. Almost all patients showed elevation of the vWF with the progression of treatment. The value of vWF of pretreatment (132.5±44.4) elevated significantly (p=0.0227) to 186±44.5 at just before second treatment. Comparing the value of vWF of pretreatment and another point, vWF elevated significantly as follows (just before 4th treatment;184.5±40.8, p=0.0117, just before 6th treatment;189.5±31.5, p=0.0117). After the treatment, the value decreased in all the patients, however there was not significant difference (p=0.0796). Six patients out of eight patients showed the progression of atherosclerosis, new plaque formation or progression of intima-media thickness by carotid artery ultrasonography. In evaluation of CAVI index, some patients showed the elevation of index with the progression of treatment. However, there was not significant elevation at all during the treatment.

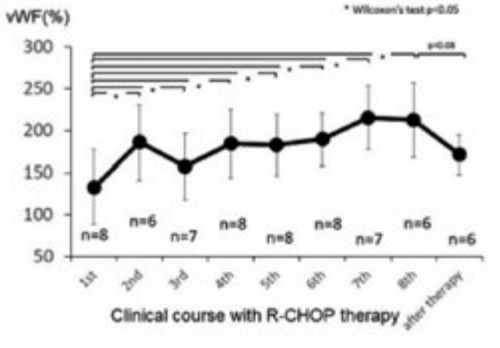


Figure 1.

Summary/Conclusion: We reported that malignant lymphoma patients showed the significant elevation of vWF during the R-CHOP therapy. We speculate that this therapy may be the risk of progression of atherosclerosis via vascular endothelial damage. Further study will be required to clarify the relationship between chemotherapy and arteriosclerosis.

PB2518

LEICINE-CONTAINING REGULATORY PEPTIDES PREVENT THE DEVELOPMENT OF TROMBOSIS

L. Lyapina*, M. Grigorjeva, T. Obergan

Moscow Lomonosov State University, Biological faculty, Moscow, Russian Federation

Background: It is known that glucine-containing peptides (glyprolines) have anticoagulant, antiplatelet and fibrindepolymerizing effects *in vitro* and *in vivo* in normal (Ashmarin *et al.*, 2008) and pathological conditions, complicated by thrombosis (metabolic disorders of fat and carbohydrate, including hyperholesterinemia, metabolic syndrome, diabetes mellitus).
Aims: To improve anticoagulation and create antithrombotic effects of glyprolines peptides by inclusion in their structure of leucine molecule from the N- or C-end in conditions of the depression of the function of the anticoagulation system in the development of metabplic syndrome, enhanced by the introduction of thromboplastin into the bloodstream.
Methods: The following methods were used in this study: registration of platelet aggregation, anticoagulant activity by test of activated partial thromboplastin time, total fibrinolytic activity (TFA), enzymatic fibrinolysis (EF), fibrindepolymerization activity (FDPA). The proline- and glycine-containing oligopeptides having a base Pro-Gly-Pro (PGP) with the addition of the amino acid leucine to N- and C- end of peptide molecule were used. All experiments were carried out on rats in accordance with the Helsinki Declaration about humane treatment of animals. The development of metabolic syndrome was modeled by keeping all animals on a high-calorie diet (HCD) for 2 months. Scheme of experiments: the experimental group of rats were intranasally administrated the peptides every 24 h for 5 days at a doses of 500μg/kg. At the same time the control group of rats were received 0.85% saline instead peptides. 18 h after the last administration of peptides rats intravenously injected 1% thromboplastin in the amount of 0.4 ml to generate thrombin in the bloodstream. Blood samples were taken 6-8 min after injection of thromboplastin.
Results: It was found that PGPL and LPGP inhibit the formation of fibrin clots in the provocation of venous thrombosis. When comparing the antithrombotic action of two peptides, it was found that the process of fibrin polymerization took place more slowly under the action of PGPL than LPGP in these conditions. The blood of animals was analyzed after the action of thromboplastin in the preliminary administration of oligopeptides PGPL and LPGP. The increase in TFA-1.7-1.6 times, EF-1.5-1.4 times, FDPA-1.9-2.7 times compared with control rats contained on the HCD and did not receive peptides were found in the blood. In these experimental conditions, in the case of the fibrin clots formation in the bloodstream, they are rapidly subjected to not only enzymatic (plasmin action), but also non-enzymatic (the action of the peptides themselves) lysis. We have shown that oligopeptides exhibit anticoagulant activity in the bloodstream. The action of PGPL is more pronounced (1.6 times increase), than anticoagulant plasma activity of LPGP (1.36 times increase). Both peptide provided in the blood antiplatelet effect (the most significant after LPGP administration). Thus, platelet aggregation decreased by 55% and 65% under the action of PGPL and LPGP respectively.
Summary/Conclusion: Thus, glyproline peptides with leucine included in their structure at both ends of the molecule activate or normalize the function of the blood anticoagulation system. They are involved in the prevention of venous thrombosis processes and contribute to the manifestation of thrombolytic effects of plasmin. They have antiplatelet effect, protecting the organism from the initial stages of thrombosis. Peptide LPGP has the most effective anticoagulant and antithrombotic action.

PB2519

ACTIVATION OF PLATELET HAEMOSTASIS AND ITS CORRECTION BY PROLINE-CONTAINING PEPTIDES IN METABOLIC DISORDERS

M. Grigorjeva*, L. Lyapina

Biology, Lomonosov Moscow State University, Moscow, Russian Federation

Background: Metabolic syndrome (MS) is characterized by dyslipidemia, hyperglycemia, insulin resistance. This pathology lead to the development of atherosclerotic changes in blood vessels and haemostasis disorders. The hyperactivity of platelets is the most important among the mechanisms involved in the processes of atherothrombosis. Also the development of MS lead to formation of large hyperactive platelets and increase of red blood cells hemolysis, which results an enhancement of the erythrocyte ADP release. This contributes to increased platelet aggregation, hypercoagulation and formation of prothrombogenic status of the organism. Directed influence of pharmacology agents may to reduce pathologically increased platelet aggregation. Earlier it was shown that regulatory oligopeptides exhibit anticoagulant and antiplatelet effects in the organism. Recently, special attention has been paid to proline-containing peptides with the addition of various amino acids to their molecules.
Aims: To study the increase of platelets aggregation function in rats with experimental metabolic syndrome and the possibility of its correction by short regulatory proline- and glycine-containing (glyproline) peptides Pro-Gly-Pro (PGP), Arg-Pro-Gly-Pro (RPGP), Pro-Gly-Pro-Leu (PGPL) and Pro-Gly-Pro-Val (PGPV).
Methods: The experiments were carried out on male adult (4 months of age) Wistar rats (350-400 g body weight). All experimental procedures were performed in accordance with the ethical principles of the Helsinki Decla-

ration. Experimental MS was induced by feeding the rats a fat-rich high-caloric diet (HCD) during 6 weeks. At the same time rats received 10% glucose ad libitum. Than MS-rats were intranasally administered peptides PGP, RPGP, PGPL and PGPV at doses 200μg/kg body weight once a day for 7 days (treated groups) and saline (control group) by simultaneous HCD continuation. ADP-induced platelet aggregation were measured in rich platelet blood plasma (Born method). Blood were collected from the jugular vena 20h and 7 days after the last peptides administration.
Results: Experiments showed that consumption of HCD by the rats over 6 weeks lead to development of lipid and carbohydrate metabolism disorders and marked hypercoagulation. First of all, platelet aggregation increased more than twofold relative to those of normal rats. The intranasal administration of PGP, RPGP, PGPL and PGPV to MS-rats resulted a reduction of ADP-induced platelet aggregation respectively by 33%, 34%, 30% and 58% compared with control group 20h after the last peptides injection. These effects were observed 7 days after peptides withdrawal too: ADP-induced platelet aggregation was decreased by 30%, 50%, 30% and 33% respectively. RPGP and PGPV had the most pronounced and stable antiplatelet effects in rats with experimental MS and these effects were long lasting.
Summary/Conclusion: Thus the presented study indicate that proline- and glycine-containing peptides have significant antithrombotis effects in rats with disorders of lipid and carbohydrate metabolism. They have antiplatelet effect and protect the organism from enhanced platelet aggregation, decrease activation of platelet haemostasis and thrombus formation. We assume that regulatory glyproline oligopeptides can be attributed to antithrombotic agents with anticoagulant and antiplatelet properties in metabolism disorders.

PB2520

EFFICIENCY OF PGPV PEPTIDE APPLICATION IN THE EVENT OF THROMBOSIS OF DANGEROUS SITUATIONS IN THE CONDITIONS OF METABOLIC SYNDROME IN RATS

T. Shubina*, L. Lyapina

Biology Department, Moscow Lomonosov State University, Biological faculty, Moscow, Russian Federation

Background: Disorders of lipid metabolism in metabolic syndrome leads to atherothrombosis, accompanied by an increase in blood clotting and a decrease in fibrinolysis. It is known that the amino acids proline, arginine, methionine, leucine and regulatory peptides Pro-Gly-Pro, Pro-Gly, Pro-Gly-Pro-Arg have hypocholesterol effect, thereby reducing the risk of developing the disease, and atherosclerosis. Endogenous tripeptide Pro-Gly-Pro has antithrombotic activity, but it is unstable in the body. Also, the essential amino acid valine is required for the growth and synthesis of body tissues. It is necessary for the normal exchange of nitrogen in the organism and is actively involved in the biosynthesis of cholesterol.
Aims: The aim of this work was to assess anticoagulant-fibrinolytic and antiplatelet effects of the peptide Pro-Gly-Pro-Val (PGPV) on the model of rats with experimental metabolic syndrome (MS).
Methods: White male rats (450-600 g) were used in the study. All experiments were conducted in accordance with ethical principles of the Helsinki Declaration. Inducing metabolic disorders caused a high-calorie diet (HCD), the energy value 130% of the standard diet and 32% fat caloric. The content of rats for 6 weeks on HCD led to the development obesity and increased blood glucose. After 1.5 months, rats were divided into groups (in each group n=10): Group 1-HCD rats treated with PGPV in dose 200 μg/kg body weight; Group 2 – untreated HCD rats were given 0.85% saline; Group 3 - healthy rats without treatment on standard feed. PGPV or saline were injected by intranasal way for 20 μl per rat once daily for 10 days. Blood samples were obtained 1 h after the last administration of drugs, after 7 and 14 days after discontinuation of the drug with continuing content on HCD (on 10^{th}, 17^{th} and 24^{th} days of experiments, respectively). ADP-induced platelet aggregation, total fibrinolytic activity (TFA), enzymatic (EFA) and non-enzymatic (NFA) fibrinolytic activity on plates of non-stabilized fibrin and anticoagulant activity (APTT test) were determined in blood plasma.
Results: It was found that the 10-fold intranasal treatment of rats with PGPV peptide led to a significant lengthening of the clot formation time by 2.2 times (by APTT test), an increase in TFA by 89% is due to the growth of EFA by 2.7 times and NFA by 1.6 times compared to the Group 2. Platelet aggregation in Group 1 was lower by 58% compared to HCD rats, but was higher than in healthy animals. 7 days after termination of treatment, the significant increase in anticoagulant and fibrinolytic properties of the plasma remained in the Group 1, compared with the values in the 2nd and 3rd Groups. Thus, the APTT increased 2.6 times, TFA – 2.3 times, EFA 2 times, NFA – 1.6 times. Attention is drawn to the fact of a sharp increase in APTT and TFA, *i.e.* the ability of the drug to provide anticoagulation effect in the long-term. 24 days after the cancellation of the PGPV anticoagulant and fibrinolytic blood activity in rats of the Group1 remained significantly higher than in the 2nd. Platelets aggregation in Group 1 corresponded to normal values and were lower than in the 2nd Group.
Summary/Conclusion: Clear evidence was obtained of the benefits of the introduction of PGPV intranasal rats with metabolic disorders as a therapeutic agent that contributes to the normalization of the hemostatic system and reduces thrombotic complications, namely, reducing the increased ability of platelets to aggregate and increase anticoagulant-fibrinolytic activity.

PB2521

CHANGE OF FIBRINOGEN LEVEL AND ACTIVITY OF FXIII UNDER THE INFLUENCE OF THE REGULATORY PEPTIDES PRO-GLY-PRO AND PRO-GLY-ARG

T. Obergan*, M. Grigorjeva

Department of Biology, M.V. Lomonosov Moscow State University, Moscow, Russian Federation

Background: Fibrin, which is the basis of the blood clot, is formed from fibrinogen plasma upon the activation by the enzyme thrombin. Stabilization of fibrin in the final stage of coagulation is provided by the factor of the blood coagulation system – transglutaminase, FXIII. Increased fibrinogen concentrations, as well as elevated FXIII activity in the blood, can create dangerous thrombosis situations in the human body. In earlier studies, it was shown that short regulatory peptides, containing proline and glycine (glyprolines) exhibit antithrombotic effects in the organism. In our studies on the model of Wessler thrombosis it was shown that repeated administration of peptides Pro-Gly and Pro-Gly-Pro led to a decrease in the weight of fresh fibrin clots and a decrease in the number of cases of thrombosis. Also, glyprolines have antiplatelet, anticoagulant and fibrinolytic activity *in vitro* and in vivo, impeding the thrombus formation and slowing blood clotting. However, these effects are short-lived due to the low stability of glyprolines. Adding to the peptide molecule of the amino acid arginine increases its resistance and antithrombotic properties. Herewith the influence of glyprolines on blood coagulation factors such as fibrinogen and fibrinase (FXIIIa) has not been studied enough.
Aims: The aim of this study was to evaluate the effect of the peptides Pro-Gly-Pro (PGP) and Pro-Gly-Arg (PGR) on activity of factor XIII, fibrinogen level and anticoagulant activity of blood and their ability to prevent thrombus formation in healthy organism.
Methods: Experiments were carried out on thirty healthy male rats of Wistar strain (200–220 g body weight) in accordance with ethical principles of the Helsinki Declaration. Three groups of animals were used (in each group n=10): untreated rats as control (Group 1), PGP-treated rats (Group 2) and PGR-treated rats (Group 3). Peptide-treated rats received PGP or PGR by intranasal way daily during 5 days in doses 1 mg/kg of the body weight in the amount of 20 μL per rat. Untreated rats (control) received 0.85% saline as vehicle in the same volume by intranasal way. Blood samples were obtained 1 h after 5^{rd} administration of drugs. Anticoagulant activity (by APTT test), activity of factor FXIII and levels of fibrinogen were estimated in blood plasma by standard methods using standard assay kits (Technology Standard, Russia).
Results: The multiple 5-fold intranasal administration of PGP led to a decrease in the activity of FXIII by 15% (P <0.05), and PGR – by 25% (P <0.01) compared to control. The use of both peptides caused a reduction in fibrinogen levels almost equally by 20% and 25%, respectively, compared to values in the control group (P <0.05 in both cases). This was accompanied by an increase of anticoagulant activity in the animal blood, since APTT raised by 21% (PGP) and 27% (PGR), respectively (P <0.01 in both cases). It is noted that the effects of the peptide PGR were more pronounced.
Summary/Conclusion: The study demonstrated the inhibitory effect of peptides PGP and PGR on blood coagulation factors due to reduce the level of fibrinogen and decrease of activity of FXIII. Thus, the peptides of the glyproline family, when they appear in the bloodstream, contribute to loosen the strength of fibrin clots in case of their emergence, reduce the prothrombotic potential of the blood and restrict the probability of thrombus formation.

PB2522

ANTITHROMBOTIC ACTIVITY OF PEPTIDE SELANK AGAINST THE BACKGROUND OF EXPERIMENTALLY INDUCED ENDOTHELIAL DYSFUNCTION IN RATS

E. Rogozinskaya*

M.V. Lomonosow Moscow State University, Moscow, Russian Federation

Background: Peptides of a glyproline series, including tripeptides PRO-GLY-PRO (PGP), are formed in the body during the hydrolysis of collagen and elastin. They carry out peptidergic regulation in the body. Multiple studies have shown that glyiprolines are effective and have antiplatelet and anticoagulant properties. Recent experimental data indicate an increase in the effects of glyiprolines when different amino acids are added to the protein structure. Thus, the amino acid arginine ARG (R) increases antithrombotic effects in the body due to the release into the bloodstream of NO.
Aims: Study an influence of arginine-containing peptide Selank on the parameters of the hemostasis system against the background of endothelial dysfunction.
Methods: Endothelial dysfunction was caused by repeated administration of a solution of NO-synthase inhibitor - L-name at a concentration of 10 mg/kg. As the drug, Selank peptide THR-LYS-PRO-ARG-PRO-GLY-PRO (TKPRPGP) was intranasal used at a concentration of 100 μg/kg. All animal experiments were conducted in accordance with ethical principles and documents of the European Convention for the Protection of Vertebrates. White Wistar rats (300 g) were divided into 4 groups: 3 test groups (rats receiving Selank; L-name; Selank + L-name); 1 group - control (NaCl). The substances were administered for 7 days daily every 24 hours. On the 8th day, blood was taken from the jugular vein and a series of experiments was conducted to determine the effect of drugs on the parameters of hemostasis. After the cancellation of Selank, but the continued introduction of L-name, after 7 days, a series of experiments were repeated. The studies were performed on the APTT test (activated partial thromboplastin time) and the parameters of thromboelastogram (TEG).
Results: It was found that the intranasal administration of Selank to rats showed the presence of anticoagulant and antiplatelet activity in blood plasma under conditions of endothelial dysfunction according to APTTV and TEG parameters. So Selank increased the APTT by 30% compared with that in rats with dysfunction of the endothelium, Selank + L-name returned this parameter to the control level. After the subsequent withdrawal of the drug against the background of L-name, a similar pattern was observed: Selank increased the APTT by 20%.
Summary/Conclusion: So, arginine-containing glyprolin Selank showed anticoagulant and antiplatelet activity, and in the presence of Selank there was no endothelial dysfunction caused by L-name. Under conditions of cancellation of the oligopeptide, but continuing dysfunction, its trace positive effects on the parameters of the hemostatic system were noted.

Transfusion medicine

PB2523

A CASE-CONTROLLED STUDY OF VASOVAGAL REACTIONS IN BLOOD DONORS: INFLUENCE OF SEX, AGE, DONATION STATUS, WEIGHT, BLOOD PRESSURE, AND PULSE

T. Faiza[1,*], D. Djamel[2], N. Rachid[3]

[1]Hematology, central hospital of the army, [2]Medecine, blood transfusion station, [3]Epidemiology, Mayo Hospital, ALGER, Algeria

Background: Vasovagal reactions occur in a small, but significant number of blood donors. These reactions may decrease return donation and disrupt blood collection activities.
Aims: The purpose of this study was to define the contributory role of sex, age, weight, blood pressure in vasovagal reactions with syncope in blood donors.
Methods: Delayed adverse reactions to blood donation occur after the donor left donation site. Their intrinsic gravity and possible complications can be increased by the fact the donor is alone. on this study was to define the contributory role of sex, age, weight, blood pressure, and pulse in vasovagal reactions with syncope in blood donors. Case controls and random population controls were used in a logistic regression analysis to determine the significance of individual variables to syncopal reactions.
Results: Female donors (RR= 0.41IC (0.23-0.72, p=0.002), Young donors; <30 years: RR=1,45 IC (1.22-1.69) P=0.013 (s), first-time donors (p<.000), low-weight donors <75Kg: RR=1.22 IC (1.06-1.58, p=0.01), and donors with low predonation blood pressure (HR=0.36, p=0.001) had higher absolute donation reaction rates than other donors. as well as the amount of blood taken (fig.1): p=0.013(S)When each variable was adjusted for other variables by regression analysis, age, weight, and donation status (first-time or repeat donor), the most important variableis volume of blood (p=.000). Whenwe compare two periods: 2008-2012 then 2008-2017, we we have noted a net decrease of this type of complications over time, which suggests a better control of the sampling techniques and in particular of the measures of more rigorous selection of the donors (RR=0.45: IC (0.30-0.723), p=0.002.

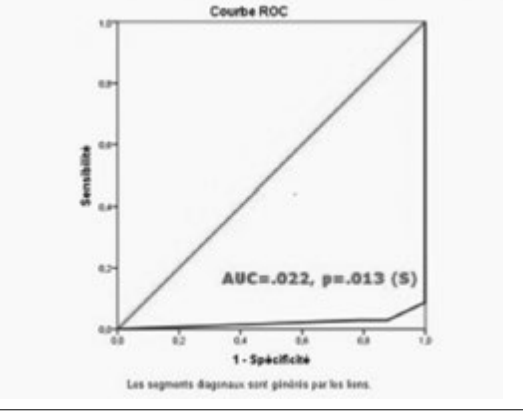


Figure 1.

Summary/Conclusion: Donation-related vasovagal syncopal reactions are a multifactorial process determined largely by age, weight, volume delayted and first-time donor status. Occurrence of a delayed donor reaction is clearly underrated in standard haemovigilance. It remains to be seen whether it have the same impact on donor return as immediate reactions. Considering that delayed reactions are much larger, it might be interesting to take them into account in the evaluation of strategies dedicated to lower immediate reactions.

PB2524

DEFERRAL FOR MALARIA RISK: AN INCREASING CONCERN ON BLOOD AVAILABILITY IN OMAN

K. Al Habsi*, A. Elkashif, S. Al Hashami, Z. Alarimi, T. Ashraf

Blood Banks Services, Ministry of Health, Muscat, Oman

Background: Malaria is an increasing concern to blood transfusion services

in non-endemic areas. Many blood donors travel to and from malaria endemic areas making it more difficult to ensure blood safety. Therefore, donor selection and deferral strategies are crucial to identify donors at risk of malaria exposure. Generally, these deferral strategies are not sensitive nor specific and can result in negative impact on the blood availability. Oman is a very low risk for malaria after an eradication program launched successfully in the 1990s. However, due to its location and historical connections with Asia and Africa many blood donors are deferred for malaria risk.
Aims: Our study aim to assess the impact of malaria deferrals on blood availability in Oman.
Methods: Records of all blood donors at the Central Blood Bank in Oman between November 2014 and October 2015 were reviewed. Archived donor forms, daily worksheets and the Information Technology "IT" system were used to collect rates of attending donors, total donations, total deferrals, positive serology and malaria-related deferrals. The percentage of donors deferred due to travel to malaria risk area, their country of origin, the areas of exposure and the duration of deferral were calculated.
Results: The total number of blood donors attended during the study period was 40285 donors. The total number of pre-donation deferrals was 12287 (30.5%) in addition to 2128 (5.3%) post-donation deferrals mainly due to reactive serology markers. The malaria risk related deferrals were 4578 donor (37.3% of total deferrals and 11.4% of total attending donors). Approximately 40% of those deferred for malaria risk were Omani nationals. The remaining 60% non-Omani deferrals were mainly Indians (79%) followed by donors from Pakistan (9%), Bangladesh (3.5%), Philippine (2%) and other nationalities. The common malaria endemic areas of exposure include India (60.1%) followed by Thailand (8%), Malaysia (7.6%), Pakistan (6.8%), Tanzania (4.1%) and several other Asian and African countries. The duration of deferral was ≥6 months in 67.1% (3074/4578) of deferred donors including 261 (5.7%) donors with permanent deferral due to previous history of malaria infection.
Summary/Conclusion: A significant number of blood donors in Oman are deferred because of travel to malaria-endemic areas. This negative impact on the blood availability requires a change in the current deferral strategy to ensure blood availability while maintaining the safety of blood products. Targeted malaria screening approach for blood donors at risk is required.

PB2525

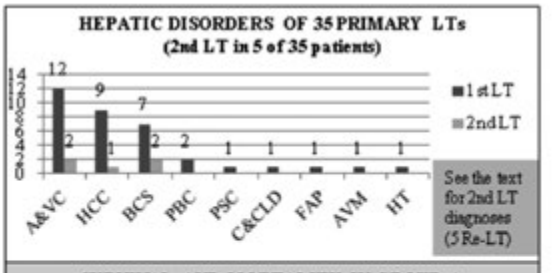
HEMATOLOGICAL DISORDERS AND LIVER TRANSPLANTATION DO PATIENTS HAVE A GOOD OUTCOME?

M.M. Campos[1,*], V. Oliveira[2], C. Gaspar[3], M.J. Marques[1], A.I. Fernandes[1], A.C. Silva[1], M.H. Alves [1]

[1]Dept of Immunohemotherapy (Head: D. Espírito Santo), Centro Hospitalar Lisboa Central, EPE (CHLC), Lisboa, [2]Dept of Transfusion Medicine, Hospital Prof. Doutor Fernando Fonseca, EPE, Amadora, [3]Dept of Clinical Hematology, Centro Hospitalar Lisboa Central, EPE (CHLC), Lisboa, Portugal

Background: In this survey we included patients with clinical indications for liver transplant (LT) and concomitant hematological disorders. End-stage liver diseases were alcoholic and viral cirrhoses (A&VC), hepatocellular carcinoma (HCC), Budd-Chiari syndrome (BCS), primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), cirrhosis and cholestatic liver disease (C&CLD), familial amyloid polyneuropathy (FAP), arteriovenous malformations (AVM), and hepatic toxicity (HT). Blood disorders were hemochromatosis, Osler-Weber-Rendu disease (OWRD), hemophilia B, myeloproliferative syndromes (MPS) including chronic myeloid leukemia (CML) submitted to bone marrow transplantation (BMT), polycythemia vera (PV) and essential thrombocythemia (ET), myelodysplastic syndrome (MDS), β-thalassemia minor, sickle cell disease (SCD), immune thrombocytopenic purpura (ITP), and acute myeloid leukemia subtype M2 (AML-M2) treated with BMT in childhood.
Aims: A series of 35 consecutive patients with hematological diseases submitted to LT was retrospectively analyzed (group A). In this set of patients, 35 primary transplants were done with grafts from different sources: cadaveric (32), related healthy living-donor (1), non-related healthy living-donor (1) and FAP carrier donor (1). A total of 5 re-transplants (all from cadaver) were performed. Main goals were to detect differences in transfusion supply comparing 127 patients with A&VC and/or HCC only (group B) with 21 hematological patients with the same liver diagnoses (group C) and their mortality rates. Consumptions and overall survival were also considered in group A.
Methods: A review of electronic clinical records was carried out in patients whose LT were comprised between 1 February 2010 and 31 January 2017 (one or more years following patient's last LT) in CHLC. Specific archives of the Department of Immunohemotherapy were assessed.
Results: The enrolled hematological patients (group A) were 21 males and 14 females (mean: 49.9; SD: 10.2 years-old). From group A and concerning A&VC and/or HCC, 18 males and 3 females (mean: 54.1; SD: 7.4 years-old) corresponded to group C which were compared to 109 males and 18 females (mean: 55.7; SD: 8.3 years-old) identified as group B during corresponding months of LT. In hematological patients, the distribution of hepatic disorders related to the 1st LT is displayed (figure 1). Diagnoses for the 2nd LT were arterial and venous thrombosis, graft fibrosing cholestatic hepatitis and acute graft failure. A&VC without HCC were clustered in one column and HCC (alone/associated with cirrhoses) in another column. Hematological diagnoses, survival and mortality are presented (figure 1). All-causes mortality rates were 25.2% and 19.0% in groups B and C respectively. Consumption medians in group B were 6 red blood cell units and 20 plasma units *versus* 3 and 14 units in group C and 4 and 18 units in group A, respectively. More products were also provided for bleeding control.

Table 1.



SURVIVAL AND MORTALITY IN GROUP A

HEMATOLOGICAL DISEASES (n = number of patients)	Patients (n)	Alive (n)	Deceased (n)
Hemochromatosis	15	11	4
MPS (PV: n=5; ET: n=3; CML: n=1)	9	4	5
OWRD (1 with ITP)	4	2	2
Hemophilia B	2	2	0
MDS	1	1	0
β-Thalassemia minor	2	1	1
SCD + β-Thalassemia minor	1	1	0
Resulted AML-M2	1	1	0
Total	35	23	12

Summary/Conclusion: Overall survival in group A corresponded to 65.7% and mortality rate reached 34.3% which was related to hepatic disorder in the majority of patients. The leading causes of death were hemorrhagic shock (all groups) followed by severe infection (group B); one case of CML relapse and another of sepsis occurred in group A. The hematological patients showed a lower blood transfusion support. MPS were associated with BCS in 7 patients. Severe diseases can be treated by a multidisciplinary management with net clinical benefits. Creating new synergies could improve outcomes of hematological patients needing LT.

PB2526

DARATUMUMAB AND BLOOD-COMPATIBILITY TESTING: EXPERIENCE IN OUR CENTER

E. Morente Constantin[1], P. Romero Garcia[2], M.P. Garrido Collado[1], A. Garcia Ruiz[1,*], E. Clavero Sanchez[1], M. Jurado Chacon[1], R. Rios Tamayo[1]

[1]Servicio de Hematología y Hemoterapia, Hospital Universitario Virgen de Las Nieves, Granada, [2]Complejo Hospitalario Soria, Soria, Spain

Background: Daratumumab is a monoclonal antibody (mAb) antiCD38, which is used in the treatment of patients with multiple myeloma. It binds to CD38, which multiple myeloma cells overexpress. Therefore, the plasma of these patients reacts with the red blood cells (RBCs) producing a panreactivity and interfering in the transfusion compatibility testing. Plasma of patients treated with daratumumab agglutinate in the Indirect Antiglobulin Test in all potentiating reagents that accelerates antibody coating on the red cells and this reaction may persist for up to 6 months after the treatment has ended.
Aims: To validate the procedure to resolve the interference of daratumumab in transfusion compatibility testing using red cells treated with Dithiothreitol (DTT), which will allow us to identify a clinically significant antibody that has been initially masked by the presence of daratumumab.
Methods: The study has been conducted in 8 patients diagnosed with multiple myeloma who have been treated with daratumumab in monotherapy. Irregular antibody screening tests and CrossMatching were performed on

all of them, being positive in the 6 cases with active treatment, and negative in the 2 in which more than six months have passed since its finalisation. We performed the technique to eliminate reactivity by treating the red cells used in compatibility tests with DTT, which negates the binding of daratumumab to CD38 on the RBC surface, but it also denatures Kell antigens, while preserving the E antigen. The material used was phosphate buffered saline (PBS) (pH 8.0), 0.2 M DTT, cells for Irregular antibody screening tests, K + and E + control cells, monospecific reagent AntiIgG and AntiIgG-C3d polyspecific. We performed the method in tubes, Biovue Column Agglutination Technology and Micro Typing System (Bio-Red).
Results: After performing the technique, we were able to eliminate panreactivity in both Irregular antibody screening tests and CrossMatching. Therefore, we consider it a very useful technique to resolve interferences produced by daratumumb with blood compatibility testing. After the treatment with DTT, Kell antigen disappears.
Summary/Conclusion: DARA causes pan reactivity *in vitro* by binding to CD38 on reagent RBCs. It is necessary to do a baseline antibody screen and Rh and Kell phenotyping (type and screen) before starting the treatment with daratumumab. Treating reagent RBCs with DTT is a useful method to mitigate the interference created by antiCD38 mAbs in pretransfusion testing, although it is not free from limitations that, in some cases, may compromise transfusion safety. Treatment with DTT leads to denaturation of the Kell protein, so that antiKell antibodies cannot be identified when the target red cells have been previously treated with DTT. It is not always possible to have RBCs of the same phenotype. We believe that it is essential to have a validated technique to resolve these discrepancies.

PB2527

SINGLE CENTRE EXPERIENCE OF THERAPEUTIC PLASMA EXCHANGE IN RENAL PATIENTS

R. Kohli[1,*], G.D. Young[2], S. Forbes[2], R. Rajakariar[2], R. Thuraisingham[2], P. MacCallum[3], L. Green[4]

[1]Haematology, Queen Mary, University of London, [2]Renal Medicine, Barts Health NHS Trust, [3]Haematology, Queen Mary, University of London; Barts Health NHS Trust, [4]Haematology, Barts Health NHS Trust; NHSBT; Queen Mary, University of London, London, United Kingdom

Background: Therapeutic plasma exchange (TPE) involves removal of plasma containing pathological circulating auto-antibodies and immune complexes from the patient and artificial replacement with a colloid solution (e.g albumin and/or plasma) or a combination with a crystalloid solution to maintain euvolaemia. Many renal disorders have an immune-mediated pathogenesis and the ability of TPE to rapidly lower serum immunoglobulins makes this treatment a first line option. The type of fluid used for performing TPE varies significantly between hospitals and clinicians due to the lack of robust evidence on optimal fluid type.
Aims: To understand TPE practice by reviewing the indications for TPE, fluid type used and number of exchange sessions and to determine if variabilities in type of fluid used in patients with similar renal conditions exist.
Methods: Four year retrospective single centre study of TPE practice between February 2014 and December 2017, at the renal unit at Barts Health NHS Trust.
Results: 134 patients underwent TPE between February 2014 and December 2017 (50% were male). The median age was 52 years (age range 19-89), and of the total, 89% received TPE for renal disease while the remainder had an underlying neurological diagnosis. The most common renal indications were vasculitis with pulmonary haemorrhage (13.4%), IgA nephropathy (7.6%), transplant rejection (6.7%) and HLA incompatibility (6.7%). The maximum number of TPE sessions received was 6 (sessions range 1-6). The majority of the patients, 53.7%, received human albumin solution (4.5% HAS) during their first session, while 29.9% received Octaplas (solvent detergent treated plasma) and 14.2% received a 50:50 mix of 4.5% HAS and Octaplas. Variability in fluid type used for patients with similar renal conditions was observed. 43.8% of patients with vasculitis and pulmonary haemorrhage received Octaplas in the first session, while 31% received 4.5% HAS and the remainder (25%) received a 50:50 mix of 4.5% HAS and Octaplas. 55.6% patients with IgA nephropathy received 4.5% HAS during their first session while 33.3% received Octaplas. In patients with HLA incompatibility, 62.5% received 4.5% HAS during session one and 37.5% received Octaplas. 50% of patients who required TPE for transplant rejection received Octaplas during the first session, 25% received 4.5% HAS and 12.5% received either a 50:50 mix or normal saline.
Summary/Conclusion: Although indications for TPE in our unit are similar to what is evidenced in literature, there is variability in type of fluid used per TPE session in patients with similar underlying renal pathology. Further prospective studies are therefore required to determine what optimal fluid type should be utilised

PB2528

HAEMOGLOBIN AND SEVERITY OF ACUTE ISCHAEMIC STROKE: REVISITING TRANSFUSION THRESHOLDS

C. Cherian Philip[1,*], J. Pandian[2], H. Khatter[2], P.N. Sylaja[3], S. Kaul[4], D. Khurana[5], M.V. Padma[6], S. Singh[1], A. Jindal[7], M.J. John[1], D. Arora[2], A.B. Singhal[8]

[1]Clinical Haematology, [2]Neurology, Christian Medical College & Hospital, [3]Neurology, Sree Chitra Tirunal Institute of Medical Sciences and Technology, Ludhiana, [4]Neurology, Nizam's Institute of Medical Sciences, Hyderabad, Hyderabad, [5]Neurology, Postgraduate Institute of Medical Education and Research, Chandigarh, [6]Neurology, All India Institute of Medical Sciences, [7]Transfusion Medicine & Immunogenetics, Christian Medical College & Hospital, Ludhiana, India, [8]Neurology, Massachusetts General Hospital, Boston, United States

Background: There exists conflicting reports on the impact of hemoglobin in stroke. Physiological data suggest an adverse impact of anaemia on stroke. Also, it is noted that in Polycythemia; thrombotic events, in particular arterial thrombosis are increased and may contribute to a heightened mortality risk in patients with PV. Available guidance based on observational data suggests maintaining a hemoglobin level above 90g/L in patients with ischaemic stroke admitted to the ICU. However such recommendations are based on evaluation of specific patient populations and carry the potential risk to harm unexplored patient subsets if generalized. The best practice in patients with stroke is unclear and the impact of hemoglobin in stroke needs to be explored.
Aims: We attempted to determine the level of hemoglobin in patients with severe acute ischaemic stroke in our tertiary care centre.
Methods: We determined the impact of hemoglobin in a discovery case-control cohort in our hospital. We also performed a validation of the findings in an independent cohort of prospectively collected dataset of the Indo-US stroke registry and add to the evidence. The discovery cohort consisted of 570 patients with acute stroke who were admitted consecutively between January, 2012 to March, 2014 to our regional tertiary center in India, with a catchment population of ≈10, 00,000. The validation cohort consisted of patient enrolled into the prospective U.S. NIH and Indian Government funded Indo-US Stroke Registry and Infrastructure Development Project register; the methods of which have been detailed previously. Briefly, this registry enrolled consecutive adult patients admitted with imaging-confirmed ischemic stroke and at two weeks after symptom onset to five academic hospitals in geographically diverse centers across India. Data was entered into a central web-based electronic database. From January, 2012 to August, 2014, data was prospectively collected for 2066 patients.
Results: The patients in the discovery cohort were matched for gender, level of hemoglobin and proportion of anemics. In this cohort on a binary logistic regression analysis of stroke severity with hemoglobin quintiles in men we did not detect any significant relation. There was a trend noted with increasing severity of stroke with lower hemoglobin quintiles in women. We then explored this trend in the validation cohort. There was a significant association with increasing severity of stroke in both men (Hb <12) and women (Hb <12.7) based on the quintiles (Table 1).

Table 1.

Table 1: Binary logistic regression analysis of stroke severity with haemoglobin quintiles in entire validation cohort (n=2066)

Analysis of stroke severity with hemoglobin quintiles in males					
HB quintile	Coefficient	Odds ratio	p-value	95% C.I.	
				Lower	Upper
1	0.531	1.701	**0.007**	1.157	2.499
2	-0.113	0.893	0.583	0.596	1.338
3	-0.366	0.694	0.094	0.452	1.065
4	-	1.000	-	-	-
5	-0.186	0.830	0.387	0.545	1.265
Analysis of stroke severity with haemoglobin quintiles in females					
HB quintile	Coefficient	Odds ratio	p-value	95% C.I.	
				Lower	Upper
1	0.022	1.022	0.934	0.608	1.718
2	-0.748	0.473	**0.015**	0.259	0.865
3	-0.865	0.421	**0.004**	0.233	0.762
4	-	1.000	-	-	-
5	0.049	1.050	0.864	0.602	1.830

The cutoff points for the quintiles are as follows: male: 12, 13.3, 14.2, and 15.2 g/dL; female: 10.7, 11.7, 12.3, and 13.6 g/dL.

Summary/Conclusion: In our analysis there appears to be a trend towards increasing severity of stroke with lower levels of hemoglobin. This is more

significant in women. Current strategy of transfusion of red cells to maintain hemoglobin above 9g/dL in patients with acute ischaemic stroke might need to be revised to higher thresholds.

PB2529

PLATELET ALLOIMMUNIZATION IN MULTITRANSFUSED PATIENTS IN MOROCCO

Z. Ouabdelmoumene[1,*], L. Blouin[2], X. Lafarge[2], F. Zarati[3], H. Mifdal[3], N. Nourichafi[3], K. Bouisk[3], M. Benajiba[4], N. Habti[1,4]

[1]Laboratory of Hematology Cell and Genetic Engineering, Faculty of Medicine and Pharmacy Casablanca University Hassan II Morocco, Casablanca, Morocco, [2]Laboratoire d'histocompatibilité, EFS Aquitaine-Limousin, Bordeaux, France, Bordeaux, France, [3]Regional blood transfusion center of Casablanca Morocco, Casablanca, [4]National Blood Transfusion Centre of Rabat Morocco, Rabat, Morocco

Background: Platelet membrane carries a large number of glycoproteins, which express the platelet's specific antigens HPA (Human Platelet Antigen) on their surface. In transfusion, anti-HPA alloimmunization can lead to many serious complications, such as multi-platelet transfusion refractoriness. However, the implication of these antigens in platel*et al.*loimmunization was not studied before in Morocco.
Aims: The aim of this study is to determine the prevalence of the anti-HPA antibodies in serum of 90 multi-transfused patients in Morocco.
Methods: The serum of 90 multi-transfused patients who received leukocyte depleted red cells component, were included in this study, the detection of HPA antibodies was performed by a qualitative solid phase enzyme linked immunesorbent assay kit (PAKPLUS).
Results: A total of 6 out of 90 (6.66%) patients were found to be immunized to platelets. 1.1% patients were found to be alloimmunized to HPA-5a, 1.1% anti-GPIIb IIIa, 2.22% anti-GPIa IIa, and 2.22% anti- GPIa IIa.
Summary/Conclusion: Using a leukocyte depleted red cells component could reduce alloimmunization to platelets. To confirm these data, it's important to study the association between platelet antigens and their clinical consequences for the proper diagnosis and a better blood transfusion safety.

PB2530

PREVALENCE OF KELL, MNS, DUFFY, KIDD, P1PK, LEWIS, LUTHERAN BLOOD GROUP SYSTEMS IN THE POPULATION OF NORTH-EASTERN REGIONS OF RUSSIAN FEDERATION

E. Butina[1,*], E. Poponina[1], A. Yovdiy[1], N. Minaeva[2], I. Paramonov[3]

[1]Laboratory of Immunohaematology, [2]Haematology, [3]Director, Federal State Institute of Science "Kirov research Institute of Hematology and Blood Transfusion of the Federal Medical and Biological Agency of Russia", Kirov, Russian Federation

Background: A study of the frequency of phenotypes of red blood cell (RBC) in the inhabitants of the region is necessary for planning the preparation and storage of blood components, the organization of prevention of post-transfusion complications and hemolytic disease of the fetus and newborn.

Table 1. Prevalence of Kell, MNS, Duffy, Kidd, P1PK, Lewis, Lutheran Blood Group Systems in the population of north-eastern regions of Russian Federation.

Blood Group System	Phenotype	Prevalence (%)
Kell	K-k+	95.1
	K+k+	4.8
	K+k-	0.1
	Kp(a+b-)	0
	Kp(a+b+)	98.2
	Kp(a-b+)	1.8
MNS	M-N-	31.3
	M+N+	56.3
	M-N+	12.4
	S+s-	18.8
	S+s+	42.1
	S-s+	39.1
Duffy	Fy(a+b-)	18.8
	Fy(a-b+)	34.4
	Fy(a+b+)	46.8
	Fy(a-b-)	0
Kidd	Jk(a+b-)	19.0
	Jk(a-b+)	25.9
	Jk(a+b+)	55.1
	Jk(a-b-)	0
P1PK	P1+	63.0
	P1-	37.0
Lewis	Le(a+b-)	7.4
	Le(a-b+)	88.9
	Le(a-b-)	3.7
	Le(a+b+)	0
Lutheran	Lu(a+b-)	1.6
	Lu(a-b+)	82.3
	Lu(a+b+)	16.1
	Lu(a-b-)	0

Aims: Determine the distribution of the phenotypes of the Kell, MNS, Duffy, Kidd, P1PK, Lewis and Lutheran Blood Group systems in donors of blood components living in north-eastern regions of the Russian Federation.
Methods: Analysis of the results of typing of RBC antigens by gel agglutination assay (BioRad, USA) in 128 donors of blood components was carried out.
Results: Frequency of occurrence of phenotypes of red blood cells is presented in the table 1.
Summary/Conclusion: In the population of north-eastern regions of Russian Federation two times less often than in European countries, there are phenotypes K+k+, M-N+, Le(a+b-), more often - P1-.

PB2531

FREQUENCY OF HPA-15A AND HPA-15B HUMAN PLATELET ALLOANTIGENS IN MOROCCAN BLOOD DONORS

Z. Ouabdelmoumene[1,*], H. Elhousse[1], N. Nourichafi[2], K. Bouisk[2], M. Benajiba[3], N. Habti[1,3]

[1]Laboratory of Hematology Cell and Genetic Engineering, Faculty of Medicine and Pharmacy Casablanca University Hassan II Morocco, [2]Regional blood transfusion center of Casablanca Morocco, Casablanca, [3]National Blood Transfusion Centre of Rabat Morocco, Rabat, Morocco

Background: HPA (Human Platel *et al.* loantigens) are polymorphisms expressed on platelet membrane glycoproteins, several studies have shown the role of antibodies against human platelet antigen (HPA)-15 in platel*et al.*loimmunisation that may show clinical manifestations, such as neonatal alloimmune thrombocytopenia, post-transfusion purpura and platelet refractoriness. Typing of HPA-15a and HPA- 15b has not been carried on the Moroccan population. This is the first study of HPA-15 system in Morocco.
Aims: The aim of this study is to determine the genotypes and allele frequencies of HPA-15a and HPA-15b among Moroccan blood donors and to compare their allele frequencies with those reported in other populations
Methods: A total of 97 Moroccan blood donor samples randomly selected, were genotyped using the polymerase chain reaction with sequence-specific primers (PCR-SSP).
Results: The results showed that the most frequent genotypes were AA (43, 29%) followed by AB (29, 89%). Allele frequencies were 0,582 and 0.417 for HPA-15a and HPA-15b, respectively. Allele frequencies were similar to those found in North Africa (Algerian). However, the B allele was less frequent in HPA-15 systems when compared with Caucasians (French) and Arabs (Saudi).
Summary/Conclusion: Differences in the distribution of HPA-15 highlight the diversity in Moroccan population. Therefore it is advantageous to establish a compatible platelet concentrates, for patients who require multiple platelets transfusions and have a higher chance of becoming alloimmunised or refractory due to anti-HPA alloantibodies. This data obtained will form an addition to the literature in transfusion research.

PB2532

FREQUENCY OF ANEMIA IN LYMPHOPROLIFERATIVE DISORDERS PATIENTS DURING ANTITUMOR THERAPY

N. Romanenko[1,*], N. Potikhonova[1], A. Chechetkin[2], S. Voloshin[1], S. Bessmeltsev[1]

[1]Clinical Hematology, [2]Transfusiology, Russian research institute of hematology and transfusiology, Saint-Petersburg, Russian Federation

Background: The anemia in lymphoproliferative disorder's (LPD) patients is a frequent symptom and can decrease the efficacy of antitumor therapy (AT-therapy), overall survival and quality of life. Anemia's pathogenesis is based on suppression by proinflammatory cytokines and decreasing erythroid precursor's sensitivity to serum erythropoietin. More over AT-therapy can play significant role in increasing of frequency and severity of anemia - chemotherapy-induced anemia.
Aims: To definite of frequency of anemia in newly diagnosticated of LPD patients and evaluate the influence of antitumor therapy on frequency and severity of anemia.
Methods: We have examined the following patients in the age of 24-85 years: multiple myeloma (MM) in II and III st. (n=119), Non-Hodgkin's lymphoma (NHL) in III-IV st. (n=72) and chronic lymphocytic leukemia (CLL) in B or C st. (n=147). Clinical blood test (hemogram) was examined more then twice before AT-therapy and during 1-3 cycles of therapy. All patients were administrated different therapy: patients with multiple myeloma – VD, V P, VMP, PAD; patients with Non-Hodgkin's lymphoma – RB, VRB, R-FC, R-CHOP, R-CVP; patients with chronic lymphocytic leukemia – RB, FC-R, R-CHOP, R-Chlorambucil.
Results: We have accessed the rate of anemia in the group of patients with

MM (n=119) was 56.3% (n=67) but after AT-therapy it significantly increased to 79.0% (n=94; p<0.01); in the group of NHL (n=72) the rate of anemia increased from 38.9% (n=28) to 65.3% (n=47; p<0.01); in the group of CLL (n=147) the rate of anemia increased from 33.3% (n=49) to 68.7% (n=101; p<0.01). Besides the grade of anemia (by WHO hematological toxicity scale) also increased. So, the number of patients with anemia (grade 1) increased from 17.5% (n=59) to 27.2% (n=92; p<0.01); the number of patients with anemia (grade 2) increased from 13.6% (n=46) to 24.9% (n=84; p<0.01); the number of patients with anemia (grade 3) increased from 7.1% (n=24) to 11.8% (n=40; p<0.05); the number of patients with anemia (grade 4) increased from 4.4% (n=15) to 7.7% (n=26; p<0.05). Too impotent to emphasize that 20.4% (n=69) of patients were administrated 1-6 dosages of red blood cells transfusions.
Summary/Conclusion: In this study were revealed the high rate of chemotherapy-induced anemia in newly diagnosed LPD patients and increasing of it during the AT-therapy that might to need administration of RBC transfusions.

PB2533

IS THERE A CHANGE IN SEROLOGICAL STATUS IN BLOOD DONORS AT THE BOUFARIK BLOOD TRANSFUSION BANK DURING THE LAST TEN YEARS?

T. Faiza[1,*], D. djamel[2], N. Rachid[3], D. Khadidja[1]

[1]Hematology, Central Hospital of the Army, [2]Medecine, blood transfusion station, [3]Epidemiology, Mayo Hospital, ALGER, Algeria

Background: Screening for infectious agents is a vital step in the process of biological qualification of blood donations in virology, preventing the transmission of viral diseases following a blood transfusion or its derivatives.
Aims: The purpose of this study is to determine the seroprevalence of infectious markers in a descriptive, retrospective and monocentric study performed at the Boufarik PTS over a 10-year period (2008-2017).
Methods: This cohort onclude 10,300 donations of blood. Donors received an interview and clinical examination before being selected for a donation. Screening for HIV, HVB and C was performed by enzyme immunoassay based on the Elisa principle and indirect haemagglutination for syphilis.
Results: The prevalence of HIV, HBS, HCV and syphilis serological markers is 0%, 24%, 0.67 and 23%, respectively. By taking two comparative periods: 2008-2012 (n=4139 donations) and 2013-2017 (n=6161 donations): there is an increase in the number of positive cases for the hepatitis B virus: n=07 (2008- 2012) *versus* n=18 (2013-2017): RR=.69 (.37-1.30), p=.13 (NS) fig.1. An increase in the number of positive cases for hepatitis C virus: n=02 (2008-2012) *vs* n=05 (20132017): RR=.89 (.21-3.73), p=.45 (NS) fig.2. A stable number of positive cases for the syphilis virus: n=12 (2008-2012) *versus* n=12 (2013-2017): RR=1.49 (.663.31), p=.16 (NS) fig.3.
Summary/Conclusion: These results show that the prevalence of viral infections is stable over time among blood donors in our region. This is due to the introduction of a quality assurance system, the choice of the collection site, the selection of donors and finally the improvement of the quality of screening, which has helped to ensure optimal blood safety.

PB2534

THE SAFETY AND EFFICACY OF THE LEAST INCOMPATIBLE CROSS-MATCH TRANSFUSION USING *IN VIVO* COMPATIBILITY TESTS

S. Maral[1,*], I. Dilek[2], S.M. Bakanay[2], A. Yıkılmaz[2], S. Akıncı[2]

[1]Hematology, Dıskapı Research and Training Hospital, [2]Hematology, Ataturk Research and Training Hospital, Ankara, Turkey

Background: Pre-transfusion compatibility testing is performed to provide appropriate blood components. Compatibility testing certainly includes the determination of ABO and Rh types, antibody screening of the patient's plasma for any unexpected anti-RBC antibodies and a crossmatching test using the patient's plasma and the donor RBCs to determine if they are compatible.If no compatible cross-matched blood can be found, clinicians may avoid transfusion and consultation by the hematology department is generally required. The majority of clinicians usually choose to transfuse group O, Rh(D)-negative.Another option is to separate the most compatible or the least incompatible blood and start the transfusion with this. Nevertheless, it is not clear how safe the least incompatible transfusion is, and whether it provides the targeted hemoglobin increments. It has also not been fully clarified whether acute hemolytic transfusion reactions can be ignored.
Aims: The aim of this study was to determine the safety and efficacy of the least incompatible cross-matched blood transfusion, through the use of biological *in vivo* cross-match testing.
Methods: The study included 18 patients with incompatible cross-matching who were transfused RBC.A total of 55 units of RBC were transfused.Red blood cell concentrates were included in saline-adenine-glucose-mannitol (SAG-M). None of the patients had any premedication such as steroids or antihistamines before transfusion. From the selected incompatible unit, 20 ml blood was collected in a 20 cc syringe(Hayat®) and infused intravenously to the patient in 10 minutes via the antecubital vein. Patients were observed for any signs of acute hemolysis for 20 minutes before the initiation of the transfusion of the residual blood. The transfusions were started from another vascular access and were completed in 2 hours. Each transfusion was observed by medical professionals to identify any evidence of acute hemolysis. Possible acute transfusion reactions were listed on a form (Figure 1: patient transfusion form) including dyspnea, headache, hypotension, back pain, fever, tachycardia, and chills, and these were recorded together with the time of occurrence by the observer.If any symptoms indicating hemolysis were observed, the transfusion was stopped and another selected unit of blood was used. Pre- and post-transfusion hemoglobin and hematocrit levels were compared to determine the increase as a result of the transfusion. Biochemical markers indicating hemolysis, such as lactate dehydrogenase and total-indirect bilirubin levels were recorded to evaluate the hemolytic state. The laboratory test results were reported within 24 hours of the transfusion.
Results: A No complications or signs were observed in any of the 55 transfusions and all the transfusions were completed succesfully.An appropriate increase in hematocrit and hemoglobin was seen post-transfusion (p:0.001). As indicators of hemolysis, lactate dehydrogenase (LDH) and bilurubin levels were determined to have significantly increased post transfusion (p:0.035, p:0.001).
Summary/Conclusion: Acute hemolytic transfusion reactions are a serious situation which can result in mortality. When pre-transfusion tests result in ABO compatibilty and are cross-match incompatible, clinicians should not avoid transfusion if it is urgently required. The biological *in vivo* compatibility test seems to be a procedure which is safe, predictive, and can be feasibly applied at the bedside, and could be life-saving for many patients.

PB2535

ANALYSIS OF RED CELL AND PLATELET TRANSFUSION IN A TERCIARY HOSPITAL. A RETROSPECTIVE CASE STUDY

R.M. Rodriguez Nuñez*, C. Fonte Feal, A. Nieto Vázquez, C. Insua Vilas, P.M. García Cereijo, M.D.C. Albo López

Hematology, Hospital Alvaro Cunqueiro, Vigo, Spain

Background: Blood transfusion is an essential treatment, sometimes the only valid one, but it always involves certain risks. Physicians should weigh in the benefits and hazards of this treatment and follow the recommendations. Most studies demonstrate that in recent years, red cell transfusion has decreased, meanwhile platelet transfusion remains practically the same. This is because of the application of smarter blood transfusion protocols.
Aims: Make a retrospective study of red cell and platelet transfusion in our hospital. We analyzed hemoglobin levels and number of platelets before and after transfusion; we also analyzed the type of surgery, medical disorder and mortality, among other variables.
Methods: We collected data of red cell and platelet transfusion at Vigo hospital, from January to June 2016. We did a descriptive analysis, presenting qualitative variables as absolute frequency and percentage, and quantitative variables as mean and standard deviation. We used Chi square test to compare qualitative variables between types of surgery and also between the various medical disorders. We used one factor ANOVA to compare quantitative variables. Data were analyzed with IBM SPSS statistics 19.0. The accepted level of statistical significance was 0.05.
Results: We transfused 7787 blood products to 1575 patients; 6175 packed red cells and 1612 platelets. 51.4% of patients were male. Mean age was 69+/-19,6. Most of red cell transfusions were to patients with medical disorders, and among them, to patients with hematological ones. In patients with non hematological medical disorders, most of transfusions were to those with GI bleeding, followed by solid organ cancer. On the other hand, most platelet transfusions were to patients with solid organ malignancies, follow by patients with non hematological anemia (Graphics 1). Patients with hematological disorders which received the most red cell transfusions were, in order: MDS, NHL, acute myeloid leukemia and allogenic stem cell transplantation. Regarding platelet transfusions, the order is the following: acute myeloid leukemia, allogenic stem transplantation, NHL and autologous stem cell transplantation. Among surgical patients, the ones which received most of red cell transfusions were trauma patients, follow by cardiac and abdominal surgeries; platelet transfusions were received mostly by patients undergoing cardiac surgery, follow by vascular surgery. Other variables, such as anticoagulation or infection, didn't have clinical significance over transfusion.

Most deaths occurred in the group of patients with medical conditions (71,2%), and among those, the ones with solid organ malignancies, follow by non hematological anemias and GI bleeding. Regarding these results we could identified inadequate transfusion behaviors.

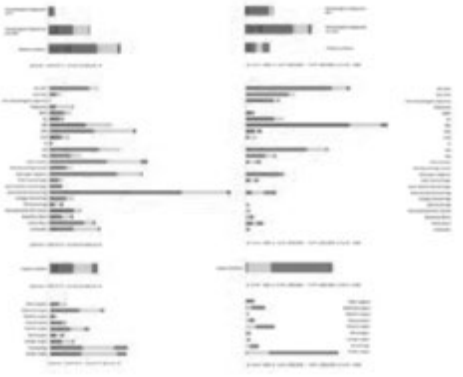


Figure 1.

Summary/Conclusion: In our hospital, we concluded that patients who received the most transfusions were those with medical conditions, and among them, the ones with hematological disorders. These results are similar to those published in other studies. Nevertheless, platelet transfusions were received in large numbers by patients with a number above 50.000, which is not what guidelines recommend. On the other hand, red cell transfusion, in our hospital, is closer to the recommendations, even if we have a liberal transfusion policy. These findings led us to apply some corrective actions in our hospital.

PB2536

HAEMOVIGILANCE REPORTS OF ADVERSE BLOOD DONOR REACTION AMONG VOLUNTARY BLOOD DONORS IN TERTIARY CARE HOSPITAL IN KATHMANDU, NEPAL

B. Nepal*

Blood Bank, Grande International Hospital, Kathmandu, Nepal

Background: Voluntary blood donation is widely considered to be safe with very minimum chance of adverse reaction, which may occur during or after the end of phlebotomy procedure.
Aims: To identify and understand the complication of adverse donor reactions though the incidence of reactions in the blood donors.
Methods: This is a prospective study done among voluntary blood donors at Grande International Hospital, Kathmandu, Nepal from February 2013 to March 2015. The outlines of reported and communicated adverse donor reaction were also collected after the blood donation from voluntary blood donors in different locations including outdoor and in-house blood donation drive.
Results: In the present study 6,955 whole blood donors were included, during the period of 2 years, 105 (1.50%) adverse donor reactions were reported. Majority 89(84.76%) of adverse donor reactions were mild in nature such as, sweating; 27(25.72%), Light headedness; 19(18.09%), nausea and vomiting; 15(14.28), allergy and bruises;11(10.47%), sore arm; 9(8.58%) and hematoma; 8(7.62%) while 16 (15.24%) were severe adverse reactions similarly, anaphylaxis;11(10.49%), loss of consciousness; 3(2.85%) and convulsive syncope;2(1.90%). Markers of the adverse donor reaction were age, sex, pulse, weight, blood pressure and donation status. Age and first time status were related with significantly higher risk of adverse reaction with 18-23 years old at higher risk compared to 24-55 years old. First time donors were at higher risk compared to repeated volunteer donors.
Summary/Conclusion: The results of the study are helpful to identify and understand the complication of adverse donor reactions though the incidence of reactions in the blood donors is lower than in other studies. Donor age and donation status were strong possibilities of complications.

PB2537

SAFE OF PLASMA EXCHANGE IN ACUTE RENAL FAILURE SECONDARY TO VASCULITIS

I.M. Parra Salinas[1,*], L. Arnaudas Casanova[2], A. Blasco Forcén[3], V.P. González Rodríguez[1], J.A. García-Erce[4]

[1]Hematology, Miguel Servet University Hospital, Zaragoza, [2]Nephrology, Reina Sofia Hospital, Tudela, [3]Nephrology, Miguel Servet University Hospital, Zaragoza, [4]Hematology, Navarra blood bank, Pamplona, Spain

Background: The benefit of Plasma Exchange (PE) in acute kidney injury secondary to vasculitis has been demonstrated both in the acute phase of these diseases, but also has been proved to decrease the dialysis-dependent rate at the first year.
Aims: The aim of this article is to evaluate the safe of PE in renal vasculitis, and secondarily the response (dialysis independence) at 30 days.
Methods: Unicentre descriptive and longitudinal study, with a prospective database of all patients with acute renal failure secondary to vasculitis treated with PE. severity of renal injury was classified according to AKIN (Acute Kidney Injury Network) scale and RIFLE (Risk, Injury, Failure, Loss y End Stage Kidney Disease risk, Injury) criteria.
Results: We analysed 13 cases, 7 women. The median age was 67.7 years, 30-day response rate were 38.5% and 92.3% had a high Charlson comorbidity index (>4). All patients needed dyalisis but only in 4 were performed before the onset of PE. Other clinical characteristics are exposed at table 1. Serum creatinine levels below 5.8 mg/dL were related with great responses (*p=0.032*). There were observed decreases of serum creatinine levels (*p=0.005*), glomerular filtrate (*p=0.003*) and random urine sample proteinuria (*p=0.045).* The devices used for PE were continous-flow cell separators. The standard replacement solution is 5% human albumin. To avoid hypocalcemia symptoms, intravenous calcium gluconate is administered as prophylaxis at the beginning of each session and is repeated if symptoms appear. In accordance with the local protocol to prevent dilutional coagulopathy, all patients receive intravenous vitamin K at the end of the procedure. A median of 6 (4-17) sessions were performed per patient and were initiated 10 (0-28) days after diagnosis. Out of the 96 sessions carried out, one (1.04%) presented a low access pressure and 4 (4.1%) clinical complications (itching in 3 and fever in 1), corresponding to two patients (table 2). None of them were serious and only one had to be suspended due to fever. Patients who experience more complications (clinical and technical) had a high number of leukocytes prior to PRT (*p=0.04*) and had undergone a higher number of sessions (*p=0.010*) as showed in table 2.

Table 1.

Summary/Conclusion: Global frequency of complications in our series was lower than published in the literature, it leads us to conclude that PRT is a safe therapeutic strategy in our centre and encourages us to postulate that our prophylactic measures. The finding of increased initial white blood cell count in patients with complications, might be investigated in studies with higher casuistry.

PB2538

FREQUENCY OF REACTIVE BLOOD DONORS IN A TERTIARY CARE HOSPITAL, KARACHI, PAKISTAN

B. Nepal*

Blood Bank, Grande International Hospital, Kathmandu, Nepal

Background: In recent years, number of patients infected with HBV or HCV or HIV or co-infected with either of the two viruses, has increased tremendously in Karachi population. IDUs (intravenous drug users), MSM (Men who have Sex with Men) and individuals having unsafe sex are among the

people who are identified as groups at higher risk of contracting these infections than others. But these studies does not give an exact picture of prevalence and frequency of these infection in Karachi's population as focus of most of these studies were individuals already involved in behaviours (intravenous drug use and unsafe sex) regarded as high risk behaviours.
Aims: To find the frequency of different types of reactive healthy blood donors at a tertiary care hospital, Karachi, Pakistan.
Methods: The retrospective observational study carried out on both male and female healthy blood donors. Data from complete blood screening from January 2013 to December 2014 were collected and frequency of various types of reactive blood donors was sorted out to get an actual picture. All the blood products were screened for HBV and HIV Using enzyme linked immunosorbent assay (Elisa plate washer version 3 and Elisa plate reader stat fax 3200). HCV screening was performed on Architect 2000 SR Chemiluminescent micro plate immune assay (CMIA). Malarial parasite tested by making thick and thin smear seen under microscope. Syphilis was tested by ICT method.
Results: A total number of 6996 healthy donors were received and about 624 were found to have blood screening positive in various combination. The highest numbers of isolates was HbsAg reactive 214, HCV 213, VDRL 170, HIV 26 and 1 case of malarial parasite. More prevalent in male population. In this study there were seven donors found with HCV –VDRL co infection and five co-infected with HCV and HbsAg two donors with HIV and HbsAg infected and two donors were HbsAg and VDRL reactive.
Summary/Conclusion: This study supports that HBV, HIV and syphilis prevalence is high and HIV prevalence is low in healthy blood donors.

PB2539

FIVE YEARS OF HEMOVIGILANCE REPORTS OF COMPLICATIONS OF THE BLOOD DONATION REPORTED AT A TERTIARY CARE CENTRE IN KARACHI

B. Nepal*

Blood Bank, Grande International Hospital, Kathmandu, Nepal

Background: There is a minor chance of risk among blood donors. Even though blood donors are usually screened for the presence of risk factors, sometimes blood donations can put a person at panic.
Aims: The safety of the blood supply depends on the actions to protect both; blood transfusion recipient and the blood donor. Hemovigilance practice of learning of complications of blood donation and protecting them from such complications is the best way to minimize the risk to blood donor.
Methods: Comprehensive blood donor hemovigilance program was studied at Dr. Ishratul Ebad Khan Institute of blood diseases, Karachi from 2010 to 2015. Outlines of reported and communicated complications were collected after whole blood donation. Analysis was done by general logistic regression.
Results: Complications after 30,000 Whole blood donation procedures calculated1620total .(54 per 1,000 donations). The majority of the complications were faint and pre-faint reaction with light headedness (58.6%), Sore arm (24%), Bruises and hematoma (14.4%). Minor complications were Agitation/sweating (2%) and arterial puncture(1%). Markers of the complications were age, sex, race, weight, blood pressure and donation status. All associated independently after whole blood donation. Age and first-time status were associated with a significantly higher risk of complications with 18-22 years old at higher risk compared to 23 to 50 years old. First-time donor were at higher risk compared to repeat donor.
Summary/Conclusion: The results of this study are helpful in identifying and understanding the promoter to complication of blood donation. Donor age and status were strong predictors of complications. The remedies and specific areas of care should be provided.

PB2540

KNOWLEDGE ATTITUDE AND PRACTICE OF BLOOD DONATION AMONGST MEDICAL STUDENTS IN NIGERIA

C. efobi[1,*], A.O. Ugwu[2], H.C. Okoye[2]

[1]Haematology, Chukwuemeka Odumegwu Ojukwu University, Awka, [2]Hematology, University of Nigeria Enugu Campus, Enugu, Nigeria

Background: There is currently a dire need for blood donors to meet the increasing demand for blood and blood products. Information on the perception and practice of blood donation among potential donors may help in designing policies for improved services in low income countries.
Aims: This study assesses the knowledge, attitude and practice of blood donation among medical students in Nigeria.
Methods: A descriptive cross sectional study carried out among different classes of medical students at two medical schools in south-eastern Nigeria in 2017. The sociodemographic characteristics, knowledge, attitude and practice of blood donation were sought from of the respondents.
Results: The age range was 19–46 years (mean=24.2±3.2) years with 169(43.7%) respondents having good knowledge and 37(9.6%) with poor knowledge of blood donation. There was a strong association between level of knowledge and respondents' year of study (p=0.001) but no significant association between knowledge and practice of blood donation (p=0.23). The donation rate was found to be 31.0% whereas 267 (69.0%) respondents had never donated blood. While a tenth (10.3%) of the respondents are regular blood donors, less than one third (31%) had ever donated blood. Majority of both the donors (85%) and non-donors (92.1%) opined that blood donation should be voluntary and non-remunerated.
Summary/Conclusion: Nigerian medical students have fair knowledge but a favourable attitude toward blood donation. Policy makers in the health sector could utilize this interesting observation in designing strategies for improving the quantity and quality of blood donation in Nigeria.

PB2541

REASONS FOR BLOOD DONOR DEFERRAL AMONG VOLUNTARY BLOOD DONORS IN A TERTIARY CARE HOSPITAL IN KATHMANDU, NEPAL

B. Nepal*

Blood Bank, Grande International Hospital, Kathmandu, Nepal

Background: Ensuring the wellness of the voluntary blood donor is the main purpose of the donor deferral. It also minimizes the unwanted symptoms that may appear among blood donor recipient.
Aims: To assess the Reasons for blood donor deferral among voluntary blood donors in a tertiary care hospital in Kathmandu, Nepal
Methods: This is the retrospective study carried out among voluntary blood donors at Grande International Hospital, a tertiary care hospital in Kathmandu, Nepal from January 2015 to January 2017.
Results: The data were collected from previous records of the blood donor history forms. From a total of 8,550 blood donations, 302(3.53%) blood donor were deferred due to various reasons. Among all the deferred blood donors 189(62.58%) were female where as 113(37.42%) were male. Furthermore, 289(95.69%) were temporarily deferred and 13(4.31%) were permanently deferred. The mean age of deferred blood donor was 35 years. Out of total blood donor deferral; 101(33.48%) donor were rejected because of bed side hypertension (*i.e.* blood pressure- systolic >140 and diastolic >90 mm hg) which was followed by anaemia(*i.e.* haemoglobin <12 gm/dl) 94(31.12%), vaccinations history 43(14.23%), hypotension (*i.e.* Systolic <90mm hg and diastolic <60 mm hg) 35(11.58%), dental examination 10(3.32%) and medication history 6(1.98%). Permanent deferral namely, risk factor involving transfusion transmitted infections and chronic disease were 5(1.65%) and 8(2.64%) respectively. The prime cause of permanent deferral was risk factor involving transfusion transmitted infections while the temporary deferral was bed side hypertension. Gender wise, the leading cause of donor deferral in male was bed side hypertension and anaemia was the major cause in female.
Summary/Conclusion: The findings of the survey aid to evaluate the significant causes of blood donor deferral. This study suggests that the restrictive criteria can be used for blood donor selection. This will in turn increase the blood supply of tertiary care hospital.

PB2542

DISTRIBUTION OF ABO AND RH BLOOD GROUP IN NEPALESE POPULATION

B. Nepal*

Blood Bank, Grande International Hospital, Kathmandu, Nepal

Background: ABO and RH are the major blood grouping system .which can effective transfusion compatibility .The distribution of blood group ABO and RH varies across the world according to the population
Aims: To get the knowledge of distribution of ABO and RH blood group for effective management of inventory
Methods: This is retrospective study conducted at Nepal Red Cross Society Central Blood Transfusion Service. Data from January 2010 to January 2016 were collected from donor management software .The data includes socio demographic data and Transfusion Transmissible infectious and other laboratory defects of blood donors.

Results: Out of 440720 blood donor, 384308 (87.20%) were male and 56412 (12.8%) were female. The commonest ABO blood group present was A (31.64%) followed by O (30.79%), B (25.94%) and AB (8.88%) in blood donors; while in Rhesus system, 428600 (97.25%) donors were Rh-Positive and 12120 (2.75%) donors were Rh-Negative.
Among the various ABO blood group "A" is the commonest and Rh Positive nearly equal to 97%.
Summary/Conclusion: Similarity of distribution of ABO and Rh blood group were reported in south Asian countries. This indicates the influence factor of genetic drift and ancestral link with Indian sub continent. These studies has a important proposition regarding the inventory management of blood bank and transfusion services.

PB2543

DISTRIBUTION OF BLOOD DONOR IN DIFFERENT AGE GROUP IN KATHMANDU NEPAL

B. Nepal*

Blood Bank, Grande International Hospital, Kathmandu, Nepal

Background: Voluntary non-remunerated blood donor consists of 78% blood donor's population in Nepal. Therefore demographic about the distribution of blood donors according to the age group is important to achieve 100% Voluntary non-remunerated blood donors in Nepal.
Aims: To explore the demographic distribution of the blood donor in different age group in the Kathmandu Nepal.
Methods: This is retrospective study conducted at Nepal Red Cross Society Central Blood Transfusion Service. Data from January 2013 to January 2017 were collected from donor management software .The data includes socio demographic data .Data has been process with SPSS version -17
Results: During 4 years study period, total of 276,290 Blood donation happened from both mobile blood collection and in-house blood collection. Out of 276,290 Collection, 48351 (17.5%) are from 18-24 age group; 106924 (38.7%) are from 25-31 age group 53324 (19.3%) are from 32-38 age group; 34536 (12.5%) are from group 39-45 age group; 23761 (8.6%) are from age group 46-52 and 9394 (3.4%) from age group above 53 respectively.
Summary/Conclusion: The distribution of ABO blood group varies regionally and from one population to another. In Kathmandu, Nepal 18-38 years age group is the most common age group encountered donating blood. The data generated in the present study and several other studies of different geographical region of India will be useful to health planners and future health challenges in the region.

PB2544

PLASMAPHERESIS: AN EXPERIENCE IN BLOOD BANK OF A TERTIARY CARE HOSPITAL IN PESHAWAR

H. Khan*

Hematology, Nowshera Medical colleg nowshera Pakistan, Nowshera, Pakistan

Background: plasmapheresis for therapeutic purposes is less practiced in Pakistan. It has a therapeutic as well as diagnostic importance and present study is a continuation of the above statement
Aims: To determine the frequency of major indications require plasmapheresis in blood bank of Lady Reading Hospital Peshawar.
Methods: This cross sectional study was conducted in blood bank of Lady Reading Hospital Peshawar from june 2010 to June 2012. Relevant information's were recorded on a pre-designed questionnaire prepared in accordance with the objectives of the study
Results: A total of 54 patients were enrolled in the trial. 23 (42.59%) were females, and 31(57.41%) were males. Males to females ration was 1.4:1. We received patients for plasmaphersis in age ranging from 15 to 74 years. Majority were young patients in age range 15 to 34 years age. We received majority of the patients for the subject procedure from ICU(Intensive care unit) 20(73%), followed by cardiothoracic unit 17(31.48%). We also receive two volunteers during study. The frequency of various indications for plasmapheresis were; myasthenia gravis 29(53.7%), Guillen barre syndrome 20(37.04%) and thrombocytopenic purpura 3(5.56%).
Summary/Conclusion: From this study we concluded that the autoimmune diseases are common in younger age which is a very serious concern for our society. Plasmapheresis is a therapeutic procedure as well and patients with autoimmune disorders get relieved with it symptomatically as autoimmune antibodies are removed. Myasthenia gravis was counted as major disease followed by GBS and thrombocytopenic purpura in our population.

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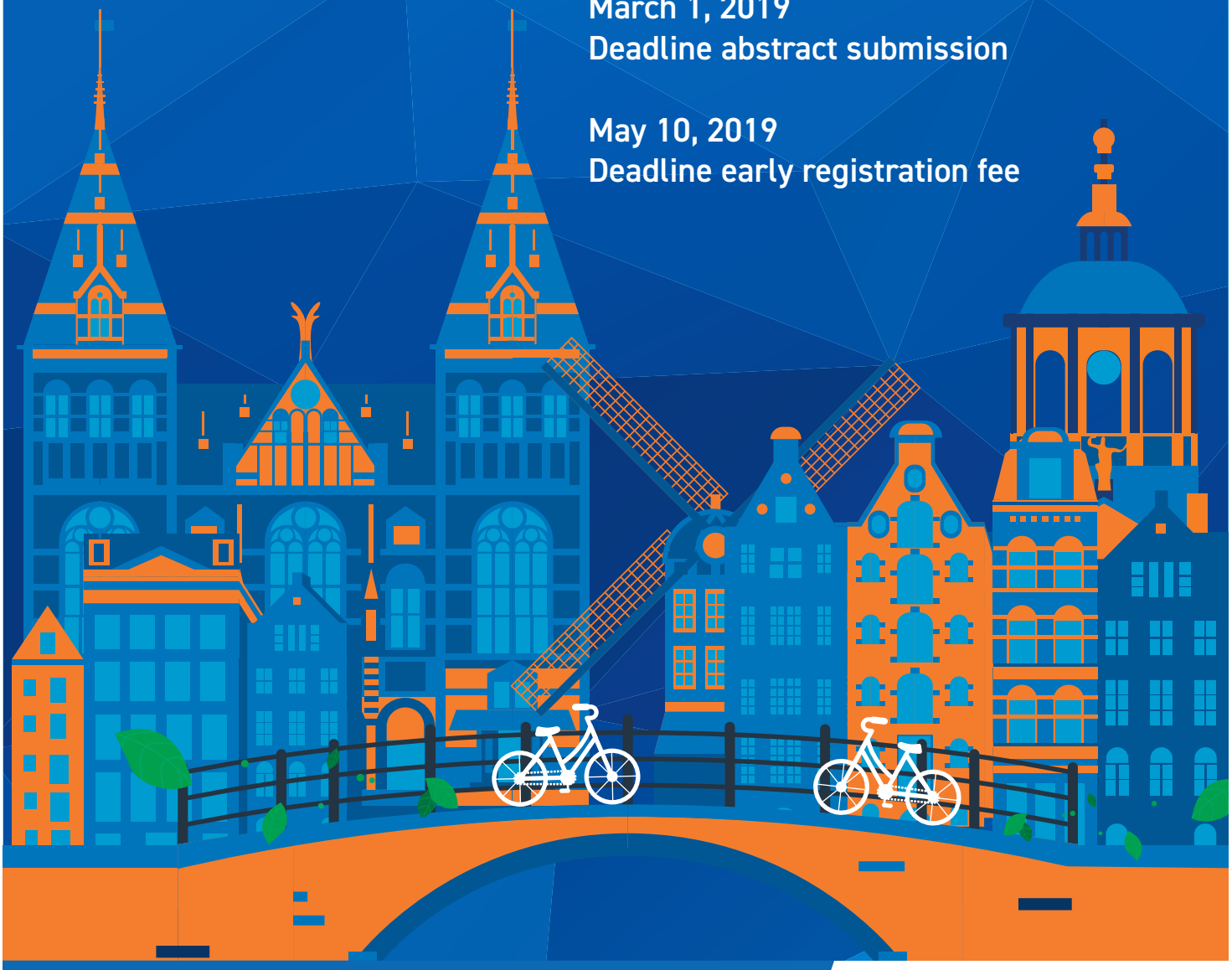

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